STRUCTURAL CHARACTERISATION OF TENDONS

Uncovering Characteristics of Human Tendons and Ligaments Commonly Injured or Used as Grafts in Orthopaedic Surgery.

HOLY TENDON, BATSAM!

Faculty of Medicine and Health

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HOLY TENDON, BATSAM!

STATEMENT OF ORIGINALITY

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged. The contributions of other researchers, assistance received in preparing this thesis and all sources have been acknowledged. The major contributions due acknowledgement are as follows:

I hereby acknowledge that the intellectual content of this thesis is my own. Project design was facilitated by collaboration with my supervisors, A/Prof. Elizabeth Clarke (EC), Prof. Christopher Little (CL) and Dr. Carina Blaker (CB), along with Dr. Dylan Ashton (DA). I conducted review of the literature, with preparation of the manuscript presented in Chapter 2 aided by CB, EC, CL and DA. Dr. Patrick Haubruck (PH), Dr. David O'Briain (DO) and Dr. David Chang (DC) performed the retrieval of the specimens included in this study, with myself, DA, EC and CB aiding with specimen preparations. I prepared all histological samples included in this thesis. Ms. Susan Smith (SS) conducted all histological processing of the tissues. I performed all histological sectioning and staining with guidance from SS. Ms. Yolanda Liu (YL) scanned all slides for archival records and histological scoring. Miss Elizabeth Faulkner (EF) and Mr. Thomas Fong (TF) aided with blinding of histological slides. I developed the new scoring system and refined this in consultation with CL, CB, EC and DA. Myself, CL, CB and EC performed histological scoring to test the new scoring system. I performed all subsequent histological scoring for data acquisition. I performed all GraphPad Prism analyses with assistance from CB, and CB wrote the code for all other analyses performed in Stata. I produced all data figures and tables. I wrote this thesis with feedback from my supervisors, EC, CL and CB.

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ABSTRACT

Tendons play a major role in musculoskeletal functioning. Tendon and ligament disease (including degeneration, rupture and traumatic injury) affects multiple different tendons throughout the body and is increasing in prevalence. Many patients require surgical reconstruction or tendon/ligament replacement with graft tissue, but long-term outcomes of such treatment is poor. The key to improving treatment outcomes is knowledge of underlying differences in physiology of different tendons/ligaments that are commonly injured and/or used for grafting. As knowledge of "normal" tendon structure is lacking, the aim of this project was to compare the structures of normal (healthy) human cadaveric male and female tendons and ligaments of the upper and lower limbs using consistent methodologies and donor tissue source. However, as current histopathological scoring systems for tendon research focus on classification of pathology rather than utility as a generalised histo-morphologic characterisation tool, this project also aimed to develop a novel scoring system suitable for charactering differences between normal tendons/ligaments.

This study incorporated histological and ultrastructural investigation conducted on 48 different tendons and ligaments harvested from left or right (randomised), upper and lower limbs of 8 human cadaveric tissue donors. These specimens form one component of the host labs' broader project to create an open access database of normal characteristics of different joint critical tissues in the human appendicular musculoskeletal system. Initially, these tendon/ligament specimens – as well as other archival human/animal histological tendon samples available from the host lab – were utilised to develop and test a new histological scoring system for tendon/ligament characterisation. The outcome was the proposal of a novel system (a "standardised scoring suite") that forms the foundation to improving future practice in tendon histological exploration.

This new scoring system was then applied to characterise the structural properties of the full dataset of human tendons and ligaments. Numerous and varied observations were made in relation to differences/similarities in tendon/ligament structural characteristics. Cell characteristics, architectural features, proteoglycan content, interfascicular regions and crimp properties all exhibited variations between different tissues. Notably, tendons of the shoulder – particularly those of the rotator cuff complex – as well as ligaments of the knee commonly presented with varied characteristics compared to other tendons of the upper and lower limbs respectively, including greater proteoglycan presence and shorter crimp wavelength. Evidently, the rotator cuff tendons showed similar characteristic patterns to those of the knee ligaments. Furthermore, the current research facilitated an improved appreciation of the variations and differences between the interfascicular regions and how these may influence the overall characteristics of tendon/ligament hierarchical structure. A new structural classification was proposed and opens scope for future research to further explore and define tendons differences and structural niches.

The findings from the characterisation element of this investigation provide critical information for understanding the normal structure and physiology of tendons and ligaments, aiding research

into appropriate prevention and repair strategies for various injuries or pathologies. Furthermore, the data collected contributes to the host project, which aims to create an extensive, open access database of the morphometric, biomechanical, biochemical, histological and ultrastructural properties of normal human upper and lower limb joint tissues, including cartilage and enthesis tissue, as well as tendons and ligaments that are either commonly reconstructed or used as graft tissues for reconstruction.

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I dedicate this work to Godma Dianne.

You were the first person to be excited about my decision to take on this journey, and the only one who didn't get to see me through to the end. I will think of you every time I pick up a needle and stitch.

STATEMENT OF CONTRIBUTIONS

The preparation work for this thesis was conducted by the following personnel. Contributors are identified by initials in underlined-type. See *Contributors' Names* for identifications. All major aspects of the research for this thesis were conducted by the author (**SH**), with contribution and collaboration work acknowledged where appropriate.

- Project design was conceptualised by **SH**, EC, CB, CL, DA and NH.
- Review of the literature for all chapters was conducted by **SH**.
- Literature Review Manuscript (Chapter 2): manuscript conception, literature review and drafting of the manuscript was conducted by **SH**, CB, DA, CL, and EC.
- Retrieval of the tendons was conducted by PH, DO and DC. Specimen preparation for research outcomes conducted by **SH**, DA, CB, and EC with assistance from prior research students. Sample preparation for histological outcome was conducted by **SH**.
- Histological processing and embedding was conducted by SS.
- Sectioning, staining and mounting of tissues was conducted by **SH**.
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- Blinding of histological slides was conducted by EF and TF. Automated blinding of digital scans/images was carried out by **SH**.
- Development of the new histological scoring system was conducted by **SH** in collaboration with CL, CB, EC and DA.
- Scoring for testing the new system was conducted by **SH**, EC, CB and CL.
- All scoring for data acquisition in Chapters 5 and 6 was conducted by **SH**.
- All statistical analyses were conducted by CB and **SH**.
- All other content in this thesis was conceptualised and prepared by **SH**. All figures, tables and diagrams were prepared by **SH**. Hand-drawn sketched were created by **SH**. All histological and electron microscopy images of in-house samples were captured by **SH**. BW aided stitching of electron microscopy images (project in collaboration with AT, with assistance from CH).
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AUTHORSHIP ATTRIBUTION STATEMENT

This thesis contains material published in the *Journal of Orthopaedic Research* (JOR).

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I conducted the literature search and initial review. All authors contributed equally to manuscript conception, literature review and drafting of the manuscript. All authors were involved in the identification of gaps in knowledge at the time of submission and discussed directions for future research to address these deficiencies. Permission to include the manuscript material in this thesis was granted by the corresponding author (Prof. Christopher Little).

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As the supervisors for the candidature upon which this thesis is based, we can confirm that the authorship attribution statement is correct.

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OVERVIEW OF THESIS

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THE MUSCULO-TENDINO-SKELETAL SYSTEM

Tendons In Focus – Background Information.

~ Chapter 2 ~

TENDONS IN REVIEW

Structural Variations of Tendons: A Systematic Search and Narrative Review of Differences Between Tendons, Regions Within Tendons, Sex and Age

~ Chapter 3 ~

UNDER THE MICROSCOPE

Protocols and Methodology

~ Chapter 4 ~

STANDARDISED SCORING SUITE

A Review of Published Scoring Systems & The Proposal of a Novel Scoring System for the Histological Assessment of Tendons/Ligaments

~ Chapter 5 ~

GAINING THE UPPER HAND IN TENDON RESEARCH

Histological Characterisation of Human Tendons of the Upper Limb

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GETTING ANOTHER LEG UP IN TENDON RESEARCH

Histological Characterisation of Human Tendons (and Ligaments) of the Lower Limb

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I'LL BE AT THE BACK

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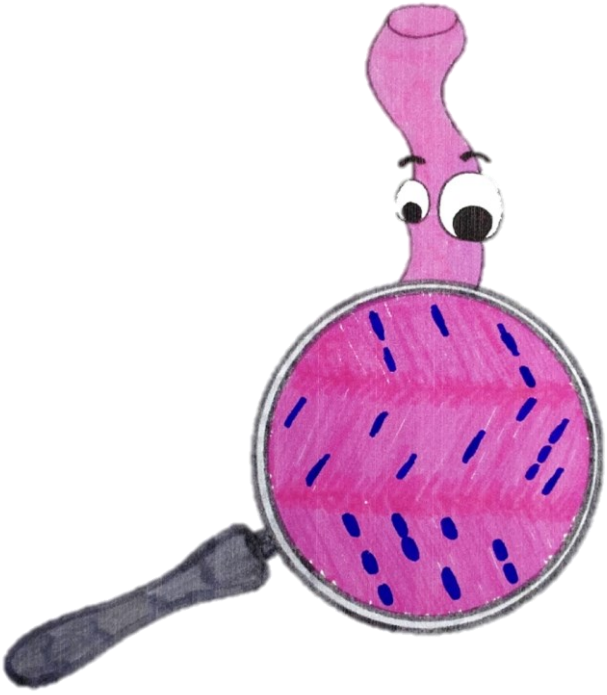

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LIST OF ABBREVIATIONS

Tendon/Ligament Tissues

ACL	Anterior Cruciate Ligament
APL	Abductor Pollicis Longus
AT	Achilles Tendon
BPTB	Bone-Patellar-Tendon-Bone
BrR	Brachioradialis
CaCL	Caudal Cruciate Ligament
CDET	Common Digital Extensor Tendon
CrCL	Cranial Cruciate Ligament
DBT	Distal Biceps Tendon
DDFT	Deep Digital Flexor Tendon
DTT	Distal Triceps Tendon
ECRB	Extensor Carpi Radialis Brevis
ECRL	Extensor Carpi Radialis Longus
ECU	Extensor Carpi Ulnaris
EDC-2/3/4/5	Extensor Digitorum Communis – 2^{nd} / 3^{rd} / 4^{th} / 5^{th} Digit
EDM	Extensor Digiti Minimi
EHL	Extensor Hallucis Longus
EIP	Extensor Indicis Proprius
EPB	Extensor Pollicis Brevis
EPL	Extensor Pollicis Longus
FB	Fibularis (Peroneus) Brevis
FCR	Flexor Carpi Radialis
FCU	Flexor Carpi Ulnaris
FDL	Flexor Digitorum Longus
FDP-2/3/4/5	Flexor Digitorum Profundus – 2^{nd} / 3^{rd} / 4^{th} / 5^{th} Digit
FDS-2/3/4/5	Flexor Digitorum Superficialis – 2^{nd} / 3^{rd} / 4^{th} / 5^{th} Digit
FHL	Flexor Hallucis Longus
FL	Fibularis (Peroneus) Longus
FPL	Flexor Pollicis Longus
GT	Gracilis Tendon
IS	Infraspinatus
LCL	Lateral Collateral Ligament
LDET	Long Digital Extensor Tendon
LHBT	Long Head Of Biceps Tendon
MCL	Medial Collateral Ligament
PCL	Posterior Cruciate Ligament
PL	Palmaris Longus
Plt	Plantaris Tendon
PT	Patellar Tendon
QT	Quadriceps Tendon
RFT	Rectus Femoris Tendon
SDFT	Superficial Digital Flexor Tendon
SSC	Subscapularis
SSP	Supraspinatus
ST	Semitendinosus Tendon
TA	Tibialis Anterior
TP	Tibialis Posterior
VIT	Vastus Intermedius Tendon

Other Abbreviations

AB/PAS	Alcian Blue / Periodic acid schiff
AI	Artificial Intelligence
BMI	Body Mass Index
BPTB	Bone-Patellar-Tendon-Bone
COMP	Cartilage Oligomeric Matrix Protein
CSA	Cross-sectional Area
DIC	Differential Interference Contrast Microscopy
ECM	Extracellular Matrix
EM	Electron Microscope
FM	Fascicular Matrix
GAG	Glycosaminoglycan
H&E	Haematoxylin & Eosin
HALT	Histological Assessment of Ligament(s) and Tendon(s)
HPF	High-Power Field
HMDS	Hexamethyldisilazane
hvEM	High Voltage Electron Microscopy
ICC	Intraclass Correlation Coefficients
IFM	Interfascicular Matrix
IFR	Interfascicular Region
IFS	Interfascicular Space
IHC	Immunohistochemistry/Immunohistochemical
JOR	Journal of Orthopaedic Research
MCPJ	Metacarpophalangeal Joint
micro CT	Micro Computed Tomography
MLM	Multiphoton Laser Microscopy
MTJ	Musculotendinous Junction
N_{aq}	Liquid Nitrogen
O.C.T.	Optimal Cutting Temperature
OM	Optical Microscopy (e.g. light, polarised, confocal, etc.)
ORS	Orthopaedic Research Society
PG	Proteoglycan
pSHG	Polarisation-resolved Second Harmonic Generation Microscopy
PSR	Picrosirius Red
ROI	Region(s) of Interest
SEM	Scanning Electron Microscope
SFM	Scanning Force Microscopy
SHG	Second Harmonic Generation Microscopy
SSS	Standardised Scoring Suite
TB	Toluidine Blue
TEM	Transmission Electron Microscopy
UTS	Ultimate Tensile Strength
αSMA	Alpha-smooth Muscle Actin
κ	Kappa

Scoring System Outcomes (Chapter 4)

b1CS	Cell Density Score (box 1)
b2CS	Cell Density Score (box 2)
b3CS	Cell Density Score (box 3)
CSpool	Cell Density Score (pooled)
CDenS	Cell Density Score (average)
CC1Sc	Cell Count-to-Score conversion (box 1)
CC2Sc	Cell Count-to-Score conversion (box 2)
CC3Sc	Cell Count-to-Score conversion (box 3)
CCSpool	Cell Count-to-Score conversion (pooled)
b1CMor	Cell Morphology (box 1)
b2CMor	Cell Morphology (box 2)
b3CMor	Cell Morphology (box 3)
CMorpool	Cell Morphology (pooled)
CMor	Cell Morphology (mode)
CArr	Cell Arrangement
FMOrg	FM Organisation
FlBun	Fibrillar Bundling
FrSepF	Fibre Separations Frequency
FrSepT	Fibre Separations Type
IFRTypSs	IFR Proportion Score (scored)
IFRTypS	IFR Proportion Score (count-to-score conversion)
IFMDen	IFM Density
AdiPres	Adipocyte Presence
AdiLoc	Adipocyte Location
VasLoc	Vascular Location
FMPG	FM Proteoglycan Presence
IFRPG	IFR Proteoglycan Presence
PGIntAv	Average PG Staining Intensity
PGIntMx	Maximal PG Staining Intensity
PGMxLoc	Maximal PG Staining Location
ChPre	Chondrocyte-Like Cell Presence
ColAliNP	Collagen Alignment (Non-Polarised)
ColAliP	Collagen Alignment (Polarised)
CriPre	Crimp Presence
CriAli	Crimp Alignment
CriWav	Crimp Wavelength (predominant)
CriAre	Crimp Wavelength Area (predominant)
b1CC	Cell Count (box 1)
b2CC	Cell Count (box 2)
b3CC	Cell Count (box 3)
CDen	Cell Density (average)
CDis	Cell Distribution
IFMC	IFM Count
IFSC	IFS Count
IFRC	IFR Count (sum IFM+IFS Counts)
IFMF	IFM Frequency
IFSF	IFS Frequency
IFRF	IFR Frequency
IFRTyp	IFR Type Proportion (IFM/IFR Counts)
VasPres	Vascular Presence (count)
VasPresP	Vascular Presence (% positive)

~ CHAPTER 1 ~

THE MUSCULO-TENDINO-SKELETAL SYSTEM

TENDONS IN FOCUS – BACKGROUND INFORMATION

"You're seriously saying that there's nothing special about us?"

~ Joker, The Lego Batman Movie (2017) ~

1.1 Overview

The following chapter outlines the basic structure of tendon and outlines the topics of subsequent chapters and introduces the themes covered in this thesis.

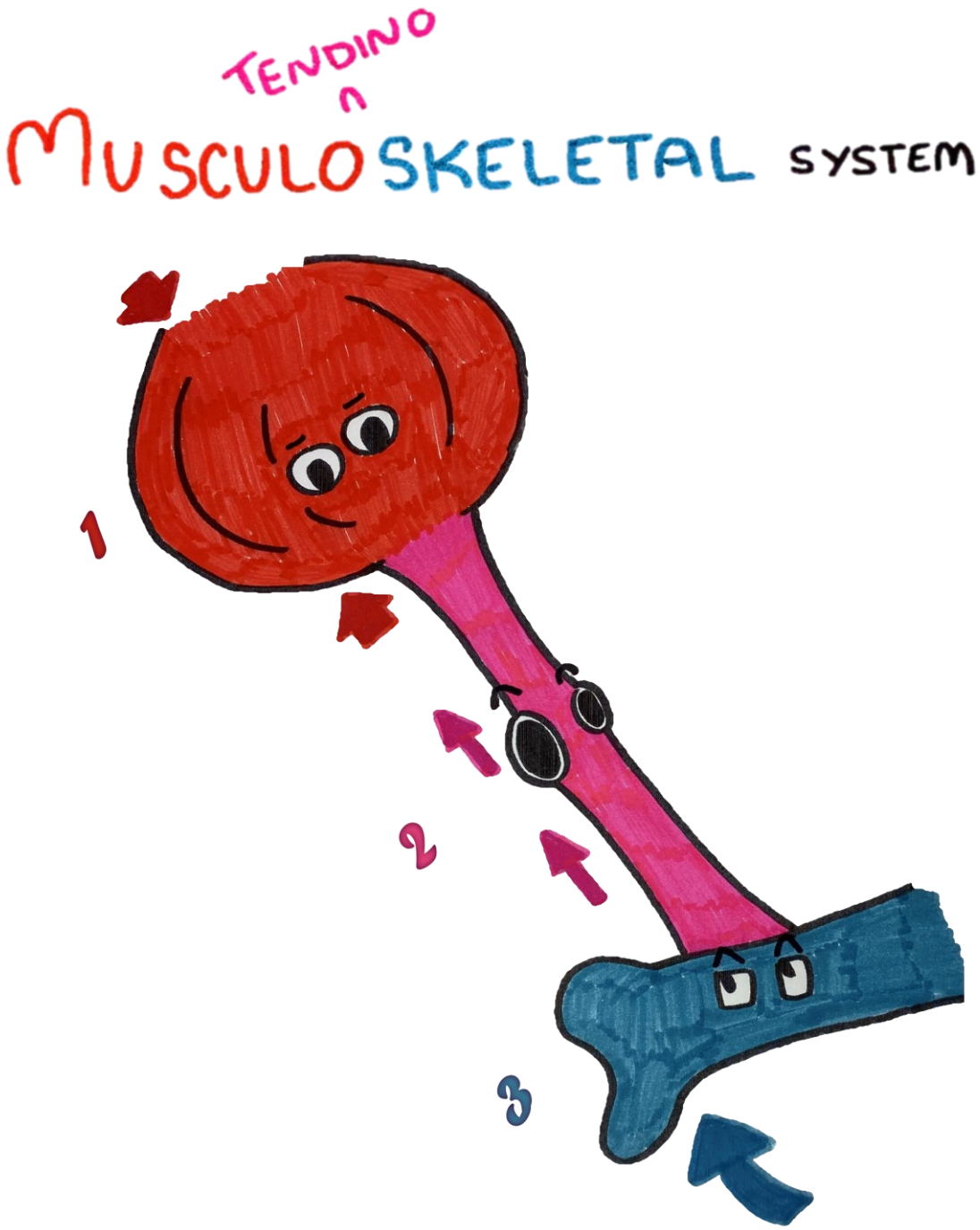


Figure 1.1.A The "Musculotendinoskeletal" System. Contraction of muscle (1) loads tendon which transfers force (2) to the skeletal system (3) for movement.

1.2 Introduction

1.2.1 Tendons in the Musculoskeletal System

Tendons transmit force from muscle to bone, playing an important role in the musculoskeletal system[1-6]. Different anatomical tendons exhibit different roles and experience varied loading environments. Appendicular tendons in particular have different functional requirements and vary in morphology and gross anatomical structure throughout the body as well as between species. The unique and intricate structure of the tendons of the hand exemplify tendons of differing functional mechanisms. The long digital flexors and the flexors and extensors of the wrist act in a 'simple' manner, exhibiting typical 'rope-like' morphology – muscular contraction pulls the associated tendon's insertion towards the muscular origin and shortens the angle of intermediary skeletal joints[5]. The digital extensors (primarily, Extensor Digitorum Communis), on the other hand, have much more complex anatomical structures, containing cross 'slips' between the separate digit tendons, regions where the tendons fan out into aponeurosis-like structures that have offshoot slips of insertions and receive insertion from other tendons – all resulting in complex force transference patterns occurring throughout the tendon[5]. However, the assessment of distinctly different tendons in humans extend little beyond gross morphology and anatomical function. The concept of 'functionally distinct' tendons is prominent in work on quadruped animals, particularly in equines. Primarily, the distinction between "energy-storing" versus "positional" tendons is widely reported[1,7-10]. Numerous investigations reveal that functionally distinct tendons in fact exhibit different material properties[11-14] and metabolic responses[15,16] likely contributing to differing underlying structural and compositional variations[11,14,16-18]. Surprisingly, the structural delineation between tendons and ligaments (structures which connect bone-to-bone and restrict skeletal displacement in joints) is also limited. Although some structural and proteomic differences have been explored[19,20], these tissues are often compared without recognition of their major biomechanical differences. Specific exploration of tendon versus ligament structure is beyond the scope of this thesis.

Whether occurring in acute or chronic scenarios – be it due to physical activity, traumatic events, or progressive degeneration – there is a high incidence of injury across many different tendons/ligaments[21-28]. For instance, the Accident Compensation Corporation in Aotearoa/New Zealand reported over a 6-year period a total of 1,112,007 claims regarding tendons/ligament injuries, costing over $1.4 billion NZD[23]. The shoulder region incurred the most tendon/ligament injuries (33% of claims), followed by the knee (25% of claims) and then the finger, thumb and wrist (19% of claims)[23]. Thorpe *et al.* (2010)[29] addresses the injury incidence discrepancy between functionally distinct equine tendons and highlights that deeper understanding is needed to explore different mechanisms which underpin this. Furthermore, demographic considerations are also important to explore, where age, sex and lifestyle influences likely prove significant in the epidemiology of tendon/ligament injury. Evidently, tendon structure changes with maturation and aging[30-33] – such as a decline in cell population[34] and collagen integrity[35] – although the incidence of injury not only follows trends of degradation with aging but also sees greater prevalence pertaining to injury in response to activity-induced scenarios (sports, physical labour,

etc.) and less sedentary lifestyles/life stages[21-23,25,26,36]. Despite varied tissues being affected by different presentations of injury, disease or pathology, knowledge on the aetiological causes for different disease progression and susceptibility amongst different tendons is lacking due to a poor conception of the normal/healthy status of the tissue and in response to different factors.

With low innate regenerative capabilities[37-39], tendons/ligaments that do experience severe injury, pathology or disease may require surgical repair or reconstruction with a tendon graft in order to reinstate normal function[40-46]. For example, in Australia, Anterior Cruciate Ligament (ACL) reconstruction alone had an incident of over 77 per 100,000 population. While many different tendons are enlisted as grafts for the reconstruction of other soft-tissues[47-65], the success of different tendon grafts varies greatly, particularly for those that are used in human ACL reconstruction[66,67]. The underlying tissue properties which may explain graft suitability or clinical outcomes are underexplored. Considering the importance of structure-function relationships in the study of biomechanics, appreciation of the native structure of the tendon graft should be a consideration. Until recently[68], a wholistic appreciation of known structural variations of different tendons has not been explored, with much of the literature focusing on only a few tendons or limited characteristics. Overall, there is little known about variations between functionally different tendons/ligaments and what implications such variability has on clinical outcomes in relation to injury and disease, or its performance with or as a replacement graft.

Despite knowing there are variations in physiological, biomechanical and clinical functions, the unique structural characteristics of the tissues performing these have not previously been systematically evaluated across a wide range of unique anatomical tendons or across a range of species with differing biomechanical demands, or humans with differing physiological status. Defining the normal, healthy structure of tendons with consideration of potentially influential variables (specific tendon, region within tendon, age, sex, species, or other biological differences) is critical for understanding tendon biomechanical properties, physiological mechanisms, and pathophysiology and associated clinical implications.

1.2.1.1 *The Basic Tendon*

In vertebrates, tendons are said to have one of the highest extracellular-matrix (ECM) to cell ratios of all tissues[69]. The ECM structure of tendon (and ligament) tissue is responsible for it's specialised mechanical role in musculoskeletal functioning. The dry weight of tendon is 65-80% collagen, typically 90% of which on a weight/mass basis is fibrillar collagen type-I[70]. This fibril-forming collagen contributes to tendon tensile strength/load resistance and limited stretch/elastic capacity[3,69,71-74]. The remaining matrix components include other fibrillar and fibril-associated collagens, elastin, and a variety of glycoproteins (proteins covalently bonded to carbohydrates) and proteoglycans (PGs; a subclass of glycoproteins containing glycosaminoglycan [GAG] chains). These components play essential roles in tendon structure-function properties including viscoelastic behaviours of the tissue as well as physiological processes such as fibrillogenesis, matrix interaction, organisation and function, and mechanisms of pathology/healing[69,75-84].

Examples of "minor" tendon constituents include collagens type-III and -V which are involved in higher order collagen structural formation/organisation (fibrillogenesis) and healing processes[75,76]. Glycoproteins are found throughout the tendon hierarchy and influence tendon

structure and function[69,85-87], e.g. cartilage oligomeric matrix protein (COMP) is abundant throughout tendon structure and aids in collagen fibrillogenesis[85,88,89]. Proteoglycans constitute 1-5% of tendon dry-weight[77], the most abundant of which (≈ 80%) is decorin[76,78], which regulates fibril formation and structure[75,79]. Another proteoglycan, lubricin, facilitates fascicular sliding, and is mostly expressed in regions such as the interfascicular matrix (IFM)[69,76,80,81,90]. In general, GAG content can be predictive of mechanical properties[82,83] and contributes to fibrillogenesis and fibril remodelling, interaction, organisation and functional attributes such as compression resistance in specific tendon regions[84,91].

The prominent cell population in tendons consist of fibroblasts – known as tenocytes – which are long, spindle-shaped cells arranged longitudinally between the fibres of the tendon and in the surrounding IFM[74,92,93]. Other cells are present within tendon, with recent single cell RNA sequencing (scRNA-seq) studies shedding more light on the varied cell populations and their role in tendon growth, maturation, injury and healing[94,95]. De Micheli *et al.* (2020)[96] identified 11 distinct cell populations within mouse Achilles tendon, including three sub-populations of fibroblasts. Single-nucleus RNA sequencing (snRNA-seq), spatial transcriptomics, and imaging exploration of cell profiles in the human Semitendinosus Tendon unveiled 12 distinct cell types with in the tendon structure, including various supply structure cells (nerve cells and lymphatic and vascular endothelium), other support cells (adipocytes, immune cells, satellite cells, mural cells), skeletal muscle cells (fast-twitch, slow twitch and transitional), as well as two distinct fibroblastic cells (MKX+ and PDGFRA+)[97], with spatiotemporal localisation of these cell clusters noted within the tendon area.

1.2.1.2 *Structural Hierarchy*

The tendon structure itself is hierarchically organised, and although there are differing theories on the exact organisation of the collagenous architecture, they are typically divided into six levels describing groupings of increasingly smaller subunits (Figure 1.2.A). In descending order of size these are: the tendon unit; fascicles; fibres; fibrils; microfibrils; and the collagen molecule, tropocollagen[29,69,74,98-100]. The tendon structure is covered in a connective tissue membrane, the epitenon, which is continuous with the endotenon surrounding the fascicles, often referred to as the aforementioned IFM[101-103]. Crimp, a waveform characteristic of both collagen fibres and fibrils, is a unique feature of the tendon structure that contributes to the loading mechanics of the tissue[73,100,104-108] (Figure 1.2.A). Overall, this complex structural architecture provides tendons with their specialised functional properties.

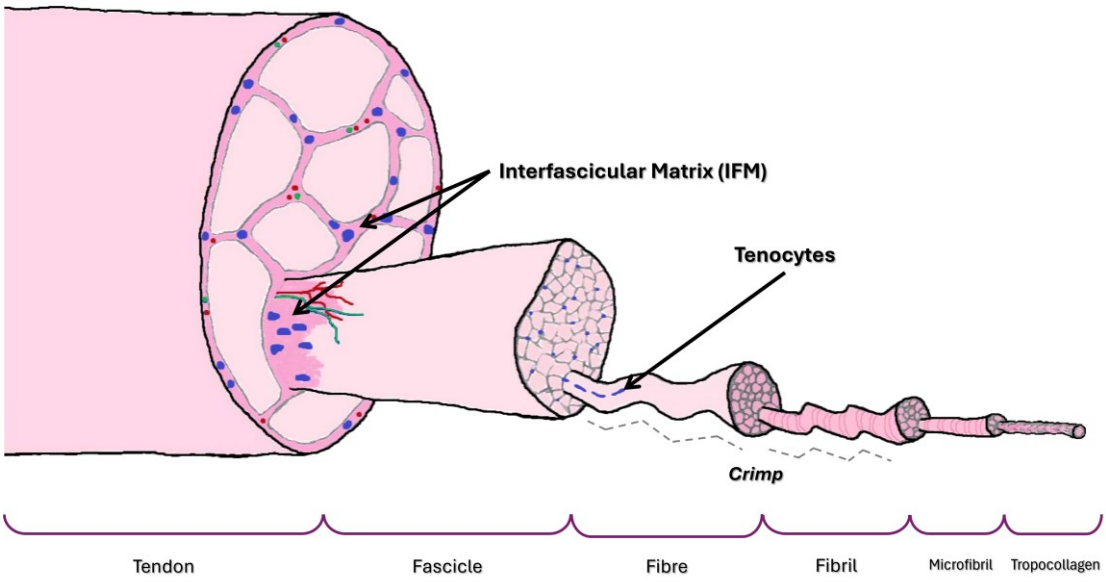
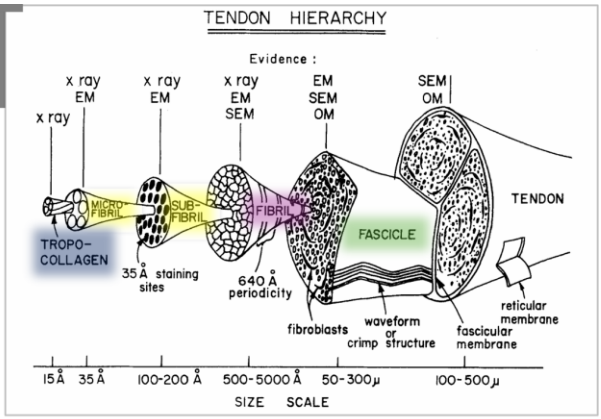


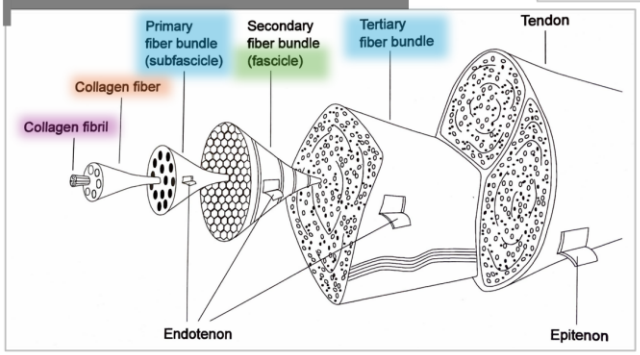
Figure 1.2.A Typical depiction of the tendon hierarchical structure. Navy blue indicates cells, including fascicular (primarily tenocytes) and interfascicular populations. Red and green lines indicate presence of neurovascular structures (interfascicular). Pink stripes on micro-/fibrillar levels depicts the characteristic 64-68nm[6] banding of Type-1 collagen fibrils.

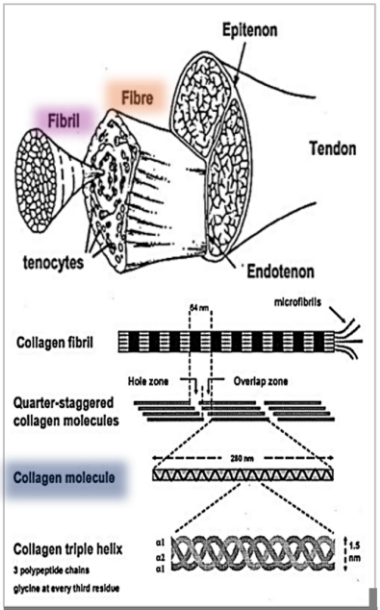
The prevalence of interchanging terminology used to describe the hierarchical levels of the tendon structure within the literature is important to note. Common discrepancies in nomenclature include: 1) "endotenon" versus "endotendon" versus "endotendiuneum" versus "peritendineum internum" versus "fascicular membrane" versus "interfascicular matrix"; 2) "fascicle" versus "sub-tendon"; 3) "fibre bundle" versus "sub-fascicle"; 4) "fibre bundle" versus "fascicle"; and 5) "fibril subunit" versus "microfibril"[6,29,69,70,74,98-100,106,109-116]. Figure 1.2.B identifies some of these nomenclature variations, but also indicates different perceptions of the overall hierarchical organisation. Some discrepancies likely arise from reflection on different tendons or species from which the structures have been defined. For example, the term "sub-tendon" is reserved for tendon such as the Achilles tendon which contains the anastomosis of two or more tendons arising from multiple muscle bellies. Furthermore, there are often discrepancies in describing and classifying the various levels of connective tissue sheaths that surround a tendon or tendon group – namely the terms "reticular membrane", "epitenon/epitendon", "paratenon/paradendineum" and "peritenon/peritendon/peritendineum externum" – which are poorly defined and often used interchangeably or in reference to different hierarchical levels[70,101,106,112-114,117-119]. For clarity and simplicity, the term ***paratenon*** is adopted in this thesis as a generalised terminology for tissue structures external to the tendon unit itself (inclusive of any structures external to the tendon-proper), while the following other hierarchy terminologies used are: "***tendon***", "***interfascicular matrix***" (*see caveat/note below; *Section 1.2.1.2.1*), "***fascicle***", "***fibre***", "***fibril***", "***microfibril***", and "***tropocollagen***." The general definitions of these hierarchical levels are outlined below. It is noted that further exploration into tendon hierarchical organisation occurs throughout this thesis which further informs these definitions, some of which are also detailed below.

i – Kastelic et al. (1978)
Generalised tendon structure following detailed exploration of rat tail tendons

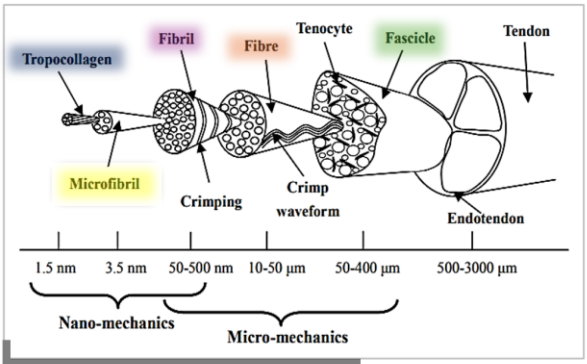


ii – Józsa and Kannus (1997)
Generalised tendon structure

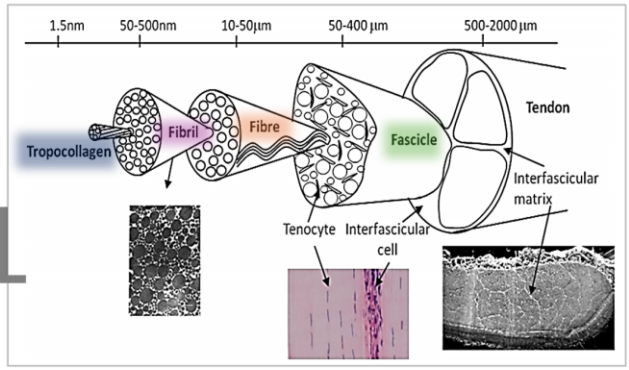




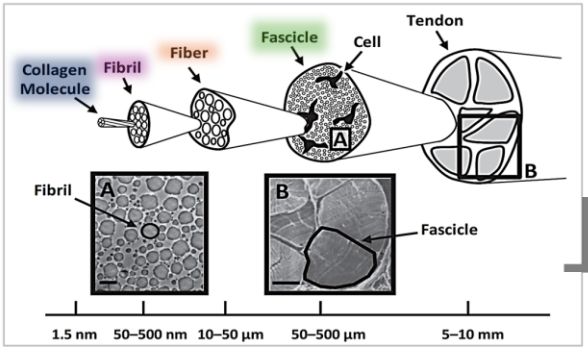
iii – Riley (2004)
Generalised tendon structure



iv – Screen (2009)
Generalised tendon structure



v – Screen et al. (2015)
Generalised tendon structure



vi – Szczesny and Elliott (2014)
Generalised tendon structure

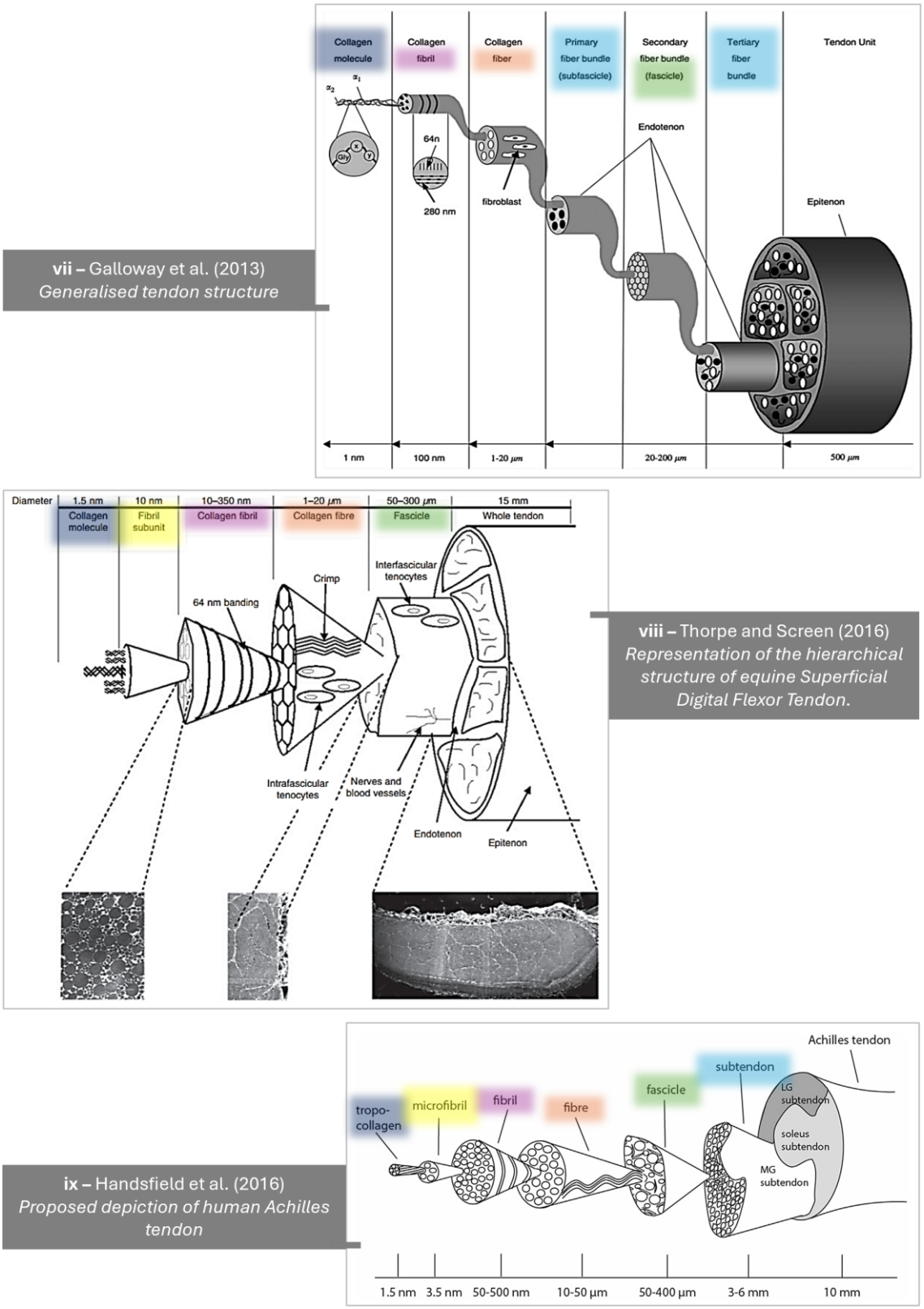


Figure 1.2.B Examples of previously published depictions of the tendon hierarchical structure[6,69,74,98-100,112,120,121]. Notice discrepancies in nomenclature such as: "microfibril" (**i**, **iv**, **ix**; yellow)[74,112,121] and "subfibril" (**i**; yellow)[112] versus "fibril subunit" (**viii**; yellow)[120]; "endotenon" (**iv**,**viii**)[74,120] versus "interfascicular matrix" (**vi**)[69] despite being between similar authors; depiction of additional levels such as "nth fibre bundle" (**ii**,**vii**; blue)[6,99] and "sub-tendon" (**ix**; blue)[121]; and even similarly depicted structures being denoted "fibril" (**i**; yellow)[112], "fascicle" (**ii**; green)[6] or "fibril" (**iii**; pink)[100] across representations. *All imaged reprinted with permission; see Appendix Section 9.2.1).*

1.2.1.2.1 Defining Hierarchical Levels

One novel terminology adopted for this project was the generalisation of inter-fascicle areas as the newly coined "interfascicular region (IFR)" *in place of "interfascicular matrix" to distinguish between inter-fascicular areas with notable matrix/tissue structures versus those which were observed to have negligible or apparently absent matrix/tissue structures between fascicles (Figure 1.2.C). The latter, defined as interfascicular space (IFS), is a new concept explored and discussed in this thesis. In brief, such instances of IFS were observed to be indicative of structural characteristics not previously explored or explicitly defined and are likely tied to tissue or species differences present in tendon hierarchical organisation. However, it is recognised that these features may also be instances of indistinguishable matrix presence or artefacts such as tissue splitting or loss of matrix during tissue processing.

Henceforth, this thesis defines the structure of the tendon-proper (also referred to as the "tendon area") as being comprised of two primary sub-regions (excluding paratenon):

(1) The fascicular matrix (FM): comprised of fascicle units which are hierarchically organised, forming a highly dense connective tissue acting as the primary load-bearing tendon structure. The collagenous FM is hierarchically structured in subunits of increasing size as follows[121-123]:

- The triple helical collagen molecule (tropocollagen) – primarily fibrillar collagen type-I
- The microfibril – aggregates of collagen molecules
- The fibril – formed by collections of microfibrils; typically 50-500 nm in diameter
- The fibre – bundles of fibrils; typically around 10 μm in diameter
- The fascicle – bundles of fibres; the major hierarchical unit second to the tendon itself.

(2) The interfascicular region (IFR): the regions between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance, and thus further categorised as either:

- Interfascicular matrix (IFM), IFRs with the presence of discernible connective tissue between/dividing fascicle units.
- Interfascicular space (IFS), IFRs with an absence of discernible connective tissue between/dividing fascicle units; noting that interfascicular cells or supply structures may still be present.

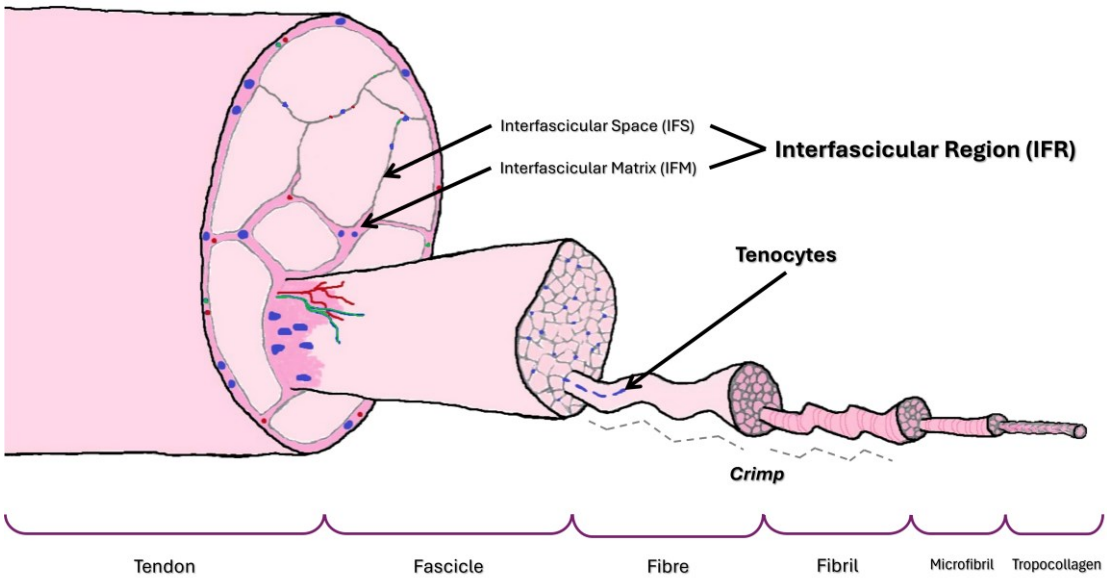


Figure 1.2.C Representative depiction of the tendon hierarchical structure as appreciated in this thesis, including the newly coined terminology for the Interfascicular Region (IFR). Navy blue indicates cells, including fascicular (primarily tenocytes) and interfascicular populations. Red and green lines indicate presence of neurovascular structures (interfascicular). Pink stripes on micro-/fibrillar levels depicts the characteristic 64-68nm[6] banding of Type-1 collagen fibrils.

1.3 Overarching Aims and Directions

"Variability is the law of life, and as no two faces are the same, so no two bodies are alike, and no two individuals react alike and behave alike under the abnormal conditions which we know as disease" ~ William Osler (Johns Hopkins Hospital). In parallel with embracing the importance of organism-level variability, it is critical to understand variations in different tendon structures to effectively understand the unique adaptive changes that occur in response to injury, disease and intervention of these dynamically important structures.

The overall aim of this thesis is to develop and apply a novel histological scoring system to characterise the structure of different human tendons/ligaments.

Chapter 2 aimed to conduct an in-depth exploration of the field to highlight current understanding of tendon variability and the prevalent gaps in knowledge with a focus on tendon structural characterisation via microscopy modalities. A systematic search with narrative review of current literature revealed the need for a comprehensive and standardised study of normal/healthy tendon characteristics using a broad range of outcomes across a large array of different tendons. This finding underpins the rationale for the overarching aim of this thesis: to conduct a standardised histological exploration of the structure of a large range of normal human appendicular tendons.

Chapter 3 details the protocols used to collect and process the wide array of human tendon/ligament samples for histological studies in this thesis.

Chapter 4 includes further literature review, which revealed that currently available scoring systems for tendon histological assessment focus on classification of disease state and are

inadequate for differentiating normal variation. Consequently, this chapter aimed to develop a new histological scoring system capable of differentiating structural variation between normal/healthy tendon/ligament tissue.

Chapter 5 and ***Chapter 6*** aimed to characterise the structural properties of different upper limb tendons and lower limb tendons, respectively, using the newly defined histological scoring system.

Finally, ***Chapter 7*** brings together findings from this thesis and outlines areas requiring further attention and exploration in order to advance research and understanding in future.

~ CHAPTER 2 ~

TENDONS IN REVIEW

STRUCTURAL VARIATIONS OF TENDONS: A SYSTEMATIC SEARCH AND NARRATIVE REVIEW OF DIFFERENCES BETWEEN TENDONS, REGIONS WITHIN TENDONS, SEX AND AGE

"With all those compartments on your belt, you'd think there would be one for tissues."

~ Alfred, Batman: The Animated Series (1992-95) ~

2.1 Overview

The following chapter consists of a review paper manuscript as submitted to the *Journal of Orthopaedic Research (JOR)* in September 2024. The paper explores the literature revealed from a systematic search conducted between 2022-2024 and addresses gaps in knowledge around the structural (microscopy) comparison of different tendons, intra-tendon heterogeneity, sex differences, age-related changes, and other variables of structural differences.

The systematic search and review of literature was conducted by the student of this thesis (**SH**), with manuscript preparation prepared by **SH** with guidance and assistance from all co-authors. The review was drafted in manuscript form and submitted to the *Journal of Orthopaedic Research* as an invited article. Status of the manuscript review stage as at the time of original submission of this thesis (end of October 2024): *Under Review*.

Amendment: Following initial thesis examination, the manuscript has since been accepted *(28-Jan-2025)*, proofed and published *(26-Feb-2025)* in the *Journal of Orthopaedic Research.*

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DOI: *https://doi.org/10.1002/jor.26060*

Figure 2.1.A Exploration into different tendons of the musculotendinoskeletal system.

2.2 Manuscript

The following pages include the manuscript published with *JOR:*

Hefferan, S.A., Blaker, C.L., Ashton, D.M., Little, C.B. and Clarke, E.C. (2025), **Structural Variations of Tendons: A Systematic Search and Narrative Review of Histological Differences Between Tendons, Tendon Regions, Sex, and Age.** *Journal of Orthopaedic Research. DOI: 10.1002/jor.26060*

The article can be accessed at: https://onlinelibrary.wiley.com/doi/full/10.1002/jor.26060

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Note: At the time of original submission and examination of this thesis (end of October 2024), the manuscript was submitted and under peer review – this version has been relocated to the appendix (*Appendix Section 9.3*).

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Structural Variations of Tendons: A Systematic Search and Narrative Review of Histological Differences Between Tendons, Tendon Regions, Sex, and Age

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ABSTRACT

Tendons are force-transmitting structures which facilitate musculoskeletal functioning. Characterizing variations between different anatomical tendons, regions within tendons, as well as between the sexes and with age can improve understanding of tendon physiology and pathology. A systematic search of the literature was conducted to identify and summarize microscopic structural (histological) variations in normal/healthy tendons in relation to these variables (Tendon, Region, Age, Sex, and Other). Regional differences within individual tendons have been investigated in numerous studies, however investigations comparing histological variations between a range of different tendons are sparse, with most focusing on a few select tendons. When injured, ageing tendons typically have a greater degree of pathological changes than younger tendons, but few studies have documented variations in tendon histology throughout typical (uninjured) ageing or across large age spans. Similarly, sex-related observations of tendon structure are underreported. This narrative review summarizes studies on these topics and explores interactions between these variables, as well as the implications of these in the context of selecting control samples for studies of tendon pathology. Future studies should endeavour to improve knowledge of tendon structural variations—specifically focusing on normal tendons—to facilitate understanding of tendon structure-function relationships, physiological mechanisms involved in tendon damage/healing, and to aid clinical research and practice.

1 | Introduction

Tendons are force transmitting units that facilitate muscular movement of bone and joints, playing an important role in biomechanical and locomotor function [1] contributing to musculoskeletal and overall well-being. Different anatomical tendons exhibit different roles and experience varied loading environments. For example, the distinction between "energy-storing" tendons versus "positional" tendons identifies unique roles for locomotor efficiency in quadrupeds and human/bipedal lower limbs [1–5]. Conversely, upper limb tendons in humans/bipeds play an important role in finer manipulation/control and may portray different gross morphologies dependent on their anatomical role, such as the rope-like digital flexors versus the flatter extensors

Christopher B. Little and Elizabeth C. Clarke contributed equally to this study.

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which fan out into aponeurosis-like structures and exhibit "offshoot slips" between neighboring tendons [6].

Tendons are prone to injury, pathology and degenerative processes [7–9]. Despite the advent of different imaging modalities, histology remains a gold standard for assessing tissue health and disease [10–16]. Understanding normal tendon histological features is critical for gaining insight into the mechanisms of tendon damage, disease, degeneration, and repair [17, 18]. While "healthy tendon" histology is often used as a baseline/control for studies of diseased tendons, knowledge about what constitutes "normal" histology and variations in multiscale aspects particularly of human tendons, are poorly explored. This review examines what is known and knowledge gaps on tendon histological characteristics assessed using microscopy modalities, with particular focus on variation between different tendons, tendon regions, ages, and sexes. A systematic literature search was conducted to identify published records which compared multiscale histological structural characteristics of normal/healthy tendons.

2 | Systematic Literature Search: Structural Variations of Normal Tendons

An electronic database search of PubMed (MEDLINE) was conducted with a focus on comparisons between "variables of interest": *Tendon*; *Region*; *Age*; *Sex*; and *Other*. Keyword search terms were: (tendon OR tendons OR tend*) AND (histolog* OR histochem* OR microscop*) AND (normal OR healthy OR "non-pathological" OR "non pathological") AND (structu*) AND (compar* OR differen* OR variation OR variations). Supporting Information S1: Material A contains the full list of generated search terms and MeSH terms, indexed according to the terms list from the National Library of Medicine. The initial search (September 30, 2022) found 2411 records, including 2 duplicate pairs (2409 unique records). The search was re-run at two subsequent timepoints during manuscript preparation, using the full list of MeSH terms each time to ensure consistency: September 22, 2023 (78 new additional unique articles identified) and August 27, 2024 (73 new additional unique articles identified). A final total of 2560 unique records were identified from the PubMed literature searches (Supporting Information S2: Table 1, Figure 1). Cross-checking of citations/reference-lists in the publications found by the systematic search criteria and alternative search strategies/engines (e.g., Google Scholar) identified a further 109 records for review.

All 2669 publications were screened by a single reviewer (S. H.) as per the workflow in Figure 1. Articles were first screened by title and abstract and excluded as per Table 1. The 216 articles passing title/abstract screening had the full text assessed for exclusion/eligibility as per Table 1 criteria. The 105 eligible papers underwent final review according to the criteria in Table 2 to remove publications with "qualitative-only" ($n = 27$) or "data-only" ($n = 19$) comparisons (these are recorded/summarized in Supporting Information S2: Table 1 for additional reference).

3 | Results

A total of 59 records representing studies which explicitly performed quantitative statistical comparisons for the "variables of interest" were included and summarized in Tables 3 and 4. Table 5 outlines acronyms referred to in this manuscript, and the Tables and Supporting Information Tables.

Of the species utilized, 19 studies investigated human specimens, 23 large quadrupeds (15 equine, 4 bovine, 2 canine, 1 ovine, 1 caprine), 16 murine (12 rat, 4 mouse), and 3 other (2 avian, 1 leporine [rabbit]). Only two studies included more than one species [75, 77] (Table 3). Only Gsell et al. [75] compared species but even here direct comparability was limited as different anatomical tendons were utilized across species.

In terms of tendons/ligaments studied, 34 publications investigated a single tendon, 14 investigated two different tendons/ligaments, 5 investigated three, 3 investigated four, 2 investigated five, and 1 investigated six. Considering the *variables of interest* (Table 4), 20 studies compared different anatomical tendons/ligaments (Supporting Information S3: Table 2a), 22 studies compared within-tendon *Region* differences (Supporting Information S3: Table 2b), 30 *Age*-related differences (Supporting Information S3: Table 2c), 8 *Sex*-differences (Supporting Information S3: Table 2d), and 16 explored *Other* variables (Supporting Information S3: Table 2e).

4 | Between-Tendon Differences

Direct comparison of tendons is limited. Two studies which passed initial eligibility but not second screening, included a large range of tendons but without any quantitative comparisons. Kannus and Jozsa [12] (Supporting Information S2: Table 1; *Noted Exclusions A*) investigated 16 "tendon-groups" (individual tendons not specified) and presented only descriptive findings, while another study comparing 24 different appendicular tendons [79] focussed on fibrocartilaginous presence and did not report statistical analyses regarding similarities/difference (Supporting Information S2: Table 1; *Noted Exclusions B*).

Most studies with quantitative analysis of normal tendon structure (34/59, Table 3) only investigated a single tendon. Where at least two tendons (or tendon/ligament) were included ($n = 25$), only 20 explored specific *Tendon* differences (Table 4, Supporting Information S3: Table 2a). Of these, three papers incorporated only one tendon and compared this with ligament tissue(s) [45, 69, 70]. Weiss et al. [44] compared five tendons and the anterior cruciate ligament (ACL) but did not explicitly perform direct tendon-versus-tendon statistical comparisons and instead reported similarities/differences in correlated variables (e.g., crimp-frequency vs. cell-number). Of the 16 papers that made direct tendon-to-tendon statistical comparisons, the majority (10/16) compare only two tendons, 5 studies comparing three, and 1 study each comparing four/five tendons (Supporting Information S3: Table 2a).

Most studies evaluating between-tendon differences use animals (13/20: equine, bovine, canine, rat), and primarily focus on the comparison of energy-storing versus positional tendons. There are clear differences reported, with extensor/positional tendons generally having lower cell density, larger collagen fibers, larger crimp wavelength, and different protein makeup

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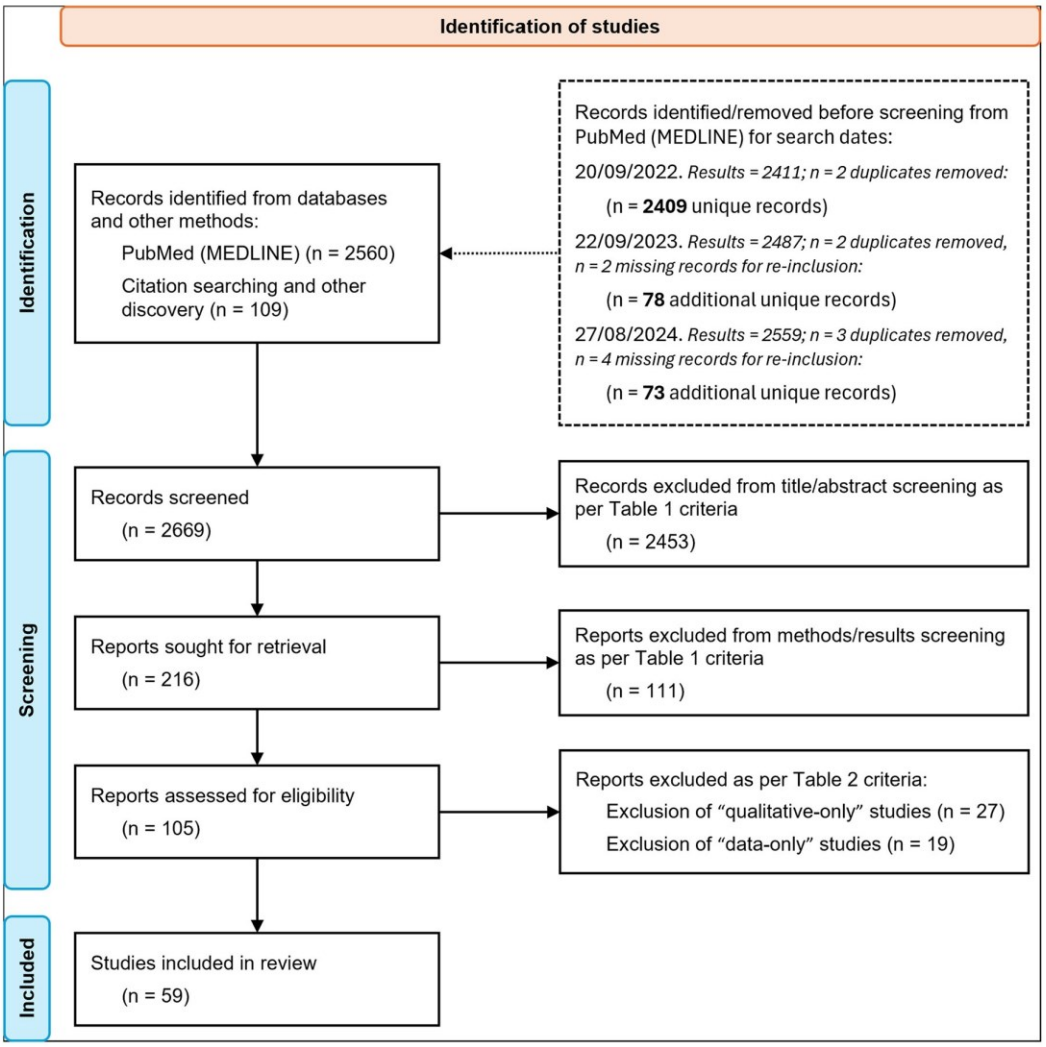

FIGURE 1 | Flow diagram of literature inclusion and screening workflow. Diagram is modified from a readily accessible template [19].

within the interfascicular and fascicular matrices (IFM and FM, respectively) in comparison to flexor/energy-storing tendons (Supporting Information S3: Table 2a). Compared with flexors, positional extensors also have overall, a more organized structure, containing relatively less IFM area [58], less randomly distributed elastin fibers [58], and larger collagen fibrils [53, 75], reflected in an overall greater total birefringence in picrosirius red (PSR) stained sections [66].

Only 7/20 *Tendon*-comparison papers (Supporting Information S3: Table 2a) evaluated *human* tissues, with the majority (6/7) primarily focused on differences between tendons commonly used as ACL-reconstruction grafts and three making direct comparisons with the ACL. These studies identified notable differences (e.g., collagen type and fibril size, vascularity, cell density) between the Quadriceps and Patellar [31, 34, 44], the hamstring and Patellar [32, 34, 44], and the Gracilis and Semitendinosus tendons [34, 35, 44] (Supporting Information S3: Table 2a). Interestingly, across all studies and species, the ACL has unique characteristics, differing both from tendons [34, 45, 63, 70] and other ligaments [63, 70]. The seventh human study by Tilley et al. [51] investigating disease progression of rotator cuff tendons, included comparisons of the "normal/control" Supraspinatus and Subscapularis tendons. However, different age cohorts (mean 23.3 years for Subscapularis and 74.6 years for Supraspinatus) confounds direct comparisons.

4.1 | Current Literature Limitations

Studies which histologically explore different tendons generally lack comprehensive exploration into quantitative between-tendon differences for a broad range of tendons and/or outcomes and are also often limited by inherent challenges due to the nature of the studies. The limited number of studies and often low sample size (one to three donors [44, 45, 80]), reflect challenges in obtaining human specimens. A general limitation of current microscopy-based studies is the largely descriptive nature of the reports. For example, observations of collagenous structure commonly include descriptions such as "homogenous," "planar waveform," or "differentially orientated," while those relating to

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TABLE 1 | Eligibility and exclusion criteria for systematic search strategy (screening stages).

Exclusion: Criterion for the exclusion of studies from further screening

Nonprimary research (e.g., reviews, letters, editorials, etc.)

Full text not available through open access, institution subscriptions/resources or not written in English.

Studies *isolated* to any of the following:

- Ligaments or ligament comparisons.
- Tendon macro-structure characterization (e.g., anatomical morphology, macroscopic exploration, etc.).
- Characterization of non-appendicular tendons (e.g., spinal, cranial, ocular, auditory, respiratory, etc.).
- Enthesis (tendon-bone junction), musculotendinous junction, aponeurosis, or tendon sheath (epitenon) characterization.
- Utilization of medical imaging techniques (e.g., MRI or ultrasound) for structural assessment.

Eligibility: Criterion for considering studies for assessment.

a. Types of studies

Primary research studies addressing the comparison of normal/healthy tendon micro-structure, including pathological/other studies offering comparative analysis of normal/healthy tendons utilized as control tissue

Full manuscript text available through open access or institution resources and written in English

b. Types of tissue explored

Tendons of the appendicular system of any species, sex, or age (excluding prenatal)

Tendon mid-substance (i.e., regions apart from enthesis (tendon-bone junction) or musculotendinous junction)

c. Types of outcome measures

Utilization of microscopy techniques (e.g., variants of optical microscopy, electron microscopy, atomic force microscopy, etc.)

At least one of the following "variables of interest" explored regarding structural comparisons of normal/healthy tendon mid-substance specimens:

i. *Tendon*: Differences between tendons (or tendons and ligaments)
ii. *Region*: Differences between tendon/ligament mid-substance regions
iii. *Age*: Differences in relation to age
iv. *Sex*: Differences between males and females
v. *Other*: Other identified differences comparing states of "normal" tendon, for example, lifestyle differences (exercise, diet, etc.), species differences, and so forth.

TABLE 2 | Final inclusion criteria for publications to be reviewed. Excluded records ("noted exclusions") remain identified in Supporting Information S2: Table 1 as additional supplementary knowledge explored in the field.

***Additional exclusion criterion A: Exclusion of "qualitative-only" studies**

Studies which solely reported qualitative or descriptive comparisons for the "variables of interest"

* A list of these publications (n = 27) can be found in Supporting Information S2: Table 1 ("Noted Exclusions A")

****Additional exclusion criterion B: Exclusions of "data-only" studies**

Studies which conducted objective measures in relation to any of the "variables of interest" but did not explicitly report statistical comparisons for these variables

** These publications ($n = 19$) are summarized and can be found in Supporting Information S2: Table 1 ("Noted Exclusions B")

Inclusions: Criterion for inclusion in summary Table 3

Studies which provided objective comparisons and reported statistical analysis for at least one of the variables of interest (Tendon, Region, Age, Sex, or Other).

cell populations include terms like "slightly elongated," "plump," "acellular areas," and "regularly arranged" [12, 14, 16, 45, 81–86]. This descriptive/qualitative approach allows for greater subjective interpretation, and limits capacity to standardize current knowledge and understanding of normal tendon structure, particularly differences between specific normal/healthy tendons. As an example, two investigations by the same group separately evaluated human Tibialis Anterior (TA) [85] and Tibialis Posterior (TP) [83] tendon structure. The descriptive "positive" Alcian-Blue staining reported, gives no indication of spread or intensity, and may inadvertently suggest similarities when comparing these two tendons. In contrast, quantitative evaluation of the thickness of the fibrocartilaginous layer at the gliding zone of the respective malleoli presents clearly defined differences: TA = 30–50 μm [85] and TP = 100–200 μm [83].

5 | Within-Tendon Variations

A total of 22 publications assessed within-tendon *Regional* variations (Table 4, Supporting Information S3: Table 2b). Mostly these studies explore variations across the longitudinal and transverse (cross-sectional) planes of the tendon/ligament, although some evaluate differences between the IFM versus FM [55, 56, 66] or between individual bodies of tendons grouped together by a shared insertion point for example, subtendons forming the Quadriceps [38]. Only 4/22 studies explored regional variations within human tendons [27, 30, 40, 48]. No studies have reported a systematic investigation of regional variations across a range of normal tendons (human or animal) to determine whether regional patterns are consistent or different between tendons. Regional differences in a single anatomical tendon were evaluated in 15/22 papers (Supporting Information S3: Table 2b), and in those that evaluated more than one tendon-type, regional differences were typically described independently. Only three studies directly compared *Region* and *Tendon*: Stanley et al. [36] studied equine superficial digital flexor tendons (SDFT) and common digital extensor tendons (CDET), however central versus core regions were

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TABLE 3 | Summary of papers exploring statistical comparisons relating to *Tendon*, *Region*, *Age*, *Sex*, or *Other* "variables of interest." Tendons/Ligaments (T/L) included in the study but not statistically assessed for any of the variables are grayed-out. Y = statistical analysis is included for the relevant variable. D = data/graphical representation of data is included for the relevant variable.

References	Species	Tendons (T) | Ligaments (L)		Microscopy modality	Tendon	Region	Age	Sex	Other
Ingelmark [20]	Murine (Rat)	1T	Achilles	TEM	—	—	**Y**	—	**Y**
Wilmink et al. [21]	Equine	1T	SDFT	OM	—	**Y**	**Y**	—	—
Patterson-Kane et al. [22]	Equine	1T	SDFT	OM, TEM	—	**Y**	**Y**	—	—
Patterson-Kane et al. [23]	Equine	1T	SDFT	OM	—	**Y**	D	—	D
Birch et al. [24]	Equine	2T	DDFT, SDFT	TEM	**Y**	**Y**	**Y**	—	—
Odetti et al. [25]	Murine (Rat)	1T	Tail	SFM	—	—	—	—	**Y**
Gibson et al. [26]	Equine	1T	SDFT	OM, TEM	—	**Y**	—	—	—
Cook et al. [27]	Human	1T	Patellar	OM	—	**Y**	**Y**	—	**Y**
Edwards et al. [28]	Equine	1T	CDET	TEM	—	—	**Y**	—	**Y**
Watanabe et al. [29]	Equine	1T	SDFT	TEM, hvEM	—	**Y**	—	—	—
Prado et al. [30]	Human	1T	Tibialis Posterior	OM	—	**Y**	**Y**	**Y**	**Y**
Hadjicostas et al. [31]	Human	2T	Patellar, Quadriceps	OM, TEM	**Y**	—	**Y**	**Y**	—
Hadjicostas et al. [32]	Human	3T	Gracilis, Semitendinosus, Patellar	OM, TEM	**Y**	—	**Y**	**Y**	—
Funakoshi et al. [33]	Caprine	2T	Infraspinatus, Patellar	OM	—	**Y**	—	—	**Y**
Hadjicostas et al. [34]	Human	4T|1L	Gracilis, Semitendinosus, Patellar, Quadriceps | ACL	OM, TEM	**Y**	—	**Y**	**Y**	—
Hadjicostas et al. [35]	Human	2T	Gracilis, Semitendinosus	OM, TEM	**Y**	—	—	—	—
Stanley et al. [36]	Equine	2T	CDET, SDFT	OM	**Y**	**Y**	D	—	**Y**
Williams et al. [37]	Leporine (Rabbit)	1T	Patellar	TEM	—	**Y**	—	—	—
Franchi et al. [38]	Murine (Rat)	3T	Patellar, Rectus Femoris, Vastus Intermedius	OM	**Y**	**Y**	—	—	—
Hansen et al. [39]	Human	1T	Patellar	TEM	—	—	—	—	**Y**
Hansen et al. [40]	Human	1T	Patellar	TEM	—	**Y**	—	—	—
Hosaka et al. [41]	Equine	3T	CDET, DDFT, SDFT	OM	**Y**	—	—	—	—
Frizziero et al. [42]	Murine (Rat)	1T	Patellar	OM	—	—	—	—	**Y**
Hu et al. [43]	Murine (Rat)	1T	*Unnamed*	SHG	—	—	**Y**	—	—

(Continues)

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TABLE 3 | (Continued)

References	Species		Tendons (T) | Ligaments (L)	Microscopy modality	Tendon	Region	Age	Sex	Other
Weiss et al. [44]	Human	5T|1L	Gracilis, Semitendinosus, Patellar, Quadriceps, Tibialis Anterior | ACL	OM	**Y**	—	—	—	—
Zhu et al. [45]	Human	*1T|1L	"Hamstring" (*Gracilis or Semitendinosus)|ACL	SEM, TEM	**Y**	—	—	—	—
Dunkman et al. [46]	Murine (Mouse)	1T	Patellar	OM, TEM	—	—	**Y**	—	—
Lavagnino et al. [47]	Murine (Rat)	1T	Tail	OM	—	—	**Y**	—	—
Mazzocca et al. [48]	Human	1T	LHBT	OM	—	**Y**	—	—	—
Thorpe et al. [49]	Equine	1T	SDFT	SEM	—	—	**Y**	—	—
Legerlotz et al. [50]	Murine (Mouse)	1T	Tail	OM	—	—	**Y**	—	—
Tilley et al. [51]	Human	2T	Subscapularis, Supraspinatus	OM, AFM	**Y**	—	**Y**	—	—
Lenskjold et al. [52]	Human	1T	Achilles	TEM	—	—	—	—	**Y**
Herod et al. [53]	Bovine	2T	CDET, SDFT	SEM	**Y**	—	—	—	—
Pardes et al. [54]	Murine (Rat)	1T	Achilles	OM	—	—	—	**Y**	**Y**
Thorpe et al. [55]	Equine	2T	CDET, SDFT	OM	**Y**	**Y**	—	—	—
Thorpe et al. [56]	Equine	1T	SDFT	OM	—	**Y**	**Y**	—	—
Biasutti et al. [57]	Ovine	1T	SDFT	OM	—	**Y**	—	—	—
Godinho et al. [58]	Equine	2T	CDET, SDFT	OM	**Y**	D	**Y**	—	—
Marquez-Arabia et al. [59]	Human	1T	Gluteus Medius	OM	—	—	**Y**	—	—
Pardes et al. [60]	Murine (Rat)	1T	Achilles	OM	—	—	**Y**	—	—
Sarver et al. [61]	Murine (Mouse)	1T	Achilles	OM	—	—	—	**Y**	—
Gagliano et al. [62]	Human	2T	Gracilis, Semitendinosus	OM	—	—	**Y**	—	—
Kharaz et al. [63]	Canine	2T|2L	LDET, SDFT|ACL, MCL	OM	**Y**	**Y**	—	—	—
Mazon et al. [64]	Murine (Rat)	1T	Calcaneal (Achilles)	OM	—	—	—	—	**Y**
Shu et al. [65]	Murine (Mouse)	2T	Achilles, Tail	TEM	D	—	**Y**	—	—
Spiesz et al. [66]	Equine	2T	CDET, SDFT	OM	**Y**	**Y**	**Y**	—	—
Takahashi et al. [67]	Bovine	1T	SDFT	OM, TEM	—	**Y**	—	—	—
Feo et al. [68]	Avian (Pedrês chicken)	1T	SDFT	OM	—	**Y**	**Y**	—	—
Hayashi et al. [69]	Canine	1T|3L	LDET | CaCL, CrCL, MCL	OM	**Y**	—	**Y**	**Y**	**Y**

(Continues)

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TABLE 3 | (Continued)

References	Species		Tendons (T) | Ligaments (L)	Microscopy modality	Tendon	Region	Age	Sex	Other
Ristaniemi et al. [70]	Bovine	1T|4L	Patellar | ACL, LCL, MCL, PCL	OM	**Y**	—	—	—	—
Asai et al. [71]	Human	1T	Semitendinosus	OM, TEM	—	—	**Y**	**Y**	**Y**
Bolam et al. [72]	Murine (Rat)	1T	Achilles	OM, TEM	—	—	—	—	**Y**
Naot et al. [73]	Human	1T	Semitendinosus	TEM/SEM	—	—	**Y**	—	—
Fjordbakk and Marques-Smith [74]	Equine	3T	Medial Patellar, Intermediate Patellar, Lateral Patellar	OM	**Y**	—	**Y**	—	—
Gsell et al. [75]	Bovine, Murine (Rat)	3T	LDET [bovine], SDFT [bovine], Tail [rat]	AFM	**Y**	—	—	—	**Y**
Johnson et al. [76]	Human	4T	Infraspinatus, Subscapularis, Supraspinatus, Teres Minor	OM	D	—	**Y**	—	—
Tinguely et al. [77]	Murine (Rat), Human	2T	Achilles [rat; human], Patellar [rat]	OM	D	—	**Y**	—	D
Zheng et al. [78]	Avian (Turkey)	1T	Gastrocnemius (Achilles)	sPHG	—	**Y**	—	—	—
$N = 59$			*Total variable (Y) count =*		20	22	30	8	16

Abbreviations: ACL = anterior cruciate ligament, CaCL = caudal cruciate ligament, CDET = common digital extensor tendon, CrCL = cranial cruciate ligament, DDFT = deep digital flexor tendon, hvEM = high voltage electron microscopy, IHC = immunohistochemistry, LCL = lateral collateral ligament, LDET = long digital extensor tendon, LHBT = long head of biceps [brachii] tendon, MCL = medial collateral ligament, OM = optical microscopy (e.g., light, polarized, confocal, etc.), PCL = posterior cruciate ligament, pSHG = polarization-resolved second harmonic generation microscopy, SDFT = superficial digital flexor tendon, SEM = scanning electron microscopy, SFM = scanning force microscopy, SHG = second harmonic generation microscopy, TEM = transmission electron microscopy.

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TABLE 4 | Summary of papers by variable (statistical comparisons).

Comparisons (variable)	Number of papers	Number of papers by species										Tendons/ligaments
		Human	Equine	Bovine	Canine	Ovine	Caprine	Rat	Mice	Avian	Leporine	
Tendon See Supporting Information S3: Table 2a for details	20	7	7	3	2	—	—	2	—	—	—	Tendons = 17 CDET, DDFT, LDET, SDFT, Patellar, Rectus Femoris, Vastus Intermedius, Quadriceps, Gracilis, Semitendinosus, Tibialis Anterior, Subscapularis, Supraspinatus, Medial Patellar Ligament, Intermediate Patellar Ligament, Lateral Patellar Ligament, Tail *Ligaments = 6* *ACL, LCL, MCL, PCL, CaCL, CrCL*
Region See Supporting Information S3: Table 2b for details	22	4	10	1	1	1	1	1	—	2	1	Tendons = 11 CDET, DDFT, LDET, SDFT, Patellar, Rectus Femoris, Vastus Intermedius, Tibialis Posterior, Infraspinatus, LHBT, Achilles/ Gastrocnemius
Age See Supporting Information S3: Table 2c for details	30	12	9	—	1	—	—	5	3	1	—	Tendons = 19 CDET, DDFT, LDET, SDFT, Patellar, Medial Patellar Ligament, Intermediate Patellar Ligament, Lateral Patellar Ligament, Quadriceps, Gracilis, Semitendinosus, Tibialis Posterior, Gluteus Medius, Infraspinatus, Subscapularis, Supraspinatus, Teres Minor, Achilles, Tail *Ligaments = 4* *ACL, MCL, CaCL, CrCL*
Sex See Supporting Information S3: Table 2d for details	8	5	—	—	1	—	—	1	1	—	—	Tendons = 7 LDET, Patellar, Quadriceps, Gracilis, Semitendinosus, Tibialis Posterior, Achilles *Ligaments = 4* *ACL, MCL, CaCL, CrCL*

(Continues)

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TABLE 4 | (Continued)

Comparisons (variable)	Number of papers	Number of papers by species									Tendons/ligaments
		Human	Equine	Bovine	Canine	Ovine	Caprine	Rat	Mice	Avian	Leporine	
Other See Supporting Information S3: Table 2e for details	16	5	2	1	1	—	1	7	—	—	—	Tendons = 9 CDET, LDET, SDFT, Patellar, Semitendinosus, Tibialis Posterior, Infraspinatus, Achilles/ Calcaneal, Tail *Ligaments = 3* *MCL, CaCL, CrCL*

Abbreviations: ACL = anterior cruciate ligament, CaCL = caudal cruciate ligament, CDET = common digital extensor tendon, CrCL = cranial cruciate ligament, DDFT = deep digital flexor tendon, LCL = lateral collateral ligament, LDET = long digital extensor tendon, LHBT = long head of biceps [brachii] tendon, MCL = medial collateral ligament, PCL = posterior cruciate ligament, SDFT = superficial digital flexor tendon.

TABLE 5 | List and definition of acronyms included in manuscript and tables.

Tendon/ligament names	
CDET	Common Digital Extensor Tendon
DDFT	Deep Digital Flexor Tendon
LDET	Long Digital Extensor Tendon
SDFT	Superficial Digital Flexor Tendon
LHBT	Long Head of Biceps [brachii] Tendon
RFT	Rectus Femoris Tendon
VIT	Vastus Intermedius Tendon
TA	Tibialis Anterior
TP	Tibialis Posterior
FHL	Flexor Hallucis Longus
ACL	Anterior Cruciate Ligament
LCL	Lateral Collateral Ligament
MCL	Medial Collateral Ligament
PCL	Posterior Cruciate Ligament
CaCL	Caudal Cruciate Ligament
CrCL	Cranial Cruciate Ligament
Outcomes	
ECM	Extracellular matrix
GAG	Glycosaminoglycan
IFM	Interfascicular matrix
FM	Fascicular matrix
IHC	Immunohistochemistry
αSMA	Alpha-smooth muscle actin
CSA	Cross-sectional area
TB	Toluidine Blue
PSR	Picrosirius Red
Microscopy Modalities	
hvEM	High voltage electron microscopy
IHC	Immunohistochemistry
MLM	Multiphoton laser microscopy
OM	Optical microscopy (e.g. light, polarized, confocal, etc.)
pSHG	Polarization-resolved second harmonic generation microscopy
SEM	Scanning electron microscopy
SFM	Scanning force microscopy
SHG	Second harmonic generation microscopy
TEM	Transmission electron microscopy

independently assessed only in SDFT (Supporting Information S3: Tables 2a and 2b); Thorpe et al. [55] and Spiesz et al. [66] compared IFM and FM structure within equine SDFT and CDET (Supporting Information S3: Table 2b) and also between tendons (Supporting Information S3: Table 2a).

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5.1 | Transverse Differences

Cross-sectional variations within tendons (e.g., central-vs.-peripheral, palmar-vs.-dorsal, medial-vs.-lateral, etc.) likely result in response to and modulate the tissue's capacity to function under variable loading (tensile or compressive) conditions. A variety of cross-sectional differences have been reported (Supporting Information S3: Table 2b) including: greater posterior versus anterior fibril density in human Patellar tendons [40], longer crimp wavelengths in superficial versus deep zones of caprine infraspinatus tendons [33], and more ovoid cells in the central versus peripheral equine SDFT [36]. One of the most common cross-sectional regional comparisons were central (core) versus peripheral in the equine SDFT (Supporting Information S3: Table 2b), with findings often contradictory, for example crimp angle and wavelength were variably greater or lesser in central regions [21–23, 26]. Factors, such as *Age* [22, 23] or *Lifestyle* [23, 36] have been suggested to play a role in these disparate *Regional* structural variations. Cross-sectional differences may also vary with longitudinal location, for example, Williams et al. [37] found medial/lateral fibril diameters and fibril-occupied areas varied between middle and distal rabbit Patellar tendon regions.

5.2 | Longitudinal Differences

Proximal-versus-distal differences could play an important role in appendicular tendon function as they traverse a joint or in the transition of tendon to muscle and bone, reflecting changes in loading and biomechanical properties in these areas. Differences identified in the literature include significantly greater GAG content in the bone-versus muscle-end of ovine SDFT [57] and human long head of biceps tendon (LHBT) [48], and greater GAG chain length in distal (bone-end) bovine SDFT [67] (Supporting Information S3: Table 2b). Mazzocca et al. [48] also found greater fibrillar organization in the distal (muscle-end) compared to proximal (bone-end) LHBT. This is consistent with more unorganized collagenous structures closest to the bone in the human Patellar tendon [27], suggesting commonality of less organized collagen at tendon-bone junctions. Conversely, vascular density was reported to increase from proximal to distal in human TA tendons [30], while in ovine SDFT the middle portion had higher vascular density relative to proximal or distal regions [57]. Interestingly in canine Long Digital Extensor Tendon (LDET), SDFT, ACL, and Medial Collateral Ligament (MCL), Kharaz et al. [63] noted no longitudinal variation for any histological measurements. Collectively these and other studies [29, 67], suggest tendon- and species-specific variability in longitudinal characteristics of normal tendons that require systematic exploration.

5.3 | Hierarchy-Related Differences

Three studies investigated differences between hierarchical tendon structures, specifically IFM and FM (Supporting Information S3: Table 2b). IFM had higher cell density in equine SDFT [56], while Spiesz et al. [66] found lower collagen content/organization (birefringence) in IFM in both equine CDET and SDFT with no tendon-specific differences. Conversely, equine CDET and SDFT had tendon-specific IFM and FM protein compositions [55]: CDET IFM had significantly higher lumican immuno-staining than FM, while SDFT IFM had greater biglycan, lubricin, and elastin staining than FM. As discussed in a later section, the older age of the horses in these studies may contribute to tendon-specific differences in IFM versus FM compositions [58].

5.4 | Future Study Design Considerations

The aforementioned examples highlight that it is critical to explicitly state the region of sample collection and have region-matched controls to evaluate pathological change. As an example, Cetti et al. [87] compared calcaneal-insertional, rupture, and proximal (10 cm from calcaneus) regions in ruptured Achilles tendons but only a single control sample from the uninjured, contralateral tendon selected between 2.2 and 8.4 cm from the calcaneal insertion. The rupture site showed greater pathology (collagen degeneration, tenocyte necrosis, inflammation) than other regions in the injured tendon, and interestingly proximal-tendon had greater pathology than distal-tendon. However, as control samples were from various regions along the length of the uninjured tendon, no conclusions can be made about underlying regional differences that might alter injury susceptibility or changes postrupture. Our own preliminary analysis of normal/healthy human cadaveric tendons [88, 89], identified clear longitudinal regional differences in crimp wavelength (Figure 2a,b) and proteoglycan content (Figure 2c,d), reaffirming the importance of region-matching control tissue samples.

In a series of studies in different animal models [17, 57, 90–92] we assessed topographical-response to injury highlighting the importance of region-matched controls. For example, Jacobson et al. [17] collected 12 proximal/distal and medial/lateral defined regional samples from each injured/pathological and contralateral/uninjured SDFT in horses, identifying distinct regionally-dependent histological differences. Cellularity, cell morphology, vascularity, cellular infiltration, collagen alignment, and proteoglycan content scores were significantly increased in injured versus control samples in the most proximal medial (*except collagen alignment score) and lateral (*except proteoglycan score) regions, while in the most distal regions, injury only increased cell morphology, vascularity and proteoglycan scores medially, and cell morphology and proteoglycan scores laterally. Detailed analysis regarding variations between control sample regions were not reported, except for highlighting the expected increase in proteoglycan content near bony-attachment sites [17, 91] as identified in other studies [48, 57] (Supporting Information S3: Table 2b). A systematic study on normal (rather than pathological) regional histological characteristics in healthy tendons would increase understanding of how and why tendon response to injury or treatment differs.

6 | Age-Related Differences

We identified 30 publications which statistically assessed *Age* effects, with 12 exploring human tissue (Table 4, Supporting Information S3: Table 2c). It is important to note that these include studies evaluating histological changes in tendon occurring with increasing biological age both postnatally (i.e., during growth/maturation) and postmaturation (i.e., across the

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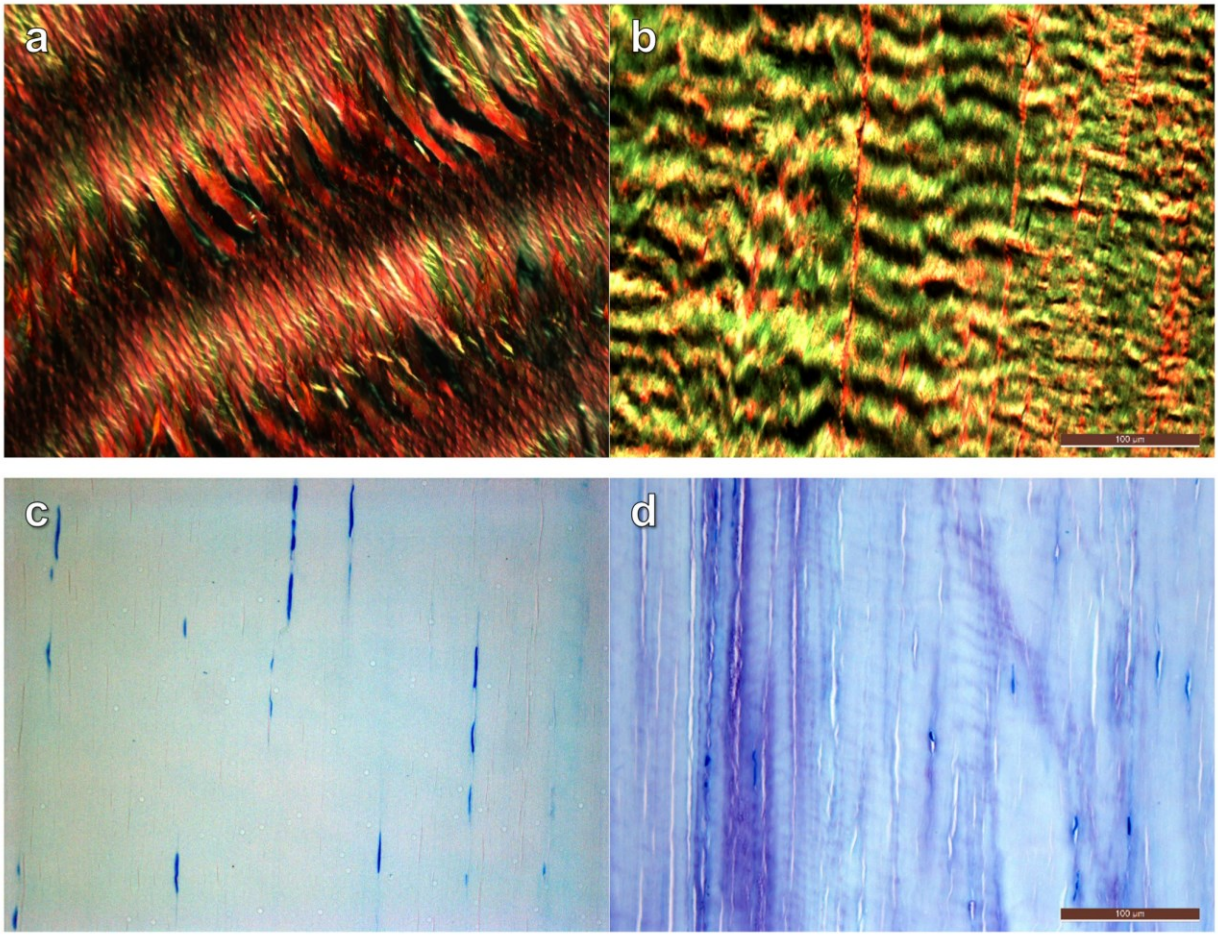

FIGURE 2 | Light microscopy images showing variation in "normal" human tendons. Histological samples are from cadaveric lower-leg tendons of female donors aged 71 (a and b) and 78 (c and d) years old. Polarized Picrosirius Red stained sections (a and b) depicting regional differences in crimp wavelength between matched proximal (a) and distal (b) regions of the Tibialis Anterior tendon. Toluidine Blue stained sections (c and d) depicting regional differences in proteoglycan content (purple staining) between matched proximal (c) and distal (d) regions of the Achilles tendon. Longitudinally orientated, formalin-fixed, paraffin embedded histological sections at 4.5 μm thick. All images: ×200 magnification; scale bars = 100 μm (applies to all images of corresponding stains).

adult lifespan). It is recognized that the changes occurring in these different life phases may differ [93, 94], with such processes likely variable between tendons, species, or even the sexes. However, the inconsistency in postnatal times at which species and sexes reach maturity hinders comparison between different animal or human models and limits the ability to separately evaluate current literature across these different age/life-stages. Additionally, the majority of studies which study *age*-related changes utilize only two differently aged cohorts and compare between them rather than assessing continuous changes throughout the maturational/aging processes. Future work to better identify and define different life stages and understand specific event-related structural homeostasis or associated changes is required.

The largest specified age span in the human studies was 66 years [51], while most spanned 29–36 years. In 6 of the 10 human studies which specified ages (two did not specify), donor average age was > 45 years, while one investigated child and adolescent cohorts [71]. In consideration of the preceding discussion of tendon variables, 5/12 human studies utilized only one anatomical tendon, and 4/12 only two. Two studies with > 2 tendons [32, 34] analyzed *Age*-effects for only 2 of the study outcomes (collagen fibril thickness and density; no correlations found). Lastly, Johnson et al. [76] statistically assessed total histopathological score but not individual structural parameters across four rotator cuff tendons.

Age-associated changes have been reported for tendon cellular content/features, structural characteristics, and compositional makeup (Supporting Information S3: Table 2c). Reduced cellularity [46, 60, 68, 69, 71] and increased cellular rounding [60, 69] have been observed with increasing age in various animal tendons. We identified similar decreasing cell density with aging (Figure 3) in cadaveric human lower-leg tendons from male and female donors aged 68–99 years [88, 89]. Importantly as discussed above, changes with biological age may not be linear, for example Legerlotz et al. [50] found cell density

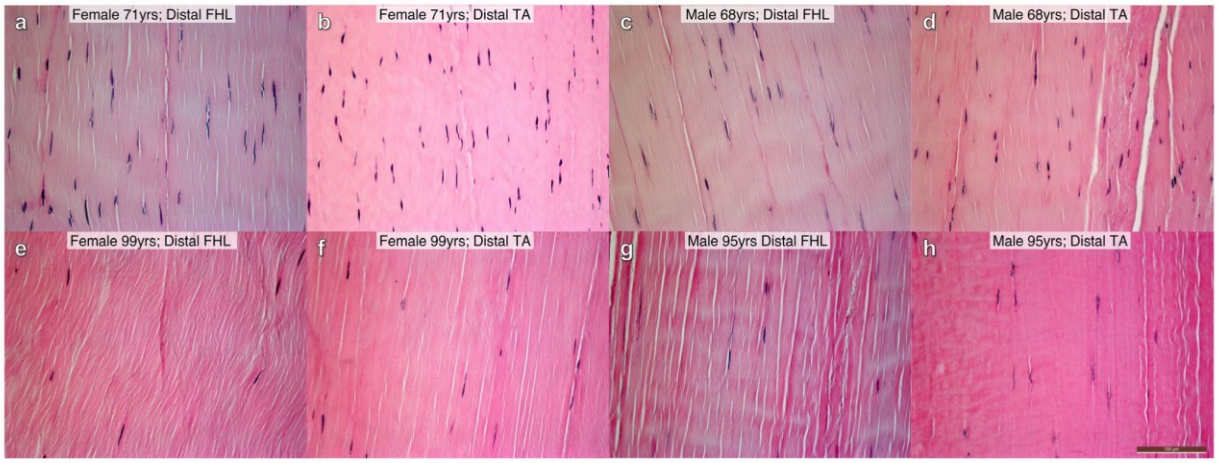

FIGURE 3 | Light microscopy images showing cell density variation in "normal" human tendons. Histological samples are from the distal region of cadaveric lower-leg Flexor Hallucis Longus (FHL; a, c, e, g) and Tibialis Anterior (TA; b, d, f, h) tendons. Female donors aged 71 (a and b) and 99 (e and f) years old, and male donors aged 68 (c and d) and 95 (g and h) years old. Note differences in relative cell density between younger (a–d) and older (e–h) donors. Longitudinally orientated, formalin-fixed, paraffin embedded histological sections at 4.5 μm thick; Haematoxylin and Eosin staining. All images: ×200 magnification; scale bar = 100 μm (applies to all images).

drastically decreased after 1 week of age in mice, but then gradually increased again from 6 weeks to 6 months of age likely reflecting changes associated with stages of maturation versus aging. Likewise, cell nuclear aspect ratios increased from 1 to 8 and 8 to 19 months in rats and then became more rounded between 19 and 28 months of age [77]. Furthermore, age-related changes may differ between tendons for example, vascular density had a quadratic relationship across ages (18–68 years) in human Gluteus Medius tendons [59], while Prado et al. [30] reported no age:vascular-density correlation in human TA tendons.

Many studies report no correlations/differences in fibril diameter with age [22, 24, 31, 32, 34, 73]. However, Shu et al. [65] reported both Achilles and tail tendons have significantly larger fibril diameters in skeletally mature (12 week-old) compared to young (3 week-old) mice. Similarly, Asai et al. [71] found "adult" (average 35 years) human Semitendinosus tendons had significantly larger fibril mean diameters compared to "immature" tendons (average 10 years), revealing changes in the finer collagenous organization between pre- and post-maturation. Disparate crimp wavelength variations with age were reported in equine SDFT with shorter wavelength in older (average 11 years) compared to younger (average 2.75 years) [21] versus mouse tail tendon (shortest crimp in youngest 3 week-old compared to 6 week-, 9 week-, 12 week-, and 6 month-old cohorts) [50].

While these studies reveal some *Age*-associated cellular and structural differences, the heterogeneities reflect limitations in exploring these characteristics across different tendons and age brackets. Furthermore, direct comparison between studies is hampered by differing methodologies and potential species-specific differences in chronological- versus biological-age relationships. There is a major gap in knowledge of normal tendon aging throughout the human lifespan, particularly considering the narrow focus of specimens and outcome measures.

6.1 | Age Versus Pathology

Histological research on aging of human tendons primarily focuses on injured/pathological samples with an unclear distinction between age-related versus injury/disease-driven changes. As an example of this poor division, the concept of "degeneration" is often used for both aging [95–97] and pathology [10, 98, 99] despite the potential for disparate mechanistic pathways in these two processes [100, 101]. Furthermore, older age groups generally exhibit more severe pathological change in diseased/injured tendons than younger ages for example, in rotator cuff tears [99, 102], Supraspinatus tendinitis [103], and Achilles tendinopathy [104]. While age was significantly correlated with histopathology-score in a study of 155 Achilles tendon biopsies from individuals with symptomatic tendinopathy, age was also inversely correlated to lifestyle activity levels [104]. Differences in older populations could indicate "normal" physiological processes including those reflecting more sedentary lifestyles. It is important to consider differences between healthy or "normal" age-related changes or whether aging causes pathology, notwithstanding such a division remains difficult to define.

6.2 | Intersection of Age and Anatomical Tendon

While age evidently influences tendon properties, the interaction between age and different anatomical tendons requires consideration, as functionally distinct tendons can be affected differently. In horses for example, the energy-storing SDFT contains significantly more elastin (predominantly in the IFM) than the positional CDET, but IFM elastin content and FM elastin organization decreased with age only in SDFTs, while FM elastin content increased significantly with age only in CDETs [58]. Age also led to the accumulation of partially degraded collagen [98] as well as increased IFM-stiffness [49] in SDFTs and not CDFTs (potentially explaining the age-related prevalence of injury in the SDFT). Unfortunately, less than half of the studies exploring

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Age-related differences (13/30; Supporting Information S3: Table 2c) assessed more than one tissue/tendon type. It is important for future histological studies on different tendons to consider age as a factor, particularly when existing knowledge for those specific tendons, species and ages is absent.

6.3 | Study Design and Methodology Considerations

The typically older age of human cadavers is a limiting factor for investigations utilizing "normal" controls or investigating normal tendon structure. When control specimens are taken from deceased patients [16, 105–111], it is not uncommon for the age difference from the often younger, more active and injury-prone cohort-of-interest to be in the order of 20 years [105–107, 110, 111]. For example, a histological study of quadriceps tendon ruptures compared biopsies from patients aged 28–81 years (mean 51.3) to cadaveric controls aged 66–91 years (mean 74.5) [107]. Comparative patient biopsies may also be from different age cohorts due to circumstances/opportunities for tissue collection for example, Zhu et al. [45] compared normal hamstring tendon grafts from patients undergoing surgery for ACL rupture (average age 25.3 ± 7.3 years) to "normal" ACL tissue from osteoarthritis patients undergoing total knee arthroplasty (average age 65.3 ± 5.6 years). It is critical that future studies better characterize "normal" tendon across a wide age-range which will help overcome issues of age-matching control and experimental samples.

Another confounding factor for *age*-effects is the potential for *species*-related differences. Conflicting results have been reported for collagen fibril diameter, which was not correlated with age in many (but not all) human studies [31, 34, 73], but was affected in equine [28], rat [20], and mouse [65] tendons. Whether this reflects bona fide species-specific differences, the particular tendons evaluated, other environmental influences (e.g., activity [20, 28]), or older chronological age in human studies is unclear. Study methodology may also affect outcomes as shown by Ingelmark [20] who used electron microscopy of fresh versus osmium tetroxide (OsO_4) impregnated samples to assess fibril diameters in old and young rats in response to different activity levels. OsO_4 treatment significantly reduced fibril diameters compared to fresh samples, however fresh samples showed no difference in fibril diameters between groups, while OsO_4 showed larger diameters for the old trained versus young untrained rats [20]. The complex age- and exercise-related differences in response to OsO_4 undoubtedly reflect changes in tendon biology but may not actually be indicative of altered fibril size in vivo. Likewise, tissue orientation affected significant findings of histological vascular density in the human Gluteus Medius tendon where correlations between density and age were observed in longitudinal but not transverse sections [59].

7 | Sex-Related Differences

A noticeable gap in the literature is understanding of *sex*-related effects on tendon structure. Only 8 papers explored *Sex* as a variable microscopically, with over half (5/8) investigating human tendons (Table 4, Supporting Information S3: Table 2d). Of the eight studies only one found a significant correlation between sex and structural characteristics: greater cellularity in the Achilles tendons of female compared to male mice [61]. No other studies reported sex-differences in cell density or morphology in various tendon/ligament models [54, 69, 71]. Pardes et al. [54] also found no differences between male, female or ovariectomized-female rat Achilles tendon proteoglycan staining. No correlations were found between sex and thickness or density of collagen fibrils in tendons commonly used for human grafts [31, 32, 34], and Prado et al. [30] identified no sex-related differences in the vascular densities of human TP tendon.

The scope of research available on *Sex*-related differences in microscopic tendon structural properties is limited, with the major focus being on cellular features and very few studies reporting on these or other structural characteristics (Supporting Information S3: Table 2d). It is common for studies investigating animal tendons to omit reporting sex or to use mixed (pooled) populations [14, 22, 55, 63, 112–114], often because it is unknown to the researcher (e.g., tendons from discarded limbs at an abattoir). Surprisingly, these issues are also observed in many human studies, where sex is either not disclosed [62, 99, 109, 115, 116] (potentially as a result of ethical/privacy issues), or *sex* as a variable is not included in the analyses due to limited sample availability [10, 27]. While the few studies available suggest limited differences between sexes, it is important to continue to fill this gap to fully understand the effects that patient demographics have on tendon structure. From our own study exploring a range of histological outcomes in nine different cadaveric human lower-leg tendons (donors aged 68–99 years; five males, four females), females had significantly longer crimp wavelengths relative to males (manuscript in preparation). These types of structural differences may be implicated in reported sexually dimorphic tendon mechanical attributes [23, 117–119], which could influence the intervention approach or outcomes of tendon related injury. Large-scale studies exploring tendon variations and demographic differences including *Sex*-specific analyses are needed.

8 | Other Normal/Healthy Tendon Variabilities

In addition to the primary "variables of interest," 16 papers assessed *Other* factors such as species or lifestyle differences (e.g., exercise, diet, etc.) on tendon structure (Table 4, Supporting Information S3: Table 2e).

8.1 | Species

Of the initial 105 eligible studies (Supporting Information S2: Table 1), only four incorporated more than one species, with three not conducting statistical *Species* comparisons. Aune et al. [120] used a rat model and human biopsies to study nerve regeneration following ACL reconstruction with Patellar tendon autografts but only observational between-species comparisons were made. Likewise, Tinguely et al. [77] reported only graphical data comparing rat and human aging and injury responses in tendons with no direct statistical comparisons. Sato et al. [121] compared the tendon healing of mice to that of

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Iberian ribbed newts, however control/normal tendons were not statistically compared. Gsell et al. [75] was the only paper specifically comparing tendons from different *Species*, exploring collagen fibril properties from bovine and rat tendons. However, the primary purpose of the study was investigating differences between positional (bovine LDET and rat Tail tendon) and energy storing (bovine SDFT) tendons. While differences were observed between the three tendon-types, species-specific comparisons were not a focus and use of different anatomical tendons limits interrogation of species-specific influences on functionally "like-for-like" tendons.

8.2 | Other Comparisons

Other variables' effects on normal/healthy tendon structural characteristics were explored in 15 papers (Supporting Information S3: Table 2e), with 6 of these studies having this as the only "variable of interest" assessed. Variables explored included: *Lifestyle* differences (e.g., level of physical activity/training/exercise [20, 27, 28, 36, 42, 52, 64], or diet [72]); *Comorbidities*/general health status (e.g., diabetic vs. nondiabetic conditions [25] or unrelated musculoskeletal injuries [27]); *Demographic* information (e.g., weight [27, 69, 71], body mass index [71], and height [27, 71]); or *Other* correlations (e.g., hormonal status [39, 54], left vs. right limbs [30], or even specifics of individual subjects [33]). Unsurprisingly considering the load bearing role of tendons, exercise-related (*Lifestyle*) comparisons predominate the *Other* variables that significantly modulated tendon histological structural measures. For example, increased training/exercise was found to be related to a reduction in the proportion of spindle-shaped tenocyte nuclei [36], and an increase in collagen alignment [42]. However, opposing results for fibril diameter changes following training (studies reporting an increase in diameter [20] compared to a decrease in diameter [28]), and the response of differing training conditions (strength, resistance, hypertrophy, or no training) causing different effects on collagenous structure (birefringence properties) [64] highlight the multifactorial influences which may predict structural variations.

9 | Conclusions

It is self-evident that knowledge of normal variations in tendon histo-anatomical structure is essential for conducting studies on tendon structure-function relationships, clinical pathology, and response to treatment; particularly when current practice in clinical and research settings utilizes histopathological scoring systems which grade characteristics relative to a normal/control reference [27, 122, 123]. To date, no studies have systematically investigated structural variations between a range of different normal tendons using consistent methodologies. This is a major limiting factor in current tendon research and is critical foundational knowledge for future studies.

Tendons have a complex and varied structure, resulting in many considerations when it comes to understanding the structure-function relationships of the tissue. Although a considerable amount of literature is available on tendon structural/compositional variations, this knowledge is predominantly derived from studies which: (1) focus on pathological conditions/structural changes; (2) report only descriptive classifications; (3) pertain to only one or two distinct tendons and offer limited comparability to other investigations; or (4) assess limited outcomes, for example, only biochemical data with no other studies performed on the tissues for more comparative analysis. Accordingly, current knowledge of tendon structural characteristics and normal variations in response to variables such as the specific anatomical *Tendon*, *Region*, *Age*, and *Sex* is limited. As a result, other areas of tendon research such as investigations into tendon physiological and pathophysiological mechanisms, biomechanical properties, or clinical outcomes, are inhibited by a lack of understanding of structural "baselines" and natural normal/healthy tissue variations for different tendons.

BOX 1 | Summary of knowledge gaps in the histological analysis of tendons.

Currently, there are limited studies which compare the multi-scale structural variations of normal (healthy) tendons. Further structural (e.g., histological, microscopy) investigations are needed to:

1. Systematically compare variations between a wide range of different anatomical tendons,
2. Characterize variations within tendons, for example, within the longitudinal or cross-sectional planes, and to determine whether any such variations exist in a range of different tendons,
3. Understand the effects of age on tendon structure, and the interaction between "normal" aging and sedentary lifestyle versus injury, pathology, or disease.
4. Compare the above outcomes between the sexes,
5. Appreciate species-specific variations as well as other variables that may influence tendon structure, for example, lifestyle, demographics, comorbidities, and so forth.
6. Understand how confounding factors might result in differences for example, specific tendons exhibiting different regional, age or other variations.
7. Appreciate how differing methodologies may influence research findings and translatability across the field.

Characteristics that have been identified as important and widely explored features of tendons, that should be considered for assessment in future tendon research include, but are not limited to:

i. Cell populations (e.g., number/density, morphology etc.)
ii. Compositional make-up (e.g., location, amount, spread etc. of different components)
iii. Overall structural architecture (e.g., fibril/fiber/fascicle organization, collagen alignment, crimp patterns etc.)

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A structure must be known before a structure-function relationship can be fully understood. Similarly, normal must be known before an appreciation of abnormal can be achieved. This review has highlighted that further research is needed to quantitatively characterize normal tendon structure in greater detail and identify variations across a range of different tendons with consideration of influential variables. Box 1 identifies the key gaps in current knowledge of tendon structural variations that need to be addressed in future studies.

Author Contributions

The first author conducted the literature search described in this review. All authors contributed to the writing and preparation of this manuscript. All authors have read and approved the final submitted manuscript.

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Ethics Statement

The histological images depicted in Figures 2 and 3 were obtained as part of a project approved by Northern Sydney Local Health District Human Research Ethics Committee (NSLHD HREC, Sydney, Australia) in accordance with the National Health and Medical Research Council (NHMRC) Act 1992. Human ethics approval code: 2019/ETH08332.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Additional Supporting Information may be found in the online version of this article.

References

1. H. L. Birch, "Tendon Matrix Composition and Turnover in Relation to Functional Requirements," *International Journal of Experimental Pathology* 88 (2007): 241–248.

2. E. L. Batson, R. J. Paramour, T. J. Smith, H. L. Birch, J. C. Patterson-kane, and A. E. Goodship, "Are the Material Properties and Matrix Composition of Equine Flexor and Extensor Tendons Determined by Their Functions?," *Equine Veterinary Journal* 35 (2003): 314–318.

3. C. T. Thorpe, C. P. Udeze, H. L. Birch, P. D. Clegg, and H. R. C. Screen, "Specialization of Tendon Mechanical Properties Results From Interfascicular Differences," *Journal of the Royal Society Interface* 9 (2012): 3108–3117.

4. H. R. C. Screen, S. Toorani, and J. C. Shelton, "Microstructural Stress Relaxation Mechanics in Functionally Different Tendons," *Medical Engineering & Physics* 35 (2013): 96–102.

5. A. Zuskov, B. R. Freedman, J. A. Gordon, J. J. Sarver, M. R. Buckley, and L. J. Soslowsky, "Tendon Biomechanics and Crimp Properties Following Fatigue Loading Are Influenced by Tendon Type and Age in Mice," *Journal of Orthopaedic Research* 38 (2020): 36–42.

6. R. Owen, J. Goodfellow, and P. G. Bullough, *Scientific Foundations of Orthopaedics and Traumatology* (Saunders, 1980).

7. S. T. Clark, M. Zhu, G. D. Gamble, et al., "Epidemiology of Tendon and Ligament Injuries in Aotearoa/New Zealand Between 2010 and 2016," *Injury Epidemiology* 7 (2020): 5.

8. N. Maffulli, J. Wong, and L. C. Almekinders, "Types and Epidemiology of Tendinopathy," *Clinics in Sports Medicine* 22 (2003): 675–692.

9. R. A. E. Clayton and C. M. Court-Brown, "The Epidemiology of Musculoskeletal Tendinous and Ligamentous Injuries," *Injury* 39 (2008): 1338–1344.

10. L. Józsa, A. Réffy, P. Kannus, S. Demel, and E. Elek, "Pathological Alterations in Human Tendons," *Archives of Orthopaedic and Trauma Surgery* 110 (1990): 15–21.

11. W. J. Schmidt, "Neuere polarisationsoptische Arbeiten auf dem Gebiete der Biologie," *Protoplasma* 29 (1938): 300–312.

12. P. Kannus and L. Józsa, "Histopathological Changes Preceding Spontaneous Rupture of a Tendon. A Controlled Study of 891 Patients," *Journal of Bone & Joint Surgery* 73 (1991): 1507–1525.

13. J. P. Watkins, J. A. Auer, S. Gay, and S. J. Morgan, "Healing of Surgically Created Defects in the Equine Superficial Digital Flexor Tendon: Collagen-Type Transformation and Tissue Morphologic Reorganization," *American Journal of Veterinary Research* 46 (1985): 2091–2096.

14. P. M. Webbon, "A Histological Study of Macroscopically Normal Equine Digital Flexor Tendons," *Equine Veterinary Journal* 10 (1978): 253–259.

15. S. Farfaras, L. E. Ejerhed, E. K. Hallström, et al., "More Histologic and Ultrastructural Degenerative Signs in the Subscapularis Tendon and the Joint Capsule in Male Patients With Shoulder Impingement," *Knee Surgery, Sports Traumatology, Arthroscopy* 26 (2018): 79–87.

16. E. Tebaa, J. Tantot, S. Isaac-Pinet, and L. Nové-Josserand, "Histologic Characteristics of the Subscapularis Tendon From Muscle to Bone: Reference to Subscapularis Lesions," *Journal of Shoulder and Elbow Surgery* 28 (2019): 959–965.

17. E. Jacobson, A. J. Dart, T. Mondori, et al., "Focal Experimental Injury Leads to Widespread Gene Expression and Histologic Changes in Equine Flexor Tendons," *PLoS One* 10 (2015): e0122220.

18. S. Arya and K. Kulig, "Tendinopathy Alters Mechanical and Material Properties of the Achilles Tendon," *Journal of Applied Physiology* 108, no. 1985 (2010): 670–675.

19. N. R. Haddaway, M. J. Page, C. C. Pritchard, and L. A. McGuinness, "PRISMA2020: An R Package and Shiny App for Producing Prisma 2020-Compliant Flow Diagrams, With Interactivity for Optimised Digital Transparency and Open Synthesis," *Campbell Systematic Reviews* 18 (2022): e1230.

20. B. E. Ingelmark, "The Structure of Tendons at Various Ages and Under Different Functional Conditions. II," *Cells Tissues Organs* 6 (1948): 193–225.

21. J. Wilmink, A. M. Wilson, and A. E. Goodship, "Functional Significance of the Morphology and Micromechanics of Collagen Fibres in Relation to Partial Rupture of the Superficial Digital Flexor Tendon in Racehorses," *Research in Veterinary Science* 53 (1992): 354–359.

22. J. Patterson-kane, E. Firth, A. Goodship, and D. Parry, "Age-Related Differences in Collagen Crimp Patterns in the Superficial Digital Flexor Tendon Core Region of Untrained Horses," *Australian Veterinary Journal* 75 (1997): 39–44.

23. J. C. Patterson-Kane, D. A. D. Parry, A. E. Goodship, and E. C. Firth, "Exercise Modifies the Age-Related Change in Crimp Pattern in the Core Region of the Equine Superficial Digital Flexor Tendon," *New Zealand Veterinary Journal* 45 (1997): 135–139.

24. H. L. Birch, J. V. B. Bailey, A. J. Bailey, and A. E. Goodship, "Age-Related Changes to the Molecular and Cellular Components of Equine Flexor Tendons," *Equine Veterinary Journal* 31 (1999): 391–396.

15544527x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jor.26060 by Samantha Heffernan - University Of Sydney , Wiley Online Library on [26/02/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

25. P. Odetti, I. Aragno, R. Rolandi, et al., "Scanning Force Microscopy Reveals Structural Alterations in Diabetic Rat Collagen Fibrils: Role of Protein Glycation," *Diabetes/Metabolism Research and Reviews* 16 (2000): 74–81.

26. K. Gibson, H. Burbidge, and I. Robertson, "The Effects of Polyester (Terylene) Fibre Implants on Normal Equine Superficial Digital Flexor Tendon," *New Zealand Veterinary Journal* 50 (2002): 186–194.

27. J. L. Cook, J. A. Feller, S. F. Bonar, and K. M. Khan, "Abnormal Tenocyte Morphology Is More Prevalent Than Collagen Disruption in Asymptomatic Athletes' Patellar Tendons," *Journal of Orthopaedic Research* 22 (2004): 334–338.

28. L. J. Edwards, A. E. Goodship, H. L. Birch, and J. C. Patterson-Kane, "Effect of Exercise on Age-Related Changes in Collagen Fibril Diameter Distributions in the Common Digital Extensor Tendons of Young Horses," *American Journal of Veterinary Research* 66 (2005): 564–568.

29. T. Watanabe, Y. Hosaka, E. Yamamoto, et al., "Control of the Collagen Fibril Diameter in the Equine Superficial Digital Flexor Tendon in Horses by Decorin," *Journal of Veterinary Medical Science* 67 (2005): 855–860.

30. M. P. Prado, A. E. de Carvalho, Jr., C. J. Rodrigues, T. D. Fernandes, A. A. M. Mendes, and O. Salomao, "Vascular Density of the Posterior Tibial Tendon: A Cadaver Study," *Foot & Ankle International* 27 (2006): 628–631.

31. P. T. Hadjicostas, P. N. Soucacos, I. Berger, N. Koleganova, and H. H. Paessler, "Comparative Analysis of the Morphologic Structure of Quadriceps and Patellar Tendon: A Descriptive Laboratory Study," *Arthroscopy: The Journal of Arthroscopic & Related Surgery: Official Publication of the Arthroscopy Association of North America and the International Arthroscopy Association* 23 (2007): 744–750.

32. P. T. Hadjicostas, P. N. Soucacos, H. H. Paessler, N. Koleganova, and I. Berger, "Morphologic and Histologic Comparison Between the Patella and Hamstring Tendons Grafts: A Descriptive and Anatomic Study," *Arthroscopy: The Journal of Arthroscopic & Related Surgery: Official Publication of the Arthroscopy Association of North America and the International Arthroscopy Association* 23 (2007): 751–756.

33. T. Funakoshi, T. Schmid, H. P. Hsu, and M. Spector, "Lubricin Distribution in the Goat Infraspinatus Tendon: A Basis for Interfascicular Lubrication," *Journal of Bone and Joint Surgery-American Volume* 90 (2008): 803–814.

34. P. T. Hadjicostas, P. N. Soucacos, N. Koleganova, G. Krohmer, and I. Berger, "Comparative and Morphological Analysis of Commonly Used Autografts for Anterior Cruciate Ligament Reconstruction With the Native ACL: An Electron, Microscopic and Morphologic Study," *Knee Surgery, Sports Traumatology, Arthroscopy* 16 (2008): 1099–1107.

35. P. T. Hadjicostas, P. N. Soucacos, N. Koleganova, G. Piecha, G. Krohmer, and I. Berger, "Comparative Analysis of the Microstructure of the Hamstring Tendons: An Electron Microscopic, Histologic, and Morphologic Study," *Journal of Surgical Orthopaedic Advances* 17 (2008): 153–158.

36. R. L. Stanley, L. J. Edwards, A. E. Goodship, E. C. Firth, and J. C. Patterson-kane, "Effects of Exercise on Tenocyte Cellularity and Tenocyte Nuclear Morphology in Immature and Mature Equine Digital Tendons," *Equine Veterinary Journal* 40 (2008): 141–146.

37. L. N. Williams, S. H. Elder, M. F. Horstemeyer, and D. Harbarger, "Variation of Diameter Distribution, Number Density, and Area Fraction of Fibrils Within Five Areas of the Rabbit Patellar Tendon," *Annals of Anatomy-Anatomischer Anzeiger* 190 (2008): 442–451.

38. M. Franchi, M. Quaranta, M. Macciocca, V. De Pasquale, V. Ottani, and A. Ruggeri, "Structure Relates to Elastic Recoil and Functional Role in Quadriceps Tendon and Patellar Ligament," *Micron* 40 (2009): 370–377.

39. M. Hansen, M. Kongsgaard, L. Holm, et al., "Effect of Estrogen on Tendon Collagen Synthesis, Tendon Structural Characteristics, and Biomechanical Properties in Postmenopausal Women," *Journal of Applied Physiology* 106, no. 1985 (2009): 1385–1393.

40. P. Hansen, B. T. Haraldsson, P. Aagaard, et al., "Lower Strength of the Human Posterior Patellar Tendon Seems Unrelated to Mature Collagen Cross-Linking and Fibril Morphology," *Journal of Applied Physiology* 108, no. 1985 (2010): 47–52.

41. Y. Z. Hosaka, H. Takahashi, T. Uratsuji, P. Tangkawattana, H. Ueda, and K. Takehana, "Comparative Study of the Characteristics and Properties of Tendinocytes Derived From Three Tendons in the Equine Forelimb," *Tissue and Cell* 42 (2010): 9–17.

42. A. Frizziero, M. Fini, F. Salamanna, A. Veicsteinas, N. Maffulli, and M. Marini, "Effect of Training and Sudden Detraining on the Patellar Tendon and Its Enthesis in Rats," *BMC Musculoskeletal Disorders* 12 (2011): 20.

43. W. Hu, H. Li, C. Wang, S. Gou, and L. Fu, "Characterization of Collagen Fibers by Means of Texture Analysis of Second Harmonic Generation Images Using Orientation-Dependent Gray Level Co-Occurrence Matrix Method," *Journal of Biomedical Optics* 17 (2012): 026007.

44. M. Weiss, F. N. Unterhauser, and A. Weiler, "Crimp Frequency Is Strongly Correlated to Myofibroblast Density in the Human Anterior Cruciate Ligament and Its Autologous Tendon Grafts," *Knee Surgery, Sports Traumatology, Arthroscopy* 20 (2012): 889–895.

45. J. Zhu, X. Zhang, Y. Ma, C. Zhou, and y Ao, "Ultrastructural and Morphological Characteristics of Human Anterior Cruciate Ligament and Hamstring Tendons," *Anatomical Record* 295 (2012): 1430–1436.

46. A. A. Dunkman, M. R. Buckley, M. J. Mienaltowski, et al., "Decorin Expression Is Important for Age-Related Changes in Tendon Structure and Mechanical Properties," *Matrix Biology* 32 (2013): 3–13.

47. M. Lavagnino, K. Gardner, and S. P. Arnoczky, "Age-Related Changes in the Cellular, Mechanical, and Contractile Properties of Rat Tail Tendons," *Connective Tissue Research* 54 (2013): 70–75.

48. A. D. Mazzocca, M. B. R. McCarthy, F. A. Ledgard, et al., "Histomorphologic Changes of the Long Head of the Biceps Tendon in Common Shoulder Pathologies," *Arthroscopy: Journal of Arthroscopic & Related Surgery* 29 (2013): 972–981.

49. C. Thorpe, C. Udeze, H. Birch, P. Clegg, and H. Screen, "Capacity for Sliding Between Tendon Fascicles Decreases With Ageing in Injury Prone Equine Tendons: A Possible Mechanism for Age-Related Tendinopathy?," *European Cells and Materials* 25 (2013): 48–60.

50. K. Legerlotz, J. Dorn, J. Richter, M. Rausch, and O. Leupin, "Age-Dependent Regulation of Tendon Crimp Structure, Cell Length and Gap Width With Strain," *Acta Biomaterialia* 10 (2014): 4447–4455.

51. J. M. R. Tilley, R. J. Murphy, S. Chaudhury, J. T. Czernuszka, and A. J. Carr, "Effect of Tear Size, Corticosteroids and Subacromial Decompression Surgery on the Hierarchical Structural Properties of Torn Supraspinatus Tendons," *Bone & Joint Research* 3 (2014): 252–261.

52. A. Lenskjold, M. Kongsgaard, J. O. Larsen, et al., "The Influence of Physical Activity During Youth on Structural and Functional Properties of the Achilles Tendon," *Scandinavian Journal of Medicine & Science in Sports* 25 (2015): 25–31.

53. T. W. Herod, N. C. Chambers, and S. P. Veres, "Collagen Fibrils in Functionally Distinct Tendons Have Differing Structural Responses to Tendon Rupture and Fatigue Loading," *Acta Biomaterialia* 42 (2016): 296–307.

54. A. M. Pardes, B. R. Freedman, G. W. Fryhofer, N. S. Salka, P. R. Bhatt, and L. J. Soslowsky, "Males Have Inferior Achilles Tendon Material Properties Compared to Females in a Rodent Model," *Annals of Biomedical Engineering* 44 (2016): 2901–2910.

55. C. T. Thorpe, K. J. Karunaseelan, J. Ng Chieng Hin, et al., "Distribution of Proteins Within Different Compartments of Tendon Varies According to Tendon Type," *Journal of Anatomy* 229 (2016): 450–458.

56. C. T. Thorpe, M. J. Peffers, D. Simpson, E. Halliwell, H. R. C. Screen, and P. D. Clegg, "Anatomical Heterogeneity of Tendon: Fascicular

15554527x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jor.26060 by Samantha Heffernan - University Of Sydney , Wiley Online Library on [26/02/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

and Interfascicular Tendon Compartments Have Distinct Proteomic Composition," *Scientific Reports* 6 (2016): 20455.

57. S. Biasutti, A. Dart, M. Smith, et al., "Spatiotemporal Variations in Gene Expression, Histology and Biomechanics in an Ovine Model of Tendinopathy," *PLoS One* 12 (2017): e0185282.

58. M. S. C. Godinho, C. T. Thorpe, S. E. Greenwald, and H. R. C. Screen, "Elastin Is Localised to the Interfascicular Matrix of Energy Storing Tendons and Becomes Increasingly Disorganised With Ageing," *Scientific Reports* 7 (2017): 9713.

59. W. H. Márquez-Arabia, J. Gómez-Hoyos, M. Gómez, et al., "Influence of Aging on Microvascular Supply of the Gluteus Medius Tendon: A Cadaveric and Histologic Study," *Arthroscopy: Journal of Arthroscopic & Related Surgery* 33 (2017): 1354–1360.

60. A. M. Pardes, Z. M. Beach, H. Raja, A. B. Rodriguez, B. R. Freedman, and L. J. Soslowsky, "Aging Leads to Inferior Achilles Tendon Mechanics and Altered Ankle Function in Rodents," *Journal of Biomechanics* 60 (2017): 30–38.

61. D. C. Sarver, Y. A. Kharaz, K. B. Sugg, J. P. Gumucio, E. Comerford, and C. L. Mendias, "Sex Differences in Tendon Structure and Function," *Journal of Orthopaedic Research* 35 (2017): 2117–2126.

62. N. Gagliano, A. Menon, F. Cabitza, R. Compagnoni, and P. Randelli, "Morphological and Molecular Characterization of Human Hamstrings Shows That Tendon Features Are Not Influenced by Donor Age," *Knee Surgery, Sports Traumatology, Arthroscopy* 26 (2018): 343–352.

63. Y. A. Kharaz, E. G. Canty-Laird, S. R. Tew, and E. J. Comerford, "Variations in Internal Structure, Composition and Protein Distribution Between Intra- and Extra-Articular Knee Ligaments and Tendons," *Journal of Anatomy* 232 (2018): 943–955.

64. J. Mazon, A. A. de Aro, P. W. Simões, and E. R. Pimentel, "Effect of Different Resistance-Training Protocols on the Extracellular Matrix of the Calcaneal Tendon of Rats," *Annals of Anatomy-Anatomischer Anzeiger* 216 (2018): 75–81.

65. C. C. Shu, M. M. Smith, R. C. Appleyard, C. B. Little, and J. Melrose, "Achilles and Tail Tendons of Perlecan Exon 3 Null Heparan Sulphate Deficient Mice Display Surprising Improvement in Tendon Tensile Properties and Altered Collagen Fibril Organisation Compared to C57BL/6 Wild Type Mice," *PeerJ* 6 (2018): e5120.

66. E. M. Spiesz, C. T. Thorpe, P. J. Thurner, and H. R. C. Screen, "Structure and Collagen Crimp Patterns of Functionally Distinct Equine Tendons, Revealed by Quantitative Polarised Light Microscopy (qPLM)," *Acta Biomaterialia* 70 (2018): 281–292.

67. N. Takahashi, T. Hirose, J. A. Minaguchi, H. Ueda, P. Tangkawattana, and K. Takehana, "Fibrillar Architecture at Three Different Sites of the Bovine Superficial Digital Flexor Tendon," *Journal of Veterinary Medical Science* 80 (2018): 405–412.

68. H. B. Fêo, A. Biancalana, W. Romero Nakagaki, A. Aparecida de Aro, and L. Gomes, "Morphological Alterations and Increased Gelatinase Activity in the Superficial Digital Flexor Tendon of Chickens During Growth and Maturation," *Anatomical Record* 302 (2019): 964–972.

69. K. Hayashi, J. Bhandal, S. Y. Kim, et al., "Comparative Histomorphometric Analysis of Cellular Phenotype in Canine Stifle Ligaments and Tendon," *Veterinary Surgery* 48 (2019): 1013–1018.

70. A. Ristaniemi, D. Regmi, D. Mondal, et al., "Structure, Composition and Fibril-Reinforced Poroviscoelastic Properties of Bovine Knee Ligaments and Patellar Tendon," *Journal of the Royal Society Interface* 18 (2021): 20200737.

71. K. Asai, J. Nakase, T. Ishikawa, et al., "Differences in Cellular and Microstructural Properties of the Semitendinosus Muscle Tendon Between Young and Adult Patients," *Journal of Orthopaedic Science* 27 (2022): 478–485.

72. S. M. Bolam, S. Konar, Y. E. Park, et al., "A High-Fat Diet Has Negative Effects on Tendon Resident Cells in an In Vivo Rat Model," *International Orthopaedics* 46 (2022): 1181–1190.

73. D. Naot, A. J. Choi, M. Street, et al., "Age-Related Differences in Hamstring Tendon Used as Autograft in Reconstructive Anterior Cruciate Ligament Surgery," *International Orthopaedics* 46 (2022): 845–853.

74. C. T. Fjordbakk and P. Marques-Smith, "The Equine Patellar Ligaments and the Infrapatellar Fat Pad—A Microanatomical Study," *BMC Veterinary Research* 19 (2023): 20.

75. K. Y. Gsell, S. P. Veres, and L. Kreplak, "Single Collagen Fibrils Isolated From High Stress and Low Stress Tendons Show Differing Susceptibility to Enzymatic Degradation by the Interstitial Collagenase Matrix metalloproteinase-1 (MMP-1)," *Matrix Biology Plus* 18 (2023): 100129.

76. J. Johnson, D. von Stade, B. Gadomski, et al., "Biomechanical and Histological Changes Secondary to Aging in the Human Rotator Cuff: A Preliminary Analysis," *Journal of Orthopaedic Research* 41 (2023): 2221–2231.

77. Y. Tinguely, V. Shi, F. Klatte-Schulz, G. N. Duda, B. R. Freedman, and D. J. Mooney, "Aging and Injury Affect Nuclear Shape Heterogeneity in Tendon," *Journal of Orthopaedic Research* 41 (2023): 2186–2194.

78. K. Zheng, J. Zhong, J. Hu, et al., "Effects of Mineralization on the Hierarchical Organization of Collagen—A Synchrotron X-Ray Scattering and Polarized Second Harmonic Generation Study," *Interface Focus* 14 (2024): 20230046.

79. M. Benjamin, S. Qin, and J. R. Ralphs, "Fibrocartilage Associated With Human Tendons and Their Pulleys," *Journal of Anatomy* 187, no. Pt 3 (1995): 625–633.

80. E. C. Schmidt, M. Chin, J. T. Aoyama, T. J. Ganley, K. G. Shea, and M. W. Hast, "Mechanical and Microstructural Properties of Pediatric Anterior Cruciate Ligaments and Autograft Tendons Used for Reconstruction," *Orthopaedic Journal of Sports Medicine* 7 (2019): 2325967118821667.

81. D. A. Edwards, "The Blood Supply and Lymphatic Drainage of Tendons," *Journal of Anatomy* 80 (1946): 147–152.

82. W. Petersen and G. Hohmann, "Collagenous Fibril Texture of the Gliding Zone of the Human Tibialis Posterior Tendon," *Foot & Ankle International* 22 (2001): 126–132.

83. W. Petersen, G. Hohmann, T. Pufe, et al., "Structure of the Human Tibialis Posterior Tendon," *Archives of Orthopaedic and Trauma Surgery* 124 (2004): 237–242.

84. W. Petersen and H. Laprell, "Insertion of Autologous Tendon Grafts to the Bone: A Histological and Immunohistochemical Study of Hamstring and Patellar Tendon Grafts," *Knee Surgery, Sports Traumatology, Arthroscopy* 8 (2000): 26–31.

85. W. Petersen, V. Stein, and T. Bobka, "Structure of the Human Tibialis Anterior Tendon," *Journal of Anatomy* 197, no. pt. 4 (2000): 617–625.

86. G. J. R. Delcroix, D. N. Kaimrajh, D. Baria, et al., "Histologic, Biomechanical, and Biological Evaluation of Fan-Folded Iliotibial Band Allografts for Anterior Cruciate Ligament Reconstruction," *Arthroscopy: Journal of Arthroscopic & Related Surgery* 29 (2013): 756–765.

87. R. Cetti, J. Junge, and M. Vyberg, "Spontaneous Rupture of the Achilles Tendon Is Preceded by Widespread and Bilateral Tendon Damage and Ipsilateral Inflammation," *Acta Orthopaedica Scandinavica* 74 (2003): 78–84.

88. S. A. Hefferan, C. L. Blaker, D. M. Ashton, et al., *Histological Variations in Aged Human Tendons*. ORS 2021 Annual Meeting. Virtual All Access. (Orthopaedic Research Scociety, 2021).

89. S. A. Hefferan, *Histological Characteristics of Different Tendons in Humans of Advanced Age* (Honours Thesis, University of Sydney, 2020).

90. R. K. Choi, M. M. Smith, J. H. Martin, et al., "Chondroitin Sulphate Glycosaminoglycans Contribute to Widespread Inferior Biomechanics in Tendon After Focal Injury," *Journal of Biomechanics* 49 (2016): 2694–2701.

91. M. M. Smith, G. Sakurai, S. M. Smith, et al., "Modulation of aggrecan and ADAMTS Expression in Ovine Tendinopathy Induced by Altered Strain," *Arthritis & Rheumatism* 58 (2008): 1055–1066.

1554527x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jor.26060 by Samantha Hefferan - University Of Sydney , Wiley Online Library on [26/02/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

92. A. S. Tsang, A. J. Dart, S. A. Biasutti, L. B. Jeffcott, M. M. Smith, and C. B. Little, "Effects of Tendon Injury on Uninjured Regional Tendons in the Distal Limb: An In-Vivo Study Using an Ovine Tendinopathy Model," *PLoS One* 14 (2019): e0215830.

93. R. Choi, M. Smith, E. Clarke, and C. Little, "Cellular, Matrix, and Mechano-Biological Differences in Load-Bearing Versus Positional Tendons Throughout Development and Aging: A Narrative Review," *Connective Tissue Research* 59 (2018): 483–494.

94. N. Andarawis-Puri, E. L. Flatow, and L. J. Soslowsky, "Tendon Basic Science: Development, Repair, Regeneration, and Healing," *Journal of Orthopaedic Research* 33 (2015): 780–784.

95. J. E. Ackerman, I. Bah, J. H. Jonason, M. R. Buckley, and A. E. Loiselle, "Aging Does Not Alter Tendon Mechanical Properties During Homeostasis, But Does Impair Flexor Tendon Healing," *Journal of Orthopaedic Research* 35 (2017): 2716–2724.

96. J. Kohler, C. Popov, B. Klotz, et al., "Uncovering the Cellular and Molecular Changes in Tendon Stem/Progenitor Cells Attributed to Tendon Aging and Degeneration," *Aging Cell* 12 (2013): 988–999.

97. R. K. Smith, H. L. Birch, S. Goodman, D. Heinegård, and A. E. Goodship, "The Influence of Ageing and Exercise on Tendon Growth and Degeneration—Hypotheses for the Initiation and Prevention of Strain-Induced Tendinopathies," *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* 133 (2002): 1039–1050.

98. C. T. Thorpe, I. Streeter, G. L. Pinchbeck, A. E. Goodship, P. D. Clegg, and H. L. Birch, "Aspartic Acid Racemization and Collagen Degradation Markers Reveal an Accumulation of Damage in Tendon Collagen That Is Enhanced With Aging," *Journal of Biological Chemistry* 285 (2010): 15674–15681.

99. S. A. Glait, S. Mahure, C. A. Loomis, et al., "Regional Histologic Differences in the Long Head of the Biceps Tendon Following Subpectoral Biceps Tenodesis in Patients With Rotator Cuff Tears and SLAP Lesions," *Knee Surgery, Sports Traumatology, Arthroscopy* 26 (2018): 2481–2489.

100. S. P. Magnusson and M. Kjaer, "The Impact of Loading, Unloading, Ageing and Injury on the Human Tendon," *Journal of Physiology* 597 (2019): 1283–1298.

101. G. Riley, "The Pathogenesis of Tendinopathy. A Molecular Perspective," *Rheumatology* 43 (2004): 131–142.

102. C. Milgrom, M. Schaffler, S. Gilbert, and M. van Holsbeeck, "Rotator-Cuff Changes in Asymptomatic Adults. The Effect of Age, Hand Dominance and Gender," *Journal of Bone and Joint Surgery. British Volume* 77 (1995): 296–298.

103. G. P. Riley, M. J. Goddard, and B. L. Hazleman, "Histopathological Assessment and Pathological Significance of Matrix Degeneration in Supraspinatus Tendons," *Rheumatology* 40 (2001): 229–230.

104. M. Aström and A. Rausing, "Chronic Achilles Tendinopathy. A Survey of Surgical and Histopathologic Findings," *Clinical Orthopaedics and Related Research* 316 (1995): 151–164.

105. C. Tallon, N. Maffulli, and S. W. B. Ewen, "Ruptured Achilles Tendons Are Significantly More Degenerated Than Tendinopathic Tendons," *Medicine & Science in Sports & Exercise* 33 (2001): 1983–1990.

106. N. Maffulli, V. Barrass, and S. W. B. Ewen, "Light Microscopic Histology of Achilles Tendon Ruptures. A Comparison With Unruptured Tendons," *American Journal of Sports Medicine* 28 (2000): 857–863.

107. N. Maffulli, A. Del Buono, F. Spiezia, U. G. Longo, and V. Denaro, "Light Microscopic Histology of Quadriceps Tendon Ruptures," *International Orthopaedics* 36 (2012): 2367–2371.

108. U. G. Longo, F. Franceschi, L. Ruzzini, et al., "Histopathology of the Supraspinatus Tendon in Rotator Cuff Tears," *American Journal of Sports Medicine* 36 (2008): 533–538.

109. N. L. Harris, D. A. B. Smith, L. Lamoreaux, and M. Purnell, "Central Quadriceps Tendon for Anterior Cruciate Ligament Reconstruction. Part I: Morphometric and Biomechanical Evaluation," *American Journal of Sports Medicine* 25 (1997): 23–28.

110. N. Maffulli, V. Testa, G. Capasso, et al., "Similar Histopathological Picture in Males With Achilles and Patellar Tendinopathy," *Medicine & Science in Sports & Exercise* 36 (2004): 1470–1475.

111. F. Klatte-Schulz, S. Minkwitz, A. Schmock, et al., "Different Achilles Tendon Pathologies Show Distinct Histological and Molecular Characteristics," *International Journal of Molecular Sciences* 19 (2018): 404.

112. R. K. Choi, M. M. Smith, S. Smith, C. B. Little, and E. C. Clarke, "Functionally Distinct Tendons Have Different Biomechanical, Biochemical and Histological Responses to In Vitro Unloading," *Journal of Biomechanics* 95 (2019): 109321.

113. R. E. Shadwick, "Elastic Energy Storage in Tendons: Mechanical Differences Related to Function and Age," *Journal of Applied Physiology* 68, no. 1985 (1990): 1033–1040.

114. P. S. Robinson, T. W. Lin, A. F. Jawad, R. V. Iozzo, and L. J. Soslowsky, "Investigating Tendon Fascicle Structure-Function Relationships in a Transgenic-Age Mouse Model Using Multiple Regression Models," *Annals of Biomedical Engineering* 32 (2004): 924–931.

115. G. A. Johnson, D. M. Tramaglini, R. E. Levine, K. Ohno, N. Y. Choi, and S. L-Y. Woo, "Tensile and Viscoelastic Properties of Human Patellar Tendon," *Journal of Orthopaedic Research* 12 (1994): 796–803.

116. R. H. Shani, E. Umpierez, M. Nasert, E. A. Hiza, and J. Xerogeanes, "Biomechanical Comparison of Quadriceps and Patellar Tendon Grafts in Anterior Cruciate Ligament Reconstruction," *Arthroscopy: Journal of Arthroscopic & Related Surgery* 32 (2016): 71–75.

117. M. R. Buckley, J. J. Sarver, B. R. Freedman, and L. J. Soslowsky, "The Dynamics of Collagen Uncrimping and Lateral Contraction in Tendon and the Effect of Ionic Concentration," *Journal of Biomechanics* 46 (2013): 2242–2249.

118. K. S. Miller, B. K. Connizzo, E. Feeney, J. J. Tucker, and L. J. Soslowsky, "Examining Differences in Local Collagen Fiber Crimp Frequency Throughout Mechanical Testing in a Developmental Mouse Supraspinatus Tendon Model," *Journal of Biomechanical Engineering* 134 (2012): 041004.

119. T. Shearer, "A New Strain Energy Function for Modelling Ligaments and Tendons Whose Fascicles Have a Helical Arrangement of Fibrils," *Journal of Biomechanics* 48 (2015): 3017–3025.

120. A. K. Aune, M. Hukkanen, J. E. Madsen, J. M. Polak, and L. Nordsletten, "Nerve Regeneration During Patellar Tendon Autograft Remodelling After Anterior Cruciate Ligament Reconstruction: An Experimental and Clinical Study," *Journal of Orthopaedic Research* 14 (1996): 193–199.

121. F. Sato, Y. Masuda, D. Suzuki, et al., "Biomechanical Analysis of Tendon Regeneration Capacity of Iberian Ribbed Newts Following Transection Injury: Comparison to a Mouse Model," *Journal of Orthopaedic Research* 42 (2024): 607–617.

122. M. Loppini, U. Longo, G. Niccoli, W. Khan, N. Maffulli, and V. Denaro, "Histopathological Scores for Tissue-Engineered, Repaired and Degenerated Tendon: A Systematic Review of the Literature," *Current Stem Cell Research & Therapy* 10 (2014): 43–55.

123. T. Movin, A. Gad, F. P. Reinholt, and C. Rolf, "Tendon Pathology in Long-Standing Achillodynia. Biopsy Findings in 40 Patients," *Acta Orthopaedica Scandinavica* 68 (1997): 170–175.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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2.3 Manuscript Supporting Information

The following appendices reflect documents submitted with the previous manuscript as supplementary material.

2.3.1 Supporting Information S1: Material A

The following details the systematic search terms as described in 'Supporting Information S1: Material A' appended to the manuscript:

Supplementary Material A – Systematic Search Terms

To explore variations in normal/healthy tendon multiscale structure, an electronic database search of PubMed (MEDLINE) was initially conducted on 30th September 2022 using the keyword search terms as follows: (tendon OR tendons OR tend*) AND (histolog* OR histochem* OR microscop*) AND (normal OR healthy OR "non-pathological" OR "non pathological") AND (structu*) AND (compar* OR differen* OR variation OR variations). During the course of reviewing the literature, the search was conducted again on 22nd September 2023 and 17th August 2024 to capture new records. To ensure results remained consistent, the full list of search and MeSH terms as per the initial keyword search in 2022 were used for subsequent searches to ensure all articles captured reflect the results generated from the indexed National Library of Medicine (NLM) terms list at the time of the initial search. The full list of terms used were: ("tendinopathy"[MeSH Terms] OR "tendinopathy"[All Fields] OR "tendonitis"[All Fields] OR "tendon s"[All Fields] OR "tendonous"[All Fields] OR "tendons"[MeSH Terms] OR "tendons"[All Fields] OR "tendon"[All Fields] OR ("tendinopathy"[MeSH Terms] OR "tendinopathy"[All Fields] OR "tendonitis"[All Fields] OR "tendon s"[All Fields] OR "tendonous"[All Fields] OR "tendons"[MeSH Terms] OR "tendons"[All Fields] OR "tendon"[All Fields]) OR "tend*"[All Fields]) AND ("histolog*"[All Fields] OR "histochem*"[All Fields] OR "microscop*"[All Fields]) AND ("normalisation"[All Fields] OR "normalisations"[All Fields] OR "normalise"[All Fields] OR "normalised"[All Fields] OR "normalises"[All Fields] OR "normalising"[All Fields] OR "normalization"[All Fields] OR "normalizations"[All Fields] OR "normalize"[All Fields] OR "normalized"[All Fields] OR "normalizer"[All Fields] OR "normalizers"[All Fields] OR "normalizes"[All Fields] OR "normalizing"[All Fields] OR "normally"[All Fields] OR "normals"[All Fields] OR "tissues"[MeSH Terms] OR "tissues"[All Fields] OR "normal"[All Fields] OR ("healthies"[All Fields] OR "healthy"[All Fields]) OR "non-pathological"[All Fields] OR "non-pathological"[All Fields]) AND "structu*"[All Fields] AND ("compar*"[All Fields] OR "differen*"[All Fields] OR ("variation"[All Fields] OR "variations"[All Fields]) OR ("variation"[All Fields] OR "variations"[All Fields])).

The document can be accessed at: https://onlinelibrary.wiley.com/doi/full/10.1002/jor.26060

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2.3.2 Supporting Information S2: Table 1

'Supporting Information S2: Table 1' appended to the manuscript consists of an excel spreadsheet containing the list of articles which were included from the systematic search and a summary of the outcomes from the review. Due to the nature and size of this document, the full sheets/tables are not included in this thesis.

The document can be accessed at: https://onlinelibrary.wiley.com/doi/full/10.1002/jor.26060

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2.3.3 Supporting Information S3: Tables 2a-e

'Supporting Information S3: Tables 2a-e' consists of five tables (a-e) appended to the manuscript as a word document. Each table (included in the following pages below) outlines the literature exploring one of the 'variables of interest' covered within the literatures: Table S-2a explores *Tendon* differences; Table S-2b explores *Region* differences; Table S-2c explores *Age* differences; Table S-2d explores *Sex* differences; and Table S-2e explores *Other* differences.

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Table S-2a. Primary findings relating to differences between tendons/ligaments. Tendons/Ligaments or Outcomes of the study not relevant or statistically assessed for the detailed variable (*Tendon*) are greyed-out. Bold **outcomes** indicate those with statistically significant findings. *Refer to Table 5 in manuscript for acronym definitions.*

Reference	Species	Tendon (T) / Ligaments (L)	Outcomes	Major (statistically significant) findings
Birch et al. (1999)	Equine	**2T** DDFT, SDFT	**Fibril diameter**	DDFT had larger fibril diameter than SDFT.
Hadjicostas et al. (2007a)	**Human**	**2T** Patellar, Quadriceps	**Collagen-fibril density**, Fibril diameter, Vascular density, **Cell density**	Quadriceps had higher fibril density (fibril:interstitium ratio) than Patellar tendon. Quadriceps had higher cell density than Patellar tendon.
Hadjicostas et al. (2007b)	**Human**	**3T** Gracilis, Semitendinosus, Patellar	**Collagen-fibril density**, Fibril diameter, Vascular density, **Cell density**	Both Gracilis and Semitendinosus had higher fibril density (fibril:interstitium ratio) than Patellar tendon. Both Gracilis and Semitendinosus had higher cell density than Patellar tendon.
Hadjicostas et al. (2008a)	**Human**	**4T** Gracilis, Semitendinosus, Patellar, Quadriceps **1L** ACL	**Collagen-fibril density**, Fibril diameter, **Vascular density**, **Cell density**, **Elastic fibril density**, **Collagen fibril-type density (Type I, Type III, Type V)**	Gracilis had higher fibril density (fibril:interstitium ratio) than Quadriceps / Semitendinosus / ACL / Patellar. Quadriceps / Semitendinosus had higher fibril density than ACL and Patellar tendon. ACL had higher vascular density than Gracilis / Semitendinosus / Quadriceps / Patellar tendons. Semitendinosus had higher vascular density than Gracilis / Quadriceps / Patellar tendons. Quadriceps had higher vascular density than Patellar tendon. ACL had higher cell density than Gracilis / Semitendinosus / Quadriceps / Patellar tendons. Gracilis / Semitendinosus / Quadriceps had higher cell density than Patellar tendon. ACL had higher elastic fibril density than Gracilis / Semitendinosus / Quadriceps / Patellar tendons. ACL had lower Collagen-I fibril density than Gracilis / Semitendinosus / Quadriceps / Patellar tendons. Gracilis had lower Collagen-I fibril density than Semitendinosus / Quadriceps / Patellar tendons. ACL had higher Collagen-III fibril density than Gracilis / Semitendinosus / Quadriceps / Patellar tendons. Gracilis / Semitendinosus / Patellar had higher Collagen-III fibril density than Quadriceps tendon. ACL had higher Collagen-V fibril density than Gracilis / Semitendinosus / Quadriceps / Patellar tendons. Gracilis had higher Collagen-V fibril density than Semitendinosus / Quadriceps tendons.
Hadjicostas et al. (2008b)	**Human**	**2T** Gracilis, Semitendinosus	**Collagen-fibril density**, Fibril diameter, **Vascular density**, Cell density, Elastic fibril density, Collagen fibril-type density (**Type I**, Type III, **Type V**)	Gracilis had higher fibril density (fibril:interstitium ratio) than Semitendinosus. Semitendinosus had higher vascular density than Gracilis. Gracilis had lower Collagen-I fibril density than Semitendinosus. Gracilis had higher Collagen-V fibril density than Semitendinosus.
Stanley et al. (2008)	Equine	**2T** CDET, SDFT	**Cell density**, **Cell morphology**	Consistently across all three different study (exercise) models: CDET had lower total cell density than SDFTc (central/core region) and SDFTp (peripheral region). CDET had lower type 1 (spindle) cell density than SDFTc and SDFTp. For Long-term exercise and Foal studies: CDET had lower type 2 (ovoid) cell density than SDFTc and SDFTp. For the Foal study: CDET had higher proportion of type 1 (spindle) cells than SDFTc and SDFTp. For short-term exercise study: CDET had lower type 2 (ovoid) cell density than SDFTc. Additionally, CDET had lower proportion of type 1 (spindle) cells than SDFTc and SDFTp.
Franchi et al. (2009)	Murine (Rat)	**3T** Patellar, RFT, VIT	**Crimp number**, Crimp angle, Crimp wavelength	VIT had a greater quantity of crimps than RFT and Patellar. VIT had smaller crimp top angle than RFT and Patellar. VIT had smaller crimp base length than RFT and Patellar.
Hosaka et al. (2010)	Equine	**3T** CDET, DDFT, SDFT	**Cell density**	SDFT had greater cell density than DDFT and CDET. DDFT had greater cell density than CDET.

Weiss et al. (2012)	Human	**5T** Gracilis, Semitendinosus, Patellar, Quadriceps, Tibialis Anterior **1L** ACL	Cell density, ***αSMA+ cell density***, ***Crimp wavelength***	There was a significant correlation of myofibroblast density and crimp frequency for ACL, Semitendinosus, Gracilis, Tibialis Anterior, Quadriceps, and Patellar tendon. Correlation strength was greatest for Patellar, followed by Semitendinosus, ACL, Quadriceps, Tibialis Anterior, and lastly Gracilis tendon.
Zhu et al. (2012)	Human	***1T** "Hamstring" (Gracilis or Semitendinosus) **1L** ACL	**Fibril diameter**, **Fibril density**	ACL had greater percentage of 0-50nm and 50-100nm fibrils than Hamstring tendon. Hamstrings had greater percentage of 100-150nm and 150-200nm fibrils than ACL. ACL had higher mean density of fibrils than Hamstrings.
Tilley et al. (2014)	Human	**2T** Subscapularis, Supraspinatus	**Fibril diameter**, Fibril angle (orientation), Crimp length, **Fibre-thickness ratios (PSR staining)**	Supraspinatus tendon (young cohort) had thinner fibril diameters than Subscapularis tendon (old cohort). Supraspinatus tendon (young cohort) had lower ratio of thick-to-thin diameter fibrils than Subscapularis tendon (old cohort).
Herod et al. (2016)	Bovine	**2T** CDET, SDFT	**Crimp wavelength**, **Fibril diameter**	CDET had longer crimp wavelength than SDFT. CDET had larger (diameter) collagen fibrils than SDFT.
Thorpe et al. (2016a)	Equine	**2T** CDET, SDFT	Elastin content, GAG content (IHC staining intensity: Biglycan, Decorin, **Fibromodulin**, Lubricin, **Lumican**)	Within the IFM, CDET had greater lumican staining than SDFT. Within the FM, CDET had greater fibromodulin staining than SDFT.
Godinho et al. (2017)	Equine	**2T** CDET, SDFT	**Elastin content** (IHC staining), **IFM:FM area ratio**, **Elastin fibre orientation/alignment**	Within the younger cohort, SDFT had higher IFM elastin content than CDET. Within both cohorts, SDFT had higher FM elastin content than CDET. Within both cohorts, SDFT had higher IFM:FM area than CDET. Within the older cohort, SDFT had more randomly distributed elastin fibres than CDET. Additionally, within the older cohort, SDFT had less overall organisation than CDET. In both cohorts, SDFT had less elastin aligned parallel to the IFM and more elastin aligned perpendicular to the IFM than CDET.
Kharaz et al. (2018)	Canine	**2T** LDET, SDFT **2L** ACL, MCL	**Microfibril (elastin) staining** (distribution, location and pericellular association), GAG staining (distribution, location, and pericellular association), Chondroid cell change, **Protein/GAG content, IHC staining** (Collagen type-I and -III, **Aggrecan**, **Versican**, Decorin, Biglycan, Elastin, Fibrillin-1, Fibrillin-2), **Collagen content (hydroxyproline)**, **Tissue architecture**, **Cell morphology**, Cell distribution, **Cell arrangement**, Vascularity, Inflammatory cell infiltration	The ACL had lower ECM organization than LDET and SDFT. MCL had lower ECM organization than LDET. ACL had more rounded cell morphology and less aligned cells than LDET. SDFT had less total collagen content than ACL, MCL, and LDET. ACL had higher GAG content than MCL, LDET, and SDFT. ACL had higher elastin content than MCL, LDET, and SDFT. ACL had higher aggrecan staining and versican staining than MCL, LDET.
Spiesz et al. (2018)	Equine	**2T** CDET, SDFT	***Collagen fibre organisation (birefringence)***, **Crimp angle**, **Crimp length**	In stained sections, CDET had higher total birefringence (higher collagen content / organisation) than SDFT. CDET had larger crimp angle than SDFT. CDET had longer crimp length than SDFT.
Hayashi et al. (2019)	Canine	**1T** LDET **3L** CaCL, CrCL, MCL	**Cell morphology (fusiform density, ovoid density, spheroid density)**, Cell density	MCL and LDET had greater densities of fusiform nuclei than in CaCL and CrCL. CaCL and CrCL had greater densities of ovoid and spheroid nuclei than in MCL and LDET. For Fusiform cell density, significant differences were observed between structures (not specified: CaCL < CrCL < MCL < LDET) For Ovoid cell density, significant differences were observed between structures (not specified: CaCL > CrCL > LDET > MCL) For Spheroid cell density, significant differences were observed between structures (not specified: CrCL > CaCL > LDET > MCL)

Ristaniemi et al. (2021)	Bovine	**1T** Patellar **4L** ACL, LCL, MCL, PCL	**Crimp angle**, Crimp length	Bovine ACL had smaller crimp angle than the MCL, PCL, and Patellar Tendon.
Fjordbakk and Marques-Smith (2023)	Equine	**3T** Medial Patellar, Intermediate Patellar, Lateral Patellar	**IFM thickness**, IFM vascularity, Adipose Infiltration	Medial PT had thicker IFM than Intermediate PT and Lateral PT. Intermediate PT had thicker IFM than Lateral PT.
Gsell et al. (2023)	**Bovine, Murine (Rat)**	**3T** LDET [Bovine], SDFT [Bovine], Tail [Rat]	**Fibril CSA**, **Fibril Aspect Ratio**	Bovine SDFT fibrils had smaller CSA than bovine LDET and rat tail fibrils. Bovine LDET fibrils had smaller Aspect Ratio (aka flatter / less plump) than bovine SDFT and rat tail fibrils.

Table S-2b. Primary findings relating to differences between regions within a tendon. Tendons/Ligaments or Outcomes of the study not relevant or statistically assessed for the detailed variable (*Region*) are greyed-out. Bold **outcomes** indicate those with statistically significant findings. *Refer to Table 5 in manuscript for acronym definitions.*

Reference	Species	Tendon (T) / Ligaments (L)	Outcomes	Major (statistically significant) findings	Regions compared
Wilmink et al. (1992)	Equine	**1T** SDFT	**Crimp wavelength**, **Crimp angle**	In the old cohort, Central fibres had smaller crimp angle than Peripheral fibres. In the old cohort, Central fibres had shorter crimp length than Peripheral fibres.	Central vs Peripheral
Patterson-Kane et al. (1997a)	Equine	**1T** SDFT	Fibril diameter, **Crimp wavelength**, **Crimp angle**	In the young cohort, Central fibres had larger crimp angle than Peripheral fibres. In the old cohort, Central fibres had smaller crimp angle than Peripheral fibres. In the young cohort, Central fibres had longer crimp length than Peripheral fibres.	Central vs Peripheral
Patterson-Kane et al. (1997b)	Equine	**1T** SDFT	Crimp wavelength, Crimp angle	For exercised equines (majority <10 years old), 6/9 horses found Central fibres had smaller crimp angle than Peripheral fibres. For non-exercised equines, 2/9 horsed found Central fibres had smaller crimp angle than Peripheral fibres, whilst 3/9 horses found Central fibres had larger crimp angle than Peripheral fibres. For exercised equines (majority <10 years old), 3/9 horses found Central fibres had shorter crimp length than Peripheral fibres, whilst 3/9 horsed found Central fibres had longer crimp length than Peripheral fibres. For non-exercised equines, 2/9 horses found Central fibres had shorter crimp length than Peripheral fibres, whilst 3/9 horsed found Central fibres had longer crimp length than Peripheral fibres.	Central vs Peripheral
Birch et al. (1999)	Equine	**2T** DDFT, SDFT	Fibril diameter		Central vs Peripheral
Gibson et al. (2002)	Equine	**1T** SDFT	* Crimp wavelength, Crimp angle		Central vs Peripheral (Proximal, middle, distal regions also included but not compared)
Cook et al. (2004)	Human	**1T** Patellar	**Cell characteristics**, **Ground substance staining**, **Collagenous structure**, Vascularity	Proximal region tendon had "worse" cell characteristics (morphological) than the Distal region. Proximal region tendon had "worse" ground substance staining than the Distal region. Proximal region tendon had "worse" collagen structure than the Distal region.	Longitudinal: Proximally (bone-attachment) vs 1cm distally
Watanabe et al. (2005)	Equine	**1T** SDFT	**Fibril diameter (mean, MAD)**, **Collagen:non-collagen ratio**	Proximal region had smaller fibril diameters than Middle region and Distal region tendon. Proximal region had greater collagen density (collagen:non-collagen ratio) than Distal region tendon.	Longitudinal: Proximal Middle, Distal
Prado et al. (2006)	Human	**1T** Tibialis Posterior	**Vascular density**	Longitudinal level (region) correlated with vascular density; Proximal tendon had lower vascular density than distal tendon.	Longitudinal: 6 segments
Funakoshi et al. (2008)	Caprine	**2T** Infraspinatus, Patellar	Fascicle diameter, **Crimp wavelength**,	Superior aspect had longer crimp wavelength than the Deep (humeral side) aspect.	Transverse (Infraspinatus): Superior / superficial vs Deep) humeral joint side compared with the superior aspect of the tendon

Stanley et al. (2008)	Equine	**2T** CDET, SDFT	Cell density, **Cell morphology**	<u>Across all three exercise study models:</u> SDFT Central region had higher type 2 (ovoid) cell density than SDFT Peripheral region. <u>In short-term exercise and foal studies:</u> SDFT Central region had lower type 1 (spindle) cell density than SDFT Peripheral region. Additionally, SDFT Central region had lower proportion of type 1 (spindle) cells than the SDFT Peripheral region. <u>In the Foal study:</u> SDFT Central region had lower type 1 (spindle) cell density than SDFT Peripheral region.	Central vs Peripheral
Williams et al. (2008)	Rabbit	**1T** Patellar	**Fibril diameter**, **Fibril-occupied area**, **Fibril density**	Significant difference was found between all regions for fibril diameter: Central-Centre > Central-Distal > Medial-Centre > Central-Proximal > Lateral-Distal. Lateral-Distal tendon had higher fibril density (number density) than Central-Distal, Central-Centre, Medial-Centre and Central-Proximal and tendon. Medial-Centre tendon had [slightly] lower fibril density than Central-Proximal tendon. Central-Centre, Central-Distal and Lateral-Distal tendon had higher fibril-occupied area (Area Fraction) than Medial-Centre and Central-Proximal tendon.	Transverse and Longitudinal: Central-Proximal (CP), Central-Centre (CC), Medial-Centre (MC), Central-Distal (CD), and Lateral-Distal (LD)
Franchi et al. (2009)	Murine (Rat)	**3T** Patellar, RFT, VIT	**Crimp number, Crimp angle, Crimp wavelength**	VIT had greater crimp number than RFT. VIT had smaller crimp angle than RFT. VIT had shorter crimp wavelength than RFT.	Sub-tendon bodies (Quadriceps)
Hansen et al. (2010)	**Human**	**1T** Patellar	Volume fraction of fibrils, **Fibril density**, Fibril diameter	Deep (posterior) tendon had higher fibril density than Superficial (anterior) tendon.	Transverse: superficial/anterior vs deep/posterior
Mazzocca et al. (2013)	**Human**	**1T** LHBT	Protein content (IHC staining: Collagen type I, Collagen type III, Tenascin C, Decorin), **Proteoglycan content (Alcian blue staining)**, **Collagen fibre orientation/organisation**	Proximal (bone-end) tendon had higher proteoglycan content than distal tendon. Proximal (bone-end) tendon had lower collagen organisation than distal tendon.	Longitudinal: Proximal vs Distal
Thorpe et al. (2016a)	Equine	**2T** CDET, SDFT	**Elastin content**, GAG content (IHC staining intensity: **Biglycan**, Decorin, Fibromodulin, **Lubricin**, **Lumican**)	For CDET, IFM had greater lumican staining than FM. For SDFT, IFM had greater biglycan staining than FM. For SDFT, IFM had greater lubricin staining than FM. For SDFT, IFM had greater elastin staining than FM.	IFM vs FM
Thorpe et al. (2016b)	Equine	**1T** SDFT	**Cell density**	IFM had higher cell density than FM.	IFM vs FM
Biasutti et al. (2017)	Ovine	**1T** SDFT	**Cellularity**, **Cell morphology**, **Vascularity**, **Inflammatory cell infiltration**, Collagen fibre alignment, **Proteoglycan content (TB staining)**	Significant (correlative) difference was found between tendon regions and cellularity (cell density): Middle tendon had the highest cell density than Proximal/Distal sites. Significant (correlative) difference was found between tendon regions and cell morphology: Middle tendon had the highest (more rounded) cell morphology than Proximal/Distal sites. Significant (correlative) difference was found between tendon regions and vascularity: Middle tendon had the highest vascular density than Proximal/Distal sites. Significant (correlative) difference was found between tendon regions and inflammatory cell infiltration: Middle tendon had the highest infiltration than Proximal/Distal sites. Significant (correlative) difference was found between tendon regions and proteoglycan content: Middle tendon had the highest proteoglycan content than Proximal/Distal sites. Distal tendon had higher proteoglycan content than the Proximal site.	Longitudinal: Proximal, Middle, Distal

Kharaz et al. (2018)	Canine	**2T** LDET, SDFT **2L** ACL, MCL	Microfibril (elastin) staining (distribution, location and pericellular association), GAG staining (distribution, location, and pericellular association), Chondroid cell change, Protein/GAG content, IHC staining (Collagen type I, Collagen type III, Aggrecan, Versican, Decorin, Biglycan, Elastin, Fibrillin-1, Fibrillin-2), Collagen content (hydroxyproline), Tissue architecture, Cell morphology, Cell distribution, Cell arrangement, Vascularity, Inflammatory cell infiltration		Longitudinal: Proximal, Middle, Distal.
Spiesz et al. (2018)	Equine	**2T** CDET, SDFT	**Collagen fibre organisation (birefringence)**	For both CDET and SDFT, IFM had lower total birefringence (lower collagen content/organisation) than FM.	IFM vs FM
Takahashi et al. (2018)	Bovine	**1T** SDFT	Fibril diameter, **Cell density**, **Collagen-fibril density**, **GAG chain length, GAG chain angle**	Distal region had higher cell density than Middle and Proximal regions. Proximal region had higher cell density than Middle region. Proximal region had higher fibril density than Middle and Distal regions. Distal region had longer GAG chain length than Proximal region. Distal region had larger GAG chain angle than Middle and Proximal regions. Middle region had larger GAG chain angle than Proximal region.	Longitudinal: near-MTJ, Middle, near-TBI (Proximal, Middle, Distal)
Feo et al. (2019)	Avian (Pedrês chicken)	**1T** SDFT	Cell density		Longitudinal: Proximal vs Distal
Zheng et al. (2024)	Avian (Turkey)	**1T** Gastrocnemius (Achilles)	**Collagen structure organisation**	LM had greater organisation than EM and NM. EM additionally had greater organisation than NM.	Longitudinal: Non- (NM; most distal), Late- (LM) and Early-mineralising (EM; most proximal) regions

Table S-2c. Primary findings relating to differences in relation to age. Tendons/Ligaments or Outcomes of the study not relevant or statistically assessed for the detailed variable (*Age*) are greyed-out. Bold **outcomes** indicate those with statistically significant findings. *Refer to Table 5 in manuscript for acronym definitions.*

Reference	Species	Tendons (T) / Ligaments (L)	Outcomes	Major (statistically significant) findings	Age Cohorts
Ingelmark (1948)	Murine (Rat)	**1T** Achilles	***Fibril diameter*, Fibril cross striation length**	In OsO4 treated samples, Young (untrained) cohort had smaller fibril diameters than old (trained) cohort. In OsO4 treated samples, Young (untrained) cohorts had narrower striation segments than the old (untrained) and old (trained) cohorts.	Young (untrained), Old (untrained) and Old (trained)
Wilmink et al. (1992)	Equine	**1T** SDFT	**Crimp wavelength, Crimp angle**	In central region, Young animals had larger crimp angle than Old animals. In peripheral region, Young animals had larger crimp angle than Old animals. In central region, Young animals had larger crimp wavelength than Old animals. In peripheral region, Young animals had larger crimp wavelength than Old animals.	Young (central and peripheral regions), Old (central and peripheral)
Patterson-Kane et al. (1997a)	Equine	**1T** SDFT	*Fibril diameter*, *Crimp wavelength*, **Crimp angle**	For central region, the Old cohort has smaller crimp angle than the Young and Middle-Aged cohorts. *[Additional pre-natal finding: Foetal cohort had larger crimp angle than all three adult groups (Young, Middle-Aged, Old)].* *[Additional pre-natal finding: Foetal cohort had longer crimp wavelength than all three adult groups (Young, Middle-Aged, Old)].* *[Additional pre-natal finding: Foetal cohort had smaller fibril diameter than young (central) cohort].*	Young (central and peripheral regions), Middle-Aged (central and peripheral), Old (central and peripheral) *[Additional Foetal cohort (central region only)]*
Birch et al. (1999)	Equine	**2T** DDFT, SDFT	Fibril diameter		Young, Old
Cook et al. (2004)	Human	**1T** Patellar	Cell characteristics, Ground substance staining, Collagenous structure, Vascularity		Grouped according to "normal" vs "abnormal" score outcomes
Edwards et al. (2005)	Equine	**1T** CDET	***Fibril diameter*, Collagen fibril density**	The Young (control of short-term exercise) cohort had greater fibril diameter than the Old (control of long-term exercise) cohort.	Young, Old
Prado et al. (2006)	Human	**1T** Tibialis Posterior	Vascular density		Young (under 40), Old (0ver 40)
Hadjicostas et al. (2007a)	Human	**2T** Patellar, Quadriceps	Collagen-fibril density, Fibril diameter, Vascular density, Cell density		Single cohort, span 35 years
(Hadjicostas et al. 2007b)	Human	**3T** Gracilis, Semitendinosus, Patellar	Collagen-fibril density, Fibril diameter, Vascular density, Cell density		Single cohort, span 44 years
Hadjicostas et al. (2008a)	Human	**4T** Gracilis, Semitendinosus, Patellar, Quadriceps **1L** ACL	Collagen-fibril density, Fibril diameter, Vascular density, Cell density, Elastic fibril density, Collagen fibril-type density (Type I, Type III, Type V)		Single cohort, span 30 years
Hu et al. (2012)	Murine (Rat)	**1T** *Unnamed*	**Collagen fibre orientation/orderliness, Fibre diameter**	The Old cohort had more orderly arranged fibre structure than the Young cohort. The Old cohort had smaller *fibres* than the Young cohort.	Young, Old

Dunkman et al. (2013)	Murine (Mice)	**1T** Patellar	Fibril diameter, **Cell morphology**, **Cellularity**, **Collagen fibre alignment**	The Old cohort had lower cell density than the Young cohort. The Young cohort had rounder cell morphology cell density than the Old cohort. The Old cohort had more aligned fibres than the Young cohort.	Young, Old
Lavagnino et al. (2013)	Murine (rat)	**1T** Tail	Cell density, Cell morphology, αSMA presence	The Young cohort had lower cell density than the Mature and Middle-Aged cohort. The Mature cohort had lower cell density than the Middle-Aged cohort. The Young cohort had rounder cell nuclei than the Mature and Middle-Aged cohort. The Mature cohort had rounder cell nuclei than the Middle-Aged cohort.	Young (1-month), Mature (3-month), Middle-Aged (12-month)
Thorpe et al. (2013)	Equine	**1T** SDFT	IFM area	Young SDFTs had greater IFM area percentage than Old SDFTs.	Young, Old
Legerlotz et al. (2014)	Murine (Mice)	**1T** Tail	**Crimp wavelength**, **Crimp height, Crimp angle**, Crimp planarity**, Cell length, Cell gap width (cell spacing), Cell density, Cell track distance (distribution)**	From both two-photon and brightfield microscopy, the 3w cohort had shorter crimp wavelength than all other cohorts (6w, 9w, 12w, 6m). For two-photon microscopy, 6w and 6m cohorts had shorter crimp wavelength than the 9w cohort. The 3w cohort had shorter crimp height than the 6w and 9w cohorts. The 6m cohort had smaller crimp angle than the 6w and 9w cohorts. The 3w cohort had shorter cell length than the 9w, 12w and 6m cohorts. The 6w cohort had shorter cell length than the 12w cohort. The 6m cohort had larger gap width than all other cohorts (3w, 6w, 9w, 12w). The 3w cohort had larger cell density than all other cohorts (6w, 9w, 12w, 6m). The 6w cohort had smaller cell density than the 12w and 6m cohorts. The 3w cohort had smaller cell track distance than the 6w and 9w cohorts.	3-weeks, 6-weeks, 9-weeks, 12-weeks and 6-months
Tilley et al. (2014)	**Human**	**2T** Subscapularis, Supraspinatus	**Fibril diameter,** Fibril angle (orientation), Crimp length, **Fibre-thickness ratios (PSR staining)**	Supraspinatus tendon (young cohort) had thinner fibril diameters than Subscapularis tendon (old cohort). Supraspinatus tendon (young cohort) had lower ratio of thick-to-thin diameter fibrils than Subscapularis tendon (old cohort).	Young (Supraspinatus), Old (Subscapularis)
Thorpe et al. (2016b)	Equine	**1T** SDFT	Cell density		Young, Old
Godinho et al. (2017)	Equine	**2T** CDET, SDFT	**Elastin content (IHC staining), IFM:FM area ratio, Elastin fibre orientation/alignment**	For IFM, elastin content increased with age in the SDFT. For FM, elastin content decreased with age in the CDET. For both SDFT and CDET, IFM:FM ratio increased with age. In SDFT, the Old cohort had more randomly distributed elastin fibres than the Young cohort. Additionally, for SDFT, the Old cohort had less overall organisation than the Young cohort.	Young, Old
Marquez-Arabia et al. (2017)	**Human**	**1T** Gluteus Medius	**Vascular density**	Vascular density was correlated with age; *convex asymmetrical quadratic correlation: density increased with age (18 to approx. 40 years) and then decreased with further aging (over 50 years).*	Single cohort, span 50 years
Pardes et al. (2017)	Murine (Rat)	**1T** Achilles	**Cell morphology**, **Cell density**, Proteoglycan content (Safranin-O/Fast Green staining)	The Old cohort had smaller dell density than Middle-Aged cohort. The Old cohort had more rounded cell morphology than the Middle-Aged and Young cohorts.	Young, Middle-Aged, Old
Gagliano et al. (2018)	**Human**	**2T** Gracilis, Semitendinosus	GAG/Proteoglycan staining intensity (alcian-blue), Sirius red staining intensity (fibrillary-collagen)		Young, Old

Shu et al. (2018)	Murine (Mice)	**2T** Achilles, Tail	**Fibril diameter**	For Both Tail and Achilles Tendons, the Old cohort had larger fibril diameter than the Young cohort.	Young, Old
Spiesz et al. (2018)	Equine	**2T** CDET, SDFT	Collagen fibre organisation (birefringence), Crimp angle, Crimp length		Young, Old
Feo et al. (2019)	Avian (Pedrês chicken)	**1T** SDFT	**Cell density**	In both regions, the Young cohort had greater cell density than the Middle and Old cohorts.	Younger (1-month), Middle (5-months), Older (8-months)
Hayashi et al. (2019)	Canine	**1T** LDET **3L** CaCL, CrCL, MCL	**Cell morphology, Cell density**	In CaCL, Cell density decreased with age. In CaCL, Fusiform cell density decreased with age.	Single cohort (canine), span 14 years
Asai et al. (2022)	**Human**	**1T** Semitendinosus	**Cell morphology, Cell density, Fibril diameter (and distribution)**	The Immature cohort had greater cell major-to-minor-length ratio (rounder cell morphology) than the Young and Adult cohorts. The Young cohort had greater cell major-to-minor-length ratio than the Adult cohort. The Immature cohort had greater cell density than the Young and Adult cohorts. The Young cohort had greater cell density than the Adult cohort. For mean fibril diameter, the Adult cohort had larger mean diameters then the Immature cohort. For 20th percentile, the Adult cohort had larger diameters than the Young and Immature cohorts. For 40th percentile, the Adult cohort had larger diameters than the Immature cohort. For 60th percentile, the Adult cohort had larger diameters than the Immature cohort. For 80th percentile, the Adult cohort had larger diameters than the Immature cohort. Additionally, fibril diameter distribution differed between all cohorts, with histograms shifted from right skewed in the Immature cohort (predominantly small-diameter fibrils) to flatter in the Adult cohort (more even spread with more larger-diameter fibrils).	Immature, Young, Adult
Naot et al. (2022)	**Human**	**1T** Semitendinosus	Fibril diameter		Young (under 20), Old (over 20)
Fjordbakk and Marques-Smith (2023)	Equine	**3T** Medial Patellar, Intermediate Patellar, Lateral Patellar	***IFM thickness, IFM vascularity,* Adipose Infiltration**	Interfascicular endotenon (IFM) characteristics had significant correlation with age for Medial and Intermediate Patellar ligaments ('tendons'). *[For both Medial and Lateral ligaments, age increased IFM size (distinction) and vasculature (prominence)].* Fatty infiltration increased with age for Medial and Intermediate ligaments.	Single cohort (equine), span 17 years.
Johnson et al. (2023)	**Human**	**4T** Infraspinatus, Subscapularis, Supraspinatus, Teres Minor	Collagen organisation, Cell appearance, Vascular presence, Collagenous architecture, GAG/Proteoglycan/ground substance presence, **Total histomophological score**	For Infraspinatus tendon, the Aged cohort had greater total histomorphological (Bonar) score than the Younger cohort.	Younger (< 36 years), Aged (> 55 years)
Tinguely et al. (2023)	**Murine (Rat), Human**	**2T** Achilles [rat; human], Patellar [Rat]	Cellularity, Segmented nuclei count per image, **Nuclear aspect ratio, individual shape mode fractions**, Proteoglycan richness (SAFO), Presence of CD31 (endothelial cells), presence of αSMA (myofibroblasts), and presence of CD146 (tendon stem/progenitor cells).	Cell morphology (nuclear aspect ratio) was correlated with age. The 1m cohort had different cell shape distributions than the 8m, 19m and 28m cohorts. The 8m and 19m cohorts had different cell shape distributions than the 29m cohort. Age had an effect on proteoglycan richness associated cell morphology.	1-month, 8-months, 19-months, 28-months

Table S-2d. Primary findings relating to differences between males and females. Tendons/Ligaments or Outcomes of the study not relevant or statistically assessed for the detailed variable (*Sex*) are greyed-out. Bold **outcomes** indicate those with statistically significant findings. *Refer to Table 5 in manuscript for acronym definitions.*

Reference	Species	Tendons (T) / Ligaments (L)	Outcomes	Major (statistically significant) findings
Prado et al. (2006)	Human	**1T** Tibialis Posterior	Vascular density	
Hadjicostas et al. (2007a)	Human	**2T** Patellar, Quadriceps	Collagen-fibril density, Fibril diameter, Vascular density, Cell density	
Hadjicostas et al. (2007b)	Human	**3T** Gracilis, Semitendinosus, Patellar	Collagen-fibril density, Fibril diameter, Vascular density, Cell density	
Hadjicostas et al. (2008a)	Human	**4T** Gracilis, Semitendinosus, Patellar, Quadriceps **1L** ACL	Collagen-fibril density, Fibril diameter, Vascular density, Cell density, Elastic fibril density, Collagen fibril-type density (Type I, Type III, Type V)	
Pardes et al. (2016)	Murine (Rat)	**1T** Achilles	Cell morphology, Cellularity, Proteoglycan content (Safranin-O/Fast Green staining)	
Sarver et al. (2017)	Murine (Mice)	**1T** Achilles	**Cell density**	Females had larger cell density than Males.
Hayashi et al. (2019)	Canine	**1T** LDET **3L** CaCL, CrCL, MCL	Cell morphology (fusiform density, ovoid density, spheroid density), Cell density	
Asai et al. (2022)	Human	**1T** Semitendinosus	Cell morphology, Cell density, Fibril diameter	

Table S-2e. Primary findings relating to differences in relation to *other* variables. Tendons/Ligaments or Outcomes of the study not relevant or statistically assessed for the detailed variable (*Other*) are greyed-out. Bold **outcomes** indicate those with statistically significant findings. *Refer to Table 5 in manuscript for acronym definitions.*

Reference	Species	Tendons (T) / Ligaments (L)	Outcomes	Major (statistically significant) findings	Variable	Variable specifics/cohorts
Ingelmark (1948)	Murine (Rat)	**1T** Achilles	**Fibril diameter,** Fibril cross striation length	In OsO4 treated fibrils of the old cohorts, the Trained cohort had larger fibril diameters than the Untrained cohort.	Lifestyle – Physical	Effect of exercise regime: Trained vs Untrained
Odetti et al. (2000)	Murine (Rat)	**1T** Tail	**Fibril diameter,** Fibril D-band interval, **Fibril D-band cleft depth**	The Diabetic cohort had larger fibril diameters than the Non-Diabetic cohort. The Diabetic cohort had larger D-Band cleft depth than the Non-Diabetic cohort.	Comorbidities – Diabetes	Effect of diabetes status: Diabetic vs Non-Diabetic
Cook et al. (2004)	**Human**	**1T** Patellar	Cell characteristics, Ground substance staining, Collagenous structure, Vascularity		Demographics Lifestyle – Physical Comorbidities – ACL Injury	Correlations made between scored outcomes (normal VS abnormal result) versus anthropometric measures of weight, height, hours per week of activity, years sport, and time from ACL rupture to reconstruction with the studied tendon
Edwards et al. (2005)	Equine	**1T** CDET	***Fibril diameter,*** Collagen fibril density	** The Short-Term Exercise (younger) cohort had greater fibril diameters than the Long-Term Exercise (older) cohort. ***Note: differences likely resulting from confounding factor of Age as an additional variable*	Lifestyle – Physical	Effect of exercise regime: Short-Term (younger cohort) vs Long-Term (older cohort) exercise regime, and respective controls
Prado et al. (2006)	**Human**	**1T** Tibialis Posterior	Vascular density		Anatomical	Comparison between left- and right-limb
Funakoshi et al. (2008)	Caprine	**2T** Infraspinatus, Patellar	**Fascicle diameter, Crimp wavelength**	Individual animal had an effect on fascicle diameter. Individual animal had an effect on fascicle diameter.	Individual Specifics	Effect of individual animal on outcome
Stanley et al. (2008)	Equine	**2T** CDET, SDFT	Cell density, **Cell morphology**	For all tendons combined (CDET, central SDFT, peripheral SDFT): The Long-Term Exercise cohort had lower proportion of type 1 (spindle) cells than the respective Control tendons.	Lifestyle – Physical	Effect of exercise regime: Long-Term, Short-Term, and Foal-Trained exercise regimes, and respective controls
Hansen et al. (2009)	**Human**	**1T** Patellar	Fibril density, Fibril-occupied area**, Fibril diameter**	ERT-use (high estrogen) cohort had greater percentage of medium (60-90nm) fibrils than the Control (low estrogen) cohort. Control cohort had a higher percentage of large (>90nm) fibrils than ERT cohort.	Biological	Comparison between different hormonal levels/interventions in post-menopausal women in response to exercise: Estrogen replacement therapy (ERT) (following hysterectomy) vs postmenopausal control

Frizziero et al. (2011)	Murine (Rat)	**1T** Patellar	**Proteoglycan (PG) content (Toluidine Blue staining intensity), Collagen fibre organisation/alignment**	** Trained tendons had higher PG staining presence than Untrained and Detrained tendons. **Note: PG content scoring was specific for tendon insertion sight (enthesis).* Trained tendons had more organised fibre organisation/alignment than Untrained and Detrained tendons.	Lifestyle – Physical	Comparison between different exercise regimes: Untrained, Trained, Detrained
Lenskjold et al. (2015)	**Human**	**1T** Achilles	Fibril density, Fibril diameter, Fibril area		Lifestyle - Physical	Effects of activity levels during youth: Athletes with prior high physical activity (HAY) versus low physical activity (LAY) during their youth
(Pardes et al. 2016)	Murine (Rat)	**1T** Achilles	Cell morphology, Cellularity, Proteoglycan content		Biological	Effects of sex-hormonal level (female vs ovariectomised female)
Mazon et al. (2018)	Murine (Rat)	**1T** Calcaneal (Achilles)	**Collagen organisation (birefringence properties)**	Strength Trained tendons had lower birefringence (less organised/aligned structure) than Untrained, Hypertrophy Trained and Resistance Trained tendons.	Lifestyle – Physical	Effect of exercise regime: Untrained, Resistance Trained, Hypertrophy Trained, Strength Trained
Hayashi et al. (2019)	Canine	**1T** LDET **3L** CaCL, CrCL, MCL	Cell morphology (fusiform density, ovoid density, spheroid density), Cell density		Demographics	Effects of body weight
Asai et al. (2022)	**Human**	**1T** Semitendinosus	Cell morphology, Cell density, Fibril diameter		Demographics	Correlation between cell density and weight, height and BMI in Young and Adult cohorts
Bolam et al. (2022)	Murine (Rat)	**1T** Achilles	Cell density, Cell morphology, Collagen fibre alignment, Fibril diameter		Lifestyle – Diet	Effect of diet: Low-Fat (control), High-Fat, High-then-Low-Fat diet regimes
Gsell et al. (2023)	**Bovine, Murine (Rat)**	**3T** LDET [Bovine], SDFT [Bovine], Tail [Rat]	Fibril CSA, Fibril Aspect Ratio	Rat Tail fibrils had larger CSA (diameter) than Bovine LDET fibrils. Rat Tail fibrils had larger Aspect Ratio (aka rounder/plumper) than Bovine LDET fibrils.	Species**	Comparison between species. ***Note: different anatomical tendons are used between species*

~ CHAPTER 3 ~

UNDER THE MICROSCOPE

PROTOCOLS AND METHODOLOGY

"I do have a plan. It's called the Justice League."

~ Batman, Justice League: Doom (2012) ~

3.1 Overview

The following chapter outlines the protocols and methodology of specimen procurement and preparation for histological assessment.

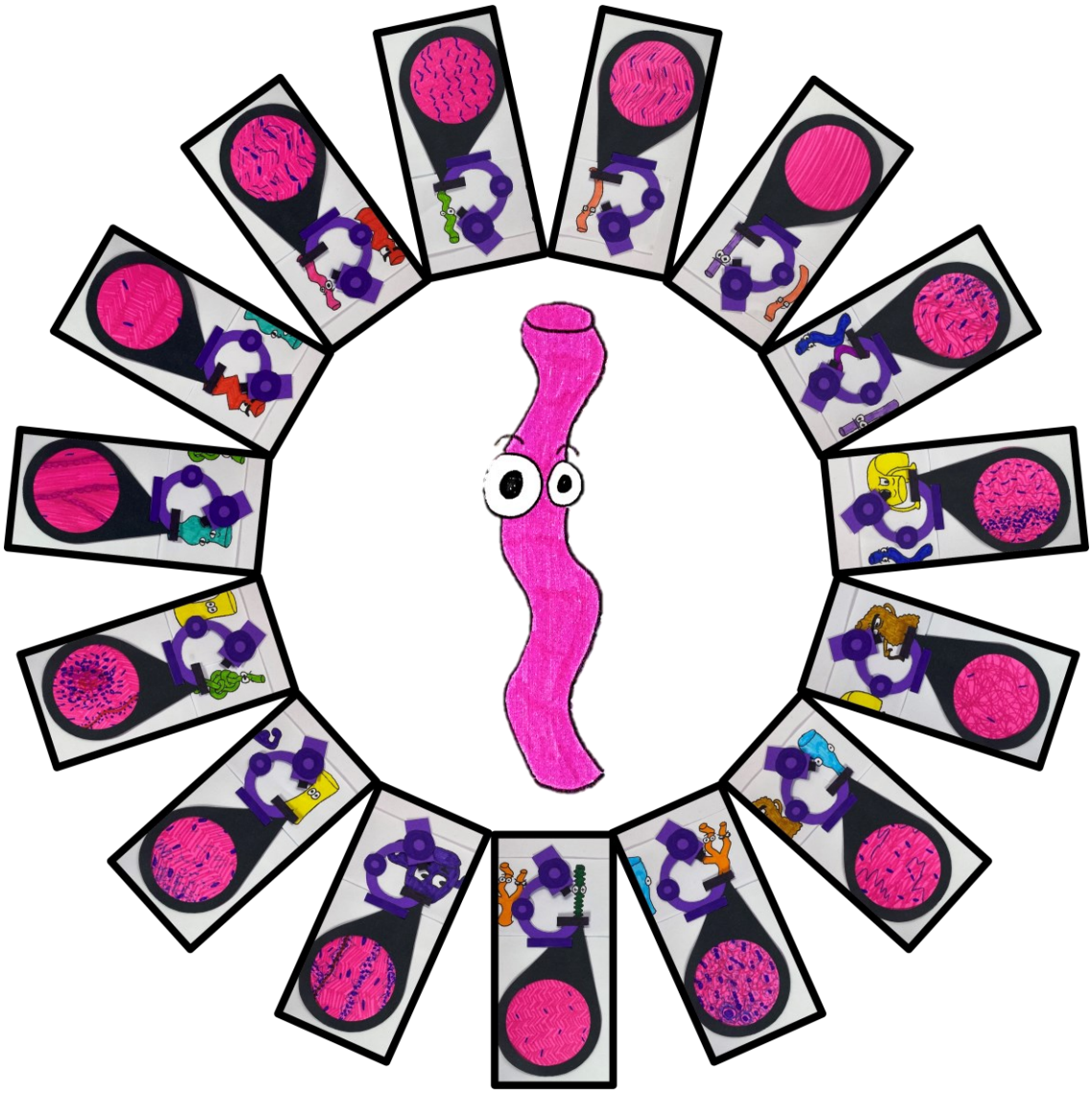


Figure 3.1.A Histological assessment of different tendons.

3.2 Donors and Specimens

3.2.1 Human Cadaveric Tissue

This study utilises tissue from eight human cadaveric donors (five male, three female) sourced fresh-frozen from a licenced donor tissue facility (Science Care Inc.), with specifications of no history of musculoskeletal injury or disease. Ethics approval to use this tissue was provided by the Northern Sydney Local Health District Human Research Ethics Committee (NSLHD HREC, Sydney, Australia. Human ethics approval code: 2019/ETH08332) in accordance with the National Health and Medical Research Council (NHMRC) Act 1992, and the research laboratory holds an Anatomy license from NSW Health to use human tissue for research. Matched left/right upper and lower limbs were obtained for each donor: bilateral clavicle to fingertip for all 8 donors, bilateral mid-femur to toe-tip for 6 donors, and bilateral femoral head to toe-tip for 2 donors.

Donor ages ranged from 49 to 65 years old, with a mean age of 56.1 ± 5.4 years (mean ± standard deviation; males = 56.2 ± 5.4 years; females = 56.0 ± 5.4 years) (Table 3.2.a). Known history and comorbidities which may affect tendon structure are listed in Table 3.2.a. Of note, the effects of chemotherapy on tendon structure are unknown and recorded for reference. A total of 44 different tendons and 4 ligaments (collectively referred to as "tissues" for simplicity) were collected from matched left and right donor limbs. The specific tissues involved in this collection are identified in Table 3.3.a. The matched left and right tissues were randomly allocated to different outcomes as part of the research laboratory's broader database of Human Musculoskeletal Tissue Characteristics (human tissue "atlas"). One side was allocated as a whole tendon to biomechanical, morphological and biochemical outcomes, while the matched contralateral tissue was sub-divided and allocated to histology (the primary outcome for this PhD thesis) and other project outcomes as identified in Figure 3.2.A. (Note: Scanning Electron Microscopy [SEM] and Differential Interference Contrast [DIC] microscopy methodology are included in *Appendix Section 9.4* as reference for images utilised in *Chapter 7).* Where contralateral tissue specimens were too short to allocate to all outcomes, outcome sample collection was prioritised in consecutive order as per the outcome list in Figure 3.2.A (bar exceptions as indicated). For histology, a 10-20mm long specimen was collected (whole-width of tendon where possible) from the tendon mid-substance. First, the midline of the free-tendon was identified, with the electron microscopy and histology samples taken proximal to this, and other outcomes taken distally as depicted in Figure 3.2.A (alternate outcome allocation are also depicted where tendon sizes/shapes differed).

All tissue retrievals were performed by the same surgeons (detailed below) for each limb region (shoulder, arm/forearm or lower limb). Limb regions were thawed at room temperature from −20°C for 24 hours. Tissue specimens were retrieved in the same sequence to allow the safe removal of each tissue and to ensure accurate identification and labelling. Unless otherwise noted, each tendon was identified and released distally then followed proximally and released at the musculotendinous junction (MTJ) (or enthesis/bone-end for shoulder tendons). Tendons were examined macroscopically by the surgeon and only included in the study if no signs of injury or pathology were present. Full retrieval methods for each limb region (shoulder, arm/forearm and lower limb) are detailed below.

Table 3.2.a List of donors and demographic details.

Donor #	Sex	Age	Height (cm)	Wright (kg)	BMI	Comments/History
1	M	50	165	59.9	21.96	Cancer; Chemotherapy
2	F	57	168	49.9	17.75	Cancer; Chemotherapy
3	M	51	168	54.4	19.37	Cancer; Chemotherapy
4	M	57	180	59.0	18.13	
5	F	62	163	54.4	20.60	
6	F	49	163	64.9	24.54	
7	M	58	168	59.0	20.98	
8	M	65	183	53.1	15.87	Metastatic Cancer
Average (All)		56.1	170	56.8	19.90	
Average (Female)		56	164	56.4	20.96	
Average (Male)		56.2	173	57.1	19.26	

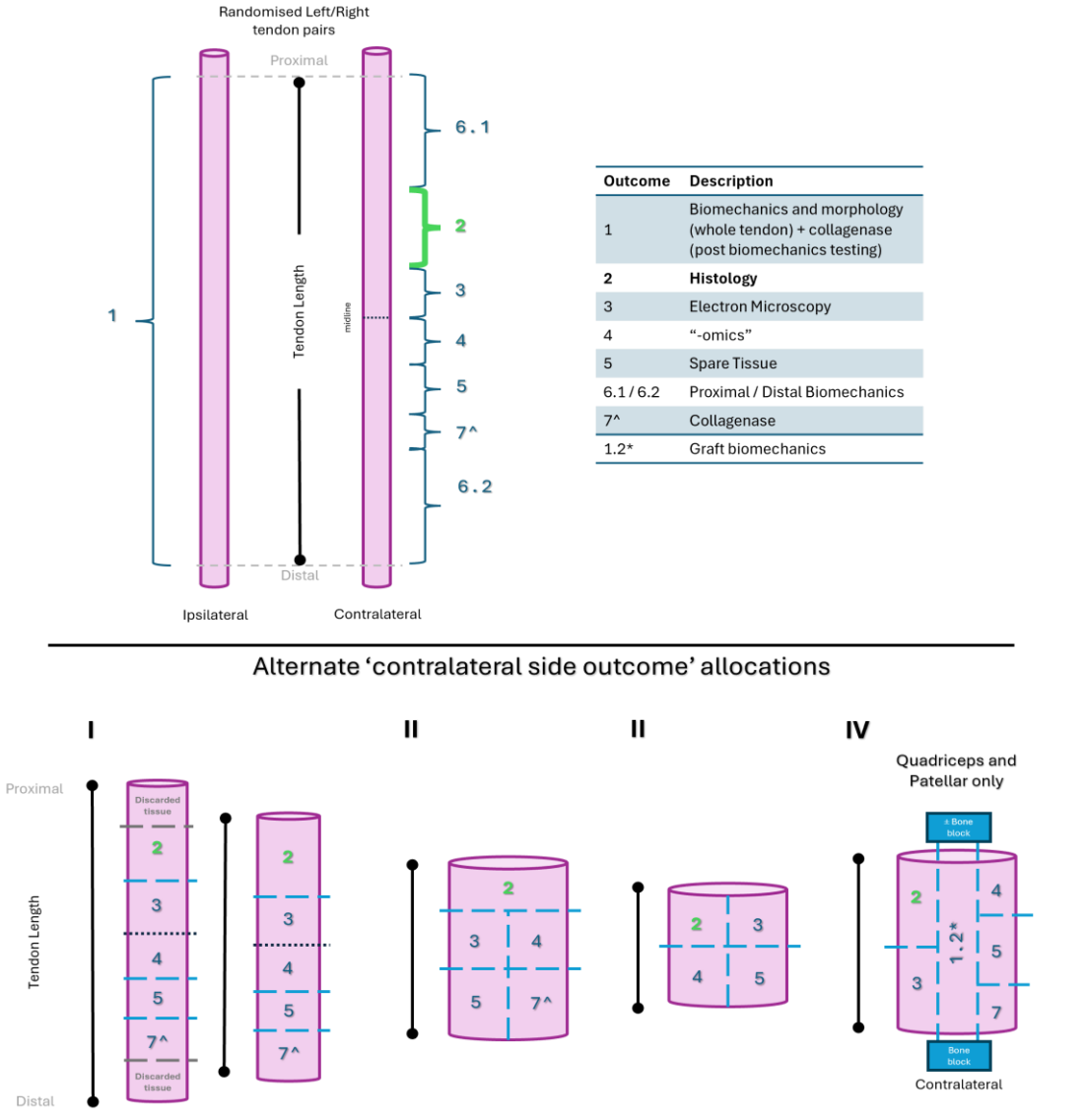


Figure 3.2.A Outcome list for tendons/ligaments of the Human Musculoskeletal Tissue Characteristics project. Outcome 2 (Histology, green) are those included in the studies of this thesis. Where specimens were short or matched left and right sides were not available, outcomes were prioritised in consecutive order as per the outcome list, bar exceptions as follows: ^contralateral tendon length (**I**,**III**) is too short to warrant outcome 6.1/6.2, thus collagenase (7) is included; or *graft specimens (1.2) collected as part of the laboratory's biomechanics study (**V**), where the middle third of the Quadriceps (with distal bone block) and Patellar (with proximal and distal bone blocks: Bone-Patellar-Tendon-Bone, BPTB) are collected as per standard graft harvest protocols. **I - V** approximately depict alternate outcome allocations dependent on differing tissue sizes/morphologies.

3.2.2 Tissue Retrieval Techniques

3.2.2.1 *Upper Limb Dissections*

Initially, the humerus was transected mid-shaft to separate the shoulder region from the rest of the arm. Shoulder dissections were performed by shoulder surgeon Dr. David O'Briain. The remaining upper limb tendons (arm tendons) were retrieved by hand surgeon Dr. David Chang. The dissection protocols were written and generously provided by the surgeon performing those respective dissections as follows:

3.2.2.1.1 Shoulder Region Tendons

Shoulder cadaver dissections began with an incision from the midpoint of the acromion in line with humeral shaft inferiorly and extended superiorly over the trapezius. Full thickness skin flaps were elevated anteriorly and posteriorly. The deltoid muscle origin was released in full from the scapular spine, acromion and lateral clavicle. Deltoid muscle was reflected inferiorly on its insertion to gain access to the underlying muscle layer. The conjoint tendon was released from coracoid process and reflected inferiorly to gain access to the subscapular fossa. The trapezius insertion was released from the scapular spine, acromion and lateral clavicle and reflected proximally to gain access the suprascapular fossa.

The Long Head of Biceps Tendon (LHBT) was identified in the bicipital groove and the transverse humeral ligament was released and the tendon mobilised. The pectoralis major muscle was released from the lateral lip of the bicipital groove of the humerus to expose the underlying biceps muscle. The convergence of the long and short head of biceps was identified inferiorly and LHBT released at this level. Proximally, the LHBT was released from supraglenoid tubercle.

For Subscapularis (SSC), the superior border was released from the rotator interval and adhesions from the coracoid were also released. Inferiorly, a plane was developed between subscapularis and latissimus dorsi and the muscle divided medially/proximally in the middle of the subscapular fossa of scapula. The SSC tendon was peeled off the lesser tuberosity insertion site.

For Infraspinatus (IS), on the inferior aspect, the plane between infraspinatus and teres minor was developed and continued laterally to greater tuberosity. The infraspinatus was released from the scapular spine and fossa. Superiorly, the scapular spine defined the superior border with Supraspinatus and this was developed laterally to the greater tuberosity. Medially/proximally, the muscle was divided in the middle of the infraspinatus fossa. The IS tendon was peeled off the greater tuberosity insertion site.

For Supraspinatus (SSP), on the posterior aspect, the scapular spine defined the posterior border with Infraspinatus and this was developed laterally to the greater tuberosity. The anterior border was released from the rotator interval. Medially/proximally, the muscle was divided in the middle of the supraspinous fossa. The SSP tendon was peeled of the greater tuberosity insertion site.

3.2.2.1.2 Arm Tendons

Exposure of the flexor surface began with a transverse incision over the flexor retinaculum, and a longitudinal incision over the carpal tunnel, extending proximally in line with Palmaris Longus (PL). PL, Flexor Carpi Radialis (FCR) and Flexor Carpi Ulnaris (FCU) were sampled first, as the

most superficial and easily identifiable structures. FCR was incised distally as it passed underneath the tubercle of the trapezium. FCU was incised distally at its insertion into pisiform, as tendon containing this sesamoid bone was not useable in this study. The carpal tunnel was incised, allowing identification of Flexor Pollicis Longus (FPL) to the thumb, and the Flexor Digitorum Superficialis (FDS) and Flexor Digitorum Profundus (FDP) to the index (2^{nd} digit, -2), middle (3^{rd} digit, -3), ring (4^{th} digit, -4) and little fingers (5^{th} digit, -5). These tendons were followed, incising the overlying skin, fat and fascia, taking great care when releasing the flexor sheath and pulley system to each finger and thumb. Multiple vincula, nutrient supplying bands of connective tissue binding flexor tendons to volar surfaces of phalanx bones, were incised. Each FDS, FDP and FPL was incised at its bony insertion to maximise tendon length.

Each extensor surface dissection began with a transverse incision over the extensor retinaculum of the wrist, with a longitudinal incision along the course of extensor digitorum to the middle finger. Tendons were excised by their extensor compartments. The first extensor compartment was released, identifying Abductor Pollicis Longus (APL) and Extensor Pollicis Brevis (EPB). The third extensor compartment was released, identifying Extensor Pollicis Longus (EPL). Each of APL, EPB and EPL were released at their bony attachments. The second compartment, now clear of the previous three tendons, was released identifying Extensor Carpi Radialis Brevis (ECRB) and Longus (ECRL). These were again released at their respective bony insertions. The fourth compartment was opened, revealing Extensor Indicis Proprius (EIP) and the Extensor Digitorum Communis (EDC) to digits -2, -3, -4 and -5. The fifth compartment was also released, revealing Extensor Digiti Minimi (EDM). EDC contains a bulk of extensor hood and tendon together overlying the metacarpophalangeal joint (MCPJ). As extensor tendon distal to this bulky area splits and reforms the tendon distal to this point was deemed not useful for the purposes of this study, hence EDC was incised distally at the level of the MCPJ. EIP and EDM were incised at the same level as EDC, as each respective tendon combined with EDC at this level. The sixth compartment was released last, revealing Extensor Carpi Ulnaris (ECU), which was incised at its bony origin at the base of the little finger metacarpal. Brachioradialis (BrR) was harvested after all other long tendons were removed, itself being removed at its broad insertion into the distal radius.

The Common (Distal) Biceps (DBT) and Triceps (DTT) tendons were harvested last. As the specimens began at the level of mid-humerus, triceps and biceps were identified in their muscle belly and released from surrounding tissue heading distally to their insertion. DBT was incised at its bony insertion into the radial tuberosity, while DTT was incised through its broad olecranon insertion.

Anatomical variants/difficulties. The PL is reported to be absent in approximately 15% of the total population, however this figure varies greatly depending on ethnic group[124]. This tendon was present in all 16 of the current specimens. FDS to the 5^{th} digit is absent in approximately 6% of people[125]. While FDS-5 size varied greatly across donors, it was not absent in any specimen from this study. EDC to the 5^{th} digit is reported to be absent from around 60% of cases[126]. In two donors, this tendon was found to be absent from both limb sides. Additionally, EDC tendons are often linked with soft tissue structures called juncturae tendinum, which can be present in highly

varied configurations[126]. Here, each of these structures was trimmed from the sides of each tendon and subsequently not relevant in the tendon analyses.

3.2.2.2 *Lower Limb Dissections*

The following protocol[127] was generously provided by orthopaedic surgeon Dr. Patrick Haubruck, who performed the retrieval of lower limb specimens used in this project:

3.2.2.2.1 Knee-region Tendons / Ligaments

Retrieval of the tendons and ligaments at the knee began with a longitudinal parapatellar incision of the skin extending proximally to the thigh and distally beyond the tibial tuberosity. The skin and underlying subcutaneous fatty tissue were bluntly mobilised and removed revealing the underlying fascia. Patellar and Quadriceps tendons (PT and QT) were sampled first as the most superficial and easily identifiable tendons. PT was released distally with a bone block of the tibial tuberosity. Thereafter, the tendon and adjacent patella were isolated from the joint capsule and fat pad. Then, the QT was identified, the surrounding muscle tissue removed, and the combined PT, patella and QT incised proximally at the musculotendinous junction (stored as a single sample wrapped in saline soaked gauze until conclusion of all tendon retrieval for the limb and separated as per the autograft harvesting techniques detailed below). Medial to the tibial tuberosity the Pes Anserinus – the conjoined anteromedial insertion site of the tendons of the sartorius, Gracilis (GT) and Semitendinosus (ST) muscle – was identified. Each muscle was followed proximally, incising the overlaying fascia. After removing the sartorius muscle and tendon, the distal insertion of the GT and ST were incised directly at the Pes Anserinus. Thereafter, the Medial Collateral Ligament (MCL) was identified and carefully harvested with bone blocks using a fine chisel both proximally and distally. Laterally, the iliotibial tract was identified and released distally to reveal the underlying Lateral Collateral Ligament (LCL) which was sampled with bone blocks both proximally and distally. Then, the remaining joint capsule and menisci were removed carefully while paying attention to not harm the cruciate ligaments. In a first step, the Anterior (ACL) and Posterior (PCL) Cruciate Ligaments were harvested together with bone blocks from the intercondylar notch and tibial plateau and then separated carefully after identifying the respective bony insertion sites.

Harvesting of autografts from QT and PT. Harvesting of autografts from the contralateral QT-patella-PT complex was conducted following all tendon retrievals for the limb. After identifying the midline of the QT, a full thickness incision (anterior-posteriorly) was made approximately 5mm medial and 5mm lateral to the midline. Thereafter, the incisions were carried proximally until the end of the sample and distally to the superior pole of the patella, which was released with a fine chisel[128]. The PT autograft was harvested in a similar manner resulting in a full thickness and approximately 10mm wide autograft extending from the inferior pole of the patella to the tibial tuberosity (BPTB autograft; bone-Patellar-Tendon-bone)[129].

3.2.2.2.2 Leg-region Tendons

Retrieval of the tendons of the lower-leg began with a dorsal longitudinal incision of the skin extending from the calcaneus to the proximal third of the lower-leg. The underlying soft tissue was mobilised in a circumferential manner and the skin and subcutaneous tissue removed on the

entire length of the incision. Retrieval began with releasing the superficial posterior compartment identifying the Achilles Tendon (AT). Medial to the AT, the Plantaris (Plt) tendon was visible. The Plt was incised distally at its insertion into the AT and followed until its musculotendinous junction and excised to maximise tendon length. Thereafter, the AT was incised distally at its calcaneus insertion and followed until its MTJ and excised to maximise tendon length. Then, the anterior compartment was released and the Tibialis Anterior (TA) and Extensor Hallucis Longus (EHL) identified. These tendons were followed, incising the overlying fascia, superior and inferior extensor retinaculum and tendon sheath. The EHL tendon was distally incised at the base of the distal phalanx of the hallux while the TA tendon was incised at its bony insertion at the medial cuneiform. Thereafter, the lateral compartment was released allowing identification of the Fibularis Longus (FL) and Brevis (FB) muscles. The tendons were followed while carefully incising the tendon sheath and superior and inferior fibular (peroneal) retinacula. While distal incising of the FB was performed at its bony insertion into the base of the fifth metatarsal, the FL was visualised and followed until the plantar crossing of the fourth metatarsal and incised. Medially, the deep posterior compartment was released revealing the Tibialis Posterior (TP), Flexor Digitorum Longus (FDL) and Flexor Hallucis Longus (FHL). The tendons were followed, incising the tendon sheath and releasing the tarsal tunnel by incising the flexor retinaculum. The TP was incised distally at its insertion into the navicular bone, the FDL was followed until its division into four individual tendons and incised proximal to this division, while the FHL was followed and incised at its insertion into the plantar distal phalanx of the hallux.

Anatomical variants/difficulties. The clinical and anatomical significance of the Plantaris muscle is highly debated with anatomical variations a prominent focus in recent literature[130-137]. It is reported to be absent in approximately 7–20% of people[134,135,137,138], likely with variance dependent on ethnicity. Tendon retrieval found the Plantaris to be absent from one limb of one donor of the current specimen, and was incorrectly identified in another donor limb (the single Plantaris tendons from each of these donors allocated to the biomechanics study of the human tissue atlas project as per the protocol described above). The prevalence of anatomical absence of the LCL is not reported, however one limb from the current study found the structure to be absent (the LCL present on the other limb was allocated to biomechanics only) – a rare finding considering that no other injury was observed on the same specimen and this ligament is seldom injured in isolation[21,139-141].

3.2.3 Dataset Numbers

In total, n = 379 specimens were collected for histological processing (48 tissues per donor, minus n = 5 missing tissues: Plantaris from two donors, EDC-5 from two donors, and LCL from one donor). Table 3.3.a in the following section outlines all the tendons/ligaments collected for the histological tissue atlas project.

3.3 Tissue Preparation – Histological Processes

This section outlines the processes used when preparing the tendon samples for histological analysis. Processing protocols were developed and optimised by Ms Susan Smith (SS) to adequately prepare the tendon tissue. Following retrieval, tendons/ligaments were immediately transected using a sharp scalpel and samples allocated to their respective outcome for further processing or further storage by the research team. Retrieved tendons collected ahead of the workflow were kept in saline soaked gauze with identification until able to be subdivided and allocated.

3.3.1 Fixation and Sample Preparation

Figure 3.2.A outlined how tendons/ligaments were divided post retrieval for samples for each outcome. For histology, 10-20 mm long portions were collected from the retrieved tissues immediately fixed in 10% neutral buffered formalin for 48 hours (room temperature) in labelled jars, before transfer into 70% v/v ethanol for storage until further processing.

Prior to processing (detailed below), the specimens collected for histology (n = 379) were prepared (by PhD student **SH**) for later embedding into blocks relative to the size of the specimen for: 1) allocating desired sectioning face; and 2) so that they would fit within the available tissue cassettes or embedding moulds. Furthermore, due to morphological and macroscopic fibre-direction variations observed, each tissue-type was individually assessed for the most suitable embedding orientation to facilitate appropriate sectioning and desired scoring outcomes. The tissue surface opposite to the desired sectioning face was marked with dye (Alcian Blue) to indicate the orientation required for embedding (and thus sectioning) and transferred into labelled cassettes which correspond to the "blocks" prepared for each tendon/ligament. All available tendons/ligaments of one type were assessed together to evaluate general sample size and morphology to achieve maximum consistency with embedding orientation for each tendon/ligament type.

Examples of embedding orientations dependent on specimen size are depicted in Figure 3.3.A (examples **I–VII**). The majority of samples were prepared for embedding such that the widest edge of the specimen was presented as the face for sectioning (**I**). Some samples required division down the midline (longitudinally following fibre direction) to reduce sectioning difficulties when cutting through tissue faces that were too wide and minimise section distortion during mounting processes (**II**). As an indication, blade travels left to right of the blue line (arrow) indicating plane of section in Figure 3.3.A examples. Samples with two (or more) distinct structures (separated fascicles/sub-tendons) were orientated to capture all bundles in the plane of section where possible (**I**). Where specimens were very thin, they were embedded 'on edge' (thinner width) to ensure security of the sample in the embedded area to prevent loss/pull-out of tissue during sectioning (**III**). Wide but thin samples were divided down the midline and "bookended" to capture two surfaces in the section area and embedded together if possible (**IV)**, else transferred to separate cassettes and thus embedded into separate blocks (**V**). Large samples (e.g. AT, rotator cuff tendons, DTT) were divided longitudinally into multiple (2-4) pieces and transferred to

separate cassettes/blocks (**V–VII**). Where tissue size differed, not all donors may have received the same number of blocks for a given tendon/ligament – primarily combinations of bookended samples (**V**) and bookends with an additional flat-face piece (**VI**) were obtained for some of the typically larger tissue-types that varied between donors (e.g. QT, PT, ACL, MCL, LCL). Multi-piece tissues were numbered consecutively for the number of pieces the sample was divided into, with orientations for respective sequential numbers kept the same if not all donors achieved the same number of pieces (for example, ".1" kept as one side of a bookend with combinations of **V** and **VI** embedding options). Tendons such as the AT, QT and PT were embedding using orientation V or VI (Figure 3.3.A) aiming to have the surface exposed between bookended blocks ".1" and ".2" include portions of the different sub-tendons with the face of the section encapsulating a superficial-to-deep view as best as achievable with the samples obtained (refer to *Section 7.6.2* for caveat discussion). The DTT was the only tendon which was embedded with four blocks (**VII**). Prepared cassettes were returned to 70% v/v ethanol for storage until processing and embedding.

In total, the Human Musculoskeletal Tissue histology project contains n = 525 tissue blocks. The number of blocks per tendon/ligament are outlined in Table 3.3.a. For those indicated by a range, the number depended on tissue size for each respective donor. All blocks were processed, sectioned and stained as described below. For this thesis, only one block per tissue sample (or one 'face' where bookended pieces were embedded together) was included for analysis (n = 379). For tendons/ligaments with multiple blocks prepared, the first ".1" block (Figure 3.3.A) was included.

3.3.2 Processing and Embedding

Specimens were processed and embedded (SS) into paraffin wax blocks (relative to their cassette allocation) following protocols adapted from those previously outlined by our laboratory[142]. In brief, specimens were dehydrated in changes of ethanol (% v/v) over 27 hours: 75% for 5 hours; then 85% = 5 hours; 95% = 5 hours; 100% = 3 hours; 100% = 3 hours; and lastly 100% for 6 hours. Clearing was performed in methyl benzoate (3 days), then infiltration with celloidin in methyl benzoate for 3 days in 1% (weight/volume) then 21 days in 4% (w/v). Specimens were then rinsed in three 10-minute changes of chloroform, before paraffin wax (Paraplast XTRA) infiltration for a total of 4 days (8 changes, under vacuum) prior to embedding in longitudinal orientation (Paraplast plain).

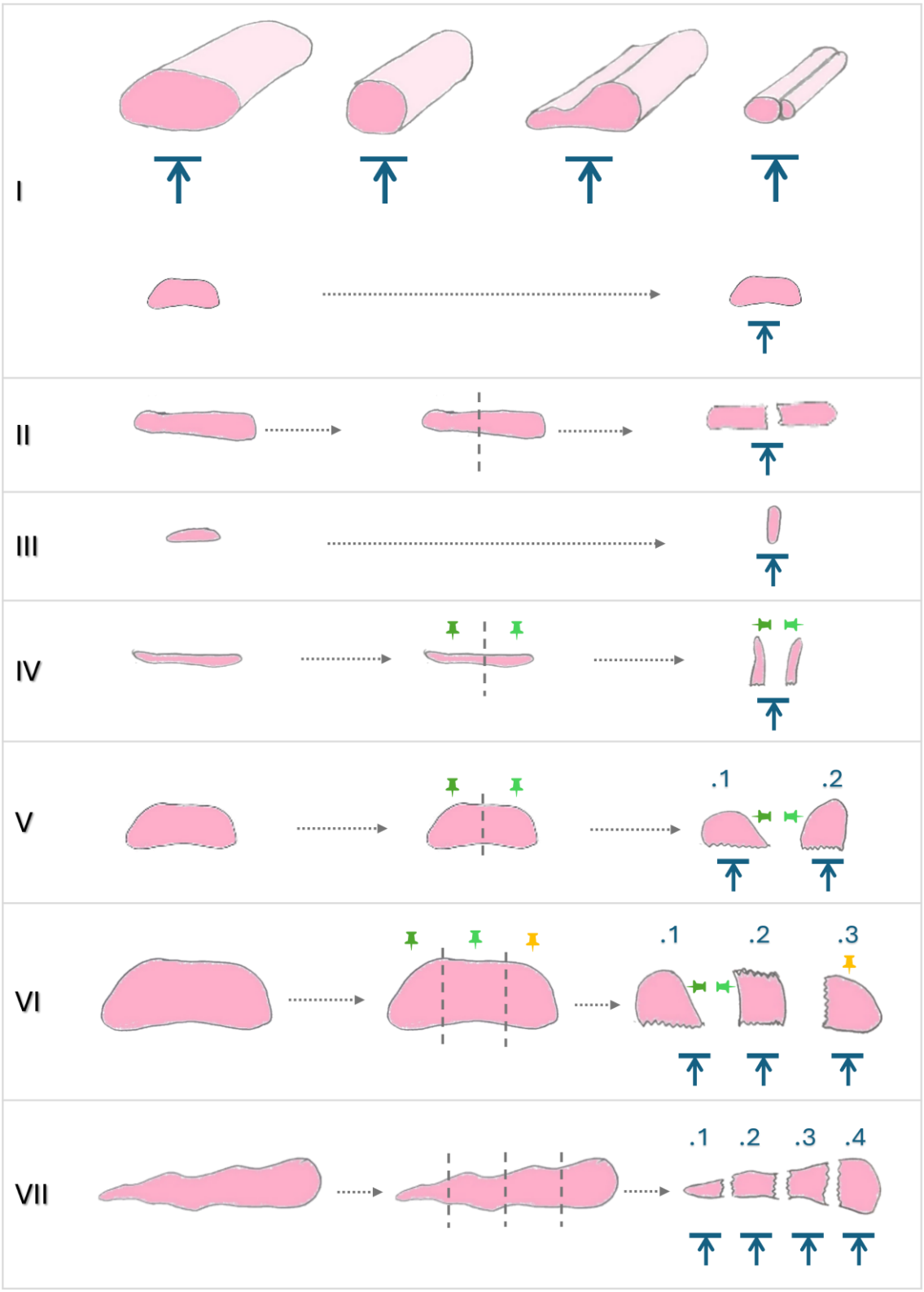


Figure 3.3.A Examples of embedding orientations for differently sized specimens (not to scale). Where possible, the widest area was embedded to present to the plane of sectioning (up-arrow) to maximise tissue area in the face of the section (**I**). Very wide sections were split before embedding to minimise sectioning difficulties and section distortion (**II**). Thin specimens were embedded on edge to ensure security in the wax block during sectioning (**III**). Larger or other-shaped specimens were divided (longitudinally with the grain of fibres) to fit within cassettes/embedding moulds (**IV**–**VII**). Pin icons indicate surface markers (Alcian Blue dye) to orientate specimens post division. Numbers indicate block-code where pieces are embedded separately. Blade during microtomy travels left to right of blue line (up-arrow = section face).

Table 3.3.a List of tendons and ligaments included for histological study from the human tissue atlas project. Number of donors samples were retrieved from, and number of paraffin embedded blocks created for histological processing/embedding are indicated.

Group	Region	Abv.	Tendon	No. Samples (donors)	No. Blocks per sample
Lower Limb Tendons	Ankle	**AT**	Achilles Tendon	8	3
		Plt	Plantaris Tendon	**6**	1
		TP	Tibialis Posterior	8	1
		TA	Tibialis Anterior	8	1
		FL	Fibularis (Peroneus) Longus	8	1
		FB	Fibularis (Peroneus) Brevis	8	1
	Foot (Toe Digits)	**FDL**	Flexor Digitorum Longus	8	1
		FHL	Flexor Hallucis Longus	8	1
		EHL	Extensor Hallucis Longus	8	1
	Knee (Tendons)	**ST**	Semitendinosus Tendon	8	1
		GT	Gracilis Tendon	8	1
		QT	Quadriceps Tendon	8	2-3
		PT	Patellar Tendon	8	2-3
	Knee (Ligaments)	**ACL**	Anterior Cruciate Ligament	8	2-3
		PCL	Posterior Cruciate Ligament	8	1
		MCL	Medial Collateral Ligament	8	2-3
		LCL	Lateral Collateral Ligament	**7**	1-3
Upper Limb Tendons	Shoulder	**SSP**	Supraspinatus	8	3
		IS	Infraspinatus	8	3
		SSC	Subscapularis	8	3
		LHBT	Long Head Of Biceps Tendon	8	1
	Elbow	**DBT**	Distal Biceps Tendon	8	1
		BrR	Brachioradialis	8	1
		DTT	Distal Triceps Tendon	8	4
	Wrist	**FCR**	Flexor Carpi Radialis	8	1
		PL	Palmaris Longus	8	1
		FCU	Flexor Carpi Ulnaris	8	1
		ECRL	Extensor Carpi Radialis Longus	8	1
		ECRB	Extensor Carpi Radialis Brevis	8	1
		ECU	Extensor Carpi Ulnaris	8	1
	Hand (Digits 2-5)	**FDS-2**	Flexor Digitorum Superficialis - 2nd Digit	8	1
		FDS-3	Flexor Digitorum Superficialis - 3rd Digit	8	1
		FDS-4	Flexor Digitorum Superficialis - 4th Digit	8	1
		FDS-5	Flexor Digitorum Superficialis - 5th Digit	8	1
		FDP-2	Flexor Digitorum Profundus - 2nd Digit	8	1
		FDP-3	Flexor Digitorum Profundus - 3rd Digit	8	1
		FDP-4	Flexor Digitorum Profundus - 4th Digit	8	1
		FDP-5	Flexor Digitorum Profundus - 5th Digit	8	1
		EIP	Extensor Indicis Proprius	8	1
		EDC-2	Extensor Digitorum Communis - 2nd Digit	8	1
		EDC-3	Extensor Digitorum Communis - 3rd Digit	8	1
		EDC-4	Extensor Digitorum Communis - 4th Digit	8	1
		EDC-5	Extensor Digitorum Communis - 5th Digit	**6**	1
		EDM	Extensor Digiti Minimi	8	1
	Thumb (Digit 1)	**FPL**	Flexor Pollicis Longus	8	1
		APL	Abductor Pollicis Longus	8	1
		EPB	Extensor Pollicis Brevis	8	1
		EPL	Extensor Pollicis Longus	8	1
		Total n =	***48*** *different tendons/ligaments*	***397*** *total collected samples*	***525*** *paraffin blocks prepared*

3.3.3 Sectioning and Staining

Paraffin blocks were cut (by **SH**) on a Leica-RM2255 rotary microtome (Leica Biosystems, Nussloch, Germany) using Feather blades N35 (Arthur Bailey Surgico, NSW, Australia). Blocks were trimmed 300-800 μm into the specimen to reveal the full face of the tissue section. Post trimming, blocks were softened in a solution of 25% glycerol / 25% ethanol / 50% water (30-60 min, 4°C) and then 4.5 μm thick sections collected onto glass slides (Crest CRE-11; Matsunami Glass Ind. Ltd., Japan) using a water-bath set at 54°C. Three slides for each specimen were prepared – one each for Haematoxylin and Eosin (H&E), Toluidine Blue with a Fast Green counter stain (TB), and Picrosirius Red (PSR) staining. Slides were dried in an oven at 80°C for 30 minutes, then overnight at 55°C.

Unstained sections were stored at room temperature for up to 6 months until all samples of a given tissue (for available donors) and tissues of given region (shoulder or upper or lower limb) were collected and could be stained in a single batch to reduce variability. PSR staining was prioritised in the workflow and done only on "recently cut" sections (< 2 weeks since sectioning) as in-house experience (SS) has found longer storage can affect staining. The staining and mounting of human tissue specimens was modified from standard procedures for tendinous tissue[142], and are detailed in Figure 3.3.B. Staining occurred in racks of 24 slides at a time with up to four racks (two only for PSR) stained at a time per stain (a 'session') with a control slide included in each batch to allow tracking of staining consistency between sessions.

3.3.3.1 *Staining Techniques for Tendon/Ligament*

3.3.3.1.1 Haematoxylin and Eosin (H&E)

Staining with H&E is a gold standard histochemical approach used for general morphological observation of tissue and cells[143,144]. The haematoxylin monohydrate (C.I. 75290) dye stains for nucleic acids (deep purple-blue) to locate cells while the eosin yellowish (C.I. 45380) counterstain dye non-selectively targets the positively charged protein matrices within tissue[143]. It is a suitable, readily available, and readily producible stain for general ECM and cellular characterisation in tendon/ligament tissue.

3.3.3.1.2 Toluidine Blue (TB)

The metachromatic Toluidine Blue O (C.I. 52040) dye allows for the selective staining of acidic tissue components[145,146] – in tendon/ligament it binds to the sulphated GAGs[147,148] allowing for identification and localisation of proteoglycan/GAG within the tissue[149]. It has been shown to be advantageous over other common stains for detecting GAGs, such as having a higher sulphate-affinity than Safranin O[149,150], and simpler procedural steps. Additionally, Toluidine Blue O also stains cell nuclei, and when combined with a counterstain can also be used for general morphological assessment and allows for more contextualised evaluation of proteoglycan presence.

3.3.3.1.3 Picrosirius Red (PSR)

The dye found in PSR stain – Sirius Red F3B / Direct Red 80 (C.I. 35780) – has a specificity for collagen[151]. Despite historical misconceptions, the use of PSR staining is not adequate for differentiating collagen types[152,153]. Rather, the elongated dye molecules, which align

longitudinally with the collagen molecule, enhances the birefringent properties of the collagen fibrils when viewed under polarised light[154-156]. In tendon/ligament tissue, the organisation and directional properties of the collagenous ECM matrix can be explored[153,157].

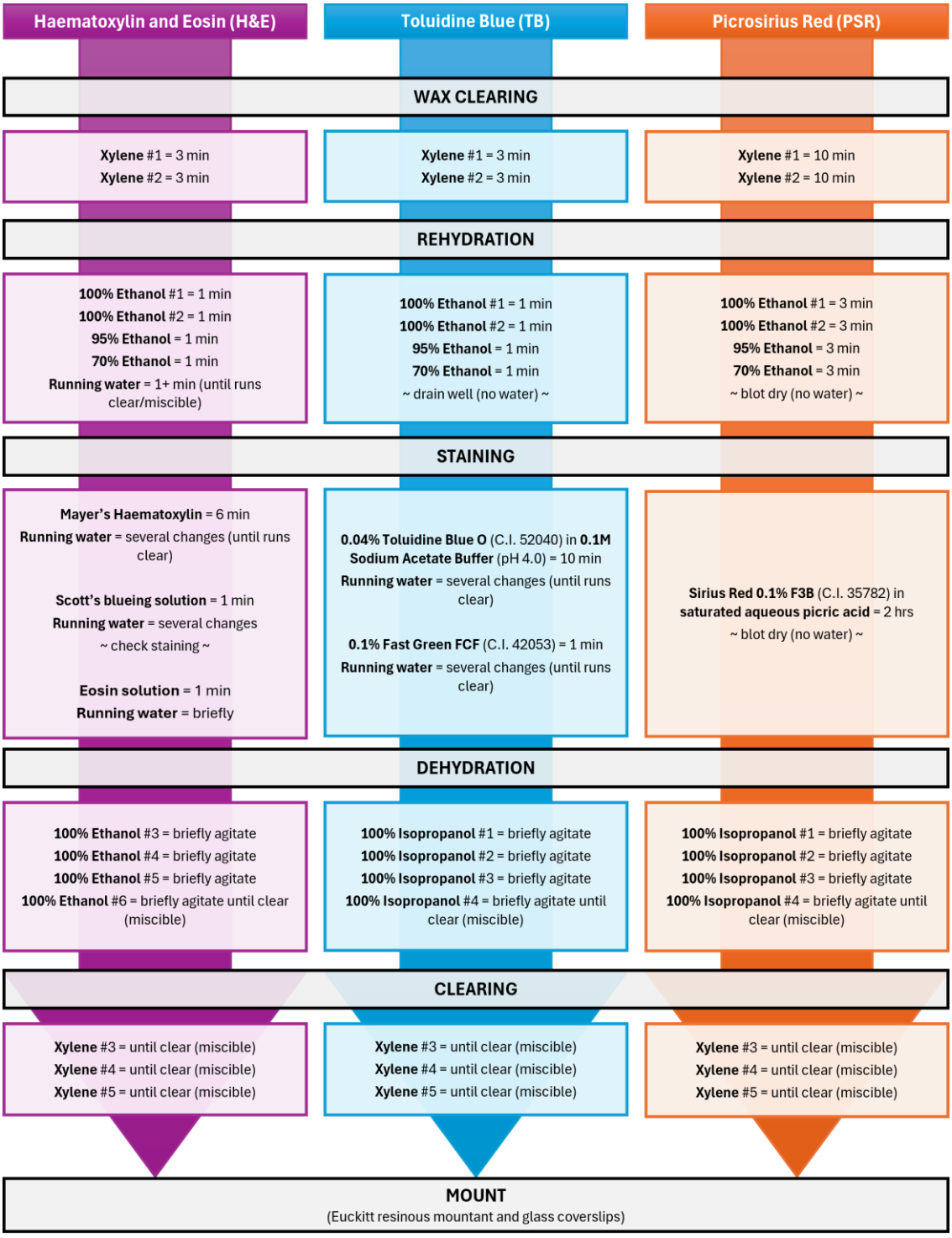


Figure 3.3.B Staining protocols for human tendon/ligament 4.5 µm sections. Note that pre- and post-staining xylene and alcohol solutions need to be kept separate to avoid wax/water contamination between stages. Slides are immersed and agitated in each solution and left for the time as specified.

3.4 Histological Observation

Histological observation was conducted as per the newly developed scoring system described in *Chapter 4* which encompasses outcomes assessed by both non-polarised (brightfield) and polarised light microscopy to explore structural properties of normal (healthy) tendons. For outcomes assessed using the brightfield light modality, scanned images of histological slides of each relevant stain were used for scoring from a computer/laptop setup (*Scanner*: NanoZoomer S210 digital slide scanner (C13239-01); HAMAMATSU PHOTONICS K.K.. *Viewing software*: NDP.view2Plus 2.9.29 (RUO); HAMAMATSU PHOTONICS K.K.). Outcomes utilising polarised light (PSR-stained sections only) were assessed directly from a microscope (Leica-DM750 light-microscope equipped with a polarising filter and Leica-DFC295 digital camera; Leica Microsystems, Wetzlar, Germany). A rotating stage enabled the slide to be actively rotated under the polariser during observation to dynamically assess the full section for birefringence changes (outcomes for collagen alignment and crimp characteristics). The accompanying Leica Application Suite (LAS) software was used to assess/measure crimp wavelength characteristics as livestreamed from the Leica-DFC295 camera setup.

3.4.1 Blinding Protocol

The primary investigator (**SH**) was blinded to samples (donor and tendon) to avoid assessment bias. Digital scans and slides were blinded with matched numbers provided for each stain of any given specimen. Blind-IDs were generated using a random-number generator. Scans were blinded by **SH** using Windows Command Prompt to rename files from a Microsoft Excel spreadsheet with the generated Blind-ID (obscured during collection and blinding). The unblinded columns were hidden at all times, and the Blind-ID list was sequentially sorted before obscuring. Physical slides were blinded with assistance from TF and EF using stickers (labelled by EF with the Blind-ID) to cover the slide label (cross-checked by TF and EF).

3.4.2 Image-Capturing and Polarisation Techniques

Histological images included in this thesis were either captured from the microscope-camera setup or obtained using the export-image feature of NDP.view2Plus (brightfield only).

Due to the nature of using polarised light, histological assessment as well as optimisation of image capturing requires careful observation to maximise consistency. This entailed a trial-and-error approach to obtain an image with the best reflection of what is seen down the microscope. The considerations for PSR image-capturing, as well as dynamic observation (live scoring) are as follows: 1) the polarising filters should be adjusted to obtain the darkest/blackest possible background (polariser and analyser perpendicular at 90° to achieve full extinction of backlight; and 2) the rotating stage is used to rotate the specimen (between polariser and analyser) to observe tissue birefringence. Tendon/ligament tissue exhibit optical colourisation appearing green (highly organised), red (highly unorganised) or yellow/orange (intermediary) dependent on the orientation of the collagen bundles relative to the polarisers[153]. Green-yellow is indicative of

tightly packed and aligned collagenous architecture, whilst orange-red indicates dispersed and unaligned structure[153].

The appearance of the crimping structure is observed as patterns of dark banding ("shadow") in contrast to the coloured regions, where light is blocked (extinction) dependant on the plane of crimp orientation relative to the polarisers. Scoring should consider birefringence patterns throughout stage rotation to grasp appreciation of the whole specimen structure and orientation which may differ across large areas. Image capturing should achieve maximum green or maximum shadow banding relative to the intent (alignment or crimping outcomes respectively) for static image capturing/scoring.

~ CHAPTER 4 ~

STANDARDISED SCORING SUITE

A REVIEW OF PUBLISHED SCORING SYSTEMS

&

THE PROPOSAL OF A NOVEL SCORING SYSTEM FOR THE HISTOLOGICAL ASSESSMENT OF TENDONS/LIGAMENTS

"The true crimefighter always carries everything he needs in his utility belt, Robin."

~ Bruce Wayne/Batman, Batman (TV series, 1966-68) ~

4.1 Overview

The following chapter explores the limitations of current histological scoring systems for tendon characterisation and proposes a new system capable of addressing gaps in knowledge and facilitating improved comparisons between different tendons and ligaments. Progression stages are outlined for the development of the system, and the final system used in subsequent chapters of this thesis is presented.

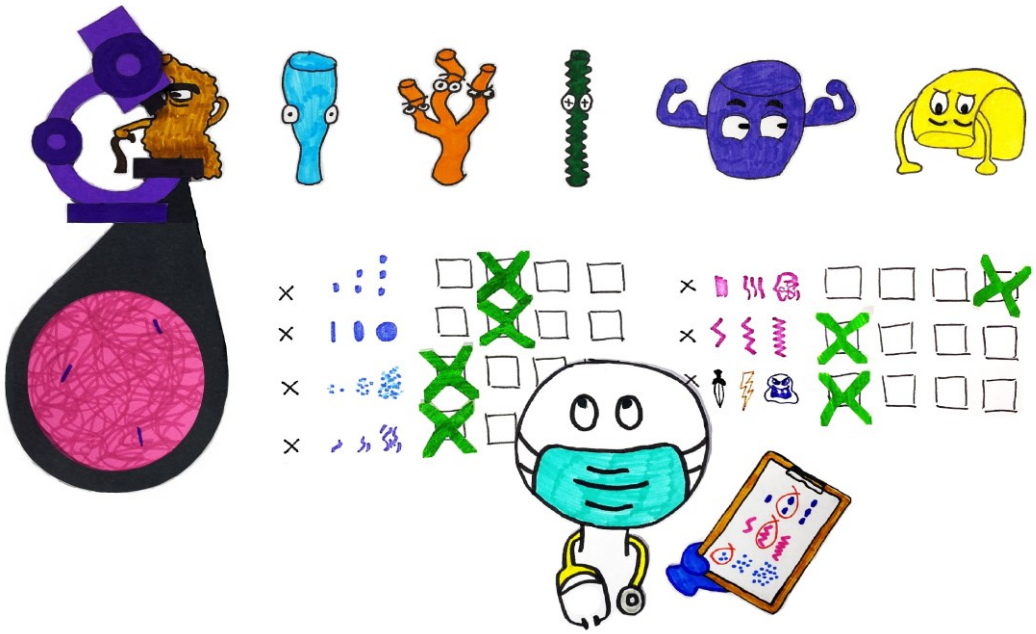


Figure 4.1.A Histological assessment of different tendons/ligaments.

4.2 Introduction

Tendons and ligaments are predominantly fibrillar collagen type-I arranged in a highly organised hierarchical structure[117] (Figure 1.2.C). They play a major role in musculoskeletal functioning and contributing to force transference in load bearing conditions[106,158,159], thus these structures are prone to damage or injury which can impair function and significantly impact an individual's lifestyle. Characterisation of "normal" tendon structure is an important consideration as tendon- or region-specific differences in "normal" architecture may have implications for understanding mechanisms and progression of pathology and healing after injury or with disease. Despite this, knowledge in this area is lacking in tendon and ligament research. Furthermore, current histopathological scoring systems are incapable of comparing between different normal (healthy) tendons or ligaments, as they are intended to assess a state of disease relative to a normal control.

This chapter reviews and assesses current histopathological scoring systems to determine their applicability to characterise differences between normal (healthy) tendons and presents recommendations for the development and application of future systems capable of comparing normal tendon or ligament tissue. A new histological scoring system targeted at the characterisation of tendons/ligaments is then proposed following these recommendations to facilitate the comparison of different tendon/ligament tissues irrespective of their condition.

PART A

4.3 Part A – Review of Current Tendon Scoring Systems

4.3.1 Overview of Histological Scoring Systems

Histology is a standard and invaluable diagnostic tool used in patient biopsy, autopsy and cadaveric investigations for clinical and research purposes[160-162]. Histological/histo*patho*logical analyses commonly enlist scoring systems as a means to translate observations – be they distinct events (e.g. presence/absence of features) or gradable differences (e.g. changes in cell number or collagen architecture) – into a numerical scale for easier interpretation and (semi)quantitative comparison. Numerous scoring systems have been developed for pathological characterisation of tendons. A systematic review by Loppini *et al.* (2015)[163] assessed 27 articles, contrasting scoring systems utilised for evaluating tendinopathy (9 systems), tendon rupture (6 systems) and tendon regeneration (12 systems). The review identified the importance of having a comprehensive system which captures a range of parameters in order to effectively assess the specimens studied.

4.3.2 Scarcity of "Normal" Scoring Systems

Unfortunately, despite the clear deficiency of research on normal tendon structure and variations between different tendons in the literature (*refer to Section 2.2*), there is no published scoring system suited to effectively target this gap in scientific knowledge in a comprehensive manner. One available system which has been applied to the characterisation of different non-pathological tendons is that of Hadjicostas *et al*.[164], whose research compared a small selection of tendons that are commonly used as graft material for surgical reconstructions. In a series of studies[164-167], the researchers applied a fully quantitative system for various light microscopy outcomes utilising histochemistry (density of elastic fibrils, fibril-interstitium ratio, density of fibroblasts) and immunohistochemistry (collagen type-I, -II and -III density, vascular density) techniques as well as electron microscopy for the assessment of fibril diameters. The benefit of objective measures is the elimination of subjective bias. Unfortunately, the Hadjicostas system does not capture the full range of structural characteristics commonly assessed when considering tendon structure, such as proteoglycan content, nuclear morphology, or those pertaining to the collagenous architecture (collagen organisation, fibre structure, crimp etc.)[168-171].

4.3.3 Prevalence and Limitations of Histopathological Systems

Although countless scoring systems have been developed for pathological assessment of a comprehensive range of structural characteristics of tendons in response to injury, disease state, or during the repair/healing process, these systems are incapable of effectively characterising differences between normal tendons. Table 4.3.a outlines some of the scored tendon outcomes in currently published literature and highlights those that are commonly evaluated (although at times with variable scoring ranges) as well as more study-specific scoring outcomes.

Table 4.3.a Examples of previously published scoring system for tendon/ligament histological assessment. Number indicates the score-range for the identified outcome category. (*) = The outcome is partially captured within another scored category. C. = Crimp characteristics. T. = Other tissue region.

	Outcome	*Watkins et al. (1985)*[172]	*Ide et al. (2009)*[173] *(modified Watkins)*	*Aström and Rausing (1995)*[174]	*Soslowsky et al. (1996)*[175]	*Dines et al. (2007)*[176] *(modified Soslowsky)*	*Movin et al. (1997)*[168]	*Lau et al. (2024)*[177] *(modified Movin)*	*Kartus et al. (2000)*[171]	*Allain et al. (2001)*[178]	*Cetti et al. (2003)*[179]	*Cook et al. (2004)*[169] *("Bonar")*	*Fearon et al. (2014)*[180] *(modifid Bonar)*	*Sano et al. (2005)*[181]	*Carvalho Pde et al. (2006)*[182]	*Murray et al. (2007)*[183]	*Glazebrook et al. (2008)*[184]	*Smith et al. (2008)*[142]	*Nixon et al. (2008)*[170]	*Schnabel et al. (2009)*[185] *(modified Nixon)*	*Peltz et al. (2012)*[186]	*Kharaz et al. (2018)*[19]
		Score Range																				
Cells	Cellularity (/density)	3	4		4			4	4				4					4	4	4	4	
	Cell morphology (shape)			4			4	4				4	4			3		4		4	4	3
	Cell phenotype or other cell-type density	3	4		4							*				3			4			
	Cellularity variation			4			4															2
	Cell arrangement															3						*
	Cell orientation	3														3						3
	Pericellular elastin staining																					4
	Pericellular PG/GAG staining																					4
	Necrosis										4											
Architecture	Fibre structure			4	4		4	4	4	4	4	4	4	3								3
	Fibre arrangement/orientation	3	4		4	4	4	4				*	*			3	5		4	4		
	Fibre diameter/size	3	4											3		3			4	4		
	Collagen-specific staining			4			4										5					
	Collagen alignment																	4				
	Fibril-Interstitium ratio																					
PG/GAGs	GAG content						4	4	4									4				2
	Interfascicular location																					4
	Inter-bundle location																					4
	FM location																					4
Elastin	Elastin content																					2
	Interfascicular location																					4
	Inter-bundle location																					4
	FM location																					4
Other Characteristics	Vascularity	3	4	4		4	4	4	4			4				3		4	4	4		2
	Vascular orientation															3						
	Vascular type/other															3				4		
	Hyalinisation/fibrosis/ granulation tissue/ cartilage formation				2	4	4		*					3								
	Haemorrhage			4															4	4		
	Inflammatory cells				2						4					3		4	4			
	Mucin/ground substance											4	4									
Other proteins etc. (IHC	Collagen-I	4																	4	4	4	
	Collagen-II																				4	
	Collagen-III	4							4										4		4	
	Collagen-IV	4																				
	Collagen-VII																				4	
	Collagen-I mRNA																		4			
	Collagen-III mRNA																		4			
	Aggrecan																				4	
	Biglycan																				4	
	Decorin																				4	
C.	Wavelength															*			4	4		
	Crimp (other)	3														3						
T.	Epitenon/Paratenon																		4	4		
	Number of (scored) outcomes:	10	5	6	6	3	8	6	5	1	3	4	4	3	0	11	2	6	13	10	9	15

4.3.3.1 *Complications with Histopathology Scoring*

Some general issues with tendon histopathology scoring are highlighted in two of the most highly cited and used systems: the Movin[168] and Bonar[169] systems. Both systems were developed for assessing tendon pathology, specifically changes in long-standing Achillodynia and asymptomatic patellar tendinosis, respectively.

4.3.3.1.1 The Movin System

The Movin system[168] (or a modification thereof) is widely applied to studies of both tendon rupture[187-191] and tendinopathy[168,189,192]. Tendons are assessed using H&E stained sections on a scale of 0 ("normal") to 3 ("markedly abnormal") for each of fibre structure, fibre arrangement, nuclei rounding, regional variations in cellularity, increased vascularity, decreased collagen stainability, non-collagenous fibrosis/hyalinisation (the "glassy" appearance of tissue[6]), and extracellular matrix (specifically GAGs, identified with Alcian Blue Periodic Acid-Schiff (AB/PAS)-stained sections), giving a total histopathological score ranging from 0 (normal tendon) to 24 (severely degenerative tendon). Additionally, volume density of GAG is estimated from AB/PAS-stained sections using a count of intersections on a square lattice. The Movin system is widely enlisted as a means to score the most pathological regions observed in a given section[168,189,190,192]. While this system quantifies the degree (magnitude) of pathological change in a tendon, it does not assess the spread of change within these tendons. Furthermore, the selection criteria for the region of "most advanced pathologic change" is not defined, introducing a level of bias based on an investigator's experience and beliefs of which parameters (fibre structure, morphology of nuclei etc.) indicate greater pathology.

4.3.3.1.2 The Bonar System

The Bonar system[169] has been adopted and modified in various recent studies, for example, to assess tendinopathy[193,194], tendon rupture[195], as well as murine models for activity-induced changes[196,197] and alcohol-inhibited healing[198]. Like the Movin system, the Bonar system also enlists a four-point scoring method, assessing four features indicative of tendinosis, including nuclear morphology (H&E; 0 = spindle-shaped, 3 = round), collagen structure with and without polarisation (H&E; 0 = dense bundles, homogenous polarisation, normal crimp, 3 = marked fibre separation / complete loss of architecture), vascularity (H&E; 0 = inconspicuous/between bundles, 3 = greater than two clusters/field of view), and ground substance/proteoglycan (Alcian-Blue/Colloidal-Iron; 0 = no staining, 3 = abundant mucin throughout). The developers of the Bonar system provided extensive justification for the scoring criteria evaluated: for example, inclusion of longitudinal collagen separation was supported by their findings that such longitudinal cleaving precedes transverse tears indicative of rupture[169]. However, an evaluation of the Bonar system by Fearon *et al.* (2014)[180] identified room for improvement in the scoring and methodology. Fearon *et al.* (2014)[180] modified the Bonar system to include a score for cellularity to address an important pathology-associated characteristic, and they also modified the vascularity score. The original method scaled vascularity on a grade from "less" to "more" vessels, where "more" indicated worse pathology[169]. However, Fearon *et al.* (2014)[180] added a classification under the highest (worst) score for "avascularity", which was not originally captured. This modification highlights the complexity of biological tissues like tendons, where

deviation from 'normal' can potentially be bidirectional, a feature that is overlooked by both Bonar and Movin's original systems.

While the Bonar system relies less on relative evaluations in comparison to the Movin system, enlisting more descriptive identifiers to the score ranges as opposed to relying on a generic "normal" to "markedly abnormal" scale, there is still an inherent association and assumption of certain tendon characteristics being indicative of pathological change. An example is portrayed by the ground substance score, which combines characteristics both for increasing Alcian Blue staining (associated with GAGs/proteoglycans) and the demarcation of fibres or "bundles"[169]. There is an assumption within the Bonar[169] system that increased ground substance (GAGs/proteoglycans) is a trend towards abnormality, and not a possible normal characteristic of certain tendons/tendon-regions.

4.3.3.2 *Grades of "Abnormality" or "Change"*

One of the major limitations of these systems (and other histopathological scores encountered within the literature) is that they focus on the *classification* of tendon disease/pathological state, most typically presenting score descriptions of "normal", "slightly abnormal", "moderately abnormal" and "markedly abnormal" for each or most of the outcomes assessed. Accordingly, all healthy ("normal") tendons would obtain a score of zero, meaning these scoring methods cannot facilitate the differentiation of histological features between different normal/healthy tendons or tendon regions. Furthermore, they can only apply to 'self-contained' studies which enlist a control (normal) tendon sample to compare 'experimental' samples against, and thus the use of different control tissue could influence the outcomes of a study and confound comparison between studies.

Other systems, which in some regards facilitate greater characterisation potential and limit associations with a level of disease state, still 'fall into the trap' of grading outcomes relative to a 'baseline' reference point – i.e. the score descriptions grade the "increase" or "decrease" of the feature evaluated[11,19,142,170-172,175,199-201]. While scoring systems are often modified for specific study cohorts, one common trend is the expected "cause-effect" nature of the grading systems. A prime example of this is the grading of cellularity on a scale of increasing or decreasing cellularity using terms such as "mild increase"/"moderate increase"/"marked increase"/"decrease". Typically this occurs where a change is expected in response to an altered clinical status or time-point, such as following injury, pathology, or treatment models[142,168,170,191,199-201]. The systems utilised in these studies assess the experimental group relative to the control group in all or most of the outcomes assessed, meaning that if the methods are applied to a study comparing between normal tendons a "control" tendon must be arbitrarily selected as there is no true standard control for comparison. To overcome this issue, future development of scoring protocols should enlist objective or semi-objective measures where appropriate. For example, instead of scores described only as mild/moderate/marked relative to an external reference, scores should be related to an "internal" standard such as percentage coverage or relative size/spacing of a specific feature.

Histopathological scoring systems tend to have an emphasis on classifying degrees of "abnormality" or "change". Few systems have been developed which assess histological outcomes independent of altering tendon status. Using the example of cell count, Kartus *et al.* (2000)[171] used one of few scored measures for cellularity that offer a "non-event-dependent"

approach, enlisting a semi-quantitative four-point score (0 = < 100 cells/high-power field [HPF]; 1 = 100–199 cells/HPF; 2 = 200–299 cells/HPF; and 3 = > 300 cells/HPF) as a means to quantify cell density without the necessity to count (which poses a time consuming task) or using image quantification software (which may not always be accessible).

4.3.3.3 *Summed Scores*

Further complications arising from this trend of *classification* versus *characterisation* is highlighted by the summing of scores. In this event, different variables/outcomes are often granted different weightings relative to their substantialness within the system parameters, as discussed by Loppini *et al.* (2015)[163]. An example can be seen when comparing the Movin and Bonar total (summed) histopathology scores. Movin's system incorporates eight outcomes (plus a quantification of GAG content)[168], while Bonar's assesses only four[169]. The Bonar (with less outcomes assessed) therefore attributed "more severe" pathology to cell morphology for example, as it makes up 25% of the total score, while in Movin's system with more outcomes this would only account for 12.5% of the summed score.

Additionally, differences in the outcomes assessed in a given study are important to consider as they provide a different picture of tendon structure. For example, in a study of ruptured versus non-ruptured supraspinatus tendons, Maffulli *et al.* (2008)[202] directly compared the Movin and Bonar systems, which generate total histopathology scores reflective of most severe abnormality, out of 24 and 12 respectively. While both methods obtained similar overall histopathological outcomes, a careful consideration of the individual characteristics that these two systems evaluate reveals the importance of resolution of the scoring system. There are several characteristics captured in the Movin system but not Bonar, in particular, inclusion of cellularity variations, collagen stainability, greater resolution of collagen architecture (structure and arrangement assessed separately) and hyalinisation[168]. This could result in dissimilarities in tendon characterisation where tendons of different type or origin display such variations. For example, while Maffulli *et al.* (2008)[202] noted the low incidence of observed hyalinisation within the tendons studied, a similar study conducted in a tendon or subject cohort more susceptible to hyalinisation changes may result in a higher total Movin score but not total Bonar score resulting in discrepancies in reports of tendon similarities or differences.

Rather than grading outcomes on a relative scale, or merging characteristics into conglomerate scores, individually characterising outcomes on objectively or semi-objectively measured scales should be the focus of histological scoring systems[203]. Realising this goal would allow for the distinction between normal tendons.

4.3.3.4 *Scope and Resolution of Scores*

Another limitation of common scoring systems is the resolution or level of detail gained from a particular scoring matrix. While the purpose of a scoring system is to translate observations into a numerical scale, published systems often limit the score scale to three or four options per outcome (refer to Table 4.3.a). Where direct objective measures are not achievable, increasing the range of scores for assessing a particular outcome can improve resolution and allow greater

discrimination between specimens. This may be particularly important where variations between normal tissues may/likely all occur within the current lowest score band.

Second to this is the tendency to limit the scope of characteristics that are assessed. Previous systems have incorporated a range of characteristics for specific features e.g. quantity/density, morphology, and distribution of tenocytes[19,142,168,170,172]. However, this practice is often limited to cellular characteristics (Table 4.3.a) while other features are rarely assessed by such a broad scope e.g. crimp characteristics of tendons are often limited to assessing wavelength. An example of a system which utilises this idea of increasing the scope of scoring is that enlisted by Kharaz *et al.* (2018)[19]. They score not only the presence of GAG staining but also the relative distribution across different hierarchical levels in the tendon, thus greater differentiation between tendons (and ligaments) could be achieved (Table 4.3.a).

4.3.3.5 *Application of the System – Intended Scoring Region*

While not explicitly an issue with the scoring matrix itself, the methodology specified by many publications often depict protocols with no clear directive for choosing *where* in the sample/specimen to score/apply the system. Current histological scoring methodologies often allude to the practice of selecting the "region of worst pathology" to be scored[168,189,190,192], or providing no indication at all[169]. Some authors have been explicit to justify regions chosen for assessment: for example, using the Bonar system Dirks *et al.* (2013)[197] choose the region to be assessed based on observations of 'worst architecture' under polarised-light. Such a specification reduces bias and allows for greater consistency. However, different approaches between studies, including those with no guidance, could provide different outcomes across studies and may lead to misinterpretation of the findings. Highlighting this, Fearon *et al.* (2014)[180] used the Bonar system to address whether an investigator's subjective interpretation of "worst pathological region" affects the outcome of a study. They found significant variations in outcomes between region-selections, where selection based on worse cell nuclei morphology correlated with the greatest Bonar (worst pathology) score. Of course, one could argue that regions of acellularity are "worse", where the loss of cell-mediated healing/regeneration capabilities could predispose a tendon to greater pathological change. Such concepts present the challenges around applying current systems, and future histological evaluation methods should be explicit in the approach used to select the scored region.

4.3.4 Limitations in Applying Current Histopathology Systems for Comparing "Normal" Tendon Characteristics

The above review highlights some general issues with previously published scoring systems for tendon histological assessment. However, the most important consideration for this thesis is the applicability, or rather lack thereof, of currently available scoring systems to the comparison and/or characterisation of normal healthy tendons. *Section 2.2* (*literature review manuscript*) identified that variables such as specific *Tendon*, *Region* within the tendon, *Sex*, *Age* as well as *Other* variables are all factors which could influence the structure of a tendon and thus contribute to the perceived definition of what is "normal".

4.3.4.1 *Variability of "Normal" Controls Depicted in the Literature*

Figure 4.3.A identifies some representative examples of control (or normal) tissue from various publications utilising histological techniques to observe different tendons and ligaments[171,198,199,202,204-208]. Despite the discrepancies in the magnification used to capture the images, wide variations in tissue structural characteristics are evident, particularly noting the collagenous architecture, even captured between images from the same article (for example, between images I-III and images XI and XII in Figure 4.3.A). While variations in tissue origin (age, species, tendon-type, tendon-region, sex etc.) and preparation (fresh, frozen, fixed etc.) could account for different structural appearances, the tendency for scoring systems to compare relative to a control source means the classification of pathology for one study may be a result of vastly different changes in tendon characteristics compared to another study. Understandably, proposing a standardised tissue as a comparator would be a difficult task. However, the development of a universally applicable and objective scoring system which does not score *relative to* a reference/control section could facilitate translatability and inter-study comparisons of future histological investigations.

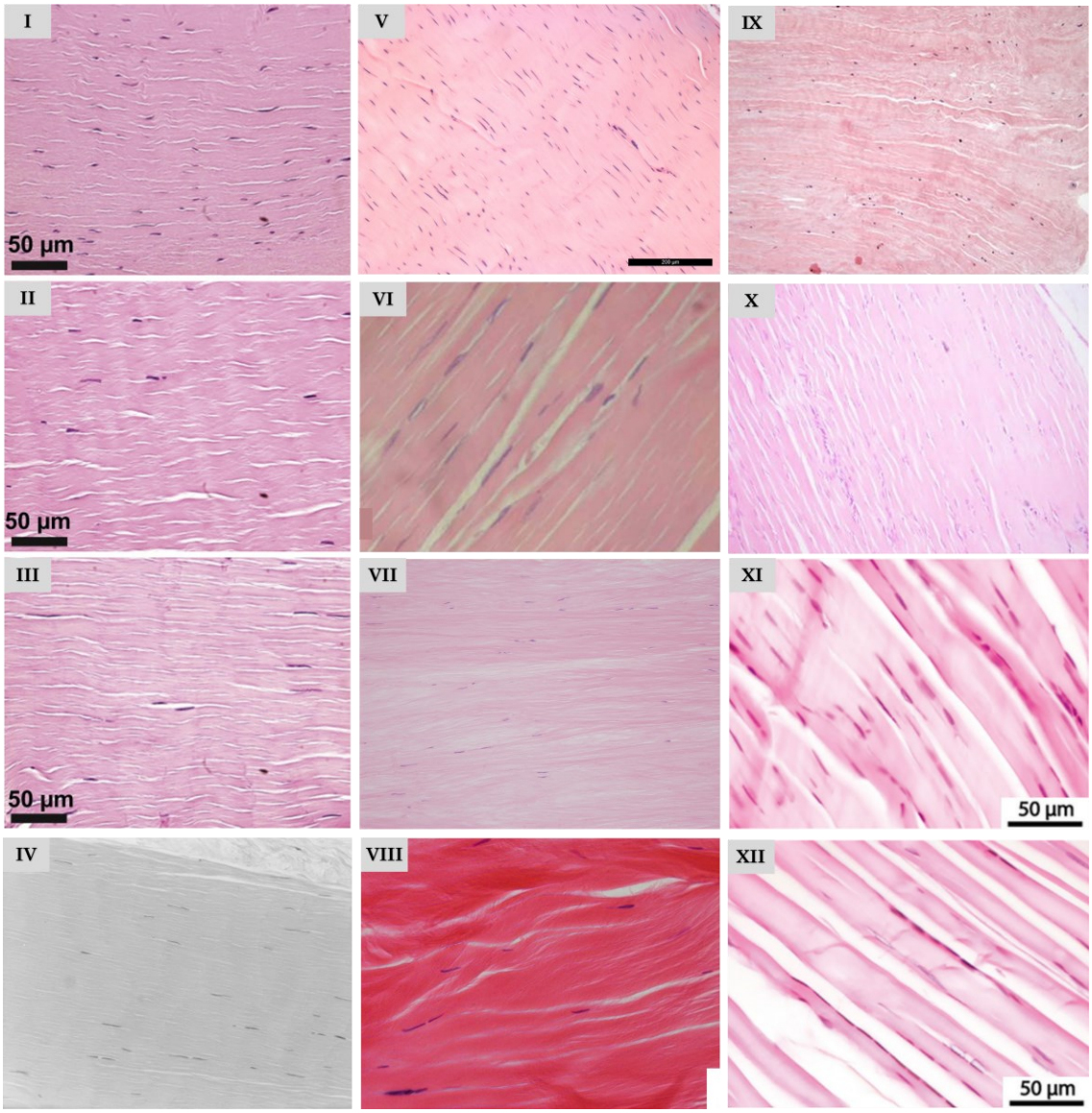


Figure 4.3.A Representative example images of varying control (or normal) tissue used for histological analysis of tendons and ligaments within the literature (*all images: H&E-stained longitudinal sections; image IV in in greyscale*). **I)** Human (mean age 41 ± 13.9 years) cadaveric Anterior Cruciate Ligament[204], *scale bar = 50 µm*. **II)** Human (mean age 41 ± 13.9 years) cadaveric Tibialis Anterior tendon[204], *scale bar = 50 µm*. **III)** Human (mean age 41 ± 13.9 years) cadaveric fan-folded Iliotibial Band allograft[204], *scale bar = 50 µm*. **IV)** Human (mean age 27 years) Patellar Tendon (central third) biopsy[171], *200x magnification*. **V)** Deep Digital Flexor Tendon from 2-year-old merino wethers (*Ovis aries*)[199], *scale bar = 200 µm*. **VI)** Achilles Tendon from skeletally mature New Zealand rabbits[205], *40x magnification*. **VII)** Human (mean age 40.4 ± 9.7 years) Achilles Tendon biopsy[206], *200x magnification*. **VIII)** Human (24-year-old male) Semitendinosus Tendon biopsy[207], *200x magnification*. **IX)** Human (68-year-old male) cadaveric Supraspinatus Tendon[202], *150x magnification*. **X)** Achilles Tendon from 6-month old female Wistar albino rats[198], *reported as 10x magnification*. **XI)** Supraspinatus Tendon from 100-day old female Sprague-Dawley rat[208], *scale bar = 50 µm*. **XII)** Supraspinatus Tendon from 100-day old male Sprague-Dawley rat[208], *scale bar = 50µm*. *All images reprinted with permission (see Appendix Section 9.5.1).*

4.3.4.2 *Dataset Variability*

The dataset (i.e. sections or images) available for investigation also plays a major role in how a particular scoring system is interpreted, used, and applied, particularly where articles do not provide detailed scoring matrices or representative examples for the scoring scale. In particular, the investigators' level of expertise in the field and understanding/familiarity with the system may see experienced versus inexperienced users developing different interpretations of the scoring matrix (particularly where subjective measures are included) or adapting their interpretations with practice and greater involvement. Experienced users may also recruit knowledge from their familiarity of the field, whilst inexperienced users may not have the expertise and prior knowledge to extrapolate from. The Movin[168] scoring methodology can be used to explore the impact of preconceived expectations of the possible range of tendon characteristics. Figure 4.3.B and Figure 4.3.C each contain a selection of four histological images from a cohort of macroscopically normal fresh-frozen human cadaveric tendons (unpublished data[209]. Lower-leg tendons; 5 male, 6 female; 83 ± 10 years old) (note that Figure 4.3.B-III and Figure 4.3.B-IV are the same as Figure 4.3.C-I and Figure 4.3.C-II, respectively). If each set of 4 images independently capture the full range of Movin *Fibre Structure* pathology scores, then the inherent variations of the tendons depicted in each dataset (Figure 4.3.B versus Figure 4.3.C) would result in very different classifications of what constitutes normal versus abnormal tissue (Table 4.3.b).

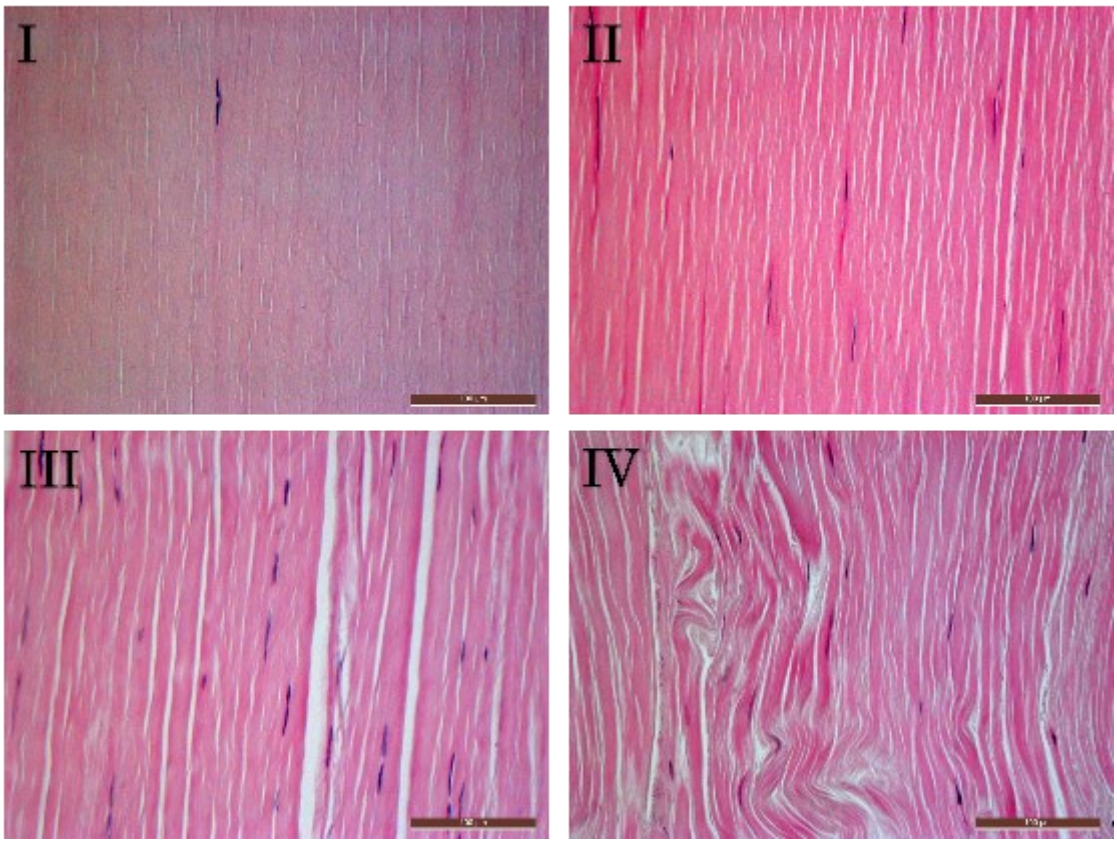


Figure 4.3.B Example Dataset 1: Various images of tendons exhibiting discrete variability within the collagenous architecture structure. *I) Proximal region Tibialis Posterior tendon from 76-year-old male. II) Proximal region Tibialis Anterior tendon from 89-year-old male. III) Distal region Fibularis Longus tendon from 89-year-old male. IV) Distal region Flexor Digitorum Longus tendon from 95-year-old male.* Note variations in the division and splitting between neighbouring collagen fibres between the images (fibre separation) and general alignment of the fibres. Images are ordered in rank from lowest to highest severity regarding the Authors' interpretation of fibre separation and general collagenous structure: **I** = normal, **II** = slightly abnormal, **III** = moderately abnormal, **IV** = markedly abnormal. *Images taken from a dataset of human cadaveric tendons[209]. H&E staining; 200x magnification, scale bars = 100 µm.*

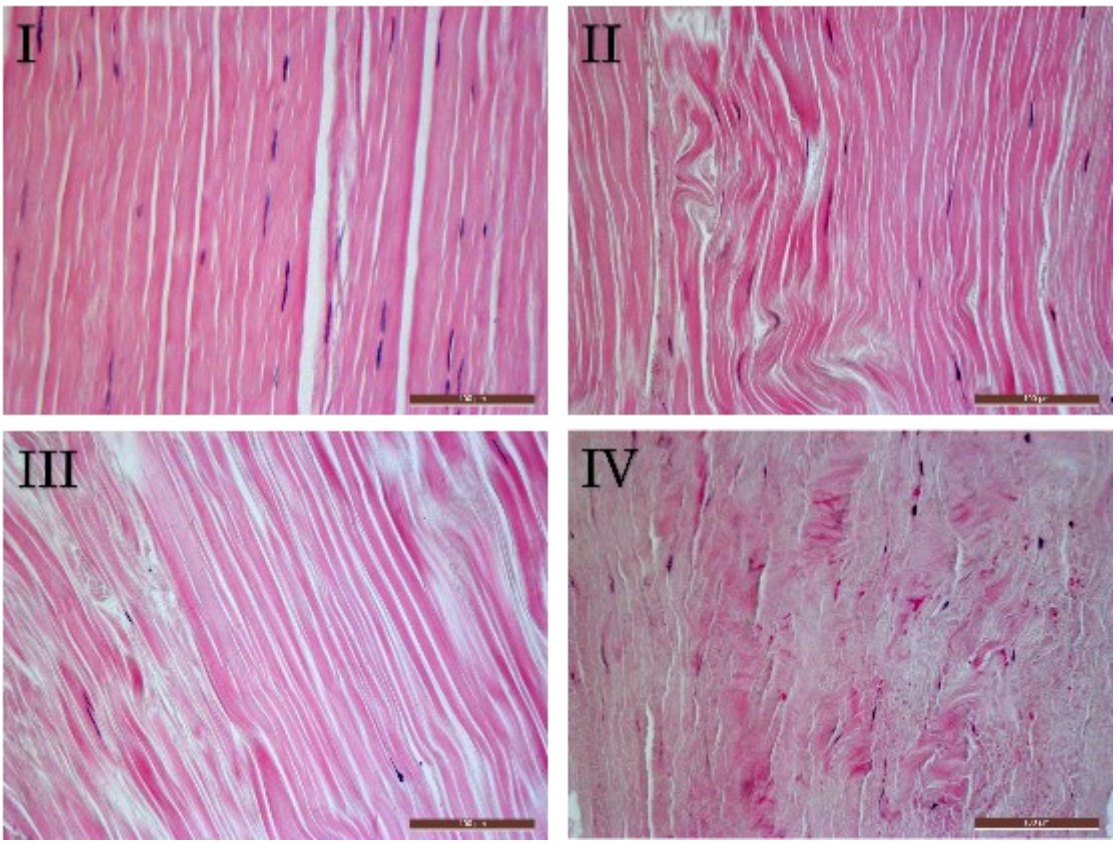


Figure 4.3.C Example Dataset 2: Various images of tendons exhibiting prominent variability within the collagenous architecture structure. *I) Distal region Fibularis Longus tendon from 89-year-old male. I and VI) Distal region Flexor Digitorum Longus tendon from 95-year-old male. III) Distal region Plantaris tendon from 71-year-old female.* Note variations in the division and separations between neighbouring collagen fibres, alignment of the fibres, and general structural organisation of the collagenous matrix between the images. Images are ordered in rank from lowest to highest severity regarding the Authors' interpretation of fibre separation and general collagenous structure: **I** = normal, **II** = slightly abnormal, **III** = moderately abnormal, **IV** = markedly abnormal. *Images taken from a dataset of human cadaveric tendons[209]. H&E staining; 200x magnification, scale bars = 100μm.*

This variability is further exaggerated if both datasets are taken together as one (Figure 4.3.D). By extending the scale to capture the scope of all images, the Author's interpretation of these examples in reference to the Movin system for Fibre Structure would then score them as: Figure 4.3.D-I,II,III = 0, normal; Figure 4.3.D-IV = 1, slightly abnormal; Figure 4.3.D-V = 2, moderately abnormal; Figure 4.3.D-VI,VII = 3, markedly abnormal (Figure 4.3.D, Table 4.3.b). An interesting example to consider are the tendons depicted in Figure 4.3.D-IV and -V, which both scored differently in all three datasets: Dataset 1 = 2 and 3 respectively; Dataset 2 = 0 and 1 respectively; and Dataset 3 = 1 and 2 respectively (Table 4.3.b). This demonstrates that when using the Movin system[168] which relies on the characteristic variability within the tissues being studied when interpreting the scoring range (Table 4.3.b), comparison of outcomes from one study to another cannot be achieved. An objective system which is not dependent on scoring *relative* to a reference could achieve more 'absolute' characterisation of tendons and be more universally applicable for differentiating variations across a range of tissues, studies, or species.

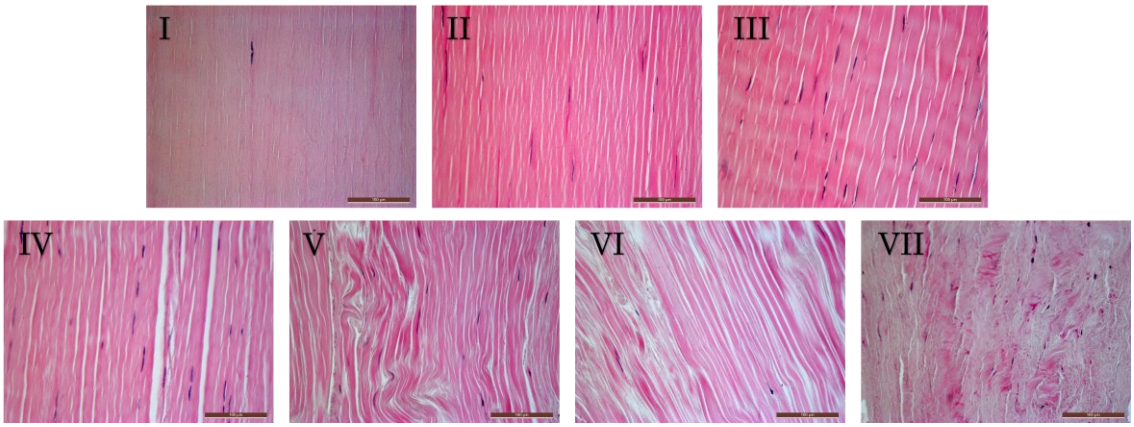


Figure 4.3.D Example Dataset 3: Various tendons exhibiting broad variability within the collagenous architecture structure. *I) Proximal region Tibialis Posterior tendon from 76-year-old male. II) Proximal region Tibialis Anterior tendon from 89-year-old male. III) Distal region Fibularis Longus tendon from 89-year-old male. III) Distal region Fibularis Brevis tendon from 81-year-old female. IV) Distal region Fibularis Longus tendon from 89-year-old male. V and VII) Distal region Flexor Digitorum Longus tendon from 95-year-old male. VI) Distal region Plantaris tendon from 71-year-old female.* Images are ordered in rank from lowest to highest severity based on the Authors' interpretation of fibre separations and general collagenous structure (I = normal, through to VII = markedly abnormal). *Images taken from a dataset of human cadaveric tendons[209]. H&E staining; 200x magnification, scale bars = 100 µm.*

Table 4.3.b Application of the Movin *Fibre Structure* score[168] for three example datasets. The four-point scoring system (range 0-3) is fitted to the extent of variability within each dataset. *0 = "normal" (deep green), 1 = "slightly abnormal" (lime green), 2 = "moderately abnormal" (orange), 3 = markedly abnormal (red). Images: H&E staining; 200x magnification, scale bars = 100 µm.*

		Image Example (identified as per Dataset 3, Figure 4.3.D)						
Movin *et al.* (1997)[168]								
Fibre Structure	**Scale**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
Figure 4.3.B (I-II, IV-V)	0-3	**0**	**1**		**2**	**3**		
Figure 4.3.C (IV-VII)	0-3				**0**	**1**	**2**	**3**
Figure 4.3.D (I-VII)	0-3	**0**	**0**	**0**	**1**	**2**	**3**	**3**

4.3.4.3 *Differentiating Between Normal vs Normal*

To assess the capacity for previously published scoring systems to characterise differences across various examples of 'normal' tendon structure, six different score matrices for the evaluation of the collagenous architecture of tendons were applied to the image examples in Dataset 3 (Figure 4.3.D). This included the Movin *Fibre Structure* and *Fibre Arrangement* scores[168], the Bonar *Collagen* score (applied without polarisation)[169], the Nixon *Linearity of Collagen Fibres* and *Uniformity of Collagen Fibres* scores[170], and the Kharaz *Extracellular Matrix Organisation* score[19] (Table 4.3.a; Table 4.3.c).

One of the biggest downfalls of currently available scoring systems in the literature is the low discrimination of tendons which may be classified as normal or healthy, but which may exhibit subtle variations. The practice of fitting the relative scoring matrices to the scope of the investigated dataset[186] – as exemplified in the previous section (*4.3.4.2 Dataset Variability*) – accentuates this by arbitrarily defining the boundaries between score classification dependent on the available dataset. As discussed previously in a review by Loppini *et al.* (2015)[163], the classification of all normal tissue into a single score value results in a generalisation of what "normal" encompasses. This can be observed by considering images I-IV in Table 4.3.c when various collagenous architecture scores were applied[19,168-170]. Ultimately these four tendon examples are classified as either normal/healthy or exhibiting slight changes towards abnormality. It can be seen that little to no discrimination is obtained from currently available scoring systems, despite careful observation of the images revealing apparent structural variations in the compactness and arrangement of the collagenous architecture (Figure 4.3.D) and potentially reflecting the range of data seen in a comparative study of normal, healthy tendons.

Table 4.3.c Application of various scoring systems to example images from Dataset 3 (Figure 4.3.D). Scores relating to the collagenous matrix of tendons from the Movin[168], Bonar[169] (scored relative to non-polarised images only), Nixon[170], and Kharaz[19] systems were applied. Note the low discrimination between images **I-IV**. *Colours reflect the range of scores for each respective system: deep green = lowest score/score of greatest normality, shades of green = below mid-range score, yellow = mid-range score (if applicable), shades of orange = above mid-range score, and deep red = highest score/score of greatest abnormality. Images: H&E staining, 200x magnification, scale bars = 100 µm.*

		Image Example (identified as per Dataset 3, Figure 4.3.D)						
Scoring System	**Scale**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
Movin: Fibre Structure	0-3	0	0	0	1	2	3	3
Movin: Fibre Arrangement	0-3	0	0	0	0	2	1	3
Bonar: Collagen (without polarization)	0-3	0	0	0	1	2	2	3
Nixon: Linearity of Collagen Fibres	1-4	1	1	1	1	3	2	4
Nixon: Uniformity of Collagen Fibres	1-4	1	1	1	1	2	2	4
Kharaz: Extracellular matrix organization	2-0	2	2	2	1	1	0	0

4.3.5 The Future of Tendon Scoring

The 'relative' and subjective nature of current histology scoring systems limits their ability to characterise "normal" tendon variability and be comparable across studies. It is acknowledged that greater resolution is required to be able to differentiate normal tendon variability, such as through greater score ranges and more outcomes being scored. Furthermore, scoring location within a sample and magnification are important considerations for a scoring protocol, and should be clearly defined to enable inter-study comparisons where possible. *Textbox 1* summarises the common limitations of published histopathological scoring systems.

Textbox 1: Summary of limitations of current tendon scoring systems.

Currently available histological scoring systems for the study of tendons exhibit inherent limitations, including their:

(1) Reliance on a control (or reference) tissue for comparison (within a subjective scoring matrix)

(2) Tendency for incorporating subjective outcomes dependent on, and influenced by:

(3) The investigator's personal experience, opinion or interpretation

(4) The perceived or provided range of variation within the tissues being studied

(5) The characteristics exhibited by the selected reference/control tissue

(6) Low resolution of scores (e.g. broad variation captured within each grade)

(7) Limited number of outcomes assessed, and the generalisation of outcomes (e.g. characterising magnitude, but not spread or location of a particular feature)

(8) Predefining pathology as a unidirectional deviation from the defined "normal" (i.e. score of zero is 'normal' and a score greater than zero is a gradation of 'abnormal')

(9) Tendency to be purpose-developed for classifying states of pathology/injury/disease, attributing variation to be a state of 'abnormality'

(10) Poor description of protocols (e.g. region of interest selection, magnification specifications, study design parameters)

Ultimately, these limitations mean such scoring systems:

(i) Have an inability to distinguish between different normal (healthy) tendons

(ii) Offer limited capacity to characterise or discern potential differences resulting from various factors such as age, sex or region within a tendon

(iii) Associate tendon variation with pathological change

PART B

4.4 Part B – Proposing A New Histological Scoring System

4.4.1 Histological Assessment of Ligament and Tendon (HALT): The Requirement for a Better Assessment Tool

A new comprehensive and objective scoring system should be developed to provide more detailed information on the characteristics of normal tendons (and ligaments), where histological assessment of these ligaments and tendons ("HALT") is the forefront to tissue structural characterisation. There is currently no single system that covers a comprehensive scope of outcomes combined with objective or semi-objective measures capable of differentiating normal tendon variations. This is reflected in a consensus statement from the Orthopaedic Research Society (ORS) 'Tendon Section' (> 100 researchers is the field of tendon scientific exploration) that "a single scoring system that could be used in all cases was not feasible at the moment, especially across different tissues states (e.g. development, homeostasis, healing, etc.) and species"[149]. In consideration of this and the previous review (*manuscript Section 2.2*), *Textbox 2* summarises recommended specifications for histological assessment approaches to tendon characterisations, including recommendations for improved standardisation, study design and reporting of results.

Textbox 2: Summary of recommended specifications for the histological assessment of ligament and tendon (HALT) structures.

Based on the limitations of current scoring systems for the histological assessment of tendons, recommendations for the development of future scoring systems include:

(1) The use of objective or semi-objective scoring matrices, encouraging reduced bias from:
   a. The investigator
   b. The range of variation within the tissues/samples being studied
   c. The need to rely on a reference/control tissue

(2) Creating a higher resolution of scores by increasing the score range to distinguish more (and more subtle/nuanced) levels of variation

(3) Including more scored outcomes, and/or the sub-classification of outcomes (e.g. characterising magnitude, spread, location, and morphology etc.)

(4) Removing associations of pathology with either a low or high score relative to the defined "normal", thus avoiding variation being defined as a state of 'abnormality'

(5) Clearly outline how the system is applied, e.g. the field of view assessed, magnification required, histological stain used, etc.

In addition, to enable translatability and comparison between studies, other important considerations for the accompanying protocols and presentation of results in tendon histological research include:

(i) Clear descriptions of tissue origin, i.e. species, sex, age, tendon, region of tendon, and other details where possible (e.g. medical history, lifestyle behaviours etc.)

(ii) The potential effects of different processing protocols, particularly when comparing results to other studies

(iii) Avoid pooling of groups where it is not necessary (e.g. combining different tendon-types, males with females, or grouping large age ranges etc.) and including analysis of different variables where possible (e.g. tendon-type, sex, age etc.)

(iv) Controlling the region of tendon used for the investigation, and providing region-match controls where required

(v) Inclusion of detailed descriptions outlining where and how the scoring is applied, e.g. the specific region(s) scored and whether it is a static (e.g. fixed image) or dynamic (e.g. scanning across whole or multiple sections) approach

This current study considered knowledge from the systematic review presented in *Chapter 2* on normal tendon characterisation as well as the recommendations of *Textbox 2* to propose a new scoring system which attempts to best address a recognised gap in the field. An interesting series of publications in the field of histological scoring systems were in a special issue of the Journal of Orthopaedic Research – Spine (JOR Spine)[210-214]. These publications initiated the step forward to producing a standardised assessment tool for intervertebral disc degeneration across a broad spectrum of models and species. In the same concept, our laboratory aimed to propose a scoring system for HALT characteristics that could capture the broad features associated with variations in normal tendon/ligament structures. In understanding that this poses a great challenge[149], this study explored the development of a scoring system consisting of a series of extensive scoring outcomes (a "standardised scoring suite"; SSS). The new scoring system could be used in full, or by selective application to characterise specific features of interest.

4.4.2 Inclusion of Scores Alongside Automated and/or Quantitative Measures

It has been well noted that achieving objective measures is critical for improving characterisation. We support the recommendation that automated measurements should be enlisted where possible for experimental studies, such as using digital image analysis tools to detect and calculate cell densities, assess staining intensities, or record direct measurements of crimp wavelengths and cell aspect ratios. However, utilisation of these tools is not always possible or may not present the most appropriate assessment protocol for the situation. Where purely objective measures are not feasible, it is important to have alternative assessment tools available. Direct histological scoring offers one such advantage of requiring few equipment/resources or system-specific expertise (e.g. software, technological tools, etc.), thereby reducing equity issues and enhancing broad applicability and uptake.

Accordingly, our laboratory aimed to propose scoring matrices for all of the outcomes to offer readily available methodologies where alternative measurement tools may not be available or readily accessible, such as in the case of clinical pathology. Similarly, where more objective 'manual counting' approaches may not be a feasible solution, some such outcomes were also accompanied by proposed scoring matrices as alternative approaches, and the reliability of both approaches were assessed as later detailed.

4.4.3 Development Processes

4.4.3.1 *Overview of the Development Processes and Stages*

The process for creating a new scoring system – or standardised scoring suite (SSS) – for tendon/ligament assessment was conducted over many stages of development. Various versions of scoring matrices were developed, modified, applied and revised by the primary laboratory residents (**SH**, CB, CBL, EC and DA), all with differing levels of expertise in histological assessment, anatomical knowledge, familiarity with the tendon field, and prior experience in evaluating tendon characteristics. The broad stages undertaken during the development and testing of the proposed system are outlined in Figure 4.4.A.

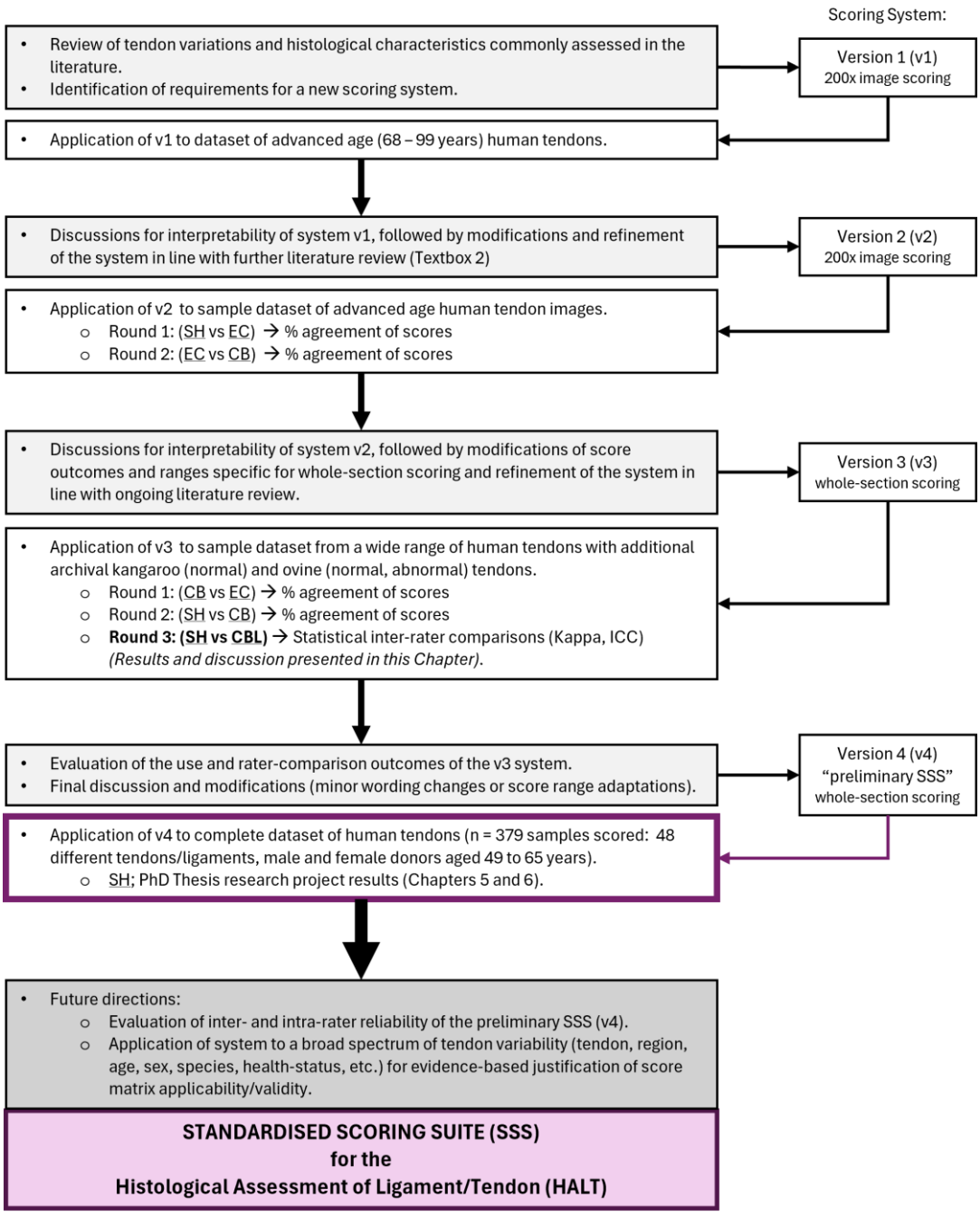


Figure 4.4.A Workflow for the development of the Standardised Scoring Suite for Histological Assessment of Ligaments and Tendons (SSS-HALT).

Initial development of a new histological scoring system ("Version 1", v1) was conducted following extensive familiarisation with histological samples, including H&E-, TB- and PSR-stained longitudinal sections of nine different lower limb tendons from male (n = 5) and female (n = 6) humans of advanced age (68-99 years), considering characterisation of both "normal" and "abnormal" tissue appearances (fresh-frozen cadaveric tissue). Further refinements were made with consideration of wider range of human tendons and ligaments (48 total tissue types; as detailed in *Section 3.2*) with additional archival kangaroo (normal) and ovine (normal, abnormal) tendons (obtained from prior studies within our laboratory).

Continuing literature review, familiarisation with published histopathological scoring systems, and discussions with the current research group facilitated modifications made to the system throughout the development stages to address noted limitations and interpretation discrepancies between lab members. Difficulties and modifications addressed during development and refinement included addressing: 1) troublesome images to score or lack of confidence with the score decision pertaining to unique features; 2) mis/variable interpretations between the scorers with defined characteristics; and 3) creating a scoring suite capable of differentiating not only normal but also "abnormal" and variable tissues with different features, in reflection of *Textbox 2* considerations.

Representative depiction of scoring rounds testing different version of the system (Figure 4.4.A) are included in *Appendix Section 9.7*: Version 2 (v2) round 1 (Supp. Figure 9.5.A) and round 2 (Supp. Figure 9.5.B) scoring; and Version 3 (v3) round 1 (Supp. Figure 9.5.C) and round 2 (Supp. Figure 9.5.D) scoring. Following the second round of v3 scoring discussions, a consensus on many of the parameters and considerations for the scoring system was establish for a modified version (v3.2). This version was then assessed by two investigators (SH vs CBL) in round 3 to explore inter-rater reliability of the system on a sample of n = 34 tendons (human and ovine samples) to capture a range of tendon variability. The results of inter-rater assessment of v3.2 are described in *Section 4.4.4* of this chapter, along with further discussion and description for development of the proposed SSS system.

4.4.3.2 *Examples of Decisions on Development and Modifications*

To elaborate on the complexity and level of detail of the development processes some examples are presented below of the modifications made to outcomes between different versions of the scoring system.

4.4.3.2.1 Characterisation Capacity / Applicability

As outlined by *Textbox 2*, primary modifications made to the system intended to improve the ability to characterise and differentiate normal tendon/ligament variation. This was achieved through improving outcome resolution (greater score range), characterisation parameters (more outcomes / specific outcome application), and objectivity. Figure 4.4.B shows an example of increased resolution in the Proteoglycan Presence outcome between v1 and v2, and an example of increased characterisation parameters between v2 and v3 where the assessment of proteoglycan presence scores were sub-divided to achieve outcomes for different tissue regions (IFR vs FM characterisation). These modifications offer greater characterisation of these regions which have been shown to differ between tendons (*refer to Section 2.3.3*).

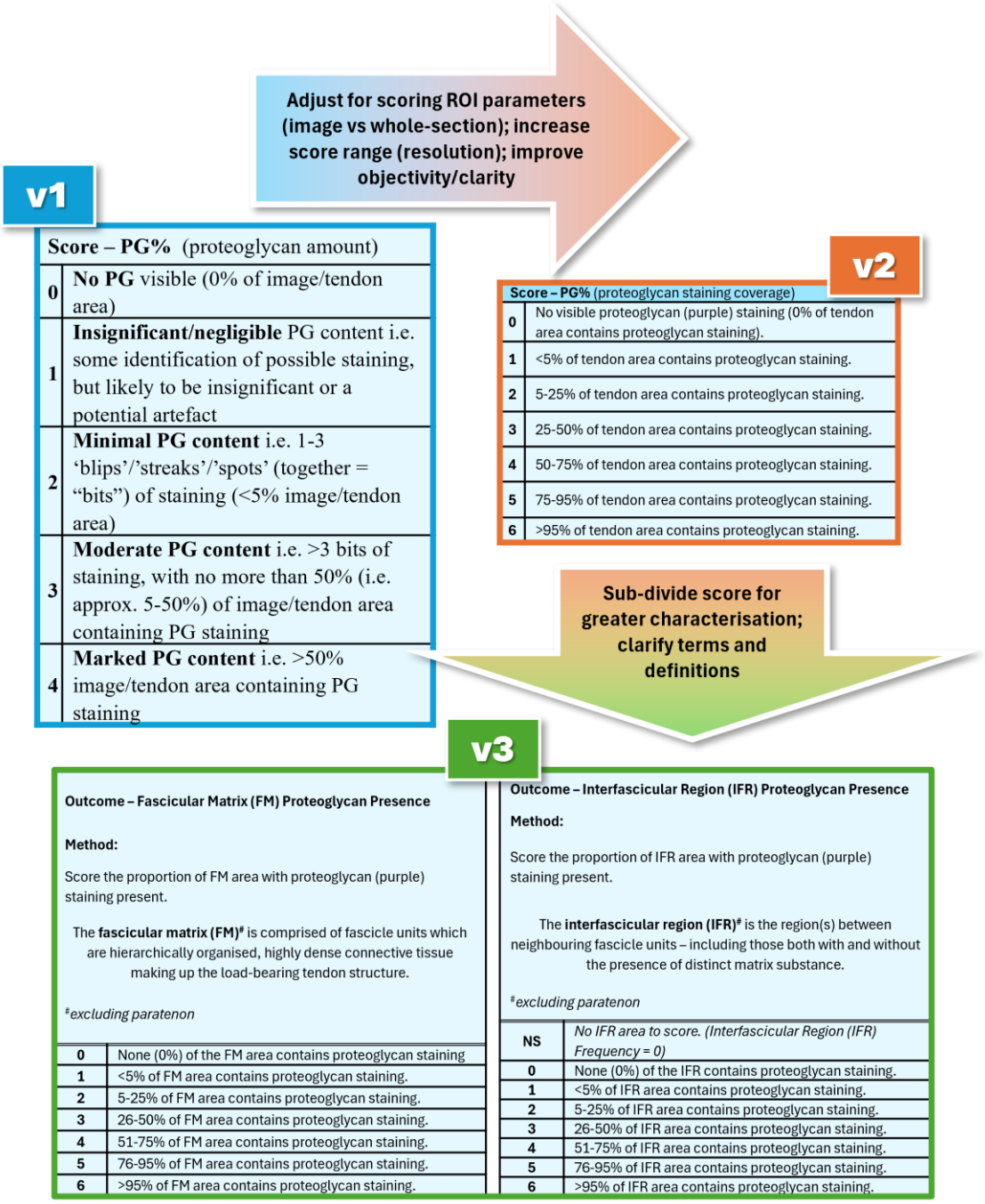


Figure 4.4.B Example of scoring matrix development for one outcome (Proteoglycan Presence) between different versions of the scoring system. Developments include modifying score specification for differing ROI (region of interest) assessments (pre-defined image vs whole-section/specified area scoring; v1/2 vs v3 respectively); including greater score range for increased resolution (v1 vs v2); and sub-dividing score into region-specific applications for improved characterisation (v3).

4.4.3.2.2 Scoring Simplicity

Another aim of the modifications to the scoring system over time was improving interpretability of the scoring matrix by identifying ways to simplify the system and to optimise workflow, particularly when grading very large datasets such as those presented in subsequent chapters of this thesis. One suggestion was the standardisation of score-ranges for similar measures – percentage-area was one particular example. In line with this, v2 used a standard

scaffold/template of scores for several outcomes based on the same percentage area coverage (Figure 4.4.C), allowing the user to keep a consistent "frame-of-mind" and reduce mistakes when swapping between different outcomes in the workflow. For example, a score of 2 for each of the outcomes corresponded to an area of 5-25%, and a score of 3 corresponded to 25-50% and so on. Further assessment and discussion saw that this approach was not always applicable depending on the nature of the outcome, but the concept was enlisted where suitable/valid in subsequent versions.

v2

Score – Orientation of collagenous structure		Score – Organisation of collagen fibrils		Score – PG% (proteoglycan staining coverage)		Score – Collagen Alignment (birefringence)		Score – Crimp Presence	
0	All of the collagen structure runs in parallel, tracking along long-axis of the tendon; collagen structure is highly ordered [**0%** vaiable orientation]	0	All collagen fibrils are organised tightly in distinct fibre structures [**0%** disorganised/separated]	0	No visible proteoglycan (purple) staining (**0%** of tendon area contains proteoglycan staining).	0	All collagen structure is highly aligned (green) [**0%** red]	0	No apparent crimp within tendon area [**0%**]
1	**<5%** of the collagen structure tracks in variable orientations	1	**<5%** of collagen fibrils are disorganised / separating into dispersed collagen structures	1	**<5%** of tendon area contains proteoglycan staining.	1	**<5%** of collagen structure is unaligned/red (>95% is aligned/green)	1	Crimp present in **<5%** of tendon area
2	**5-25%** of the collagen structure tracks in variable orientations	2	**5-25%** of collagen fibrils are disorganised / separating into dispersed collagen structures	2	**5-25%** of tendon area contains proteoglycan staining.	2	**5-25%** of collagen structure is unaligned/red (75-95% is aligned/green)	2	**5-25%** of tendon area contains apparent crimp
3	**25-50%** of the collagen structure tracks in variable orientations	3	**25-50%** of collagen fibrils are disorganised / separating into dispersed collagen structures	3	**25-50%** of tendon area contains proteoglycan staining.	3	**25-50%** of collagen structure is unaligned/red (50-75% is aligned/green)	3	**25-50%** of tendon area contains apparent crimp
4	**50-75%** of the collagen structure tracks in variable orientations	4	**50-75%** of collagen fibrils are disorganised / separating into dispersed collagen structures	4	**50-75%** of tendon area contains proteoglycan staining.	4	**50-75%** of collagen structure is unaligned/red (25-50% is aligned/green)	4	**50-75%** of tendon area contains apparent crimp
5	**75-95%** of the collagen structure tracks in variable orientations	5	**75-95%** of collagen fibrils are disorganised / separating into dispersed collagen structures	5	**75-95%** of tendon area contains proteoglycan staining.	5	**75-95%** of collagen structure is unaligned/red (5-25% is aligned/green)	5	**75-95%** of tendon area contains apparent crimp
6	[Nearly all] (**>95%**) of the collagen structure tracks in variable orientations, not following the long-axis of the tendon; collagen structure is highly haphazard/swirling/in-dissaray	6	[Nearly all] (**>95%**) of the collagen fibrils are highly dispersed and disorganised, not forming distinct fibre structures	6	**>95%** of tendon area contains proteoglycan staining.	6	Collagen structure is highly unaligned (**>95%** red); <5% of collagen structure is aligned (green)	6	Crimp present in **>95%** of tendon area

Figure 4.4.C Example of scoring matrix development for improved scoring simplicity and user workflow. Figure depicts a selection of v2 outcomes which enlist consistent score ranges for percentage-area assessment of different characteristics. Score 0 = 0% coverage of the characteristic/feature, 1 = <5%, 2 = 5-25%, 3 = 25-50%, 4 = 50-75%, 5 = 75-95%, 6 = >95%. The caveat of score-overlap typically observed in many systems (e.g. 25% included in definition for score 2 and 3) was noted for amendment in subsequent systems. *Pink column outcomes (left two) = assessed from H&E-stained sections. Blue column outcome (middle) = assessed from TB-stained sections. Orange column outcomes (right two) = assessed from PSR-stained sections.*

4.4.3.2.3 Wording and Interpretability / Objectivity

One of the primary goals discussed during the development of the scoring system was modifying wording for improved interpretability and objectivity. Selection of wording was carefully discussed to ensure it was interpreted similarly between laboratory personnel, but also to reduce subjectivity from misinterpretation. For example, the change of Fibre Separation scores between v1 (score = 2) "*Fibre separations are considerably long, arguably irregular and/or somewhat variable throughout the majority of the image/tendon; Moderate fibre splitting apparent, occurring in >25% of the image area*" and v3 (score = 3) "*Majority (> 50%) of fibres have separations that are predominantly "long", averaging 100-150 µm in length*" (Figure 4.4.D). In this modification, the addition of objective definitions for the different separation lengths (e.g. "long" averaging 100-150 µm) reduced individual user bias and facilitated improved delineation between scores. Some outcomes were also supplemented with figures to help orientate less-experienced investigators to certain features; a suggestion recommended by a group developing histopathology scoring systems for intervertebral disc degeneration[211].

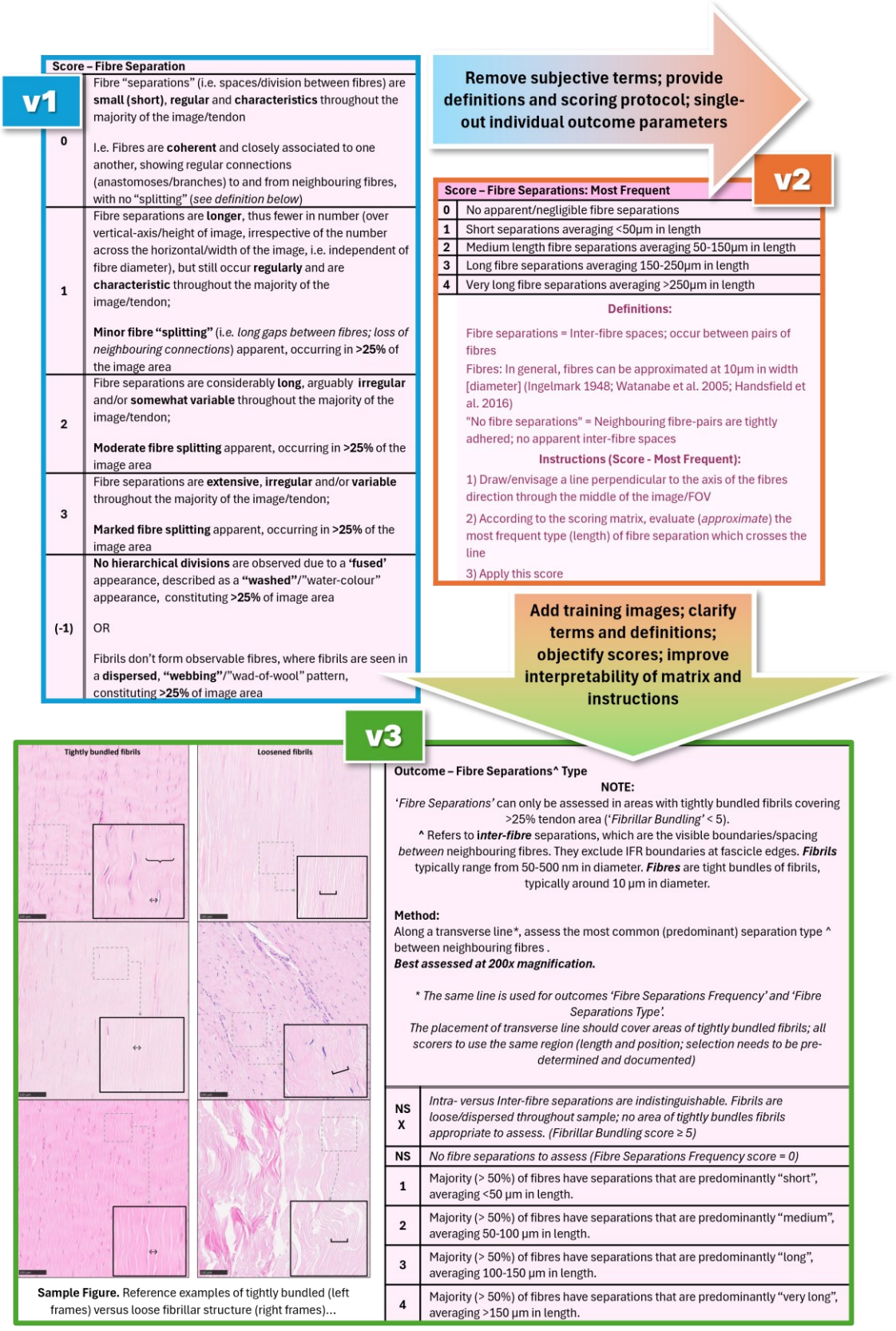


Figure 4.4.D Example of scoring matrix development for the Fibre Separations score outcome between system versions. Developments include modifying wording for improved interpretability and objectivity, plus inclusion of definitions (v2, v3) and training images (v3) for orientating the scorer/investigator to characteristic features.

4.4.4 Methodology for Validity Testing of "Version 3.2"

Having trialled and improved numerous iterations of a new scoring system, including 2 rounds of assessing the percentage agreement between scorers using v3 (Figure 4.4.A), the next stage was formally assessing inter-rater agreement for the proposed system. An amended version of v3 (i.e. v3.2; presented in *Appendix Section 9.5.3* for reference) was applied to a dataset of archival normal human cadaveric tendons to capture characteristics across a range of variables (tendon, region, age, sex, etc.). Two assessors (rater-1 = SH and rater-2 = CBL) independently scored 34 samples, blinded to the tendon variables.

Outcomes assessed (v3.2) included: Cell Density (count and score); Cell Distribution; Cell Morphology; Cell Arrangement; Fascicular Matrix (FM) Organisation; Fibrillar Bundling; Fibre Separation Frequency and Type; Interfascicular Matrix, Space and Region Frequencies; IFR Type Proportion; IFM Density; Adipocyte Presence and Localisation; Vasculature Presence and Localisation; FM and IFR Proteoglycan Presence; average and maximum Proteoglycan Staining Intensities; Proteoglycan Staining Location; Chondrocyte-Like Cell Presence; Collagen Alignment (polarised and non-polarised); Crimp Presence; Crimp Alignment; Crimp Wavelength; and Crimp Wavelength Area (*Appendix Section 9.5.3*).

Additionally, intra-rater comparisons were made on certain outcomes to assess the validity/suitability of different scoring approaches. First, Cell Density was assessed by each assessor using two approaches, conducted on three pre-selected ROIs for each sample. The initial scoring of cell density used the ordinal scoring matrix presented (*Section 4.5.1 Standardised Scoring Suite:* Table 4.5.a). Cells were then manually counted, and these values were converted to the score-range values to compare the scoring approaches for each assessor. Second, a similar comparison was made for interfascicular region (IFR) characterisation. IFR Type was initially semi-quantitatively scored on the proportion of interfascicular matrix (IFM) versus interfascicular space (IFS). These results were then compared against direct counts of IFM and IFS values that were transformed to an equivalent proportion score from IFM counts divided by IFS counts. Lastly, Collagen Alignment was scored both using polarised and non-polarised light approaches by both assessors, and intra-rater comparisons were calculated to assess the agreement between these two methods.

4.4.4.1 *Statistical Analysis: Inter-rater and Intra-rater Comparisons*

All analyses were conducted in Stata (SE 15.1, StataCorp LLC, Texas, USA). For inter-rater agreement between assessors, categorical data (ordinal and nominal scored outcomes) were analysed using Cohen's Weighted Kappa. Continuous data (count and ratio outcomes) were analysed using Intraclass Correlation Coefficients (ICC) from a two-way mixed effects model. For intra-rater comparisons of different scoring approaches for Cell Density (direct score versus count-to-score conversion), IFR Type (direct score versus count-to-score conversion) and Collagen Alignment (polarised versus non-polarised), the Weighted Kappa test was also used. Considering previous interpretations[215-218], Kappa values (κ) and Kappa percentage agreement (%, a weighted agreement of all scores) and ICC Agreements were interpreted according to the definitions in Table 4.4.a.

Table 4.4.a Kappa value (κ) and Kappa/ICC Agreement value interpretations.

Value	K-value Interpretation	Kappa % / ICC Agreement Interpretation	Colour Key
<0 - 0	total disagreement	total disagreement	
>0 - <0.1	none	none	
0.1 - <0.2	poor	negligible	
0.2 - <0.4	minimal	inadequate	
0.4 - <0.6	weak	minimal	
0.6 - <0.8	moderate	acceptable	
0.8 - <0.9	strong	good	
0.9 - <1.0	almost perfect	excellent	
1.0	perfect	perfect	

4.4.5 Results of Rater Comparisons

From the Kappa tests for scored outcomes, the % agreement between raters were mostly good or excellent despite the lower κ-values, predominated by weak comparability (Table 4.4.b). ICC Agreement for most continuous data outcomes were good to excellent (Table 4.4.c).

Table 4.4.b Cohen's Kappa inter-rater agreement for nominal/ordinal data outcomes (scoring system version 3.2)

ABBREV.	OUTCOME	NO. PAIRED SCORES	NO. POSSIBLE SCORES	K-VALUE	KAPPA % AGREEMENT	95% CONFIDENCE INTERVAL	
b1CS	Cell Density Score (box 1)	34	8	0.8158	0.9580	0.623	1.008
b2CS	Cell Density Score (box 2)	34	8	0.7974	0.9412	0.595	1.000
b3CS	Cell Density Score (box 3)	34	8	0.8102	0.9496	0.609	1.011
CSpool	Cell Density Score (pooled)	102	8	0.8083	0.9496	0.694	0.923
CDenS	**Cell Density Score (average)**	34	8	0.9279	0.9832	0.727	1.129
CC1Sc	Cell Count-to-Score conversion (box 1)	34	8	0.9116	0.9790	0.716	1.107
CC2Sc	Cell Count-to-Score conversion (box 2)	34	8	0.9422	0.9832	0.734	1.150
CC3Sc	Cell Count-to-Score conversion (box 3)	34	8	0.9582	0.9874	0.747	1.170
CCSpool	Cell Count-to-Score conversion (pooled)	102	8	0.9400	0.9832	0.824	1.056
b1CMor	Cell Morphology (box 1)	31	4	0.6485	0.9068	0.348	0.949
b2CMor	Cell Morphology (box 2)	30	4	0.8300	0.9444	0.490	1.170
b3CMor	Cell Morphology (box 3)	31	4	0.5009	0.8746	0.203	0.799
CMorpool	Cell Morphology (pooled)	92	4	0.6751	0.9082	0.494	0.856
CMor	**Cell Morphology (mode)**	33	4	0.5581	0.9259	0.221	0.896
CArr	**Cell Arrangement**	33	4	0.4801	0.8283	0.241	0.719
FMOrg	**FM Organisation**	34	7	0.5192	0.8627	0.320	0.719
FlBun	**Fibrillar Bundling**	34	6	0.5250	0.7765	0.266	0.784
FrSepF	**Fibre Separations Frequency**	20	7	0.5744	0.8500	0.304	0.845
FrSepT	**Fibre Separations Type**	14	4	0.4444	0.7619	0.052	0.837
IFRTypSs	**IFR Proportion Score (scored)**	31	7	0.0373	0.6774	-0.181	0.256
IFRTypS	IFR Proportion Score (count-to-score conversion)	31	7	-0.1344	0.7043	-0.334	0.066
IFMDen	**IFM Density**	27	4	0.3530	0.7243	0.086	0.620
AdiPres	**Adipocyte Presence**	34	7	0.4645	0.9216	0.239	0.690
AdiLoc	**Adipocyte Location**	13	7	-0.0368	0.8333	-0.412	0.339
VasLoc	**Vascular Location**	25	7	0.1379	0.8600	-0.069	0.345
FMPG	**FM Proteoglycan Presence**	32	7	0.5777	0.8594	0.377	0.779
IFRPG	**IFR Proteoglycan Presence**	31	7	0.3818	0.7742	0.202	0.561
PGIntAv	**Average PG Staining Intensity**	22	3	0.8571	0.9545	0.506	1.208
PGIntMx	**Maximal PG Staining Intensity**	22	3	0.6650	0.8636	0.347	0.983
PGMxLoc	**Maximal PG Staining Location**	22	3	0.5045	0.8295	0.144	0.865
ChPre	**Chondrocyte-Like Cell Presence**	32	4	0.5829	0.8542	0.347	0.818
ColAliNP	**Collagen Alignment (Non-Polarised)**	30	8	0.6651	0.9286	0.449	0.881
ColAliP	**Collagen Alignment (Polarised)**	30	8	0.4812	0.8857	0.281	0.682
CriPre	**Crimp Presence**	30	6	0.4379	0.8933	0.189	0.687
CriAli	**Crimp Alignment**	30	5	0.2365	0.7417	0.048	0.425
CriWav	**Crimp Wavelength (predominant)**	30	15	0.5121	0.9548	0.298	0.726
CriAre	**Crimp Wavelength Area (predominant)**	30	6	0.3168	0.8467	0.142	0.491

Table 4.4.c Interclass correlations (ICC) inter-rater agreement for continuous data outcomes (scoring system version 3.2)

ABBREV.	OUTCOME	NO. PAIRED SCORES	ICC AGREEMENT	95% CONFIDENCE INTERVAL	
b1CC	Cell Count (box 1)	34	0.9870	0.974	0.994
b2CC	Cell Count (box 2)	34	0.9825	0.965	0.991
b3CC	Cell Count (box 3)	34	0.9894	0.979	0.995
CDen	**Cell Density (average)**	34	0.9943	0.989	0.997
CDis	**Cell Distribution**	34	0.9916	0.983	0.996
IFMC	IFM Count	34	0.7662	0.581	0.876
IFSC	IFS Count	34	0.4463	0.132	0.679
IFRC	IFR Count (sum IFM+IFS Counts)	34	0.9380	0.880	0.969
IFMF	**IFM Frequency**	34	0.3350	0.001	0.602
IFSF	**IFS Frequency**	34	0.3801	0.053	0.634
IFRF	**IFR Frequency**	34	0.8655	0.748	0.930
IFRTyp	**IFR Type Proportion (IFM/IFR Counts)**	31	-0.1718	-0.492	0.189
VasPres	Vascular Presence (count)	34	0.8044	0.643	0.897
VasPresP	**Vascular Presence (% positive)**	34	0.8044	0.643	0.897

When comparing different scoring approaches, the % agreement between approaches was high for both raters, with mostly moderate or higher κ-values (Table 4.4.d). When comparing the direct scoring approach versus the conversion of counts-to-scores for Cell Density, both raters had excellent % agreement, although rater-1 had almost perfect κ-values while rater-2 had moderate to strong κ-values. Pooling all three cell regions of interest (ROIs) (rater-1: $\kappa = 0.9255$, % Agreement = 0.9790; rater-2: $\kappa = 0.7808$, % Agreement = 0.9426) shows similar findings compared to assessing ROIs individually (Table 4.4.d). Comparing direct scoring versus count-to-score conversions of IFR Type Proportions showed minimal κ (0.3722) but acceptable % agreement (0.7969) for rater-1 but moderate κ (0.7637) with excellent % agreement (0.9301) for rater-2. Lastly, comparing polarised versus non-polarised scoring approaches for Collagen Alignment assessment on PSR-stained sections saw moderate κ-values (rater-1 = 0.6787, rater-2 = 0.6254) but excellent % agreement (rater-1 = 0.9381, rater-2 = 0.9143) for both raters (Table 4.4.d).

Table 4.4.d Cohen's Kappa intra-rater agreement for scoring approach comparisons for rater-1 and rater-2 (scoring system version 3.2).

ABBREV.	OUTCOME	DESCRIPTION	NO. PAIRED SCORES	K-VALUE	KAPPA % AGREEMENT	95% CONFIDENCE INTERVAL	
Rater-1							
C1	Cell Count Score (box 1)	Score versus Count-to-Score	34	0.9826	0.9958	0.783	1.182
C2	Cell Count Score (box 2)	Score versus Count-to-Score	34	0.9006	0.9706	0.692	1.109
C3	Cell Count Score (box 3)	Score versus Count-to-Score	34	0.9011	0.9706	0.690	1.112
Cpool	Cell Count Score (pooled)	Score versus Count-to-Score	102	0.9255	0.9790	0.809	1.042
IFRTyping	IFR Type Proportion Score	Score versus Count-to-Score	32	0.3722	0.7969	0.185	0.560
ColAli	Collagen Alignment	Polarised score versus Non-polarised score	30	0.6787	0.9381	0.452	0.905
Rater-2							
C1	Cell Count Score (box 1)	Score versus Count-to-Score	34	0.8136	0.9580	0.626	1.002
C2	Cell Count Score (box 2)	Score versus Count-to-Score	34	0.7497	0.9286	0.548	0.952
C3	Cell Count Score (box 3)	Score versus Count-to-Score	34	0.7824	0.9412	0.585	0.980
Cpool	Cell Count Score (pooled)	Score versus Count-to-Score	102	0.7808	0.9426	0.667	0.894
IFRTyping	IFR Type Proportion Score	Score versus Count-to-Score	31	0.7636	0.9301	0.550	0.977
ColAli	Collagen Alignment	Polarised score versus Non-polarised score	30	0.6254	0.9143	0.398	0.853

4.4.6 Discussion of Scoring System Outcomes

The following sections discuss the results of the inter-rater and intra-rater comparisons, highlighting strengths and weaknesses of the assessed scoring system (v3.2) and identifies some final modifications made to produce the version enlisted for the purpose of subsequent results chapters in this thesis (a "preliminary" version of the proposed standardised scoring suite, v4). These chapters explore the structural characteristics of a large dataset of different human upper (*Chapter 5)* and lower (*Chapter 6*) limb tendons. Some outcome scores with apparent weaknesses/limitations, were retained where it was considered that additional results from these projects would support or negate the need to further improve/include these characteristics in the practice of HALT.

4.4.6.1 *Cell Characteristics*

Initially, cells were assessed excluding IFR cells (only FM tenocytes assessed), however this was deemed difficult in normal tendons due to challenges defining the IFR versus FM area and was thus evaluated as "all tendon-area cells". Manual counting of cell numbers within the ROI boxes had the highest agreement between raters, with similar findings for ICC and κ agreement when converting counts to the scoring matrix grades (Table 4.4.c, Table 4.4.b). While the scoring method (Cell Density Score) had slightly less agreement between raters, results saw high compatibility between each raters' (intra-rater) own scoring versus count-to-score conversion (Figure 4.4.E). Interesting to note is the disparate κ-values for these comparisons, with rater-2 having lower κ compared to rater-1 (pooled values = 0.7808 and 0.9255 respectively). Discussion

revealed the high correlation for rater-1 was likely due to a 'rough count' approach to scoring, with low numbers quickly counted at a glance and larger numbers mentally divided into quarters for rough counting and multiplying by four. Nonetheless, these results support the validity of scoring for density assessment of cells, which may pose a more time efficient approach with practice when evaluating large-scale studies or when assessing larger ROIs. For the purpose of subsequent results chapters in this thesis however, the count approach was adopted due to the minimal timing differences experienced by the rater for the current dataset. The study design also favoured the slightly improved accuracy/objectivity of the measure with greater consistency achievable when scoring over multiple days and across a large sample set.

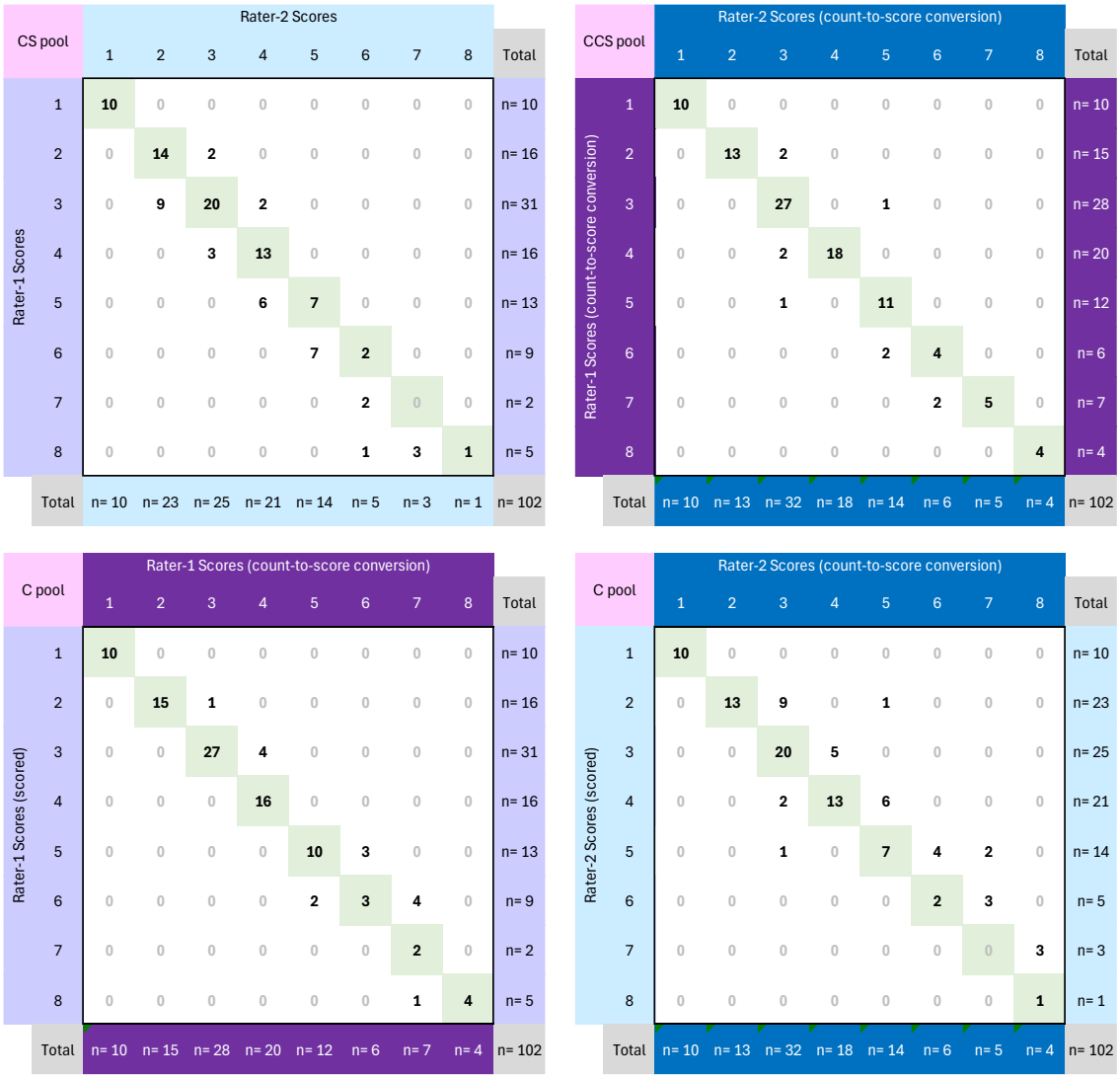


Figure 4.4.E Distribution of scores (pooled) for Cell Density Scores from rater comparisons of scoring system Version 3.2. ***Top-Left:*** inter-rater comparisons of Cell Density Score as directly scored (CS) by raters. ***Top-Right:*** inter-rater comparisons of Cell Density Score as converted from count-to-score approach (CCS). ***Bottom-Left:*** intra-rater comparisons of Cell Density Score for scored versus count-to-score conversion approaches (C) from rater-1. ***Bottom-Right:*** intra-rater comparisons of Cell Density Score for scored versus count-to-score conversion approaches (C) from rater-2.

Cell Morphology scores exhibited variable κ-values when assessing individual ROIs (box-1 = 0.6485, box-2 = 0.8300, box-3 = 0.5009), with moderate findings for pooled scores (κ = 0.6751) but weak findings when considering the mode score across the three ROI boxes (κ = 0.5581). Meanwhile, % agreement values were good or excellent across all Cell Morphology comparisons (Table 4.4.b). This discrepancy is likely a 'kappa paradox' as a result of the large uneven skew of scores (Figure 4.4.F)[219-222]. An approach the proposed SSS took for this outcome was defining the typically subjective classifications of the cell (nuclear) morphologies (Figure 4.5.A), which the group agreed was a valuable tool for training and familiarisation and perceivably reduced intra- and inter-rater inconsistencies. For the following results chapters, discussions found these results were sufficient to support the inclusion of the Cell Morphology outcome in assessment of the complete dataset for tendon-characterisation of this feature.

CMor (pool)	Rater-2 Scores 1	2	3	4	Total
Rater-1 Scores 1	**2**	**1**	**2**	0	*n= 5*
2	**4**	**51**	**2**	0	*n= 57*
3	0	**9**	**8**	**1**	*n= 18*
4	0	**1**	**3**	**8**	*n= 12*
Total	*n= 6*	*n= 62*	*n= 15*	*n= 9*	*n= 92*

CMor (mode)	Rater-2 Scores 1	2	3	4	Total
Rater-1 Scores 1	**6**	**1**	0	0	*n= 7*
2	0	**18**	**1**	0	*n= 19*
3	**1**	**3**	**1**	0	*n= 5*
4	0	0	**1**	**1**	*n= 2*
Total	*n= 7*	*n= 22*	*n= 3*	*n= 1*	*n= 33*

Figure 4.4.F Distribution of scores for Cell Morphology (CMor) pooled (left) and mode (right) results from inter-rater comparisons of scoring system Version 3.2.

Cell Arrangement scores presented with weak κ (0.4801) but good % agreement (0.8283, Table 4.4.b), again with a skew of scores (Figure 4.4.G) potentially contributing to the kappa paradox. The majority of disagreed scores differed only by a score of one (11/14 cases) although the matrix does present a relatively small score selection (4-scale range). Most of these cases are represented by discrepancies between a of score 1 (<5% of cells in chains) and a score of 2 (>5% to 1/3 of cells in chains), with primarily rater-1 typically scoring higher. The resolution threshold between these score categories may subject the raters to disagreement due to individual interpretations of the threshold between these ranges (< or > 5%). Similarly, the assessment of the outcome over the whole-section area was susceptible to disagreement due to the very large sample size of the included dataset and the challenges of interpreting % of cells across such a large section-area. Considering the justifiable κ- and good % agreement-values, the Cell Arrangement outcome was carried through to the SSS applied to proceeding chapters to explore the implications of the outcome/matrix on a larger dataset of different tendons.

CArr		Rater-2 Scores				
		1	2	3	4	Total
Rater-1 Scores	1	**10**	**2**	**1**	0	n= 13
	2	**8**	**4**	0	**1**	n= 13
	3	**1**	**1**	**3**	0	n= 5
	4	0	0	0	**2**	n= 2
	Total	n= 19	n= 7	n= 4	n= 3	n= 33

Figure 4.4.G Distribution of scores for Cell Arrangement (CArr) results from inter-rater comparisons of scoring system Version 3.2.

The scoring system also assessed the presence of 'atypical' cell morphology, where cells typically resembling chondrocytes (rounded, with pericellular GAG/proteoglycan staining and found within a lacunae) are often observed in load-compressed areas, fibrocartilaginous-like areas and even pathological regions of tendon[169,175,223,224]. The scoring matrix aimed to capture the presence of these chondrocyte-like cells, as well as other 'atypical' cells which exhibited similar characteristics but may not quite fulfil this definition entirely (i.e. only 2 of the 3 criteria met e.g. rounded and with 'halo'/in lacunae but no pericellular GAG). The inter-rater comparisons found good % agreement but weak κ for the outcome (0.8542 and 0.5826 respectively, Table 4.4.b). However, scores never differed by more than one. The approach of scoring the entire tendon section potentially influenced the raters' chance of 'catching' rare occasions (difference between 0 = no and 1 = very few cells present). Similarly, the subjective nature of the scale challenges agreeability between perceived numbers and the definitions (scores) applied by raters. Obviously, an objective density count would improve the outcome and is recommended where achievable, however this was deemed impractical for the scale of the specimens we explored. Furthermore, attributing density measurements to characteristics which are often region-specific[118,169,175,223-226] offers challenges when assessing larger sample areas which may not be consistent across specimens or laboratories due to the natural size variation of different tendons. Despite these concerns, the score was retained for subsequent chapters to identify the prevalence of chondrocyte-like cell occurrences across the large tendon dataset included, however the outcome was renamed to "Atypical-Phenotype Cell Presence". This was to avoid misconceptions that all cells captured by the defined '2-out-of-3-features' rule (Table *4.5.e*) are definitively a stage of chondrocyte-differentiation of the cell population and to allude the possibility of other cell types being captured within this definition (where further research is needed to identify particular markers for defining cell populations).

ChPre		Rater-2 Scores				
		0	1	2	3	Total
Rater-1 Scores	0	**12**	**7**	0	0	n= 19
	1	**1**	**3**	0	0	n= 4
	2	0	**5**	0	**1**	n= 6
	3	0	0	0	**3**	n= 3
	Total	n= 13	n= 15	n= 0	n= 4	n= 32

Figure 4.4.H Distribution of scores for Chondrocyte-Like Cell Presence (ChPre) results from inter-rater comparisons of scoring system Version 3.2.

4.4.6.2 *Interfascicular Regions – Spaces versus Matrix*

Defining the hierarchical structure in terms of differentiating the FM versus IFM architecture of tendons via standard histochemical stains proved to be a considerable challenge. In the current study these outcomes were assessed on H&E stained sections, however, they were conducted on PSR-stained sections for the studies presented in later chapters (*Chapter 5* and *Chapter 6*) as these were discussed and determined to provide better identification of the structures compared to H&E- or TB-stained sections.

The number of fascicle-fascicle boundaries (i.e. the interfascicular regions = IFRs) was determined by summing the separate counts for the number of IFMs (IFRs with visible collagenous matrix area) and the number of IFSs (IFRs without visible collagenous matrix) across the width of the scorable tendon area. The IFR Count found excellent ICC agreement (0.9380), where IFM Count had acceptable agreement (0.7662) whilst IFS Count was minimal (0.4463). Normalisation of these counts to frequencies (#/mm) fared poorer, showing only good agreement for IFR Frequency (0.8655) but inadequate agreement for both IFM Frequency (0.3350) and IFS Frequency (0.3801; Table 4.4.c). Further characterisation of the IFM (IFM Density score) had acceptable kappa % agreement (0.7243) but minimal κ (0.3530; Table 4.4.b). Notably, rater-2 typically assessed more higher-density IFMs (higher scores) than rater-1 (Figure 4.4.I).

IFMDen		Rater-2 Scores				
		1	2	3	4	Total
Rater-1 Scores	1	0	**1**	**1**	**1**	n= 3
	2	**1**	**5**	**2**	**3**	n= 11
	3	0	**1**	**2**	**6**	n= 9
	4	0	0	0	**4**	n= 4
	Total	n= 1	n= 7	n= 5	n= 14	n= 27

Figure 4.4.I Distribution of scores for IFM Density (IFMDen) results from inter-rater comparisons of scoring system Version 3.2.

Conversion of counts to a ratio (IFMs/IFRs) found an ICC of total disagreement (-0.1718; Table 4.4.c), where converting this value to a score as per v3.2 (*IFR Type Proportion Score*) found total disagreement of the κ-value (-0.1344) but acceptable kappa % agreement (0.7043; Table 4.4.b; IFRTypS, Figure 4.4.J). Initially, both raters directly scored the *IFR Proportion Score* prior to the counting approach (IFRTypSs, Figure 4.4.J), which revealed no κ-value agreement (0.0373) but acceptable kappa % agreement (0.6774; Table 4.4.b). Comparing the scored versus count-to-score approaches saw variable results for the two raters. Rater-1 had minimal κ-value agreement (0.3722) but acceptable kappa % agreement (0.7969), while rater-2 had much better agreeability between the two approaches, with moderate κ-value agreement (0.7636) and excellent kappa % agreement (0.9301; Table 4.4.d; Figure 4.4.J). It is notable that rater-1 had a large number (n=10) of scores generated by the 2 methods differing by 1 in the same direction (converted score = 5 and scored score = 6 [Figure 4.4.J]). This likely contributed to the very low κ-value despite a high kappa %, and may reflect a learned ability to estimate percentages which can be improved with practice (rater-2 is a more experienced histological assessor).

Interestingly, the comparison of IFR-related outcomes found greater agreement levels than individual IFM- or IFS-related outcomes, indicating the agreement came not from identifying the region of fascicle-fascicle boundaries (IFRs) but rather in characterising these regions through assessing the presence/absence and amount of matrix observed in these areas. Discussions revealed where one rater might identify loose, disorganised collagenous architecture around a perceived fascicle-fascicle boundary as an IFM, another might perceive this as the disruption of the edge of a fascicle (disorganised FM area) adjacent thus to an IFS (Figure 4.4.K). Another discrepancy was noted where one rater perceived an “IFS line” while another perceived a “highly dense IFM line” – a difference of opinion when there is a darkly stained boundary between fascicles as to whether they are just boundaries (IFS) or if there is matrix (densely packed) between the fascicles (Figure 4.4.L), likely influenced also by viewing magnification and/or monitor setup.

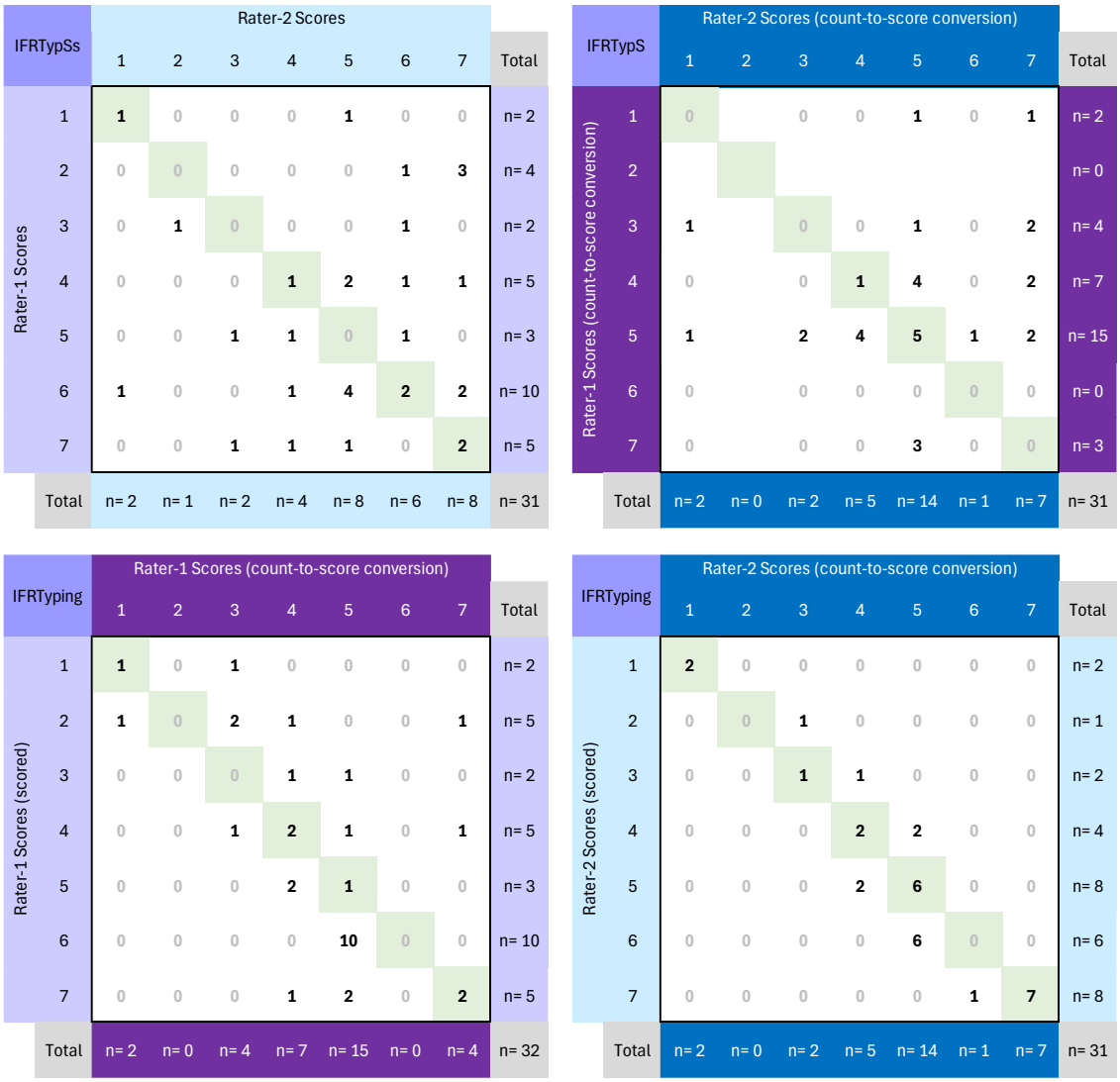


Figure 4.4.J Distribution of scores for IFR Type Proportion assessments from rater comparisons of scoring system Version 3.2. ***Top-Left:*** inter-rater comparisons of IFR Type Proportion Score as directly scored (IFRTypSs) by raters. ***Top-Right:*** inter-rater comparisons of IFR Type Proportion Score as converted from count-to-score approach (IFRTypS). ***Bottom-Left:*** intra-rater comparisons of IFR Type Proportion Score for scored versus count-to-score conversion approaches (IFRTyping) from rater-1. ***Bottom-Right:*** intra-rater comparisons of IFR Type Proportion Score for scored versus count-to-score conversion approaches (IFRTyping) from rater-2. *Cells are blank where neither rater utilised that score category.*

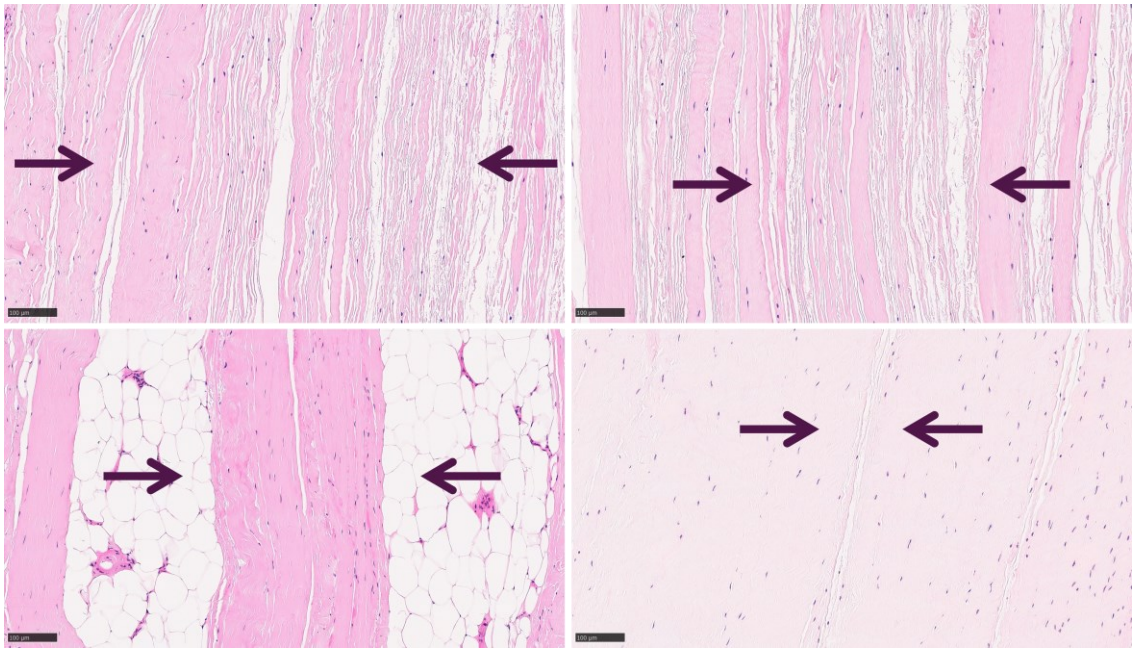


Figure 4.4.K Example of fascicles and IFR boundaries with unclear discrimination as disrupted/disorganised FM matrix versus IFM. *H&E-stained section of human tendons. All Images: 200x magnification, scale bar = 100 µm.*

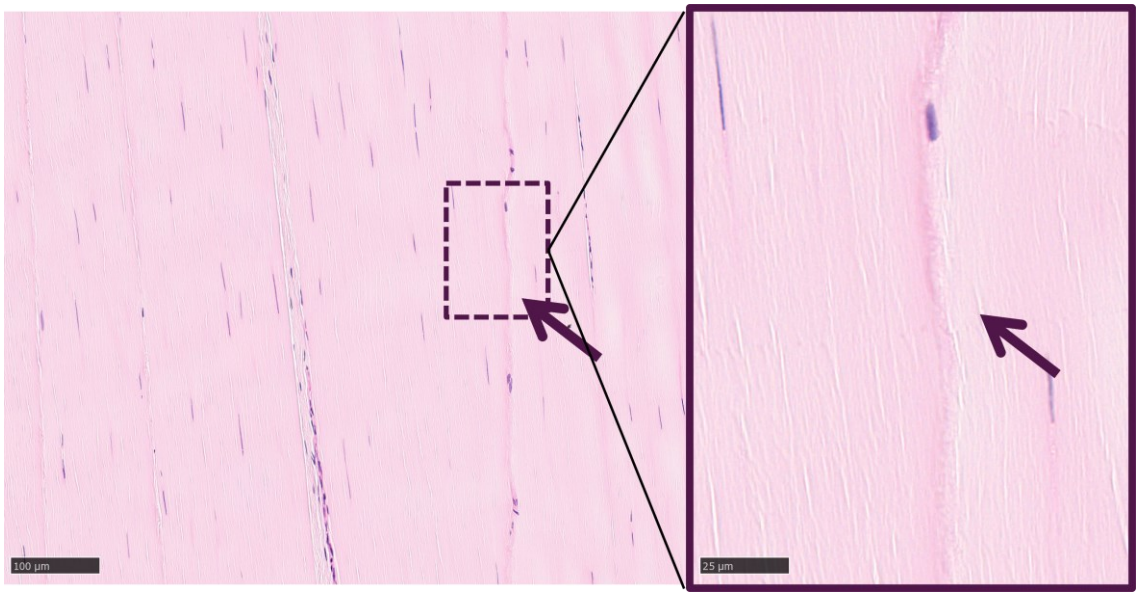


Figure 4.4.L Example of an "IFS line" (dark pink stained indiscrete fascicle-fascicle boundary) with potential definition as a "highly dense IFM line" dependent on rater interpretation or magnification of assessment. *H&E-stained section of human tendon. Image: 200x magnification, scale bar = 100 µm. Inset: 800x magnification, scale bar = 25 µm.*

Additional to perception differences is the complication of the 'set-up' or 'two-step' decision process enlisted as an aim to make the scoring matrix more objective. Defining the presence of 'matrix' or 'no matrix' to define an IFR as IFM or IFS respectively using a 50% threshold when assessing a defined ROI also leaves room for disagreement. Where the perceived threshold of matrix presence versus absence differs between raters, likely from assessing borderline quantities, the resultant conclusion (IFM versus IFS) from either rater may differ and contribute to overall

disagreement in the outcome. The concept of this caveat is discussed further in *Section 4.4.6.3 Collagenous Architecture.*

Unfortunately, the premise of many of the outcomes proposed in system v3.2 were designed to differentiate outcomes for FM and IFR/IFM areas separately to increase tendon characterisation and add/identify further discriminability between tendons based on a feature which was clearly quite variable between samples. Evidently from the above results, the detailed discrimination between FM and IFM proved a significant challenge, and (as later outlined) posed complications for scoring which revolved around being able to characterise these hierarchical structures independently. Consequently, for the purpose of assessing the datasets in later chapters in this thesis, many of these FM versus IFM scores were excluded or outcomes were assessed for the collagenous structure in general (all tendon area excluding paratenon). Nevertheless, and consistent with the importance of the IFRs and associated matrix (IFM) composition in tendon biomechanics[227-230] we stand by the notion that the classification of characteristics between or specific to FM and IFM separately could reveal interesting and important definition of tendon structure and/or variability. Future endeavours should enlist more objective measurements and/or approaches such as IHC assessment and training images, to aid identification and differentiation of these structures and provide modifications to the SSS for these elements of tendon/ligament characterisation.

Despite the limitations, it was deemed valuable to retain the score (count) of IFRs in the exploration of the datasets in subsequence chapters of this thesis to aid understanding of tendon structure and identify where differences may be present. Considering only a single rater will score those projects, a count of IFRs (with notes of IFM versus IFS classifications; "IFR Type Proportion" outcome) is expected to reveal interesting findings between the large range of tendons to support further SSS development even where objectivity of the outcome may not yet be fully achieved.

4.4.6.3 *Collagenous Architecture*

The scores assessing the collagenous structure through H&E-stained sections generally exhibited acceptable to good % agreement, however all scores – that is, Fascicular Matrix Organisation (FMOrg), Fibrillar Bundling (FlBun), Fibre Separations Frequency (FrSepF), and Fibre Separations Type (FrSepT) – all obtained weak κ-vales (Table 4.4.b). The distribution of scores for FMOrg revealed that discrimination between no (0%) versus a little (<5%) area coverage with disorganised structure was a prevalent case (n = 8) for disagreement between raters (Figure 4.4.N). This score also saw rater-1 consistently scoring higher than rater-2, likely a result of familiarity (experience) and subjective influence over the interpretation of the "organised" versus "disorganised" characteristic definitions. Nevertheless, other than one instance, raters were never more than two scores different, a fairly good outcome considering the difficult nature of the score and the challenge of differentiating FM versus IFR as previously discussed.

Fibrillar Bundling exhibited the most dispersed score distribution between raters for the H&E-assessed architecture scores (Figure 4.4.N), with rater-1 typically scoring the fibrillar structure as predominantly loose (high score) whilst rater-2 predominantly determining the fibrils to be tightly bundled (low score). Post-scoring discussion concluded there was an issue with the scorers'

"wispy threshold" perception – appearance of all loose fibrils but with denser staining appearance often could be seen as all tightly packed fibrils (solid pink staining area). The raters' perceptions, along with screen resolution and the magnification used to view the image/section, led to differing observations and resulted in scores at the extreme ends of the range between raters.

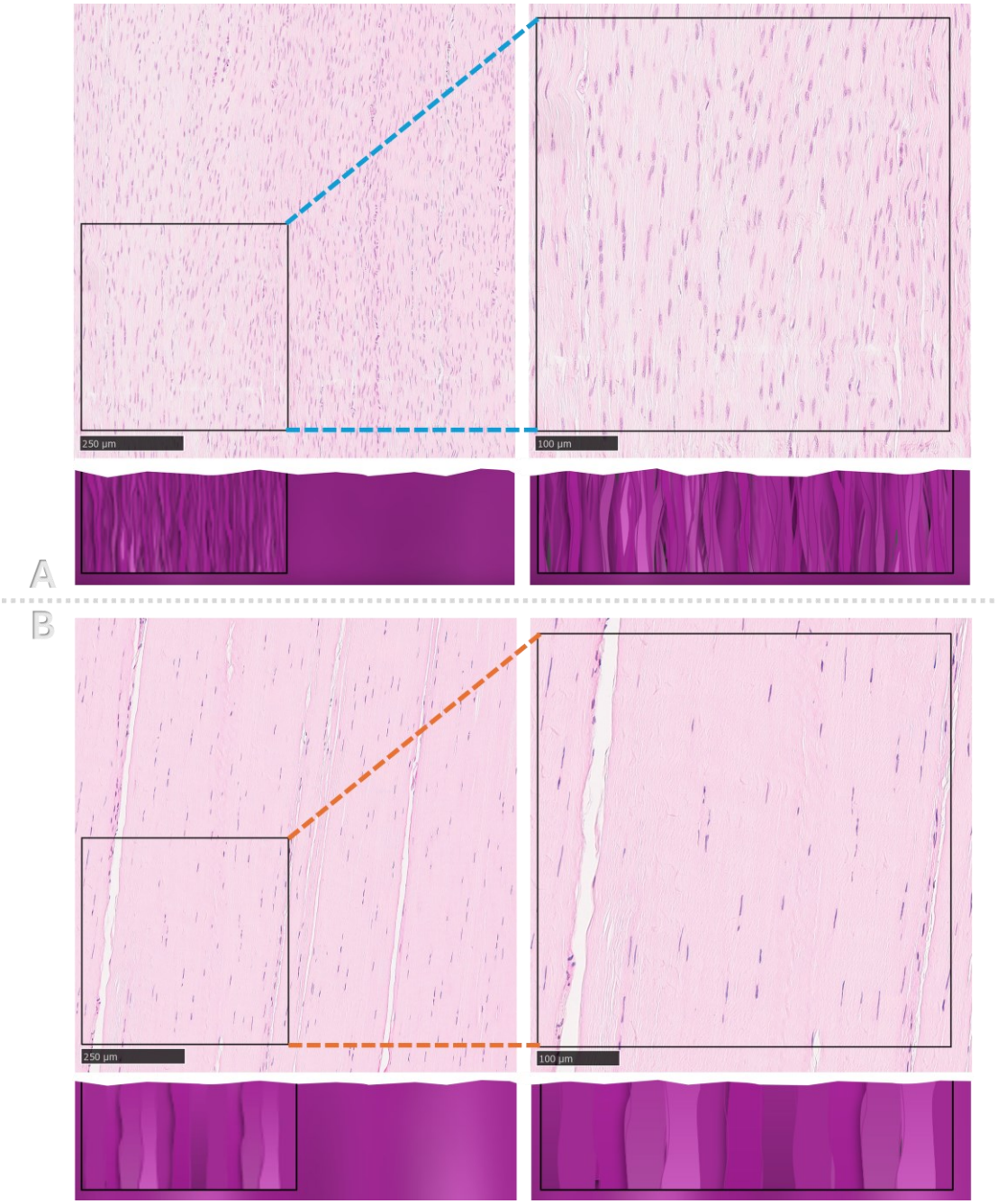


Figure 4.4.M Example of scorer subjectivity: possible differing perceptions of tissue architecture *(H&E staining, Supp. Table 9.5.b*) dependent on mode of observation, for example, magnification used. When observed at lower magnification (*100x; scale bars = 250 µm*), **left images** present regions of dense/'solid' stained areas perceived as a Fibrillar Bundling score of 1 for both examples (**A** and **B**; mostly tightly **bundled** fibrils). At higher magnification (*200x; scale bars = 100 µm*), **left images** reveal more detailed observation of the underlying fibrillar architecture and examples could arguably receive **(A,** top**)** a score of 6 (mostly **loose**/dispersed fibrils) or **(B**, bottom**)** a score of 1 (mostly tightly **bundled** fibrils). Cartoon depictions below histology images demonstrate structural architecture: example **A** = highly segregated fibrillar structuring, no clearly demarcated bundles (fibres); example **B** = tightly packed fibrillar structure presenting as clearly demarcated bundles (fibres).

The Fibre Separations scores exemplify a caveat associated with a few of the scoring matrices in the proposed scoring system: the issue of the polarising nature of the score definition. If scoring the "majority" or most common type/occurrence/etc. of a feature, this becomes a defining factor in what feature is then actually scored by the observer for the outcome. As with Cell Morphology score which uses a >50% threshold, the Fibre Separations Type assesses the "most common" length of separation. Where "short" and "long" separations are present in fairly equal numbers, the perception by different raters of which predominates could result in marked score differences. Discriminating between inter vs intra fibre spaces ("fibre separations" vs "fibrillar loosening" respectively) and identification of the collagen fibre boundaries themselves (e.g. if the space is in the middle of one fibre, or between two smaller fibres) was also found to be challenging throughout the development of these scoring matrices.

A proposed solution to some of these identified issues is for more case examples to be used as training tools, and more in-depth training of raters to elaborate architecture divisions and how to prepare the application of the system (ROI requirements, screen settings, magnification specification etc.). Rater training could improve coherence of the interpretation of the score, not only between but also within raters.

All *Collagenous Architecture* scores were retained for subsequent chapters, with a number of the identified limitations obviated by use of a single trained scorer (SH). Features of the collagenous architecture are important characteristics for tendon histological assessment (Table 4.3.a). The application of these scores to a large dataset of tendons under a single-rater dependent setting could identify tendon variability or similarities that may support or negate the need to improve/include these characteristics in the practice of HALT. The main modification made was to change the FMOrg score from being specific to the FM area only (as per v3.2) to include all collagenous architecture (FM and IFM; ColOrg, v4) of the tendon area (excluding paratenon). This change was in line with challenges as discussed previously regarding differentiating between FM and IFM/R. The Fibrillar Bundling outcome was also renamed to 'Fibrillar Loosening' to match the name reference to the association of a higher score.

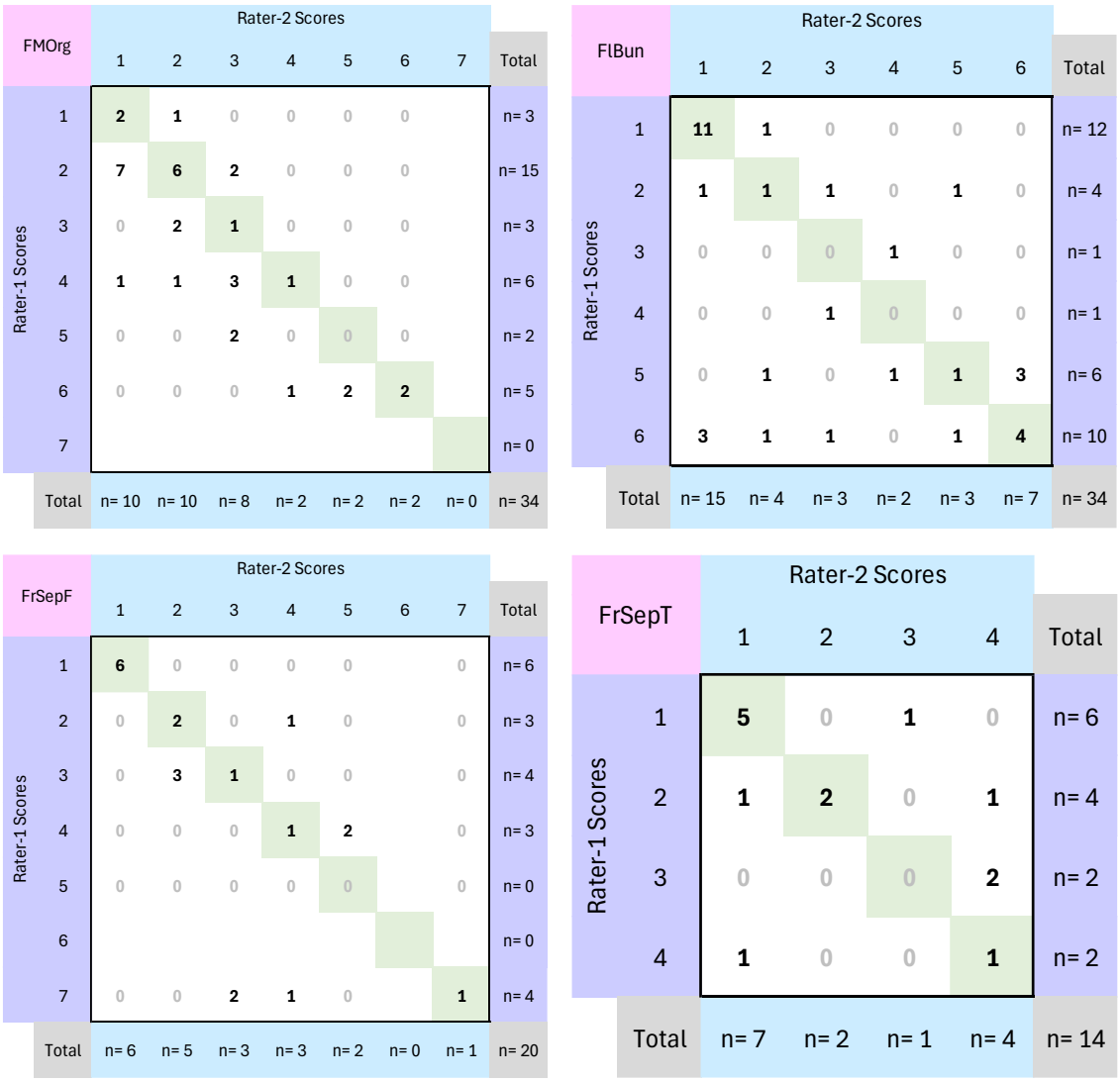


Figure 4.4.N Distribution of scores for Fascicular Matrix Organisation (FMOrg, ***Top-Left***), Fibrillar Bundling (FlBun, ***Top-Right***), Fibre Separations Frequency (FrSepF, ***Bottom-Left***), and Fibre Separations Type (FrSepT, ***Bottom-Right***) scores from inter-rater comparisons of scoring system Version 3.2. *Cells are blank where neither rater utilised that score category.*

PSR-stained sections were used to assess the alignment of the FM collagen using both brightfield (non-polarised) and polarised light approaches. Polarised light is commonly used/recommended for PSR-stained assessment of tissues[151,153-155,231,232], however, based on previous observations[142,200,201], we hypothesised that this outcome could be assessed under brightfield conditions for tendon/ligament characterisation. To test this, we scored Collagen Alignment using both approaches and compared each between raters, but also compared each raters' polarised results to their respective non-polarised results.

Both approaches achieved acceptable comparability between raters, and surprisingly, the non-polarised approach had better agreeability ($\kappa = 0.6651$ and % agreement = 0.9285) compared to the polarised approach ($\kappa = 0.4812$ and % agreement = 0.8857; Table 4.4.b). The minor disagreement between raters for both approaches (Figure 4.4.O) could in part result from the challenging discrimination of FM and IFM. However, PSR-staining was determined in earlier

scoring system development to be the easiest stain for IFR identification suggesting FM/IFM differentiation may have minimal influence for PSR-based Collagen Alignment scores. Rater-1 had slightly lower scores on average, accounted for by the greater inclination of excluding 'unaligned IFM area' from the assessment of FM area compared to rater-2.

When comparing approaches, both rater-1 and rater-2 had acceptable κ (0.6787 and 0.6252 respectively) and excellent % agreement (0.9381 and 0.9143 respectively). Non-polarised assessment proved to be a valid outcome (Figure 4.4.O) which provides an alternative where polarised microscopy may not be readily available or increases scoring time from managing the dynamic nature of adjusting the polarisers for direct microscope scoring or capturing polarised images. For subsequent chapters, the non-polarised approach was adopted in v4 of the SSS.

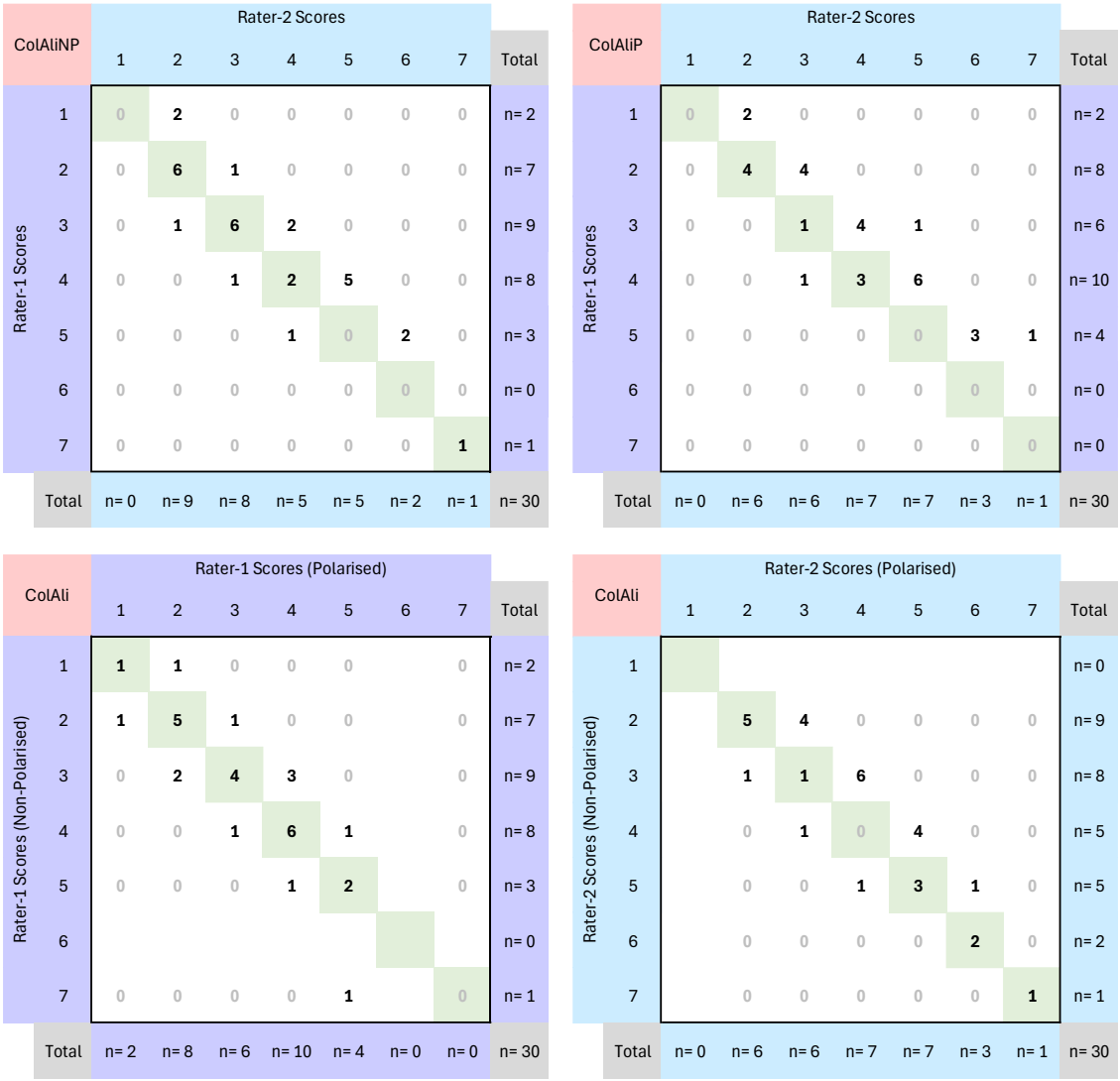
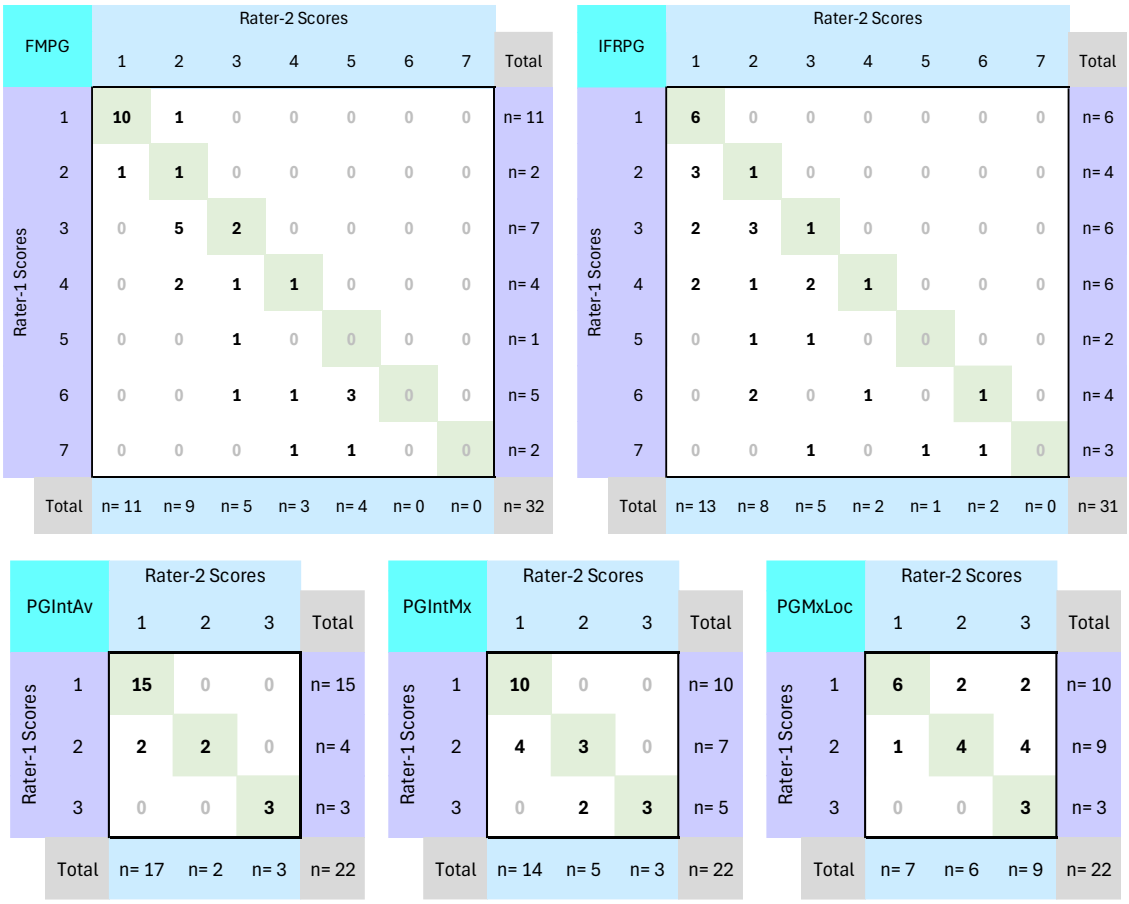


Figure 4.4.O Distribution of scores for Collagen Alignment Scores from rater comparisons of scoring system Version 3.2. ***Top-Left:*** inter-rater comparisons of Collagen Alignment (Non-Polarised, ColAliNP). ***Top-Right:*** inter-rater comparisons of Collagen Alignment (Polarised, ColAliP). ***Bottom-Left:*** intra-rater comparisons of Non-Polarised versus Polarised scoring of Collagen Alignment from rater-1. ***Bottom-Right:*** intra-rater comparisons of Non-Polarised versus Polarised scoring of Collagen Alignment from rater-2. *Cells are blank where neither rater/scenario utilised that score category.*

4.4.6.4 *Proteoglycan Content*

The scores for the assessment of proteoglycan (PG) content were modified considerably during the developmental iterations of the scoring system, with notable mention to the differing staining patterns observed in and predicted to be influenced by the presence and tendency for increased staining in the IFR areas. Accordingly, v3.2 aimed to characterise PG staining specific to the FM and IFR regions separately. Scoring PG stained area for the FM found weak κ (0.5777) but good % agreement (0.8594, Table 4.4.b), where rater-1 typically scored higher, and the < or > 5% threshold between scores 2 and 3 presenting a tendency for disagreement (Figure 4.4.P). Unsurprisingly, results for IFR PG content fared worse (κ= 0.3817, % agreement = 0.7742, Table 4.4.b), as a result of difficulty in defining IFR area. In particular, the presence of PG staining was discussed to actually influence perception of the identification of IFR in itself.

Evaluation of staining intensity achieved good agreeability despite the subjective nature of the score which would typically leave room for disagreement (Table 4.4.b), where Average intensity staining had strong κ and excellent % agreement (0.8571 and 0.9545 respectively), while Maximal intensity staining was slightly lower with moderate κ and good % agreement (0.6650 and 0.8636 respectively). As expected, localising the maximal staining fared the worst with weak κ but good % agreement (0.5045 and 0.8295 respectively, Table 4.4.b). The small score range likely contributed to the drastic drop in κ and the significance where score difference was greater than 1, but also the FM versus IFR issue again likely contributed to disagreement (Table 4.4.b).



FMPG	Rater-2 Scores							
Rater-1 Scores	1	2	3	4	5	6	7	Total
1	**10**	**1**	0	0	0	0	0	n= 11
2	**1**	**1**	0	0	0	0	0	n= 2
3	0	**5**	**2**	0	0	0	0	n= 7
4	0	**2**	**1**	**1**	0	0	0	n= 4
5	0	0	**1**	0	0	0	0	n= 1
6	0	0	**1**	**1**	**3**	0	0	n= 5
7	0	0	0	**1**	**1**	0	0	n= 2
Total	n= 11	n= 9	n= 5	n= 3	n= 4	n= 0	n= 0	n= 32

IFRPG	Rater-2 Scores							
Rater-1 Scores	1	2	3	4	5	6	7	Total
1	**6**	0	0	0	0	0	0	n= 6
2	**3**	**1**	0	0	0	0	0	n= 4
3	**2**	**3**	**1**	0	0	0	0	n= 6
4	**2**	**1**	**2**	**1**	0	0	0	n= 6
5	0	**1**	**1**	0	0	0	0	n= 2
6	0	**2**	0	**1**	0	**1**	0	n= 4
7	0	0	**1**	0	**1**	**1**	0	n= 3
Total	n= 13	n= 8	n= 5	n= 2	n= 1	n= 2	n= 0	n= 31

PGIntAv	Rater-2 Scores			
Rater-1 Scores	1	2	3	Total
1	**15**	0	0	n= 15
2	**2**	**2**	0	n= 4
3	0	0	**3**	n= 3
Total	n= 17	n= 2	n= 3	n= 22

PGIntMx	Rater-2 Scores			
Rater-1 Scores	1	2	3	Total
1	**10**	0	0	n= 10
2	**4**	**3**	0	n= 7
3	0	**2**	**3**	n= 5
Total	n= 14	n= 5	n= 3	n= 22

PGMxLoc	Rater-2 Scores			
Rater-1 Scores	1	2	3	Total
1	**6**	**2**	**2**	n= 10
2	**1**	**4**	**4**	n= 9
3	0	0	**3**	n= 3
Total	n= 7	n= 6	n= 9	n= 22

Figure 4.4.P Distribution of scores for Fascicular Matrix Proteoglycan Presence (FMPG, ***Top-Left***), Interfascicular Region Proteoglycan Presence (IFRPG, ***Top-Right***), Average Proteoglycan Staining Intensity (PGIntAv, ***Bottom-Left***), Maximal Proteoglycan Staining Intensity (PGIntMx, ***Bottom-Middle***) and Maximal Proteoglycan Staining Location (PGMxLoc, ***Bottom-Right***) scores from inter-rater comparisons of scoring system Version 3.2.

A more general note regarding the scoring of PG (purple) staining from TB-stained sections is the influence of equipment set-up. Precautions are advised regarding program or hardware settings for different setups using direct microscopy or digital scans for scoring. Our laboratory conducted all brightfield assessment digitally on personal laptops from scanned slides. Changes in software and screen-display settings altered colour differentiation between setups and influenced scorer perception, likely contributing to score discrepancies. Particularly with chromatic-dependant score outcomes, there should be an effort to standardise these parameters where possible, such as using the same software settings (e.g. standardising gamma correction etc.) or – if possible – using common hardware systems.

In line with the identified limitations, the SSS to be used in proceeding chapters (that is, v4) was modified such that the PG Presence score was changed to assess tendon area as a whole and the localisation score was excluded due to the challenge in defining the difference between FM and IFR area.

4.4.6.5 *Other Characteristics*

The presence and location of adipose tissue was assessed from standard H&E-stained sections. The Adipocyte Presence score found weak κ (0.4645) but excellent % agreement (0.9216, Table 4.4.b); with 13/15 of the disagreements being rater-1 consistently scoring one score higher than rater-2 (Figure 4.4.Q). Raters noted the difficulty in defining % area coverage of this characteristic, noting challenges where adipocytes were dispersed in clumps / isolated incidences rather than a part of "tissue-type" regions. Furthermore, the range of scores proposed – as seen in the distribution for both raters (Figure 4.4.Q) – didn't reflect the observed occurrence of the characteristic. Accordingly, the scoring matrix was modified for v4 used in later chapters to increase resolution in the lower-range scores, dropping the higher % area categories which are not expected to be identified in normal tendon/ligament tissue samples. Despite good % agreement (0.8333), the Adipocyte Location score found total disagreement from the κ-value (-0.0368, Table 4.4.b) which could be expected due to the difficulty discerning IFR region. This score was dropped from v4 of the SSS.

AdiPres	Rater-2 Scores					
Rater-1 Scores	1	2	3	4	5	Total
1	**15**	0	0	0		n= 15
2	**5**	**4**	**1**	0		n= 10
3	**1**	**6**	0	0		n= 7
4	0	0	**2**	0		n= 2
5						n= 0
Total	n= 21	n= 10	n= 3	n= 0	n= 0	n= 34

AdiLoc	Rater-2 Scores							
Rater-1 Scores	1	2	3	4	5	6	7	Total
1	**7**	0	**1**		**1**	0		n= 9
2	**2**	**1**	0		0	0		n= 3
3	0	0	0		0	0		n= 0
4								n= 0
5	0	0	0		0	0		n= 0
6	**1**	0	0		0	0		n= 1
7								n= 0
Total	n= 10	n= 1	n= 1	n= 0	n= 1	n= 0	n= 0	n= 13

Figure 4.4.Q Distribution of scores for Adipocyte Presence (AdiPres, ***Left***) and Adipocyte Location (AdiLoc, ***Right***) scores from inter-rater comparisons of scoring system Version 3.2. *Cells are blank where neither rater utilised that score category.*

Development of a scoring matrix to score vascular presence in an objective way proved difficult in a dataset of such variably sized specimens. The approach for assessing v3.2 required detailed discussion between both raters to designate the ROIs to be used for scoring presence/absence of vasculature within each region and resulted in individualised approaches taken for uniquely shaped/sized samples which limit ease of methodology communication. The ICC agreement for number (VasPres) and proportion (VasPresP) of positive regions were good (both 0.8044, Table 4.4.c), but results saw a very high proportion of regions being scored positive for vessels due to the longitudinal nature of vascular tracking within the tissue structure. We would propose future work to explore methods of more objective counts of vessel numbers taking into consideration

normalisation of tissue area, but also providing an achievable measure to be conducted in a manual-scoring setting.

As anticipated, despite good % agreement (0.8600) the Vascular Presence score found poor κ-value agreement (0.1379, Table 4.4.b), primarily attributed to the difficult definition of IFR area. The expectation of vasculature presence to occur in the IFR area also contributed to the tendency for rater-2 to score lower (Figure 4.4.R), where this characteristic – similar to PG presence – influenced the opinion of where the IFR was located. Similarly, identification of vessels in tendon/ligament tissue from standard H&E-stained sections proved to be difficult even for more experienced histopathologists and was also very time-demanding on the large sample sections.

VasLoc	Rater-2 Scores						
	1	2	3	4	5	6	Total
Rater-1 Scores 1	**8**	**1**	0	0	0		n= 9
2	**8**	**1**	**1**	0	0		n= 10
3	**1**	**2**	**1**	0	0		n= 4
4	**1**	0	0	0	0		n= 1
5	**1**	0	0	0	0		n= 1
6							n= 0
Total	n= 19	n= 4	n= 2	n= 0	n= 0	n= 0	n= 25

Figure 4.4.R Distribution of scores for Vasculature Location scores from inter-rater comparisons of scoring system Version 3.2. *Cells are blank where neither rater utilised that score category.*

Due to these challenges, and the poor discriminability of the vascularity of tendons due to most samples scoring high due to individual vessels crossing many ROIs across a section, vasculature was not assessed in SSS-v4 for the projects in subsequent chapters of this thesis. Future work should be done to enlist IHC identification of these regions and features to aid improvement of this outcome assessment.

4.4.6.6 *Crimping Structure*

The scores assessing the crimping structure through polarised PSR-stained sections found minimal or weak κ-values with acceptable to excellent % agreement (Table 4.4.b). The low κ-value (0.4379) for Crimp Presence was likely a result of the kappa paradox with results skewed towards the high end of the scale (Figure 4.4.S). Discussion of the two cases of a 3-score difference (rater-1 scoring 6; rater-2 scoring 3) found both were cases of large areas of very 'disorganised' looking FM area which could alternatively be perceived as large IFM-like regions. Perception influenced the total area considered when associating %-coverage of crimp. The very low κ-value (0.2365) for Crimp Alignment was attributed to the nature of the score and the conclusion that the outcome intended was too difficult to describe/interpret.

The low κ-value (0.5121) for Crimp Wavelength was also attributed to the kappa paradox, where the samples assessed did not include scores from the 7-15 range (Figure 4.4.S). This outcome was designed to have an open-ended range to account for variability between different tendons or other variables (e.g. age, pathology, species, etc.). The range was extended from an earlier scoring system version due to incidence of greater wavelength observed in different tendon datasets. Suggestions for future intra-/inter-rater studies would be to conduct statistical analyses relative to the utilised range of scores specific to the study investigation rather than the 'possible' range of scores. Other disagreements also arose due to the 'set-up/2-step' nature of the score focusing on the 'predominant' wavelength, where differing judgement may see raters scoring different regions of crimp patterns accordingly.

The Crimp Wavelength Area found a high proportion of ±1 score difference between raters, but also a relatively high portion of ±2 scores different (Figure 4.4.S). Again, the issue of FM versus IFR area challenged this outcome. Additionally, the perception of which crimp patterning constituted the 'predominant' crimp to be scored relative to also led to disagreement, as well as the attribution of when a perception in slight wavelength difference was or wasn't considered a different (or 'variable') wavelength to be scored.

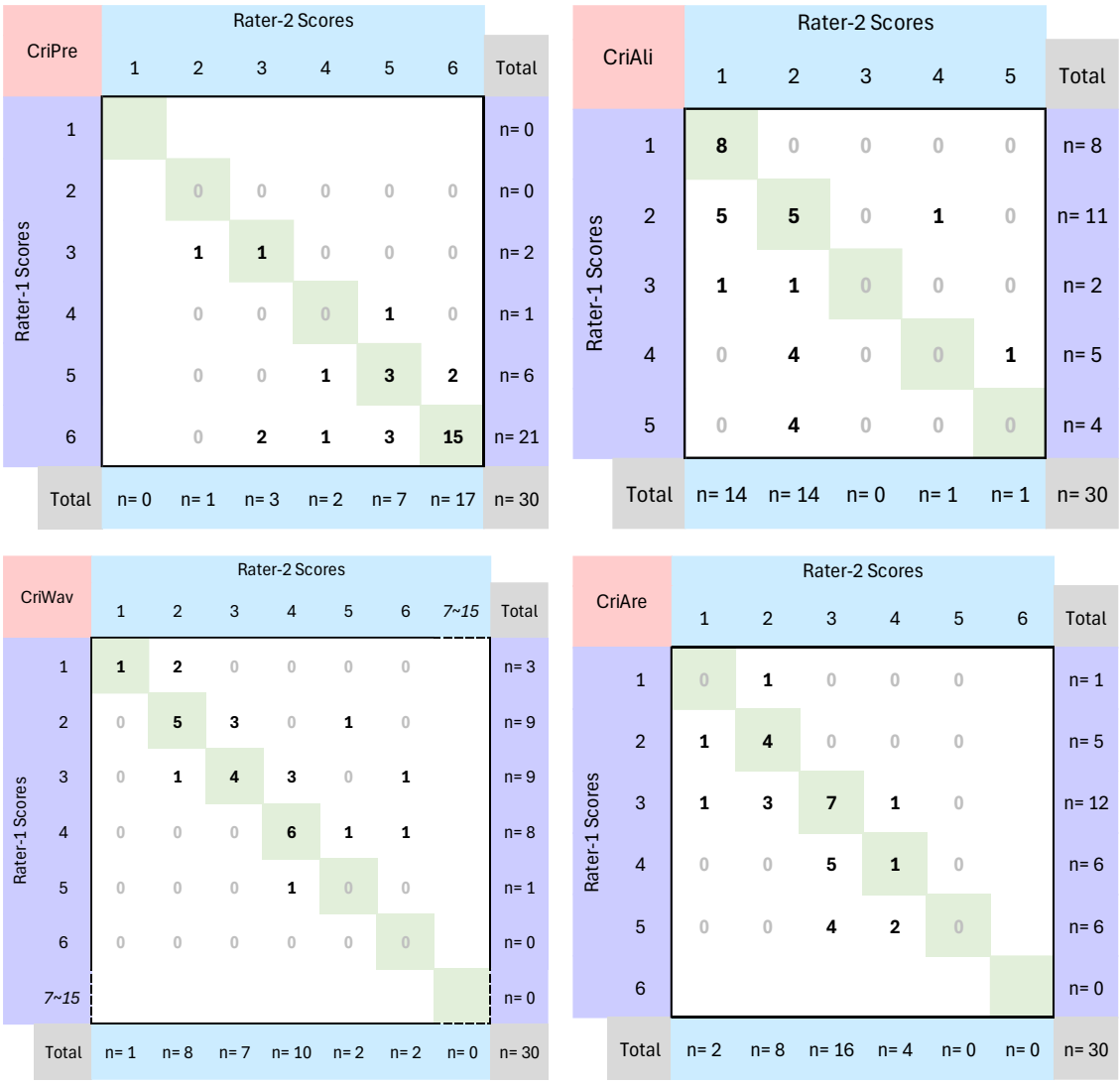


Figure 4.4.S Distribution of scores for Crimp Presence (CriPres, ***Top-Left***), Crimp Alignment (CriAli, ***Top-Right***), Crimp Wavelength (predominant; CriWav, ***Bottom-Left***), and Crimp Wavelength Area (of predominant; CriAre, ***Bottom-Right***) scores from inter-rater comparisons of scoring system Version 3.2. *Cells are blank where neither rater utilised that score category.*

For SSS-v4, the Crimp Presence outcome was renamed to 'Crimp Absence' to match the name reference to the association of a higher score, while the Crimp Wavelength Area outcome was renamed to 'Crimp Wavelength Variation'. Despite the low κ-values, both outcomes were retained for SSS-v4 scoring of the tendons in subsequent chapters, but the scores were modified to be specific to "whole tendon area (excluding paratenon)", removing associations with FM versus IFR area. Crimp Wavelength Variation also had the score range slightly adjusted (merged scores 4 and 5 of v3.2). The Crimp Wavelength outcome was retained, but Crimp Alignment was dropped due to the low agreeability and discussion that the outcome was challenging to assess and explain. As differing rater interpretations and specimen variability affected Crimp Wavelength scoring ability, exploring characteristics such as this in more detail requires further training or sample images at the very least, but would also benefit from automated image analysis of pattern variability.

PART C

4.5 Part C – The System

4.5.1 Standardised Scoring Suite (SSS)

The following tables outline the newly proposed scoring system for tendon/ligament histological characterisation which will be used in subsequent chapters of this thesis. The system presented below offers a preliminary version of the SSS (v4), noting that further minor development will be required to fully validate and improve on the system.

Table 4.5.a Scoring matrices for assessing characteristics of cell populations from H&E-stained sections.

Tenocyte Population
Haematoxylin & Eosin
The structure of the tendon-proper ('tendon area') is typically comprised of two primary sub-regions (excluding paratenon): 1. **The fascicular matrix (FM):** is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure. 2. **The interfascicular region (IFR):** the regions between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance.

Outcome – Cell Density **Method:** Count the number of cells within a region of interest (ROI) in the tendon area, and average per mm^2.	
__	cells/mm^2
Cell Density Score *– Cell density can be scored using the below scoring matrix.*	
0	No cells present.
1	1-100 cells/mm^2
2	101-250 cells/mm^2
3	251-500 cells/mm^2
4	501-750 cells/mm^2
5	751-1000 cells/mm^2
6	1001-1500 cells/mm^2
7	> 1500 cells/mm^2

Outcome – Cell Distribution **NOTE:** Cell density must be assessed across multiple ROIs to determine Cell Distribution. *Number (at least two) and placement of boxes depends on the research question explored.* **Method:** Determine the difference in cell density between ROIs (calculate as *Maximum minus Minimum* Cell Density).	
__	cells/mm^2 difference

Outcome – Cell Morphology **Method:** Assess the morphology (shape) of the tenocyte nuclei (defined below), and score the predominant shape. Cell (nuclear) morphologies are categorised into three shapes based on their aspect ratio (width:length): **Spindle-shaped nuclei** – (fusiform), long, skinny, streak-like. Aspect ratio >1:5 **Cigar-shaped nuclei** – (ovoid), elongated but plump. Aspect ratio between 1:3 and 1:5 **Spherical nuclei** – (spheroid), rounded, egg-shaped, very plump. Aspect ratio < 1:3 Illustrations of the different cell (nuclei) morphologies and their discerning aspect ratios can be seen in Figure 4.5.A.	
NS	N/A – No cells present to score *(Cell Density = 0)*
1	Mixed morphology of cells (spindle-shaped, cigar-shaped and spherical all ≤ 50% each)
2	**> 50%** of cells are **spindle-shaped**
3	**> 50%** of cells are **cigar-shaped**
4	**> 50%** of cells are **spherical**

Outcome – Cell Arrangement **Method:** Assess and score the arrangement of tenocytes relative to one-another (defined below). Cells are categorised into two major arrangement types: **Solitary** – cells show no particular arrangement relative to neighbouring cells; isolated/independent of each other. **Chains** – cells **(≥ 3)** are arranged longitudinally in-series within their inter-fibre space and are located within 2 (average) nuclei-lengths apart. Examples of the presence of tenocytes arranged in chains can be seen in Figure 4.5.B.	
NS	N/A – No cells present to score *(Cell Density = 0)*
1	Cells are solitary; only occasional (up to 5% of) cells arranged in chains.
2	**>5%** to **< 1/3** of cells arranged in **chains**.
3	**1/3** – **2/3** of cells arranged in **chains**.
4	**> 2/3** of cells arranged in **chains**.

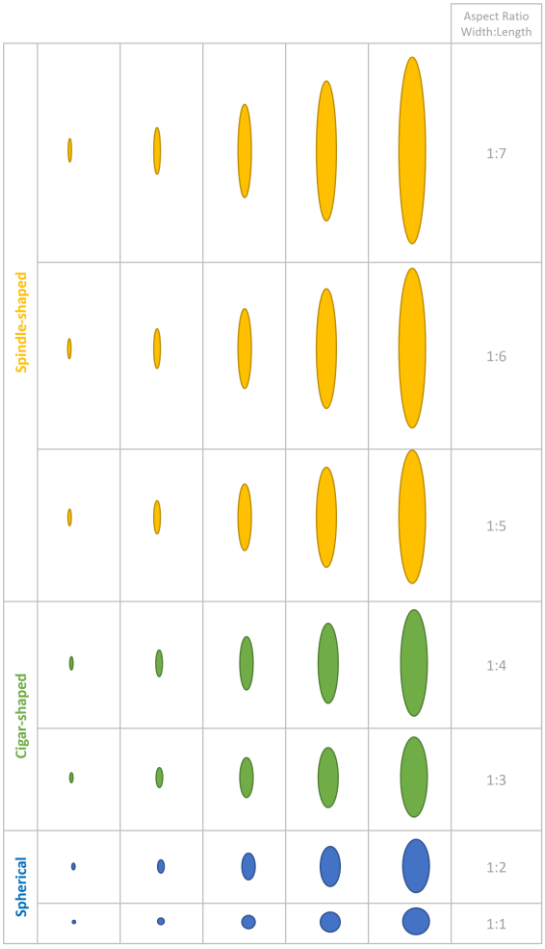


Figure 4.5.A Visual guide for the three different cell (nuclei) morphologies as determined by their aspect ratio (width:length) (Table 4.5.a).

Figure 4.5.B Example of tenocytes arranged in chains. Black boxes indicate tenocyte chains as per the defined criteria (Table 4.5.a). *H&E staining, 200x magnification; Scale bar = 100 µm.*

Table 4.5.b Scoring matrices for assessing characteristics of collagenous architecture from H&E-stained sections.

Collagenous Architecture
Haematoxylin & Eosin
The structure of the tendon-proper ('tendon area') is typically comprised of two primary sub-regions (excluding paratenon): 1. **The fascicular matrix (FM):** is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure. The collagenous FM is hierarchically structured as follows: • The *collagen molecule* (tropocollagen) – primarily fibrillar collagen type-I • The *microfibril* – aggregates of collagen molecules • The *fibril* – formed by microfibrils; fibrils typically range from 50-500 nm in diameter • The *fibre* – bundles of fibrils, typically around 10 µm in diameter • The *fascicle* – bundles of fibres; the major hierarchical unit second to the *tendon* itself. 2. **The interfascicular region (IFR):** the regions between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance: • **Interfascicular matrix (IFM)** comprises the *presence* of connective tissue between/dividing fascicle units. • **Interfascicular space (IFS)** comprises the *absence* of connective tissue between/dividing fascicle units; NOTE – includes directly adjacent fascicles with no visible white space (or matrix). Interfascicular cells or supply structure may be present.

Outcome – Collagenous Organisation

Method:
Score the proportion of collagenous area associated with organised vs disorganised structure (defined below).

Collagenous organisation is categorised into two major definitions based on the alignment/orientation of the collagenous structure:

Organised = Highly unified/parallel collagenous structure aligned along the primary tendon axis.

Disorganised = Variable or negligible alignment of the collagenous structure with the primary tendon axis; collagenous structure alignment is haphazard, swirling, squashed, in disarray etc.

Examples of organised versus disorganised collagenous structure can be seen in Figure 4.5.C.

0	All (100%) of the collagenous structure is **organised**	None (0%) of the collagenous structure is **disorganised**.
1	>95% of the collagenous structure is **organised**	<5% of the collagenous structure is **disorganised**
2	76-95% of the collagenous structure is **organised**	5-24% of the collagenous structure is **disorganised**
3	51-75% of the collagenous structure is **organised**	25-49% of the collagenous structure is **disorganised**
4	26-50% of the collagenous structure is **organised**	50-74% of the collagenous structure is **disorganised**
5	5-25% of the collagenous structure is **organised**	75-95% of the collagenous structure is **disorganised**
6	<5% of the collagenous structure is **organised**	>95% of the collagenous structure is **disorganised**

Outcome – Fibrillar Loosening ^

^ Refers to ***intra-fibre*** spaces, where spaces/separations are *between fibrils,* seen as visible spacing ***within*** *the observable fibres.* ***Fibrils*** typically range from 50-500 nm in diameter. ***Fibres*** are tight bundles of fibrils, typically around 10 µm in diameter.

Method:
Score the proportion of FM area associated with bundled vs loose packing (defined below) of the fibrillar structure.
Best assessed at 200x magnification.

Arrangement of collagen ***fibrils*** is categorised into two major definitions based on their packing density:

Bundled = neighbouring fibrils are all tightly adhered (highly dense), uniformly aligned, and form/integrate into distinct fibre structures.
Loose = neighbouring fibrils are dispersed / show spreading from one another, segregating out from the apparent fibre structure or preventing clear identification of distinct fibre-level structures within the tendon hierarchical organisation.

Examples of bundled versus loose fibrillar structure can be seen in Figure 4.5.D.

1	>95% of collagen fibrils are **bundled** tightly	<5% of collagen fibrils are **loose**/dispersed
2	76-95% of collagen fibrils are **bundled** tightly	5-24% of collagen fibrils are **loose**/dispersed
3	51-75% of collagen fibrils are **bundled** tightly	25-49% of collagen fibrils are **loose**/dispersed
4	26-50% of collagen fibrils are **bundled** tightly	50-74% of collagen fibrils are **loose**/dispersed
5	5-25% of collagen fibrils are **bundled** tightly	75-95% of collagen fibrils are **loose**/dispersed
6	<5% of collagen fibrils are **bundled** tightly	>95% collagen fibrils are **loose**/dispersed

Outcome – Fibre Separations^ Frequency

NOTE:
'*Fibre Separations'* can only be assessed in areas with tightly bundled fibrils covering >25% tendon area.

^ Refers to **i*nter-fibre*** separations, which are the visible boundaries/spacing *between* neighbouring fibres. They exclude IFR boundaries at fascicle edges. ***Fibrils*** typically range from 50-500 nm in diameter. ***Fibres*** are tight bundles of fibrils, typically around 10 µm in diameter.

Method:
Along a transverse line*, score the frequency of distinct separations between fibres intersecting the line.
Best assessed at 200x magnification.

** The same line is used for outcomes 'Fibre Separations Frequency' and 'Fibre Separations Type'.*
The placement of transverse line should cover areas of tightly bundled fibrils; all scorers to use the same region (length and position; selection to be pre-determined and documented).

NS	*Intra- versus Inter-fibre separations are indistinguishable. All fibrils are loose/dispersed within sample.*	
0	All (100%) of collagen fibres exhibit *no separations (neighbouring fibres are adjoined).*	No (0%) of collagen fibres exhibit visible separations.
1	>95% of collagen fibres exhibit *no separations*.	<5% of collagen fibres exhibit visible separations.
2	76-95% of collagen fibres exhibit *no separations.*	5-24% of collagen fibres exhibit visible separations.
3	51-75% of collagen fibres exhibit *no separations.*	25-49% of collagen fibres exhibit visible separations.
4	26-50% of collagen fibres exhibit *no separations.*	50-74% of collagen fibres exhibit visible separations.
5	5-25% of collagen fibres exhibit *no separations*.	75-95% of collagen fibres exhibit visible separations.
6	<5% of collagen fibres exhibit *no separations*.	>95% collagen fibres exhibit visible separations.

Outcome – Fibre Separations^ Type

NOTE:
'*Fibre Separations'* can only be assessed in areas with tightly bundled fibrils covering >25% tendon area.

^ Refers to ***inter-fibre*** separations, which are the visible boundaries/spacing *between* neighbouring fibres. They exclude IFR boundaries at fascicle edges. ***Fibrils*** typically range from 50-500 nm in diameter. ***Fibres*** are tight bundles of fibrils, typically around 10 µm in diameter.

Method:
Along a transverse line*, assess the most common (predominant) separation type between neighbouring fibres (where present).
Best assessed at 200x magnification.

** The same line is used for outcomes 'Fibre Separations Frequency' and 'Fibre Separations Type'.*
The placement of transverse line should cover areas of tightly bundled fibrils; all scorers to use the same region (length and position; selection needs to be pre-determined and documented).

Examples of fibre separation types can be seen in Figure 4.5.C.

NS	*Intra- versus Inter-fibre separations are indistinguishable. Fibrils are loose/dispersed throughout sample; no area of tightly bundled fibrils appropriate to assess.*
NS	*No fibre separations to assess (Fibre Separations Frequency score = 0)*
1	Majority (> 50%) of fibres have separations that are predominantly "short", averaging <50 µm in length.
2	Majority (> 50%) of fibres have separations that are predominantly "medium", averaging 50-100 µm in length.
3	Majority (> 50%) of fibres have separations that are predominantly "long", averaging 100-150 µm in length.
4	Majority (> 50%) of fibres have separations that are predominantly "very long", averaging >150 µm in length.

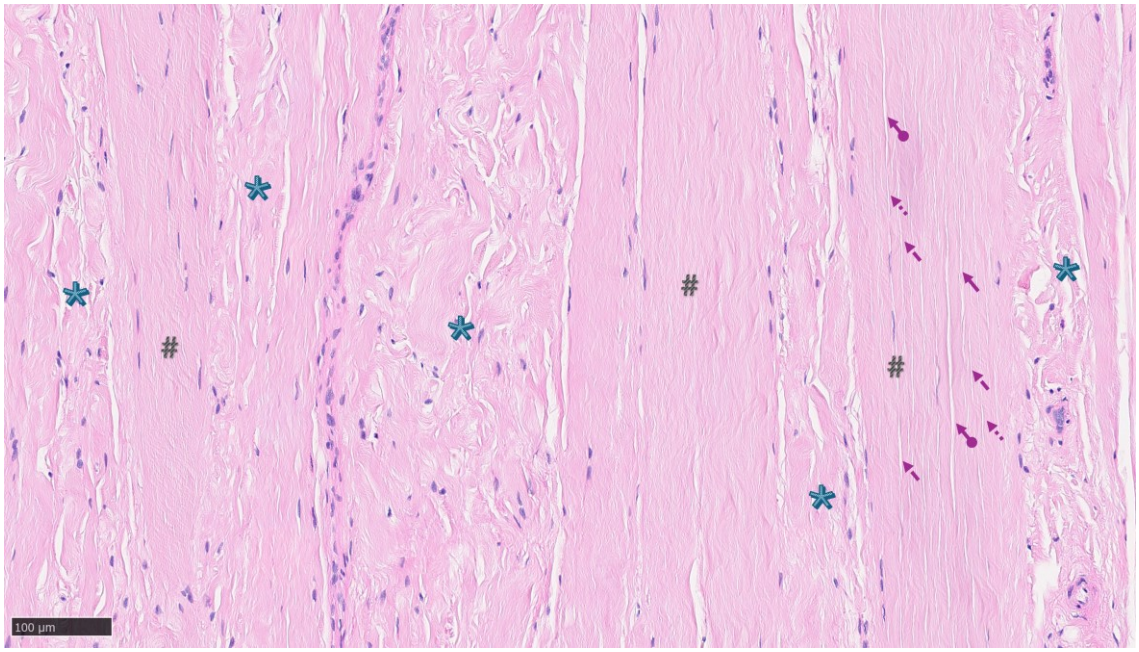


Figure 4.5.C Example of different collagenous architecture characteristics (Table 4.5.b). **Collagenous Organisation:** asterisks (*) = areas defined as *disorganised*, number-symbol (#) = areas defined as *organised*. **Fibre Separations:** arrow with circle = *"very long" (>150 µm)* separation, regular arrow = *"long" (100-150 µm)* separation, dash-arrow = *"medium" (50-100 µm)* separation, dotted-arrow = *"short" (<50 µm)* separation. *H&E staining; 200x magnification; Scale bar = 100 µm.*

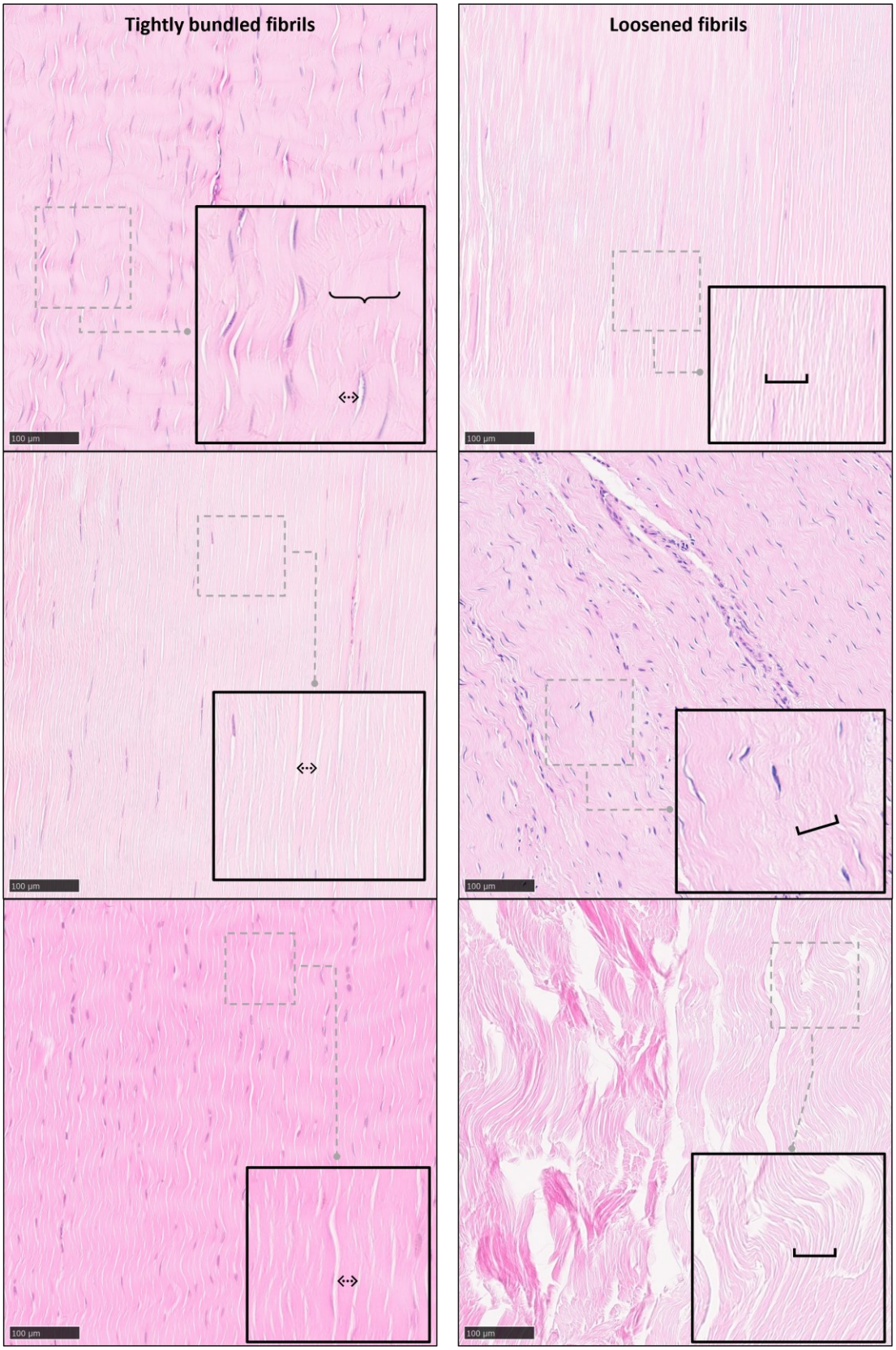


Figure 4.5.D Examples of tightly bundled (left frames) versus loose fibrillar structure (right frames) (Table 4.5.b). **Left frames:** Tightly bundled fibrils create distinct fibre structures between *inter*-fibre spaces (fibres identified by dotted lines with arrow heads) or homogenous, dense fibre groups (within braces). **Right frames:** Loosened fibrils display *intra*-fibre spacing (within square brackets), diminishing identification of fibre boundaries. *H&E staining; 200x magnification (main images); Scale bars = 100 µm.*

Table 4.5.c Scoring matrices for assessing characteristics of the tendon interfascicular region (IFR) from suitably stained sections that identify IFR features. **IFR characteristics can be differentiated from different standard histochemical stains. Selection of staining technique can be chosen as available to the laboratory. Recommendation: immunohistochemical staining identification should be enlisted where available.*

IFR Characteristics *(Suitable staining technique for identification of IFR features)**
The **interfascicular region (IFR)**[#] is the region(s) between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance, as follows: • **Interfascicular matrix (IFM)** comprises the *presence* of connective tissue between/dividing fascicle units. • **Interfascicular space (IFS)** comprises the *absence* of connective tissue between/dividing fascicle units; NOTE – includes directly adjacent fascicles with no visible white space (or matrix). Interfascicular cells or supply structures may be present. [#]*endotenon, excluding paratenon* Examples of IFR, IFM and IFS are depicted in Figure 4.5.E, Figure 4.5.F, Figure 4.5.G, and Figure 4.5.H.

Outcome – Interfascicular Matrix (IFM) Frequency **Method:** Across a transverse line, count the number of IFMs intersecting the line* and take an average per 100 µm. **Assess each respective IFR within ± 150 µm of the transverse line; count only if* ***≥ 50%*** *of the IFR within the ROI is IFM.* Examples of interfascicular regions (IFRs) classified as interfascicular matrix (IFM) can be seen in Figure 4.5.E.	
__	IFM regions / 100 µm

Outcome – Interfascicular Space (IFS) Frequency **Method:** Across a transverse line, count the number of IFSs intersecting the line* and take an average per 100 µm. **Assess each respective IFR within ± 150 µm of the transverse line; count only if* ***> 50%*** *of the IFR within the ROI is IFS.* Examples of interfascicular regions (IFRs) classified as interfascicular space (IFS) can be seen in Figure 4.5.E.	
__	IFS regions / 100µm

Outcome – Interfascicular Region (IFR) Frequency **NOTE:** IFR Frequency is determined by adding the IFS and IFM frequencies. Alternatively, it can be independently counted instead of summing the individual IFS and IFM frequencies (using the method below). **Method:** Across a transverse line, count the number of IFRs intersecting the line and take an average per 100 µm. Examples of interfascicular regions (IFR) can be seen in Figure 4.5.E.	
__	IFRs / 100µm

Outcome – Interfascicular Region (IFR) Type Proportions **Method:** Across a transverse line*, determine the proportion of IFRs represented by IFM versus IFS. (Assessed as *Percent of IFRs representing IFM, or scored according to the matrix below*). **Assess each respective IFR within ± 150 µm of the transverse line; count as IFM if* ***≥50%*** *of ROI or IFS if* ***>50%*** *of ROI.*	
__	% of IFRs are ***IFM***.

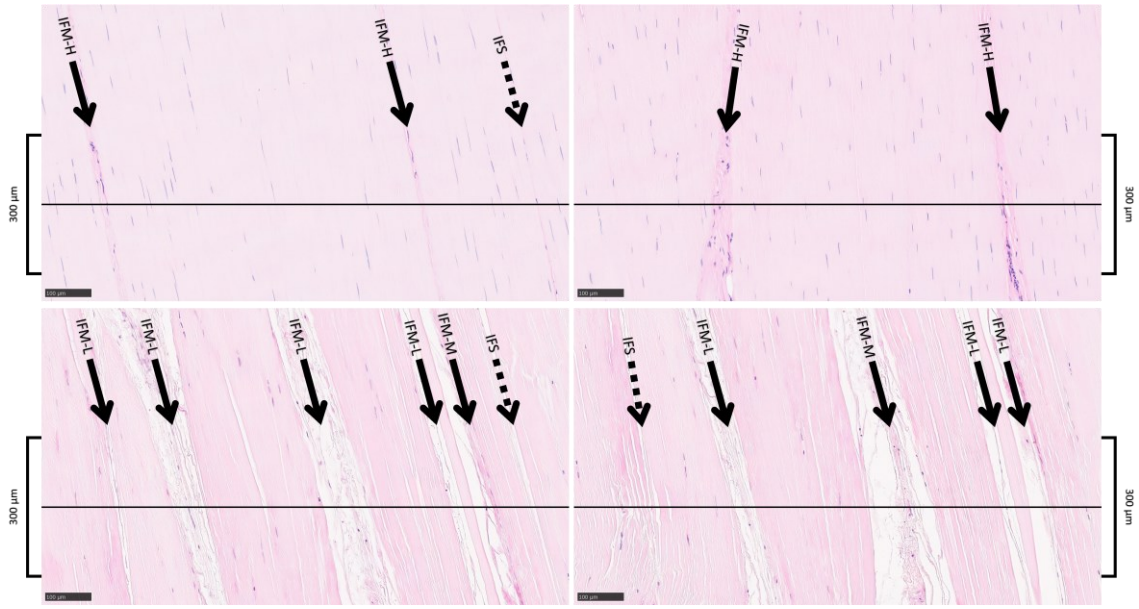


Figure 4.5.E Examples of interfascicular regions (IFRs) classified as interfascicular space (IFS, dashed arrow), or interfascicular matrix (IFM, solid arrow) with low (-L), medium (-M) or high (-H) matrix density. IFRs are classified using definitions/methods as per Table 4.5.c. Respective IFRs are assessed within a 300 µm region of interest (ROI; ± 150 µm of a transverse line) and classified as IFM if **≥50%** of ROI contains interfascicular connective tissue or IFS if **>50%** of ROI contains no discernible interfascicular connective tissue. Within images, unmarked spacings are determined to be *intra*-fascicular and thus not identified as IFRs. H*&E staining, 200x magnification; Scale bars = 100 µm.*

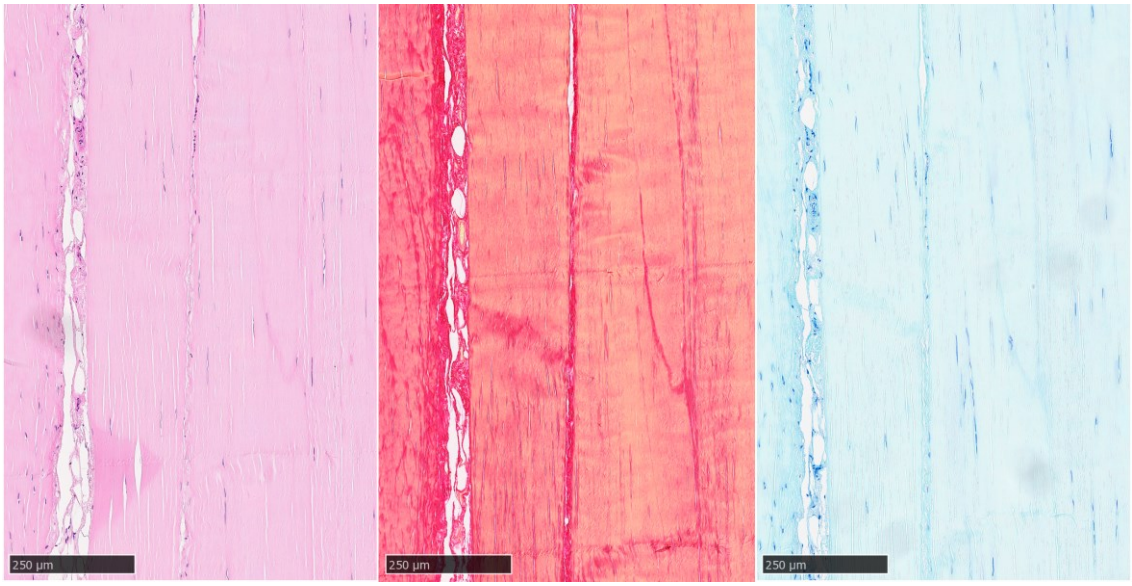


Figure 4.5.F Examples of interfascicular regions (IFRs) as observed with different three different histochemical stains of the same human tendon specimen (Table 4.5.c). **Left:** H*&E staining.* **Middle:** *PSR staining.* **Right:** *TB staining.* **All images:** 1*00x magnification; Scale bars = 250 µm.*

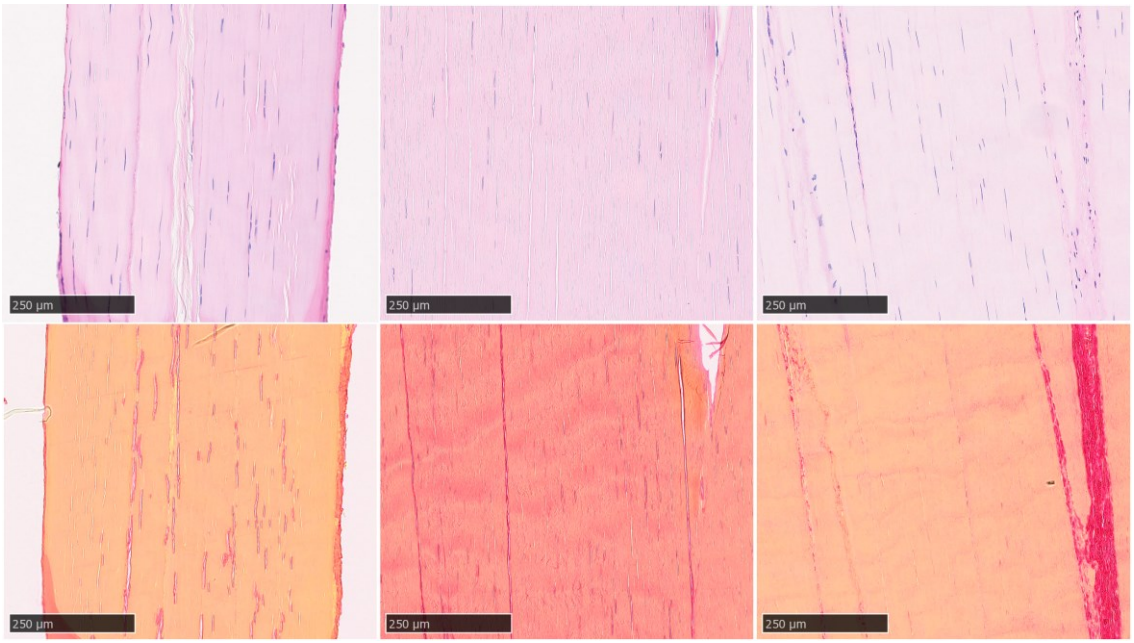


Figure 4.5.G Variable examples of interfascicular regions (IFRs) as observed with two different histochemical stains (Table 4.5.c). Three samples depicted (left, middle, right) using both H&E (top) and PSR (bottom) staining. *All images: 100x magnification; Scale bars = 250 µm.*

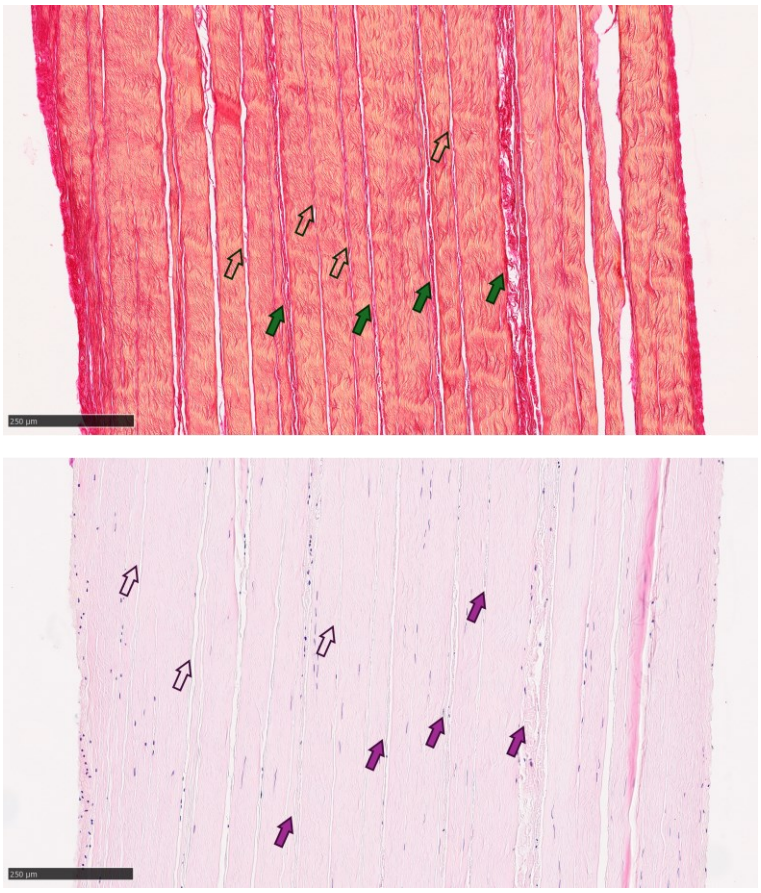


Figure 4.5.H Examples of interfascicular regions (IFRs) classified as interfascicular space (IFS, hollow-arrows) or interfascicular matrix (IFM, solid-arrows) (Table 4.5.c). The same human tendon specimen is observed using PSR (top) and H&E (bottom) staining. *Both images: 150x magnification; Scale bars = 250 µm.*

Table 4.5.d Scoring matrices for assessing general characteristics of tendon from suitably stained sections that identify adipocytes. **Characteristics can be differentiated from different standard histochemical stains. Selection of staining technique can be chosen as available to the laboratory. Recommendation: immunohistochemical staining identification should be enlisted where available.*

General Tissue Characteristics *(Suitable staining technique for identification of features)*	
Outcome – Adipose Presence **Method:** Score the proportion of tendon area containing adipocytes. Examples of adipose presence within the tendon area can be seen in Figure 4.5.I.	
0	Adipocytes absent from tendon area (0%).
1	Adipocytes are present in <5% of tendon area.
2	Adipocytes are present in 5-10% of tendon area.
3	Adipocytes are present in 10-20% of tendon area.
4	Adipocytes are present in 20-25% of tendon area.
5	Adipocytes are present in >25% of tendon area.

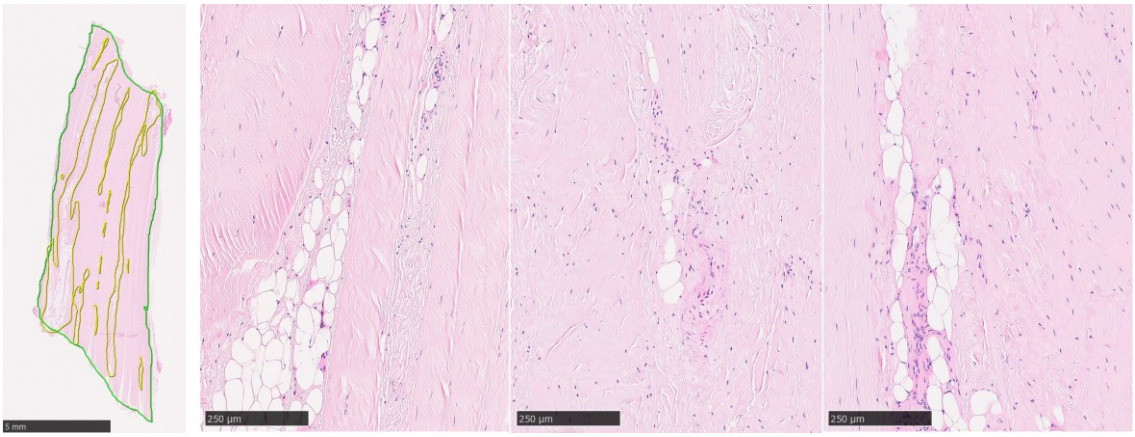


Figure 4.5.I Example of adipocyte presence within tendon (Table 4.5.d). *All scans H&E staining (different samples depicted).* **Left image** = example of Adipocyte Presence score = 5; adipocytes present in >25% (yellow regions) of tendon area (green region). *0.68x magnification; Scale bar = 5mm.* **Right images** = three examples of adipocyte regions within tendon area. *100x magnification; Scale bars = 250 µm*.

Table 4.5.e Scoring matrices for assessing proteoglycan characteristics and atypical-phenotype cell transformation of tendon from TB-stained sections.

Proteoglycan (PG) Content and Chondrocyte-Like Cells		
Toluidine Blue		
Outcome – Proteoglycan Presence **Method:** Score the proportion of tendon area[#] with proteoglycan (purple) staining present. [#]*excluding paratenon*		
0	None (0%) of the tendon area contains proteoglycan staining.	All (100%) of the tendon area is absent of proteoglycan staining.
1	<5% of the tendon area contains proteoglycan staining.	>95% of the tendon area is absent of proteoglycan staining.
2	5-25% of the tendon area contains proteoglycan staining.	75-95% of the tendon area is absent of proteoglycan staining.
3	26-50% of the tendon area contains proteoglycan staining.	50-74% of the tendon area is absent of proteoglycan staining.
4	51-75% of the tendon area contains proteoglycan staining.	25-49% of the tendon area is absent of proteoglycan staining.
5	76-95% of the tendon area contains proteoglycan staining.	5-24% of the tendon area is absent of proteoglycan staining.
6	>95% of the tendon area contains proteoglycan staining.	<5% of the tendon area is absent of proteoglycan staining.

Outcome – Proteoglycan (PG) Staining Intensity (Average) **Method:** Score the ***average (predominant)*** intensity of the proteoglycan (purple) staining within the tendon area (including IFR and FM). *Note: disregard small (approximately cell-sized) staining artefacts.* Examples of differing levels of proteoglycan (purple) staining intensities can be seen in Figure 4.5.J.	
NS	N/A – no proteoglycan staining to score. (*PG Presence score = 0*).
1	**Pale** purple staining.
2	**Moderate** purple staining.
3	**Deep** purple staining.

Outcome – Proteoglycan (PG) Staining Intensity (Maximal) **Method:** Score the ***maximal intensity*** of the proteoglycan (purple) staining within the tendon area (including IFR and FM). *Note: disregard small (approximately cell-sized) staining artefacts.* Examples of differing levels of proteoglycan (purple) staining intensities can be seen in Figure 4.5.J.	
NS	N/A – no proteoglycan staining to score. *(PG Presence score = 0).*
1	**Pale** purple staining.
2	**Moderate** purple staining.
3	**Deep** purple staining.

Outcome – Atypical-Phenotype Cell Presence

Method:
Score the presence of cells with differing/'atypical' phenotypes within the tendon area as per the definition below. (Differing cell phenotypes include tenocyte cells presenting with more 'atypical' phenotype relative to the typical tenocyte morphology, such as differentiated cells, chondrocytes/chondrocyte-like cells (commonly encountered in regions of fibrocartilaginous change), metabolically active cells, etc.).

Atypical-Phenotype Cells are defined as those with ***at least two*** of the following:

- cigar/spherical nuclei
- nuclei surrounded by a lacunae/ "halo"
- pericellular proteoglycan (purple) staining

Examples of different chondrocyte-like / 'atypical' cell phenotypes can be seen in Figure 4.5.K.

NS	N/A – no cells to score.
0	**No** atypical-phenotype cells present.
1	**Very few** atypical-phenotype cells present.
2	**Moderate number** of atypical-phenotype cells present
3	**Numerous** atypical-phenotype cells present

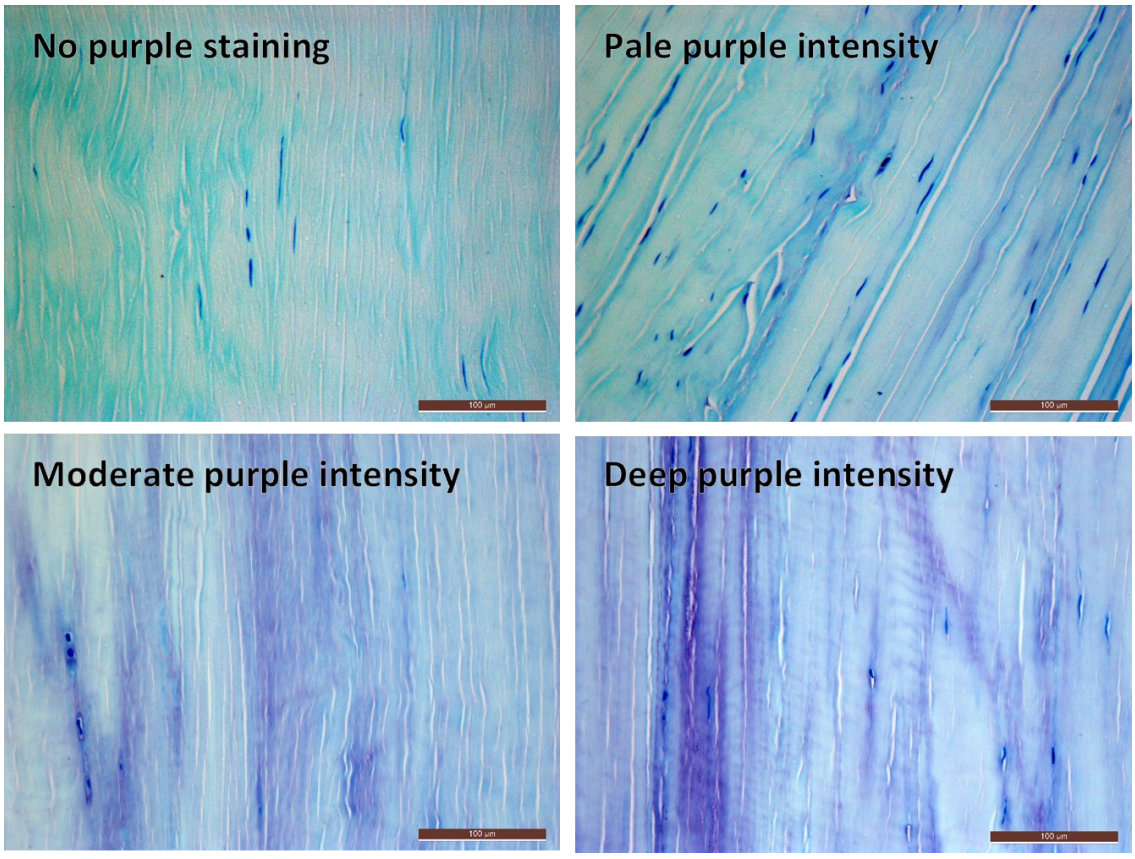


Figure 4.5.J Visual representations of differing intensity levels of the maximal proteoglycan (purple) staining (Table 4.5.e). *TB stained; 200x magnification; Scale bars = 100 µm.*

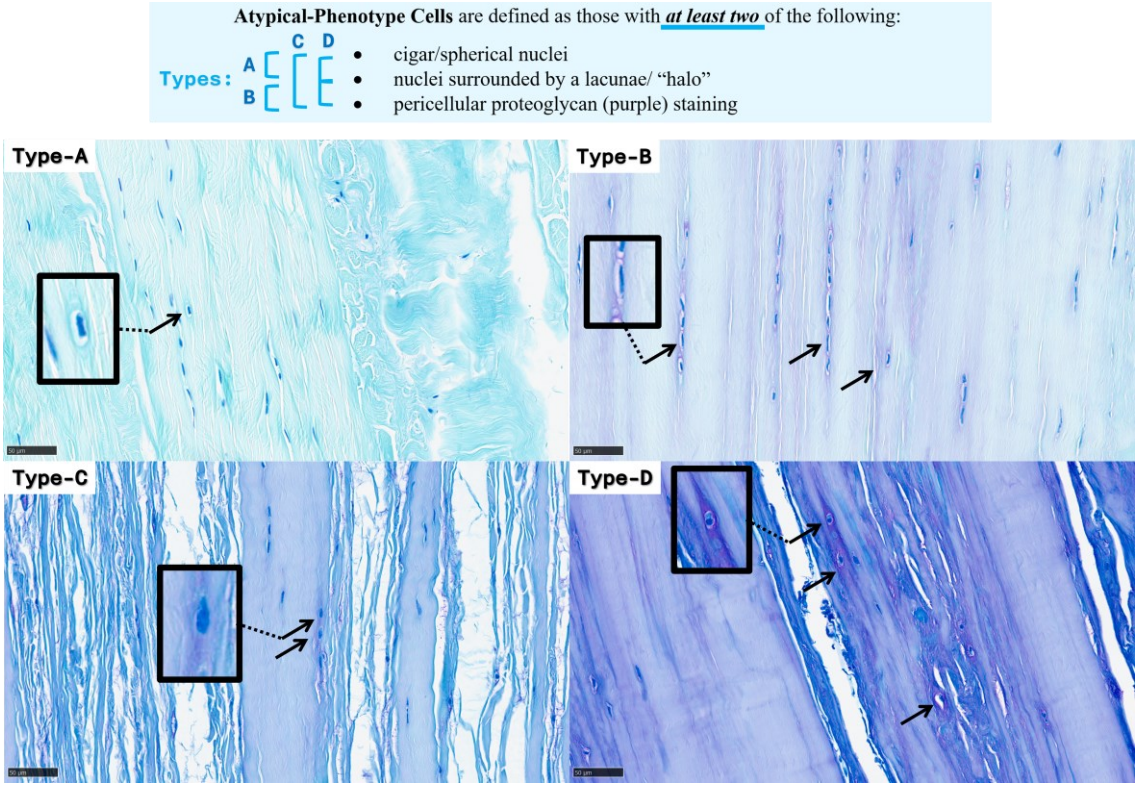


Figure 4.5.K Examples of different types of atypical-phenotype cells based on the required two-out-of-three definitions as per the scoring matrix (Table 4.5.e, ***Top panel***). Arrows indicate examples of the cell types (A-D) matching the respective image panel label. *TB staining; 400x magnification; Scale bars = 50 µm.*

Table 4.5.f Scoring matrices for assessing characteristics of tendon collagenous alignment from PSR-stained sections under non-polarised and polarised light. *Note: polarised light approach not scored for SSS-v4 in subsequent thesis chapters.*

Collagenous Alignment
Picrosirius Red; non-polarised and polarised light
Picrosirius Red histochemical staining reflects two characteristic colours of staining when viewed under ***non-polarised*** (brightfield) light: 1. **Yellow-Orange** – reflective of aligned fibrillar structure. 2. **Red** – reflective of unaligned fibrillar structure. Picrosirius Red histochemical staining reflects two characteristic colours when viewed under ***polarised*** light: 1. **Green-Yellow** – reflective of aligned fibrillar structure. 2. **Red-Orange** – reflective of unaligned fibrillar structure. ***Note: Cut ends of sections as well as certain artefacts (e.g. tissue fraying/tearing artefacts) should be excluded/ignored where possible.*** The structure of the tendon-proper is typically comprised of two primary sub-regions (excluding paratenon): 1. **The fascicular matrix (FM):** is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure. 2. **The interfascicular region (IFR):** the regions between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance.

Outcome – Collagen Alignment (Non-Polarised)

Method:
Score the proportion of tendon area[#] that is yellow-orange vs red.

[#]*excluding paratenon*

Examples of non-polarised appearance of tendon PSR staining can be seen in Figure 4.5.L.

0	None (0%) of the FM area is red	All (100%) of the FM area is yellow-orange
1	< 5% of the FM area is red	> 95% of the FM area is yellow-orange
2	5-25% of FM area is red	75-95% of the FM area is yellow-orange
3	26-50% of the FM area is red	50-74% of the FM area is yellow-orange
4	51-75% of the FM area is red	25-49% of the FM area is yellow-orange
5	76-95% of the FM area is red	5-24% of the FM area is yellow-orange
6	> 95% of the FM area is red	< 5% of the FM area is yellow-orange
7	All (100%) of the FM area is red	None (0%) of the FM area is yellow-orange

Outcome – Collagen Alignment (Polarised^)

^ For a given region of interest, rotate specimen through polariser range to observe maximal area of achievable green-yellow coverage, *minimising black/'shadowed' areas.*

Method:
Score the proportion of tendon[#] area that is green-yellow vs red-orange.

[#]*excluding paratenon*

Examples of polarised appearance of tendon PSR staining can be seen in Figure 4.5.L.

0	None (0%) of the FM area is red-orange	All (100%) of the FM area is green-yellow
1	< 5% of the FM area is red-orange	> 95% of the FM area is green-yellow
2	5-25% of the FM area is red-orange	75-95% of the FM area is green-yellow
3	26-50% of the FM area is red-orange	50-74% of the FM area is green-yellow
4	51-75% of the FM area is red-orange	25-49% of the FM area is green-yellow
5	76-95% of the FM area is red-orange	5-24% of the FM area is green-yellow
6	> 95% of the FM area is red-orange	< 5% of the FM area is green-yellow
7	All (100%) of the FM area is red-orange	None (0%) of the FM area is green-yellow

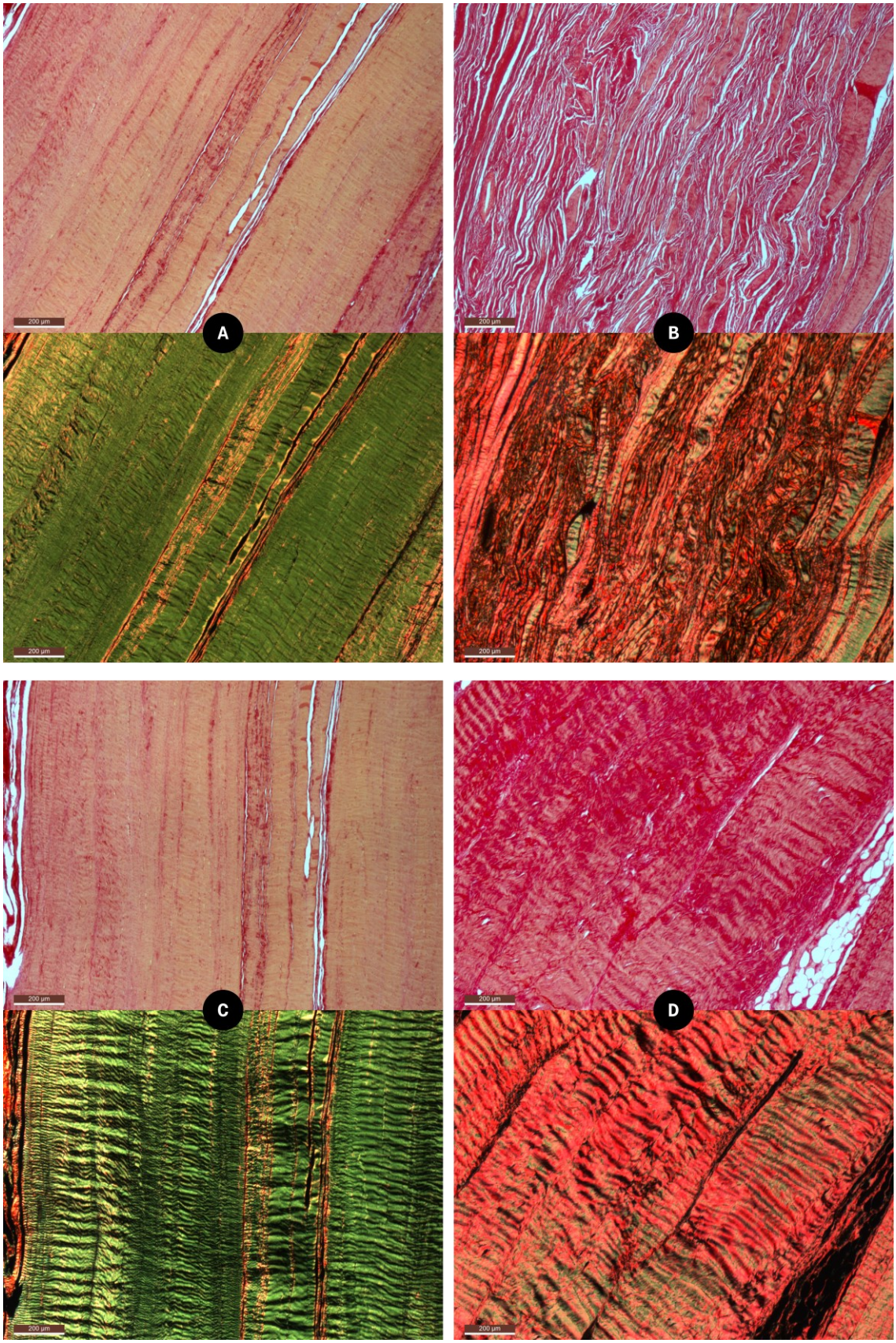


Figure 4.5.L Examples of collagen alignment characteristics as observed with brightfield/non-polarised ("NP", top frames) and polarised light ("P", bottom frames) microscopy with varying degrees of *aligned* (NP = yellow/orange, P = green-yellow) and *unaligned* (NP = red, P = red-orange) collagen (Table 4.5.f). *PSR staining, 50x magnification; Scale bars = 200 µm*.

Table 4.5.g Scoring matrices for assessing tendon crimp characteristics from PSR- stained sections under polarised light.

Crimp Structure
Picrosirius Red; polarised light
Scored using polarised light^ *^For a given region of interest, rotate specimen through polariser range to observe greatest definition of crimp waveform.* **The fascicular matrix (FM)**[#] is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure. [#]*excluding paratenon*

Outcome – Crimp Absence **Method:** Score the proportion of FM area associated with the absence versus presence of crimp (waveform pattern). Various examples of crimp appearances can be seen in Figure 4.5.M		
1	Crimp present in >90% of FM area	Crimp absent from <10% of FM area.
2	61-90% of FM area contains apparent crimp	Crimp absent from 10-39% of FM area.
3	41-60% of FM area contains apparent crimp	Crimp absent from 40-59% of FM area.
4	10-40% of FM area contains apparent crimp	Crimp absent from 60-90% of FM area.
5	Crimp present in <10% of FM area	Crimp absent from >90% of FM area.
6	No apparent crimp within FM area (0%).	Crimp absent from all (100%) of FM area.

Outcome – Crimp Wavelength (Predominant) **Method:** Measure and score the ***predominant*** crimp wavelength **(**occupying the greatest tendon area). Various examples of crimp appearances can be seen in Figure 4.5.M.	
NS	N/A – no apparent crimp within tendon area *(Crimp Presence score 0).*
1	Crimp wavelength ≤ 25 µm
2	Crimp wavelength 26-50 µm
3	Crimp wavelength 51-75 µm
4	Crimp wavelength 76-100 µm
5	Crimp wavelength 101-125 µm
6	Crimp wavelength 126-150 µm
7	Crimp wavelength 151-200 µm
8	Crimp wavelength 201-250 µm
9	Crimp wavelength 251-300 µm
10	Crimp wavelength 301-350 µm
11	Crimp wavelength 351-400 µm
12	Crimp wavelength 401-500 µm
13	Crimp wavelength 501-600 µm
14	Crimp wavelength 601-700 µm
15	Crimp wavelength >700 µm

Outcome – Crimp Wavelength Variation **Method:** Score the proportion of crimped area associated with the ***predominant*** crimp wavelength (scored above; *Outcome - Crimp Wavelength (Predominant)).* Various examples of crimp appearances can be seen in Figure 4.5.M.		
NS	N/A – no apparent crimp within tendon area *(Crimp Presence score 0).*	
0	*Predominant* crimp wavelength constitutes all (100%) of visible crimp area.	None (0%) of the crimp area contains other crimp wavelengths.
1	*Predominant* crimp wavelength constitutes >90% of visible crimp area.	<10% of the crimp area contains other crimp wavelengths.
2	*Predominant* crimp wavelength constitutes 61-90% of visible crimp area.	10-39% of the crimp area contains other crimp wavelengths.
3	*Predominant* crimp wavelength constitutes 41-60% of visible crimp area.	40-59% of the crimp area contains other crimp wavelengths.
4	*Predominant* crimp wavelength constitutes <40% of visible crimp area.	>60% of the crimp area contains other crimp wavelengths.

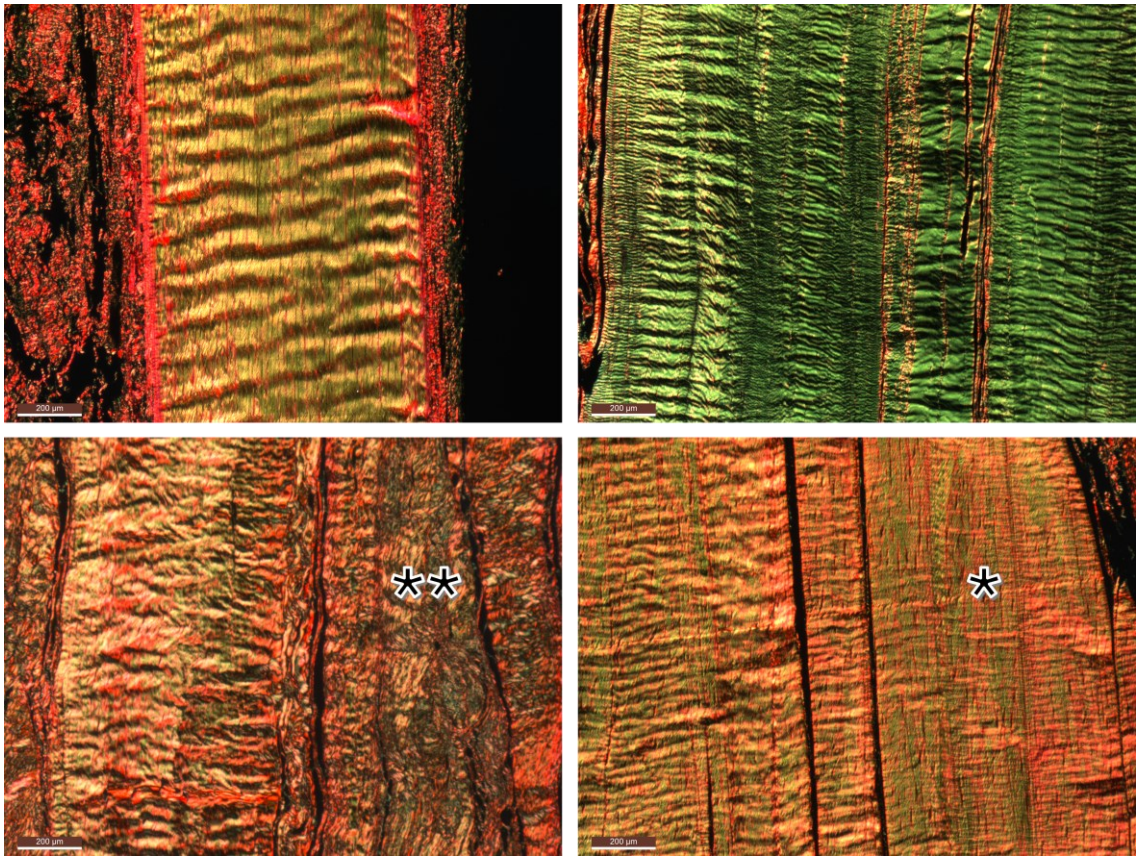


Figure 4.5.M Examples of crimp characteristics as observed with polarised light (Table 4.5.g). **Top Left** = uniform (non-variable) crimp present in all tendon area. **Top Right** = *variable* crimp wavelengths present in tendon area. **Bottom Left** = area *absent of crimp* observed due to haphazard structure (double asterisks **). **Bottom Right** = area *absent of crimp* observed due to loss of crimping structure (asterisk *). *PSR staining, polarised light; 50x magnification; Scale bars = 200 µm.*

4.5.2 Discussion of the SSS

4.5.2.1 *An Adaptive System*

It was a significant challenge to design a scoring system capable of comprehensively characterising tendon histological structure. During the project of developing a novel scoring system an article[149] was released following a meeting at the ORS Annual Meeting Conference in 2022. At this meeting, the concept of a common scoring system application for the assessment of different tendons and tendons states (e.g. development, homeostasis, healing, species, etc.) was discussed. Evidently, the discussion (which comprised around 100 members of the ORS 'Tendon Section') came to the conclusions that such a system was "not feasible"[149]. Despite this position, our laboratory continued to explore many iterations of scoring systems with the intent of identifying the plausible and the invalid outcomes which could constitute such a scoring system. The primary focus was achieving a system which could distinguish normal differences and support definitions of characteristics typical to different tendons or tendon-states.

It is hoped the proposed SSS can serve as a preliminary toolbox for further development and improvement of scoring matrices capable of differentiating tendons based on their disparate characteristic architecture through histological assessment (HALT). The SSS is not intended to be presented as restrictive methodology. It aims to provide a foundation which can be used selectively for the needs and requirements of specific research questions and experimental protocols. To this end, a number of scores were developed with reference to the % area covered to facilitate the application of these scores to different sized specimens and ROIs. While other scores were used herein on the predominant feature (e.g. crimp wavelength), the outcomes and it's descriptions have been written such that they can be applied repeatedly to distinct regions (e.g. FM vs IFM) and alternate definitions (e.g. the predominant or non-predominant characteristic, or in relation to maximum or minimum). Certain outcomes may not be relevant for all studies, while others may require finer discrimination or further characterisation to adequately capture study-specific features. Nevertheless, the spectrum of matrices proposed serve as a comprehensive foundation for future studies and considerations to be modelled from.

4.5.2.2 *Reserved For Future Development*

While some scores were excluded from v3.2 in the presented SSS (v4), it is acknowledged that many of these outcomes still offer promise with further refinement. One example is outcomes pertaining to the vascularity of the sample, an important feature when evaluating diseased/injured/pathological tendons (Table 4.3.a; [168-170,172]) but whether underlying variation in different normal tendons/tendon regions exists and may modify disease risk is unclear. Another example is the case of associating different outcomes (e.g. cell count and morphology, proteoglycan staining) with FM versus IFR regions of the tendon hierarchy. Outcomes which were specific to either region in v3.2 were collapsed for v4, targeting the outcome characteristics to the tendon (collagenous) area as a whole. However, defining within their structure how tendons or tendon regions differ may provide novel insights into tendon structure/function relationships, disease risk and/or graft suitability[80,227,228,230,233-235].

4.5.2.3 *Final Remarks*

It has been discussed that the new scoring system exhibited some limitations and room for improvement. One comment from both raters was the time-demanding nature required to capture all outcomes, particularly when scoring large samples (tissue/section size) such as the ones enlisted for this thesis, or when assessing a large number of samples. However, the time required for scoring improved markedly with familiarisation for both raters. It was agreed that the system was definitely extensive and while complex, was appropriate for gathering a level of detail that can (if proven important) identify structural variation between different 'normal' tendons not previously possible with prior scoring systems aimed at histo*patho*logy.

In the end, understanding and ease-of-use of the system was found to be largely similar between experienced and inexperienced assessors of tendon histological characterisation. Challenging outcomes were nevertheless noted where more novice raters in particular, but not exclusively, benefited from the inclusion of example images to train and familiarise scorers to the features being assessed.

Previously it has been indicated that increasing score ranges can reduce inter-rater reliability, where end range scores (i.e. "none" and "all" outcome measures) show excellent to near-perfect reliability, whilst mid-range scores only fair to moderate kappa values resultant from difficulty in delineating between finer characteristic changes[214]. Results of the current study found similar findings, however, it was deemed necessary to include more resolution in the scoring matrices to enable differentiation between normal tendons which exhibit more discrete/subtle characteristic differences. The following chapters on the characterisation of 48 different human tendons (and ligaments) of the upper and lower limb will provide more information on the ability of the current system to differentiate between normal tendons which have qualitatively been observed to exhibit variable structural features.

4.6 Conclusions

A review of the literature found that commonly used systems such as the Movin and Bonar methodologies focus on classification of tendon disease state. The relative nature of current scoring systems which rely on comparing to a reference/control tissue sample, means the characterisation of tissue achieved in one study cannot be compared to the results of another study. In addition to adopting consistent methodologies (histological preparation, staining, magnification, outcomes assessed etc.), it was identified that scoring matrices should also aim to achieve objectivity and greater resolution to limit bias and facilitate greater differentiation.

Extensive development was conducted to propose a suite of scoring matrices that facilities the characterisation of normal, healthy tendon. Validation of the system (v3.2) found suitable inter-rater reliability to warrant use of the scoring system for subsequent chapters in this thesis (histological characterisation of the 48 different human upper and lower limb tendons/ligaments). Intra-rater comparisons also gave insight into the reliability of different assessment approaches and identified new methodology for assessing outcomes (i.e. non-polarised characterisation of collagen alignment from PSR-stained sections) not previously known. Although further

improvements are required, the new proposed SSS offers a novel useful toolbox for the characterisation of tendon and ligament structure using commonly and readily available histological stains – for both normal and diseased tendons/ligaments.

~ CHAPTER 5 ~

GAINING THE UPPER HAND IN TENDON RESEARCH

HISTOLOGICAL CHARACTERISATION OF HUMAN TENDONS OF THE UPPER LIMB

"It's not exactly a normal world, is it?"

~ Batman, Batman (1989) ~

5.1 Overview

The following chapter explores the histological characterisation of thirty-one different tendons of the human upper limb, including those of the shoulder, elbow, wrist and hand regions. Specimens were assessed using the novel histological scoring system as per *Chapter 4*.

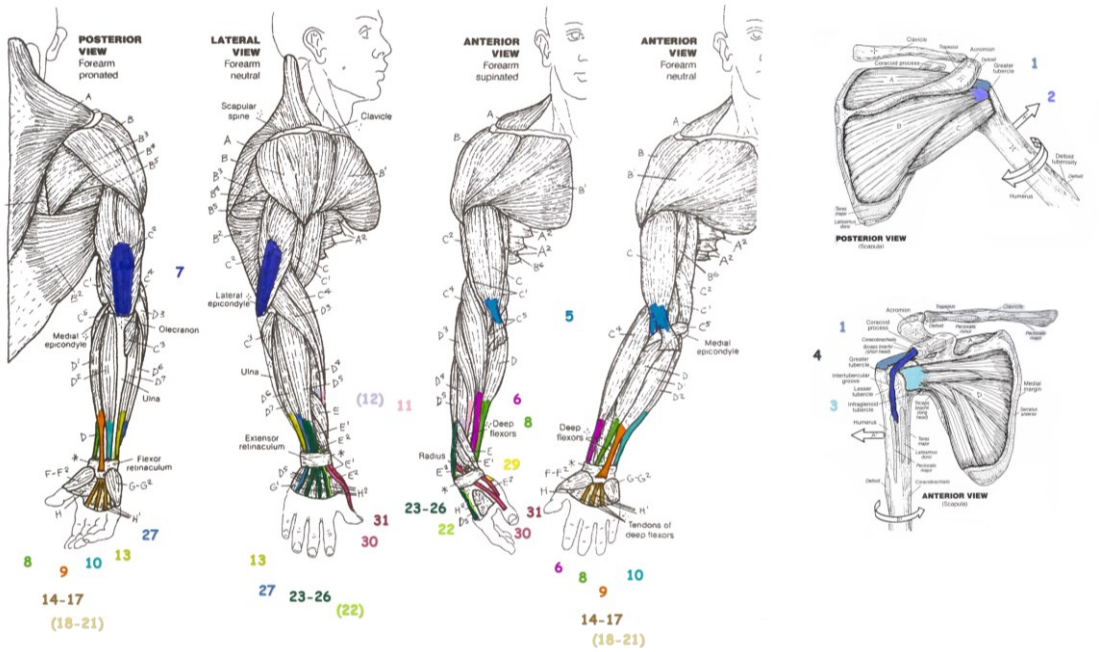


Figure 5.1.A Tendons of the upper limb. Image adapted from Kapit and Elson (2014)[236]. *Highlighted tissue reflect: 1 = Supraspinatus, 2 = Infraspinatus, 3 = Subscapularis, 4 = Long Head of Biceps Tendon, 5 = Distal Biceps Tendon, 6 = Brachioradialis, 7 = Distal Triceps Tendon, 8 = Flexor Carpi Radialis, 9 = Palmaris Longus, 10 = Flexor Carpi Ulnaris, 11 = Extensor Carpi Radialis Longus, 12 = Extensor Carpi Radialis Brevis, 13 = Extensor Carpi Ulnaris, 14-17 = Flexor Digitorum Superficialis 2nd-5th Digits, 18-21 = Flexor Digitorum Profundus 2nd-5th Digits, 22 = Extensor Indicis Proprius, 23-26 = Extensor Digitorum Communis 2nd-5th Digits, 27 = Extensor Digiti Minimi, 28 = Flexor Pollicis Longus, 29 = Abductor Pollicis Longus, 30 = Extensor Pollicis Brevis, 31 = Extensor Pollicis Longus.*

5.2 Introduction

It is pivotal to the understanding of injury, disease, pathology and healing mechanisms to first define and understand normal characteristics and variations within or between tendons. A systematic search conducted in *Chapter 2* found that current research on the structural characterisation of tendons is predominated by studies on injured or pathological/diseased tendons, with little information available on the normal structure of tendons. Even more confounding to the lack of current information on tendon structural variability is that only two studies were identified that evaluated human tendons of the upper limb regions[237,238].

5.2.1 Upper Limb Tendons

Tendons of the upper limb are no exception to the prevalence of soft-tissue injuries. In an epidemiological study of musculoskeletal soft-tissue injuries presenting to the Royal Infirmary of Edinburgh (Scotland) across a 5-year period, forearm/hand extensor tendon injuries were the second most prevalent injury of the 2794 included cases (totalling 16.8%), and the most common tendon/ligament injury[25]. Similarly, Clark *et al.* (2020)[23] found that shoulder and finger/thumb/wrist tendon injuries were the first and third most common tendon and ligament injuries respectively, in a retrospective population-based study in Aotearoa/New Zealand across a 6-year period (presenting 1,112,077 cases). A study of 464 female participants from a 20-year-old longitudinal population study on shoulder musculoskeletal findings (both shoulders included: n = 928 shoulders) revealed that 22.2% of the cohort had at least one full-thickness tear of the rotator cuff tendons, which jumps to 59.5% when including other pathologies or partial tears[239]. What these and many other studies do not address is if, beyond obvious risks such as "injury-exposure" (e.g. hand/finger lacerations, shoulder/elbow sporting strain), there are inherent biological or structural reasons certain tendons have higher prevalence of injury/disease and/or poorer outcomes following conservative or intervention approaches[25,29,239,240].

Building knowledge on the structural characteristics of tendons can aid in understanding links between structure versus functional attributes as well as mechanisms behind tissue health and pathology. This study aims to assess variations in structural characteristics of a range of different human upper limb tendons utilising histological outcomes to explore different structural features.

5.3 Methodology

Detailed methodology pertaining to specimen collection, tissue processing, and the histological scoring assessment are found in *Chapter 3* and *Chapter 4* respectively. A brief overview of these methods plus additional protocols specific to the conduct of this upper limb tendon investigation are described below.

5.3.1 Specimens

5.3.1.1 *Tendon Inclusion*

Details of the tissue procurement and associated ethics approval for the current investigation are provided in *Chapter 3*. Briefly, thirty-one (31) tendons were retrieved from fresh-frozen cadaveric upper limbs (n = 8, clavicle to fingertips) as outlined in Table 5.3.a. Donor ages range from 49 to 65 years old, with a mean age (± standard deviation) of 56.1 ± 5.8 (males = 56.2 ± 6.1; females = 56.0 ± 6.6).

Table 5.3.a List of human upper limb tissue (tendon) names and abbreviations analysed in this study. *Graph Key* indicates colour coding for results graphs presented later in this chapter.

Region	(Sub-classification)	Tissue (Tendon)	Abbreviation	Graph Key
Shoulder	*Rotator Cuff*	Supraspinatus	**SSP**	
		Infraspinatus	**IS**	
		Subscapularis	**SSC**	
	(Flexor)	Long Head of Biceps Tendon	**LHBT**	
Elbow	*Flexors*	Distal Biceps Tendon	**DBT**	
		Brachioradialis	**BrR**	
	Extensors	Distal Triceps Tendon	**DTT**	
Wrist	*Flexors*	Flexor Carpi Radialis	**FCR**	
		Palmaris Longus	**PL**	
		Flexor Carpi Ulnaris	**FCU**	
	Extensors	Extensor Carpi Radialis Longus	**ECRL**	
		Extensor Carpi Radialis Brevis	**ECRB**	
		Extensor Carpi Ulnaris	**ECU**	
Hand (Digits 2-5)	*Flexors (superficial)*	Flexor Digitorum Superficialis - 2nd Digit	**FDS-2**	
		Flexor Digitorum Superficialis - 3rd Digit	**FDS-3**	
		Flexor Digitorum Superficialis - 4th Digit	**FDS-4**	
		Flexor Digitorum Superficialis - 5th Digit	**FDS-5**	
	Flexors (deep)	Flexor Digitorum Profundus - 2nd Digit	**FDP-2**	
		Flexor Digitorum Profundus - 3rd Digit	**FDP-3**	
		Flexor Digitorum Profundus - 4th Digit	**FDP-4**	
		Flexor Digitorum Profundus - 5th Digit	**FDP-5**	
	Extensors	Extensor Indicis Proprius	**EIP**	
		Extensor Digitorum Communis - 2nd Digit	**EDC-2**	
		Extensor Digitorum Communis - 3rd Digit	**EDC-3**	
		Extensor Digitorum Communis - 4th Digit	**EDC-4**	
		Extensor Digitorum Communis - 5th Digit	**EDC-5**	
		Extensor Digiti Minimi	**EDM**	
Thumb (Digit 1)	*Flexor*	Flexor Pollicis Longus	**FPL**	
	Abductor	Abductor Pollicis Longus	**APL**	
	Extensors	Extensor Pollicis Brevis	**EPB**	
		Extensor Pollicis Longus	**EPL**	

5.3.1.2 *Specimen Preparation*

As detailed previously (*Chapter 3*) formalin-fixed, paraffin embedded histological samples (longitudinal orientation, 4.5 µm sections) of human tissue of the upper limb were used for assessment. Three sections of each tendon were utilised, one each for H&E-, TB- and PSR-stained sections, facilitating exploration of different structural tissue characteristics.

5.3.2 Histological Assessment

A novel scoring system for the histological characterisation of tendons was created as detailed in *Chapter 4* and used for the current study exploring the structural properties of different normal (healthy) tendons of the upper limb. The investigator was blinded to *Tendon* and *Donor ID* (refer to *Section 3.4.1*).

The outcomes assessed are outlined in Table 5.3.b. Scanned images of histological slides of each stain were used to score all outcomes using 'brightfield' light modality approaches (NDP.view2Plus). Outcomes utilising 'polarised' light on PSR-stained sections were assessed directly from a microscope (Leica-DFC295).

Table 5.3.b Scoring outcomes enlisted for the histological assessment of human upper limb tendons. IFM = Interfascicular Matrix. IFS = Interfascicular Space. IFR = Interfascicular Region. ROI = Region of Interest. H&E = Haematoxylin and Eosin. TB = Toluidine Blue. PSR = Picrosirius Red. *Refer to main text for clarification.

Outcome	Stain	Modality	Area Assessed	ROI (if applicable)	Protocol
Cell Density	H&E	Brightfield	3 x ROIs	200 µm x 200 µm; 0.04 mm^2	Average of ROIs
Cell Distribution			3 x ROIs	200 µm x 200 µm; 0.04 mm^2	Max. – Min. of ROIs
Cell Morphology			3 x ROIs	200 µm x 200 µm; 0.04 mm^2	Mode of ROIs
Cell Arrangement			Whole section		
Collagenous Organisation			Whole section		
Fibrillar Loosening			1 x ROI	500 µm x 500 µm; 0.25 mm^2	
Fibre Separations Frequency			1 x ROI	500 µm	
Fibre Separations Type			1 x ROI	500 µm	
IFM Frequency	PSR		1 x ROI	Width of tendon sample*	
IFS Frequency			1 x ROI	Width of tendon sample*	
IFR Frequency			1 x ROI	Width of tendon sample*	Sum of *IFM* and *IFS Frequencies*
IFR Type Proportion			1 x ROI	Width of tendon sample*	Percentage of *IFM/IFR*
Adipose Presence	H&E		Whole section		
Proteoglycan Presence	TB		Whole section		
Proteoglycan Staining Intensity (Average)			Whole section		
Proteoglycan Staining Intensity (Maximum)			Whole section		
Atypical-Phenotype Cell Presence			Whole section		
Collagen Alignment	PSR		Whole section		
Crimp Absence	PSR	Polarised	Whole section		
Crimp Wavelength			Whole section		
Crimp Wavelength Variation			Whole section		

Outcomes which assessed the 'whole section' evaluated all tendon area, excluding that determined to be paratendinous tissue (e.g. paratenon/epitenon). Outcomes which utilised specified ROIs (areas, lines etc. – refer to Table 5.3.b) were done in a manner trying to best achieve consistency across samples of different lengths, widths and section areas as detailed below.

ROIs for Cell-related characteristics were 0.04 mm^2 square areas. Three ROIs were placed within the tendon area in a diagonal fashion with respect to the long-axis of the tendon: one close to the *centre* + *midline* of the section, one at the *end* + *side* of the long-axis, and the last diagonally

opposite at the *opposing-end* + *opposing-side*. Examples of cell ROI placement are seen in Figure 5.3.A. Cell Density was determined by counting the number of cells in the ROI then normalising by the ROI area to obtain cells/mm^2 then calculating the average Cell Density across the three ROIs. Cell Distribution was calculated as the difference (maximum minus the minimum) in density values between the three ROIs. Cell Morphology was determined as the mode of the scores (as per the scoring matrix) across the three ROIs. Where no mode was achieved (i.e. three different scores given for each ROI), a score of 1 (that is, "mixed morphology") was given for the overall outcome.

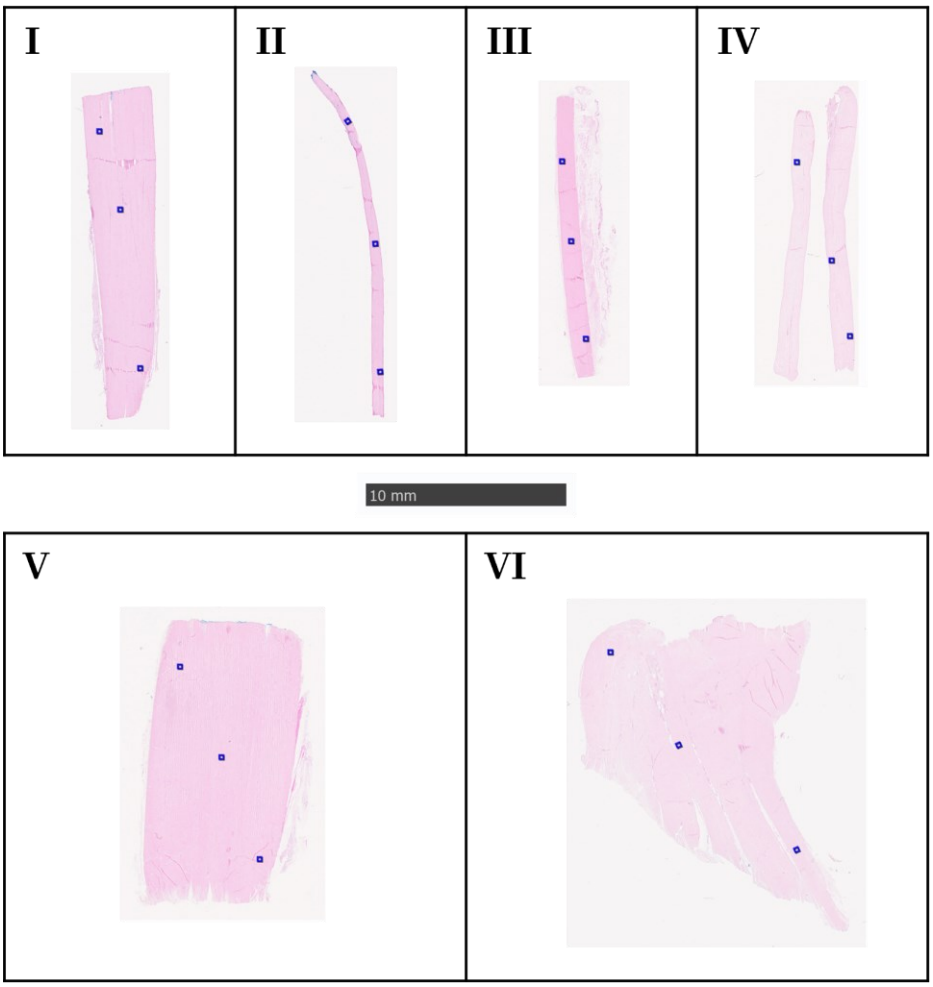


Figure 5.3.A Cell characteristic ROI selection examples for tendon sections of variable sizes. ROI-2 is placed around the centre-midline of the section. ROI-1 and ROI-3 are placed near top and bottom of section on opposing sides (where applicable). Example **IV** depicts tendon with two segregated sub-tendon pieces; ROIs are placed as best as possible considering the tendon area as one. *H&E stained, Scale bar 10mm, magnification 0.32x.*

Fibre Separations Frequency and Type scores were determined from the separations intersecting a 500 µm line (Table 5.3.b) as per the scoring matrix (*see Section 4.5.1*). The ROI line was placed in an area of the tendon which would achieve a low Fibrillar Loosening score to maximise the clarity of fibres for the identification of separations between the fibres (i.e. limiting discrepancies with intra-fibrillar spaces).

IFM and IFS Frequency scores were determined by counting the respective features intersecting a ROI line* (Table 5.3.b) that aimed to span the width of the tendon but avoid areas of obvious collagenous disorganisation and normalising to the length of the line to give number/mm. If a ROI line of at least 30% of the tendon width and a minimum of 300 μm long was not achievable across an area with clear fascicular structures, the tendon sample was excluded from the interfascicular characteristic scores. IFR frequency was calculated as the sum of the IFM and IFS counts and normalised to give number/mm. IFR Type Proportion was calculated as the percentage of IFM count over total IFR count.

5.3.3 Statistical Analysis

5.3.3.1 *Categorical Data*

Data from nominal and ordinal outcomes were analysed in GraphPad Prism (version 10.3.0 [507], GraphPad Software, LLC., California, USA). Differences between tendons were analysed using non-parametric Kruskal-Wallis tests followed by Dunn's test with a Bonferroni correction for multiple pairwise comparisons. Statistical significance was taken as an adjusted $p < 0.05$. Graphs were generated in GraphPad Prism. Due to limited donor numbers, the effects of donor variables (e.g. sex and age) was not analysable for categorical outcomes.

5.3.3.2 *Continuous Data*

Data from continuous (counted) outcomes were analysed in Stata (SE 15.1, StataCorp LLC, Texas, USA). Mixed-effects linear regression models were used to compare tendon-types, and mean-centred donor characteristics were included as covariates. These included sex (females versus males), age (unit = year), height (unit = inch), and weight (unit = pound), using the original units provided by the tissue bank. A random intercept term was included to account for the non-independence of tendons retrieved from the same donor. Specific pairwise tendon comparisons were evaluated using Sidak's adjustment for multiple comparisons. Statistical significance was taken as an adjusted $p < 0.05$. Graphs were generated in GraphPad Prism.

5.4 Results

5.4.1 Specimens and Data

Thirty-one (31) different tendons of the upper limb from 8 donors (5 male, 3 female) were included in this study. A total of n = 246 samples were included, where the Extensor Digitorum Communis tendon of the 5th digit was absent from two of the donors (both male). All other tendons were included from each donor (n = 8). Unless otherwise specified, outcomes included all n = 246 datapoints. A breakdown of n-numbers per tendon per outcome is provided in Table 5.4.a. Within graphs, tendons were grouped and colour coded as per the regions specified in Table 5.3.a. Flexor tendons are indicated by solid bars, whilst extensor tendons (including the similarly posteriorly located APL) are indicated by diagonal striped bars. Rotator cuff tendons are indicated by horizontal stripped bars (Table 5.3.a). Tables of pairwise tendon comparisons in the main text show significant differences with adjusted P-values for each. The complete list of pairwise tendon comparisons including non-significant comparisons can be found in *Appendix Section 9.6*.

Table 5.4.a Number of data points (n=) for each upper limb tendon by outcome. Refer to Table 5.3.a for tendon acronym identification. In general, all tendons n = 8 except $n_{EDC\text{-}5}$ = 6. Bold numbers in pink-highlighted cells indicate reduced datapoints included in analysis because of reasons such as exclusion due to artefacts or "NS" (no score) as per scoring matrix guidelines, for example.

	SSP	IS	SSC	LHBT	DBT	BrR	DTT	FCR	PL	FCU	ECRL	ECRB	ECU	FDS-2	FDS-3	FDS-4	FDS-5	FDP-2	FDP-3	FDP-4	FDP-5	EIP	EDC-2	EDC-3	EDC-4	EDC-5	EDM	FPL	APL	EPB	EPL
Cell Density	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Cell Distribution	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Cell Morphology	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Cell Arrangement	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Collagenous Organisation	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Fibrillar Loosening	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Fibre Separations Frequency	**4**	8	8	8	8	8	8	**7**	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Fibre Separations Type	**2**	**4**	**7**	**5**	8	**5**	**6**	**4**	8	**5**	**4**	**3**	**5**	**6**	8	**7**	**7**	8	**7**	**7**	8	**4**	**7**	**5**	**7**	6	**7**	**7**	**4**	**2**	**2**
Adipose Presence	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Proteoglycan Presence	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Proteoglycan Staining Intensity (Average)	8	8	**7**	**7**	**3**	**2**	**5**	**1**	**0**	**1**	**0**	**4**	**4**	**2**	**3**	**3**	**1**	**4**	**3**	**4**	**3**	**1**	**3**	**2**	**1**	**2**	**3**	**5**	**0**	**5**	**0**
Proteoglycan Staining Intensity (Maximum)	8	8	**7**	**7**	**3**	**2**	**5**	**1**	**0**	**1**	**0**	**4**	**4**	**2**	**3**	**3**	**1**	**4**	**3**	**4**	**3**	**1**	**3**	**2**	**1**	**2**	**3**	**5**	**0**	**5**	**0**
Atypical-Phenotype Cell Presence	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Collagen Alignment	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Crimp Absence	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Crimp Wavelength	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
Crimp Wavelength Variation	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
IFR Frequency	**4**	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	6	8	8	8	8	8
IFR Type Proportion	**4**	8	8	8	8	**5**	8	8	**2**	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	**6**	**5**	8	8	8	8	8

5.4.2 Cell Characteristics

5.4.2.1 *Cell Density and Cell Distribution*

The Cell Density and Cell Distribution outcomes evaluated the average density and difference in densities of the cell population within the tendon area, respectively, from H&E-stained sections (Figure 5.4.A). Cell Density was taken as the average of three separate ROIs within the tendon/ligament area; a higher value indicates a greater number of cells per mm^2. Cell Distribution was the difference between the maximum and minimum densities in the 3 ROIs; a higher value indicates a greater difference in density values across the ROIs assessed.

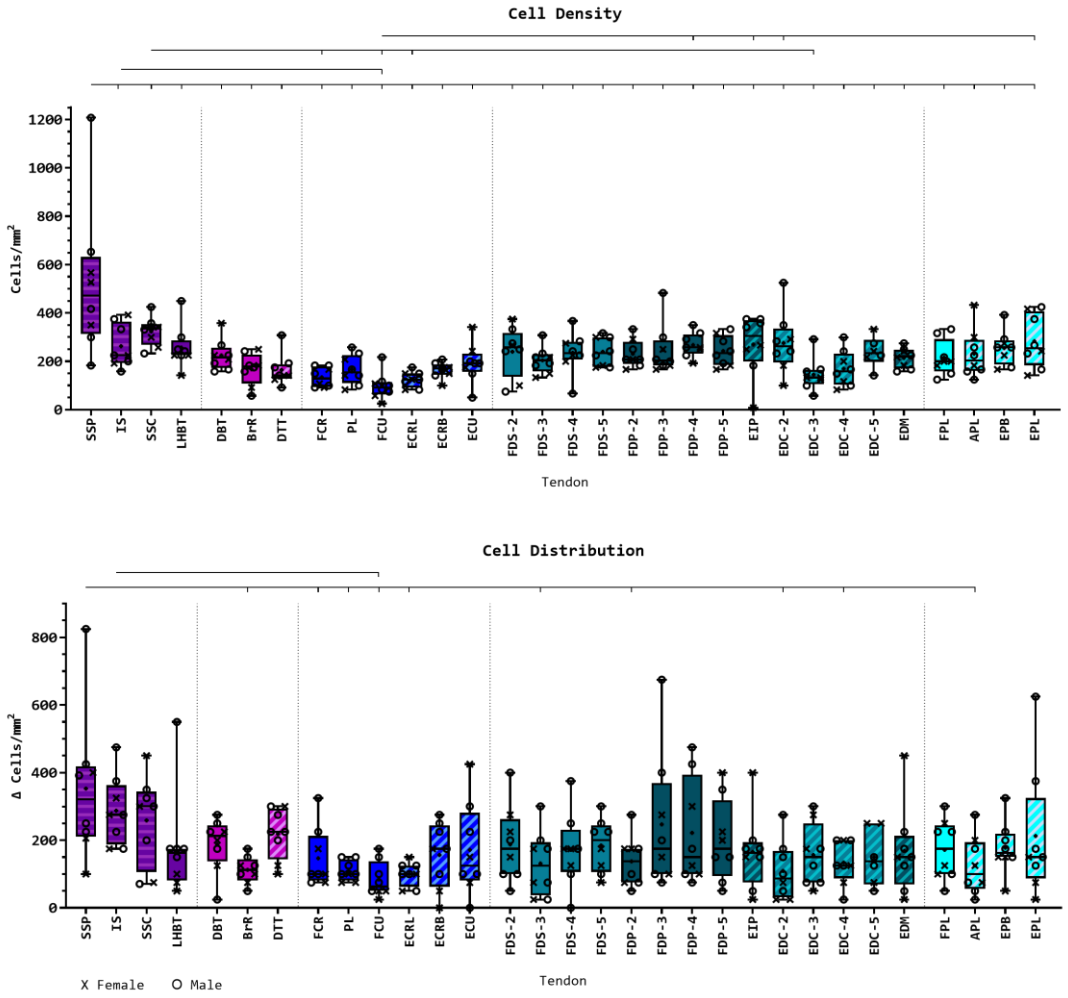


Figure 5.4.A Cell Density (top) and Cell Distribution (bottom) of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

There was a strong significant effect of *Tendon* on Cell Density ($p < 0.0001$). When comparing between specific tendons, the SSP had a significantly higher cell density (mean 525.6 cells/mm^2) compared to all other tendon types (Figure 5.4.A; $p = 0.0010$ vs SSC, $P < 0.0001$ for all other comparisons, Table 5.4.b). Conversely, the FCU had the lowest cell density (mean 97.9 cells/mm^2) and was significantly different compared to IS (mean 262.5 cells/mm^2, $p = 0.0495$), SSC (mean 323.8 cells/mm^2, $p = 0.0001$), FDP-4 (mean 267.7 cells/mm^2, $p = 0.0301$), EIP (mean 269.8 cells/mm^2, $p = 0.0245$), EDC-2 (mean 276.0 cells/mm^2, $p = 0.0130$) and EPL (mean 283.3 cells/mm^2, $p = 0.0061$) in addition to the SSP (Table 5.4.b). Additionally, the SSC also had a significantly higher cell density compared to the FCR (mean 134.4 cells/mm^2, $p = 0.0039$), ECRL (mean 126.0 cells/mm^2, $p = 0.0015$) and EDC-3 (mean 145.8 cells/mm^2, $p = 0.0132$, Table 5.4.b).

Likewise, there was a strong significant effect of *Tendon* on Cell Distribution ($p < 0.0001$) although there were fewer individual tendon differences. Besides the IS which had a significantly

higher Cell Distribution difference (287.5 Δ cells/mm^2) compared to the FCU (84.4 Δ cells/mm^2, p = 0.0474), the SSP was the only other tendon to have significantly higher cell density difference (Cell Distribution) relative to other tendons (Table 5.4.c). The SSP (352.9 Δ cells/mm^2) was significantly higher than the BrR (112.5 Δ cells/mm^2, p = 0.0020), FCR (146.9 Δ cells/mm^2, p = 0.0379), PL (109.4 Δ cells/mm^2, p = 0.0015), FCU (84.4 Δ cells/mm^2, p = 0.0001), ECRL (100.0 Δ cells/mm^2, p = 0.0006), FDS-3 (131.3 Δ cells/mm^2, p = 0.0106), FDP-2 (137.5 Δ cells/mm^2, p = 0.0179), EDC-2 (109.4 Δ cells/mm^2, p = 0.0015), EDC-4 (134.4 Δ cells/mm^2, p = 0.0138), and APL (125.0 Δ cells/mm^2, p = 0.0062; Table 5.4.c).

Table 5.4.b Cell Density pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Sidak correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis accounts for model covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.a.

Graph Position	Tendon Comparison	Contrast	Summary	Adjusted P Value	95% Confidence Interval	
1 vs. 2	SSP vs. IS	-263.101	****	<0.0001	-427.585	-98.617
1 vs. 3	SSP vs. SSC	-201.793	**	0.0010	-366.277	-37.309
1 vs. 4	SSP vs. LHBT	-268.310	****	<0.0001	-432.793	-103.826
1 vs. 5	SSP vs. DBT	-300.601	****	<0.0001	-465.085	-136.117
1 vs. 6	SSP vs. BrR	-357.893	****	<0.0001	-522.377	-193.409
1 vs. 7	SSP vs. DTT	-361.018	****	<0.0001	-525.502	-196.534
1 vs. 8	SSP vs. FCR	-391.226	****	<0.0001	-555.710	-226.742
1 vs. 9	SSP vs. PL	-358.935	****	<0.0001	-523.418	-194.451
1 vs. 10	SSP vs. FCU	-427.685	****	<0.0001	-592.168	-263.201
1 vs. 11	SSP vs. ECRL	-399.560	****	<0.0001	-564.043	-235.076
1 vs. 12	SSP vs. ECRB	-362.060	****	<0.0001	-526.543	-197.576
1 vs. 13	SSP vs. ECU	-332.893	****	<0.0001	-497.377	-168.409
1 vs. 14	SSP vs. FDS-2	-286.018	****	<0.0001	-450.502	-121.534
1 vs. 15	SSP vs. FDS-3	-321.435	****	<0.0001	-485.918	-156.951
1 vs. 16	SSP vs. FDS-4	-290.185	****	<0.0001	-454.668	-125.701
1 vs. 17	SSP vs. FDS-5	-286.018	****	<0.0001	-450.502	-121.534
1 vs. 18	SSP vs. FDP-2	-294.351	****	<0.0001	-458.835	-129.867
1 vs. 19	SSP vs. FDP-3	-278.726	****	<0.0001	-443.210	-114.242
1 vs. 20	SSP vs. FDP-4	-257.893	****	<0.0001	-422.377	-93.409
1 vs. 21	SSP vs. FDP-5	-282.893	****	<0.0001	-447.377	-118.409
1 vs. 22	SSP vs. EIP	-255.810	****	<0.0001	-420.293	-91.326
1 vs. 23	SSP vs. EDC-2	-249.560	****	<0.0001	-414.043	-85.076
1 vs. 24	SSP vs. EDC-3	-379.768	****	<0.0001	-544.252	-215.284
1 vs. 25	SSP vs. EDC-4	-355.810	****	<0.0001	-520.293	-191.326
1 vs. 26	SSP vs. EDC-5	-278.783	****	<0.0001	-456.790	-100.775
1 vs. 27	SSP vs. EDM	-311.018	****	<0.0001	-475.502	-146.534
1 vs. 28	SSP vs. FPL	-309.976	****	<0.0001	-474.460	-145.492
1 vs. 29	SSP vs. APL	-294.351	****	<0.0001	-458.835	-129.867
1 vs. 30	SSP vs. EPB	-270.393	****	<0.0001	-434.877	-105.909
1 vs. 31	SSP vs. EPL	-242.268	****	<0.0001	-406.752	-77.784
2 vs. 10	IS vs. FCU	-164.583	*	0.0495	-329.067	-0.099
3 vs. 8	SSC vs. FCR	-189.433	**	0.0039	-353.917	-24.949
3 vs. 10	SSC vs. FCU	-225.891	***	0.0001	-390.375	-61.407
3 vs. 11	SSC vs. ECRL	-197.766	**	0.0015	-362.250	-33.282
3 vs. 24	SSC vs. EDC-3	-177.975	*	0.0132	-342.459	-13.491
10 vs. 20	FCU vs. FDP-4	169.792	*	0.0301	5.308	334.276
10 vs. 22	FCU vs. EIP	171.875	*	0.0245	7.391	336.359
10 vs. 23	FCU vs. EDC-2	178.125	*	0.0130	13.641	342.609
10 vs. 31	FCU vs. EPL	185.417	**	0.0061	20.933	349.901

Table 5.4.c Cell Distribution pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Sidak correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis accounts for model covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.a.

Graph Position	Tendon Comparison	Contrast	Summary	Adjusted P Value	95% Confidence Interval	
1 vs. 6	SSP vs. BrR	-240.375	**	0.0020	-442.796	-37.954
1 vs. 8	SSP vs. FCR	-206.000	*	0.0379	-408.421	-3.579
1 vs. 9	SSP vs. PL	-243.500	**	0.0015	-445.921	-41.079
1 vs. 10	SSP vs. FCU	-268.500	***	0.0001	-470.921	-66.079
1 vs. 11	SSP vs. ECRL	-252.875	***	0.0006	-455.296	-50.454
1 vs. 15	SSP vs. FDS-3	-221.625	*	0.0106	-424.046	-19.204
1 vs. 18	SSP vs. FDP-2	-215.375	*	0.0179	-417.796	-12.954
1 vs. 23	SSP vs. EDC-2	-243.500	**	0.0015	-445.921	-41.079
1 vs. 25	SSP vs. EDC-4	-218.500	*	0.0138	-420.921	-16.079
1 vs. 29	SSP vs. APL	-227.875	**	0.0062	-430.296	-25.454
2 vs. 10	IS vs. FCU	-203.125	*	0.0474	-405.546	-0.704

5.4.2.1.1 Donor Characteristics

Linear predictions found that Cell Density was statistically different between the *sexes* (p = 0.0443), where males exhibited significantly higher cell density relative to females (contrast = 44.40, Table 5.4.d). Similarly, Cell Distribution was statistically different between the *sexes* (p = 0.0036), where males exhibited significantly greater difference in densities relative to females (contrast = 55.36, Table 5.4.d).

In contrast, linear predictions found that *age*, *height* or *weight* did not affect Cell Density (p = 0.4928, p = 0.2297 and p = 0.7532 respectively, Table 5.4.e), or Cell Distribution (p = 0.1444, p = 0.1485 and p = 0.7334 respectively, Table 5.4.e).

Table 5.4.d Cell Density and Cell Distribution main effects linear predictions for *sex* from mixed-effects linear regression analysis of 31 different human upper limb tendons.

Outcome	Comparison	Contrast	p-value	95% Confidence Interval	
Cell Density	Female (reference) vs. Male	44.403	**0.0443**	1.125	87.681
Cell Distribution	Female (reference) vs. Male	53.362	**0.0036**	17.405	89.321

Table 5.4.e Cell Density and Cell Distribution main effects linear predictions for *age*, *height* and *weight* from mixed-effects linear regression analysis of 31 different human upper limb tendons.

Outcome	Variable	*dx*	dy/dx	p-value	95% Confidence Interval	
Cell Density	Age	*Years*	1.526	0.4928	-2.835	5.888
	Height	*Inches*	-5.307	0.2297	-13.967	3.352
	Weight	*Pounds*	-0.320	0.7532	-2.315	1.675
Cell Distribution	Age	*Years*	2.700	0.1444	-0.926	6.327
	Height	*Inches*	-5.306	0.1485	-12.504	1.891
	Weight	*Pounds*	0.288	0.7334	-1.367	1.942

5.4.2.2 *Cell (Nuclear) Morphology*

The Cell Morphology score assessed the predominant morphology (shape: spindle, cigar, spherical) of tenocyte nuclei present within the tendon sample area from H&E-stained sections (Figure 5.4.B). It should be noted that the score is not a purely ordinal scale: a score of one (1) indicates approximately evenly mixed morphology between two or more phenotypes while a score within the 2-4 range indicates a change from a predominant spindle shape (score 2), to increasingly more rounded (cigar [score 3] to spherical [score 4]) phenotypes being the dominant cell type.

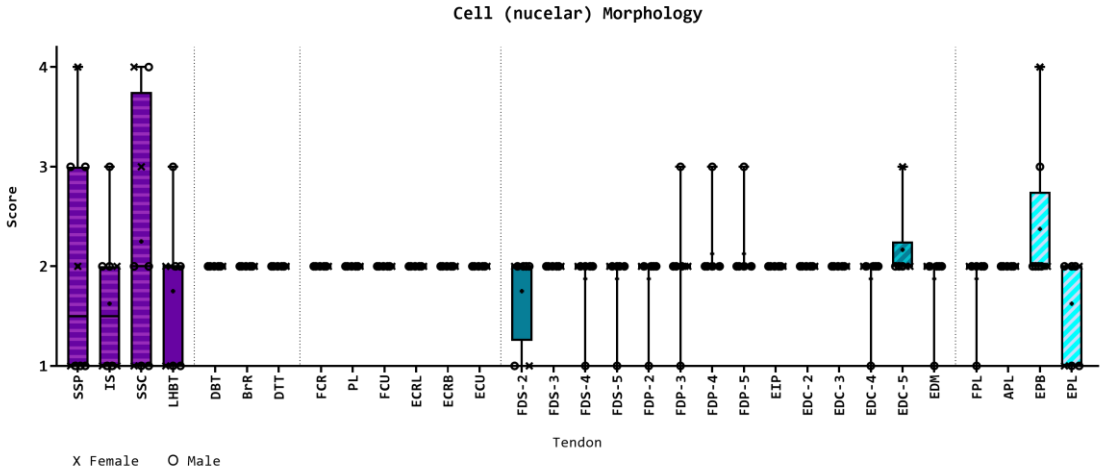


Figure 5.4.B Cell Morphology scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

In general, most tendons had predominantly spindle-shaped nuclei (scores of 2; Figure 5.4.B). This was confirmed with Kruskal-Wallis analysis which revealed no significant effect of *Tendon* on Cell Morphology score ($p = 0.5424$). The shoulder tendons (SSP, IS, SSC, and LHBT) generally displayed great variability across the donors and contained more cigar-shaped or spherical cell nuclei, scoring either specific to these morphologies (3 or 4 respectively) or mixed morphologies (score of 1, Figure 5.4.B). Hand and thumb tendons also incurred scores indicating more cigar-shaped or rounded nuclei, whilst the elbow and wrist tendons only incurred scores of 2 (Figure 5.4.B), having predominantly spindle-shaped nuclei. However, there were no significant differences when comparing between tendons ($p > 0.05$, Supp. Table 9.6.a).

5.4.2.3 *Cell Arrangement*

The Cell Arrangement score assessed the relative positioning of cells with respect to one another from H&E-stained sections (Figure 5.4.C). A greater score indicates a greater proportion of cells arranged longitudinally in chains (*refer to Figure 4.5.B*). A score of one (1) indicates cells are dispersed and show no relative arrangement to neighbouring cells, whilst a score of four (4) indicates most cells are aligned in series with one another between fibres.

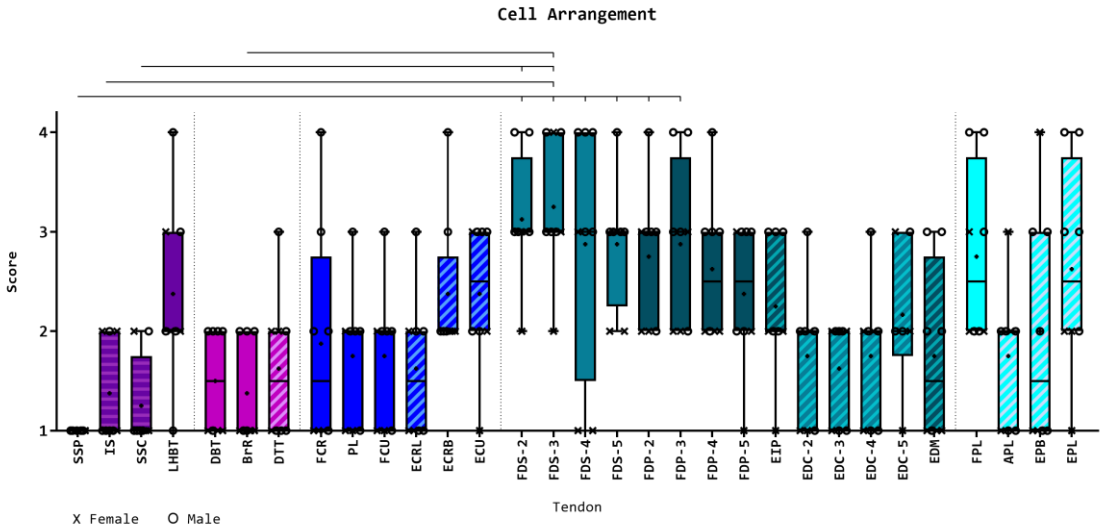


Figure 5.4.C Cell Arrangement scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tendon* on Cell Arrangement score ($p < 0.0001$). The SSP exhibited the lowest level of cell arrangement (cells were isolated and show little to no alignment in chains; Figure 5.4.C). When comparing between tendons, SSP had significantly less cell chaining compared to all of the superficial digital flexors (FDS-2, FDS-3, FDS-4, FDS-5; all $p < 0.05$, Table 5.4.f) and two of the deep digital flexors (FDP-2, $p = 0.0305$; FDP-3, $p = 0.0162$). The IS also had significantly less cell chaining than the FDS-2 ($p = 0.0403$), as did the SSC compared to the FDS-2 ($p = 0.0206$) and FDS-3 ($p = 0.0108$). Similarly, BrR had significantly less cell chaining than the FDS-3 ($p = 0.0403$; Table 5.4.f).

Table 5.4.f Cell Arrangement pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.a.

Graph Position	Tendon Comparison	Mean rank difference	Summary	Adjusted P Value
1 vs. 14	SSP vs. FDS-2	-159.1	**	0.0012
1 vs. 15	SSP vs. FDS-3	-164.1	***	0.0006
1 vs. 16	SSP vs. FDS-4	-133.9	*	0.0359
1 vs. 17	SSP vs. FDS-5	-144.6	**	0.0091
1 vs. 18	SSP vs. FDP-2	-135.2	*	0.0305
1 vs. 19	SSP vs. FDP-3	-140.2	*	0.0162
2 vs. 15	IS vs. FDS-3	-132.9	*	0.0403
3 vs. 14	SSC vs. FDS-2	-138.3	*	0.0206
3 vs. 15	SSC vs. FDS-3	-143.3	*	0.0108
6 vs. 15	BrR vs. FDS-3	-132.9	*	0.0403

5.4.2.4 *Atypical-Phenotype Cell Presence*

The Atypical-Phenotype Cell Presence score assessed the presence of "atypical" cell phenotypes within the tendon area from TB-stained sections (Figure 5.4.D). "Atypical" cells included those that may not be properly described as chondrocytes/'chondrocyte-like' (as they do not display all 3 criteria; *refer to Figure 4.5.K*) but also do not resemble typical tenocyte morphology. A higher score indicates a greater presence of these cells, where a score of zero (0) indicates none are present and a score of three (3) indicates numerous are present.

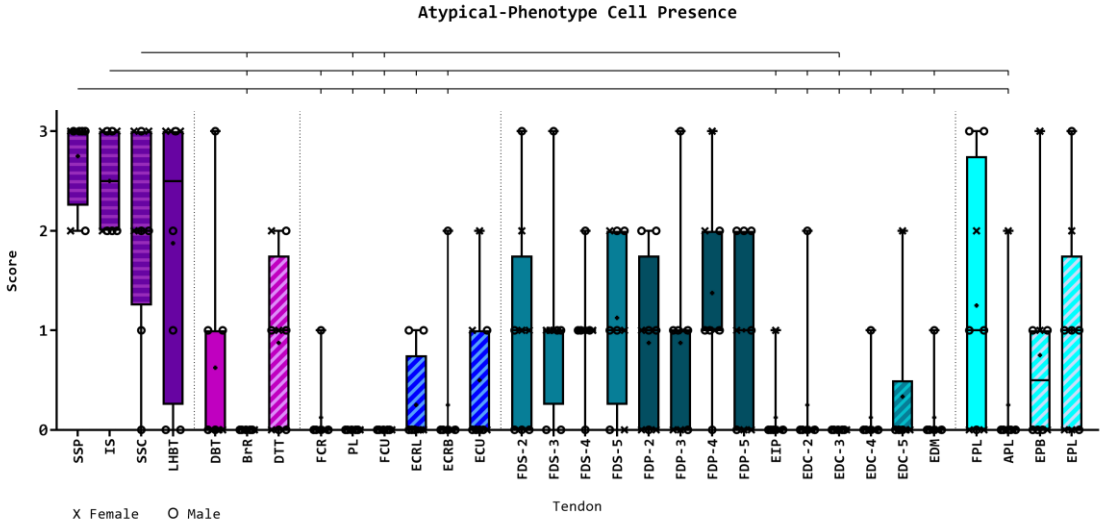


Figure 5.4.D Atypical-Phenotype Cell Presence scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tendon* on Atypical-Phenotype Cell Presence score ($p < 0.0001$). The rotator cuff tendons (SSP, IS and SSC) exhibited the highest presence of atypical-phenotype/chondrocyte-like cells (Figure 5.4.D). When comparing between tendons, the SSP and IS had significantly higher presence than the BrR ($p = 0.0004$ and $p = 0.0013$ respectively), five of the wrist tendons (FCR, PL, FCU, ECRL, ECRB; all $p < 0.05$, Table 5.4.g), five of the digital extensors (EIP, EDC-2, EDC-3, EDC-4 and EDM; all $p < 0.05$, Table 5.4.g), as well as the APL ($p = 0.0056$ for SSP and $p = 0.0151$ for IS). The SSP also had significantly more atypical-phenotype/chondrocyte-like cell presence compared to the EDC-5 ($p = 0.0470$, Table 5.4.g). Meanwhile, the SSC had significantly higher presence than the BrR, PL, FCU and EDC-3 ($p = 0.0484$ for all, Table 5.4.g).

Table 5.4.g Atypical-Phenotype Cell Presence pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.a.

Graph Position	Tendon Comparison	Mean rank difference	Summary	Adjusted P Value
1 vs. 6	SSP vs. BrR	157.9	***	0.0004
1 vs. 8	SSP vs. FCR	146.1	**	0.0026
1 vs. 9	SSP vs. PL	157.9	***	0.0004
1 vs. 10	SSP vs. FCU	157.9	***	0.0004
1 vs. 11	SSP vs. ECRL	134.3	*	0.0140
1 vs. 12	SSP vs. ECRB	140.8	**	0.0056
1 vs. 22	SSP vs. EIP	146.1	**	0.0026
1 vs. 23	SSP vs. EDC-2	140.8	**	0.0056
1 vs. 24	SSP vs. EDC-3	157.9	***	0.0004
1 vs. 25	SSP vs. EDC-4	146.1	**	0.0026
1 vs. 26	SSP vs. EDC-5	135.1	*	0.0470
1 vs. 27	SSP vs. EDM	146.1	**	0.0026
1 vs. 29	SSP vs. APL	140.8	**	0.0056
2 vs. 6	IS vs. BrR	150.8	**	0.0013
2 vs. 8	IS vs. FCR	138.9	**	0.0073
2 vs. 9	IS vs. PL	150.8	**	0.0013
2 vs. 10	IS vs. FCU	150.8	**	0.0013
2 vs. 11	IS vs. ECRL	127.1	*	0.0362
2 vs. 12	IS vs. ECRB	133.7	*	0.0151
2 vs. 22	IS vs. EIP	138.9	**	0.0073
2 vs. 23	IS vs. EDC-2	133.7	*	0.0151
2 vs. 24	IS vs. EDC-3	150.8	**	0.0013
2 vs. 25	IS vs. EDC-4	138.9	**	0.0073
2 vs. 27	IS vs. EDM	138.9	**	0.0073
2 vs. 29	IS vs. APL	133.7	*	0.0151
3 vs. 6	SSC vs. BrR	124.9	*	0.0484
3 vs. 9	SSC vs. PL	124.9	*	0.0484
3 vs. 10	SSC vs. FCU	124.9	*	0.0484
3 vs. 24	SSC vs. EDC-3	124.9	*	0.0484

5.4.3 Proteoglycan/GAG Characteristics

5.4.3.1 *Proteoglycan Presence*

The Proteoglycan Presence score assessed the presence of proteoglycan/GAG staining (purple chromatic appearance), irrespective of intensity, within the tendon area from TB-stained sections (Figure 5.4.E). A higher score indicates more presence of proteoglycan staining, where a score of zero (0) indicates no staining present (0% of tendon area contains staining) and a score of six (6) indicates > 95% of the tendon area contains staining.

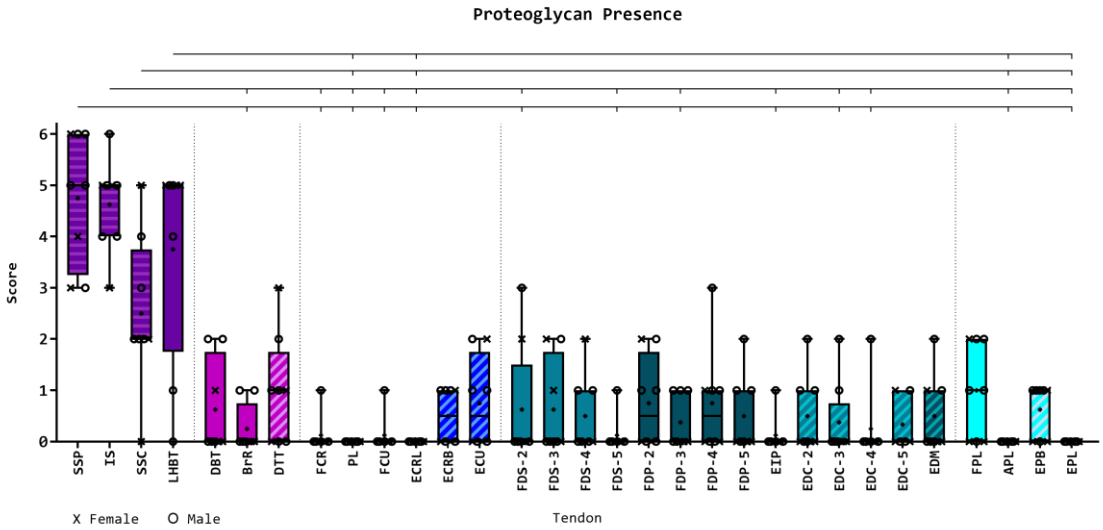


Figure 5.4.E Proteoglycan Presence scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tendon* on Proteoglycan Presence score ($p < 0.0001$). The shoulder tendons (SSP, IS, SSC and LHBT) exhibited the highest presence of proteoglycan staining, while PL, ECRL, APL and EPL were all completely void of proteoglycan throughout the sections scored for each donor (Figure 5.4.E). When comparing between tendons, the SSP and IS scored significantly higher than BrR, FCR, PL, FCU, ECRL, FDS-2, FDS-5, FDP-3, EIP, EDC-3, EDC-4, APL and EPL (all $p < 0.05$, Table 5.4.h), while SSC and LHBT scored significantly higher than PL, ECRL, ALP and EPL (all $p = 0.0357$ for SSC and all $p = 0.0107$ for LHBT, Table 5.4.h).

Table 5.4.h Proteoglycan Presence pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.b.

Graph Position	Tendon Comparison	Mean rank difference	Summary	Adjusted P Value
1 vs. 6	SSP vs. BrR	134.4	**	0.0070
1 vs. 8	SSP vs. FCR	146.6	**	0.0011
1 vs. 9	SSP vs. PL	158.8	***	0.0001
1 vs. 10	SSP vs. FCU	146.6	**	0.0011
1 vs. 11	SSP vs. ECRL	158.8	***	0.0001
1 vs. 14	SSP vs. FDS-2	124.2	*	0.0296
1 vs. 17	SSP vs. FDS-5	146.6	**	0.0011
1 vs. 19	SSP vs. FDP-3	122.3	*	0.0385
1 vs. 22	SSP vs. EIP	146.6	**	0.0011
1 vs. 24	SSP vs. EDC-3	130.3	*	0.0128
1 vs. 25	SSP vs. EDC-4	142.4	**	0.0021
1 vs. 29	SSP vs. APL	158.8	***	0.0001
1 vs. 31	SSP vs. EPL	158.8	***	0.0001
2 vs. 6	IS vs. BrR	133.2	**	0.0084
2 vs. 8	IS vs. FCR	145.4	**	0.0013
2 vs. 9	IS vs. PL	157.6	***	0.0002
2 vs. 10	IS vs. FCU	145.4	**	0.0013
2 vs. 11	IS vs. ECRL	157.6	***	0.0002
2 vs. 14	IS vs. FDS-2	122.9	*	0.0351
2 vs. 17	IS vs. FDS-5	145.4	**	0.0013
2 vs. 19	IS vs. FDP-3	121	*	0.0455
2 vs. 22	IS vs. EIP	145.4	**	0.0013
2 vs. 24	IS vs. EDC-3	129	*	0.0152
2 vs. 25	IS vs. EDC-4	141.2	**	0.0025
2 vs. 29	IS vs. APL	157.6	***	0.0002
2 vs. 31	IS vs. EPL	157.6	***	0.0002
3 vs. 9	SSC vs. PL	122.8	*	0.0357
3 vs. 11	SSC vs. ECRL	122.8	*	0.0357
3 vs. 29	SSC vs. APL	122.8	*	0.0357
3 vs. 31	SSC vs. EPL	122.8	*	0.0357
4 vs. 9	LHBT vs. PL	131.5	*	0.0107
4 vs. 11	LHBT vs. ECRL	131.5	*	0.0107
4 vs. 29	LHBT vs. APL	131.5	*	0.0107
4 vs. 31	LHBT vs. EPL	131.5	*	0.0107

5.4.3.2 *Proteoglycan Staining Intensity [Average and Maximum]*

The Proteoglycan Staining Intensity scores [Average and Maximum] assessed the intensity of the purple chromatic staining appearance within the tendon area from TB-stained sections (Figure 5.4.F). As there were 151 samples with a Proteoglycan Presence score of 0 (no proteoglycan staining present), only n = 95 datapoints (samples) were included for Proteoglycan Staining Intensity Average and Maximum score analysis. A greater score indicates greater staining intensity reflective of greater proteoglycan content. A score of one (1) indicates pale purple appearance, while a score of three (3) indicates deep purple staining.

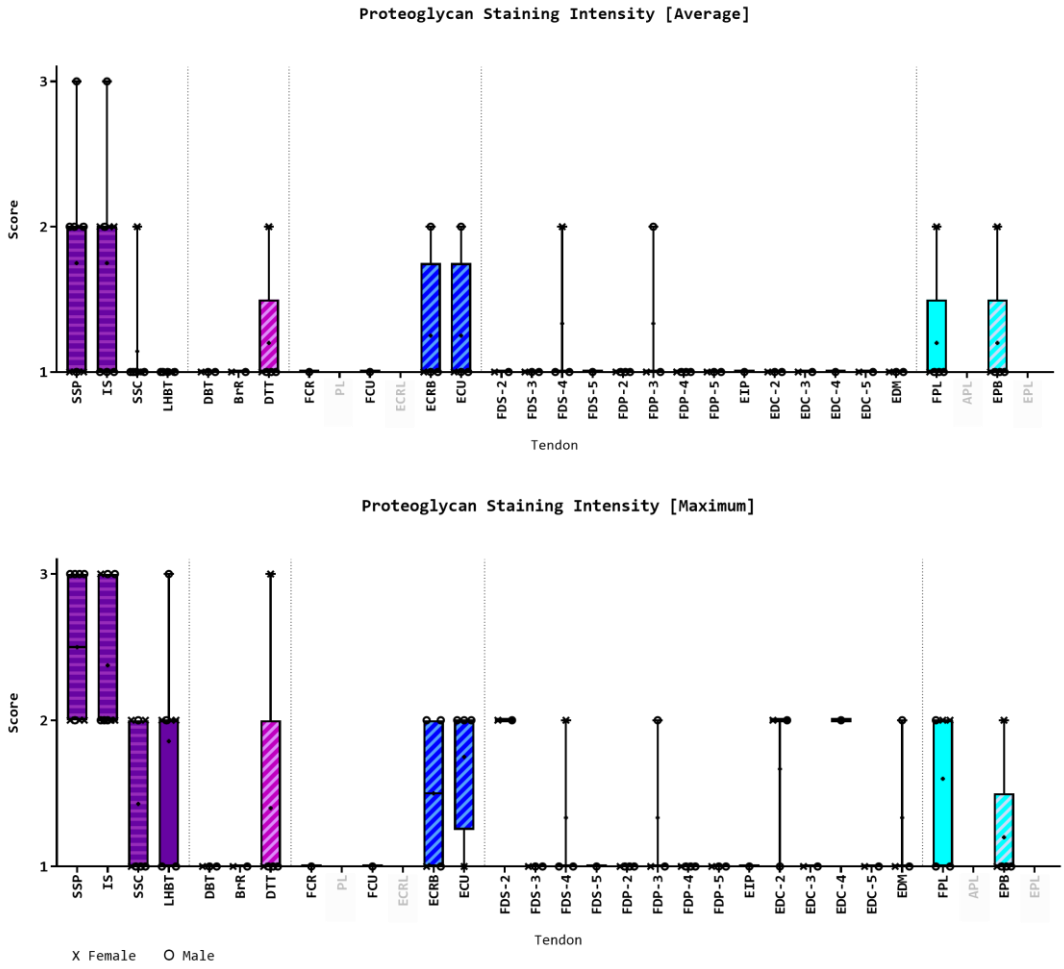


Figure 5.4.F Proteoglycan Staining Intensity Average (top) and Maximum (bottom) scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Note: indicated in grey, the PL, ECRL, APL and EPL contain no datapoints (no scores) as they were absent of any proteoglycan staining. Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was no significant effect of *Tendon* on Proteoglycan Staining Intensity Average score ($p = 0.2167$), however there was significant effect of *Tendon* on Proteoglycan Staining Intensity Maximum score ($p = 0.0009$). From the Average intensity score, the majority of upper limb tendons were found to have pale proteoglycan staining (score of 1) with the PL, ECRL, APL and EPL having no positive staining at all (no scores, Figure 5.4.F). Conversely, there were a number of tendons exhibiting localised areas of greater intensity staining (Maximum score), such as the SSP, IS, LHBT, ECU, FDS-2 and EDC-2 which all had majority of donors (individual data points) present with Maximum scores greater than the Average intensity score. When comparing between tendons, there were no significant differences for either Average or Maximum scores (all comparisons $p > 0.05$, Supp. Table 9.6.b).

5.4.4 Architectural Characteristics

5.4.4.1 *Collagenous Organisation*

The Collagenous Organisation score assessed the general structural appearance of the collagenous matrix within the tendon area from H&E-stained sections (Figure 5.4.G). A higher score indicates a more disorganised collagenous architecture within the tendon area. A score of zero (0) indicates the collagenous structure is completely organised with highly aligned/orientated collagen, whilst a score of six (6) indicates variable or unaligned collagenous matrix constituting nearly all of the tendon area.

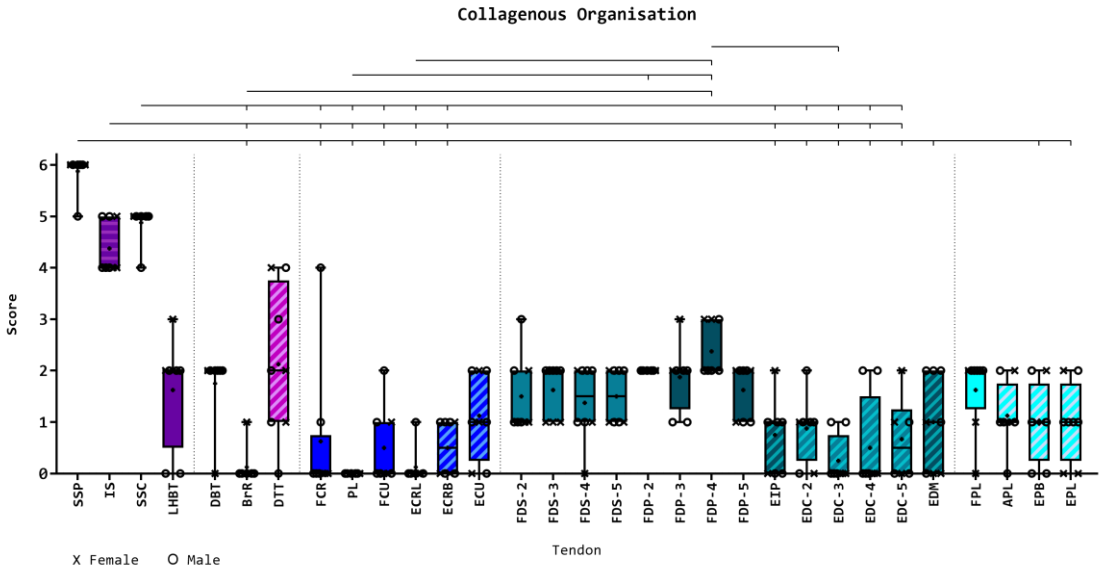


Figure 5.4.G Collagenous Organisation scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tendon* on Collagenous Organisation score ($p < 0.0001$). The rotator cuff tendons (SSP, IS, and SSC) exhibited the highest level of disorganisation (high score) within their collagenous architecture, while the BrR, PL, ECRL and EDC-3 exhibited the greatest levels of organisation (low score) relative to other tendons (Figure 5.4.G). When comparing between tendons, the rotator cuff tendons (SSP, IS and SSC) had significantly higher disorganisation than the BrR ($p < 0.0001$ for all), five of the wrist tendons (FCR, PL, FCU, ECRL and ECRB; all $p < 0.05$, Table 5.4.i), and four of the digital extensors (EIP, EDC-3, EDC-4 and EDC-5; all $p < 0.05$, Table 5.4.i). The SSP and SSC were also significantly more disorganised than the remaining digital extensor (EDC-2; $p = 0.0105$ for SSP and $p = 0.0333$ for SSC), while the SSP furthermore had significantly greater collagenous disorganisation than the EDM ($p = 0.0323$), EPB ($p = 0.0315$) and EPL ($p = 0.0315$).

Additionally, the FDP-4 tendon was significantly more disorganised than BrR (p = 0.0114), PL (p = 0.0037), ECRL (p = 0.0114) and EDC-3 (p = 0.0333), and the FDP-2 was significantly more disorganised than the PL tendon (p = 0.0279, Table 5.4.i).

Table 5.4.i Collagenous Organisation pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.c.

Graph Position	Tendon Comparison	Mean rank difference	Summary	Adjusted P Value
1 vs. 6	SSP vs. BrR	196.4	****	<0.0001
1 vs. 8	SSP vs. FCR	173	***	0.0002
1 vs. 9	SSP vs. PL	204.9	****	<0.0001
1 vs. 10	SSP vs. FCU	170.7	***	0.0003
1 vs. 11	SSP vs. ECRL	196.4	****	<0.0001
1 vs. 12	SSP vs. ECRB	170.9	***	0.0003
1 vs. 22	SSP vs. EIP	153.7	**	0.0034
1 vs. 23	SSP vs. EDC-2	145.2	*	0.0105
1 vs. 24	SSP vs. EDC-3	187.9	****	<0.0001
1 vs. 25	SSP vs. EDC-4	170.5	***	0.0003
1 vs. 26	SSP vs. EDC-5	159.3	**	0.0078
1 vs. 27	SSP vs. EDM	136.3	*	0.0323
1 vs. 30	SSP vs. EPB	136.5	*	0.0315
1 vs. 31	SSP vs. EPL	136.5	*	0.0315
2 vs. 6	IS vs. BrR	182.3	****	<0.0001
2 vs. 8	IS vs. FCR	158.9	**	0.0016
2 vs. 9	IS vs. PL	190.8	****	<0.0001
2 vs. 10	IS vs. FCU	156.6	**	0.0023
2 vs. 11	IS vs. ECRL	182.3	****	<0.0001
2 vs. 12	IS vs. ECRB	156.8	**	0.0022
2 vs. 22	IS vs. EIP	139.6	*	0.0216
2 vs. 24	IS vs. EDC-3	173.8	***	0.0002
2 vs. 25	IS vs. EDC-4	156.4	**	0.0023
2 vs. 26	IS vs. EDC-5	145.2	*	0.0408
3 vs. 6	SSC vs. BrR	187.3	****	<0.0001
3 vs. 8	SSC vs. FCR	163.9	***	0.0008
3 vs. 9	SSC vs. PL	195.8	****	<0.0001
3 vs. 10	SSC vs. FCU	161.6	**	0.0011
3 vs. 11	SSC vs. ECRL	187.3	****	<0.0001
3 vs. 12	SSC vs. ECRB	161.8	**	0.0011
3 vs. 22	SSC vs. EIP	144.6	*	0.0114
3 vs. 23	SSC vs. EDC-2	136.1	*	0.0333
3 vs. 24	SSC vs. EDC-3	178.8	****	<0.0001
3 vs. 25	SSC vs. EDC-4	161.4	**	0.0012
3 vs. 26	SSC vs. EDC-5	150.2	*	0.0231
6 vs. 20	BrR vs. FDP-4	-144.6	*	0.0114
9 vs. 18	PL vs. FDP-2	-137.5	*	0.0279
9 vs. 20	PL vs. FDP-4	-153.1	**	0.0037
11 vs. 20	ECRL vs. FDP-4	-144.6	*	0.0114
20 vs. 24	FDP-4 vs. EDC-3	136.1	*	0.0333

5.4.4.2 *Fibrillar Loosening*

The Fibrillar Loosening score assessed the 'packing density' of fibrils within a single ROI on H&E-stained sections (Figure 5.4.H). A higher score indicates a greater proportion of area with loose fibril arrangement / poorly defined fibre structures. A score of one (1) indicates all/nearly all of the fibrils in the ROI are bundled into distinct fibre structures, whilst a score of six (6) indicates all/nearly all of the area contains loose/dispersed fibrils without clear/distinct fibre structures.

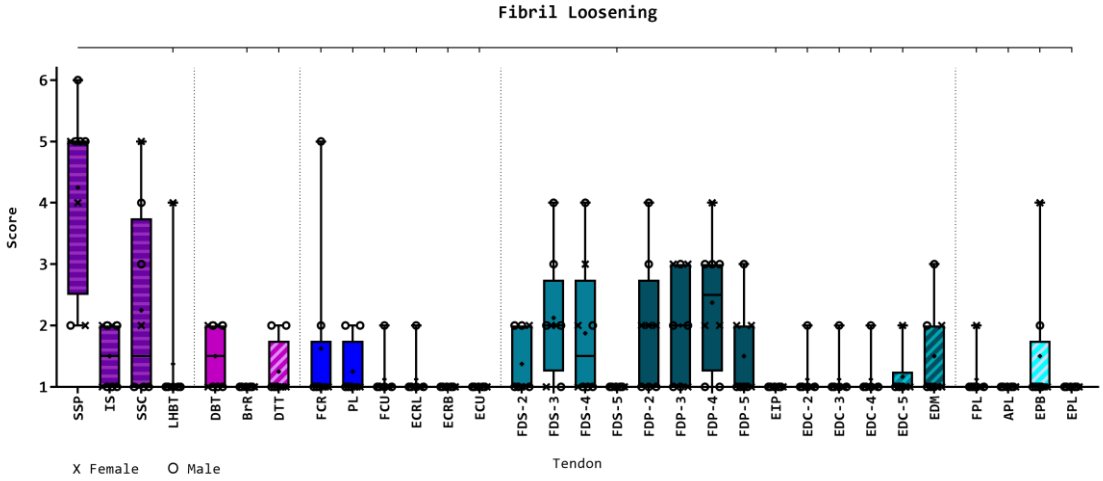


Figure 5.4.H Fibrillar Loosening scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tendon* on Fibrillar Loosening score ($p < 0.0001$). Tendons of the upper limb predominantly show < 24% of fibrils being loose/dispersed (score of 1 or 2), whereas the SSP exhibited the highest proportion of loose fibrils and was the only tendon to show a significant difference relative to other tendons (Figure 5.4.H). When comparing between tendons, SSP had significantly higher proportion of loosened fibrils compared to the LHBT ($p = 0.0053$), BrR ($p = 0.0002$), DTT ($p = 0.0214$), all six wrist tendons (FCR, PL, FCU, ECRL, ECRB, and ECU; all $p < 0.05$), FDS-5 ($p = 0.0002$) , EIP ($p = 0.0002$), all Extensor Digitorum branches (EDC-2, EDC-3, ECD-4, EDC-5; all $p < 0.05$), and all four thumb tendons (FPL, APL, EPB, EPL; all $p < 0.05$, Table 5.4.j).

Table 5.4.j Fibrillar Loosening pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.c.

Graph Position	Tendon Comparison	Mean rank difference	Summary	Adjusted P Value
1 vs. 4	SSP vs. LHBT	124.4	**	0.0053
1 vs. 6	SSP vs. BrR	142.8	***	0.0002
1 vs. 7	SSP vs. DTT	115.4	*	0.0214
1 vs. 8	SSP vs. FCR	109.8	*	0.0494
1 vs. 9	SSP vs. PL	115.4	*	0.0214
1 vs. 10	SSP vs. FCU	129.1	**	0.0024
1 vs. 11	SSP vs. ECRL	129.1	**	0.0024
1 vs. 12	SSP vs. ECRB	142.8	***	0.0002
1 vs. 13	SSP vs. ECU	142.8	***	0.0002
1 vs. 17	SSP vs. FDS-5	142.8	***	0.0002
1 vs. 22	SSP vs. EIP	142.8	***	0.0002
1 vs. 23	SSP vs. EDC-2	129.1	**	0.0024
1 vs. 24	SSP vs. EDC-3	129.1	**	0.0024
1 vs. 25	SSP vs. EDC-4	129.1	**	0.0024
1 vs. 26	SSP vs. EDC-5	124.6	*	0.0218
1 vs. 28	SSP vs. FPL	129.1	**	0.0024
1 vs. 29	SSP vs. APL	142.8	***	0.0002
1 vs. 30	SSP vs. EPB	110.7	*	0.0435
1 vs. 31	SSP vs. EPL	142.8	***	0.0002

5.4.4.3 *Fibre Separations [Frequency and Type]*

The Fibre Separations scores [Frequency and Type] assessed characteristics of separations between neighbouring fibres in a defined ROI from H&E-stained sections (Figure 5.4.I). The SSP samples of four donors were not able to have an appropriate fibre separation ROI placed for assessment due to high fibrillar loosening across the whole tendon area, therefore only n = 242 datapoints were included for the Fibre Separations Frequency outcome. A further 67 datapoints were missing from the Fibre Separations Type outcome due to a Frequency score of 0 (no separations), resulting in a total of n = 175 datapoints being included for analysis. The Fibre Separations Frequency score assessed the proportion of fibres which were visibly separated from neighbouring fibres, with a higher score indicating a greater proportion of fibre separations across the area assessed. A score of zero (0) indicates no separations are present (all fibres are tightly adhered/adjacent to one another), while a score of six (6) indicates nearly all/nearly all of the fibres are visibly separated from one another. The Fibre Separations Type score assessed the predominant length of the separations before fibres diverge/converge. A higher score indicates longer fibre separations present in the area assessed (indicative of fewer fibre-fibre connections/anastomoses within the tissue structure). A score of one (1) indicates separations were predominantly < 50 µm in length, while a score of four (4) indicates separations were predominantly > 150 µm long.

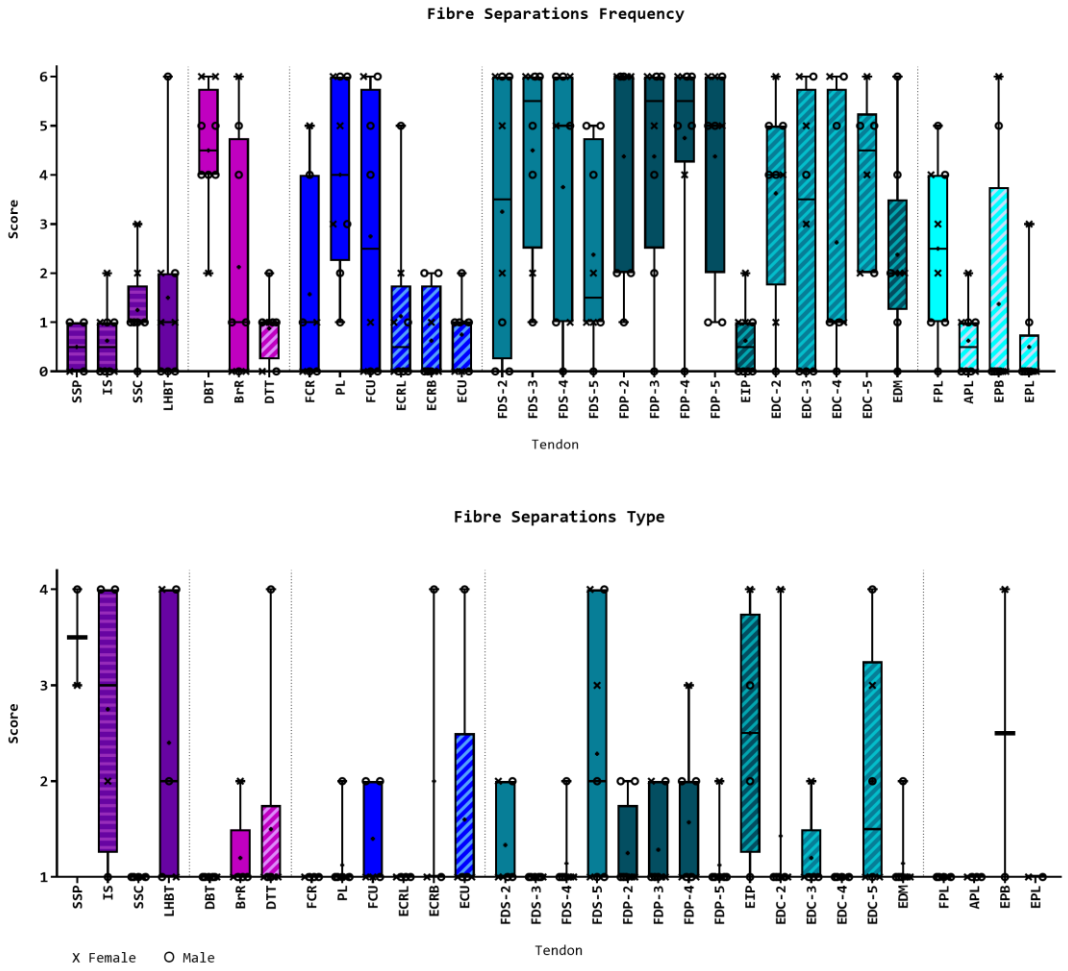


Figure 5.4.I Fibre Separations Frequency (top) and Type (bottom) scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a significant effect of *Tendon* on Fibre Separations Frequency ($p < 0.0001$) and Type ($p = 0.0032$) scores. Frequency of fibre separations was highly variable for most tendons, although the shoulder stabilisers (SSP, IS SSC and LHBT), elbow extensor (DDT), wrist extensors (ECRL, ECRB and ECU) as well as some of the digital/thumb extensors (namely EIP, APL and EPL) exhibited some of the most consistent and lowest frequency of separations (Figure 5.4.I). On the other hand, trends of separation Type were not evident, with all tendons of the upper limb displaying varied lengths of separations (Figure 5.4.I). When comparing between tendons, there were no significant differences for either Frequency or Type scores (all $p > 0.05$, Supp. Table 9.6.c).

5.4.4.4 *Collagen Alignment*

The Collagen Alignment score assessed the alignment characteristic of the collagenous matrix within the tendon area utilising standard brightfield light on PSR-stained sections (Figure 5.4.J). A higher score indicates less aligned collagen within the tendon area. A score of zero (0) indicates all of the tendon area contains highly aligned collagenous structure, whilst a score of seven (7) indicates none of the tendon area contains aligned collagenous structure (where all the collagenous matrix is unaligned).

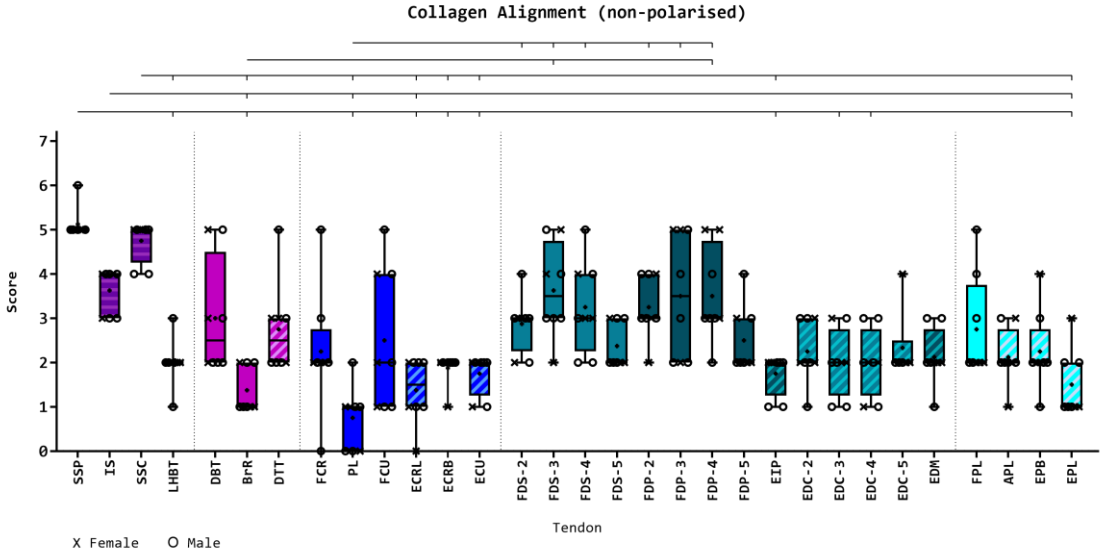


Figure 5.4.J Collagen Alignment scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tendon* on Collagen Alignment score ($p < 0.0001$). The SSP exhibited the greatest proportion of unaligned collagen relative to other tendons, while the PL exhibited the greatest proportion of aligned collagen (Figure 5.4.J). When comparing between tendons, the SSP and SSC had a significantly higher proportion of unaligned collagen than the LHBT ($p = 0.0168$ and $p = 0.0458$ respectively), BrB and PL (all comparisons $p < 0.0001$), ECRL ($p < 0.0001$ and $p = 0.0002$ respectively), ECRB ($p = 0.0046$ and $p = 0.0134$ respectively), ECU and EIP (both $p = 0.0013$ for SSP, and both $p = 0.0041$ for SSC), and EPL ($p = 0.0001$ and $p = 0.0004$ respectively). The SSP additionally had a higher proportion of unaligned collagen compared to the EDC-3 and EDC-4 (both $p = 0.0190$). The IS tendon was significantly more unaligned than the BrR ($p = 0.0090$), PL ($p = 0.0003$), ECRL ($p = 0.0207$) and EPL ($p = 0.0317$). In addition to the three rotator cuff tendons (SSP, IS and SSC), the BrR had significantly more aligned collagen than the FDS-3 ($p = 0.0254$) and FDP-4 ($p = 0.0444$). Furthermore, in addition to the three rotator cuff tendons, the PL was significantly more aligned than the FDS-2, -3 and -4, and FDP-2, -3 and -4 ($p = 0.0448$, $p = 0.0009$, $p = 0.0096$, $p = 0.0039$, $p = 0.0084$, and $p = 0.0016$ respectively) (Table 5.4.k).

Table 5.4.k Collagen Alignment pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.c.

Graph Position	Tendon Comparison	Mean rank difference	Summary	Adjusted P Value
1 vs. 4	SSP vs. LHBT	139.8	*	0.0168
1 vs. 6	SSP vs. BrR	184.8	****	<0.0001
1 vs. 9	SSP vs. PL	209.8	****	<0.0001
1 vs. 11	SSP vs. ECRL	178.4	****	<0.0001
1 vs. 12	SSP vs. ECRB	149.6	**	0.0046
1 vs. 13	SSP vs. ECU	158.4	**	0.0013
1 vs. 22	SSP vs. EIP	158.4	**	0.0013
1 vs. 24	SSP vs. EDC-3	138.9	*	0.0190
1 vs. 25	SSP vs. EDC-4	138.9	*	0.0190
1 vs. 31	SSP vs. EPL	175.1	***	0.0001
2 vs. 6	IS vs. BrR	144.6	**	0.0090
2 vs. 9	IS vs. PL	169.5	***	0.0003
2 vs. 11	IS vs. ECRL	138.2	*	0.0207
2 vs. 31	IS vs. EPL	134.8	*	0.0317
3 vs. 4	SSC vs. LHBT	131.8	*	0.0458
3 vs. 6	SSC vs. BrR	176.8	****	<0.0001
3 vs. 9	SSC vs. PL	201.8	****	<0.0001
3 vs. 11	SSC vs. ECRL	170.4	***	0.0002
3 vs. 12	SSC vs. ECRB	141.6	*	0.0134
3 vs. 13	SSC vs. ECU	150.4	**	0.0041
3 vs. 22	SSC vs. EIP	150.4	**	0.0041
3 vs. 31	SSC vs. EPL	167.1	***	0.0004
6 vs. 15	BrR vs. FDS-3	-136.6	*	0.0254
6 vs. 20	BrR vs. FDP-4	-132.1	*	0.0444
9 vs. 14	PL vs. FDS-2	-132	*	0.0448
9 vs. 15	PL vs. FDS-3	-161.5	***	0.0009
9 vs. 16	PL vs. FDS-4	-144.1	**	0.0096
9 vs. 18	PL vs. FDP-2	-150.8	**	0.0039
9 vs. 19	PL vs. FDP-3	-145.1	**	0.0084
9 vs. 20	PL vs. FDP-4	-157	**	0.0016

5.4.4.5 *Adipose Presence*

The Adipose Presence score assessed the presence of fatty (adipose) tissue/cells within the tendon area from H&E-stained sections (Figure 5.4.K). A higher score indicates a greater area of tissue containing adipocytes, where a score of zero (0) indicates no adipose present and a score of five (5) indicates > 25% of the tendon area contains adipose.

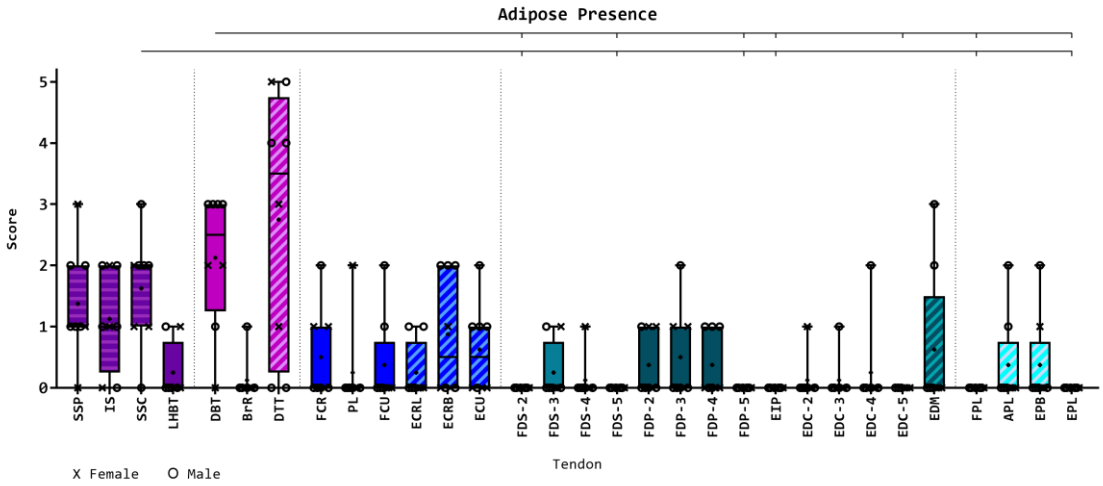


Figure 5.4.K Adipose Presence scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tendon* on Adipose Presence score ($p < 0.0001$). The DTT exhibited the largest spread of scores between the donors, whilst the FDS-2, FDS-5, FDP-5, EIP, EDC-5, FPL and EPL all exhibited no (score = 0) adipocytes in any of the donor samples (Figure 5.4.K). When comparing between tendons, the SSC and DBT had significantly higher presence of adipose/adipocytes than these latter mentioned tendons (SSC all with $p = 0.0352$, all with $p = 0.0082$) except the EDC-5 with was only significantly different to the DBT ($p = 0.0327$; Table 5.4.l).

Table 5.4.l Adipose Presence pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.c.

Graph Position	Tendon Comparison	Mean rank difference	Summary	Adjusted P Value
3 vs. 14	SSC vs. FDS-2	113.3	*	0.0352
3 vs. 17	SSC vs. FDS-5	113.3	*	0.0352
3 vs. 21	SSC vs. FDP-5	113.3	*	0.0352
3 vs. 22	SSC vs. EIP	113.3	*	0.0352
3 vs. 28	SSC vs. FPL	113.3	*	0.0352
3 vs. 31	SSC vs. EPL	113.3	*	0.0352
5 vs. 14	DBT vs. FDS-2	122.9	**	0.0082
5 vs. 17	DBT vs. FDS-5	122.9	**	0.0082
5 vs. 21	DBT vs. FDP-5	122.9	**	0.0082
5 vs. 22	DBT vs. EIP	122.9	**	0.0082
5 vs. 26	DBT vs. EDC-5	122.9	*	0.0327
5 vs. 28	DBT vs. FPL	122.9	**	0.0082
5 vs. 31	DBT vs. EPL	122.9	**	0.0082

5.4.5 Interfascicular Characteristics

5.4.5.1 *Interfascicular Region Frequency*

The Interfascicular Region (IFR) Frequency outcome evaluated the frequency of IFRs across the width of the tendon area from PSR-stained sections (brightfield microscopy; Figure 5.4.L). The SSP samples of four donors were not able to have an appropriate IFR ROI (*refer to Section 5.3.2*) placed for assessment due to low collagenous organisation throughout the tendon area, therefore only n = 242 datapoints were included for analysis. IFR Frequency was taken as the number of IFRs per mm of tendon width; a higher value indicates a greater incidence of IFRs indicative of smaller fascicles across the tendon area.

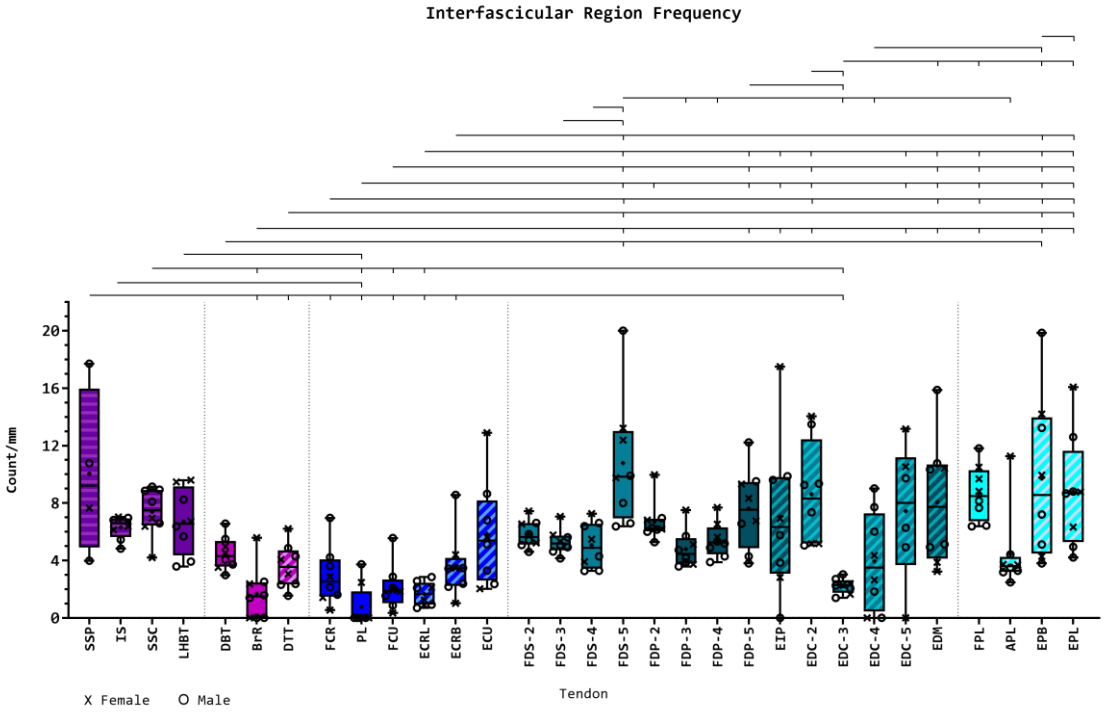


Figure 5.4.L Interfascicular Region Frequency of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Mixed-effects linear regression analysis with Sidak's adjustment. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

There was a strong significant effect of *Tendon* on IFR Frequency ($p < 0.0001$). Results were variable across the tendons, with extensor tendons (Table 5.3.a; bars coloured with diagonal stripes in Figure 5.4.L) tending to have more variability in IFR frequency (Figure 5.4.L). The SSP (mean 10.03 IFRs/mm), FDS-5 (mean 10.78 IFRs/mm), and EPB (mean 9.70 IFRs/mm) had the three highest IFR frequencies, whilst the PL (mean 0.78 IFRs/mm), ECRL (mean 1.67 IFRs/mm) and BrR (mean 1.68 IFRs/mm) had the lowest frequencies (Figure 5.4.L). When comparing between tendons, varied and numerous significant differences in IFR Frequency across the upper limb tendons were found as detailed in Table 5.4.m.

Table 5.4.m Interfascicular Region Frequency pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Sidak correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis accounts for model covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.d.

Graph Position	Tendon Comparison	Contrast	Summary	Adjusted P Value	95% Confidence Interval	
1 vs. 6	SSP vs. BrR	-8.397	***	0.0001	-14.677	-2.117
1 vs. 7	SSP vs. DTT	-6.491	*	0.0295	-12.771	-0.211
1 vs. 8	SSP vs. FCR	-7.152	**	0.0049	-13.432	-0.872
1 vs. 9	SSP vs. PL	-9.304	****	<0.0001	-15.584	-3.024
1 vs. 10	SSP vs. FCU	-7.949	***	0.0005	-14.229	-1.669
1 vs. 11	SSP vs. ECRL	-8.406	***	0.0001	-14.686	-2.126
1 vs. 12	SSP vs. ECRB	-6.465	*	0.0314	-12.745	-0.185
1 vs. 24	SSP vs. EDC-3	-7.859	***	0.0006	-14.139	-1.579
2 vs. 9	IS vs. PL	-5.521	*	0.0131	-10.622	-0.421
3 vs. 6	SSC vs. BrR	-5.711	**	0.0069	-10.811	-0.610
3 vs. 9	SSC vs. PL	-6.618	***	0.0002	-11.718	-1.518
3 vs. 10	SSC vs. FCU	-5.263	*	0.0303	-10.363	-0.162
3 vs. 11	SSC vs. ECRL	-5.720	**	0.0067	-10.820	-0.620
3 vs. 24	SSC vs. EDC-3	-5.173	*	0.0401	-10.273	-0.073
4 vs. 9	LHBT vs. PL	-5.923	**	0.0033	-11.023	-0.822
5 vs. 17	DBT vs. FDS-5	6.297	***	0.0008	1.196	11.397
5 vs. 30	DBT vs. EPB	5.222	*	0.0344	0.121	10.322
6 vs. 17	BrR vs. FDS-5	9.094	****	<0.0001	3.994	14.194
6 vs. 21	BrR vs. FDP-5	5.916	**	0.0034	0.816	11.017
6 vs. 22	BrR vs. EIP	5.359	*	0.0223	0.259	10.459
6 vs. 23	BrR vs. EDC-2	6.927	***	0.0001	1.827	12.028
6 vs. 26	BrR vs. EDC-5	5.635	*	0.0358	0.118	11.152
6 vs. 27	BrR vs. EDM	6.390	***	0.0006	1.290	11.491
6 vs. 28	BrR vs. FPL	6.995	***	0.0001	1.895	12.096
6 vs. 30	BrR vs. EPB	8.019	****	<0.0001	2.919	13.119
6 vs. 31	BrR vs. EPL	7.115	****	<0.0001	2.015	12.216
7 vs. 17	DTT vs. FDS-5	7.188	****	<0.0001	2.088	12.288
7 vs. 30	DTT vs. EPB	6.113	**	0.0017	1.013	11.213
7 vs. 31	DTT vs. EPL	5.209	*	0.0358	0.109	10.310
8 vs. 17	FCR vs. FDS-5	7.849	****	<0.0001	2.749	12.950
8 vs. 23	FCR vs. EDC-2	5.682	**	0.0076	0.582	10.783
8 vs. 27	FCR vs. EDM	5.146	*	0.0436	0.045	10.246
8 vs. 28	FCR vs. FPL	5.750	**	0.0060	0.650	10.851
8 vs. 30	FCR vs. EPB	6.774	***	0.0001	1.674	11.875
8 vs. 31	FCR vs. EPL	5.871	**	0.0040	0.770	10.971
9 vs. 17	PL vs. FDS-5	10.001	****	<0.0001	4.901	15.102
9 vs. 18	PL vs. FDP-2	5.924	**	0.0033	0.824	11.024
9 vs. 21	PL vs. FDP-5	6.824	***	0.0001	1.724	11.924
9 vs. 22	PL vs. EIP	6.266	***	0.0009	1.166	11.367
9 vs. 23	PL vs. EDC-2	7.835	****	<0.0001	2.734	12.935
9 vs. 26	PL vs. EDC-5	6.542	**	0.0021	1.025	12.060
9 vs. 27	PL vs. EDM	7.298	****	<0.0001	2.198	12.398
9 vs. 28	PL vs. FPL	7.903	****	<0.0001	2.802	13.003
9 vs. 30	PL vs. EPB	8.926	****	<0.0001	3.826	14.027
9 vs. 31	PL vs. EPL	8.023	****	<0.0001	2.923	13.123
10 vs. 17	FCU vs. FDS-5	8.646	****	<0.0001	3.546	13.746
10 vs. 21	FCU vs. FDP-5	5.468	*	0.0156	0.368	10.569
10 vs. 23	FCU vs. EDC-2	6.479	***	0.0004	1.379	11.580
10 vs. 27	FCU vs. EDM	5.942	**	0.0031	0.842	11.043
10 vs. 28	FCU vs. FPL	6.547	***	0.0003	1.447	11.648
10 vs. 30	FCU vs. EPB	7.571	****	<0.0001	2.471	12.671
10 vs. 31	FCU vs. EPL	6.668	***	0.0002	1.567	11.768

11 vs. 17	ECRL vs. FDS-5	9.103	****	<0.0001	4.003	14.204
11 vs. 21	ECRL vs. FDP-5	5.926	**	0.0033	0.825	11.026
11 vs. 22	ECRL vs. EIP	5.368	*	0.0216	0.268	10.469
11 vs. 23	ECRL vs. EDC-2	6.937	***	0.0001	1.836	12.037
11 vs. 26	ECRL vs. EDC-5	5.644	*	0.0349	0.127	11.162
11 vs. 27	ECRL vs. EDM	6.400	***	0.0006	1.299	11.500
11 vs. 28	ECRL vs. FPL	7.005	***	0.0001	1.904	12.105
11 vs. 30	ECRL vs. EPB	8.028	****	<0.0001	2.928	13.129
11 vs. 31	ECRL vs. EPL	7.125	****	<0.0001	2.024	12.225
12 vs. 17	ECRB vs. FDS-5	7.162	****	<0.0001	2.062	12.263
12 vs. 30	ECRB vs. EPB	6.087	**	0.0018	0.987	11.188
12 vs. 31	ECRB vs. EPL	5.184	*	0.0387	0.083	10.284
15 vs. 17	FDS-3 vs. FDS-5	5.463	*	0.0159	0.363	10.564
16 vs. 17	FDS-4 vs. FDS-5	5.717	**	0.0068	0.616	10.817
17 vs. 19	FDS-5 vs. FDP-3	-5.973	**	0.0028	-11.073	-0.873
17 vs. 20	FDS-5 vs. FDP-4	-5.348	*	0.0231	-10.448	-0.248
17 vs. 24	FDS-5 vs. EDC-3	-8.556	****	<0.0001	-13.657	-3.456
17 vs. 25	FDS-5 vs. EDC-4	-6.830	***	0.0001	-11.931	-1.730
17 vs. 29	FDS-5 vs. APL	-6.329	***	0.0007	-11.429	-1.228
21 vs. 24	FDP-5 vs. EDC-3	-5.379	*	0.0209	-10.479	-0.278
23 vs. 24	EDC-2 vs. EDC-3	-6.390	***	0.0006	-11.490	-1.289
24 vs. 27	EDC-3 vs. EDM	5.853	**	0.0042	0.752	10.953
24 vs. 28	EDC-3 vs. FPL	6.458	***	0.0005	1.357	11.558
24 vs. 30	EDC-3 vs. EPB	7.481	****	<0.0001	2.381	12.582
24 vs. 31	EDC-3 vs. EPL	6.578	***	0.0003	1.477	11.678
25 vs. 30	EDC-4 vs. EPB	5.755	**	0.0059	0.655	10.856
29 vs. 30	APL vs. EPB	5.254	*	0.0311	0.153	10.354

5.4.5.1.1 Donor Characteristics

Linear predictions found that IFR Frequency was not affected by any donor variables i.e. *sex* (p = 0.2987, Table 5.4.n), *age*, *height* or *weight* (p = 0.5171, p = 0.8568 and p = 0.4858 respectively, Table 5.4.o).

Table 5.4.n Interfascicular Region Frequency main effects linear predictions for *sex* from mixed-effects linear regression analysis of 31 different human upper limb tendons.

Outcome	Comparison	Contrast	p-value	95% Confidence Interval	
IFR Frequency	Female (reference) vs. Male	-0.505	0.2987	1.125	87.681

Table 5.4.o Interfascicular Region Frequency main effects linear predictions for *age*, *height* and *weight* from mixed-effects linear regression analysis of 31 different human upper limb tendons.

Outcome	Variable	*dx*	dy/dx	p-value	95% Confidence Interval	
IFR Frequency	Age	*Years*	-0.032	0.5171	-0.128	0.064
	Height	*Inches*	-0.018	0.8568	-0.209	0.173
	Weight	*Pounds*	-0.016	0.4858	-0.059	0.028

5.4.5.2 *Interfascicular Region Type Proportion*

The Interfascicular Region Type Proportion outcome evaluated the proportion of IFRs defined as Interfascicular Matrix (IFM, an IFR containing distinct matrix substance) versus Interfascicular Space (IFS, a visible IFR/fascicle border containing no distinct matrix substance) from PSR-stained sections (brightfield microscopy; Figure 5.4.M). The SSP samples of four donors were

unable to have appropriate IFR ROIs placed for assessment (refer to *Section 5.4.5.1*). A further 12 datapoints were missing from the IFR Type Proportion outcome due to a IFR Frequency score of 0 (no IFRs), resulting in a total of n = 230 datapoints being included for analysis. IFR Type Proportion was taken as proportion of IFMs over the total number of IFRs; a higher percentage indicates a greater proportion of matrix-containing IFRs across the tendon area.

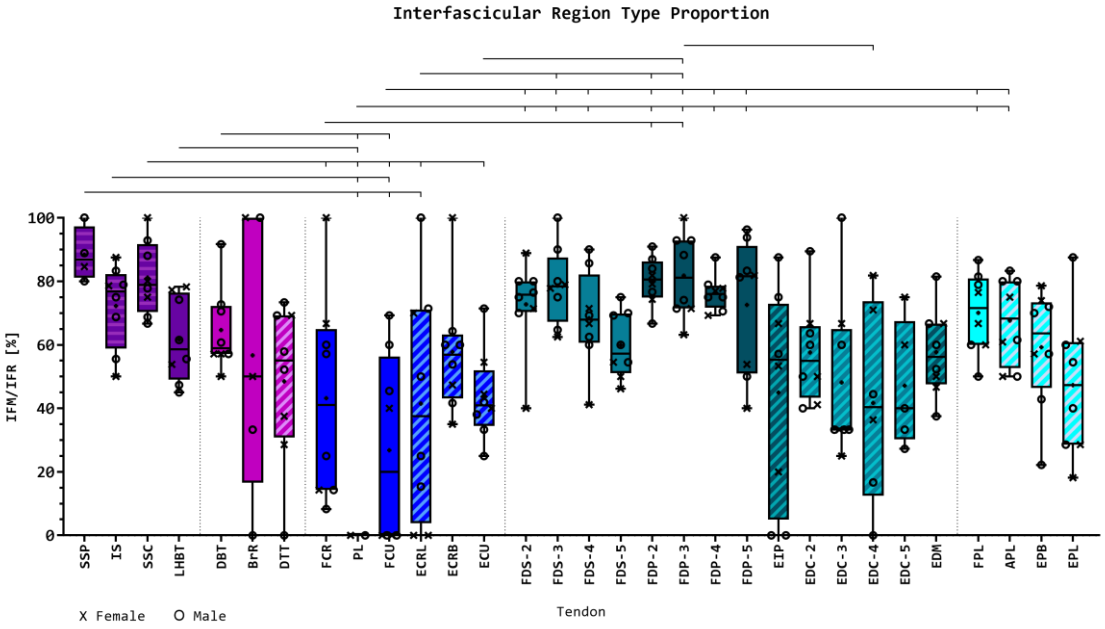


Figure 5.4.M Interfascicular Region Type Proportion of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Mixed-effects multilevel linear regression analysis with Sidak's adjustment. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

There was a strong significant effect of *Tendon* on IFR Type Proportion ($p < 0.0001$). Results were variable across the tendons, with the shoulder tendons and digital flexors showing some of the highest IFM proportions (Figure 5.4.M). The SSP had the highest IFM/IFR proportion (mean 88.38%), while the PL shows no IFM presence (mean 0.0 %, Figure 5.4.M). When comparing between tendons, varied and numerous significant differences in IFR Type Proportion across the upper limb tendons were found as detailed in Table 5.4.p.

Table 5.4.p Interfascicular Region Type Proportion pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Sidak correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis accounts for model covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.d.

Graph Position	Tendon Comparison	Contrast	Summary	Adjusted P Value	95% Confidence Interval	
1 vs. 9	SSP vs. PL	-85.631	***	0.0001	-149.775	-21.487
1 vs. 10	SSP vs. FCU	-61.246	***	0.0001	-106.619	-15.873
1 vs. 11	SSP vs. ECRL	-46.605	*	0.0326	-91.978	-1.232
2 vs. 9	IS vs. PL	-69.756	**	0.0021	-128.548	-10.965
2 vs. 10	IS vs. FCU	-45.372	***	0.0009	-82.209	-8.534
3 vs. 8	SSC vs. FCR	-37.917	*	0.0315	-74.755	-1.080
3 vs. 9	SSC vs. PL	-78.681	***	0.0001	-137.472	-19.890
3 vs. 10	SSC vs. FCU	-54.296	****	<0.0001	-91.133	-17.458
3 vs. 11	SSC vs. ECRL	-39.655	*	0.0145	-76.492	-2.817
3 vs. 13	SSC vs. ECU	-37.534	*	0.0372	-74.371	-0.696
4 vs. 9	LHBT vs. PL	-59.179	*	0.0451	-117.970	-0.388
5 vs. 9	DBT vs. PL	-62.190	*	0.0198	-120.981	-3.399
5 vs. 10	DBT vs. FCU	-37.805	*	0.0331	-74.643	-0.967
8 vs. 18	FCR vs. FDP-2	36.844	*	0.0499	0.007	73.682
8 vs. 19	FCR vs. FDP-3	38.541	*	0.0240	1.703	75.378
9 vs. 14	PL vs. FDS-2	70.273	**	0.0018	11.482	129.064
9 vs. 15	PL vs. FDS-3	76.166	***	0.0003	17.375	134.957
9 vs. 16	PL vs. FDS-4	65.889	**	0.0068	7.098	124.680
9 vs. 18	PL vs. FDP-2	77.608	***	0.0002	18.817	136.399
9 vs. 19	PL vs. FDP-3	79.304	***	0.0001	20.513	138.095
9 vs. 20	PL vs. FDP-4	73.920	***	0.0005	15.129	132.711
9 vs. 21	PL vs. FDP-5	70.086	**	0.0019	11.295	128.877
9 vs. 28	PL vs. FPL	67.578	**	0.0041	8.787	126.369
9 vs. 29	PL vs. APL	65.142	**	0.0085	6.351	123.933
10 vs. 14	FCU vs. FDS-2	45.888	***	0.0007	9.050	82.726
10 vs. 15	FCU vs. FDS-3	51.781	****	<0.0001	14.943	88.618
10 vs. 16	FCU vs. FDS-4	41.504	**	0.0061	4.666	78.342
10 vs. 18	FCU vs. FDP-2	53.223	****	<0.0001	16.385	90.061
10 vs. 19	FCU vs. FDP-3	54.919	****	<0.0001	18.082	91.757
10 vs. 20	FCU vs. FDP-4	49.535	***	0.0001	12.698	86.373
10 vs. 21	FCU vs. FDP-5	45.701	***	0.0007	8.863	82.539
10 vs. 28	FCU vs. FPL	43.193	**	0.0027	6.356	80.031
10 vs. 29	FCU vs. APL	40.757	**	0.0087	3.919	77.595
11 vs. 15	ECRL vs. FDS-3	37.140	*	0.0440	0.302	73.977
11 vs. 18	ECRL vs. FDP-2	38.582	*	0.0236	1.744	75.420
11 vs. 19	ECRL vs. FDP-3	40.278	*	0.0109	3.441	77.116
13 vs. 19	ECU vs. FDP-3	38.157	*	0.0284	1.319	74.995
19 vs. 25	FDP-3 vs. EDC-4	-40.372	*	0.0406	-80.212	-0.531

5.4.5.2.1 Donor Characteristics

Linear predictions found that IFR Type Proportion was not affected by any donor variables i.e. *sex* (p = 0.1631, Table 5.4.q), *age*, *height* or *weight* (p = 0.3674, p = 0.9672 and p = 0.4692 respectively, Table 5.4.r).

Table 5.4.q Interfascicular Region Type Proportion main effects linear predictions for *sex* from mixed-effects linear regression analysis of 31 different human upper limb tendons.

Outcome	Comparison	Contrast	p-value	95% Confidence Interval	
IFR Frequency	Female (reference) vs. Male	4.832	0.1631	-1.959	11.622

Table 5.4.r Interfascicular Region Type Proportion main effects linear predictions for *age*, *height* and *weight* from mixed-effects linear regression analysis of 31 different human upper limb tendons.

Outcome	Variable	*dx*	dy/dx	p-value	95% Confidence Interval	
IFR Type Proportion	Age	*Years*	0.321	0.3674	-0.378	1.021
	Height	*Inches*	-0.029	0.9672	-1.412	1.354
	Weight	*Pounds*	0.116	0.4692	-0.198	0.429

5.4.6 Crimp Characteristics

5.4.6.1 *Crimp Absence*

The Crimp Absence score assessed the presence or loss of crimping structure within the tendon sample area utilising polarised light on PSR-stained sections (Figure 5.4.N). A higher score indicates a greater area of the tendon with an absence of crimp. A score of one (1) indicates a crimping waveform was present in nearly all the tendon area, whilst a score of six (6) indicates a complete absence of crimp.

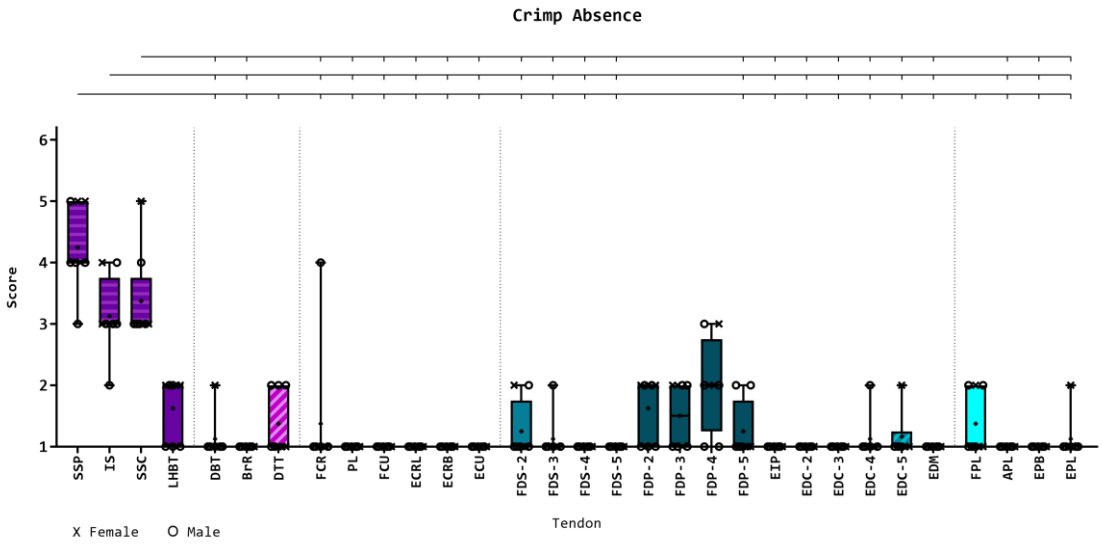


Figure 5.4.N Crimp Absence scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tendon* on Crimp Absence score ($p < 0.0001$). The majority of tendons showed a strong presence of crimp throughout (score 1; Figure 5.4.N). In contrast, the rotator cuff tendons (SSP, IS and SSC)

exhibited the highest absence of crimp of all tendons. When comparing between tendons, all three rotator cuff tendons had significantly higher absence of crimping compared to 22 other tendons of the upper limb (two elbow tendons: DBT, BrR; all six wrist tendons: FCR, PL, FCU, ECRL, ECRB, ECU; all superficial digital flexors: FDS-2, FDS-3, FDS-4, FDS-5; one deep digital flexor: FDP-5; all digital extensors: EIP, EDC-2, EDC-3, EDC-4, EDC-5, EDM; and three thumb tendons: APL, EPB and EPL. $p < 0.05$ for all comparisons, Table 5.4.s). The SSP additionally had significantly higher crimp absence compared to the elbow DDT and the thumb FPL tendons ($p = 0.0425$ for both, Table 5.4.s).

Table 5.4.s Crimp Absence pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.e.

Graph Position	Tendon Comparison	Mean rank difference	Summary	Adjusted P Value
1 vs. 5	SSP vs. DBT	132.1	***	0.0004
1 vs. 6	SSP vs. BrR	145.9	****	<0.0001
1 vs. 7	SSP vs. DTT	104.6	*	0.0425
1 vs. 8	SSP vs. FCR	127.8	***	0.0008
1 vs. 9	SSP vs. PL	145.9	****	<0.0001
1 vs. 10	SSP vs. FCU	145.9	****	<0.0001
1 vs. 11	SSP vs. ECRL	145.9	****	<0.0001
1 vs. 12	SSP vs. ECRB	145.9	****	<0.0001
1 vs. 13	SSP vs. ECU	145.9	****	<0.0001
1 vs. 14	SSP vs. FDS-2	118.4	**	0.0044
1 vs. 15	SSP vs. FDS-3	132.1	***	0.0004
1 vs. 16	SSP vs. FDS-4	145.9	****	<0.0001
1 vs. 17	SSP vs. FDS-5	145.9	****	<0.0001
1 vs. 21	SSP vs. FDP-5	118.4	**	0.0044
1 vs. 22	SSP vs. EIP	145.9	****	<0.0001
1 vs. 23	SSP vs. EDC-2	145.9	****	<0.0001
1 vs. 24	SSP vs. EDC-3	145.9	****	<0.0001
1 vs. 25	SSP vs. EDC-4	132.1	***	0.0004
1 vs. 26	SSP vs. EDC-5	127.5	**	0.0047
1 vs. 27	SSP vs. EDM	145.9	****	<0.0001
1 vs. 28	SSP vs. FPL	104.6	*	0.0425
1 vs. 29	SSP vs. APL	145.9	****	<0.0001
1 vs. 30	SSP vs. EPB	145.9	****	<0.0001
1 vs. 31	SSP vs. EPL	132.1	***	0.0004
2 vs. 5	IS vs. DBT	120	**	0.0034
2 vs. 6	IS vs. BrR	133.8	***	0.0003
2 vs. 8	IS vs. FCR	115.6	**	0.0071
2 vs. 9	IS vs. PL	133.8	***	0.0003
2 vs. 10	IS vs. FCU	133.8	***	0.0003
2 vs. 11	IS vs. ECRL	133.8	***	0.0003
2 vs. 12	IS vs. ECRB	133.8	***	0.0003
2 vs. 13	IS vs. ECU	133.8	***	0.0003
2 vs. 14	IS vs. FDS-2	106.3	*	0.0330
2 vs. 15	IS vs. FDS-3	120	**	0.0034
2 vs. 16	IS vs. FDS-4	133.8	***	0.0003
2 vs. 17	IS vs. FDS-5	133.8	***	0.0003
2 vs. 21	IS vs. FDP-5	106.3	*	0.0330
2 vs. 22	IS vs. EIP	133.8	***	0.0003

2 vs. 23	IS vs. EDC-2	133.8	***	0.0003
2 vs. 24	IS vs. EDC-3	133.8	***	0.0003
2 vs. 25	IS vs. EDC-4	120	**	0.0034
2 vs. 26	IS vs. EDC-5	115.4	*	0.0300
2 vs. 27	IS vs. EDM	133.8	***	0.0003
2 vs. 29	IS vs. APL	133.8	***	0.0003
2 vs. 30	IS vs. EPB	133.8	***	0.0003
2 vs. 31	IS vs. EPL	120	**	0.0034
3 vs. 5	SSC vs. DBT	123.8	**	0.0017
3 vs. 6	SSC vs. BrR	137.5	***	0.0001
3 vs. 8	SSC vs. FCR	119.4	**	0.0037
3 vs. 9	SSC vs. PL	137.5	***	0.0001
3 vs. 10	SSC vs. FCU	137.5	***	0.0001
3 vs. 11	SSC vs. ECRL	137.5	***	0.0001
3 vs. 12	SSC vs. ECRB	137.5	***	0.0001
3 vs. 13	SSC vs. ECU	137.5	***	0.0001
3 vs. 14	SSC vs. FDS-2	110	*	0.0181
3 vs. 15	SSC vs. FDS-3	123.8	**	0.0017
3 vs. 16	SSC vs. FDS-4	137.5	***	0.0001
3 vs. 17	SSC vs. FDS-5	137.5	***	0.0001
3 vs. 21	SSC vs. FDP-5	110	*	0.0181
3 vs. 22	SSC vs. EIP	137.5	***	0.0001
3 vs. 23	SSC vs. EDC-2	137.5	***	0.0001
3 vs. 24	SSC vs. EDC-3	137.5	***	0.0001
3 vs. 25	SSC vs. EDC-4	123.8	**	0.0017
3 vs. 26	SSC vs. EDC-5	119.2	*	0.0172
3 vs. 27	SSC vs. EDM	137.5	***	0.0001
3 vs. 29	SSC vs. APL	137.5	***	0.0001
3 vs. 30	SSC vs. EPB	137.5	***	0.0001
3 vs. 31	SSC vs. EPL	123.8	**	0.0017

5.4.6.2 *Crimp Wavelength*

The Crimp Wavelength score assessed the length of the predominant crimping structure within the tendon sample area utilising polarised light on PSR-stained sections (Figure 5.4.O). A higher score indicates a longer crimp wavelength, where a score of one (1) indicates crimp ≤ 25 µm in length, while greater scores indicate longer waves (non-linear range brackets).

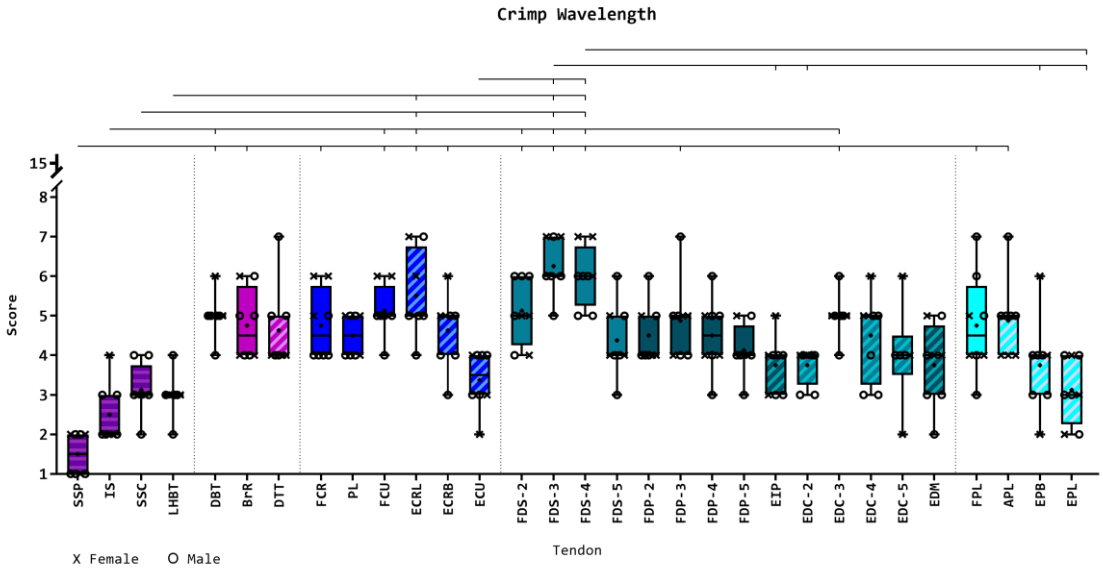


Figure 5.4.O Crimp Wavelength scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female O = Male. *Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tendon* on Crimp Wavelength score ($p < 0.0001$). The human upper limb tendon dataset achieved Crimp Wavelength scores between 1 (≤ 25 µm) and 7 (151-200 µm), with no tendons scoring higher than a 7 indicating a maximum crimp wavelength of 200 µm for the included human upper limb tendon dataset (Figure 5.4.O). The SSP had the lowest mean score value of 1.5 indicating average crimp lengths ranging between < 25 µm to 50 µm long. The SSP was also the only tendon to achieve a score of 1 indicating crimp ≤ 25 µm long (Figure 5.4.O). The shoulder tendons (SSP, IS, SSC and LHBT) and thumb extensors (EPB, EPL) generally exhibited some of the shortest wavelengths, while the 3rd and 4th digit superficial flexors (FDS-3, FDS-4) exhibited some of the longest wavelengths (Figure 5.4.O). When comparing between tendons, the shoulder tendons had significantly shorter ($p < 0.05$) wavelengths compared to 13, 7, 3 and 3 other tendons respectively for the SSP, IS, SSC and LHBT, while the FDS-3 and FDS-4 had significantly longer ($p < 0.05$) wavelengths compared to 9 and 6 other tendons respectively (Figure 5.4.O, Table 5.4.t).

Table 5.4.t Crimp Wavelength pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.e.

Graph Position	Tendon Comparison	Mean rank difference	Summary	Adjusted P Value
1 vs. 5	SSP vs. DBT	-162.8	**	0.0010
1 vs. 6	SSP vs. BrR	-140.4	*	0.0210
1 vs. 8	SSP vs. FCR	-140.4	*	0.0210
1 vs. 10	SSP vs. FCU	-168.7	***	0.0004
1 vs. 11	SSP vs. ECRL	-179.5	****	<0.0001
1 vs. 12	SSP vs. ECRB	-136.4	*	0.0341
1 vs. 14	SSP vs. FDS-2	-165.2	***	0.0007
1 vs. 15	SSP vs. FDS-3	-215.1	****	<0.0001
1 vs. 16	SSP vs. FDS-4	-206.8	****	<0.0001
1 vs. 19	SSP vs. FDP-3	-146.3	**	0.0098
1 vs. 24	SSP vs. EDC-3	-162.8	**	0.0010
1 vs. 28	SSP vs. FPL	-135.4	*	0.0388
1 vs. 29	SSP vs. APL	-146.3	**	0.0098
2 vs. 5	IS vs. DBT	-140.3	*	0.0211
2 vs. 10	IS vs. FCU	-146.3	**	0.0099
2 vs. 11	IS vs. ECRL	-157.1	**	0.0023
2 vs. 14	IS vs. FDS-2	-142.8	*	0.0155
2 vs. 15	IS vs. FDS-3	-192.7	****	<0.0001
2 vs. 16	IS vs. FDS-4	-184.3	****	<0.0001
2 vs. 24	IS vs. EDC-3	-140.3	*	0.0211
3 vs. 11	SSC vs. ECRL	-136.6	*	0.0333
3 vs. 15	SSC vs. FDS-3	-172.3	***	0.0003
3 vs. 16	SSC vs. FDS-4	-163.9	***	0.0009
4 vs. 11	LHBT vs. ECRL	-144.1	*	0.0131
4 vs. 15	LHBT vs. FDS-3	-179.7	****	<0.0001
4 vs. 16	LHBT vs. FDS-4	-171.3	***	0.0003
13 vs. 15	ECU vs. FDS-3	-157.4	**	0.0022
13 vs. 16	ECU vs. FDS-4	-149	**	0.0069
15 vs. 22	FDS-3 vs. EIP	137.3	*	0.0309
15 vs. 23	FDS-3 vs. EDC-2	139.3	*	0.0241
15 vs. 30	FDS-3 vs. EPB	134.6	*	0.0428
15 vs. 31	FDS-3 vs. EPL	168.1	***	0.0005
16 vs. 31	FDS-4 vs. EPL	159.7	**	0.0016

5.4.6.3 *Crimp Wavelength Variation*

The Crimp Wavelength Variation score assessed the proportion of tendon area exhibiting different crimp wavelength patterns relative to the predominant wavelength scored previously (Crimp Wavelength, *Section 5.4.6.2)*, utilising polarised light on PSR-stained sections (Figure 5.4.P). A higher score indicates a greater area of the tendon with differing crimp wavelengths. A score of zero (0) indicates only one crimp wavelength (as per score ranges for Crimp Wavelength; *refer to Table 4.5.g*) is present in the tendon area, whilst a score of four (4) indicates that the predominant crimp wavelength (as scored) constitutes < 40% of the tendon area.

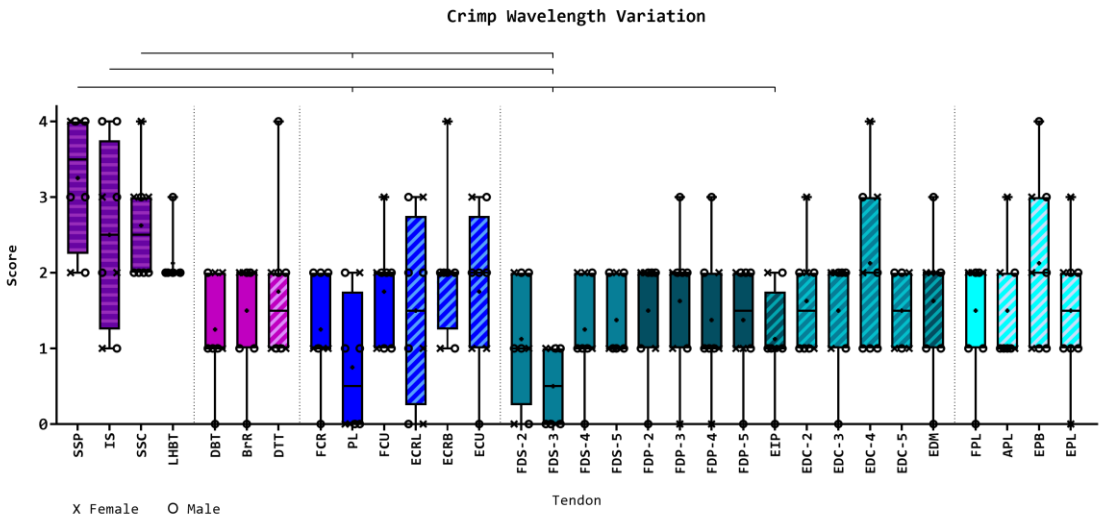


Figure 5.4.P Crimp Wavelength Variation scores of 31 different human upper limb tendons. *Refer to Table 5.3.a for identification of tendon acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon differences indicated by overhead horizonal lines with the tendon at the left origin significantly different to all tendons marked by downward ticks. All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 5.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a significant effect of *Tendon* on Crimp Wavelength Variation score ($p = 0.0001$). The rotator cuff tendons (SSP, IS, and SSC) exhibited the highest variation of crimp wavelength (Figure 5.4.P). When comparing between tendons, the SSP, IS and SSC all had significantly higher proportion of variable crimp than FDS-3 ($p < 0.0001$, $p = 0.0349$ and $p = 0.0021$ respectively). The SSP and SSC also had significantly higher levels of variable crimp wavelengths than the PL ($p = 0.0026$ and $p = 0.0455$ respectively). Furthermore, the SSP had significantly higher variability than the EIP ($p = 0.0415$; Table 5.4.u).

Table 5.4.u Crimp Wavelength Variation pairwise comparisons between 31 different human upper limb tendons for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 5.3.a for identification of tendon acronyms*. Full list of pairwise comparisons can be found in Supp. Table 9.6.e.

Graph Position	Tendon Comparison	Mean rank difference	Summary	Adjusted P Value
1 vs. 9	SSP vs. PL	152.4	**	0.0026
1 vs. 15	SSP vs. FDS-3	175.6	****	<0.0001
1 vs. 22	SSP vs. EIP	131.4	*	0.0415
2 vs. 15	IS vs. FDS-3	132.8	*	0.0349
3 vs. 9	SSC vs. PL	130.6	*	0.0455
3 vs. 15	SSC vs. FDS-3	153.9	**	0.0021

5.5 Discussion

The current study uncovered interesting findings relating to variations in human upper limb tendons as explored through histological assessment. While outcomes are varied and diverse across the tissues, this work highlights some trends not previously captured. Notably, there seems to be a disagreement between accepted definitions of "normal" tendon and the characteristics observed in the current investigation exploring typical features in normal (healthy, non-pathological) tendons of the upper limb.

5.5.1 Structural Variation of Upper Limb Tendons

5.5.1.1 *Cell Characteristics*

Generally, the literature defines tenocyte populations as relatively sparse, elongated, spindle-shaped cells regularly arranged longitudinally between the parallel fibres[101,120,241,242]. From the current investigation, it is interesting to note that this definition is not applicable to all tendons / tendon groups. In clinically normal upper limb tendons, there were instances of varied cellular morphologies and phenotypes (Figure 5.4.B and Figure 5.4.D), as well as diverse arrangement patterns of cells (Figure 5.4.C).

Tendons conforming to "typical" as per standard literature definitions include those of the elbow, wrist and digital extensors of the hand, most of which portrayed relatively low but consistent cell densities (Figure 5.4.A) with dispersed arrangements (Figure 5.4.C), predominated by spindle-shaped nuclei (Figure 5.4.B) and with fewer incidences of atypical/chondrocyte-like cell phenotypes (Figure 5.4.D). Conversely, tendons of the rotator cuff strayed from the 'normal' spindle-shape population, containing higher levels of cigar-shaped or rounded cells and with a much higher prevalence of atypical/chondrocyte-like cell phenotypes (Figure 5.4.B and Figure 5.4.D).

An interesting observation is the lack of rounded nuclei and atypical-phenotype/chondrocyte-like cells within the digital extensor tendons. However, this might be a limitation of the tissue procurement methods and the region at which the samples were collected from the tendon. Protocols adopted had the respective tendons incised between the MTJ and metacarpal-phalangeal joint, excluding the region of tendon as it passes along the phalanges and traverses over the interphalangeal joints where it experiences compressive loading. Should the tissue from the EDC digital branches have been collected from the phalangeal region as opposed to the metacarpal region, structural characteristics pertaining to cellular properties may have found different results due to the compressive and shear loads experienced in this region[118,226,243]. Conversely, the high fibrocartilaginous-like features present in the rotator cuff tendons (being the aforementioned cellular feature along with higher proteoglycan presence) could be expected considering the short length of these tendons and that these features are typically near bony-attachments as the tendon transitions to fibrocartilage near the enthesis[201,237].

5.5.1.2 *Architectural and Crimp Characteristics*

Tendon has been typically defined by its dense, regular collagenous architecture – a structure consisting of homogenous collagen bundles and expressing a planar waveform known as crimp[70,74,120,169]. However, histological assessment of a wide range of normal upper limb tendons found various depictions of tendon structural attributes.

The majority of the upper limb tendons fit this generalised definition with the collagenous architecture predominantly reflecting "highly unified/parallel collagenous structure aligned along the primary tendon axis" (*refer to Table 4.5.b*), scoring between zero and two (0-2; i.e. <25% of the collagenous structure is disorganised) in the Collagenous Organisation outcome (Figure 5.4.G). Standouts scoring much higher on the scale were the rotator cuff tendons (Figure 5.4.G), which also had higher Collagen Alignment scores (Figure 5.4.J) indicating loss of alignment of the collagenous matrix, as well as higher Crimp Absence scores (Figure 5.4.N) indicating greater areas of tendon not containing the typical waveform characteristic. These features may indicate different structure-function attributes of these tendons, however there might be introduced bias due to the difficulty differentiating between FM and IFR, where IFRs with prominent matrix (that is, IFM) were unavoidably captured in overall scoring of "tendon area" (*refer to Section 4.4.6.2*). Notably, these rotator cuff tendons do present with relatively higher presence of IFRs (high IFR Frequency outcome, Figure 5.4.L) with a higher IFM:IFS ratio (IFR Type Proportion outcome, Figure 5.4.M), likely contributing the aforementioned architecture scores more to IFR related biomechanical influences[8,227,228,230,244] as opposed to FM variations.

One of the most widely used scoring system for the assessment of tendinopathy (the Movin system[169]) proposes that the longitudinal segregation of fibres from one another (defined here as 'fibre separations') precedes the appearance of transverse tearing of fibres (as would be expected to occur in the case of a ruptured tendon)[159]. These separations are considered a foundation of tendinopathy, which in itself is a precursor for tendon rupture[162]. Whilst Longo *et al.* (2008)[190] found ruptured SSP tendons (biopsy samples of macroscopically intact portion of ruptured tendon; patient mean age = 58.2 years; females and males) to have greater prevalence of scoring higher for abnormal fibre structure (inclusive of the fibre separation characteristic) with a modified Movin system. They also observed that control SSP tendons (post-mortem collection of tendon from deceased patients; mean age = 69.6 years; males only) presented with cases of separations between the fibre structures, not always scoring zero (0) as "normal fibre structure", possibly alluding to degeneration of the structure with the older age of the control tendons. Although interestingly, the rotator shoulder tendons actually present with some of the fewest fibre separations (Figure 5.4.I) despite these tendons ranking high in regard to the incidence of tendinopathy/injury[23]. In contrast, extensor tendon of the hand, which are more commonly associated with traumatic injury as opposed to progressive pathological change[245-247], typically presented with a higher frequency of fibre separations (Figure 5.4.I). With the "pathology prone" rotator cuffs scoring lowest in the current study, there lies room for challenging current perceptions of typically defined "abnormal" features actually being indications of pathology, and highlighting the importance of defining "normal" in different tendons, or in association with other variables such as normal aging processes.

Where the current investigation stands above previous research is the inclusion of more quantitative individual outcomes which allows us to isolate different features observed in tendon structure, rather than grouping general features in a scale with descriptive trends alluded to by the different scores. In the current study of normal tendons, the SSP stood out and scored highest for Fibrillar Loosening, reflecting loss of the distinct fibre structure and separation of the fibrils from one another. This was also described by Longo *et al.* (2008)[190] as being prevalent in the pathological SSP, however this outcome is only descriptively alluded to along with descriptions of fibre separations and waviness (crimp) in the discussion of their 'Fibre Structure' score the scaled from '0 = normal appearance' to '3 = markedly abnormal appearance'. By segregating different features into separate scored/semi-quantified outcomes, the current scoring system allows for more in-depth characterisation of tendons variability, but also for later exploration into correlations with particular features versus tendon attributes, or between outcomes themselves. Unfortunately, observations between Fibre Separation and Fibril Loosening trends from the current investigation cannot be explored due to the use of different ROI locations for these two scores. While small ROIs were chosen for the current investigation due to the very large size of some of the tissue sections being explored, it would be useful for studies to include as much tissue area as practical (where time restraints etc. permit) to achieve a better assessment of typical features within the tendon area. This would also allow for ROI matching to explore links between different observations of the hierarchical organisation of the multi-level collagen bundles.

5.5.2 Cuffing the Culprits

Understanding the implications of variability between human upper limb tendons is an extensive topic which would require further exploration to unveil structure-function implications and the relevance of these results to clinical outcomes. However, one observation from the current study is noteworthy for highlighting. In most outcomes, the rotator cuff tendons (SSP, IS and SSC) – sometimes with the addition of the LHBT as the fourth shoulder tendon – tended to stand out as distinctly different structures from the rest of the upper limb tendons. This study is not the first to allude to this being so, where Blevins *et al.* (1997)[242] extensively describes the unique structural appearance of the rotator cuff tendon group against the typical understanding of tendon structure. The intricate nature of the cuff structure[242,248-250] accompanied by its unique and complex biomechanical role in shoulder stability and motion[251] is possibly one contributor to the rotator cuff tendons exhibiting such unique structural characteristics. For example, the layered nature of the tendon structures likely contributes to the higher incidence of IFM structures between the segregated FM regions[242,248]. Similarly, the interwoven nature of the four adjacent rotator cuffs (SSC, IS, SSC and Teres Minor; the latter was not included in the present investigation) made orientating the tissue for histological embedding particularly challenging and almost certainly affected assessment of the collagenous architecture outcomes. Although, the higher Collagenous Organisation (Figure 5.4.G) and Collagen Alignment (Figure 5.4.J) scores of the rotator cuff tendons may not only be linked to their anatomical arrangement where sections capture the overlapping structural layers, but also tied to their functional requirements. These tendons not only engage shoulder motion (as typical of tendons) but also act to stabilise the shoulder joint (as typical of ligaments)[252,253]. This ball-and-socket joint exhibits spherical and rotational mobility,

and the 'disorganised' and 'unaligned' – or rather, multidirectional – microstructure of the collagenous matrix may support the rotator cuff complex in its requirement to guide and resist forces in varying and multiple directions. Additionally, the shorter length of these tendons relative to others of the upper limb meant the histology samples were relatively close to the enthesis zone at the bone attachment, possibly at least in part explaining the fibrocartilaginous architecture observed with the higher presence of proteoglycan staining (Figure 5.4.E) and atypical/chondrocyte-like cell phenotypes (Figure 5.4.D). (Note: another perspective of the shoulder tendon observations is presented in *Chapter 7 ~ Section 7.4.1.2* regarding a caveat of the study design during sample retrieval relating to the proximal-distal origin and insertion of the shoulder tendons [rotator cuffs and LHBT] versus the other tendons).

The high prevalence of rotator cuff disease/injury is a consideration for further exploration of these the results obtained from the current investigation[23,27,239,254]. Particularly as the incidence of rotator cuff injury/disease is present both in symptomatic and non-symptomatic shoulders[239,255], understanding the implications of the unique structural attributes of these tendons is important to defining causal relationships between structural changes and the risk of pathology. While the tissue for the current project was collected from a licenced donor tissue facility (Science Care Inc) with a proviso of no history of musculoskeletal injury or disease and were checked microscopically by the surgeon for signs of damage/injury/disease, there is no guarantee that the samples present what is "normal" for shoulder tendons or perhaps include asymptomatic pathological tendon without macroscopic manifestation. Additionally, the age of the donors included in this study (*refer to Table 3.2.a*) match the cohort range typically expected for increased pathophysiological findings of rotator cuff disease[255] and greater incidence of tears[239,256]. However, association between pathological change and normal age-associated changes is poorly understood. Ultimately, a wider range of donors would be needed for this assessment.

Another important consideration is the tissue collection of the specimens for this study. While trained orthopaedic surgeons with expert anatomical knowledge (DO) isolated the tendon-muscle structure from the cadavers, the structural complexity of the rotator cuff remains a challenge to isolating the tendons as distinct structures. As the full connective tissue structure of the cuff were included in histological processing for each divided tendon (SSP, IS and SSC), the incorporation of other fibrous tissue such as capsular tissue or fused ligaments in the samples used for assessment was unavoidable due to the integration of these structure within one another. Where histological observations may have been a tool to distinguish the precise tendon region within the section, the investigator was blinded to sample ID and was thus not at liberty to exclude fibrous collagenous architecture in regions where this may be expected, which may or may not have been designated as 'proper' tendinous tissue.

5.5.3 Future Improvements

5.5.3.1 *Regional Variations*

As biomechanical actions at different anatomical regions differ – for example, loading differences of digital tendons in the wrist region verses as they track over the digital joints – associating the structural variations throughout different regions of individual tendons also plays an important

role in understanding function of the tissue. Differing incidence of positive long-term outcomes from tendon repair between proximal (63-83% of cases) versus distal (<50%, significance level $p < 0.001$) zones identifies the non-uniform response of the EDC tendons to injury/repair[257]. While biomechanical influence may contribute to explaining these findings, variations in underlying structural properties of the tendon in these regions may also be a factor for understanding responses of the tissue to clinical injury/response scenarios.

The current study design did not facilitate exploration into broader regional variations as the protocols only allowed for a single sample (approximately 10-20mm long x the width of the tendon) to be collected from each tendon. Even within these single samples of tendon however, the novel scoring matrix identified the non-homogenous nature of specific aspects of tendon structure such as Cell Distribution (scored from dispersed ROIs), and Crimp Wavelength Variation assessed across whole-sections. Full exploration into structure-function relationships would benefit from future studies characterising the full tendon structure of a range of tendons using consistent methodology.

5.5.3.2 *Histological Limitations*

A primary limitation of histological evaluation is the ability to assess 3-dimentional (3D) structure within a 2D histological section[258]. For example, scoring the presence (Fibre Separations Frequency) and length (Fibre Separations Type) of separations between fibres (Figure 5.4.I) is complicated by sectioning orientation. Longitudinal separations between fibres – especially in cases where the tendon exhibits distinct crimping waveforms – has the potential to appear in and out of the plane of section and influence assessment of the structure. Furthermore, Elliott (1965)[70] expressed theories on the twisting nature of collagen bundles (fibres) and the criss-crossing nature of the fibrils intertwining between the fibre structures, features that would influence the ability to capture the true nature of the separations between fibres in a two-dimensional histological section. Further work is needed to explore the 3D arrangement of the tendon structure to inform better histological evaluation. Exploration of serial sections, utilisation of other modalities (such as scanning electron microscope [SEM], micro-computed tomography [micro CT]), or the exploration of alternate planes (transverse observations) could aid definition of the normal and varied 3D structures of these tissue to advise better 2D characterisation.

5.5.4 Conclusion

The current study explored structural variations of thirty-one human upper limb tendons using histological assessment. Findings uncovered heterogeneity between the structure of these tissues not previously identified in current literature. This was particularly evident for rotator cuff tendons which often present with quite distinct characteristics. Importantly, widely accepted definitions – particularly those pertaining to features of the cellular population and collagenous architecture – do not capture the scope of characteristics observed in the current study of apparently healthy tendons. While deeper exploration into intra-tendon heterogeneity and detailing region-specific variations is needed, this study challenges current standards of “normal” tendon characteristics.

~ CHAPTER 6 ~

GETTING ANOTHER LEG UP IN TENDON RESEARCH

HISTOLOGICAL CHARACTERISATION OF HUMAN TENDONS (AND LIGAMENTS) OF THE LOWER LIMB

"That's one trouble with dual identities, Robin. Dual responsibilities."

~ Bruce Wayne/Batman, Batman (TV series, 1966-68) ~

6.1 Overview

The following chapter explores the histological characterisation of seventeen different tendons and ligaments of the human lower limb, including those of the knee, ankle and foot regions. Specimens are assessed using the novel histological scoring system as per *Chapter 4*.

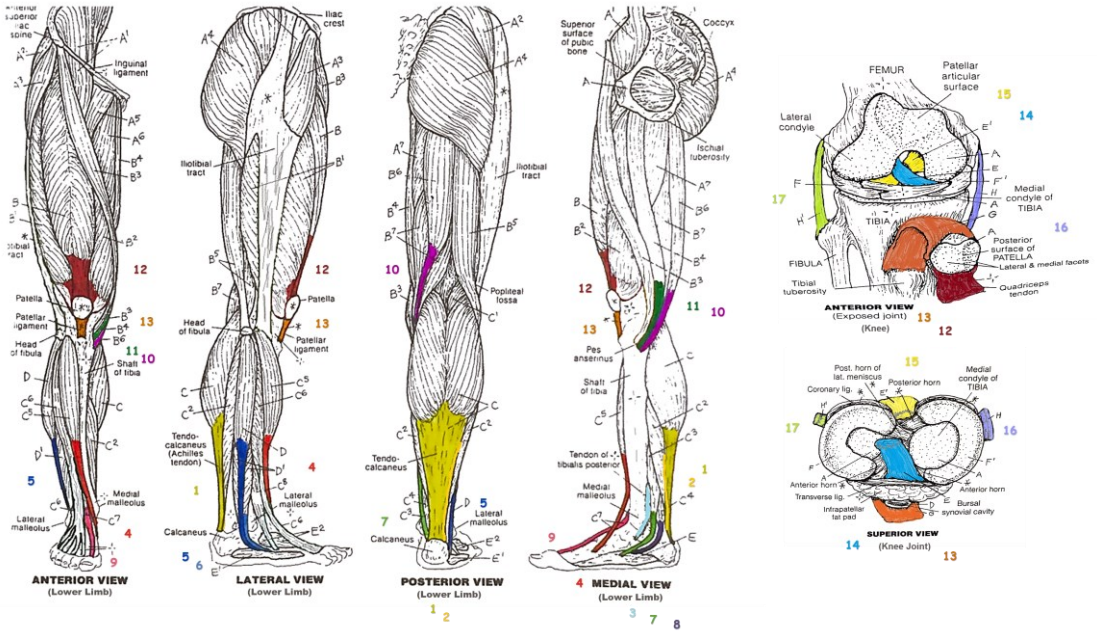


Figure 6.1.A Tendons of the lower limb. Image adapted from Kapit and Elson (2014)[236]. *Highlighted tissue reflect: 1 = Achilles Tendon, 2 = Plantaris tendon, 3 = Tibialis Posterior, 4 = Tibialis Anterior, 5 = Fibularis (peroneus) Longus, 6 = Fibularis (peroneus) Brevis, 7 = Flexor Digitorum Longus, 8 = Flexor Hallucis Longus, 9 = Extensor Hallucis Longus, 10 = Semitendinosus Tendon, 11 = Gracilis Tendon, 12 = Quadriceps Tendon, 13 = Patellar Tendon, 14 = Anterior Cruciate Ligament, 15 = Posterior Cruciate Ligament, 16 = Medial Collateral Ligament, 17 = Lateral Collateral Ligament.*

6.2 Introduction

Tendons play a major role in normal musculoskeletal functioning. Injury, pathology or disease of tendons can impair function and may require surgical repair or reconstruction. Characterisation of "normal" tendon structure is important for understanding mechanisms and progression of pathology and healing, but also for understanding native properties to aid the selection or development of biological or biomimetic grafts. While *Chapter 2* found that current research on the structural characterisation of human tendons chiefly explores tendons of the lower as opposed to upper limb, it is noted that these studies predominantly focus on those tendons that are most commonly used as grafts in ACL reconstruction (Patellar, Quadriceps, and Hamstring tendons). Thus, exploration into a broader range of human lower leg tendons will not only increase fundamental knowledge of tendon physiology but potentially expand the pool of tendons considered for use as grafts[127,259].

6.2.1 Lower Limb Tendons (and Ligaments)

As with the upper limb (*Chapter 5*), tendons of the lower limb are prone to injury, pathology and disease[23,25,160,162]. Clark *et al.* (2020)[23] reported 43.7% of cases from a 6-year retrospective population-based study (during 2011-2016) of tendon/ligament injuries in Aotearoa/New Zealand were attributed to lower limb regions, costing over 42% of total claim costs. Two studies which examined diagnoses of tendon injuries presented to the National Institute of Traumatology, Budapest, Hungary, between the late 1960's to 1990 saw spontaneous rupture of the Achilles Tendon to be the most common scenario[160,162]. Similarly, Clayton and Court-Brown (2008)[25] saw Achilles Tendon rupture as the most prevalent lower limb tendon/ligament injury presenting to the Royal Infirmary of Edinburgh, Scotland, between 1996 and the year 2000, constituting 10.7% of all soft tissue injuries, with ACL (7.6%) and MCL (4.9%) injury being the next highest for lower limb cases. Unlike tendons of the shoulder region in the upper limb, the Achilles, ACL and MCL injuries typically present at a younger age[25].

Tendon and ligament injury can cause detrimental consequences to the proper functioning and mobility of joints and the musculoskeletal system. Tendon grafts are used in the orthopaedic field for the replacement of tendons and ligaments lost to rupture[40,41], lesions, traumatic injury[42,43] or cancer of or near these tissues[44-46]. While there are many operations of the lower limb which utilise tendon grafts, including Patellar[40], Quadriceps[260] and Achilles[61] tendon ruptures, one of the most common orthopaedic operations utilising graft replacements is anterior cruciate ligament (ACL) reconstruction surgery[261,262]. Australia has one of the highest ACL reconstruction rates globally, with over 10 000 primary reconstructions per year, costing in excess of AUD $75 million annually[263]. In the past couple of decades, this trend had been increasing[264].

There are a variety of lower limb tendons that are used as grafts in tendon/ligament reconstructive surgery. The Semitendinosus (ST)[47,48], Gracilis (GT)[48], Quadriceps (QT)[49-51], Patellar (PT)[50,52] tendons are most commonly used as graft tissues for ACL reconstruction. However, many other tendons have also been enlisted as either autografts (self-tissue) or allografts (human donor tissue), including the Achilles Tendon (AT)[53-55], Tibialis Posterior (TP)[56], Tibialis Anterior (TA)[54],

Fibularis [Peroneus] Longus (FL)[57], Fibularis [Peroneus] Brevis (FB)[58], Flexor Digitorum Longus (FDL)[59,60], Flexor Hallucis Longus (FHL)[56,61], Extensor Hallucis Longus (EHL)[62], and even the Plantaris (Plt) tendon[63-65].

Unfortunately, with the predominant focus of literature being skewed towards pathological assessment, and the emphasis of tendon characterisation research in the lower limb favouring exploration into the tissues most commonly used as grafts in ACL reconstructive surgery (i.e. PT, QT, ST and GT), there is a lack of information regarding the characterisation of other tendons in the lower limb. Not only is healthy tissue characterisation important to understand the pathology of these structures, but it is also critical to define normal structure and physiology of involved tissues to develop appropriate injury prevention and repair strategies.

Furthermore, the narrowed focus of normal tendon characterisation limits research supporting the inclusion of other tendons in clinical applications such as being used as grafts. Previous work from our lab has opened scope for the inclusion of more tendons in the pool of suitable graft tissues following biomechanical, morphological and biochemical assessment[127]. The research in the current chapter aims to expand on this previous work, by comparing histological structural characteristics of a range of different human tendons and ligaments of the lower limb.

6.3 Methodology

Detailed methodology pertaining to specimen collection, tissue processing, and the histological scoring assessment are found in *Chapter 3* and *Chapter 4* respectively. Additional information for the specific application of the scoring system is explained in *Chapter 5*. A brief overview of these methods plus protocols specific to the conduct of this investigation in the lower limb are described below.

6.3.1 Specimens

6.3.1.1 *Tissue (Tendon or Ligament) Inclusion*

Details of the tissue procurement and associated ethics approval for the current investigation are provided in *Chapter 3*. Briefly, thirteen (13) tendons and four (4) ligaments were retrieved from fresh-frozen cadaveric lower limbs (n = 8, mid-femur to toe) as outlined in Table 6.3.a. Donor ages ranged from 49 to 65 years old, with a mean age (± standard deviation) of 56.1 ± 5.8 years (males = 56.2 ± 6.1 years; females = 56.0 ± 6.6 years).

Table 6.3.a List of human lower limb tissue (tendon or ligament) names and abbreviations analysed in this study. *Graph Key* indicates colour coding for results graphs presented later in this chapter.

Region	(Sub-classification)	Tissue (Tendon or Ligament)	Abbreviation	Graph Key
Ankle	*Posterior Compartment Plantar Flexors*	Achilles Tendon	**AT**	
		Plantaris tendon	**Plt**	
		Tibialis Posterior	**TP**	
	Dorsi Flexor	Tibialis Anterior	**TA**	
	Lateral Compartment Plantar Flexors	Fibularis (Peroneus) Longus	**FL**	
		Fibularis (Peroneus) Brevis	**FB**	
Foot (Toe Digits)	*Flexors*	Flexor Digitorum Longus	**FDL**	
		Flexor Hallucis Longus	**FHL**	
	Extensor	Extensor Hallucis Longus	**EHL**	
Knee (Tendons)	*Flexors*	Semitendinosus Tendon	**ST**	
		Gracilis Tendon	**GT**	
	Extensors	Quadriceps Tendon	**QT**	
		Patellar Tendon	**PT**	
Knee (Ligaments)	*Anterior-Posterior Stabilisers*	Anterior Cruciate Ligament	**ACL**	
		Posterior Cruciate Ligament	**PCL**	
	Medial-Lateral Stabilisers	Medial Collateral Ligament	**MCL**	
		Lateral Collateral Ligament	**LCL**	

6.3.1.2 *Specimen Preparation*

As detailed previously (*Chapter 3*) formalin -fixed, paraffin embedded histological samples (longitudinal orientation, 4.5µm sections) of human tissue of the lower limb were used for assessment. Three sections of each tendon or ligament were utilised, one each for H&E-, TB- and PSR-staining, facilitating exploration of different structural tissue characteristics.

6.3.2 Histological Assessment

A novel scoring system for the histological characterisation of tendons (and ligaments) was developed as detailed in *Chapter 4* and used for the current study exploring the structural properties of different normal (healthy) tendons and ligaments of the lower limb. The investigator was blinded to *Tendon* and *Donor ID* (*refer to Section 3.4.1*).

The outcomes assessed are outlined in Table 6.3.b. Scanned images of histological slides of each stain were used to score all outcomes using 'brightfield' light modality approaches (NDP.view2Plus). Outcomes utilising 'polarised' light modality on PSR-stained sections were assessed directly from a microscope (Leica-DM750).

Table 6.3.b Scoring outcomes enlisted for the histological assessment of human lower limb tissues. IFM = Interfascicular Matrix. IFS = Interfascicular Space. IFR = Interfascicular Region. ROI = Region of Interest. H&E = Haematoxylin and Eosin. TB = Toluidine Blue. PSR = Picrosirius Red. * Refer to the main text within *Chapter 5 ~ Section 5.3.2* for clarification of ROI details.

Outcome	Stain	Modality	Area Assessed	ROI (if applicable)	Protocol
Dell Density	H&E	Brightfield	3 x ROIs	200 µm x 200 µm; 0.04 mm^2	Average of ROIs
Cell Distribution			3 x ROIs	200 µm x 200 µm; 0.04 mm^2	Max. – Min. of ROIs
Cell Morphology			3 x ROIs	200 µm x 200 µm; 0.04 mm^2	Mode of ROIs
Cell Arrangement			Whole section		
Collagenous Organisation			Whole section		
Fibrillar Loosening			1 x ROI	500 µm x 500 µm; 0.25 mm^2	
Fibre Separations Frequency			1 x ROI	500 µm	
Fibre Separations Type			1 x ROI	500 µm	
IFM Frequency	PSR		1 x ROI	Width of tendon sample*	
IFS Frequency			1 x ROI	Width of tendon sample*	
IFR Frequency			1 x ROI	Width of tendon sample*	Sum of *IFM* and *IFS Frequencies*
IFR Type Proportion			1 x ROI	Width of tendon sample*	Percentage of *IFM/IFR*
Adipose Presence	H&E		Whole section		
Proteoglycan Presence	TB		Whole section		
Proteoglycan Staining Intensity (Average)			Whole section		
Proteoglycan Staining Intensity (Maximum)			Whole section		
Atypical Phenotype Cell Presence			Whole section		
Collagen Alignment	PSR		Whole section		
Crimp Absence	PSR	Polarised	Whole section		
Crimp Wavelength			Whole section		
Crimp Wavelength Variation			Whole section		

Outcomes which assessed the 'whole section' evaluated all tendon/ligament tissue area, excluding that determined to be paratendinous tissue (e.g. paratenon/epitenon). Outcomes which utilised specified ROIs (areas, lines etc. – refer to Table 6.3.b) were done in a manner trying to best achieve consistency across samples of different lengths, widths and section areas. The reader is referred to *Chapter 5 ~ Section 5.3.2* for clarification of details regarding ROI placement and specifics.

6.3.3 Statistical Analysis

6.3.3.1 *Categorical Data*

Data from nominal and ordinal outcomes were analysed in GraphPad Prism (version 10.3.0 [507], GraphPad Software, LLC., California, USA). Differences between tendons were analysed using non-parametric Kruskal-Wallis tests followed by Dunn's test with a Bonferroni correction for multiple pairwise comparisons. Statistical significance was taken as an adjusted $p < 0.05$. Graphs were generated in GraphPad Prism. Due to limited donor numbers, the effects of donor variables (e.g. sex and age) was not analysable for categorical outcomes.

6.3.3.2 *Continuous Data*

Data from continuous (counted) outcomes were analysed in Stata (SE 15.1, StataCorp LLC, Texas, USA). Mixed-effects linear regression models were used to compare tendon-types, and

mean-centred donor characteristics were included as covariates. These included sex (females versus males), age (unit = year), height (unit = inch), and weight (unit = pound), using the original units provided by the tissue bank. A random intercept term was included to account for the non-independence of tendons retrieved from the same donor. Specific pairwise tendon comparisons were evaluated using Sidak's adjustment for multiple comparisons. Statistical significance was taken as an adjusted $p < 0.05$. Graphs were generated in GraphPad Prism.

6.4 Results

6.4.1 Specimens and Data

Seventeen (17) different tendons/ligaments of the lower limb from 8 donors (5 male, 3 female) were included in this study. A total of n = 133 samples were assessed as the Plt tendon was absent from two of the donors (one male, one female) and the Lateral Collateral Ligament (LCL) was absent from one donor (male). All other tissues were included from each donor (n = 8 per tendon/ligament). Unless otherwise specified, outcomes included all n = 133 datapoints. A breakdown of n-numbers per tendon/ligament per outcome is provided in Table 6.4.a. Within graphs, tendons and ligaments were grouped and colour coded as per the regions specified in Table 6.3.a. Plantar flexors, flexors, and ligaments are indicated by solid bars, whilst dorsi flexors and extensors are indicated by diagonal striped bars (Table 6.3.a). Tables of pairwise tissue comparisons in the main text show significant differences with adjusted p-values for each outcome. The complete list of pairwise tissue comparisons including non-significant comparisons can be found in *Appendix Section 9.7.*

Table 6.4.a Number of data points (n=) for each lower limb tendon/ligament by outcome. Refer to Table 6.3.a for tissue acronym identification. In general, all tissues n = 8 except n_{Plt} = 6 and n_{LCL} = 7. Bold numbers in pink-highlighted cells indicate reduced datapoints included in analysis because of reasons such as exclusion due to artefacts or "NS" (no score) as per scoring matrix guidelines, for example.

	AT	Plt	TP	TA	FL	FB	FDL	FHL	EHL	ST	GT	QT	PT	ACL	PCL	MCL	LCL
Cell Density	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Cell Distribution	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Cell Morphology	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Cell Arrangement	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Collagenous Organisation	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Fibrillar Loosening	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Fibre Separations Frequency	8	6	8	8	8	8	8	8	8	8	8	8	8	**7**	**7**	**7**	7
Fibre Separations Type	**7**	6	**4**	**6**	**5**	**7**	**4**	**7**	**5**	8	**5**	**7**	**7**	**0**	**4**	**6**	**2**
Adipose Presence	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Proteoglycan Presence	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Proteoglycan Staining Intensity (Average)	**6**	**1**	**7**	**3**	**5**	**6**	**2**	**6**	**1**	**7**	**3**	8	**7**	8	8	**7**	**5**
Proteoglycan Staining Intensity (Maximum)	**6**	**1**	**7**	**3**	**5**	**6**	**2**	**6**	**1**	**7**	**3**	8	**7**	8	8	**7**	**5**
Atypical-Phenotype Cell Presence	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Collagen Alignment	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Crimp Absence	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Crimp Wavelength	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
Crimp Wavelength Variation	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
IFR Frequency	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7
IFR Type Proportion	8	**2**	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7

6.4.2 Cell Characteristics

6.4.2.1 *Cell Density and Cell Distribution*

The Cell Density and Cell Distribution outcomes evaluated the average density and difference in densities of the cell population within the tendon area, respectively, from H&E-stained sections (Figure 6.4.A). Cell Density was taken as the average of three separate ROIs within the tendon/ligament area; a higher value indicates a greater number of cells per mm^2. Cell Distribution was the difference between the maximum and minimum densities in the 3 ROIs; a higher value indicates a greater difference in density values across the ROIs assessed.

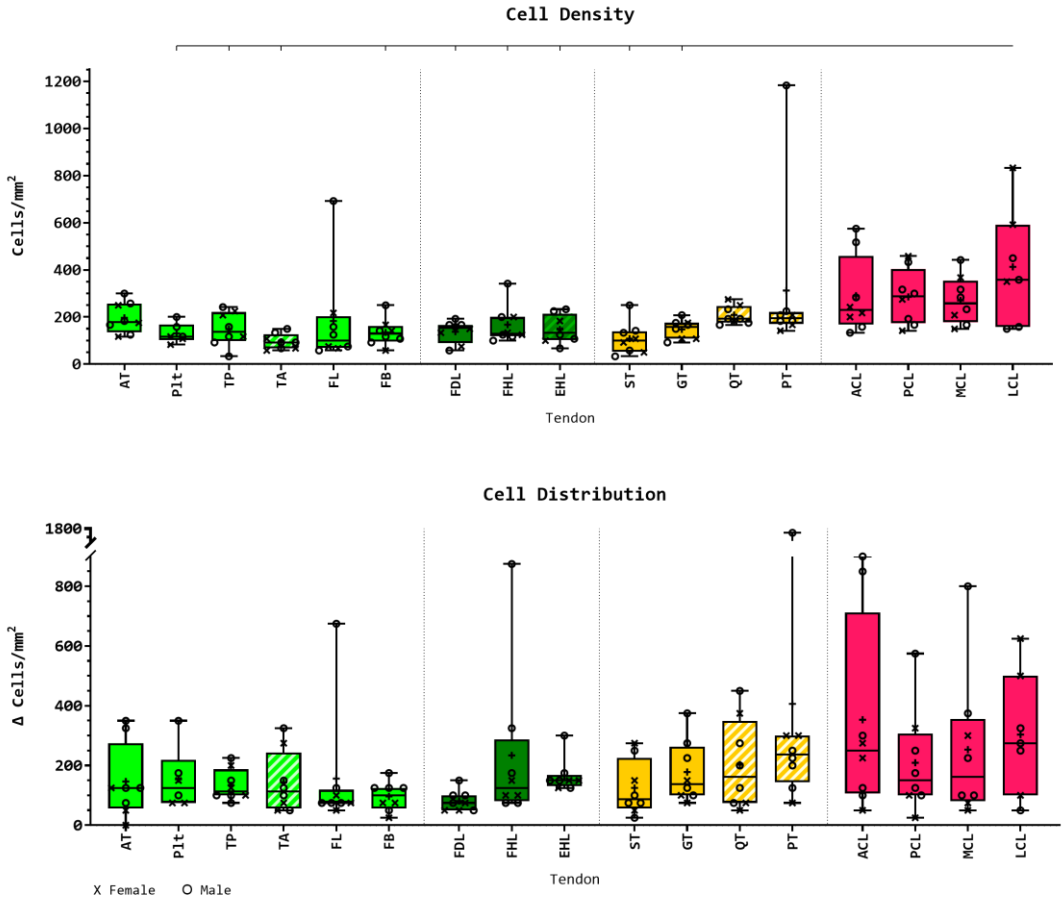


Figure 6.4.A Cell Density (top) and Cell Distribution (bottom) of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tendon at the right origin. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

There was a strong significant effect of *Tissue* on Cell Density ($p < 0.0001$). The four knee ligaments (ACL, PCL, MCL and LCL) had some of the highest cell densities relative to the other tissues (Figure 6.4.A). When comparing between specific tissues the LCL had a significantly higher cell density (mean 413.0 cells/mm^2) compared to nine other tendons/ligaments, being the Plt (mean 130.5 cells/mm^2, $p = 0.0132$), TP (mean 149.0 cells/mm^2, $p = 0.0073$), TA (mean 95.88 cells/mm^2, $p = 0.0002$), FB (mean 134.5 cells/mm^2, $p = 0.0027$), FDL (mean 137.5 cells/mm^2, $p = 0.0034$), FHL (mean 167.8 cells/mm^2, $p = 0.0240$), EHL (mean 147.9 cells/mm^2, $p = 0.0068$), ST (mean 108.3 cells/mm^2, $p = 0.0004$), and GT (mean 147.9 cells/mm^2, $p = 0.0068$) (Table 6.4.b).

There was also a significant effect of *Tissue* on Cell Distribution ($p = 0.0296$). While the knee ligaments trended to have donor samples with greater differences in cell densities relative to the other tendons (Figure 6.4.A), there were no significant differences when comparing between tendons/ligaments (all comparison $p > 0.05$, Supp. Table 9.7.a).

Table 6.4.b Cell Density pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Sidak correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis accounts for model covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.a.

Graph Position	Tissue Comparison	Contrast	Summary	Adjusted P Value	95% Confidence Interval	
2 vs. 17	Plt vs. LCL	271.286	*	0.0132	23.663	518.909
3 vs. 17	TP vs. LCL	260.249	**	0.0073	31.123	489.376
4 vs. 17	TA vs. LCL	313.374	***	0.0002	84.248	542.501
6 vs. 17	FB vs. LCL	274.833	**	0.0027	45.706	503.959
7 vs. 17	FDL vs. LCL	271.708	**	0.0034	42.581	500.834
8 vs. 17	FHL vs. LCL	241.499	*	0.0240	12.373	470.626
9 vs. 17	EHL vs. LCL	261.291	**	0.0068	32.165	490.417
10 vs. 17	ST vs. LCL	300.874	***	0.0004	71.748	530.001
11 vs. 17	GT vs. LCL	261.291	**	0.0068	32.165	490.417

6.4.2.1.1 Donor Characteristics

Linear predictions found that neither Cell Density nor Cell Distribution were affected by *sex* ($p = 0.8602$ and $p = 0.1429$ respectively; Table 6.4.c). Similarly, linear predictions found that neither Cell Density nor Cell Distribution were affected by *age*, *height* or *weight* ($p = 0.5335$, $p = 0.2000$ and $p = 0.1658$ respectively for Cell Density; $p = 0.7001$, $p = 0.6688$ and $p = 0.3674$ respectively for Cell Distribution; Table 6.4.d).

Table 6.4.c Cell Density and Cell Distribution main effects linear predictions for *sex* from mixed-effects linear regression analysis of 17 different human lower limb tendons/ligaments.

Outcome	Comparison	Contrast	p-value	95% Confidence Interval	
Cell Density	Female (reference) vs. Male	-5.241	0.8602	-63.554	53.072
Cell Distribution	Female (reference) vs. Male	68.149	0.1429	-23.025	159.323

Table 6.4.d Cell Density and Cell Distribution main effects linear predictions for *age*, *height* and *weight* from mixed-effects linear regression analysis of 17 different human lower limb tendons/ligaments.

Outcome	Variable	*dx*	dy/dx	p-value	95% Confidence Interval	
Cell Density	Age	*Years*	1.853	0.5335	-3.979	7.684
	Height	*Inches*	7.613	0.2000	-4.031	19.257
	Weight	*Pounds*	1.913	0.1658	-0.792	4.619
Cell Distribution	Age	*Years*	1.792	0.7001	-7.325	10.909
	Height	*Inches*	3.974	0.6688	-14.232	22.180
	Weight	*Pounds*	1.945	0.3674	-2.285	6.176

6.4.2.2 *Cell (nuclear) Morphology*

The Cell Morphology score assessed the predominant shape (spindle, cigar, spherical) of tenocyte nuclei present within the tendon sample area from H&E-stained sections (Figure 6.4.B). It should be noted that the score is not a purely ordinal scale: a score of one (1) indicates approximately evenly mixed morphology between two or more phenotypes while a score within the 2-4 range indicates a change from a predominant spindle shape (score 2), to increasingly more rounded (cigar [score 3] to spherical [score 4]) phenotypes being the dominant cell type.

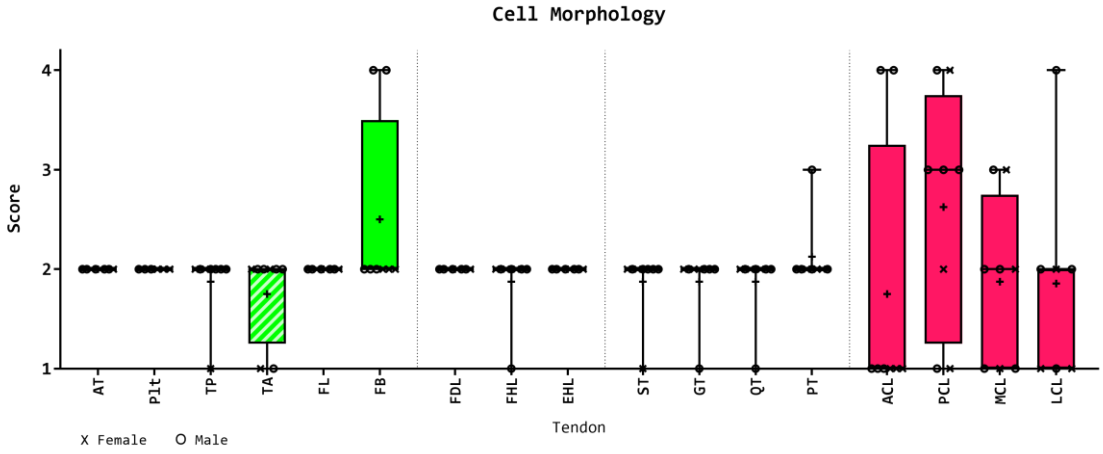


Figure 6.4.B Cell Morphology scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was no significant effect of *Tissue* on Cell Morphology score ($p = 0.2300$). It was observed that most tendons predominantly had scores of 2 (majority spindle-shaped nuclei, Figure 6.4.B), whilst the knee ligaments (ACL, PCL, MCL and LCL) displayed great variability across the donors and contained more cigar-shaped or spherical cell nuclei (scoring either 3 or 4 respectively) or mixed morphologies (score of 1, Figure 6.4.B). However, there were no significant differences when comparing between tendons/ligaments ($p > 0.05$, Supp. Table 9.7.a).

6.4.2.3 *Cell Arrangement*

The Cell Arrangement score assessed the relative positioning of cells with respect to one another from H&E-stained sections (Figure 6.4.C). A higher score indicates a greater proportion of cells arranged longitudinally in chains (*refer to Figure 4.5.B*). A score of one (1) indicates cells are dispersed and show no relative arrangement to neighbouring cells, whilst a score of four (4) indicates most cells are aligned in series with one another between fibres.

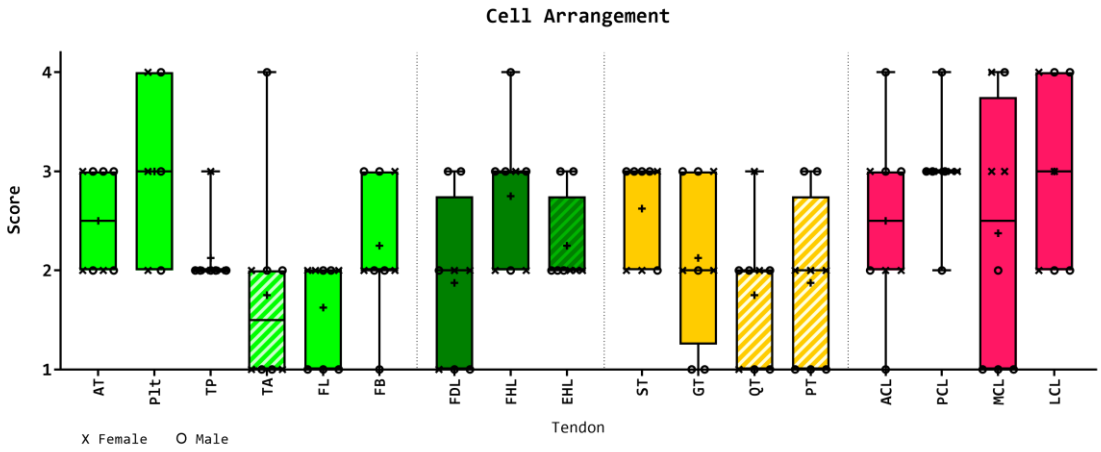


Figure 6.4.C Cell Arrangement scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a significant effect of *Tissue* on Cell Arrangement score ($p = 0.0039$). There was variability in the arrangement of cells in isolated (score = 1) or chain (greater scores) placements across the specimens (Figure 6.4.C), with no significant differences found when comparing between tissues ($p > 0.05$, Supp. Table 9.7.a).

6.4.2.4 *Atypical-Phenotype Cell Presence*

The Atypical-Phenotype Cell Presence score assessed the presence of "atypical" cell phenotypes within the tendon area from TB-stained sections (Figure 6.4.C). "Atypical" cells included those that may not be properly described as 'chondrocyte-like' (as they do not display all 3 criteria; *refer to Figure 4.5.K*) but also do not resemble typical tenocyte morphology. A higher score indicates a greater presence of these cells, where a score of zero (0) indicates none are present and a score of three (3) indicates numerous are present.

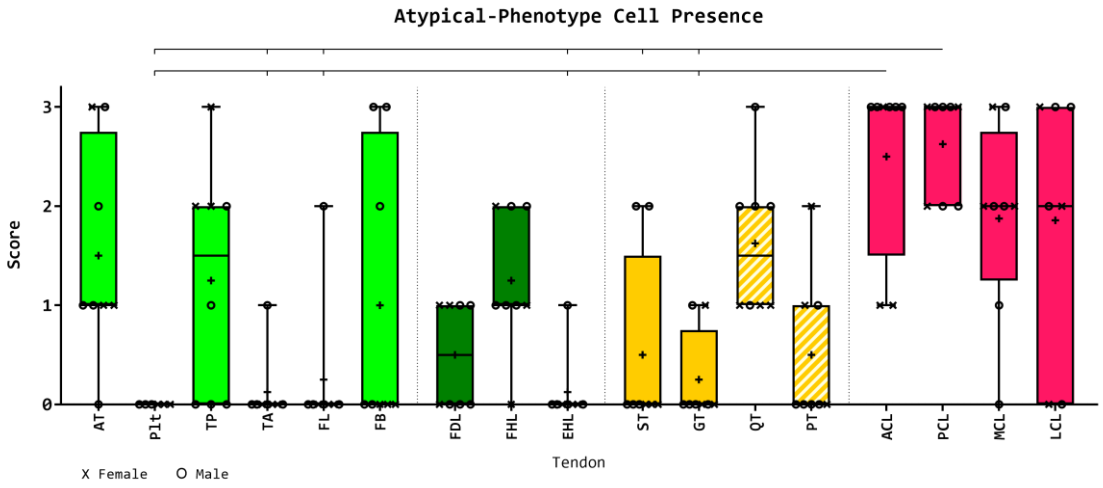


Figure 6.4.D Atypical-Phenotype Cell Presence scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tissue* on Atypical-Phenotype Cell Presence score ($p < 0.0001$). The cruciate ligaments (ACL and PCL) exhibited the highest presence of atypical-phenotype/chondrocyte-like cells, whilst the Plt, TA, FL and EHL exhibited some of the lowest (Figure 6.4.D). When comparing between tissues, both the ACL and PCL had significantly more atypical-phenotype cells compared to the Plt ($p = 0.0067$ and $p = 0.0032$ respectively), TA ($p = 0.0060$ and $p = 0.0027$ respectively), FL ($p = 0.0125$ and $p = 0.0057$ respectively), EHL ($p = 0.0060$ and $p = 0.0027$ respectively), and GT ($p = 0.0210$ and 0.0098 respectively; Table 6.4.e). The PCL also had significantly more atypical-phenotype cells compared to the ST ($p = 0.0392$; Table 6.4.e).

Table 6.4.e Atypical-Phenotype Cell Presence pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.a.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
2 vs. 14	Plt vs. ACL	-79.5	**	0.0067
2 vs. 15	Plt vs. PCL	-82.88	**	0.0032
4 vs. 14	TA vs. ACL	-74.06	**	0.0060
4 vs. 15	TA vs. PCL	-77.44	**	0.0027
5 vs. 14	FL vs. ACL	-70.94	*	0.0125
5 vs. 15	FL vs. PCL	-74.31	**	0.0057
9 vs. 14	EHL vs. ACL	-74.06	**	0.0060
9 vs. 15	EHL vs. PCL	-77.44	**	0.0027
10 vs. 15	ST vs. PCL	-65.75	*	0.0392
11 vs. 14	GT vs. ACL	-68.63	*	0.0210
11 vs. 15	GT vs. PCL	-72	**	0.0098

6.4.3 Proteoglycan/GAG Characteristics

6.4.3.1 *Proteoglycan Presence*

The Proteoglycan Presence score assessed the presence of proteoglycan/GAG staining (purple chromatic appearance), irrespective of intensity, within the tendon area from TB-stained sections (Figure 6.4.E). A higher score indicates more presence of proteoglycan staining, where a score of zero (0) indicates no staining present (0% of tendon area contains staining) and a score of six (6) indicates > 95% of the tendon area contains staining.

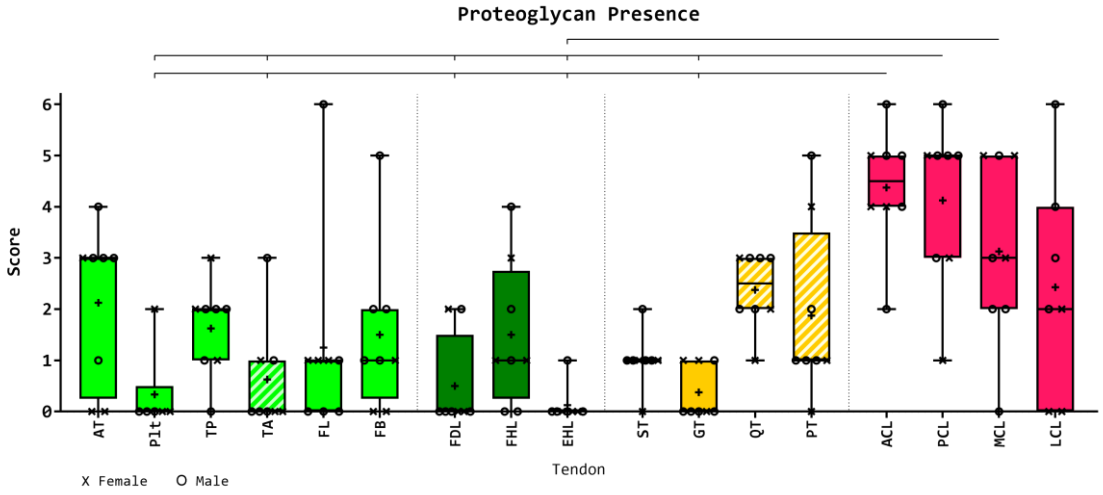


Figure 6.4.E Proteoglycan Presence scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tissue* on Proteoglycan Presence score ($p < 0.0001$). The knee ligaments had the largest presence of proteoglycan staining, with mean scores of 4.375, 4.125, 3.125 and 2.429 respectively for the ACL, PCL, MCL and LCL. The QT had the highest proteoglycan presence for the tendons with a mean score of 2.375. No lower limb tendons were completely void of proteoglycan staining, although the Plt and EHL both only had one donor sample presenting with proteoglycan staining each (Figure 6.4.E). When comparing between tissues, the ACL and PCL contained significantly more proteoglycan content compared to the Plt ($p = 0.0050$ and $p = 0.0137$ respectively), TA ($p = 0.0092$ and $p = 0.0265$ respectively), FDL ($p = 0.0039$ and $p = 0.0119$ respectively), EHL ($p = 0.0003$ and $p = 0.0009$ respectively) and GT ($p = 0.0026$ and $p = 0.0081$ respectively; Table 6.4.f). The MCL also had significantly more proteoglycan than the EHL ($p = 0.0307$; Table 6.4.f).

Table 6.4.f Proteoglycan Presence pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.b.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
2 vs. 14	Plt vs. ACL	-83.65	**	0.0050
2 vs. 15	Plt vs. PCL	-78.77	*	0.0137
4 vs. 14	TA vs. ACL	-74.75	**	0.0092
4 vs. 15	TA vs. PCL	-69.88	*	0.0265
7 vs. 14	FDL vs. ACL	-78.44	**	0.0039
7 vs. 15	FDL vs. PCL	-73.56	*	0.0119
9 vs. 14	EHL vs. ACL	-89.44	***	0.0003
9 vs. 15	EHL vs. PCL	-84.56	***	0.0009
9 vs. 16	EHL vs. MCL	-69.19	*	0.0307
11 vs. 14	GT vs. ACL	-80.19	**	0.0026
11 vs. 15	GT vs. PCL	-75.31	**	0.0081

6.4.3.2 *Proteoglycan Staining Intensity [Average and Maximum]*

The Proteoglycan Staining Intensity scores [Average and Maximum] assessed the intensity of the purple chromatic staining appearance within the tendon area from TB-stained sections (Figure 5.4.F). As there were 43 samples with a Proteoglycan Presence score of 0 (no proteoglycan staining present), only n = 90 datapoints (samples) were included for Proteoglycan Staining Intensity Average and Maximum score analysis. Note that the Plt and EHL only contained one sample each. (Figure 5.4.F). A greater score indicates greater straining intensity reflective of greater proteoglycan content. A score of one (1) indicates pale purple appearance, while a score of three (3) indicates deep purple staining.

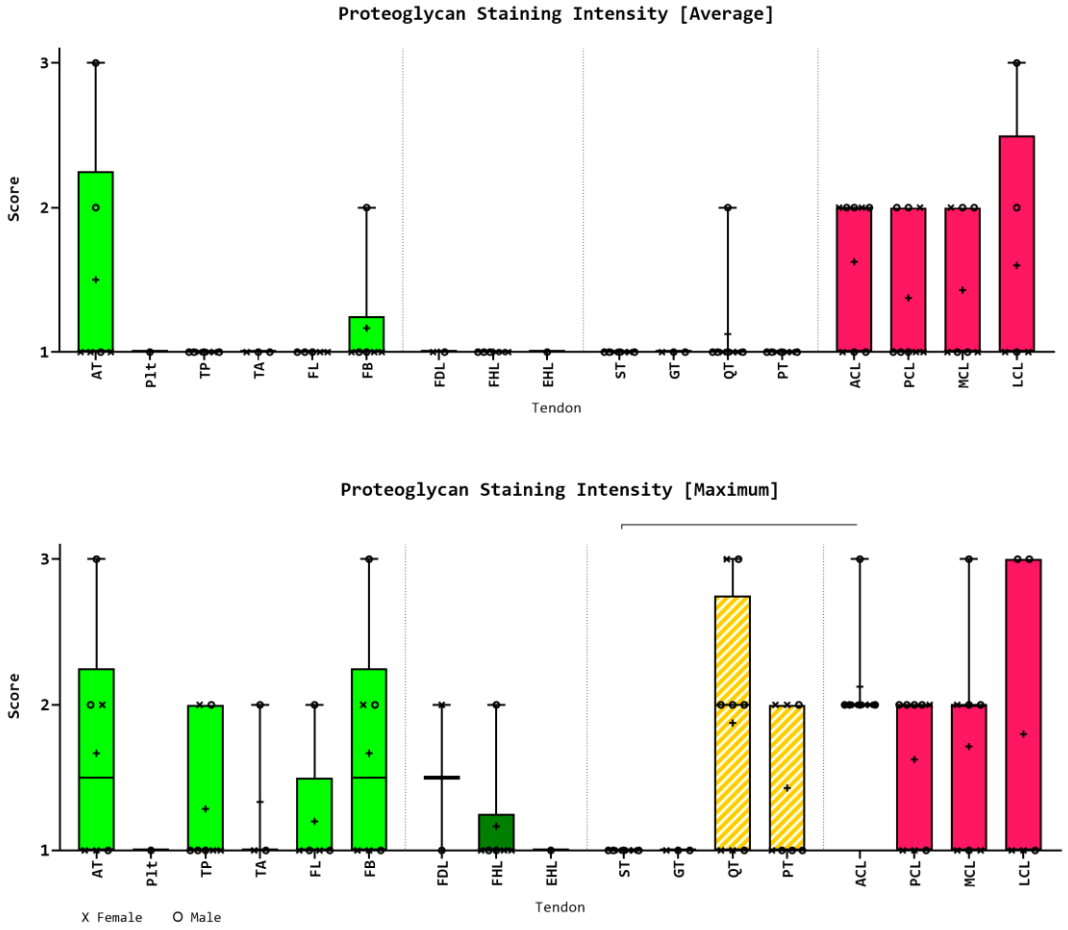


Figure 6.4.F Proteoglycan Staining Intensity Average (top) and Maximum (bottom) scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was no significant effect of *Tissue* on Proteoglycan Staining Intensity Average score ($p = 0.0541$) or Proteoglycan Staining Intensity Maximum score ($p = 0.0711$). From the Average intensity score, the majority of lower limb tendons (tendon tissue) were found to have pale proteoglycan staining (score of 1), whilst the knee ligaments commonly had more intense staining indicative of greater relative proteoglycan/GAG content (Figure 5.4.F). Conversely, there was a diverse spread with most tissues (including ligaments) exhibiting localised areas of greater intensity staining (Maximum score), with multiple donors (individual data points) presenting with Maximum scores greater than the Average intensity score (Figure 5.4.F). When comparing between tissues, there were no significant differences for Average Proteoglycan Staining Intensity score (all comparisons $p > 0.05$, Supp. Table 9.7.b), whilst for Maximum Proteoglycan Staining Intensity score the ACL had significantly more intense staining compared to just the ST ($p = 0.0363$, Table 6.4.g). It is likely that the absence of significance

between the ACL and the EHL/GT (which scored similar to the ST) were due to limited samples sizes of these tendons.

Table 6.4.g Proteoglycan Staining Intensity [Maximum] pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.b.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
10 vs. 14	ST vs. ACL	-43.44	*	0.0363

6.4.4 Architectural Characteristics

6.4.4.1 *Collagenous Organisation*

The Collagenous Organisation score assessed the general structural appearance of the collagenous matrix within the tendon area from H&E-stained sections (Figure 6.4.G). A higher score indicates a more disorganised collagenous architecture within the tendon area. A score of zero (0) indicates the collagenous structure is completely organised with highly aligned/orientated collagen, whilst a score of six (6) indicates variable or unaligned collagenous matrix constituting nearly all of the tendon area.

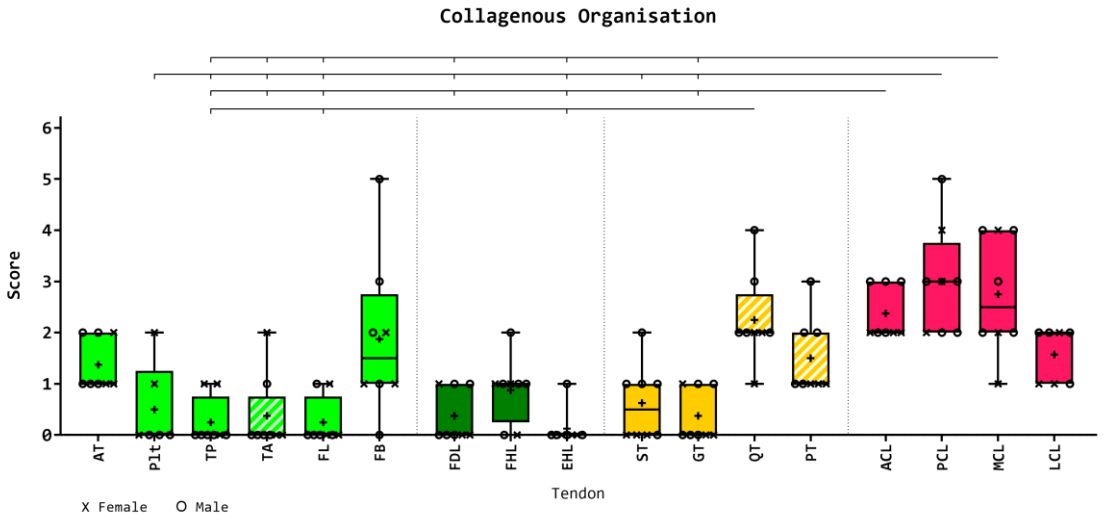


Figure 6.4.G Collagenous Organisation scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tissue* on Collagenous Organisation score ($p < 0.0001$). The knee ligaments (ACL, PCL, MCL and LCL), knee extensor tendons (QT and PT), AT and FB exhibited the highest level of disorganisation

(high score) within their collagenous architecture. Although some individual samples of other tissues scored higher, the PCL achieved the highest mean score (3.000) indicating only up to half the ligament area had disorganised collagenous architecture on average (Figure 6.4.G). When comparing between tissues, the ACL, PCL and MCL all had significantly more disorganised structure compared to the TP, TA, FL, FDL, EHL and GT (all comparison $p < 0.05$, Table 6.4.h). The PCL additional had significantly more disorganisation relative to the Plt and ST ($p = 0.0375$ and $p = 0.0394$ respectively), while the QT also had significantly more disorganisation compared to the TP, FL (both $p = 0.0283$) and EHL ($p = 0.0087$, Table 6.4.h).

Table 6.4.h Collagenous Organisation pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.c.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
2 vs. 15	Plt vs. PCL	-72.63	*	0.0375
3 vs. 12	TP vs. QT	-68.56	*	0.0283
3 vs. 14	TP vs. ACL	-74.56	**	0.0075
3 vs. 15	TP vs. PCL	-82	**	0.0012
3 vs. 16	TP vs. MCL	-75.56	**	0.0059
4 vs. 14	TA vs. ACL	-70.19	*	0.0199
4 vs. 15	TA vs. PCL	-77.63	**	0.0036
4 vs. 16	TA vs. MCL	-71.19	*	0.016
5 vs. 12	FL vs. QT	-68.56	*	0.0283
5 vs. 14	FL vs. ACL	-74.56	**	0.0075
5 vs. 15	FL vs. PCL	-82	**	0.0012
5 vs. 16	FL vs. MCL	-75.56	**	0.0059
7 vs. 14	FDL vs. ACL	-69.25	*	0.0244
7 vs. 15	FDL vs. PCL	-76.69	**	0.0046
7 vs. 16	FDL vs. MCL	-70.25	*	0.0197
9 vs. 12	EHL vs. QT	-73.88	**	0.0087
9 vs. 14	EHL vs. ACL	-79.88	**	0.0021
9 vs. 15	EHL vs. PCL	-87.31	***	0.0003
9 vs. 16	EHL vs. MCL	-80.88	**	0.0017
10 vs. 15	ST vs. PCL	-67	*	0.0394
11 vs. 14	GT vs. ACL	-69.25	*	0.0244
11 vs. 15	GT vs. PCL	-76.69	**	0.0046
11 vs. 16	GT vs. MCL	-70.25	*	0.0197

6.4.4.2 *Fibrillar Loosening*

The Fibrillar Loosening score assessed the 'packing density' of fibrils within a single ROI on H&E-stained sections (Figure 6.4.H). A higher score indicates a greater proportion of area with loose fibril arrangement / poorly defined fibre structures. A score of one (1) indicates all/nearly all of the fibrils in the ROI are bundled into distinct fibre structures, whilst a score of six (6) indicates all/nearly all of the area contains loose/dispersed fibrils without clear/distinct fibre structures.

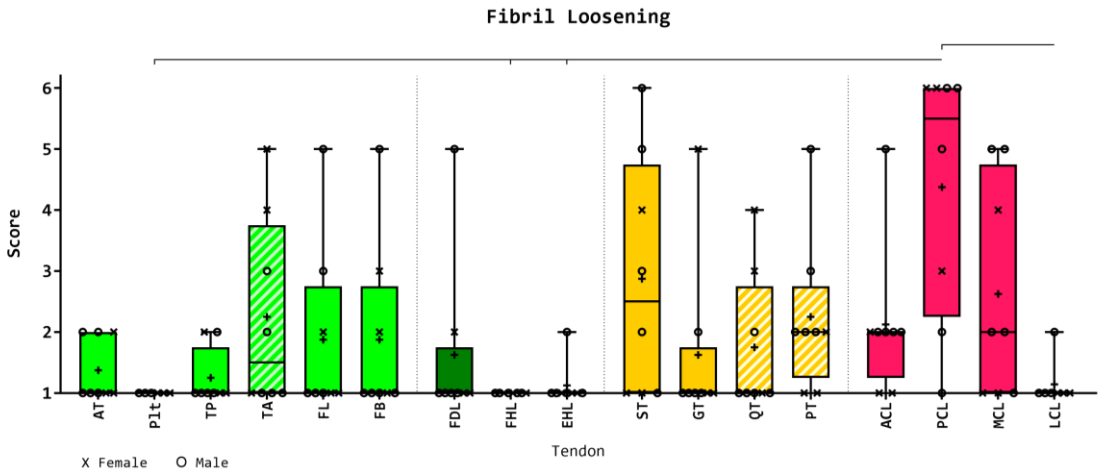


Figure 6.4.H Fibrillar Loosening scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tissue* on Fibrillar Loosening score ($p = 0.0009$). Tendons and ligaments of the lower limb shows great variability in the proportion of loosened fibrils within their structure. The PCL exhibited the highest proportion of loose fibrils (mean 4.375, indicating more than 50% of fibrils are loosened) and was the only tissue to show a significant difference relative to the others (Figure 6.4.H). When comparing between tissues, the PCL had significantly higher proportion of loosened fibrils compared to the Plt, FHL, EHL and LCL ($p = 0.0148$, $p = 0.00040$, $p = 0.0205$ and $p = 0.0421$ respectively; Table 6.4.i).

Table 6.4.i Fibrillar Loosening pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.c.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
2 vs. 15	Plt vs. PCL	-70.88	*	0.0148
8 vs. 15	FHL vs. PCL	-70.88	**	0.0040
9 vs. 15	EHL vs. PCL	-64.25	*	0.0205
15 vs. 17	PCL vs. LCL	63.3	*	0.0421

6.4.4.3 *Fibre Separations [Frequency and Type]*

The Fibre Separations scores [Frequency and Type] assessed characteristics of separations between neighbouring fibres in a defined ROI from H&E-stained sections (Figure 6.4.I). There was one ACL, one PCL and one MCL sample that were not able to have an appropriate fibre separation ROI placed for assessment due to high fibrillar loosening of the whole tissue area, therefore only n = 130 datapoints were included for the Fibre Separations Frequency outcome. A further 40 datapoints were missing from the Fibre Separations Type outcome due to a Frequency score of 0 (no separations), resulting in a total of n = 90 datapoints being included for analysis. Note that all scorable ACL samples (n = 7) received a Fibre Separation Frequency score of zero (0) and thus there were no ACL samples included in the n = 90 datapoints for the Fibre Separations Type score (Figure 6.4.I). The Fibre Separations Frequency score assessed the proportion of fibres which were visibly separated from neighbouring fibres, with a higher score indicating a greater proportion of fibre separations across the area assessed. A score of zero (0) indicates no separations are present (all fibres are tightly adhered/adjacent to one another), while a score of six (6) indicates all/nearly all of the fibres are visibly separated from one another. The Fibre Separations Type score assessed the predominant length of the separations before fibres diverge/converge. A higher score indicates longer fibre separations present in the area assessed (indicative of fewer fibre-fibre connections/anastomoses within the tissue structure). A score of one (1) indicates separations were predominantly < 50 μm in length, while a score of four (4) indicates separations were predominantly > 150 μm long.

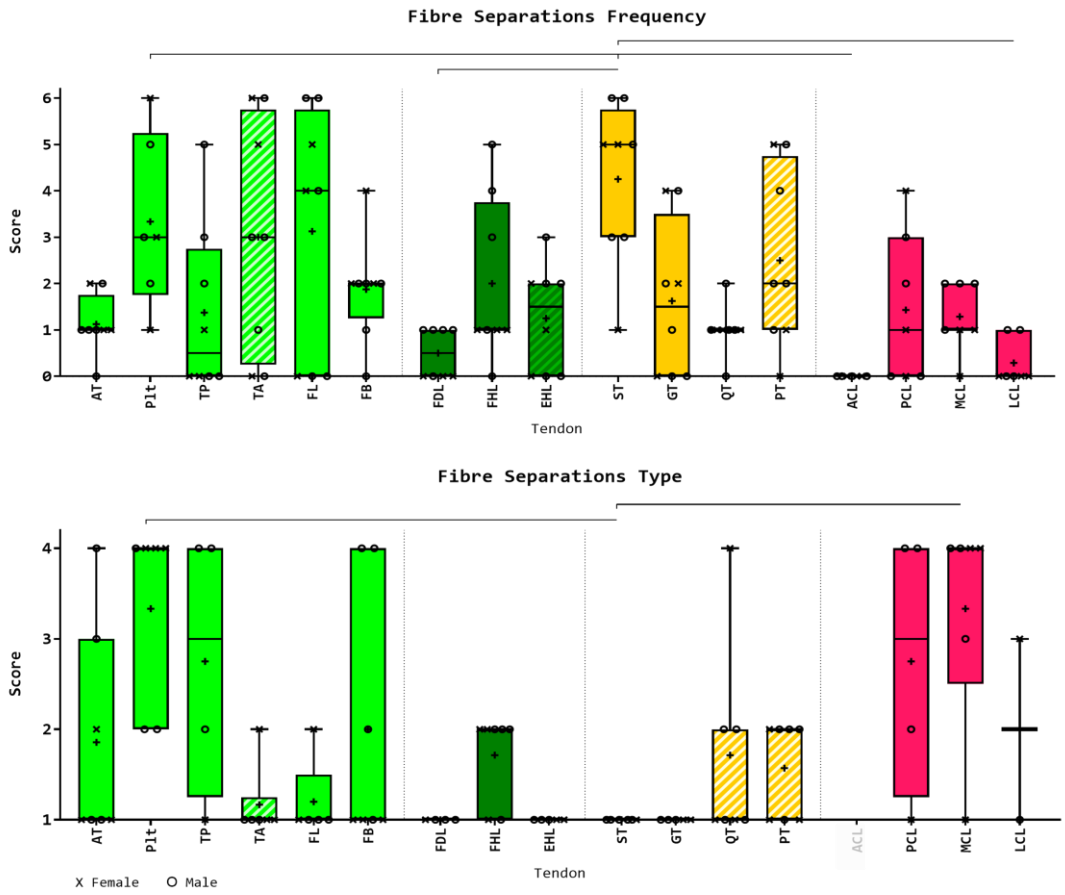


Figure 6.4.I Fibre Separations Frequency (top) and Type (bottom) scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Note: for the Type score (bottom graph), ACL (indicated in grey) contains no datapoints (no scores) as it lacked any fibre separations to assess (all samples scored 0 in Fibre Separation Frequency score [top graph]). Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tissue* on Fibre Separations Frequency ($p = 0.0002$) and Type ($p = 0.0002$) scores. Frequency of fibre separations was variable for most tissues (large spread of scores for the particular tissue). The knee ligaments (ACL, PCL, MCL and LCL) along with the AT, TP, FLD EHL and QT tended to have fewer separations between fibres (Figure 6.4.I). For the Fibre Separation Type score, there was varied results across the tendon/ligament, with some tissues exhibiting varied lengths of separations (large spread for the tissue), while others achieved the same/similar score for all samples. The Plt, TP, PCL and MCL had some of the longest separations, while the FDL, EHL, ST and GT consistently exhibited only short separations < 50 µm in length (Figure 6.4.I). When comparing between tendons for Fibre Separation Frequency, the ST had significantly more separations compared to the FDL ($p = 0.0204$), ACL ($p = 0.0005$) and LCL ($p = 0.0061$), while the ACL also had significantly fewer separations relative to the Plt ($p = 0.0191$; Table 6.4.j). When comparing between tendons for Fibre Separation Type, the ST has significantly shorter separations in comparison to the Plt ($p = 0.0108$) and MCL ($p = 0.0397$; Table 6.4.k).

Table 6.4.j Fibre Separations Frequency pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.c.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
2 vs. 14	Plt vs. ACL	77.67	*	0.0191
7 vs. 10	FDL vs. ST	-69.5	*	0.0204
10 vs. 14	ST vs. ACL	88	***	0.0005
10 vs. 17	ST vs. LCL	77.43	**	0.0061

Table 6.4.k Fibre Separations Type pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.c.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
2 vs. 10	Plt vs. ST	49.17	*	0.0108
10 vs. 16	ST vs. MCL	-45.08	*	0.0397

6.4.4.4 *Collagen Alignment*

The Collagen Alignment score assessed the alignment characteristic of the collagenous matrix within the tendon area utilising standard brightfield light on PSR-stained sections (Figure 6.4.J). A higher score indicates a less aligned collagen within the tendon area. A score of zero (0) indicates all of the tendon area contains highly aligned collagenous structure, whilst a score of seven (7) indicates none of the tendon area contains aligned collagenous structure (where all the collagenous matrix is unaligned).

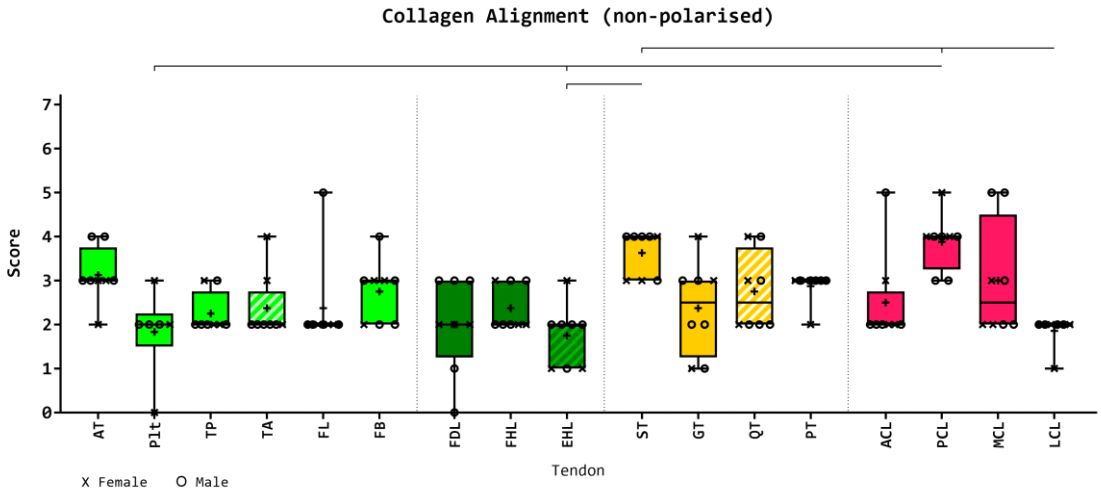


Figure 6.4.J Collagen Alignment scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tissue* on Collagen Alignment score ($p < 0.0001$). Alignment was fairly consistent (low score spread) within most tissues. The PCL exhibited the highest proportion of unaligned collagen (higher score; mean 3.875, indicating typically between 26-75% of collagen was unaligned [score 3 or 4]), while the EHL had the highest proportion of aligned collagen (lower score; mean 1.750 indicating typically between only 5-25% of collagen was unaligned [score 1 or 2]) (Figure 6.4.J). When comparing between tissues, the PCL had significantly more unaligned collagen compared to the Plt ($p = 0.0296$), EHL ($p = 0.0011$), and LCL ($p = 0.0032$), and the ST also had significantly more unaligned collagen than the LCL ($p = 0.0100$; Table 6.4.l). In addition to the PCL, the EHL also had significantly more aligned collagen relative to the ST ($p = 0.0037$; Table 6.4.l).

Table 6.4.l Collagen Alignment pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.c.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
2 vs. 15	Plt vs. PCL	-71.75	*	0.0296
9 vs. 10	EHL vs. ST	-75.38	**	0.0037
9 vs. 15	EHL vs. PCL	-80.31	**	0.0011
10 vs. 17	ST vs. LCL	73.74	**	0.0100
15 vs. 17	PCL vs. LCL	78.68	**	0.0032

6.4.4.5 *Adipose Presence*

The Adipose Presence score assessed the presence of fatty (adipose) tissue/cells within the tendon area from H&E-stained sections (Figure 6.4.K). A higher score indicates a greater area of tissue containing adipocytes, where a score of zero (0) indicates no adipose present and a score of five (5) indicates > 25% of the tendon area contains adipose.

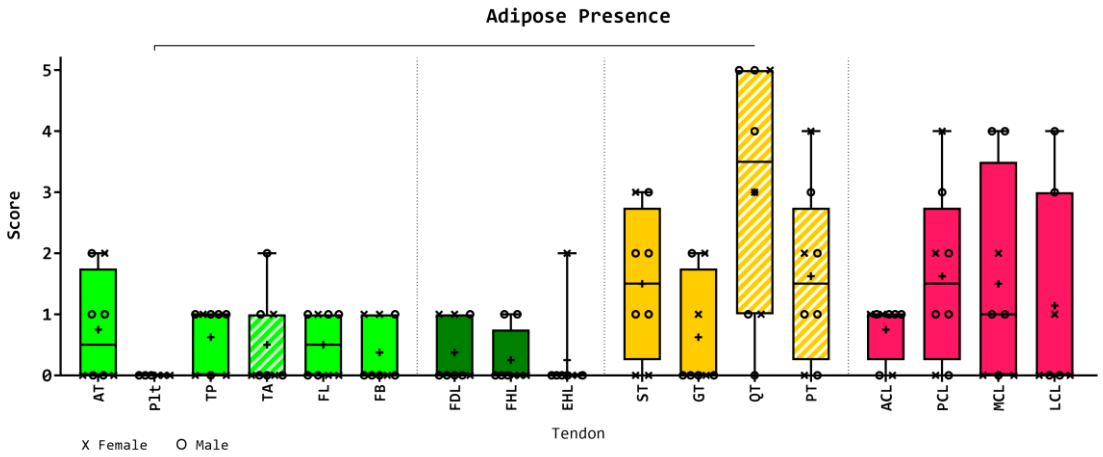


Figure 6.4.K Adipose Presence scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a significant effect of *Tissue* on Adipose Presence score ($p = 0.0036$). The QT had the greatest presence of adipocytes/adipose tissue (mean score 3.000, adipose present in 10-20% of tendon area) whilst the Plt had the lowest (mean score 0.000) with no presence of adipose in any sample (Figure 6.4.K). When comparing between tissues, it was only this difference that was significant, with the QT having significantly more adipose compared to the Plt ($p = 0.0204$; Table 6.4.m).

Table 6.4.m Adipose Presence pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.c.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
2 vs. 12	Plt vs. QT	-72.81	*	0.0204

6.4.5 Interfascicular Characteristics

6.4.5.1 *Interfascicular Region Frequency*

The Interfascicular Region (IFR) Frequency outcome evaluated the frequency of IFRs across the width of the tendon area from PSR-stained sections (brightfield microscopy; Figure 6.4.L). All tendons achieved an appropriate IFR ROI placement (*refer to Section 5.3.2*) so all n = 133 datapoints were included for analysis. IFR Frequency was taken as the number of IFRs per mm of tendon width; a higher value indicates a greater incidence of IFRs indicative of smaller fascicles across the tendon area.

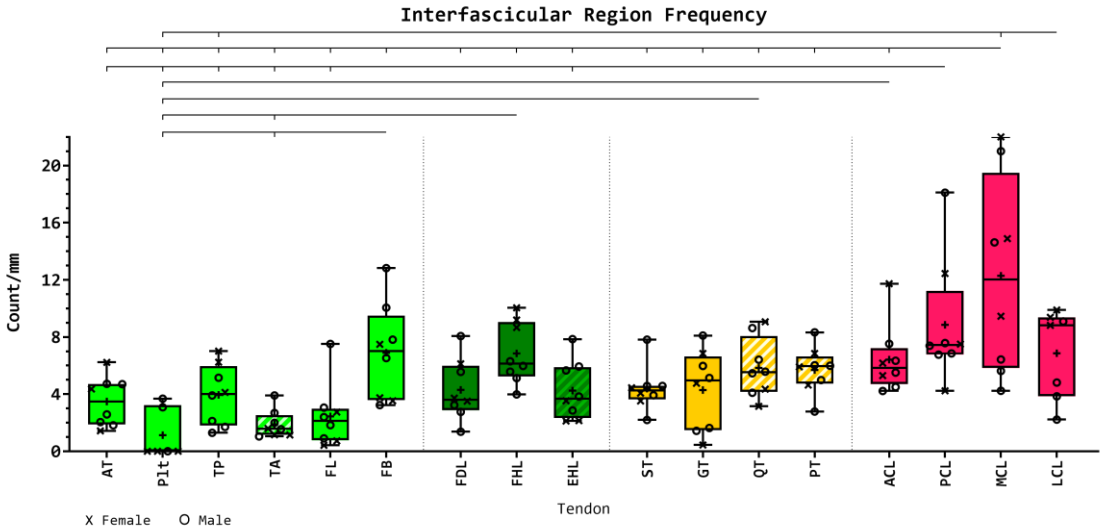


Figure 6.4.L Interfascicular Region Frequency of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Mixed-effects linear regression analysis with Sidak's adjustment. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

There was a strong significant effect of *Tissue* on IFR Frequency ($p < 0.0001$). Results were variable between the tissues. The ankle tendons (excluding the FB) had the lowest IFR frequencies, with the Plt containing the fewest IFRs proportional to tendon width, averaging a mean of 1.130 IFRs/mm. The FB along with the foot/toe tendons, the knee tendons, and the ACL and LCL had relatively moderate IFR frequencies, ranging from a mean of 2.244 IFRs/mm for the EHL to 6.903 IFRs/mm for the FB. The PCL and MCL had the two highest frequencies, having a mean of 8.859 IFRs/mm and 12.28 IFRs/mm respectively (Figure 6.4.L). When comparing between tissues, varied and numerous significant differences in IFR Frequency across the lower limb tendons and ligaments were found as detailed in Table 6.4.n.

Table 6.4.n Interfascicular Region Frequency pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Sidak correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis accounts for model covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.d.

Graph Position	Tissue Comparison	Contrast	Summary	Adjusted P Value	95% Confidence Interval	
1 vs. 15	AT vs. PCL	5.372	**	0.0044	0.775	9.968
1 vs. 16	AT vs. MCL	8.792	****	<0.0001	4.195	13.389
2 vs. 6	Plt vs. FB	6.092	**	0.0019	1.107	11.077
2 vs. 8	Plt vs. FHL	6.048	**	0.0022	1.063	11.032
2 vs. 12	Plt vs. QT	5.034	*	0.0439	0.049	10.019
2 vs. 14	Plt vs. ACL	5.603	**	0.0087	0.618	10.588
2 vs. 15	Plt vs. PCL	8.047	****	<0.0001	3.062	13.032
2 vs. 16	Plt vs. MCL	11.467	****	<0.0001	6.483	16.452
2 vs. 17	Plt vs. LCL	6.193	**	0.0026	1.045	11.340
3 vs. 15	TP vs. PCL	4.913	*	0.0195	0.316	9.510
3 vs. 16	TP vs. MCL	8.334	****	<0.0001	3.737	12.930
4 vs. 6	TA vs. FB	5.018	*	0.0140	0.422	9.615
4 vs. 8	TA vs. FHL	4.974	*	0.0161	0.377	9.571
4 vs. 15	TA vs. PCL	6.973	****	<0.0001	2.376	11.570
4 vs. 16	TA vs. MCL	10.394	****	<0.0001	5.797	14.990
4 vs. 17	TA vs. LCL	5.119	*	0.0179	0.356	9.882
5 vs. 15	FL vs. PCL	6.405	***	0.0001	1.809	11.002
5 vs. 16	FL vs. MCL	9.826	****	<0.0001	5.229	14.423
6 vs. 16	FB vs. MCL	5.375	**	0.0044	0.778	9.972
7 vs. 16	FDL vs. MCL	7.986	****	<0.0001	3.389	12.582
8 vs. 16	FHL vs. MCL	5.420	**	0.0037	0.823	10.017
9 vs. 15	EHL vs. PCL	4.615	*	0.0475	0.018	9.211
9 vs. 16	EHL vs. MCL	8.035	****	<0.0001	3.438	12.632
10 vs. 16	ST vs. MCL	7.890	****	<0.0001	3.293	12.486
11 vs. 16	GT vs. MCL	7.987	****	<0.0001	3.390	12.584
12 vs. 16	QT vs. MCL	6.434	***	0.0001	1.837	11.031
13 vs. 16	PT vs. MCL	6.588	****	<0.0001	1.991	11.184
14 vs. 16	ACL vs. MCL	5.865	***	0.0008	1.268	10.461
16 vs. 17	MCL vs. LCL	-5.275	*	0.0111	-10.038	-0.512

6.4.5.1.1 Donor Characteristics

Linear predictions found that IFR Frequency was not affected by *sex* (p = 0.1628, Table 6.4.o) nor *age* (p = 0.7960), however there was a small but significant reduction in IFR Frequency with increasing donor *height* (-0.330 frequency/inch, p = 0.0076) and increasing donor *weight* (-0.058 frequency/lbs, p = 0.0439; Table 6.4.p).

Table 6.4.o Interfascicular Region Frequency main effects linear predictions for *sex* from mixed-effects linear regression analysis of 17 different human lower limb tendons/ligaments.

Outcome	Comparison	Contrast	p-value	95% Confidence Interval	
IFR Frequency	Female (reference) vs. Male	0.863	0.1628	-0.349	2.076

Table 6.4.p Interfascicular Region Frequency main effects linear predictions for *age*, *height* and *weight* from mixed-effects linear regression analysis of 17 different human lower limb tendons/ligaments.

Outcome	Variable	*dx*	dy/dx	p-value	95% Confidence Interval	
IFR Frequency	Age	*Years*	-0.016	0.7960	-0.137	0.105
	Height	*Inches*	-0.330	0.0076	-0.572	-0.088
	Weight	*Pounds*	-0.058	0.0439	-0.114	-0.002

6.4.5.2 *Interfascicular Region Type Proportion*

The Interfascicular Region Type Proportion outcome evaluated the proportion of IFRs defined as Interfascicular Matrix (IFM, an IFR containing distinct matrix substance) versus Interfascicular Space (IFS, a visible IFR/fascicle border containing no distinct matrix substance) from PSR-stained sections (brightfield microscopy; Figure 6.4.M). The Plt samples of four donors were not included due a IFR Frequency score of 0 (no IFRs), resulting in only n = 2 datapoints for the Plt and a total of n = 129 datapoints in total being included for analysis. IFR Type Proportion was taken as proportion of IFMs over the total number of IFRs; a higher percentage indicates a greater proportion of matrix-containing IFRs across the tendon area.

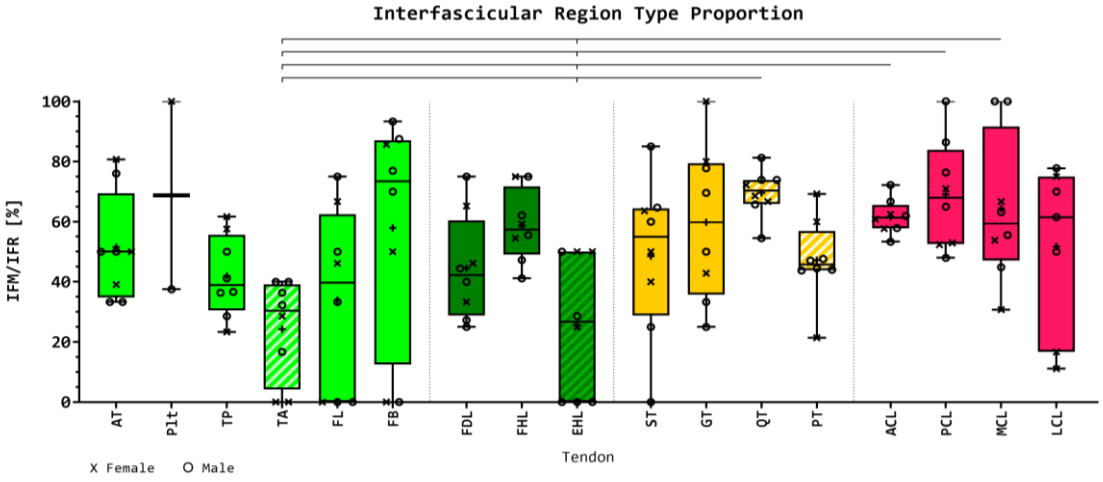


Figure 6.4.M Interfascicular Region Type Proportion of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Mixed-effects multilevel linear regression analysis with Sidak's adjustment. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

There was a strong significant effect of *Tissue* on IFR Type Proportion ($p < 0.0001$). Results were variable across the tissues, but all specimens exhibited some proportion of IFM within their IFR presence (Figure 6.4.M). The TA and EHL has the two lowest IFM/IFR proportions (mean of 24.23% and 25.45% respectively), whilst the Plt, PCL and QT had the three highest proportions (mean of 68.75%, 69.02% and 69.60% respectively), noting that the Plt only had two donor samples with IFR frequency above 0 (Figure 6.4.M). When comparing between tissues, the QT, ACL, PCL and MCL all had significantly greater IFM:IFS ratios (higher IFM/IFR proportion) compared to the TA ($p = 0.0011$, $p = 0.0316$, $p = 0.0015$ and $p = 0.0108$ respectively) and the EHL ($p = 0.0019$, $p = 0.0496$, $p = 0.0025$ and $p = 0.0176$ respectively; Table 6.4.q).

Table 6.4.q Interfascicular Region Type Proportion pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Sidak correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis accounts for model covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.d.

Graph Position	Tissue Comparison	Contrast	Summary	Adjusted P Value	95% Confidence Interval	
4 vs. 12	TA vs. QT	45.367	**	0.0011	9.198	81.536
4 vs. 14	TA vs. ACL	37.403	*	0.0316	1.234	73.572
4 vs. 15	TA vs. PCL	44.782	**	0.0015	8.614	80.951
4 vs. 16	TA vs. MCL	40.120	*	0.0108	3.952	76.289
9 vs. 12	EHL vs. QT	44.153	**	0.0019	7.984	80.322
9 vs. 14	EHL vs. ACL	36.189	*	0.0496	0.020	72.358
9 vs. 15	EHL vs. PCL	43.569	**	0.0025	7.400	79.737
9 vs. 16	EHL vs. MCL	38.906	*	0.0176	2.738	75.075

6.4.5.2.1 Donor Characteristics

Linear predictions found that IFR Type Proportion was not affected by any donor variables – that being *sex* (p = 0.8898, Table 6.4.r), *age*, *height* or *weight* (p = 0.3815, p = 0.3970 and p = 0.4614 respectively, Table 6.4.s).

Table 6.4.r Interfascicular Region Type Proportion main effects linear predictions for *sex* from mixed-effects linear regression analysis of 17 different human lower limb tendons/ligaments.

Outcome	Comparison	Contrast	p-value	95% Confidence Interval	
IFR Type Proportion	Female (reference) vs. Male	0.685	0.8898	-9.001	10.370

Table 6.4.s Interfascicular Region Type Proportion main effects linear predictions for *age*, *height* and *weight* from mixed-effects linear regression analysis of 17 different human lower limb tendons/ligaments.

Outcome	Variable	*dx*	dy/dx	p-value	95% Confidence Interval	
IFR Type Proportion	Age	*Years*	0.435	0.3815	-0.539	1.408
	Height	*Inches*	-0.836	0.3970	-2.772	1.099
	Weight	*Pounds*	0.169	0.4614	-0.281	0.620

6.4.6 Crimp Characteristics

6.4.6.1 *Crimp Absence*

The Crimp Absence score assessed the presence or loss of crimping structure within the tendon/ligament sample area utilising polarised light on PSR-stained sections (Figure 6.4.N). A higher score indicates a greater area of the tendon with an absence of crimp. A score of one (1) indicates a crimping waveform was present in nearly all the tendon area, whilst a score of six (6) indicates a complete absence of crimp.

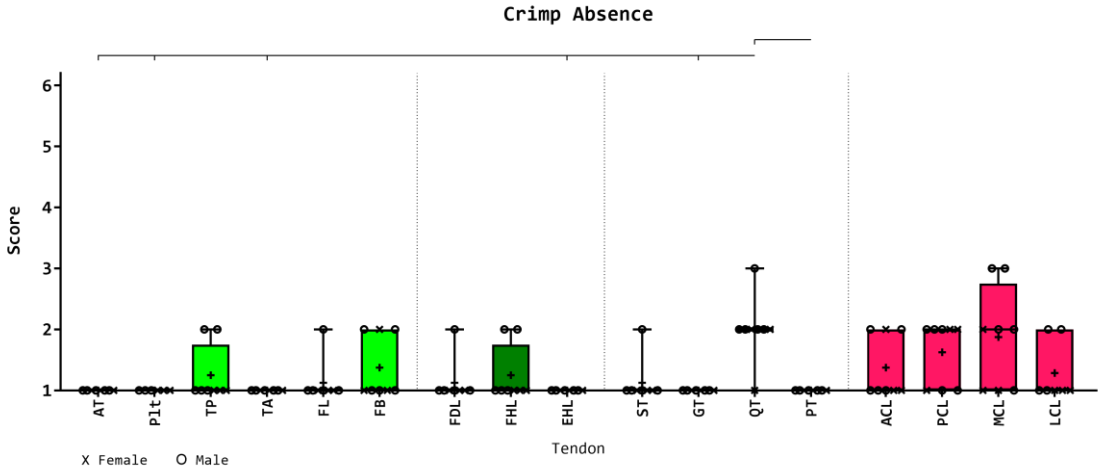


Figure 6.4.N Crimp Absence scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tissue* on Crimp Absence score ($p < 0.0001$). The QT had the highest proportion of tendon area with an absence of crimp (mean score 2.000, crimp absent from < 40% of tendon area), while the AT, Plt, TA, EHL, GT and PT all had crimp absent from no more than 10% of the area as a maximum (Figure 6.4.N). When comparing between tissues, it was these tendons that had a significant difference, where the QT had significantly more crimp absence compared to the AT, Plt, TA, EHL, GT and PT ($p = 0.0190$ for Plt; $p = 0.0053$ for all other comparisons; Table 6.4.t).

Table 6.4.t Crimp Absence pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.e.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
1 vs. 12	AT vs. QT	-58.88	**	0.0053
2 vs. 12	Plt vs. QT	-58.88	*	0.019
4 vs. 12	TA vs. QT	-58.88	**	0.0053
9 vs. 12	EHL vs. QT	-58.88	**	0.0053
11 vs. 12	GT vs. QT	-58.88	**	0.0053
12 vs. 13	QT vs. PT	58.88	**	0.0053

6.4.6.2 *Crimp Wavelength*

The Crimp Wavelength score assessed the length of the predominant crimping structure within the tendon/ligament sample area utilising polarised light on PSR-stained sections (Figure 6.4.O). A higher score indicates a longer crimp wavelength, where a score of one (1) indicates crimp ≤ 25 µm in length, while greater scores indicate longer waves (non-linear range brackets).

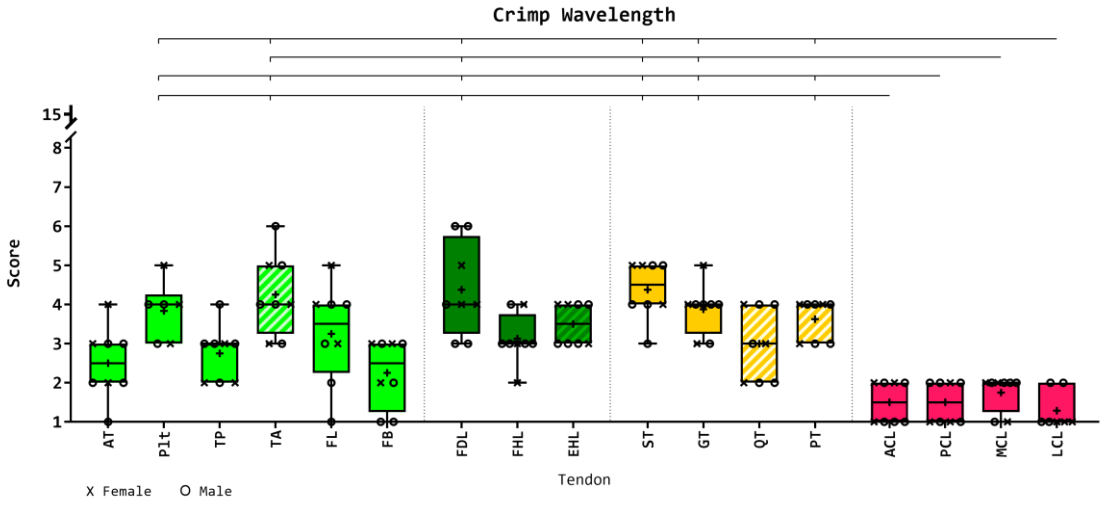


Figure 6.4.O Crimp Wavelength scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a strong significant effect of *Tissue* on Crimp Wavelength score ($p < 0.0001$). The human lower limb tendon dataset had Crimp Wavelength scores between 1 (≤ 25 µm) and 6 (126-150 µm). No tendons scored higher than a 6, indicating crimp wavelengths reach up to 150 µm in length for the included human tendons/ligaments of the lower limb. The FDL and ST had the highest mean score (both 4.375) indicating average crimp lengths ranging between 76-125 µm (score 4 or 5; Figure 6.4.O). Conversely, the knee ligaments

(ACL, PCL, MCL and LCL) had the shortest wavelengths, all having scores of 2 or below (means of 1.500, 1.500, 1.750 and 1.286 respectively), indicating crimp wavelengths < 50 µm long (Figure 6.4.O). The ACL, PCL and LCL all had significantly shorter crimp wavelengths compared to the Plt, TA, FDL, ST, GT and PT (all $p < 0.05$, Table 6.4.u), while the MCL had significantly shorter crimps compared to the TA, FDL, ST and GT (all $p < 0.05$, Table 6.4.u).

Table 6.4.u Crimp Wavelength pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.e.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
2 vs. 14	Plt vs. ACL	72.33	*	0.0482
2 vs. 15	Plt vs. PCL	72.33	*	0.0482
2 vs. 17	Plt vs. LCL	77.48	*	0.0278
4 vs. 14	TA vs. ACL	80.94	**	0.0022
4 vs. 15	TA vs. PCL	80.94	**	0.0022
4 vs. 16	TA vs. MCL	74.94	**	0.0087
4 vs. 17	TA vs. LCL	86.08	**	0.0012
7 vs. 14	FDL vs. ACL	81.75	**	0.0018
7 vs. 15	FDL vs. PCL	81.75	**	0.0018
7 vs. 16	FDL vs. MCL	75.75	**	0.0073
7 vs. 17	FDL vs. LCL	86.89	**	0.0010
10 vs. 14	ST vs. ACL	87.5	***	0.0004
10 vs. 15	ST vs. PCL	87.5	***	0.0004
10 vs. 16	ST vs. MCL	81.5	**	0.0019
10 vs. 17	ST vs. LCL	92.64	***	0.0002
11 vs. 14	GT vs. ACL	74.38	**	0.0099
11 vs. 15	GT vs. PCL	74.38	**	0.0099
11 vs. 16	GT vs. MCL	68.38	*	0.0361
11 vs. 17	GT vs. LCL	79.52	**	0.0057
13 vs. 14	PT vs. ACL	67	*	0.0479
13 vs. 15	PT vs. PCL	67	*	0.0479
13 vs. 17	PT vs. LCL	72.14	*	0.0274

6.4.6.3 *Crimp Wavelength Variation*

The Crimp Wavelength Variation score assessed the proportion of tendon area exhibiting different crimp wavelength patterns relative to the predominant wavelength scored previously (Crimp Wavelength, *section 6.4.6.2)*, utilising polarised light on PSR-stained sections (Figure 6.4.P). A higher score indicates a greater area of the tendon with differing crimp wavelengths. A score of zero (0) indicates only one crimp wavelength (as per score ranges for Crimp Wavelength; *refer to Table 4.5.g*) is present in the tendon area, whilst a score of four (4) indicates that the predominant crimp wavelength (as scored) constitutes < 40% of the tendon area.

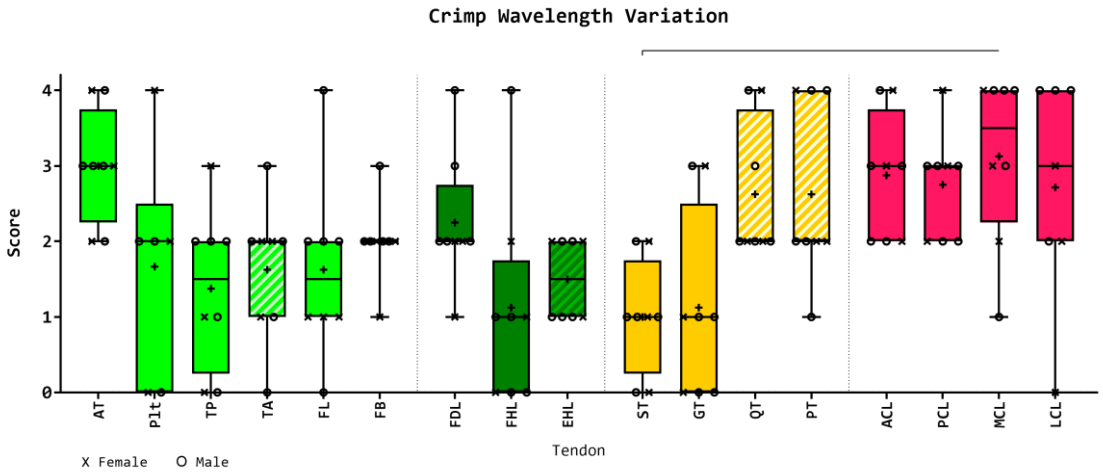


Figure 6.4.P Crimp Wavelength Variation scores of 17 different human lower limb tendons/ligaments. *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Box-and-whiskers respectively indicate the interquartile range, and minimum and maximum values. Mean indicated by plus (+) symbol. Significant tendon/ligament differences indicated by overhead horizonal lines with all tissues marked by downward ticks significantly different to the tissue at the origin point (right; no tick). All pairwise analyses performed after Kruskal-Wallis test with Dunn's adjustment for multiple comparisons. ✕ = Female ○ = Male. *Refer to Table 6.4.a for n-number of each tendon.*

Kruskal-Wallis analysis revealed that there was a significant effect of *Tissue* on Crimp Wavelength Variation score ($p = 0.0001$). There was variability across the tendons/ligaments in relation to crimp variation. The AT along with the QT and two knee ligaments (ACL, PCL) had the highest proportion of crimped tendon area exhibiting differing wavelengths (scores of 2 or above only, indicating always >10% of crimp is variable; Figure 6.4.P). When comparing between tissues, the ST versus the MCL was the only significant difference, where the MCL had significantly more area with variable crimp wavelengths compared to the ST ($p = 0.0486$; Table 6.4.v).

Table 6.4.v Crimp Wavelength Variation pairwise comparisons between tissues of 17 different human lower limb tendons/ligaments for significantly different comparisons only. Adjustment for multiple comparisons performed using a Bonferroni correction. Asterisks indicate strength of significance: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$. Analysis does not account for donor covariates (sex, age, height, and weight). *Refer to Table 6.3.a for identification of tendon/ligament acronyms.* Full list of pairwise comparisons can be found in Supp. Table 9.7.e.

Graph Position	Tissue Comparison	Mean Rank Difference	Summary	Adjusted P Value
10 vs. 16	ST vs. MCL	-66.25	*	0.0486

6.5 Discussion

The current study uncovered new findings relating to structural variations in human lower limb tendons and ligaments as explored through histological assessment. While findings are often specific to differences between the tendon specimens versus the ligament specimens, this work highlights structural characteristics and variability between tendons, not previously captured. Notably, the typical selection of tendon grafts for the surgical reconstruction of ligaments, do not always reflect the closet match to the native ligament characteristics being replaced.

6.5.1 Structural Variations of Lower Limb Tendons (and Ligaments)

6.5.1.1 *Cell Characteristics*

The scores assessing the cellular properties explore characteristics not previously evaluated in a quantitative manner for this range of human lower limb tendons. While cell density has been commonly examined for typical ACL graft substitutes (*refer to Section 2.3.3 Supporting Information S3*: Tables 2a-e)[68], cell distribution (or variation in cell density), predominating cell morphology, arrangement of cells within the tendon structure, as well as the presence of atypical-phenotype or chondrocyte-like cells which differ from the usual understanding of tenocyte phenotypes, have not previously been evaluated.

Differences in cellular properties may imply differing physiological statuses/capabilities of different tendons, giving rise to variances in cellular mechanotransduction, metabolic activity and synthesis of the extracellular matrix within the tissue[265,266]. However, cellular morphology, arrangement and the presence of atypical-phenotype cells were not significantly different between tendons, and although the ligaments tended to have more rounded nuclear morphologies (Figure 6.4.B) and more atypical cell types (Figure 6.4.D), only the ACL and PCL had significantly more atypical cells compared to other tissues (Figure 6.4.D). The LCL was the only tissue which had significantly greater cell density (Figure 6.4.A).

Interestingly, the current study found conflicting results to a previous research group (Hadjicostas *et al.*) that assessed cellular densities in the four common graft choices in ACL reconstruction (QT, PT, ST and GT) across multiple studies. In one study, Hadjicostas *et al.* (2007)[165] reported the QT (17.4 ± 3.25 cells/mm^2) had significantly higher cell density than the PT (10.65 ± 2.48 cells/mm^2), while Hadjicostas *et al.* (2007)[167] found both hamstring tendons to have higher densities than the PT (ST = 23.25 ± 4.41 cells/mm^2, GT = 21.9 ± 3.41 cells/mm^2, PT = 12.80 ± 2.09 cells/mm^2). In a third investigation, Hadjicostas *et al.* (2008)[164] found the ACL (34.4.5 ± 10.2 cells/mm^2) had significantly higher cell density than the four commonly grafted tendons. The ST (21.5 ± 4.4 cells/mm^2), GT (19.9 ± 3.6 cells/mm^2) and QT (17.4 ± 3.4 cells/mm^2) were furthermore also significantly higher than the PT (12.7 ± 2.1 cells/mm^2)[164]. While the difference was not statistically significant, the ST cell density was relatively larger than the GT (ST = 21.5 ± 4.4 cells/mm^2, GT = 19.9 ± 3.6 cells/mm^2) in a similar investigation[166] (however caution should be used because the same means and standard deviations are reported in both studies, despite reporting different sample sizes). Notwithstanding this caveat, overall, these studies indicate the

order of tendons/ligaments ranking as the ACL with the highest cell density, followed by the ST, GT, QT and then the PT with the lowest density.

Conversely, in the current study of human lower limb tendons/ligaments – while no significant differences were found between these tissues – the order of higher cell density started with the PT (312.4 ± 352.8 cells/mm^2), followed by the ACL (290.6 ± 165.0 cells/mm^2), QT (208.4 ± 39.25 cells/mm^2), GT (147.9 ± 40.99 cells/mm^2) and then ST with the lowest relative density (108.3 ± 69.37 cells/mm^2) (Figure 6.4.A). However, the PT has one donor sample with an evident outlier, where excluding this datapoint places the PT as the 3rd highest density (middle rank) with 188.0 ± 28.21 cells/mm^2 (that is, ACL > QT > PT > GT > ST).

Something to point out is the vastly different cell densities between the current study and the Hadjicostas *et al.* group[164-167], being an order of 10x higher for the present study. Hadjicostas *et al.* evaluated cell density by counting the number of cells (fibroblasts) in the "whole area section" and normalised to number per mm^2 of tendinous tissue area. On the other hand, our approach evaluated cell density by averaging the normalised counts of cells from three separate ROIs of 0.04 mm^2. While they did not statistically compare densities, Weiss *et al.* (2012)[267] recorded the QT with the highest density in their study (118.2 cells/mm^2), followed by the ACL (88.7 cells/mm^2), PT (80.8 cells/mm^2), GT (58.9 cells/mm^2), ST (52.7 cells/mm^2) as well as the TA (43.6 cells/mm^2) when averaging 10 ROIs of 1 mm^2 area, observing densities still lower than the current study, but also much higher than the Hadjicostas *et al.* group, however only one donor was used for tendon tissue while the ACL was collected from three donors, and multiple segments along the length of each tissues was assessed and (presumably) averaged. Although there were different approaches taken to calculate cell density, the normalisation to the same scale (cells per mm^2) should allow for comparability between the investigations across research groups, with examples from the current presented in Figure 6.5.A showing much higher densities than those reported by Hadjicostas *et al.* respective to a representative 1 mm^2 area.

Other methodological factors were also considered. Both the Hadjicostas *et al.* and our research groups collected similarly sized samples from male and female donors with similar indications of musculoskeletal health history. The Hadjicostas *et al.* group investigated younger cohorts available in some of their investigations (mean age of 36.4 years[164] or 36.6 years[166]) but similar in others (mean age of 56.6 years[165,167]). However, cell density is expected to decrease with age[34,268-271]. Unfortunately, the sample processing specifications are limited in the published articles, stating only that histological samples were "formalin-fixed (4% formalin in phosphate-buffered saline solution) and paraffin-embedded via the routine procedure."[164-167]. In contrast, our samples were fixed in 10% neutral buffered formalin, a routine procedure known to preserve the cellular components of tissue[272], although it is possible that the solution specified by Hadjicostas et al. is actually 4% *formaldehyde*, an equivalent name for 10% formalin, although Weiss *et al.* (2012)[267] had also reported using a 5% formalin fixation process. Other unknown details from Hadjicostas' studies include the thickness of the evaluated sections which may influence the number of cells captured in a given section; and the inclusion or exclusion of IFR-related cells in the cell count, which were ultimately included in our cell counts (*refer to Section 4.4.6.1*) and typically have a higher packing density than fascicular cells[244].

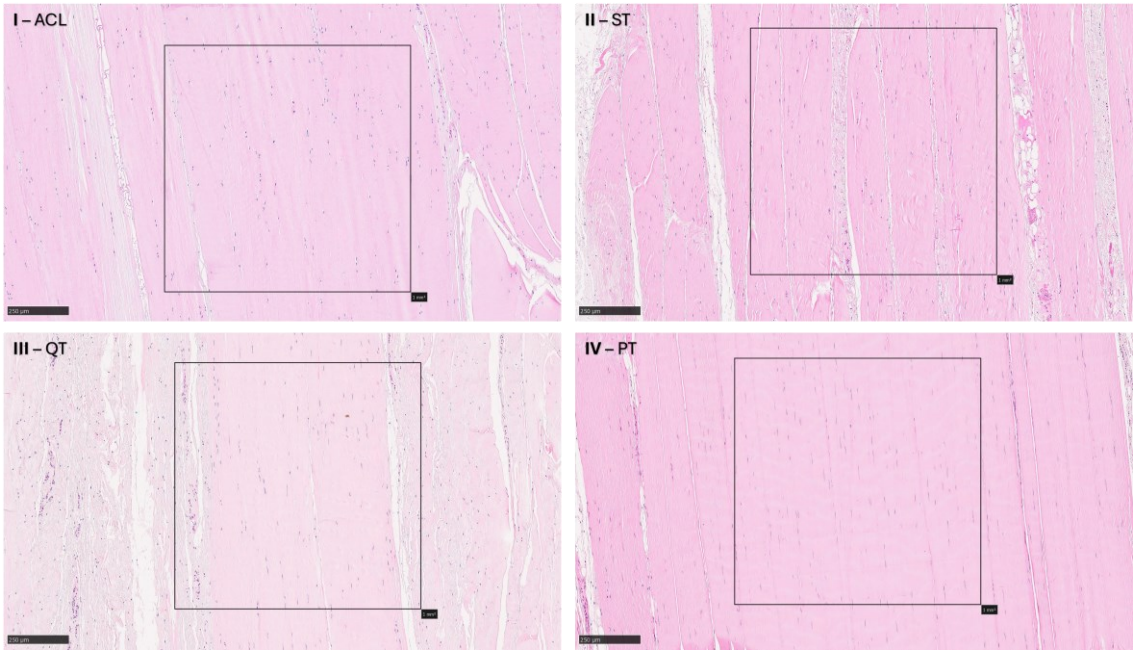


Figure 6.5.A Representative images showing cell densities respective to a 1 mm^2 ROI (square) for the Anterior Cruciate Ligament (ACL: **I**), Semitendinosus tendon (ST: **II**), Quadriceps tendon (QT: **III**), and Patellar tendon (PT: **IV**). *Hematoxylin & Eosin; 100x magnification, scale bars = 250 µm.*

The large difference between these two investigations, without a clear cause, accentuates the necessity for more detailed reporting and standardised methodological practices to be applied across tendon comparisons. In the current absence of this across the literature, large-scale studies exploring a wider range of tendons – as opposed to individual groups exploring select few specimens – is the most valuable way to fill current gaps in knowledge of tendon characterisation.

6.5.1.2 *Proteoglycan Content*

The presence of proteoglycan within tendon/ligament structure contributes to the composition of the tendon extracellular matrix, properties of which is also associated with the mechanical environment of the tissue and is important for tissue physiology[69,118,273]. For example, decorin helps regulates fibril formation and structure[75,76,78,79], while lubricin facilitates fibril sliding[69,76,80,81] and GAGs in general can be predictive of mechanical properties[77,82-84,91,274] such as compression resistance in specific tendon regions. In the current study, no significant differences in proteoglycan content were observed between tendon types, while the ACL, PCL and MCL were the only tissues to have significantly greater proteoglycan content compared to other tendons (Figure 6.4.E), typically scoring higher also with the average staining intensity (Figure 5.4.F) as observed through assessment of Toluidine Blue histochemistry. The ACL exhibited the highest proteoglycan content within the lower limb tissues, typically outranking the levels exhibited by the tendons commonly used as grafts to replace them.

Higher proteoglycan content in the ligaments may impact the mechanical properties of the tissues or indicate unique loading environments which should be considered in reconstructive surgery. Our group have reported on biochemical analysis of donor-matched tendons (opposing side limb) to the current histological investigations (*refer to Figure 3.2.A*) that had undergone mechanical testing to failure[127]. Sulphated GAG (sGAG, an indication of proteoglycan content) was

determined for the 13 tendons and 4 ligaments presented in this study, as well as samples from the middle-third ("graft" samples) of the QT (mQT) and PT (mPT) from the same tendon used for histological sampling. While direct parallels are not the case, the general trends of PG content are similar. The ACL and PCL in both investigations stood out with having significantly higher proteoglycan content relative to many of the other tendons[127] (Figure 6.4.E). Furthermore, across all tendons and ligaments, higher sGAG content was associated with lower strength (ultimate tensile strength, UTS) and elastic modulus.

Our group also previously saw no notable differences (no statistics reported) in histological evaluation of proteoglycan content between ovine energy-storing (SDFT and DDFT) and positional (medial extensor) tendons[11] – functionally-distinct tendons akin to the human Achilles (energy-storing) and Tibialis Anterior (positional) – while sGAG significantly differed between the ovine tendons being of higher content in the energy-storing tendons[11]. Again, the energy-storing SDFT with higher sGAG had significantly lower strength (UTS) and elastic modulus[11].

Thus, our prior investigations reveal a negative correlation of proteoglycan (sGAG) content with biomechanical outcomes[11,127]. Greater UTS and elastic modulus of tendons was associated with significantly lower proteoglycan content (sGAG), supporting the notion that structural changes associated with pathology (such as increased proteoglycan content) that precede rupture contribute to the loss in mechanical integrity of the tissue[160,162,174,179,200,275]. With this consideration, it could be speculated that perhaps replacing the ACL with a graft exhibiting natively low sGAG content (hence higher strength and elastic modulus) could support more resistance to mechanical insult as the tissue integrates and remodels to the new environment.

6.5.1.3 *Collagenous Organisation*

Interestingly, the current investigation of human lower limb tendons and ligaments found some ligaments have significantly more disorganised collagenous architecture compared to the majority of tendons (Figure 6.4.G). It is not clear whether this is a true difference in collagen fibrillar matrix organisation in ligaments versus tendons as a result of differing mechanical load-resistance requirements[106,276] or pertains to the comparably higher IFR counts of some of the ligaments (Figure 6.4.L) mostly presenting with interfascicular matrix (IFM; Figure 6.4.M). Much like the current findings, a study on canine tendons and ligaments recorded ligaments to have lesser collagen organisation. Kharaz *et al.* (2018)[19] found that the ACL had significantly lower extracellular matrix organisation than the LDET and SDFT, while the MCL had lower significantly lower organisation than the LDET[19]. The study also reported the two tendons to have more compact fascicles with narrower IFM compared to the ligaments[19]. These discoveries from the published canine work and from the current human investigation are noteworthy, where previous correspondence in the scientific community alluded to ligaments not exhibiting interfascicular regions, presumably through expectation implied by the multidirectional nature of ligament collagen fibres[106,276] which in argument could reduce fascicle formation and thus IFR/M. However, our findings and previous work[19,109,277] has clearly identified IFR, and a higher proportion of these being IFM, in ligaments.

Nonetheless, collagenous organisation is often linked to tissue function[8,19,278,279]. As with the rotator cuff tendons of the shoulder (*see Section 5.5.2*), the ligaments of the knee exhibited

comparably higher Collagenous Organisation scores relative to most other lower limb tendons (Figure 6.4.G), reflective of more haphazard orientation of the collagenous matrix. This characteristic evidently associates with ligamentous structures[276], supporting a range of pull directions necessary to allow the ligament to withstand the varied tensile forces experienced in the act of stabilising joints. The higher incidence if IFRs (Figure 6.4.L) and those with IFM presence (Figure 6.4.M) in the ligaments also supports the notion that these tissues require particular hierarchical organisation with greater interfascicular sliding capacity to facilitate the multidirectional loads and non-uniform strain within the tissue. Likewise, in the current study there was a tendency for plantar-surface / posterior tendons of the leg/ankle region, and extensors / anterior tendons of the knee to exhibit comparably higher incidence of IFR and IFM proportion compared to their opposing counter-part. It would be interesting to explore potential connection to the requirement of these tissue to stretch further over joints as they track over the external surface of their respective joint angles. It could be hypothesis that IFRs and specialised IFM constituents exhibit correlation to greater strain (elongation) capacity of these structures.

The requirement of biomechanical efficiency of tendons/ligaments opens discussion for uncovering structural attributes which support functional needs. As with quadruped SDFT versus CDET tendons, the AT and TA tendons of the human lower limb also engage as energy-storing and position tendons in the generation of locomotion, respectively[280].The relatively high IFM proportion of identified IFRs of the Achilles Tendon compared to the Tibialis Anterior tendon in the current study reflects similarly to observations by Patel *et al.* (2021)[280]. The protein composition associated with the IFM of energy-storing tendons reflect reduced hysteresis (thus greater energy transfer efficiency) and reduced force relaxation (thus resists loss in tension)[280] and facilitate effective tendon recoil and thus locomoting propulsion. An interesting observation from the current study is the similar relationship between the AT and TP as to the FHL and ELH, where the FHL supports locomotive plantar flexion alongside the AT (contributing the “toe-off” moment of propulsion in the walking cycle), while the EHL facilitates ground clearance in dorsiflexion[281]. With current tendon-based research typically restricted to only a few different tendons, the current study exploring a larger range of tissue types reflects structural trends which may support future classification of tendon functional distinctions following further research and exploration.

In the context of grafts, a histological focus on the structural appearance of the collagenous architecture is commonly used to assess the progress of graft remodelling, often termed “ligamentisation”, in the assessment of tendon grafts used for ACL reconstruction[52,282-287]. However, this feature is often underreported and granted limited emphasis relative to the other two features commonly assessed, being cellular and vascularisation characteristics[287,288]. Irrespective, as it has been reported that tendon grafts never remodel fully to reflect the native characteristics of the reconstructed ligament[283,287], it may be beneficial to select graft tendons that already most closely resemble/approximate the tissues to be repaired. Interesting to note is the generalisation of similar outcomes reported for the PT as to the knee ligaments in the current study. In part this could be expected due to the functionally unique position of the PT, which is short and flanked by two bony attachments (as exhibited by ligaments). However, it retains

function closer to the role of tendons, transferring tensile force to enact skeletal motion, giving it unique structure-function considerations requiring further investigation.

6.5.1.4 *Adipose Presence*

For the included donors, end-of-life (body-mass index) BMIs ranged from 15.87 to 24.54 (average 19.9 ± 2.5). Notably, no single donor predominated the highest scoring samples, implying little effect of a specific donor (with e.g. higher BMI and systemic adiposity or age/senile-associated change) on the outcomes.

In regard to the presence of adipocytes/adipose tissue scored within the tendon area, only the QT was significantly different to other tissues, having greater adipose presence compared to the Plt which was completely absent of any adipose/adipocytes (Figure 6.4.K). In general, the knee region was observed to have much "fattier" structures compared to the ankle and foot regions (Figure 6.4.K). The ST, QT and PT, along with the PCL, MCL and LCL all presenting with greater adipose presence, while the AT scored the highest of the ankle/foot region tendons.

Proximity to fat pads of the AT (Kager's fat pad), PT (Hoffa's fat pad) and QT (Quadriceps fat pad)[101] likely justify the increased adipose present within these tendons. These fat pad structures are known to integrate into the tendon structure, accounting for the increased adipose observed histologically[289-291]. The close proximity of the ligaments and flexor tendons (ST, GT) of the knee to the surrounding fat pads – as well as the adjacent Pes Anserine Bursa[292] – could implicate the higher incidence of adipocyte presence in these structures. Functionally speaking – while also offering more complex and not fully understood attributes – fat pads/bursae broadly enact to provide support/lubrication around joints for smooth and efficient movement[253,293]. Likewise, greater adipose presence in tendons/ligaments may develop as a cushioning feature to the tissue structure due to their loading environments. The shorter length of the extensor tendons and ligaments and thus proximity to the enthesis regions may imply biomechanical contribution of the adipose tissue at the insertion points/points close to compressive points around joints, as well as aiding dissipation of load throughout other regions of the tendon[294]. The energy-storing nature of the AT and the dynamic loading conditions of the knee extensors[295,296] and structures of the Pes Anserinas[297,298] may implicate the requirements for increase load dispersion via adipose cushioning. Interestingly, the ACL was the least fatty structure of the ligaments, setting it apart from both the other knee ligaments and the typical graft choices from the knee region.

Adipose is rarely quantified in histopathological scoring systems, with mucoid degeneration more commonly assessed[168,169]. This is surprising considering clinically, the presence of adipocytes or lipid deposits is one underlying pathology present in cases of tendinopathy[162,299-302]. Likely, the biopsy-style nature of typical histopathology assessments of patients limits sample size and reduces the overall findings of adipose structure within the tendon whole. Regardless, assessment of specific tissues needs to consider the significance of adipocyte presence where certain tendons may naturally contain more fatty infiltration under normal conditions. Fat pad associated adipose may also implicate increased vascular and neural supplies of the tissue[290,294,303] with intra-tissue adipose presence likely alluding to potential healing capacity but also pain in injury and chronic disease of tendons/ligaments.

6.5.1.5 *Crimp*

Crimp wavelength reflects one structural feature of tendons that is closely linked to the mechanical attributes of the tissue, influencing the transfer of force under tensile loading of the tissue[107,304,305]. The ligaments of the knee were found to have the shortest (predominantly < 50 μm in length) and most consistent wavelengths across all specimens differing significantly from many of the other tendons (Figure 6.4.O), but had relatively higher intra-specimen variability (Figure 6.4.P). The differing roles of tendons versus ligaments may also necessitate their structural differences regarding crimp. The main function of tendons is to transmit force between muscles and bones, while ligaments provide stability and act to resist undesired joint movement[1-3], they need to resist excessive motions while the function of tendons is to drive movement. One theory behind the shorter wavelength of ligaments is the necessity to improve shock-absorption of the tissue (dampening loads experienced at the entheses)[6,104,107,267,306] and how quickly load needs to be dissipated[307] while still facilitating action of the ligament to resist (stabilise) skeletal motion. In tendons, the pliable muscle would aid in this dampening for tendons. We have shown that crimp wavelength is significantly shorter in the distal (bone) end of tendons compared to the proximal (MTJ) end[209,308], suggesting the bone-insertion of these structures favours finer crimp for improved load transference. Furthermore, we previously found males to have significantly shorter crimp wavelength compared to females[209,308], a characteristic likely adapted to the typically larger and stronger muscle architecture of males[309] thus facilitating the tendon to enact load improved dampening. The shorter wavelength of energy-storing tendons[17,18], including the AT in the present study, also points to viscoelastic considerations of crimp for high energy tendons. Our laboratory previously assessed the mechanical attributes of tendons tensile tested to failure[127] using donor-matched tendons (opposing side limb) to those in the current histological investigations (*refer to Figure 3.2.A*). While no correlative analysis has been performed to date, future research could explore the relationship between failure strain (% deformation before failure) and crimp wavelength to explore the mechanical significance of crimp to resisting (or benefiting) stretch and deformation.

A consideration that may cause shorter crimp wavelength is the contraction of tissue due to increased elastin content. Hadjicostas *et al.* (2008)[164] found elastin content of the ACL to be significantly higher than the ST, GT, QT and PT tendons. Similarly, elastin content has also been shown to be significantly greater in the ACL compared to other canine tissues[19], however the MCL elastin content was low and similar to the LDET and SDFT tendons, not reflecting the crimp trend of the current study. Additionally, the density of cells positive for actin isoform α-smooth muscle actin has been shown to have strong correlation to crimp frequency[267]. IHC assessment on the current dataset might reveal whether there is a similar greater incidence of α-smooth muscle actin positive cells in ligaments versus tendons, particularly given that the ligaments in the current study had, on average, higher cell densities than tendons (Figure 6.4.A).

Considering the importance of biomechanics in tendon and ligament function, it is intriguing that the common ACL graft options (knee tendons) stand out as some of the most different in terms of crimp wavelength compared to the ACL itself. Rather, the AT, TP and FB most closely match the ACL.

6.5.2 Future Directions

6.5.2.1 *Clinical Implications (Grafting)*

The current study explored a large range of thirteen tendons and four ligaments using consistent methodologies, assessing not only the commonly grafted tendons (ST, GT, QT and PT) and injured ligament (ACL), but also many other tissues which are either commonly injured or enlisted as grafts in reconstructive surgery.

Despite the varied clinical outcomes (success rates, rupture, comorbidities, patient satisfaction, etc.) associated with differing ACL reconstruction approaches or graft choices[310-316], there is little evidence to support why one tendon graft might outrank another[311,317]. Considering the structural changes that occur during the ligamentisation process of grafting in the case of ligament reconstruction[183,286-288,311,318] – or the potential adaptions of a tendon to new loading environments when grafted to replace anther tendon[319,320] – it may be beneficial to understand not only the native properties of the tissue being replaced by the graft, but also the characteristics of the graft tissue itself. In other words, what characteristics are paramount to support effective and complete ligamentisation – or "re-tendonisation" – and allow adaptation to the new environment. Few studies have explored underlying differences of different graft tissues and the potential implications for clinical outcomes. Sometimes, only the native ACL (or ligament to be reconstructed) is used as a control without consideration of the native tendon tissue[321], likely due to the prospective nature of the study and unavailability of donor specimens.

Outcome-outcome correlations may have important clinical implications, particularly for the selection of grafts or the design of biomimetic materials[322,323] based on specific native tissue properties. Most studies focus on correlations between mechanical outcomes and other characteristics[127,275,324,325], though rarely with histological findings. It is more prevalent in the literature for investigations to consider the effects of the mechanical properties of tendons – as well the implications of biomechanical considerations for surgical techniques – on the clinical outcome of reconstructive surgery[313]. On the other hand, to the best of our knowledge, no studies have directly explored the relationships between structural properties of involved tissues and clinical results (success rates, rupture, comorbidities, patient satisfaction, etc.), while Mayr *et al.* (2012)[288] is one of few that correlated histology of the ligamentised tissue to mechanical integrity of the reconstruction.

Appreciating outcome-outcome correlations is linked to better understanding of structure-function relationships. Previous work on equine tendons[7,15,18,29,227,228,230,244,326-330] have collectively explored concepts across multiple outcome measures and built foundational knowledge of this space, but translation to the human situation is lacking. The work in the current chapter on structural features, along with earlier studies of differing biomechanical and biochemical properties[127,259] of a range of human tendons identify variations in the 'baseline' properties of tendons that may prove important for selecting potential graft tissue sources. Future work of our laboratory is to assess the correlations between biomechanical, compositional (biochemical) and structural (histological, electron microscopy) outcome of donor matched tendons to explore differences between tendons and the correlations between tissue properties. This information will

support further research into defining the important consideration for graft selection or tissue engineering and provide important data towards enabling the idea of "personalised" graft selection.

6.5.2.2 *Donor Variables*

The strength of the current study lies in comparing a range of tendons and ligaments from the same set of donors within a defined age bracket (49-65 years old), with no known history of musculoskeletal conditions and BMIs < 25 kg/m^2. While this minimises the effects of systemic factors (e.g. age, sex, obesity, etc.) that could confound comparisons, the restrictive donor criteria and limited number of donors (while greater than most normal comparative studies) constrains the statistical power needed to analyse the effects of donor variables in depth. Nevertheless, the present study identified no broad relationship between donor variables and Cell Density (Table 6.4.c; Table 6.4.d), Cell Distribution or IFR Type Proportion (Table 6.4.r; Table 6.4.s) for the lower limb tendons and ligaments. However, IFR Type Frequency was shown to be affected by donor height and weight (Table 6.4.p), where the number of IFRs decreased with increasing height and weight. An increase in cross-sectional area and maximum load to failure of the AT with increasing donor height[259] possibly suggests the contribution of the mechanically important FM area is prioritised in increasing tendon size (thus lower IFR frequency) for load resistance within larger body structures. Considering the implications of sex, where although males have a slightly larger (but non-significant) IFR frequency than females (Table 6.4.o), the typically larger musculoskeletal structures of men – including tendon morphology[259] – also points to increasing tendon size being predominated by larger fascicular structure (few IRFs). However, further evaluation of IFR versus FM contribution to tendon mechanical dynamics with altering tendon or organism size (or biology) is required, particularly considering the IFR is an important structure in tendon mechanics[8,227,228,230,244].

Although more work is needed to fully characterise individual or cohort-specific variation within these musculoskeletal tissues, this research provides important groundwork for defining and understanding tendon properties and possible links to donor characteristics. Donor variables have previously been associated with changes in tissue properties (refer to *Section 2.3.3 Supporting Information S3*: Tables 2a-e)[68], and these have been shown to be tendon specific. Previously we found that only the AT, TP and TA were significantly affected by donor variables (age or height) for certain biomechanical outcomes[259], a study which included tendons from the same donors of the current study as well as an additional cohort of older donors (full cohort ranges: age = 49-99 years, height = 145-216 cm). Similarly, tendon-specific age effects have been identified in equine tissues. Godinho *et al.* (2017)[234] concluded that age related loss of IFR elastin organisation seemed specific to the equine SDFT and not the CDET, while Fjordbakk and Marques-Smith (2023)[303] reported on age significantly increasing vasculature and IFM size in the Medial and Intermediate Patellar Ligaments, but not the Lateral.

6.5.3 Conclusion

The current study explored structural variations of thirteen human lower limb tendons and four ligaments using histological assessment. Findings uncovered heterogeneity between the structure of these tissues, furthering the scope of knowledge which was previously limited to a few select tendons in current literature. Histological explorations using the novel scoring system (*Section 4.5.1*) found knee ligaments to exhibit comparably different and often diverse structural properties relative to other lower limb tendons. Furthermore, the typical graft tendons used for ACL reconstruction (i.e. PT, QT, ST and GT) do not always reflect the best structural match to the native ACL for all outcomes.

While deeper exploration into inter-tendon heterogeneity and defining structure-function relationships is needed, this study challenges current graft selection choices where future research could identify additional tendons suitable for use as grafts or as reference for the development of biomimetic tissues. Further exploration is needed to implicate the structural variations against potential differences in mechanical properties[127,259] and grafting suitability between tissues.

~ CHAPTER 7 ~

TO END ON A LIGHT STATEMENT

TENDONS BACK IN FOCUS – DISCUSSION OF NEW DISCOVERIES AND DIRECTIONS FOR FURTHERING THE FIELD

"The world only makes sense when you force it to."

~ Bruce Wayne/Batman, The Dark Knight Returns #4 (1986) ~

7.1 Overview

Overall, the work presented in this thesis addressed prevalent gaps in knowledge in the field of tendon structural exploration. This chapter discusses the topics covered and addresses points for future work to advance the field of tendon characterisation.

Figure 7.1.A Tendons and their clinical relevance.

7.2 Summary of Thesis Topics

Systematic exploration into current literature on tendon structural characterisation (as assessed through microscopy modalities) found that current understanding of variations between different tendons or ligaments is insufficient (*Chapter 2*).

Further exploration into tendon histological characterisation has been stalled by a lack of histological scoring systems able to quantitatively capture the variability across normal, healthy tendons. Accordingly, a new scoring system was developed (the Standardised Scoring Suite; SSS) for the Histological Assessment of Ligament and Tendon (HALT; *Chapter 4*).

The SSS identified variation across 48 different tendons/ligaments of the human appendicular skeleton (upper and lower limb), collected from human cadavers from donors with no history of musculoskeletal injury or disease **(***Chapter 5* and *Chapter 6*).

Application of the SSS to a variety of tendons demonstrated the need for such a system and also highlighted areas for improvement, including strengthening the guidelines for implementation, ensuring awareness of limitations and addressing further knowledge gaps in tendon structure.

7.3 Discussion of the SSS

7.3.1 Achieving Recommendation for HALT

As identified by *Textbox 2* of *Chapter 4*, recommended specifications for tendon and ligament histological assessment were presented. Summarised below are examples of the achievements of the newly proposed scoring system in line with these recommendations (Figure 7.3.A). However, despite this, there is room for further work in the refinement of the HALT-SSS system presented in this thesis.

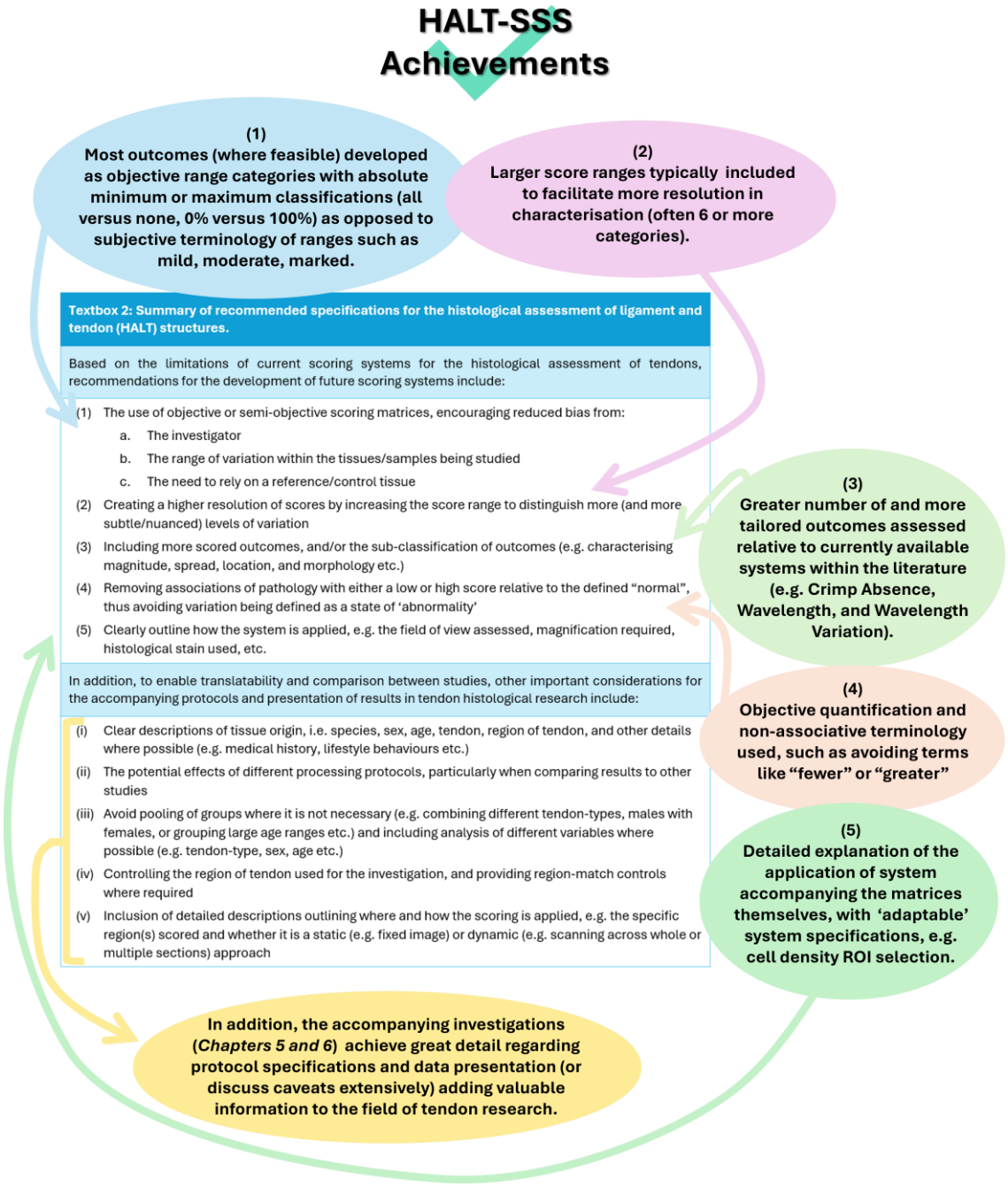


Figure 7.3.A Examples of the recommended specifications (*Chapter 4*, *Textbox 2*) achieved by the newly developed tool (Standardised Scoring Suite, SSS) for the Histological Assessment of Ligament and Tendon tissue (HALT) presented in *Chapter 4* and explored in *Chapters 5 and 6*.

7.3.2 Considerations for HALT

Throughout the development and application of the HALT-SSS to analyse a wide range of tendons of the upper and lower limb, a number of additional considerations were identified, as described below.

7.3.2.1 *Artefacts.*

Histology is not a perfect science. Dense connective tissue such as tendon and ligament prove particularly challenging to section, often resulting in the introduction of artefacts. Determining what is an artefact versus real/true histological findings can be difficult and requires experience and/or training. It is recommended that if artefacts are observed during initial observations, these should be noted in detail to facilitate future scoring and comparability to other observers and not confuse artefacts with characteristic changes.

For example, cutting chatter/venetian-blind effects[331] – resulting in varying section thicknesses parallel to the knife edge throughout the section – may influence staining intensity, structural appearances and alignment characteristics (Figure 7.3.B). Utilising recorded notes, it may be possible to justify certain scores despite up-front effects of the artefacts, such as defining consistent staining within the tissue despite the changes resulting from the "corrugated" chatter effect if 'background staining' is perceived to be consistent. Another example may be the decision to ignore holes/tearing artefacts when considering scores. Documentation during scoring was important and will be critical for the ongoing use and development of the SSS to track intra- and inter-scorer consistency over time. These artefact examples are also important considerations for implementing automated image analysis protocols which could over- or under-estimate characteristics (such as staining intensity, collagenous organisation) when applied to artefact-affected regions. This may then require additional steps to ensure appropriate ROI selection or training/validation testing to detect and exclude artefacts.

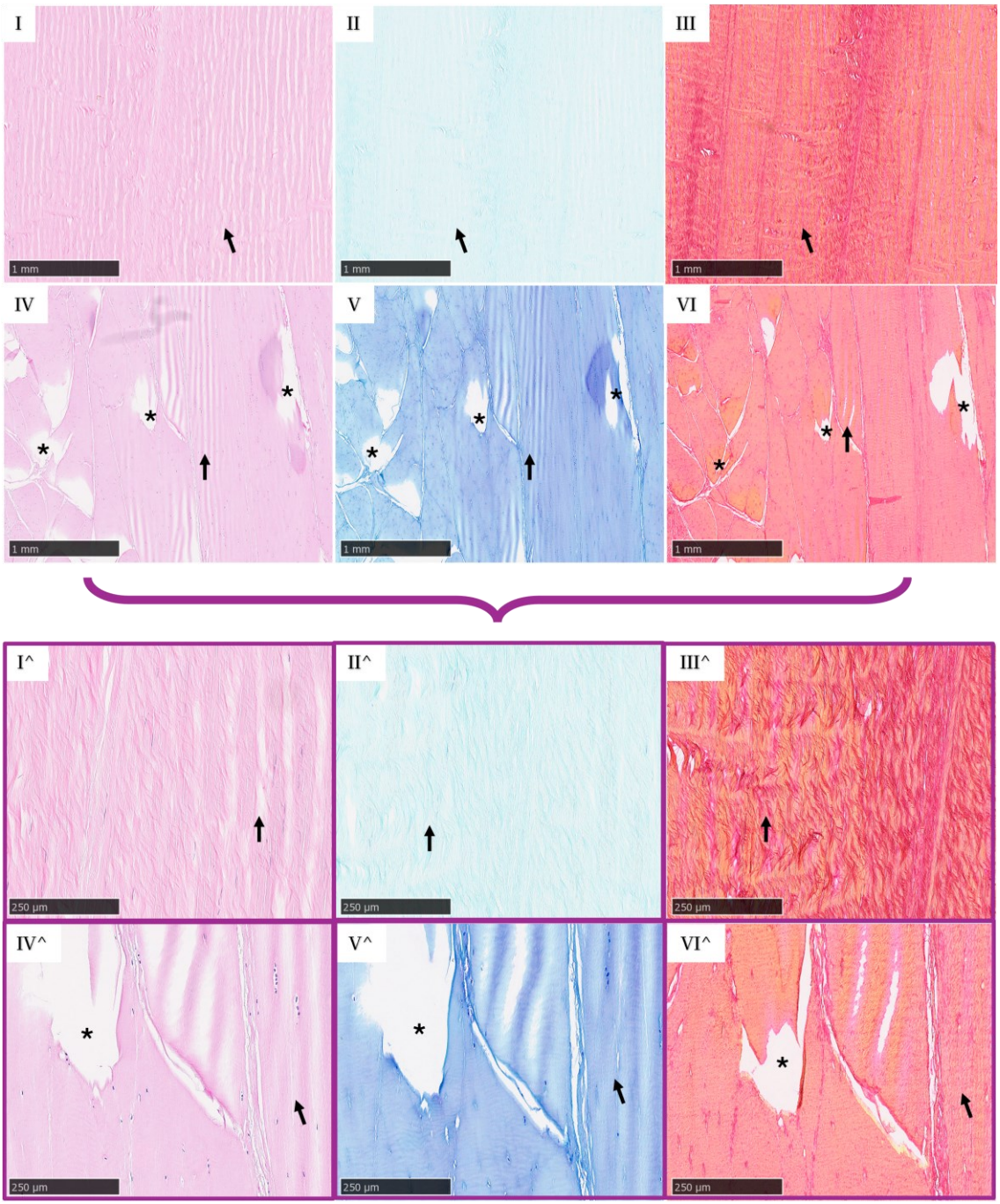


Figure 7.3.B Examples of different histological artefacts encountered, such as chatter-effects (arrows) or tears/holes in the tissue (asterisks *). **I/IV** = Haematoxylin and Eosin stained. **II/V** = Toluidine Blue stained. **III/VI** = Picrosirius Red stained. **Top frames** (I-VI): *25x magnification, scale bars = 1 mm*. **Bottom frames** (^): *100x magnification depicting centre of respective lower magnification frame, scale bars = 250 µm*.

In addition, chatter-effects also highlight a consideration for histological preparation of tissues like tendon/ligament. Arguably, this type of cutting artefact may be induced by the tissue crimp causing inconsistent 'pressure' against the blade during sectioning. Our laboratory has extensive experience working with the histological processing of tendons[11,142,199-201,332] and note the difficulties in cutting tendons in particular, with orientation of the specimen relative to the cutting direction being an important consideration. Cutting longitudinal sections with the edge of the

blade running parallel to the fibre direction reduces snagging and pulling-out of the fibres/fascicles and limits blade movement induced by the crimp (which would occur if orientated parallel to crimp / perpendicular to fibres) (Figure 7.3.C).

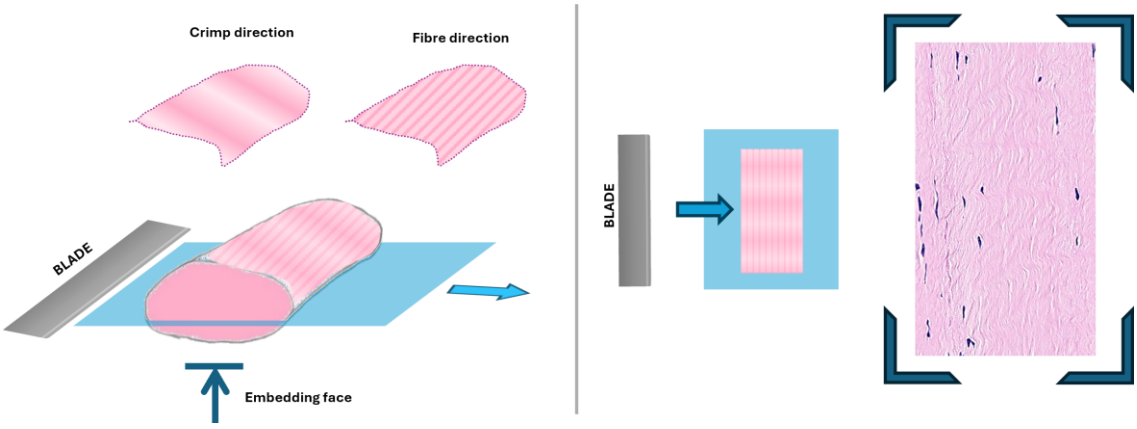


Figure 7.3.C Recommended cutting orientation for histological sectioning of tendon/ligament tissue.

7.3.2.2 *Sample Variability*

Ironically, one of the biggest challenges in developing an all-use scoring system to characterise the natural variability between different tendons was the scope of the variability such datasets present. It was difficult to develop objective measures and parameter definitions that clearly and easily captured the full range of characteristics encountered across different tendons.

As one example, in early versions of the system, cell density was assessed specific to the FM (excluding cells within the IFR). The dimensions of a pre-specified square ROI intended to ensure only FM inclusion were set using a selection of representative samples from across the full range of tendons available. However, upon application to the larger dataset samples were encountered with fascicles smaller than the ROI, causing unavoidable IFR inclusion. As mentioned in *Chapter 2*, the use of IHC or specialised histochemical stains to differentiate IFR from FM could aid in excluding IFR area and cells from density calculations.

Similar challenges were faced with the vascular density outcome, where designing a scoring matrix capable of objectively assessing vascular presence across samples of such extensive variability in size and structural features proved challenging without relying on time-consuming methods of manual counting across the whole sample or resorting to less widely available automated image analysis methods. Again, IHC could aid in identification of vasculature, and perhaps the use of a lattice for scoring positive regions may be better suited for variably sized samples. Elsewise, it may be suitable to utilise ROIs with variable dimensions (set a defined area required but not pre-define the shape) to allow exclusion of IFR areas.

Additionally, while not explored in the scope of this thesis, further variability in the overall tissue structure and presentation of particular features will undoubtably be encountered with different pathological conditions or tendon health states. Exploration of how the system is applied and potential need for study-specific modifications should be tested and validated in the future.

7.3.3 General Caveats

Some common limitations of the system were covered in the discussion of the scoring matrix outcomes (*refer to Section 4.4.6*), such as the "two-step setup" to some scores which first require the assessor to define the majority/most prominent characteristics to be scored, and the challenge in assessing characteristics separately for FM vs IFR areas. However, other general histology considerations/caveats were encountered, as discussed below.

7.3.3.1 *Unavoidable Subjectivity*

Inevitably, the nature of scoring a descriptive characteristic, even on a graded scale, will always result in some level of subjectivity. One example where objectivity is hard to achieve in manual scoring approaches is the assessment of staining intensity. As with many prior systems, the proposed system grades Toluidine Blue (TB) metachromatic (purple) staining intensity as an indication of relative proteoglycan content (amount). Being of a qualitative nature, objectifying this is near impossible without the use of image analysis software. However, other solutions should be explored in line with the goal to provide a system that is usable and readily available even with limited equipment and resources. The level of variability in interpretations may be mitigated through the provision of a set of standards or representative images to "calibrate" all users to the same range. This approach was taken in the SSS for TB staining intensities as well as other scores including cell morphology and phenotype depictions (Figure 4.5.J; Figure 4.5.B; Figure 4.5.K). As addressed in the field of histology/histopathology, the practice of providing training tools is important, particularly for introducing novice investigators to the characteristics to be scored[149,333].

It was also challenging to develop clear, concise and objective score descriptors that did not introduce more room for subjective interpretation. As an example, the definition of cells arranged into chains for the Cell Arrangement score was as follows: *chains = cells (≥ 3) are arranged longitudinally in-series within their inter-fibre space and are located within 2 (average) nuclei-lengths apart*. Despite the objective nature of the definition (cells must be within "2 (average) nuclei-lengths apart"), subjective judgement may still occur based on what is considered the "average" distance between nuclei. Additionally, this distance is influenced by factors such as the plane of section, variability across large sections, or even regional variations across the tissue. In general, the development of clear and non-subjective definitions to facilitate objective measurement was challenging. However, the advantages of providing more objective descriptors was considered greater than the disadvantage of possibly introducing additional subjectivity, thus these descriptors were maintained.

7.3.3.2 *Scoring Approach*

During testing of v3.2 of the scoring system (*Section 4.4.4*), the approach taken by each rater was discussed. Rater-1 scored by an outcome-by-outcome approach (one outcome assessed for all samples, then the next outcome, etc.) except where outcomes were explicitly linked (e.g. scores relating to PG staining). Conversely, rater-2 scored using a sample-by-sample approach, assessing all outcomes for an individual sample before progressing to the next sample. When transitioning between different outcomes and stains, rater-2 noted that having a wholistic view of

an individual sample influenced their perception of individual outcomes and led to adjustments being made to previously assessed outcomes before progressing to the next sample. On the other-hand, rater-1 developed familiarity with dataset-wide variability of an individual outcome allowing for refinement and an "informed" appreciation of the thresholds for each score grade. The greater intra-rater reliability of rater-2's scored versus counted IFR Type Proportions (Figure 4.4.J) may suggest, at least for this outcome, better appreciation of IFR characteristics through exposure to observing matched samples across the three stains. However, there is insufficient data available across the multitude of scoring parameters to support recommendation of one approach over the other.

The concept of human judgment and discretion plays a pivotal role in histological assessment, with the approach (outlined above) being just one example to highlight the challenge of achieving a truly objective and unbiased assessment tool. At the very least, scoring systems should be pushed towards objective scoring parameters to reduce bias and improve reliability and validity, though it has been emphasised that systems should ideally be replaced by automated image analyses[149]. While this is potentially a valuable step for future development, there remains the issue that understanding of normal variation is limited (*Chapter 2*), and thus the information required to support the development of these automated assessment tools is lacking.

7.3.4 Future Developments

7.3.4.1 *System Refinement and Validation*

It is acknowledged that the current SSS contains an extensive number of outcome measures which were necessary for the comprehensive assessment of an under-researched area. These may be further reduced in the future following additional evaluations to establish whether some outcomes are 'redundant' in the sense that they assess similar/related/overlapping variables or evaluate a feature not pivotal to characterising tendon heterogeneity or other adaptations. For example, exploration into outcome-outcome correlations could justify reducing the outcome list to improve efficiency where scores may provide the same information or interpretations.

Some opportunities for potential refinement were identified in using the SSS. Not explored in the current study but noted for future assessment, is the suspected link between the PSR collagen alignment outcome and the H&E evaluation of fibrillar loosening (with likely/potential ties also to fibre separations). Consistent with this, we observed greater "red" colouration (brightfield and polarised; "unaligned structure") in areas of fibres with more loosely organised collagen architecture (fibrillar loosening). Similarly, observations of an "edge-effect" where borders of fibre structures appear red likely indicate loosened fibrillar structure that may be expected at the edge of a fibre (that is, bundle of fibrils). These observations reflect this notion of the red colouration aiding the delineation of the unaligned/unorganised structural arrangement of the collagenous architecture[11,142,153,157,177,334,335]. Considering the difficulty experienced with defining a scoring matrix and assessing fibrillar loosening in H&E sections (*Section 4.4.6.3*), an alternate approach of evaluating this feature which is more objectively defined and evaluated such as with PSR, could improve the system usability. Unfortunately, the areas assessed for the two outcomes

differed in the current projects (designated ROI used for H&E; whole section for PSR) and thus correlation analysis is not possible using the current data.

Furthermore, additional testing of the system is also required to assess validity and reproducibility. While beyond the scope of the current project, intra-rater agreement assessed by comparing repeated scores from the same rater after a period of time (longitudinal consistency) would provide insight into the level of subjectivity versus objectivity achieved by the scoring matrices.

7.3.4.2 *Recommendations*

Based on the preceding discussion identifying specific issues with and opportunities for improving the SSS, a number of research directions and strategies for future work are highlighted below:

- Exploring correlations between the histology outcomes and with outcomes from other modalities (morphological, biomechanical, compositional, ultrastructural etc.) – to reduce system use burden, provide possible surrogate outcomes for more demanding/specialised methods, and enhance understanding of tendon structure/function relationships;
- Using the SSS to evaluate a larger range of tissue origins (anatomical tendons, defined tendon regions, multiple species, greater age/life-stage range, additional sex/biological factors) and especially health status (healthy, diseased, injured, active/non-active) – to increase the system utility and potentially provide a unified approach and improve cross-study comparison;
- Defining investigation-specific technical and personnel influences e.g. tissue processing protocols, staining specificity/interpretation for different outcomes, rater experience/training levels, and ROI selection and application specifics for the system – to increase consistency, reliability and repeatability of tendon research;
- Validation of inter- and intra-rater reliability across a greater number of scorers and developing unified training for application of the system to enhance this – reducing the need for multiple scorers while still providing repeatable and reproducible data, and increasing research efficiency and throughput.

While contributing valuable work into the field of tendon histological assessment, the views and recommendations presented regarding the current SSS development are that of one research group with varying levels of expertise in the field and techniques explored. The review by Chatterjee *et al.* (2023)[149], as well as previous work in intervertebral disc degeneration histopathology[210-214], emphasised the importance of collaboration and discussion with the broader scientific community involved in the field, and ultimately adoption of unified, reproducible and comparable methods and research outcomes.

7.3.4.3 *Evaluating Histopathology*

One of the trends of existing histopathology scoring systems is the summing of scores to give an overall histopathology score/rating[168,169]. While this was deemed unsuitable for the main goal of characterising normal tendon (*Chapter 4*), there is merit in considering certain outcomes in conjunction with others in line with the concept of developing a scoring suite capable of

differentiating not only normal but also "abnormal". As the current proposed SSS system includes cases of multiple scored outcomes assessing the same general characteristic – for example, number and distribution of cells; or presence (%) and intensity of proteoglycan staining – certain outcomes may offer the opportunity to define a "pathology" threshold/grading scale when considering a summed score for a set of tendon features.

Contradictory to the common practice of summing scores for a "pathological assessment"[142,168,169], arguments are made in other tissues[203] that a single summed score does a disservice to pathology evaluation. Defining disease sub-types/phenotypes that might have the same summed score but driven by entirely different individual pathological features[202,203] limits the potential to realise the likely requirement for different therapeutic approaches. Understanding the normal physiology and pathophysiology experienced by different tendons and conditions requires a deeper understanding of the unique mechanistic pathways, which may be better identified by the assessment of individual elements. Future exploration of the utility of more generalised characterisation through use of a summed histology score versus scores for feature-clusters or individual features is required and may depend on the individual investigation and its objective.

7.3.4.4 *Automated Outcomes*

The primary intention of the developed SSS was to provide a readily available system to support tendon characterisation no matter the situation or setting. The development/identification and implementation of automated tools for all of the outcomes was beyond the scope of this thesis. However, undoubtably the best way to achieve objective measures when 'scoring' histological characteristics is to enlist measured-outcomes, with automated analyses offering the highest level of objectivity and consistency with the greatest level of characterising resolution. A bonus is reducing an individual's scoring time and improving research efficiency.

There are numerous published systems where automated image analysis tools have been enlisted to assess objective measurements (instead of scoring scales or manual objective measurements). These include: Toluidine Blue or Alcain Blue metachromasia (PG staining) image colour thresholding[177,336]; cell density[164,209] and nuclear morphology[209,337] particle analysis; collagenous structure/organisation/alignment[149,177,208] using Fourier transform modelling or other measured outcomes; vascular density/endothelial cell presence evaluation[32,338]; and collagen type or elastin staining immunohistochemical quantification[19]. Many of these tools require a specific set of setup parameters and often rely on less-readily available stains or more costly immunohistochemical detections for such characteristic assessment[32,164,338], an approach which was beyond the scope of this thesis's goals of using stains and methods routinely, readily and widely available. Existing tools may also be limited by the specific datasets they were originally developed for, again highlighting the difficulties in developing broadly applicable systems when current knowledge of tendon characteristics and the range of their variations are limited.

7.3.5 Artificial Intelligence (AI)

Despite the growing emergence of artificial intelligence (AI) in all aspect of science, technology, and health, the role it can/will play in histological evaluation is unrealised. The potential for implementing AI in histological assessments was raised in discussion with the Head of Group Technology (AM) at Datacom, one of Australia's leading Information Technology Services companies. In particular, whether AI can exercise discretion or perform classification, which is ultimately a judgement call. In legal and bureaucratic sectors instigating AI, discretion relies on consulting experts in the field to provide AI with the tools to automate a decision-making process, including constructing rules and parameters that can replicate an expert's decision-making process[339,340]. In this way, topical issues and limitations can be addressed as part of the development process, and the implementation of critical thinking and research at the start of the machine learning workflow will result in better outcomes.

The implementation of AI algorithms for histological evaluation in other fields has gained traction in recent years[214,341-346]. Providing the system with histological images and developing assessment workflows through supervised and unsupervised machine learning algorithms has shown promising results[214,346], including the ability to characterise intervertebral disc degeneration in a mouse model[214]. However, there is a necessity for AI algorithms to be trained on extensive and unbiased datasets that accurately capture the state of tissue characterisation[345]. As highlighted in this thesis, that is something the field of tendon/ligament histological assessment is far from. Notably, *Chapter 2* identified the major gaps in current knowledge of tendon variability, while *Chapter 4* revealed the lack of characterisation tools available to broaden the field and address those gaps. Furthermore, considerations of different sectioning and staining protocols, sample dimensions, tissue/sectioning artefacts, and parameters defining the conditions of the input data (tissue specific variability, disease/health status, etc.), pose challenges for training and replicating the level of discretion achieved by higher-order human judgment. The practice of implementing AI in histology is still in early stages. To the best of our knowledge, extension of this practice to the field of HALT has not yet been explored.

It is without a doubt that future directions of histological and histopathological evaluation – including that of HALT – would greatly benefit from the application of AI, both in the collection of data itself using automated systems, but also in the processing of ever-increasing datasets to improve visualisation and dissemination of information. The proposed SSS presented in this thesis (*Chapter 4*) was shown beneficial to the field due to its expanded and extensive outcome assessment compared to previously published scoring systems for HALT (Figure 7.3.A), unveiling new insights on tendon structural variability (*Chapter 5; Chapter 6*). However, as previously reported in the development of a comprehensive scoring system for intervertebral disc degeneration[214] and experienced in the current work on tendon scoring, the implementation of more complex scoring matrices can lead to reductions in data reliability/reproducibility as well as challenges analytical tactics. Within machine and deep learning, the technique of dimensional reduction – such as Principal Component Analysis, t-Distributed Stochastic Neighbour Embedding, Uniform Manifold Approximation and Projection, and the use of auto-encoders – can improve exploration of larger, more complex datasets[347-350] and improve data visualisation

and interpretation and can significantly increase the magnitude of information/data explorable[341,343,344]. Implementing AI for histological evaluation benefits research and clinical advancements through saving time and expenses and allows for larger tissue samples and extensive dataset to be explored in depth where this may not be feasible for manual assessment without compromise of quality or the effects of human error[341]. Beyond just single-source inputs, the large computational capacity of AI can also allow histological data to be combined with ultrastructural, biomechanical, biochemical, demographical and various other dataset to identify relationships with higher dimensional capacity than simple analytical tests. However, training of AI requires resources and input data to become effective, which ultimately originates from traditional human-driven exploration and understanding.

A key advantage of AI is the capacity to learn and detect multiple complex features and patterns that are often outside of the bounds of existing tools. AI offers the potential for seamless unbiased histological assessment with improved accuracy, reliability and efficiency. However, this cannot occur until expert opinion in the field is extended and consolidated, and the challenges and natural inconsistencies/variability associated with histologic methodology for tendons/ligaments are addressed. Standardising quantitative discriminatory manual histological approaches as initiated in this thesis marks the beginning of this journey, with dissemination of knowledge the next recognised critical step in progress[149].

7.4 New Insights on Tendon Differences

While *Chapter 2* revealed that there exists an understanding and appreciation of structural variations between different tendons, information is sparse relative to the wider range of different tendons present in the appendicular (and axial) system. Further, microscopic observations of structural differences are often secondary outcomes addressed with limited statistical comparisons. Across the 59 articles which statistically explored *'variables of interest'* through microscopy evaluation, only 14 human-specific tissues (13 tendons, 1 ligament) were explored across the publications, and only 7 tendons and the ACL have been assessed with consideration of specific tissue-versus-tissue comparisons. The studies conducted in this thesis significantly contribute to knowledge in the field, investigating an extensive list of 48 different tissues (44 tendons and 4 ligaments; Table 3.3.a), addressing gaps by defining tendon structural variability which facilitates exploration into structure-function relationships and mechanisms fundamental to the prevention, treatment and management of tendon injury and disease.

One direction for future consideration is more in-depth evaluation of the difference between upper and lower limb tendons. In the current thesis the upper and lower limb tendon analyses were conceived and conducted independently in recognition of statistical constraints. The current studies explored n = 31 tissues for the upper limb project and n = 17 tissues for the lower limb project. Respectively, these studies entailed 465 and 136 pairwise comparisons, with Sidak and Bonferroni α significance levels for the upper limb analyses being 0.0001103 and 0.0001075 respectively, and 0.0003771 and 0.0003680 for the lower limb respectively. In contrast, a study to compare across all n = 48 tissues would entail a whopping 1128 comparisons, with Sidak and

Bonferroni tests requiring α significance levels of 0.0000455 and 0.0000443 respectively. Accordingly, this task was deemed unfeasible for the current thesis.

While statistically evaluating upper versus lower limb tissue differences was beyond the scope of this PhD, qualitative comparison reveals interesting trends that warrant future statistical analysis (see below).

7.4.1 SSS Outcome Examples – A Closer Look

7.4.1.1 *Fibrillar Loosening and Fibre Separations*

The dispersion (separation or longitudinal cleaving) of the collagenous structure is a feature often included in the assessment of tendon histopathology[169,171]. Here, associations between neighbouring fibres were characterised by defining the presence and "type" of separation between fibre pairs. That is, determining the existence and the length of disconnection between neighbouring fibres. Observable splitting (exposing white/background space) between the collagen structures of tendon (fascicles/fibres/fibrils) may be a result of histological processing artefacts. However, all scores were performed blinded, on tendons and ligaments from the same set of donors with the same treatment of all samples. With the effects of tissue handling held consistent, the observable differences even if exacerbated/highlighted by tissue processing likely reflect underlying variability in the *in vivo* structure/composition of the tissues. "Longer" separations are indicative of lower adherence or fewer cross-connections/branches/anastomoses between fibre structures along their length, while the size of fibres – which are known to vary considerably between species[70,117,121,351] – may indirectly alter the observed frequency of fibre separations. Such occurrences could implicate variation between the fibrillar structure or presence of other structural proteins contributing to the complex hierarchical structure of the collagenous FM matrix[6,73,117,352-354], such as the presence of elastin which aids resistance of transverse and shear deformation of the fibre structures[352].

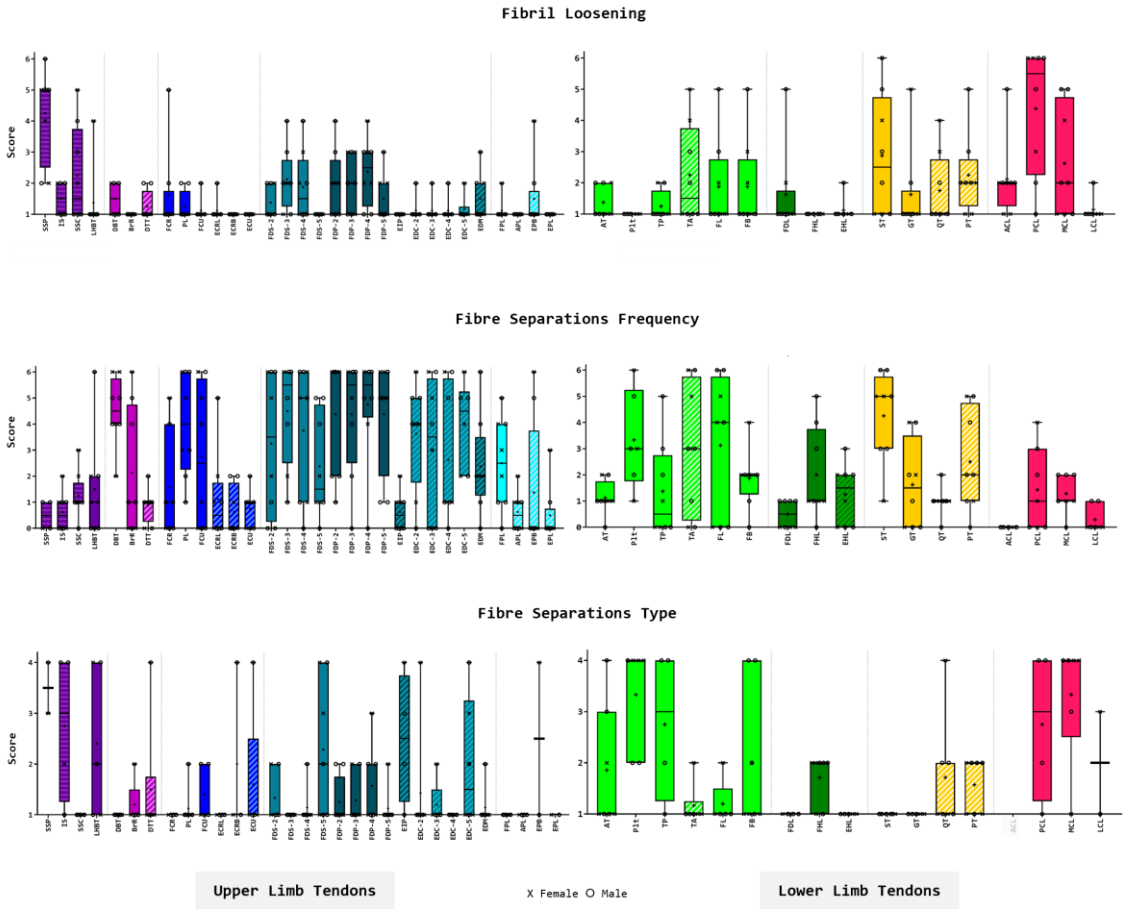


Figure 7.4.A Results from Fibrillar Loosening (top), Fibre Separations Frequency (middle) and Fibre Separations Type (bottom) scores of 31 different human upper limb (left) and 17 different human lower limb (right) tendons/ligaments. In order from left to right, upper limb tendons are as follows: SSP, IS, SSC, LHBT, DBT, BrR, DTT, FCR, PL, FCU, ECRL, ECRB, EDU, FDS-2 to FDS-5, FDP-2 to FDP-5, EIP, EDC-2 to EDC-5, EDM, FPL, APL, EPB, EPL. Lower limb tendons are as follows (left to right): AT, Plt, TP, TA, FL, FB, FDL, FHL, EHL, ST, GT, QT, PT, ACL, PCL, MCL, LCL. *Refer to Table 3.3.a for identification of tendon acronyms, and graphs presented in Chapter 5 and Chapter 6 for full legend details.*

Figure 7.4.A shows the results of the Fibrillar Loosening and Fibre Separations Frequency and Type scores for both the upper limb (*Chapter 5*) and lower limb (*Chapter 6*) projects in this thesis, side-by-side with the same graph scales. Similarities between the knee ligaments (ACL, PCL, MCL and LCL) and the stabilising rotator cuff tendons of the shoulder (SSP, IS, and SSC) tending to have higher fibrillar loosening but fewer fibre separations and typically longer in nature compared with other tendons. One theory behind the more dispersed fibrillar structures in these tissues could be their short length and the proximity of samples to the bone-attachment. O'Brien (1992)[278] highlights the plaited nature of collagen fibrils at the bone attachment of tendons which experience tension exerted in multi-directional planes, a feature likely contributing to the histological observations achieved (Figure 7.4.B). Conversely, Figure 7.4.C shows the densely packed fibrils of the long rope-like ECRB tendon of the wrist, exhibiting distinguishable fibre structure in a highly organised collagenous architecture. Interestingly, the PT counters this – where being a short tendon flanked by two enthesis (similar to ligaments, a naming convention sometimes adopted to the patellar structure[355]) it would be expected to exhibit similar structural characteristics to the ligaments and rotator cuff tendons. Rather the PT presents with higher

frequencies of fibre separation, likely aiding the non-uniform translation[295] of the tendon architecture during knee flexion (tendon stretch), which has shown to be greater than non-uniform displacement of the AT[295,356] consistent with its reduced fibre separations (thus presumably larger fibres) compared with the PT.

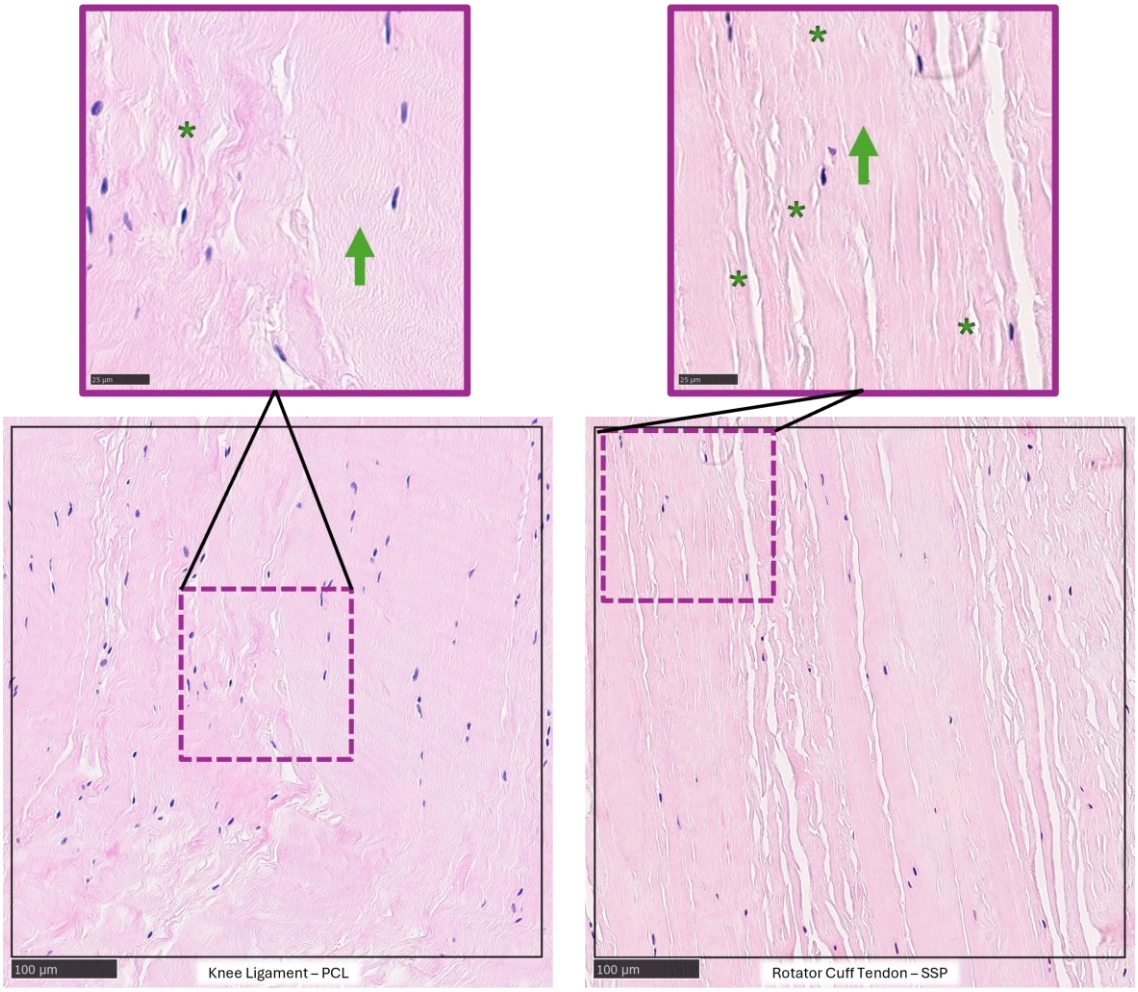


Figure 7.4.B Examples of a knee ligament (Posterior Cruciate Ligament, PCL; left) and rotator cuff tendon (Supraspinatus, SSP; right) obtaining high Fibrillar Loosening score. Inset images (top frames) show areas of dispersed (low density) fibrils with poor fibre delineation [arrows] and multi-directional fibril tracking [asterisks]. *Image (bottom frames) scale bar = 100 µm, 200x magnification. Inset (top) scale bar = 25 µm, 800x magnification. H&E-stained sections.*

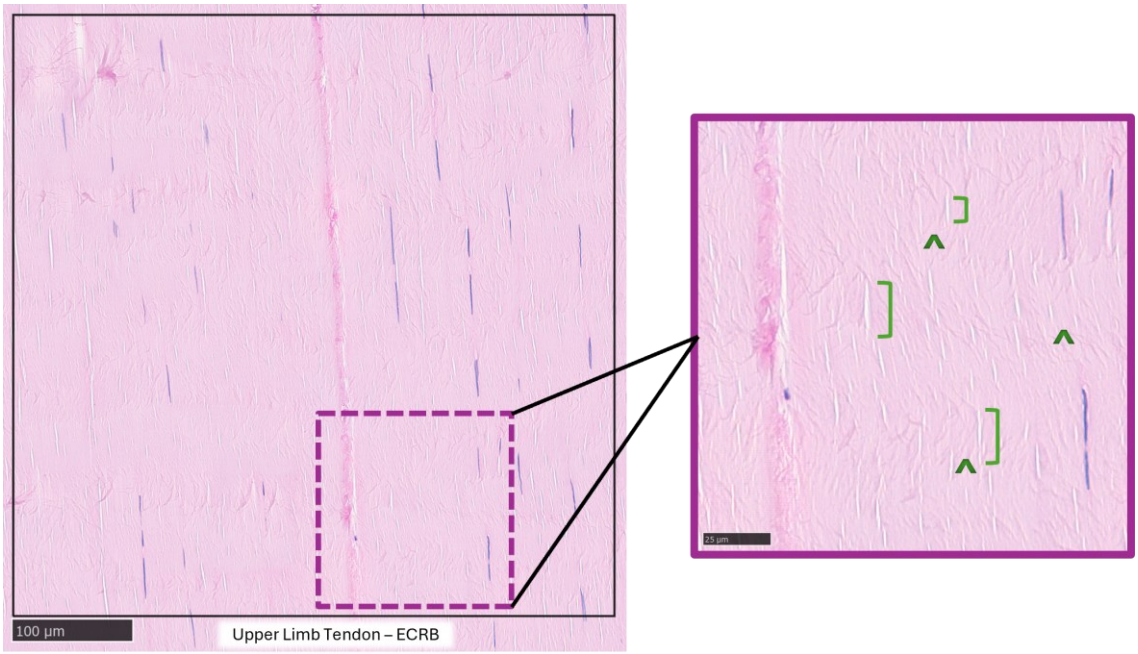


Figure 7.4.C Examples of an upper limb tendon (Extensor Carpi Radialis Brevis, ECRB) obtaining low Fibrillar Loosening score. Inset image (right frame) shows areas of bundled (high density) fibrils with clear fibre delineation [^carets] between short fibre separations [square brackets]. *Image (left frame) scale bar = 100 µm, 200x magnification. Inset (right frame) scale bar = 25 µm, 800x magnification. H&E-stained sections.*

7.4.1.2 *Cell (Nuclear) Morphology / Phenotype*

Cell morphology has only been statistically compared between different tendons in three prior publications (*refer to Section 2.3.3*; [19,269,357]). Considering the significance of cell populations on the regulation of the extracellular matrix[117], understanding the basic morphological/phenotypic variations of cells in different tendons paves the way for future work on identifying the roles of specific cell types in relation to tissue mechanics and physiological responses to injury including healing[358].

Figure 7.4.D shows the results of the Cell Morphology, Atypical-Phenotype Cell Presence and Proteoglycan Presence scores for both the upper limb (*Chapter 5*) and lower limb (*Chapter 6*) projects in this thesis. Again, similarities between the rotator cuff tendons and knee ligaments are evident in these 3 outcomes, with increased nuclear rounding, atypical-phenotype cell presence and greater proteoglycan presence. The presence of atypical-phenotypes characteristic of chondrocyte-like cells with all three defining features – rounded morphology, lacunae 'halos' and pericellular proteoglycan staining – are expected in areas of normal tendon exposed to compressive loads[223,224,357,359] as well as in the pathogenesis of some tendinitis/tendinopathy cases[142,169,200,201]. The results are in line with the expected compression experienced by the knee ligaments, where the collateral ligaments are under tension but wrap around the convex surface of the knee joint, and the crossing nature of the ACL and PCL within the joint causes ligament-ligament compression. Similarly, the anatomical positioning of the rotator cuff over the underlying convex surface of the shoulder likely causes compression to the structures. Additionally, the proximity of the ligament samples to the bone-insertion sites predispose the

microstructures to complex forces including increased compressive and shear loads at these regions[360]. A similar argument could be made in the short and interwoven rotator cuff tendons, as tendon near the enthesis exhibits these fibrocartilaginous features in response to the different loading requirements in the area[200,237,361,362]. This understanding is important for the consideration of tissue (tendon/ligament) comparisons where anatomically short structures may exclude the tissue from exhibiting "mid-substance" characteristics, and highlighting the importance of considering region selection and proximity to adjacent structures for all investigations.

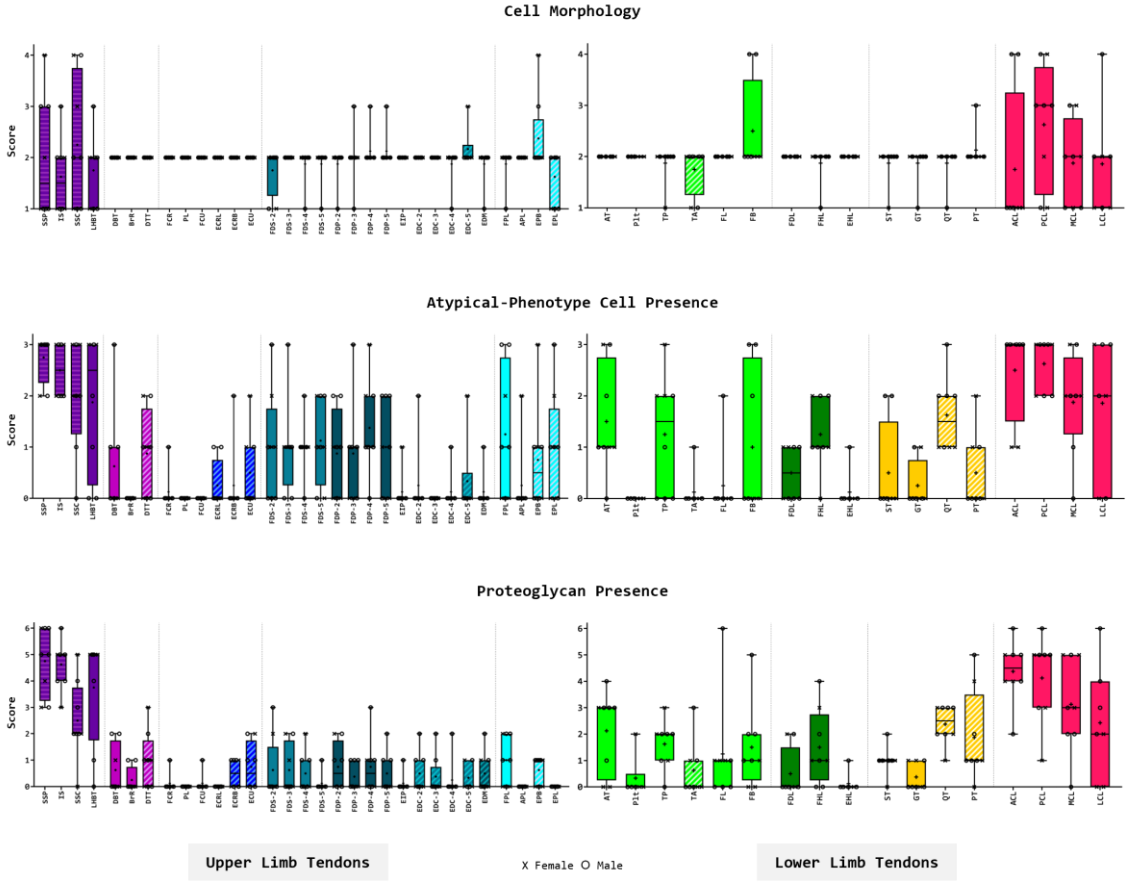


Figure 7.4.D Results from Cell (Nuclear) Morphology (top), Atypical-Phenotype Cell Presence (middle) and Proteoglycan Presence (bottom) scores of 31 different human upper limb (left) and 17 different human lower limb (right) tendons/ligaments. In order from left to right, upper limb tendons are as follows: SSP, IS, SSC, LHBT, DBT, BrR, DTT, FCR, PL, FCU, ECRL, ECRB, EDU, FDS-2 to FDS-5, FDP-2 to FDP-5, EIP, EDC-2 to EDC-5, EDM, FPL, APL, EPB, EPL. Lower limb tendons are as follows (left to right): AT, Plt, TP, TA, FL, FB, FDL, FHL, EHL, ST, GT, QT, PT, ACL, PCL, MCL, LCL. *Refer to Table 3.3.a for identification of tendon acronyms, and graphs presented in Chapter 5* and *Chapter 6 for full legend details.*

Conversely and at odds with the above proposed effects of tissue length and enthesis proximity, the PT had relatively lower PG presence, fewer atypical-phenotype cells, and primarily spindle-shape nuclear morphologies (Figure 7.4.D). While the PT is a shorter structure traversing the knee joint (contralateral limb free-tendon length mean ± SD = 33 ± 8 mm)[127] with entheses at both ends similar to the ligaments (ACL = 34 ± 6 mm; PCL = 40 ± 4 mm; MCL = 57 ± 14 mm; LCL = 29 ± 4 mm)[127], the underlying Hoffa's fat pad[101] as opposed to bone may limit compressive forces, suggesting that the compressed nature of the other structures may be more important in

driving the fibrocartilaginous changes exhibited by some tissues. Furthermore, the PT being a continuation of the QT (52 ± 8 mm)[127] presents the unique consideration that the compressed region of the joint PT/QT structure is localised to the sesamoid bone (patella) which acts to distribute load during joint articulation.

A caveat of the current study was the standardised 'anatomical plane of reference' used for allocating tissue from the whole tendon to different outcomes (*Chapter 3*), where the histology sample was collected from the more proximal aspect of the tendon. Discounting the ligaments or patellar tendons which contain bone-insertions at both ends, all tendons retrieved from the lower limb and arm (mid-humerus to fingertip) (*Section 3.2.2*) have their MTJ located proximally and bone-insertion distally. The tendons collected from the shoulder, however, have their proximal origin as the bone-end, with the MTJ located distally. The rotator cuff tendons are quite short and the whole tendon length could be argued as being comparably close to the bone attachment regardless of where samples are collected (free-tendon length mean ± SD [unpublished data from the contralateral limbs]: SSP = 14 ± 2 mm; IS = 15 ± 3 mm; SSC = 13 ± 3 mm). The LHBT, which is the only "long" tendon in the same orientation (89 ± 7 mm), still followed trends similar to the short (thus enthesis-close) rotator cuff tendons and the knee ligaments, particularly for the outcomes presented in Figure 7.4.D. This suggests that proximity to the bone-attachment is a strong factor regarding the chondroid metaplasia-like characteristics observed. However as noted above, the exception is the patellar tendon which has similar bone-attachment proximity but does not cluster consistently with rotator cuff tendons and the ligaments. This suggests that potentially a combination of bone proximity and compressive loading is important. Further regional assessments along the length of different anatomical tendons should be conducted to explore whether regions adjacent to the MTJ in the short rotator cuff tendons emulates that of longer tendons where the bone enthesis structures are not in close proximity.

Interestingly, some tendons – namely digital flexor tendons of the hand (Figure 7.4.D) – with comparatively low proteoglycan content and predominant cell morphology depicting 'typical' spindle-shaped nuclei still presented with relatively high numbers of atypical-phenotype cells. Here, the "atypical" cells are likely including those less aligned with the chondrocyte-like definition as above and rather resembling different cell phenotypes; possibly something more akin to metabolically active tenoctyes[117,269,357,358,363], with a rounded morphology and visible 'halo' of cytoplasmic area (reduced nucleus:cytoplasm ratio) (Figure 4.5.K). These tendons contrast to the digital extensors of the hand, which while also exhibiting predominantly spindle-shape tenocytes and comparably low PG presence, the extensors had fewer atypical-phenotype cell presence compared to the flexors. The more power-driven hand flexors (responsible for the power-grip) may necessitate the more metabolically active cells[364]. This pattern is also somewhat replicated in some of the flexor/extensor tendon pairs of the lower limb, with the EHL less likely to exhibit atypical-phenotype cells than the FDL/FHL tendons. Recent discoveries of the heterogeneous fibroblast populations in tendon possibly[96,97,365] support the idea that phenotypically different cells possibly reflect a distinct population with physiological implications beneficial to a given tendon. Conversely, exploration of cell populations in diseased tendon have shown links between cellular profiles and progression of tendinopathy[94,95,366].

Further exploration into the phenotypic differences of the cell populations, as well as their physiological significance, is required, particularly with focus on highlighting regional or tendon-specific differences and well as demographic influences. One consideration for future development of the SSS would be to differentiate population densities for cells with different phenotypes defined using immunostaining. This could not only reveal important tendon versus tendon differences but could also be used to attribute certain phenotype densities to differing physiological or mechanical statuses of the tissue in more detail. However, use of improving research tools such as scRNA-seq is further necessary to aid deeper understanding of cellular control over tendon development, maturation, aging and pathophysiology and variations of such amongst different tendons or demographic states[95]. Not only will recent discoveries around the heterogenic nature of tendon cell populations[94-97] support the development of medical interventions and therapies for diseased or injured tendons[94,366], but it will also aid continuing development of assessment tools such as the HALT-SSS to provide readily available diagnosis tools in clinical and laboratory settings.

7.4.1.3 *Crimping Architecture*

Crimp is an important structural feature for the mechanics of tendons/ligaments during motion of the musculoskeletal system. The waveform structure of the collagenous architecture promotes the low-stiffness "toe" region of the dynamic stress-strain response of tendons under tensile loading[106-108,304,367]. It has been reported that energy-storing tendons have shorter wavelength compared to positional tendons[17,18], which is reflected in differing stress-strain curves in response to mechanical testing[368].

Figure 7.4.E shows the results of the Cell Density count and Crimp Wavelength score for both the upper limb (*Chapter 5*) and lower limb (*Chapter 6*) projects in this thesis. Except for the distinct observation of the TA (typical positional tendon) having longer wavelength compared to the AT (typical energy-storing tendon), there was no clear crimp-wavelength association with other functionally opposing flexor/extensor or energy-storing/positional tendons apparent in the upper or lower limb across the broad range human tendons explored. However, an apparent inverse association between cell density and crimp wavelength was evident in both upper and lower limbs. While conflicting results have previously been reported[369], recent data supports a correlation between shorter crimp wavelengths and the number of cells positive for αSMA[267], and the active contribution of tenocytes to crimp formation has been modelled[370]. These are both consistent with our finding of tendons with higher cell density having shorter crimp wavelengths. The association between cells and crimp also identifies a consideration for the development of bioengineered grafts, where inducing crimp[370] via cellular influences may allow for the tweaking of necessary structural elements for biomechanical properties.

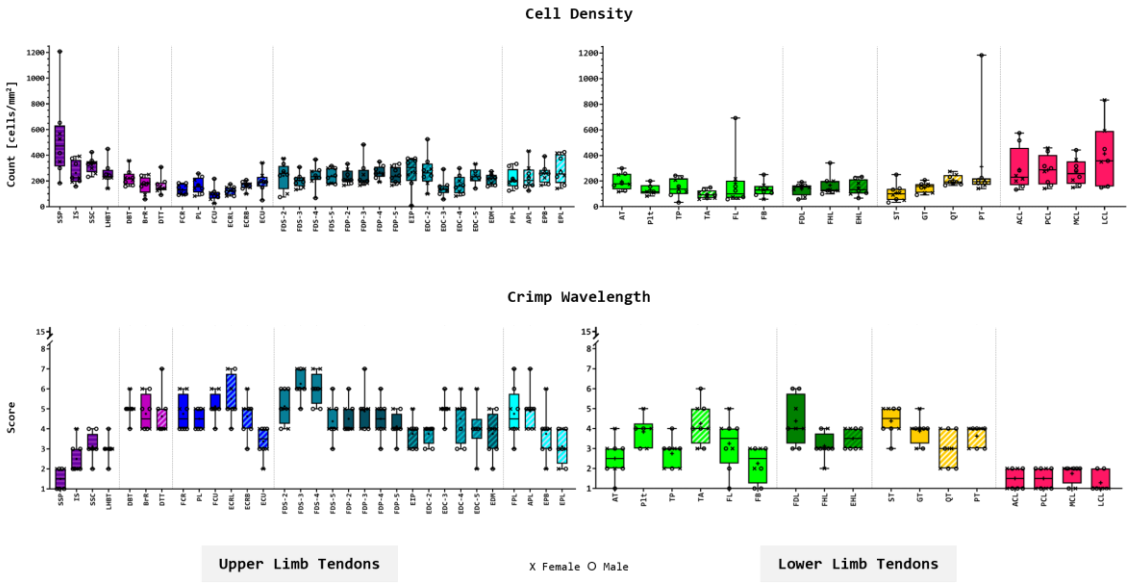


Figure 7.4.E Results from Cell Density count (top) and Crimp Wavelength score (bottom) of 31 different human upper limb (left) and 17 different human lower limb (right) tendons/ligaments. In order from left to right, upper limb tendons are as follows: SSP, IS, SSC, LHBT, DBT, BrR, DTT, FCR, PL, FCU, ECRL, ECRB, EDU, FDS-2 to FDS-5, FDP-2 to FDP-5, EIP, EDC-2 to EDC-5, EDM, FPL, APL, EPB, EPL. Lower limb tendons are as follows (left to right): AT, Plt, TP, TA, FL, FB, FDL, FHL, EHL, ST, GT, QT, PT, ACL, PCL, MCL, LCL. *Refer to Table 3.3.a for identification of tendon acronyms, and graphs presented in Chapter 5 and Chapter 6 for full legend details.*

Future work should endeavour to explore correlations between outcomes to investigate links between characteristics of the tendon/ligament structure to support observations with statistical evidence. Furthermore, there is room for further tendon differentiation using complementary methods to obtain more specific detail, such as cell typing and determining molecular compositions. Such differentiation may also aid the broader anatomical and orthopaedic sectors by outlining parameters for tendon classification. The concept of 'energy-storing' versus 'positional' tendons are often reserved to animal models[1,7-10], although similar functional classification of human tendons relative to functional attributes have been explored in relation to human AT and TA tendons[280], with the energy-storing AT exhibiting more elastic and fatigue resistant IFM than the positional AT tendon IFM. However, the link between structure-function characteristics and being akin to energy-storing or positional biomechanical roles has not been explored across a larger range of tendons – insight which could prove enlightening for future laboratory and clinical research. Understanding the functional assignment of a tendon may inform of underlying structural features induced by the loading environment of the tissue, or conversely, appreciation of likely embryological preprograming responsible in developing function-specific tissues.

7.4.2 Interfascicular Uncertainties

One of the biggest challenges with characterising different tendons was determining the overall hierarchical structure. While the typical 6-level hierarchy is the most common depiction[29,69,74,98-100,371], there has been inconsistency in hierarchical nomenclature throughout the decades of tendon research[121], with Elliott (1965)[70] stating: *"There is no standard nomenclature for the aggregations of collagen fibrils within the tendon, perhaps because of their great variability."*

Handsfield *et al.* (2016)[121] reviewed tendon nomenclature and despite noting expectations that the tendon hierarchy will differ between tendons or species, they proposed the use of size-based classifications to more objectively define the tendon hierarchy in the following ascending order: tropocollagen, microfibril, fibril, fibre, fascicle, (± fascicle bundle), (± sub-tendon), tendon. The proposed fascicle nomenclature was defined to incorporate structures ranging from 50-400 μm[121]. *Fascicle bundles* were reported to be observed in their preliminary investigations and thus included as a proposed hierarchical level, whilst *sub-tendons* were noted to be specific to tendons such as the Achilles or Quadriceps tendons which constitute fused tendon structures arising from separate muscle bellies.

Figure 7.4.F shows the results of the IFR Frequency and IFR Type Proportion counts for both the upper limb (*Chapter 5*) and lower limb (*Chapter 6*) projects in this thesis. Notably, the frequency of IFRs greatly differ between tendons across the human appendicular system, ranging from 0 to 22 IFRs/mm. While the outcome itself should be specifically measured for a true indication, these findings can be interpreted as an approximate indication of fascicle size as IFRs delineate the boundaries between neighbouring fascicles and scores have been normalised to a standardised unit (count/mm). Higher IFR frequency implies more fascicle-fascicle boundaries occurring per mm and thus smaller fascicles. Although this calculation can be influenced by the section plane and relative three-dimensional shape of the fascicles, the variations in fascicle sizes identified by this thesis fall within the expected range defined by Handsfield *et al.* (2016)[121]. Using Handsfield's[121] fascicle classifications, it would be expected that the frequency of IFRs would range from approximately 19 IFRs/mm (for small 50 μm fascicles) to 1.5 IFRs/mm (for large 400 μm fascicles), a range well reflected by the results of the upper and lower limb human tendons explored here (Figure 7.4.F).

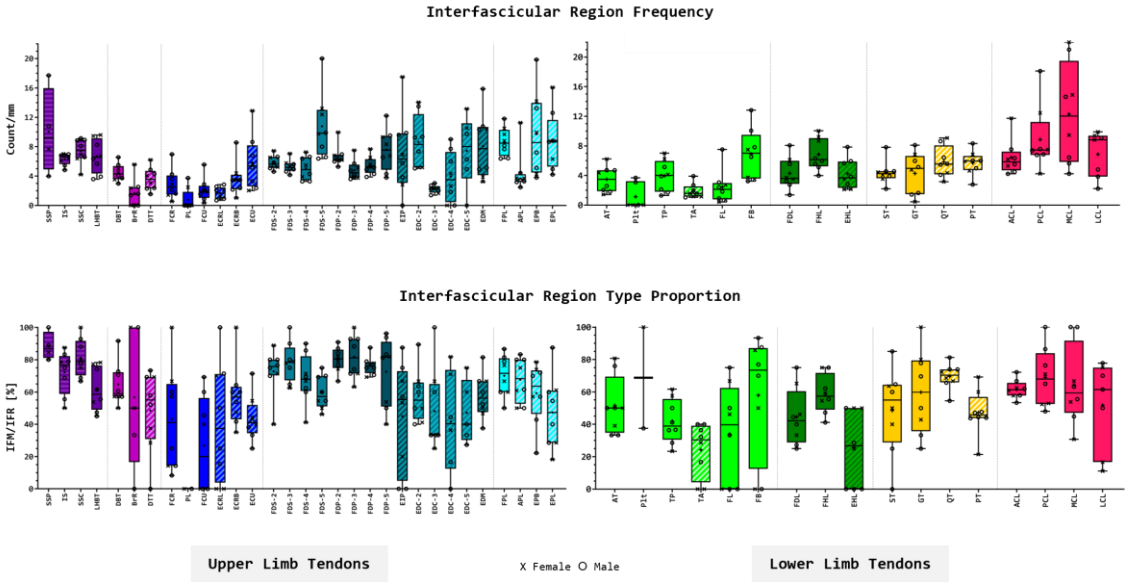


Figure 7.4.F Results from Interfascicular Region (IFR) Frequency (top) and IFR Type Proportion (bottom) counts of 31 different human upper limb (left) and 17 different human lower limb (right) tendons/ligaments. In order from left to right, upper limb tendons are as follows: SSP, IS, SSC, LHBT, DBT, BrR, DTT, FCR, PL, FCU, ECRL, ECRB, EDU, FDS-2 to FDS-5, FDP-2 to FDP-5, EIP, EDC-2 to EDC-5, EDM, FPL, APL, EPB, EPL. Lower limb tendons are as follows (left to right): AT, Plt, TP, TA, FL, FB, FDL, FHL, EHL, ST, GT, QT, PT, ACL, PCL, MCL, LCL. *Refer to Table 3.3.a for identification of tendon acronyms, and graphs presented in Chapter 5* and *Chapter 6 for full legend details.*

However, this estimation does not capture those tendons which exhibited no IFRs in the ROI assessed, or conversely presented as "single" fascicles. The lines used to assess the IFR outcomes in these tendons (n = 16 samples) ranged from 315 µm to 1310 µm (average 716.25 ± 271.38 µm). Using the previous estimation approach, this suggests that in 14 of the 16 samples, the "fascicle" was larger than the upper 400 µm width defined by Handsfield's fascicle classification[121]. These current findings may justify the need for a reclassification of the tendon hierarchy and the definitions or parameters for each structural level to be evaluated using a larger scope of tendons. Additionally, Elliott (1965)[70] presented theories on the non-homogeneous fascicle size along the tendon length, accounting for the merging of smaller fascicles to form bigger bundles throughout the structure, and that this differs between tendons. Handsfield *et al.* (2016)[121] acknowledged that further discussion is needed to discern whether the multiscale hierarchy of all tendons should or should not be defined by a single standard.

As mentioned previously, Figure 7.4.F demonstrates the range of IFRs present across the upper and lower limb tissues. While the rotator cuff tendons and knee ligaments continue to show similarities, the digital tendons of the upper limb also show a relatively higher IFR frequency (thus implying smaller fascicle sizes) compared to the wrist region and lower limb tendons. Figure 7.4.G highlights this, showing the IFR Frequency scores for typical lower limb energy-storing versus positional tendons and a hand digital flexor and extensor, alongside Scanning Electron Microscopy (SEM) images of a transverse section of the FDP-2, EDC-2, AT and TA tendons (tendon and donor matched to the histological tissues as presented in this thesis). The SEM images exemplify the "greater" structural division and arrangement of the upper limb tendons with more distinct fascicles and clear IFR/IFM.

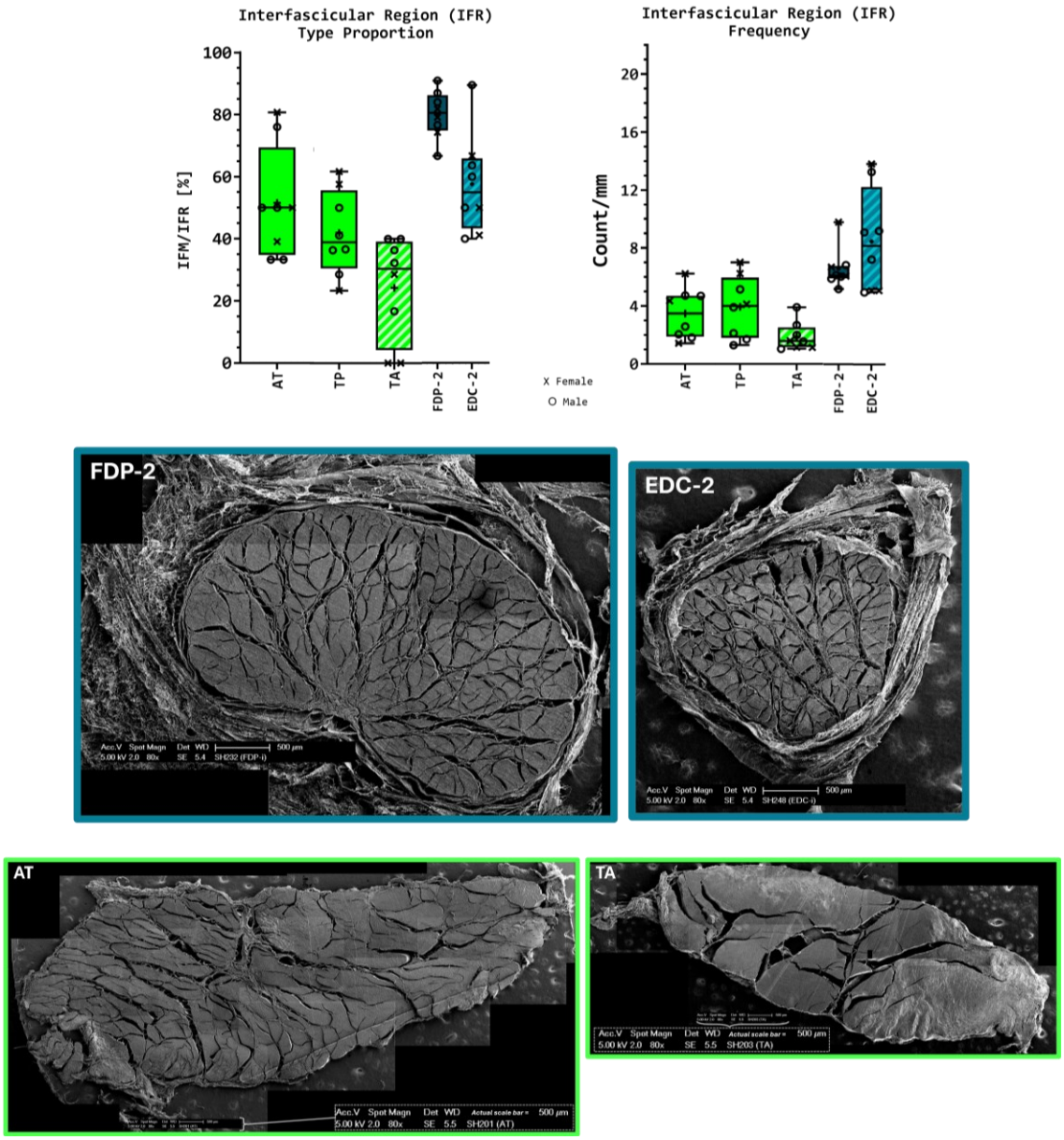


Figure 7.4.G Interfascicular Region (IFR) appearances of examples of human lower limb energy-storing versus positional tendons and upper limb flexor versus extensor tendons from a single donor. **Graphs:** Histological IFR Frequency (left) and Type Proportion (right) counts of 5 different human tendons (AT = Achilles Tendon, TP = Tibialis Posterior, TA = Tibialis Anterior, FDS-2 = Flexor Digitorum Profundus – 2nd digit, EDC-2 = Extensor Digitorum Communis – 2nd Digit). **Images:** Representative Scanning Electron Microscopy (SEM) images of transverse section of FDP-2 (upper limb flexor), EDC-2 (upper limb extensor), AT (lower limb energy-storing) and TA (positional) tendons. *SEM image scale bars = 500 µm*. **Note** differences in fascicular structures and IFR appearance between tendon examples. *SEM methodologies are outlines in Appendix Section 9.4.1.*

It is well conceptualised that the IFR is important for fascicle sliding, aiding the loading response of the tendon unit[117,230,295,356]. The ECU also stands out in the wrist, portraying a relatively higher IFR frequency. Kalson *et al.* (2012)[354] proposed the spiralling nature of the ECU fascicular and fibre structures likely aids the strength and preservation of the tendon during loaded rotation of the forearm – with IFRs allowing sliding between fascicles during power grip which actively involves rotation of the forearm. In the lower limb, the helical structure of the fascicular

arrangement and supporting IFR/IFM aids elastic recoil and load transference in energy-storing tendons more distinctly than in positional tendons[87,230,372], reflected in the current study on human tendons by the higher IFR frequency and IFM proportion of the energy-storing AT and TP tendons compared to the positional TA tendon. Godinho *et al.* (2017)[234] likewise saw greater IFM:FM ratio in energy-storing SDFT versus the positional CDET in equine tendons. Interestingly, flexor tendons tend to have higher proportions of IFM:IFS compared to their extensor counterparts irrespective of limb, such as flexors versus extensors of the hand digit tendons, toe digit tendons, and ankle plantar-flexors versus dorsi-flexors (complement to the "extensors"). Again, this is possibly associated with facilitating greater fascicle sliding to support the dynamic loading of the power-grip initiators in the hand and energy-storing locomotive tendons in the ankle. Interestingly, from our published lower limb[127] and unpublished upper limb biomechanics data on matched tissues from the contralateral limbs, flexor tendons on average have weaker UTS than the corresponding extensors, likely exhibiting a functional trade-off for heterogenous intra-tendon mobility (fascicle sliding) versus material strength. This has also been reflected in equine SDFT versus CDET, with the flexor having a significantly weaker UTS[8] compared to the extensor. Contradictory to this, however, our biomechanical data also revealed the UTS of the aforementioned upper limb tendons to be higher than many of the lower limb tendons. While this mimics the same relationship between IFR frequency from the current study (being higher in the upper limb versus lower limb on average), it goes against the previous relationship between functionally distinct (energy-storing/positional or flexor/extensor) that saw greater IFM proportion reflecting weaker UTS, possibly identifying unique associations with FM and IFR structures versus biomechanical function between limbs.

7.4.2.1 *Redefining the Tendon Hierarchy*

In the future, more comprehensive exploration into the hierarchical organisation of the tendons presented in this thesis could aid in defining general and/or specialised tendon structures. In using multiscale imaging approaches, such as combining histology with electron microscopy (EM), further light could be shed on the inconsistency of tendon structural divisions between different tissue types[373], as well as facilitate more in-depth assessment of the presented scoring matrices within the SSS. As addressed by Handsfield *et al.* (2016)[121] and other published depictions of the tendon hierarchy[6,99,278], additional hierarchical levels 'either side' of the typically defined fascicle ("primary fibre bundles"/"sub-fascicles" and "tertiary fibre bundles") are often-reported in different tendons. In the current studies, the identification of these structural divisions was challenging in the longitudinal histological sections. While the novel concept of IFS presented in this thesis could account for the boundaries between these structural divisions, further exploration is needed to validate these predictions. Furthermore, observations made through preliminary assessment of SEM and Differential Interference Contrast (DIC) microscopy images of transverse sections of tendon- and donor-matched tissues to the histological samples presented in this thesis highlight the variability and ambiguous nature of these hierarchical divisions. Figure 7.4.H depicts SEM and DIC images of the upper limb EDC-2 tendon, portraying different bundling groups surrounded by distinct IFM substance. Representative histological examples of different IFRs are juxtaposed to convey the variable IFR appearances even within a single sample, likely reflecting boundaries between different 'levels' of the hierarchy. Meanwhile, Figure 7.4.I

portrays the lower limb TA, which exhibits much larger and less clearly demarcated fascicle structures with minimal IFM / indiscrete IFRs.

While future work is required to systematically assess the multiscale structure of tendons, we highlight the necessity to redefine the "typical" tendon structure to improve knowledge dissemination and support understanding that not all tendons are alike. The IFR characteristics alone present interesting differences between upper and lower limb tendons, and it is noted that the hierarchical organisation may also differ for the limbs of humans versus animals. For example, the upper limb tendons of bipeds such as humans may be distinctly different to the forelimbs of quadrupeds due to differences in functional demands. Furthermore, distinct tendon differences may also be founded in the overall hierarchical structure.

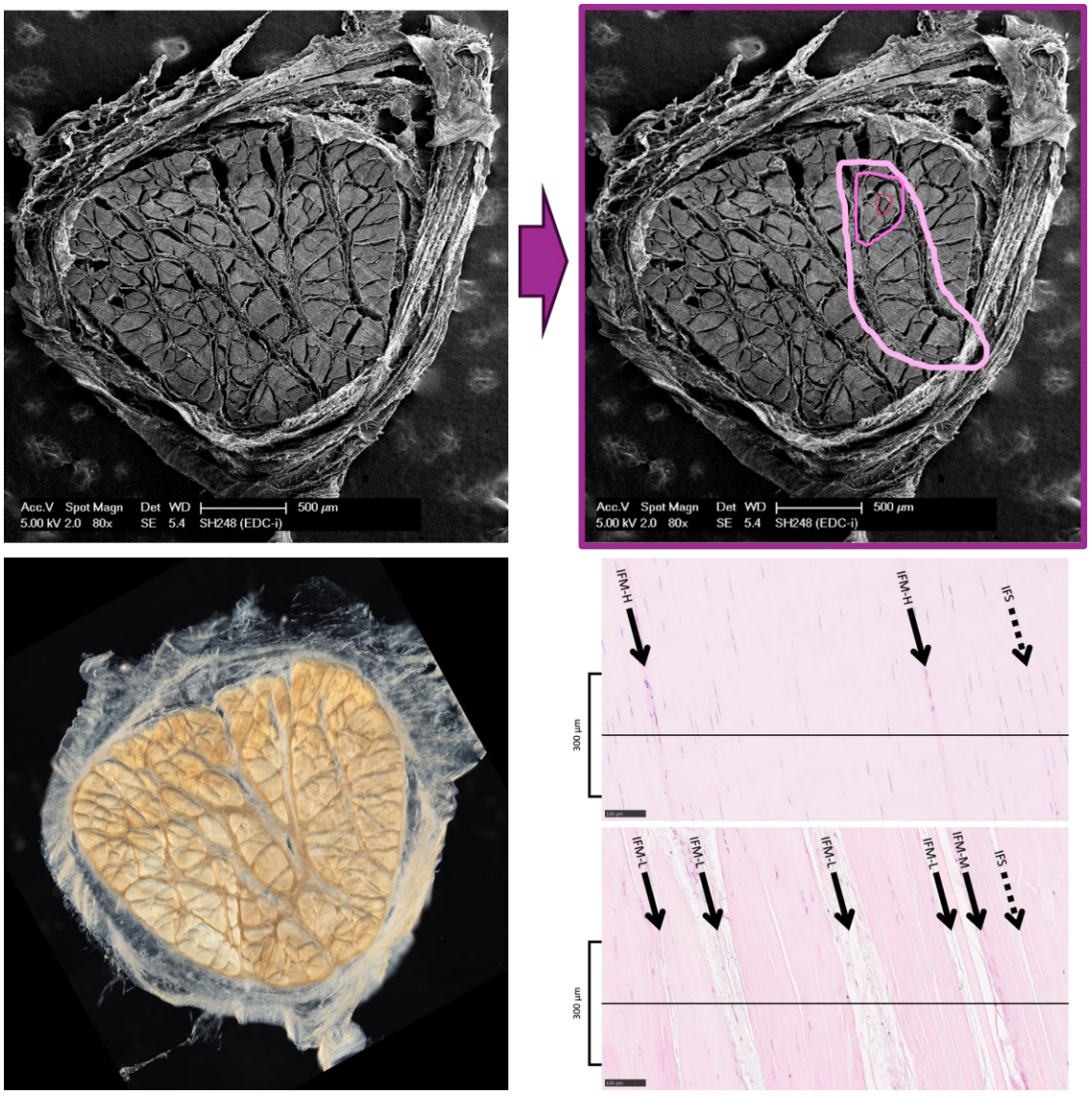


Figure 7.4.H Representative depictions of tendon fascicular matrix (FM) versus interfascicular regions (IFR). **Top-left:** Representative SEM image of a transverse section of EDC-2. *Scale bar = 500 µm*. **Top-right:** Identification of different structural "fibre bundle" levels of the EDC-2. *Transverse SEM section: Scale bar = 500 µm*. **Bottom-left:** Representative DIC image of a transverse section of the same EDC-2 sample (different section). *No scale bar available*. **Bottom-right**: Reference examples of IFRs classified as interfascicular space (IFS, dashed arrow), or interfascicular matrix (IFM, solid arrow) with low (-L), medium (-M) or high (-H) matrix density. *H&E-stained longitudinal sections. Scale bars (solid black bars) = 100 µm.* **Note** the differing fascicular appearances throughout the EDC-2 samples, including variability with IFR characteristics at different 'levels' of the hierarchy depicted by the graded pink outlines in the top-right image. These are likely associated with the examples of different IFR types in the H&E histological section. *EDC-2 = Extensor Digitorum Communis – 2nd Digit. SEM = Scanning Electron Microscopy. DIC = Differential Interference Contrast microscopy. IFR = Interfascicular Region. H&E = Haematoxylin and Eosin. SEM/DIC methodologies are outlines in Appendix Section 9.4.1.*

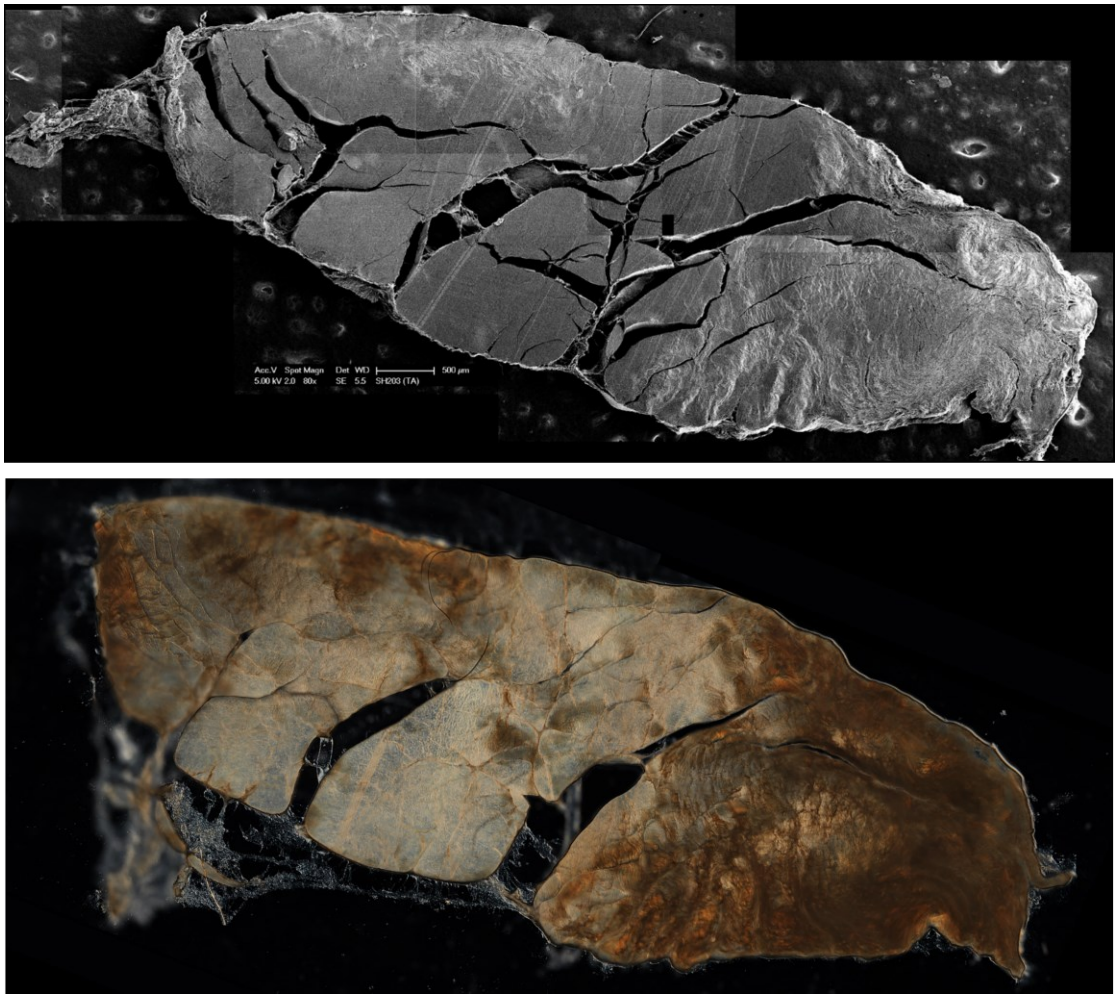


Figure 7.4.I Top: Representative SEM image of a transverse section of TA. *Scale bar = 500 µm*. **Bottom:** Representative DIC image of a transverse section of the same TA sample (different section). *No scale bar available*. **Note** the appearance of fascicular matrix (FM) and IFR within the TA samples, including the absence of distinctly prominent IFR areas. *TA = Tibialis Anterior. SEM = Scanning Electron Microscopy. DIC = Differential Interference Contrast microscopy. IFR = Interfascicular Region. SEM/DIC methodologies are outlines in Appendix Section 9.4.1.*

As exemplified by Handsfield *et al.* (2016)[121], a 'conditional' hierarchical classification can aid in capturing different representations of the tendon structure. To avoid misconceptions about hierarchical nomenclature and confusion with the terms "nth fibre bundle" at different levels of the structure, we propose a modification to the hierarchical classification of tendon collagen amalgamations, with "Segments" being grouped Fascicles (previously "Tertiary Fibre Bundles"), and "Sub-Fascicles" being the alternatively called "Primary Fibre Bundles". We also recommend against delineating structures based on size. While approximate ranges can aid wholistic interpretation, size should not be a defining factor for classifying different structural levels due to potential variability in general morphology between different tendons[117,121]. Lastly, we support the addition of the "sub-tendons" level applying to those such as the Achilles or Quadriceps tendons that arise from multiple muscle bellies. Figure 7.4.J portrays a conceptual depiction of a potential proposal for a new hierarchical classification, where future studies exploring a wide range of tendons (tendon type, species, regional alterations, etc.) could support further classification, as well as necessary amendments specific to ligaments.

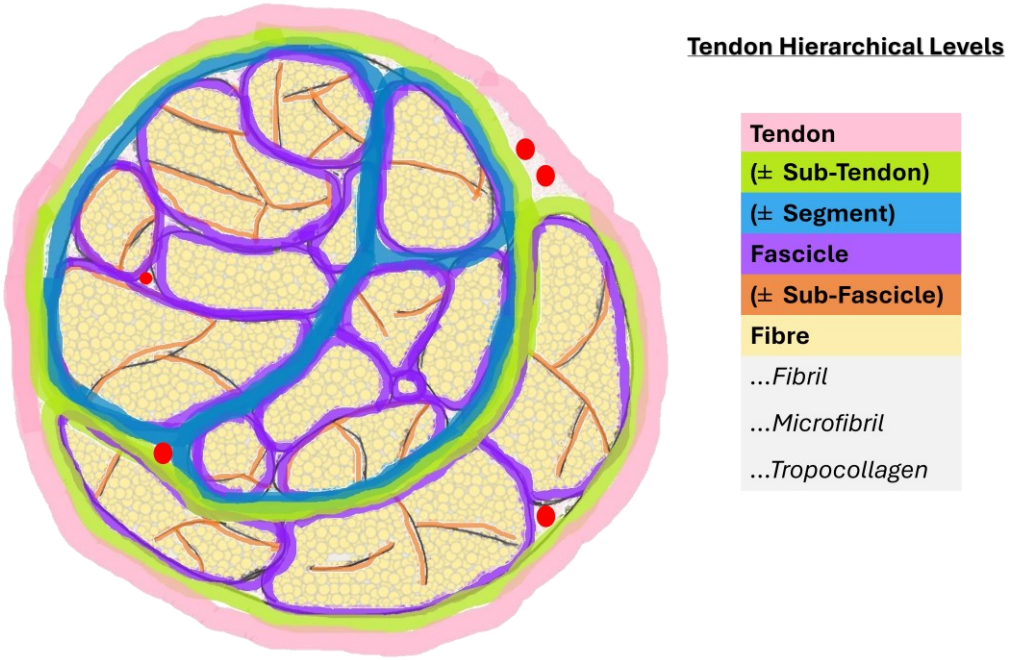


Figure 7.4.J Example of potential tendon hierarchical classifications. Listed structural levels correspond to the coloured feature within the representative tendon cross-section. Sub-Tendons, Segments and Sub-Fascicles may be tissue-specific elements. Fibrils, Microfibrils and Tropocollagen are not depicted in the sketch. Red dots indicate present of supply structures (e.g. vasculature) within the surrounding interfascicular regions and loose connective tissue sheaths.

7.5 Notes on Clinical Implications

The tendons included in the current studies were chosen in consultation with orthopaedic surgeons (NH, PH, DC, DO) as being tendons of clinical significance, such as being commonly injured, important structures in orthopaedic surgery, or enlisted as grafts for the reconstruction of other tissues[23,25,43,46,48,50,54,56,127,160,162,239]. The limited scope of tendon characterisation in current literature limits knowledge on mechanisms of injury, pathology, disease, or healing/repair and if pathophysiology differs with tendon type. Likewise, limited appreciation of variable tendon structure-function attributes impedes appropriate selection of grafts or the development of new biomimicking grafts in the context of reconstructive surgery. Biomechanical data in the field of tendon comparisons is much more prevalent[127,204,249,259,324,374-377] despite the inherent understanding that a tissues' mechanical properties arise from its structural organisation.

The current study is the first to explore the structure of a large range of human tendons (and ligaments), which are significant to the orthopaedic setting, using consistent assessment protocols. *Chapter 6* discussed that there is limited support in the literature to preference one graft option over the other in orthopaedic reconstructive surgery in relation to their native tissue characteristics. This is surprising considering many of the commonly enlisted graft tendons exhibit distinct characteristics to the ACL (*Chapter 6;* [164,267,324,378]), one of the most commonly reconstructed tissues[264,379]. Selection of grafts is largely driven by surgeon preference, the ease of surgical implementation and/or the consideration of the perceived 'surgical success'[50,261,313,317,380,381]. However, structural differences of different tendons (or graft choices) influence tissue mechanical

properties and likely the ability of the tissue to adapt and integrate into the new environment (i.e. "ligamentise"), and could thereby underly 'surgical success' parameters[66]. Likewise, *Chapter 5* discussed how definition of "normal" is challenged by appreciation of the tendon variability exhibited by the current studies, highlighting limitations of previous exploration into tendon pathophysiology. For example, different structures might influence responses to exercise and load and thus injury and tendinopathy risk, as seen in functionally distinct equine tendons[1,8,15,29,382].

If we consider the notion of Wolff's Law, which states "*every change in the form and function of bone or of their function alone is followed by certain definite changes in their internal architecture, and equally definite alteration in their external confirmation,*"[383] we could argue the same may apply to tendons and ligaments. How much of the structural difference identified might be modified by exercise and loading? Is tendon pathology a result of particular tendons not being able to readily adapt to changing loading environments? Is the selection of tendons for grafts dependant more on the native structure of the tendon, or the new loading environment and its effects on structural adaptation?

The findings from the current projects underpin the beginning to discovering the answers to these questions, identifying variations between tendons/ligaments that will facilitate further appreciation of the tissues structure-function relationships.

7.5.1 Future Exploration – "Mi Tendon, Su Tendon" … or is it?

Unfortunately, due to small donor numbers, evaluation of variables such as *age*- and *sex*-related differences were not feasible for the scored (categorical) data, and statistics for the continuous (counted) data outcomes should be considered carefully due to the narrow age range and low n-value. Furthermore, the collection of only a single tendon histology sample from one region of the whole tendon structure meant that these projects could not explore *region*-specific differences. In the future, exploration into variables which affect tendon characteristics should be explored with larger donor numbers, ideally across the full scope of tendons explored in the present studies. However, the current study would also enable the selection of tendons representative of different structural phenotypes/clusters (including but beyond flexors/extensors, upper/lower limb), to facilitate increasing the n-value of a fewer number of tendons for such detailed time-consuming analysis. Some considerations for studies of particular variable influences on tendon properties are discussed below.

7.5.1.1 *"That's Tendon For You, Old Chum"*

Both in Australia and globally we live in an aging population[384,385]. A review by Choi *et al.* (2018)[386] revealed that mechanical and biological studies on 'aged' tendons are in minority, with a dominant focus on paediatrics and adulthood. The systematic review in *Chapter 2* found that structural characterisation of tendon age-related differences is limited to a selection of 19 tendons and 4 ligaments, with only 11 of these in humans. The incidence of tendon injury increases with age[22], with differences associated with sex[26] and ethnicity[23]. Since structural changes have been shown to precede rupture[179], there is a need to better understand tendon structural changes with aging, which may precede, or predispose the tendon to injury, and how this may differ between anatomical tendons. However, changes reported in response to age may not necessarily be

indicative of "pathology" per se, but rather 'normal/healthy' age-remodelling processes[169,361]. Without deeper understanding of normal remodelling process, it is hard to attribute structural/compositional changes to pathology or typical/normal physiological processes.

In the current studies, age was not found to be associated with changes in cell density, cell distribution, IFR frequency or IFR type proportion for either the upper limb or lower limb tendon groups. The small cohort and relatively narrow age range limits the strength of the conclusions that can be drawn from the current observations of age-related effects however it is important to continue to consider factors that have previously been shown to have an effect. For example, cell density variations are a commonly observed feature significantly affected by age[31,34,269-271]. A greater understanding of tendon structure and variations in response to aging (as well as other factors like species, anatomical tendon etc.) would facilitate improved research into tendon patho-physiological mechanisms and more accurately define those features that are indicative, predictive or causative of pathology.

7.5.1.2 *"Fe Male or Not Fe Male"*

While there are differences in the incidence of tendon injury between men and women in some cases[174,387-389], it is unclear if these are caused by underlying tendon structural differences between sexes due to limited knowledge in the field relating to females[390], or a result of lifestyle differences and number of change-inducing exposure events[388] within the demographics of the populations studied. Regardless, the limited scope of research on sex-related differences in tendon structure (*Chapter 2*) constrains understanding of the potential physiological and pathophysiological implications. While *Chapter 2* revealed isolated evidence for tendon structural differences relating to sex[391], compositional and biomechanical analysis has uncovered additional support for differences between the sexes[391-395] – characteristics which may point to potential hormonal/biological influences on tendon physiology or other multifactorial influences influencing tendon mechano-biological processes.

Interestingly, the current thesis found sex-related differences in cell density and distribution in the upper limb, but not the lower limb tendons, with males exhibiting greater cell density but also greater distribution of cells compared to females. While the data from the current projects did not support statistical exploration into sex for categorical (scored) outcomes, we previously revealed crimp wavelength to be significantly longer in females compared to males in advanced-age human lower leg tendons[209].

7.5.1.3 *"Here, There, or Everywhere"*

The functional capacity of a tendon to tolerate loading and strain is dependent on structure-function interactions in both a longitudinal and cross-sectional (transverse) direction[396]. Variations in intra-tendon structure across these same domains/directions may also reflect adaptations to accommodate the mechanical requirements in the transition of tendon to muscle and bone, as well as interactions with the surrounding environment as it traverses across or through other anatomical structures such as joints. While potentially driven by adaptation to loading, the structural differences between tendons are also important in understanding different responses to pathological stimuli and regional tendon pathophysiological mechanisms.

The ***mechanical implications*** of an important structure in musculoskeletal function such as tendon are plentiful. Examples include fibrocartilaginous tissues adaptations[224 ,359 ,397], region-related tissue zones[77,123,237,361,398-400,401] , and specific sub-tendon variations[80,355,402-405].

However, it can't be ignored that differences throughout a tendon might also have ***pathophysiological implications*** and potentially explain differing injury incidences relative to tendon region[388,406], with intra-tendon variations also associated with hallmark characteristics of tendon pathology[142,168,200,201,224,237,329,330,357,359,399,407-410]. Understanding whether such structural characteristics which are reported in normal/healthy tissue are indicative of pathological predisposition, or warrant redefinition of pathological parameters for certain tendons/regions, requires further research. Regardless, regional variations play an important role in understanding tendon (or ligament) physiological and metabolic potential.

Importantly, ***consideration of prior research conclusions*** also needs attention, where discrepancies in findings may be underpinned by differences such as the particular tendon explored, regions included, tissue origin (species, age, sex, etc.) or even the experimental protocols enlisted. For example, crimp wavelength has shown varied differences between central and peripheral regions of equine SDFT. There are conflicting reports of greater wavelength observed in both the central[305,411] and peripheral[33,305] regions, or no significant differences at all[412]. The current study reported variation in crimp wavelength (Figure 5.4.O and Figure 6.4.O) even within the same isolated tendon sample used for histology outcomes. Localisation of these differences relative to proximal/distal, bone/muscle proximity, central/peripheral were not explored, but are an interesting consideration for further investigation, particularly considering the change in crimp over the longitudinal length of a tendon[209,308]. Exploring regional variations in a range of tendons using identical protocols can aid consolidation of previous findings.

Interestingly, our review of the literature (*Chapter 2*) found *Region*-related comparisons to be more prominent in the literature compared to *Tendon*-related comparisons (*refer to Section 2.3.3*). While histological differences as well as other compositional findings relating to regional variations have been reported for a selection of tendons, there has not been a systematic investigation of variations using consistent methodologies across a range of normal tendons – particularly regarding human tendons – to determine if there is a consistent pattern.

7.5.1.3.1 "To Infinity and Beyond"

The primary focus of this thesis was on characterising tendon mid-substance structure, hence considerations of structural variations outside of the 'tendon area' were beyond the scope of this project. However, it is still worth a brief mention to explore other areas important to the wholistic tendon structure as considerations for future work in the field. These include characterisation of paratenon, entheses, musculotendinous junction (MTJ) or aponeuroses. Similarly, the literature review presented in *Chapter 2* only included studies on ligaments if directly compared with an appendicular tendon, and the current investigation only incorporated four ligaments of the knee region due to restraints and limitations experienced during the experimental design of the laboratory's tissue atlas projects and specimen retrievals.

7.5.1.4 *"Putting Connective Back into Tissue"*

We previously reported tendon specific differences in relation to donor variables (e.g. sex, age, height, etc.) for biomechanical outcomes[259]. This study highlighted and cautioned against the over generalisation of variable effects across all tendons as erroneous interpretations may occur without structured investigation of tendon-specific interactions, particularly when assessing wide-ranging tissue datasets. It will be important to bear this in mind when conducting future research correlating different histological outcomes in the current study both with each other and with prior data from the same tendons[127,259]. Critical to identifying correlations specific to individual tendon or tendon-phenotype (e.g, anatomic region or function cluster) is to expand the number of replicates of these. Results of the upper (*Chapter 5*) and lower (*Chapter 6*) limb HALT projects demonstrated differences not previously known of normal tissues, but also highlights the need to explore tendon/ligament structural variations in more detail. These projects also support the necessity of having tools that are capable of capturing these differences, such as the novel SSS (*Chapter 4 ~ Section 4.5.1*).

The literature review in *Chapter 2* identified seven publications which compared *Tendon*-related differences in human tissue. While providing insight into the field in some contexts, all of these studies identify shortfalls in the research. In particular, inadequate detail in reported methodologies[164], pooling of different tissues[378], non-quantifiable assessment limiting comparability across studies/research groups[378], influence of confounding variables[238], and lack of exploration into normal-versus-normal characterisation[267] are key issues. Where studies explore experimental models (investigating pathophysiology or lifestyle influences, for example), reporting and ideally comparisons of the normal tendon (control) characteristics should be given greater priority than currently practiced given that histological parameters remain a gold standard for assessing tissue health and disease[160-162,172,250,327,413]. At the very least, where samples are available even if analysis cannot be conducted, dissemination of data to facilitate further exploration is essential to address the lack of knowledge in the field on normal tendon-tendon and tendon-region differences. *"The only source of knowledge is experience" ~ Albert Einstein.*

7.6 Sample Caveats

Unfortunately, the ambitious scope of the laboratory's human tissue atlas and difficulty in conducting investigations with human cadavers (e.g. tissue procurement, uncontrolled variability, etc.; *Section 4.5.2*), meant some limitations experienced in the current PhD studies date back to the design and preparation of the broader project. Some examples where such limitations occurred, or challenges were experienced, are described below.

7.6.1 Normal VS "Normal"

The tissue for the projects in this thesis were sourced from fresh-frozen cadavers obtained from a licenced donor tissue facility (Science Care Inc) with specifications of no history of musculoskeletal injury or disease. During sample retrieval, tendons/ligaments were examined macroscopically by the surgeon and only included in the study if no signs of injury or pathology were present. While this satisfied the definition of "normal" for the purpose of this project, there

is a possibility that donors included in the study had either asymptomatic or unreported tendon disorders (injury/pathology/disease) which were not apparent macroscopically.

The issue of defining "normal" is exemplified in human rotator cuff tendons, where the incidence of injury/disease is high both in symptomatic and non-symptomatic shoulders[239,255]. Riley *et al.* (1994)[361] conducted biochemical analysis on human rotator cuff tendons collected under the proviso (similar to the current work) of "no history of tendonitis or pathology". Despite this, macroscopically "abnormal" tendons were identified, and grouped separately (further split into 'ruptured', 'thin/degenerate', 'calcified', and 'miscellaneous' abnormalities) and compared to the macroscopically "normal" cadaver tendons as well as the clinically impacted injury/pathology biopsy samples from patients. Interestingly, the Supraspinatus tendon biochemical compositions (GAG, uronic acid, chondroitin sulphate and dermatan sulphate) were not significantly different between the "normal" or any of the macroscopically "abnormal" cadaver specimens observed. However, the "normal" cadaver tendon showed significantly lower uronic acid, chondroitin sulphate and dermatan sulphate compared to chronic tendinitis biopsy samples[361]. This study highlights that rather than a simple dichotomy, normality may lie on a spectrum, and clearly defining factors such as tendon type and region, donor age and sex that may have distinct "normal" is critical to advancing knowledge of tendon health and disease.

"I have a problem when people say something's real or not real, or normal or abnormal. The meaning of those words for me is very personal and subjective. I've always been confused and never had a clearcut understanding of the meaning of those kinds of words" ~ Tim Burton.

7.6.2 Sample Blinding VS Inherent Variation

The majority of outcomes assessed by the SSS were applied to the 'whole tendon area' presented in the histological sections. Unfortunately, defining structural areas for samples which are clearly anatomically different on a macroscopic scale (such as those of variable size, with or without paratenon or adhered connective sheaths, presence of sesamoid regions, or are adjacent to bursae, capsules, fat pads, etc.) under a truly blinded scenario is challenging. Further, the present SSS is potentially not ideal for different regions of the tendon-whole such as paratenon, enthesis, MTJ, etc. Application of the SSS has not been explicitly tested on these areas but is likely not optimal due to the presence of distinct tissues and cell types in these different tissues, and these "tendon-approximate regions" need to be considered in a different context with tailored scoring matrices.

Upon retrospective consideration of the tendon types included in the study, and a brief unblinded exploration during the production of this thesis, it is evident that our sample dataset included specimens which contained many of the aforementioned tendon-approximate tissue, such as: regions particularly close/inclusive of the enthesis; areas of MTJ transitional tissue; areas of joint capsule or additional adjoining ligamentous/tendinous tissue structures either adjacent or adhered to the tendon/ligament proper; loose and closely adhered paratendinous sheaths; and adjacent structures such as nerves and large vessels traversing between segregated fascicular structures. Whether due to differences in structures lost during tissue retrieval or processing, sectioning orientation (which was considered relative to overall sample morphology) or other stages of the workflow, these "alternate structures" were not consistently apparent or present in all samples of

the same tendon/ligament. This can cause issues in blinded histological scoring in terms of justification to exclude regions of section without biasing assessment. It may be important for multiple investigators to first assess samples collaboratively to describe the tissue sections and identify the characteristics observed in the samples to be able to agree on and define the tendon mid-substance for consistent characterisation approaches. It would be interesting in future analyses to highlight samples which have retrospectively been structurally described to contain alternate structures and explore agreement of the scoring system rater comparison for these.

Second to the inclusion of non-mid-substance tendon area is the distinctive variability of certain tendon/ligament types purely from an anatomical sense. Clark and Harryman (1992)[248] detailed the complex and integrated structure of the rotator cuff. The samples of the 'tendons' of the rotator cuff inevitably contain portions of the anastomosed structures that form the rotator cuff and capsule of the glenohumeral joint. Histological observation of the supraspinatus tendon[248], for example, revealed a layered structure comprised of the conjoined tendon, capsule and coracohumeral ligament, which present with varying directionality and differ throughout the tendon. Similar observations were seen in the samples of the current projects, however, due to the blinded nature of the study and randomisation of different tendons, considerations of interwoven structures such as these were not known to the scorers.

Anatomical cardinal planes were not recorded during tendon (or ligament) retrieval in the current study, where the surgeon excised and handed-over the whole tendon (plus some of the respective muscle belly for gross orientation) and the primary investigator team completed division and separation into relevant outcome samples. This not only impacted orientation of layered/multidirectional structures such the rotator cuffs or QT and PT, but future exploration into transverse regional variations in all tendons/ligaments, as these cannot be attributed to anatomical planes (superficial, deep, medial, lateral) which were not identified or recorded as unknown. While tendon samples such as the QT and PT has observable direction relative to their layered structure and these layers (and sub-tendon accordingly) were captured in the section face (*Section 3.3.2)*, directionality was not always clearly identified with tendons such as the AT. However, it is worth noting that the embedding approach taken with some of the samples (*refer to Section 3.3.2*; particularly the larger samples such the rotator cuff tendons and AT, QT and PT, which portray this layered or sub-tendon structure) means that differently orientated samples are available for future work to explore generalised comparisons of whether embedding orientation has a significant effect on results.

7.6.3 Samples Numbers VS Statistical Strength

As previously addressed, the limited donor numbers in the present study unfortunately restrained the potential to explore their effects of donor variables (e.g. sex and age) on the histological outcomes assessed. A common limitation with studies comparing human specimens is low sample size with studies typically enlisting only one to three donors[267,325,378] due to ethical and logistical challenges. The current study was no exception, where even the planned donor procurement of n = 12 was restricted to n = 8 by supply limitations caused by the global COVID-19 pandemic.

7.6.4 Project Design VS Retrospective Discovery

The overall Human Musculoskeletal Tissue atlas project was designed to support further characterisation of musculoskeletal tissues via biomechanical, morphological, composition and structural exploration. The primary outcome was biomechanical testing and thus the whole tendon (first limb side, randomised) as well as proximal and distal regions from the opposing limb were collected for the biomechanics projects. While histological samples took precedence over proximal/distal biomechanics samples (Figure 3.2.A), this was only in the case where the free-tendon length (excluding muscle and bone) did not allow for all outcomes to be collected. Consequently, the proximity of the histology sample differed with regard to distance to the proximal end of the tendon (typically muscle-end, discussed previously) depending on whether these additional biomechanics samples were collected or not. Similarly, there were origin-insertion differences (bone versus muscle) for the shoulder tendons as discussed in *Section 7.4.1.2*.

Considering the numerous histological differences that have been observed between proximal and distal regions not only by our previous work[209,308] (conducted post commencement of the current sample retrieval processes) but also in current literature (*refer to Section 2.3.3*), the sampling consistency between tissue-types in the current project is not ideal, and regional difference may play a role in the outcomes we observed. Regional variations in cell characteristics, crimp characteristics and proteoglycan content reflect different attributes associated with bone vs muscle ends of the lower leg tendons in advanced aged human tendons[209,308], characteristics which might explain differences observed in the shoulder tendons in the current study. These limitations within our own study highlight that current practice in tendon structural evaluation lack consistency and reproducibility (where dissemination of assessment protocols is absent) – a vital workspace for further improvement.

7.6.5 *In Vivo* VS *In Vitro*

Following from previous discussion of histologically induced artefacts (*Section 7.3.2.1*), an important consideration when studying the structure of tissues is the impacts that processing of tissue has on the observed characteristics and tissue properties. Histological methodologies undeniably alter tissue properties relative to their *in vivo* state. This may be due to either 'false' attributes being evoked in the structure due to artefacts alone (such as physical damage), or accentuation of features in response to tissue preparations.

The presence and appearance of the newly defined IFS features of tendon/ligament tissue (that is, IFRs with imperceptible/indistinguishable matrix within the region) is one example of an observed characteristic that has the potential to be altered or influenced by tissue processing procedures. In some instances, these IFS "features" are hard to discern from fascicular matrix components, presenting as slightly densely staining 'lines' within otherwise solid FM areas. In others, they present as actual 'white spaces' (absence of tissue within the section area) otherwise described as physical gaps between neighbouring fascicles. These perceived gaps are likely a matter of how the tissue responds to histological processing (shrinkage and separation due to water loss) causing segregation at these boundary marks between fascicles, and do not reflect the

same as in *in vivo* situations. Another consideration is the potential misconception of gaps between matrix that were induced by true artefacts being perceived as IFS; possible causes being shrinkage or tearing within the FM structure or actual loss of the loosely situated matrix (IFM) during tissue processing. Nonetheless, these considerations are important points for discussion in the endeavour to better explore and define these newly described characteristics of the tendon hierarchy. As explored in Figure 4.4.L, there also remains another possibility that there is in fact matrix within these IFS regions, either missed by the observer or of a scale too small for the current histological observation approaches. Figure 7.6.A identifies different observations of IFRs, including an example of a perceived artefact. Future work is needed to truly understand the structural elements and their inherent *in vivo* state.

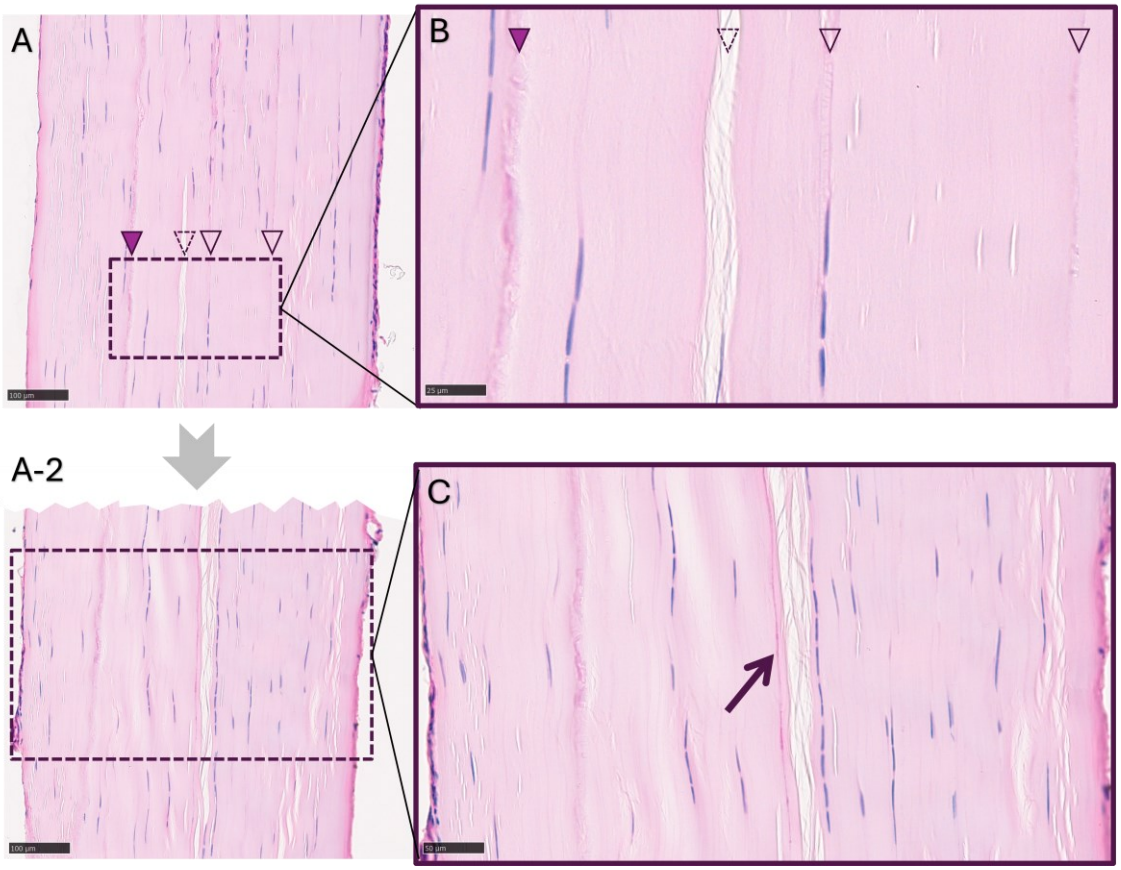


Figure 7.6.A Examples of different IFM and IFS case-scenarios. **A and inset-B)** Four identifications of perceivable IFRs: ***solid-triangle*** = small/discrete IFM line; ***dashed-hollow-triangle*** = tearing artefact, however, may be perceived as disrupted/disorganised FM matrix beside an IFS or loose IFM; ***hollow-triangles (x2)*** = IFS lines. **A-2 and inset-C)** Different region of same tendon as (A) reveals an IFS line near the suspected tearing artefact (arrow) . *All images H&E-staining. Images A/A-2: 200x magnification, scale bar = 100 µm. Image inset B: 800x magnification, scale bar = 25 µm. Image inset C: 400x magnification, scale bar = 50 µm.*

As another example, tissue handling procedures prior to and during histological processing may also further alter tissue properties from the *in vivo* situation. The tissues utilised in the current studies had undergone one freeze-thaw cycle; having been frozen and stored at −20°C upon collection from the donor facility prior to thawing for in-house tendon retrieval (note that conditions/exposure prior to our laboratory obtaining the tissue are unknown).

Our group has previously shown that biomechanical properties of kangaroo tail tendon were not affected by increasing freeze-thaw cycles or different storage temperatures, however, longer storage duration were found to have increased UTS[414]. Notably, findings relating to number of freeze-thaw cycles and biomechanical properties show varying conclusions[415,416], highlighting the need for consistent handling within study projects to reduce sample discrepancies. The effects of tissue freezing temperatures and number of freeze-thaw cycles on structural integrity has also been explored. Quirk *et al.* (2018)[416] observed changes in rat Achilles Tendon enthesis regions with freezing causing reduced proteoglycans and chondrocyte cytoplasm and nuclear size compared to fresh samples – characteristics which worsened with more freeze-thaw cycles. Interestingly though is that the tendon mid-substance exhibited no pronounced histological changes[416].

While the tissue utilised in this thesis were frozen specimens, the consistent protocols/treatment of the tissue means the relative comparisons of characteristics allow for valid inter-study comparisons. Nevertheless, the handling and processing of the samples should be considered when inferring *in vivo* properties. Beyond the need for more in-depth exploration into the effects of procedural handling of tissue throughout an investigation, an important point for future exploration is the applicability of the new HALT-SSS in more clinically relevant setting, such as performing pathological assessment on fresh biopsy samples.

7.7 Final Remarks – "Holy Tendon, Batman!"

Tendons (and ligaments) are a unique structural tissue somewhat underappreciated even within the musculoskeletal research community. Current research on tendons predominantly focuses on characterising tendon pathology, injury, or disease, with limited literature on normal (healthy) tendons, particularly in humans. This represents a notable deficit in knowledge considering that tendons play such a crucial role in musculoskeletal function across a spectrum of mechano-biological environments. An in-depth literature review on tendon microscopic structure identified that knowledge on normal tendon characteristics and associated variations across a range of distinct tendons, regions within tendons, ages and between the sexes is limited and needs further investigation. Other cases which present compositional variability (biochemical evaluation) support the need for structural assessments such as microscopy evaluation to further understanding of differences (such as localisation, distribution, etc.), and aid clinical practice such as biopsy assessment via histopathology. Thus, understanding and defining "normal/healthy" anatomy and physiology is crucial to understanding multifaceted pathology and providing suitable treatment/management strategies, or understanding implications relating to graft selection in the case of orthopaedic surgery.

Unfortunately, the limited dissemination of knowledge around tendon structural variation inhibits development of expertise in the field and further exploration in the effects of structural changes on tendon mechanics, physiology or clinical implications. Critically, there is currently no publicly available histological scoring system capable of extensively characterising normal variation in tendons. While further development is needed, this thesis proposes a new system capable of

normal tendon characterisation which offers the foundation for future improvement of research practices and knowledge dissemination in the field.

To the best of our knowledge, this is the first in-depth exploration into structure of the widest ever range of tendons using consistent and extensive methodology, revealing similarities and differences between human upper and lower limb tendons never before explored. While the scope of the investigation in this thesis saw the need to consider the upper and lower limb tendon data as two studies, the data remains standardised across both limbs and in general contributes to our laboratories' undertaking to develop an open access tendon and ligament atlas of biomechanical, morphological, compositional and structural/ultrastructural data for an extensive list of tendons and other donor-matched joint tissue. Once complete, the atlas project will provide a comprehensive database of tendon characteristics to further explore outcome correlations and the interactions between different variables.

Inevitably, demographic source as well as study-specific methodology are variables that may contribute to similarities and differences in the observed structural make-up of tendons. The investigations conducted during the research period are not without these and other limitations. Nonetheless, the ability to cross-compare research between laboratories/studies becomes ever more important when it comes to research on human tissues. Obtaining and utilising human tissue is often difficult and optimising internal and external use of the information/data achieved by human-tissue research (such as openly providing data or results useful for additional analyses) offers invaluable benefits for scientific knowledge and clinical translation – a point our laboratory aims to stand by and facilitate with the human tissue atlas project.

Tendon is a dynamic tissue ripe with variability. Expectedly, further work is needed. The research and findings presented in this thesis just scratch the surface of an astonishingly under researched field. Only when we know what healthy is, can we recognise and start to understand disease.

As cartoonist Scott Adams once put, "normal people believe that if it ain't broke, don't fix it. Engineers believe that if it ain't broke, it doesn't have enough features yet."

Histologists believe if it ain't broke, we should learn even more about it so when it is broke, we know why and how to fix it.

~ Samantha Hefferan ~

~ CHAPTER 8 ~

THE TENDON LEAGUE

REFERENCES

"For once I'm stuck without a punchline."

~ The Joker, Batman: Mask of the Phantasm (1993) ~

8.1 Overview

The following listed references concern those cited in Chapter 1 and Chapters 3-8. References for Chapter 2 are contained within Chapter 2 (within the submitted manuscript and supporting Appendix).

8.2 References

1 Birch H.L. (**2007**). Tendon matrix composition and turnover in relation to functional requirements. *Int J Exp Pathol*. 88(4): 241-248. DOI:10.1111/j.1365-2613.2007.00552.x

2 Benjamin M. & Ralphs J.R. (**1997**). Tendons and ligaments--an overview. *Histol Histopathol*. 12(4): 1135-1144.

3 Mienaltowski M.J. & Birk D.E. (**2014**). In: *Progress in Heritable Soft Connective Tissue Diseases* (ed Halper J.) 5-29 (Springer Netherlands).

4 Viidik A. (**1990**). In: *Biomechanics of Diarthrodial Joints.* (eds Ratcliffe A. *et al.*) 3-38 (Springer New York).

5 Owen R., Goodfellow J. & Bullough P.G. (**1980**). *Scientific foundations of orthopaedics and traumatology*. (Saunders).

6 Józsa L.s.G. & Kannus P. (**1997**). *Human tendons : anatomy, physiology, and pathology*. (Human Kinetics).

7 Batson E.L., Paramour R.J., Smith T.J., Birch H.L., Patterson-Kane J.C. *et al.* (**2003**). Are the material properties and matrix composition of equine flexor and extensor tendons determined by their functions? *Equine Vet J*. 35(3): 314-318. DOI:10.2746/042516403776148327

8 Thorpe C.T., Udeze C.P., Birch H.L., Clegg P.D. & Screen H.R. (**2012**). Specialization of tendon mechanical properties results from interfascicular differences. *J R Soc Interface*. 9(76): 3108-3117. DOI:10.1098/rsif.2012.0362

9 Screen H.R.C., Toorani S. & Shelton J.C. (**2013**). Microstructural stress relaxation mechanics in functionally different tendons. *Med Eng Phys*. 35(1): 96-102. DOI:https://doi.org/10.1016/j.medengphy.2012.04.004

10 Zuskov A., Freedman B.R., Gordon J.A., Sarver J.J., Buckley M.R. *et al.* (**2020**). Tendon Biomechanics and Crimp Properties Following Fatigue Loading Are Influenced by Tendon Type and Age in Mice. *J Orthop Res*. 38(1): 36-42. DOI:10.1002/jor.24407

11 Choi R.K., Smith M.M., Smith S., Little C.B. & Clarke E.C. (**2019**). Functionally distinct tendons have different biomechanical, biochemical and histological responses to in vitro unloading. *J Biomech*. 95(109321. DOI:10.1016/j.jbiomech.2019.109321

12 Shepherd J.H., Legerlotz K., Demirci T., Klemt C., Riley G.P. *et al.* (**2014**). Functionally distinct tendon fascicles exhibit different creep and stress relaxation behaviour. *Proc Inst Mech Eng H*. 228(1): 49-59. DOI:10.1177/0954411913509977

13 Quigley A.S., Bancelin S., Deska-Gauthier D., Legare F., Kreplak L. *et al.* (**2018**). In tendons, differing physiological requirements lead to functionally distinct nanostructures. *Sci Rep*. 8(1): 4409. DOI:10.1038/s41598-018-22741-8

14 Eekhoff J.D., Fang F., Kahan L.G., Espinosa G., Cocciolone A.J. *et al.* (**2017**). Functionally Distinct Tendons From Elastin Haploinsufficient Mice Exhibit Mild Stiffening and Tendon-Specific Structural Alteration. *J Biomech Eng*. 139(11). DOI:10.1115/1.4037932

15 Birch H.L., McLaughlin L., Smith R.K. & Goodship A.E. (**1999**). Treadmill exercise-induced tendon hypertrophy: assessment of tendons with different mechanical functions. *Equine Vet J Suppl*30): 222-226. DOI:10.1111/j.2042-3306.1999.tb05222.x

16 Birch H.L., Worboys S., Eissa S., Jackson B., Strassburg S. *et al.* (**2008**). Matrix metabolism rate differs in functionally distinct tendons. *Matrix Biol*. 27(3): 182-189. DOI:10.1016/j.matbio.2007.10.004

17 Herod T.W., Chambers N.C. & Veres S.P. (**2016**). Collagen fibrils in functionally distinct tendons have differing structural responses to tendon rupture and fatigue loading. *Acta Biomater*. 42(296-307. DOI:10.1016/j.actbio.2016.06.017

18 Spiesz E.M., Thorpe C.T., Thurner P.J. & Screen H.R.C. (**2018**). Structure and collagen crimp patterns of functionally distinct equine tendons, revealed by quantitative polarised light microscopy (qPLM). *Acta Biomater*. 70(281-292. DOI:10.1016/j.actbio.2018.01.034

19 Kharaz Y.A., Canty-Laird E.G., Tew S.R. & Comerford E.J. (**2018**). Variations in internal structure, composition and protein distribution between intra- and extra-articular knee ligaments and tendons. *J Anat*. 232(6): 943-955. DOI:10.1111/joa.12802

20 Kharaz Y.A., Tew S.R., Peffers M., Canty-Laird E.G. & Comerford E. (**2016**). Proteomic differences between native and tissue-engineered tendon and ligament. *Proteomics*. 16(10): 1547-1556. DOI:10.1002/pmic.201500459

21 Swenson D.M., Collins C.L., Best T.M., Flanigan D.C., Fields S.K. *et al.* (**2013**). Epidemiology of knee injuries among U.S. high school athletes, 2005/2006-2010/2011. *Med Sci Sports Exerc*. 45(3): 462-469. DOI:10.1249/MSS.0b013e318277acca

22 Tashjian R.Z. (**2012**). Epidemiology, Natural History, and Indications for Treatment of Rotator Cuff Tears. *Clin Sports Med*. 31(4): 589-604. DOI:10.1016/j.csm.2012.07.001

23 Clark S.T., Zhu M., Gamble G.D., Naot D., Paine S.-J. *et al.* (**2020**). Epidemiology of tendon and ligament injuries in Aotearoa/New Zealand between 2010 and 2016. *Injury Epidemiology*. 7(1). DOI:10.1186/s40621-020-0231-x

24 Debski T. & Noszczyk B.H. (**2020**). Epidemiology of complex hand injuries treated in the Plastic Surgery Department of a tertiary referral hospital in Warsaw. *Eur J Trauma Emerg Surg*. DOI:10.1007/s00068-020-01312-5

25 Clayton R.A.E. & Court-Brown C.M. (**2008**). The epidemiology of musculoskeletal tendinous and ligamentous injuries. *Injury*. 39(12): 1338-1344. DOI:10.1016/j.injury.2008.06.021

26 Lantto I., Heikkinen J., Flinkkila T., Ohtonen P. & Leppilahti J. (**2015**). Epidemiology of Achilles tendon ruptures: increasing incidence over a 33-year period. *Scand J Med Sci Sports*. 25(1): e133-138. DOI:10.1111/sms.12253

27 Littlewood C., May S. & Walters S. (**2017**). Epidemiology of Rotator Cuff Tendinopathy: A Systematic Review. *Shoulder & Elbow*. 5(4): 256-265. DOI:10.1111/sae.12028

28 Maffulli N., Wong J. & Almekinders L.C. (**2003**). Types and epidemiology of tendinopathy. *Clin Sports Med*. 22(4): 675-692. DOI:10.1016/s0278-5919(03)00004-8

29 Thorpe C.T., Clegg P.D. & Birch H.L. (**2010**). A review of tendon injury: why is the equine superficial digital flexor tendon most at risk? *Equine Vet J*. 42(2): 174-180. DOI:10.2746/042516409X480395

30 Marquez-Arabia W.H., Gomez-Hoyos J., Gomez M., Florez I., Gallo J.A. *et al.* (**2017**). Influence of Aging on Microvascular Supply of the Gluteus Medius Tendon: A Cadaveric and Histologic Study. *Arthroscopy*. 33(7): 1354-1360. DOI:10.1016/j.arthro.2017.01.036

31 Legerlotz K., Dorn J., Richter J., Rausch M. & Leupin O. (**2014**). Age-dependent regulation of tendon crimp structure, cell length and gap width with strain. *Acta Biomater*. 10(10): 4447-4455. DOI:10.1016/j.actbio.2014.05.029

32 Tinguely Y., Shi V., Klatte-Schulz F., Duda G.N., Freedman B.R. *et al.* (**2023**). Aging and injury affect nuclear shape heterogeneity in tendon. *J Orthop Res*. 41(10): 2186-2194. DOI:10.1002/jor.25649

33 Wilmink J., Wilson A.M. & Goodship A.E. (**1992**). Functional significance of the morphology and micromechanics of collagen fibres in relation to partial rupture of the superficial digital flexor tendon in racehorses. *Res Vet Sci*. 53(3): 354-359. DOI:https://doi.org/10.1016/0034-5288(92)90139-S

34 Asai K., Nakase J., Ishikawa T., Yoshimizu R., Kimura M. *et al.* (**2022**). Differences in cellular and microstructural properties of the semitendinosus muscle tendon between young and adult patients. *J Orthop Sci*. 27(2): 478-485. DOI:10.1016/j.jos.2021.01.012

35 Thorpe C.T., Streeter I., Pinchbeck G.L., Goodship A.E., Clegg P.D. *et al.* (**2010**). Aspartic acid racemization and collagen degradation markers reveal an accumulation of damage in tendon collagen that is enhanced with aging. *J Biol Chem*. 285(21): 15674-15681. DOI:10.1074/jbc.M109.077503

36 Granan L.P., Forssblad M., Lind M. & Engebretsen L. (**2009**). The Scandinavian ACL registries 2004-2007: baseline epidemiology. *Acta Orthop*. 80(5): 563-567. DOI:10.3109/17453670903350107

37 Sharma P. & Maffulli N. (**2006**). Biology of tendon injury: healing, modeling and remodeling. *J Musculoskelet Neuronal Interact*. 6(2): 181-190.

38 Asahara H., Inui M. & Lotz M.K. (**2017**). Tendons and Ligaments: Connecting Developmental Biology to Musculoskeletal Disease Pathogenesis. *J Bone Miner Res*. 32(9): 1773-1782. DOI:10.1002/jbmr.3199

39 Andarawis-Puri N., Flatow E.L. & Soslowsky L.J. (**2015**). Tendon basic science: Development, repair, regeneration, and healing. *J Orthop Res*. 33(6): 780-784. DOI:10.1002/jor.22869

40 Chen B., Li R. & Zhang S. (**2012**). Reconstruction and restoration of neglected ruptured patellar tendon using semitendinosus and gracilis tendons with preserved distal insertions: two case reports. *Knee*. 19(4): 508-512. DOI:10.1016/j.knee.2011.07.007

41 Chen C. & Hunt K.J. (**2019**). Open Reconstructive Strategies for Chronic Achilles Tendon Ruptures. *Foot Ankle Clin*. 24(3): 425-437. DOI:10.1016/j.fcl.2019.04.002

42 Duarte M. & Fradinho N. (**2021**). A Combined Surgical Approach for Extensor Hallucis Longus Reconstruction: Two Case Reports. *Indian J Plast Surg*. 54(2): 225-228. DOI:10.1055/s-0040-1721525

43 Kurashige T. (**2019**). Chronic extensor hallucis longus tendon rupture treated with double-bundle autograft of extensor hallucis capsularis: A case report. *SAGE Open Med Case Rep*. 7(2050313X19841962. DOI:10.1177/2050313X19841962

44 Omori S., Hamada K., Outani H., Oshima K., Joyama S. *et al.* (**2015**). Intraoperative extracorporeal autogenous irradiated tendon grafts for functional limb salvage surgery of soft tissue sarcomas of the wrist and hand. *World J Surg Oncol*. 13(179. DOI:10.1186/s12957-015-0588-4

45 Muller D.A., Beltrami G., Scoccianti G., Cuomo P., Totti F. *et al.* (**2018**). Allograft Reconstruction of the Extensor Mechanism after Resection of Soft Tissue Sarcoma. *Adv Orthop*. 2018(6275861. DOI:10.1155/2018/6275861

46 Mundinger G.S., Prucz R.B., Frassica F.J. & Deune E.G. (**2014**). Concomitant upper extremity soft tissue sarcoma limb-sparing resection and functional reconstruction: assessment of outcomes and costs of surgery. *Hand (N Y)*. 9(2): 196-204. DOI:10.1007/s11552-013-9567-9

47 Dierckman B.D. & Ferkel R.D. (**2015**). Anatomic Reconstruction With a Semitendinosus Allograft for Chronic Lateral Ankle Instability. *Am J Sports Med*. 43(8): 1941-1950. DOI:10.1177/0363546515593942

48 Edgar C.M., Zimmer S., Kakar S., Jones H. & Schepsis A.A. (**2008**). Prospective comparison of auto and allograft hamstring tendon constructs for ACL reconstruction. *Clin Orthop Relat Res*. 466(9): 2238-2246. DOI:10.1007/s11999-008-0305-5

49 Harris N.L., Smith D.A., Lamoreaux L. & Purnell M. (**1997**). Central quadriceps tendon for anterior cruciate ligament reconstruction. Part I: Morphometric and biomechanical evaluation. *Am J Sports Med*. 25(1): 23-28. DOI:10.1177/036354659702500105

50 Ajrawat P., Dwyer T., Whelan D., Theodoropoulos J., Murnaghan L. *et al.* (**2019**). A Comparison of Quadriceps Tendon Autograft With Bone-Patellar Tendon-Bone Autograft and Hamstring Tendon Autograft for Primary Anterior Cruciate Ligament Reconstruction: A Systematic Review and Quantitative Synthesis. *Clin J Sport Med*. DOI:10.1097/JSM.0000000000000765

51 Lippe J., Armstrong A. & Fulkerson J.P. (**2012**). Anatomic guidelines for harvesting a quadriceps free tendon autograft for anterior cruciate ligament reconstruction. *Arthroscopy*. 28(7): 980-984. DOI:10.1016/j.arthro.2012.01.002

52 Abe S., Kurosaka M., Iguchi T., Yoshiya S. & Hirohata K. (**1993**). Light and electron microscopic study of remodeling and maturation process in autogenous graft for anterior cruciate ligament reconstruction. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*. 9(4): 394-405. DOI:https://doi.org/10.1016/S0749-8063(05)80313-5

53 Chun C.H., Han H.J., Lee B.C., Kim D.C. & Yang J.H. (**2004**). Histologic findings of anterior cruciate ligament reconstruction with Achilles allograft. *Clin Orthop Relat Res*421): 273-276. DOI:10.1097/01.blo.0000126332.91435.76

54 Kim S.J., Bae J.H. & Lim H.C. (**2014**). Comparison of Achilles and tibialis anterior tendon allografts after anterior cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthrosc*. 22(1): 135-141. DOI:10.1007/s00167-012-2334-6

55 Kumar A., Rutherford-Davies J., Thorpe P. & Newson A. (**2020**). Combined quadriceps lengthening (using an external ring fixator) and patellar tendon reconstruction (using a tendoachilles allograft) in a case of chronic patellar tendon rupture: A case report. *The Knee*. 27(2): 598-606. DOI:10.1016/j.knee.2019.12.014

56 Meter J., Bascharon R., Amesur A., Gupta S., Arias D. *et al.* (**2022**). Revision Achilles Tendon Repair Using Posterior Tibial Tendon Allograft and Flexor Hallucis Longus Transfer. *Arthrosc Tech*. 11(3): e359-e363. DOI:10.1016/j.eats.2021.11.001

57 He J., Tang Q., Ernst S., Linde M.A., Smolinski P. *et al.* (**2021**). Peroneus longus tendon autograft has functional outcomes comparable to hamstring tendon autograft for anterior cruciate ligament reconstruction: a systematic review and meta-analysis. *Knee Surg Sports Traumatol Arthrosc*. 29(9): 2869-2879. DOI:10.1007/s00167-020-06279-9

58 Pérez Teuffer A. (**1974**). Traumatic rupture of the Achilles Tendon. Reconstruction by transplant and graft using the lateral peroneus brevis. *Orthop Clin North Am*. 5(1): 89-93.

59 Guyton G.P., Jeng C., Krieger L.E. & Mann R.A. (**2001**). Flexor digitorum longus transfer and medial displacement calcaneal osteotomy for posterior tibial tendon dysfunction: a middle-term clinical follow-up. *Foot Ankle Int*. 22(8): 627-632. DOI:10.1177/107110070102200802

60 DiPaola M. & Raikin S.M. (**2007**). Tendon transfers and realignment osteotomies for treatment of stage II posterior tibial tendon dysfunction. *Foot Ankle Clin*. 12(2): 273-285, vi. DOI:10.1016/j.fcl.2007.03.001

61 Wapner K.L., Pavlock G.S., Hecht P.J., Naselli F. & Walther R. (**1993**). Repair of chronic Achilles tendon rupture with flexor hallucis longus tendon transfer. *Foot Ankle*. 14(8): 443-449. DOI:10.1177/107110079301400803

62 Alayane A., Moussa M.K., Vieira T.D. & Lopes R. (**2024**). Arthroscopic Anatomic Lateral Ankle Reconstruction Using Allograft: A Simplified Approach. *Arthroscopy Techniques*. DOI:10.1016/j.eats.2024.103063

63 Alva A., Kumar V., Sreekumar R., Ng C.Y. & Talwalkar S.C. (**2013**). Autologous Tendon Grafts Used in Upper Limb Surgery. *Open Journal of Orthopedics*. 03(02): 74-78. DOI:10.4236/ojo.2013.32014

64 Pagenstert G.I., Valderrabano V. & Hintermann B. (**2005**). Lateral Ankle Ligament Reconstruction With Free Plantaris Tendon Graft. *Techniques in Foot & Ankle Surgery*. 4(2): 104-112. DOI:10.1097/01.btf.0000152574.09654.80

65 Josipovic M., Vlaic J., Serdar J., Simunovic M., Nizic D. *et al.* (**2020**). Plantaris tendon: a novel graft for anterolateral ligament reconstruction and additional reinforcement for anterior cruciate ligament autografts in combined reconstructive procedures. *Knee Surg Sports Traumatol Arthrosc*. 28(8): 2604-2608. DOI:10.1007/s00167-020-05885-x

66 Runer A., Keeling L., Wagala N., Nugraha H., Ozbek E.A. *et al.* (**2023**). Current trends in graft choice for anterior cruciate ligament reconstruction - part I: anatomy, biomechanics, graft incorporation and fixation. *J Exp Orthop*. 10(1): 37. DOI:10.1186/s40634-023-00600-4

67 Runer A., Keeling L., Wagala N., Nugraha H., Ozbek E.A. *et al.* (**2023**). Current trends in graft choice for primary anterior cruciate ligament reconstruction - part II: In-vivo kinematics, patient reported outcomes, re-rupture rates, strength recovery, return to sports and complications. *J Exp Orthop*. 10(1): 40. DOI:10.1186/s40634-023-00601-3

68 Hefferan S.A., Blaker C.L., Ashton D.M., Little C.B. & Clarke E.C. Structural Variations of Tendons: A Systematic Search and Narrative Review of Histological Differences Between Tendons, Tendon Regions, Sex, and Age. *J Orthop Res*. n/a(n/a). DOI:https://doi.org/10.1002/jor.26060

69 Screen H.R., Berk D.E., Kadler K.E., Ramirez F. & Young M.F. (**2015**). Tendon functional extracellular matrix. *J Orthop Res*. 33(6): 793-799. DOI:10.1002/jor.22818

70 Elliott D.H. (**1965**). Structure and Function of Mammalian Tendon. *Biol Rev Camb Philos Soc*. 40(392-421. DOI:10.1111/j.1469-185x.1965.tb00808.x

71 Liu S.H., Yang R.S., al-Shaikh R. & Lane J.M. (**1995**). Collagen in tendon, ligament, and bone healing. A current review. *Clin Orthop Relat Res*318): 265-278.

72 Ishizaki Y., Wang J., Kim J., Matsumoto T. & Maeda E. (**2024**). Contributions of collagen and elastin to elastic behaviours of tendon fascicle. *Acta Biomater*. 176(334-343. DOI:10.1016/j.actbio.2024.01.014

73 Franchi M., Trire A., Quaranta M., Orsini E. & Ottani V. (**2007**). Collagen structure of tendon relates to function. *ScientificWorldJournal*. 7(404-420. DOI:10.1100/tsw.2007.92

74 Screen H.R.C. (**2009**). Hierarchical Approaches to Understanding Tendon Mechanics. *Journal of Biomechanical Science and Engineering*. 4(4): 481-499. DOI:10.1299/jbse.4.481

75 Svensson R.B., Heinemeier K.M., Couppe C., Kjaer M. & Magnusson S.P. (**2016**). Effect of aging and exercise on the tendon. *J Appl Physiol (1985)*. 121(6): 1237-1246. DOI:10.1152/japplphysiol.00328.2016

76 Thorpe C.T., Birch H.L., Clegg P.D. & Screen H.R.C. (**2015**). In: *Tendon Regeneration* (eds Gomes M.E. *et al.*) 3-39 (Academic Press).

77 Rigozzi S., Müller R. & Snedeker J.G. (**2009**). Local strain measurement reveals a varied regional dependence of tensile tendon mechanics on glycosaminoglycan content. *J Biomech*. 42(10): 1547-1552. DOI:10.1016/j.jbiomech.2009.03.031

78 Samiric T., Ilic M.Z. & Handley C.J. (**2004**). Characterisation of proteoglycans and their catabolic products in tendon and explant cultures of tendon. *Matrix Biol*. 23(2): 127-140. DOI:10.1016/j.matbio.2004.03.004

79 Rees S.G., Flannery C.R., Little C.B., Hughes C.E., Caterson B. *et al.* (**2000**). Catabolism of aggrecan, decorin and biglycan in tendon. *The Biochemical journal*. 350(Pt 1): 181-188.

80 Funakoshi T., Schmid T., Hsu H.P. & Spector M. (**2008**). Lubricin distribution in the goat infraspinatus tendon: a basis for interfascicular lubrication. *J Bone Joint Surg Am*. 90(4): 803-814. DOI:10.2106/JBJS.G.00627

81 Kohrs R.T., Zhao C., Sun Y.L., Jay G.D., Zhang L. *et al.* (**2011**). Tendon fascicle gliding in wild type, heterozygous, and lubricin knockout mice. *J Orthop Res*. 29(3): 384-389. DOI:10.1002/jor.21247

82 Robinson P.S., Lin T.W., Jawad A.F., Iozzo R.V. & Soslowsky L.J. (**2004**). Investigating tendon fascicle structure-function relationships in a transgenic-age mouse model using multiple regression models. *Ann Biomed Eng*. 32(7): 924-931. DOI:10.1023/b:abme.0000032455.78459.56

83 Redaelli A., Vesentini S., Soncini M., Vena P., Mantero S. *et al.* (**2003**). Possible role of decorin glycosaminoglycans in fibril to fibril force transfer in relative mature tendons—a computational study from molecular to microstructural level. *J Biomech*. 36(10): 1555-1569. DOI:10.1016/s0021-9290(03)00133-7

84 Graham H.K., Holmes D.F., Watson R.B. & Kadler K.E. (**2000**). Identification of collagen fibril fusion during vertebrate tendon morphogenesis. The process relies on unipolar fibrils and is regulated by collagen-proteoglycan interaction. *J Mol Biol*. 295(4): 891-902. DOI:10.1006/jmbi.1999.3384

85 DiCesare P., Hauser N., Lehman D., Pasumarti S. & Paulsson M. (**1994**). Cartilage oligomeric matrix protein (COMP) is an abundant component of tendon. *FEBS Lett*. 354(2): 237-240. DOI:https://doi.org/10.1016/0014-5793(94)01134-6

86 Elliott D.M., Robinson P.S., Gimbel J.A., Sarver J.J., Abboud J.A. *et al.* (**2003**). Effect of altered matrix proteins on quasilinear viscoelastic properties in transgenic mouse tail tendons. *Ann Biomed Eng*. 31(5): 599-605. DOI:10.1114/1.1567282

87 Zhang S., Ju W., Chen X., Zhao Y., Feng L. *et al.* (**2022**). Hierarchical ultrastructure: An overview of what is known about tendons and future perspective for tendon engineering. *Bioact Mater*. 8(124-139. DOI:10.1016/j.bioactmat.2021.06.007

88 Sodersten F., Hultenby K., Heinegard D., Johnston C. & Ekman S. (**2013**). Immunolocalization of collagens (I and III) and cartilage oligomeric matrix protein in the normal and injured equine superficial digital flexor tendon. *Connect Tissue Res*. 54(1): 62-69. DOI:10.3109/03008207.2012.734879

89 Smith R.K., Zunino L., Webbon P.M. & Heinegård D. (**1997**). The distribution of cartilage oligomeric matrix protein (COMP) in tendon and its variation with tendon site, age and load. *Matrix Biol*. 16(5): 255-271. DOI:10.1016/s0945-053x(97)90014-7

90 Sun Y.L., Wei Z., Zhao C., Jay G.D., Schmid T.M. *et al.* (**2015**). Lubricin in human achilles tendon: The evidence of intratendinous sliding motion and shear force in achilles tendon. *J Orthop Res*. 33(6): 932-937. DOI:10.1002/jor.22897

91 Siadat S.M., Zamboulis D.E., Thorpe C.T., Ruberti J.W. & Connizzo B.K. (**2021**). In: *Adv Exp Med Biol* Vol. 1348 45-103.

92 Kjaer M. (**2004**). Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiol Rev*. 84(2): 649-698. DOI:10.1152/physrev.00031.2003

93 Durgam S. & Stewart M. (**2016**). Tendon-derived progenitor cells: In vitro characterization and clinical applications for tendon repair. *Journal of Stem Cell Research and Medicine*. 1(1): 8-17. DOI:10.15761/jscrm.1000102

94 Wang T., Wang L., Zhang L., Long Y., Zhang Y. *et al.* (**2023**). Single-cell RNA sequencing in orthopedic research. *Bone Res*. 11(1): 10. DOI:10.1038/s41413-023-00245-0

95 Lin P., Gan Y.B., He J., Lin S.E., Xu J.K. *et al.* (**2024**). Advancing skeletal health and disease research with single-cell RNA sequencing. *Mil Med Res*. 11(1): 33. DOI:10.1186/s40779-024-00538-3

96 De Micheli A.J., Swanson J.B., Disser N.P., Martinez L.M., Walker N.R. *et al.* (**2020**). Single-cell transcriptomic analysis identifies extensive heterogeneity in the cellular composition of mouse Achilles tendons. *Am J Physiol Cell Physiol*. 319(5): C885-C894. DOI:10.1152/ajpcell.00372.2020

97 Mimpen J.Y., Ramos-Mucci L., Paul C., Kurjan A., Hulley P.A. *et al.* (**2024**). Single nucleus and spatial transcriptomic profiling of healthy human hamstring tendon. *FASEB J*. 38(10): e23629. DOI:10.1096/fj.202300601RRR

98 Szczesny S.E. & Elliott D.M. (**2014**). Interfibrillar shear stress is the loading mechanism of collagen fibrils in tendon. *Acta Biomater*. 10(6): 2582-2590. DOI:10.1016/j.actbio.2014.01.032

99 Galloway M.T., Lalley A.L. & Shearn J.T. (**2013**). The role of mechanical loading in tendon development, maintenance, injury, and repair. *J Bone Joint Surg Am*. 95(17): 1620-1628. DOI:10.2106/JBJS.L.01004

100 Riley G. (**2004**). The pathogenesis of tendinopathy. A molecular perspective. *Rheumatology (Oxford)*. 43(2): 131-142. DOI:10.1093/rheumatology/keg448

101 Benjamin M., Kaiser E. & Milz S. (**2008**). Structure-function relationships in tendons: a review. *J Anat*. 212(3): 211-228. DOI:10.1111/j.1469-7580.2008.00864.x

102 Thorpe C.T., Birch H.L., Clegg P.D. & Screen H.R. (**2013**). The role of the non-collagenous matrix in tendon function. *Int J Exp Pathol*. 94(4): 248-259. DOI:10.1111/iep.12027

103 Edwards D.A. (**1946**). The blood supply and lymphatic drainage of tendons. *J Anat*. 80(147-152.142.

104 Franchi M., Raspanti M., Dell'Orbo C., Quaranta M., De Pasquale V. *et al.* (**2008**). Different crimp patterns in collagen fibrils relate to the subfibrillar arrangement. *Connect Tissue Res*. 49(2): 85-91. DOI:10.1080/03008200801913635

105 Franchi M., De Pasquale V., Martini D., Quaranta M., Macciocca M. *et al.* (**2010**). Contribution of glycosaminoglycans to the microstructural integrity of fibrillar and fiber crimps in tendons and ligaments. *ScientificWorldJournal*. 10(1932-1940. DOI:10.1100/tsw.2010.192

106 Butler D.L., Grood E.S., Noyes F.R. & Zernicke R.F. (**1978**). Biomechanics of ligaments and tendons. *Exerc Sport Sci Rev*. 6(125.

107 Hansen K.A., Weiss J.A. & Barton J.K. (**2002**). Recruitment of tendon crimp with applied tensile strain. *J Biomech Eng*. 124(1): 72-77. DOI:10.1115/1.1427698

108 Wang J.H. (**2006**). Mechanobiology of tendon. *J Biomech*. 39(9): 1563-1582. DOI:10.1016/j.jbiomech.2005.05.011

109 Amiel D., Kleiner J.B. & Akeson W.H. (**1986**). The natural history of the anterior cruciate ligament autograft of patellar tendon origin. *Am J Sports Med*. 14(6): 449-462. DOI:10.1177/036354658601400603

110 Edama M., Kubo M., Onishi H., Takabayashi T., Inai T. *et al.* (**2015**). The twisted structure of the human Achilles tendon. *Scand J Med Sci Sports*. 25(5): e497-503. DOI:10.1111/sms.12342

111 Szaro P., Witkowski G., Śmigielski R., Krajewski P. & Ciszek B. (**2009**). Fascicles of the adult human Achilles tendon - an anatomical study. *Annals of Anatomy - Anatomischer Anzeiger*. 191(6): 586-593. DOI:10.1016/j.aanat.2009.07.006

112 Kastelic J., Galeski A. & Baer E. (**1978**). The multicomposite structure of tendon. *Connect Tissue Res*. 6(1): 11-23. DOI:10.3109/03008207809152283

113 Dyer R.F. & Enna C.D. (**1976**). Ultrastructural features of adult human tendon. *Cell Tissue Res*. 168(2): 247-259. DOI:10.1007/BF00215881

114 Strocchi R., Leonardi L., Guizzardi S., Marchini M. & Ruggeri A. (**1985**). Ultrastructural aspects of rat tail tendon sheaths. *J Anat*. 140 (Pt 1)(Pt 1): 57-67.

115 Duance V.C., Restall D.J., Beard H., Bourne F.J. & Bailey A.J. (**1977**). The location of three collagen types in skeletal muscle. *FEBS Lett*. 79(2): 248-252. DOI:10.1016/0014-5793(77)80797-7

116 Herrmann H., Dessau W., Fessler L.I. & von der Mark K. (**1980**). Synthesis of types I, III and AB2 collagen by chick tendon fibroblasts in vitro. *Eur J Biochem*. 105(1): 63-74. DOI:10.1111/j.1432-1033.1980.tb04474.x

117 Kannus P. (**2000**). Structure of the tendon connective tissue. *Scand J Med Sci Sports*. 10(6): 312-320. DOI:10.1034/j.1600-0838.2000.010006312.x

118 Benjamin M. & Ralphs J.R. (**1998**). Fibrocartilage in tendons and ligaments--an adaptation to compressive load. *J Anat*. 193 (Pt 4)(Pt 4): 481-494. DOI:10.1046/j.1469-7580.1998.19340481.x

119 Viidik A. (**1966**). In: *Studies on the Anatomy and Function of Bone and Joints* (ed Evans F.G.) 17-39 (Springer Berlin Heidelberg).

120 Thorpe C.T. & Screen H.R. (**2016**). Tendon Structure and Composition. *Adv Exp Med Biol*. 920(3-10. DOI:10.1007/978-3-319-33943-6_1

121 Handsfield G.G., Slane L.C. & Screen H.R.C. (**2016**). Nomenclature of the tendon hierarchy: An overview of inconsistent terminology and a proposed size-based naming scheme with terminology for multi-muscle tendons. *J Biomech*. 49(13): 3122-3124. DOI:10.1016/j.jbiomech.2016.06.028

122 Ingelmark B.E. (**1948**). The Structure of Tendons at Various Ages and Under Different Functional Conditions. II. *Cells Tissues Organs*. 6(3): 193-225.

123 Watanabe T., Hosaka Y., Yamamoto E., Ueda H., Sugawara K. *et al.* (**2005**). Control of the collagen fibril diameter in the equine superficial digital flexor tendon in horses by decorin. *J Vet Med Sci*. 67(9): 855-860. DOI:10.1292/jvms.67.855

124 Ioannis D., Anastasios K., Konstantinos N., Lazaros K. & Georgios N. (**2015**). Palmaris Longus Muscle's Prevalence in Different Nations and Interesting Anatomical Variations: Review of the Literature. *J Clin Med Res*. 7(11): 825-830. DOI:10.14740/jocmr2243w

125 Townley W.A., Swan M.C. & Dunn R.L. (**2010**). Congenital absence of flexor digitorum superficialis: implications for assessment of little finger lacerations. *J Hand Surg Eur Vol*. 35(5): 417-418. DOI:10.1177/1753193409358523

126 Zilber S. & Oberlin C. (**2004**). Anatomical variations of the extensor tendons to the fingers over the dorsum of the hand: a study of 50 hands and a review of the literature. *Plast Reconstr Surg*. 113(1): 214-221. DOI:10.1097/01.PRS.0000091163.86851.9C

127 Ashton D.M., Blaker C.L., Hartnell N., Haubruck P., Liu Y. *et al.* (**2024**). The Biomechanical, Biochemical, and Morphological Properties of 19 Human Cadaveric Lower Limb Tendons and Ligaments: An Open-Access Data Set. *Am J Sports Med*. 52(9): 2391-2401. DOI:10.1177/03635465241260054

128 Mehran N., Damodar D. & Shu Yang J. (**2020**). Quadriceps Tendon Autograft in Anterior Cruciate Ligament Reconstruction. *JAAOS - Journal of the American Academy of Orthopaedic Surgeons*. 28(2): 45-52. DOI:10.5435/jaaos-d-19-00032

129 Frank R.M., Higgins J., Bernardoni E., Cvetanovich G., Bush-Joseph C.A. *et al.* (**2017**). Anterior Cruciate Ligament Reconstruction Basics: Bone-Patellar Tendon-Bone Autograft Harvest. *Arthrosc Tech*. 6(4): e1189-e1194. DOI:10.1016/j.eats.2017.04.006

130 Olewnik L., Zielinska N., Karauda P., Tubbs R.S. & Polguj M. (**2020**). A three-headed plantaris muscle: evidence that the plantaris is not a vestigial muscle? *Surg Radiol Anat*. 42(10): 1189-1193. DOI:10.1007/s00276-020-02478-8

131 Spina A.A. (**2007**). The plantaris muscle: anatomy, injury, imaging, and treatment. *J Can Chiropr Assoc*. 51(3): 158-165.

132 Gonera B., Kurtys K., Paulsen F., Polguj M., LaPrade R.F. *et al.* (**2021**). The plantaris muscle - Anatomical curiosity or a structure with important clinical value? - A comprehensive review of the current literature. *Ann Anat*. 235(151681. DOI:10.1016/j.aanat.2021.151681

133 Silver R., Garza J.d.l. & Rang M. (**1985**). The myth of muscle balance. A study of relative strengths and excursions of normal muscles about the foot and ankle. *The Journal of Bone & Joint Surgery British Volume*. 67-B(3): 432-437. DOI:10.1302/0301-620x.67b3.3997956

134 Olewnik L., Wysiadecki G., Polguj M. & Topol M. (**2017**). Anatomic study suggests that the morphology of the plantaris tendon may be related to Achilles tendonitis. *Surg Radiol Anat*. 39(1): 69-75. DOI:10.1007/s00276-016-1682-1

135 Park J.H., Cho J., Kim D., Kwon H.W., Lee M. *et al.* (**2022**). Anatomical Classification for Plantaris Tendon Insertion and Its Clinical Implications: A Cadaveric Study. *Int J Environ Res Public Health*. 19(10). DOI:10.3390/ijerph19105795

136 Kurtys K., Gonera B., Olewnik L., Karauda P., Tubbs R.S. *et al.* (**2021**). Is the plantaris muscle the most undefined human skeletal muscle? *Anat Sci Int*. 96(3): 471-477. DOI:10.1007/s12565-020-00586-4

137 Olewnik L., Wysiadecki G., Podgorski M., Polguj M. & Topol M. (**2018**). The Plantaris Muscle Tendon and Its Relationship with the Achilles Tendinopathy. *Biomed Res Int*. 2018(9623579. DOI:10.1155/2018/9623579

138 Simpson S.L., Hertzog M.S. & Barja R.H. (**1991**). The plantaris tendon graft: An ultrasound study. *The Journal of Hand Surgery*. 16(4): 708-711. DOI:https://doi.org/10.1016/0363-5023(91)90198-K

139 Yaras R.J., O'Neill N., Mabrouk A. & Yaish A.M. (**2024**). In: *StatPearls* (StatPearls Publishing, Treasure Island (FL)).

140 Ramos L.A., Zogbi T., de Andrade E.F., Taniguti de Oliveira G., Nicolini A.P. *et al.* (**2019**). Treatment and outcomes of lateral collateral ligament injury associated with anterior and posterior cruciate ligament injury at 2-year follow-up. *J Orthop*. 16(6): 489-492. DOI:10.1016/j.jor.2019.05.010

141 Rodriguez A.N., Falaas K.L. & LaPrade R.F. (**2024**). In: *Orthopaedic Sports Medicine* Ch. Chapter 38-1, 1-11.

142 Smith M.M., Sakurai G., Smith S.M., Young A.A., Melrose J. *et al.* (**2008**). Modulation of aggrecan and ADAMTS expression in ovine tendinopathy induced by altered strain. *Arthritis Rheum*. 58(4): 1055-1066. DOI:10.1002/art.23388

143 Fischer A.H., Jacobson K.A., Rose J. & Zeller R. (**2008**). Hematoxylin and eosin staining of tissue and cell sections. *CSH Protoc*. 2008(pdb.prot4986. DOI:10.1101/pdb.prot4986

144 Feldman A.T. & Wolfe D. (**2014**). Tissue processing and hematoxylin and eosin staining. *Methods Mol Biol*. 1180(31-43. DOI:10.1007/978-1-4939-1050-2_3

145 Sridharan G. & Shankar A.A. (**2012**). Toluidine blue: A review of its chemistry and clinical utility. *J Oral Maxillofac Pathol*. 16(2): 251-255. DOI:10.4103/0973-029X.99081

146 Vidal B.C. & Mello M.L.S. (**2019**). Toluidine blue staining for cell and tissue biology applications. *Acta Histochem*. 121(2): 101-112. DOI:10.1016/j.acthis.2018.11.005

147 Maccari F. & Volpi N. (**2002**). Glycosaminoglycan blotting on nitrocellulose membranes treated with cetylpyridinium chloride after agarose-gel electrophoretic separation. *Electrophoresis*. 23(19): 3270-3277. DOI:10.1002/1522-2683(200210)23:19<3270::AID-ELPS3270>3.0.CO;2-I

148 Volpi N., Maccari F. & Titze J. (**2005**). Simultaneous detection of submicrogram quantities of hyaluronic acid and dermatan sulfate on agarose-gel by sequential staining with toluidine blue and Stains-All. *J Chromatogr B Analyt Technol Biomed Life Sci*. 820(1): 131-135. DOI:10.1016/j.jchromb.2005.03.028

149 Chatterjee M., Evans M.K., Bell R., Nguyen P.K., Kamalitdinov T.B. *et al.* (**2023**). Histological and immunohistochemical guide to tendon tissue. *J Orthop Res*. DOI:10.1002/jor.25645

150 Schmitz N., Laverty S., Kraus V.B. & Aigner T. (**2010**). Basic methods in histopathology of joint tissues. *Osteoarthritis Cartilage*. 18 Suppl 3(S113-116. DOI:10.1016/j.joca.2010.05.026

151 Gupta S., Aggarwal R., Gupta V., Vij R., Tyagi N. *et al.* (**2017**). Picrosirius red: a better polarizing stain. *Journal of Histotechnology*. 40(2): 46-53. DOI:10.1080/01478885.2017.1325111

152 Lopez De Padilla C.M., Coenen M.J., Tovar A., De la Vega R.E., Evans C.H. *et al.* (**2021**). Picrosirius Red Staining: Revisiting Its Application to the Qualitative and Quantitative Assessment of Collagen Type I and Type III in Tendon. *J Histochem Cytochem*. 69(10): 633-643. DOI:10.1369/00221554211046777

153 Lattouf R., Younes R., Lutomski D., Naaman N., Godeau G. *et al.* (**2014**). Picrosirius red staining: a useful tool to appraise collagen networks in normal and pathological tissues. *J Histochem Cytochem*. 62(10): 751-758. DOI:10.1369/0022155414545787

154 Courtoy G.E., Leclercq I., Froidure A., Schiano G., Morelle J. *et al.* (**2020**). Digital Image Analysis of Picrosirius Red Staining: A Robust Method for Multi-Organ Fibrosis Quantification and Characterization. *Biomolecules*. 10(11). DOI:10.3390/biom10111585

155 Junqueira L.C.U., Bignolas G. & Brentani R.R. (**1979**). Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *The Histochemical Journal*. 11(4): 447-455. DOI:10.1007/BF01002772

156 Constantine V.S. & Mowry R.W. (**1968**). Selective staining of human dermal collagen. II. The use of picrosirius red F3BA with polarization microscopy. *J Invest Dermatol*. 50(5): 419-423. DOI:10.1038/jid.1968.68

157 Dayan D., Hiss Y., Hirshberg A., Bubis J.J. & Wolman M. (**1989**). Are the polarization colors of Picrosirius red-stained collagen determined only by the diameter of the fibers? *Histochemistry*. 93(1): 27-29. DOI:10.1007/BF00266843

158 Lichtwark G.A. & Barclay C.J. (**2010**). The influence of tendon compliance on muscle power output and efficiency during cyclic contractions. *J Exp Biol*. 213(5): 707-714. DOI:10.1242/jeb.038026

159 Ker R. (**2007**). Mechanics of tendon, from an engineering perspective. *International Journal of Fatigue*. 29(6): 1001-1009. DOI:10.1016/j.ijfatigue.2006.09.020

160 Józsa L., Réffy A., Kannus P., Demel S. & Elek E. (**1990**). Pathological alterations in human tendons. *Arch Orthop Trauma Surg*. 110(1): 15-21. DOI:10.1007/BF00431359

161 Schmidt W.J. (**1938**). Neuere polarisationsoptische Arbeiten auf dem Gebiete der Biologie. *Protoplasma*. 29(1): 300-312. DOI:10.1007/BF01602507

162 Kannus P. & Jozsa L. (**1991**). Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. *J Bone Joint Surg Am*. 73(10): 1507-1525.

163 Loppini M., Longo U.G., Niccoli G., Khan W.S., Maffulli N. *et al.* (**2015**). Histopathological scores for tissue-engineered, repaired and degenerated tendon: a systematic review of the literature. *Curr Stem Cell Res Ther*. 10(1): 43-55. DOI:10.2174/1574888x09666140710110723

164 Hadjicostas P.T., Soucacos P.N., Koleganova N., Krohmer G. & Berger I. (**2008**). Comparative and morphological analysis of commonly used autografts for anterior cruciate ligament reconstruction with the native ACL: an electron, microscopic and morphologic study. *Knee Surg Sports Traumatol Arthrosc*. 16(12): 1099-1107. DOI:10.1007/s00167-008-0603-1

165 Hadjicostas P.T., Soucacos P.N., Berger I., Koleganova N. & Paessler H.H. (**2007**). Comparative analysis of the morphologic structure of quadriceps and patellar tendon: a descriptive laboratory study. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association*. 23(7): 744-750. DOI:10.1016/j.arthro.2007.01.032

166 Hadjicostas P.T., Soucacos P.N., Koleganova N., Piecha G., Krohmer G. *et al.* (**2008**). Comparative analysis of the microstructure of the hamstring tendons: an electron microscopic, histologic, and morphologic study. *J Surg Orthop Adv*. 17(3): 153-158.

167 Hadjicostas P.T., Soucacos P.N., Paessler H.H., Koleganova N. & Berger I. (**2007**). Morphologic and histologic comparison between the patella and hamstring tendons grafts: a descriptive and anatomic study. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association*. 23(7): 751-756. DOI:10.1016/j.arthro.2007.02.002

168 Movin T., Gad A., Reinholt F.P. & Rolf C. (**1997**). Tendon pathology in long-standing achillodynia. Biopsy findings in 40 patients. *Acta Orthop Scand*. 68(2): 170-175. DOI:10.3109/17453679709004002

169 Cook J.L., Feller J.A., Bonar S.F. & Khan K.M. (**2004**). Abnormal tenocyte morphology is more prevalent than collagen disruption in asymptomatic athletes' patellar tendons. *J Orthop Res*. 22(2): 334-338. DOI:10.1016/j.orthres.2003.08.005

170 Nixon A.J., Dahlgren L.A., Haupt J.L., Yeager A.E. & Ward D.L. (**2008**). Effect of adipose-derived nucleated cell fractions on tendon repair in horses with collagenase-induced tendinitis. *Am J Vet Res*. 69(7): 928-937. DOI:10.2460/ajvr.69.7.928

171 Kartus J., Movin T., Papadogiannakis N., Christensen L.R., Lindahl S. *et al.* (**2000**). A radiographic and histologic evaluation of the patellar tendon after harvesting its central third. *Am J Sports Med*. 28(2): 218-226. DOI:10.1177/03635465000280021301

172 Watkins J.P., Auer J.A., Gay S. & Morgan S.J. (**1985**). Healing of surgically created defects in the equine superficial digital flexor tendon: collagen-type transformation and tissue morphologic reorganization. *Am J Vet Res*. 46(10): 2091-2096.

173 Ide J., Kikukawa K., Hirose J., Iyama K.-i., Sakamoto H. *et al.* (**2009**). The effect of a local application of fibroblast growth factor-2 on tendon-to-bone remodeling in rats with acute injury and repair of the supraspinatus tendon. *J Shoulder Elbow Surg*. 18(3): 391-398. DOI:10.1016/j.jse.2009.01.013

174 Aström M. & Rausing A. (**1995**). Chronic Achilles tendinopathy. A survey of surgical and histopathologic findings. *Clin Orthop Relat Res*316): 151-164.

175 Soslowsky L.J., Carpenter J.E., DeBano C.M., Banerji I. & Moalli M.R. (**1996**). Development and use of an animal model for investigations on rotator cuff disease. *J Shoulder Elbow Surg*. 5(5): 383-392. DOI:10.1016/s1058-2746(96)80070-x

176 Dines J.S., Weber L., Razzano P., Prajapati R., Timmer M. *et al.* (**2007**). The effect of growth differentiation factor-5–coated sutures on tendon repair in a rat model. *J Shoulder Elbow Surg*. 16(5): S215-S221. DOI:10.1016/j.jse.2007.03.001

177 Lau Y.K., Iyer K., Shetye S., Friday C.S., Dodge G.R. *et al.* (**2024**). Evaluation of tendon and ligament microstructure and mechanical properties in a canine model of mucopolysaccharidosis I. *J Orthop Res*. 42(7): 1409-1419. DOI:10.1002/jor.25813

178 Allain J., Goutallier D. & Voisin M.C. (**2001**). Macroscopic and histological assessments of the cruciate ligaments in arthrosis of the knee. *Acta Orthop Scand*. 72(3): 266-269. DOI:10.1080/00016470152846592

179 Cetti R., Junge J. & Vyberg M. (**2003**). Spontaneous rupture of the Achilles tendon is preceded by widespread and bilateral tendon damage and ipsilateral inflammation. *Acta Orthop Scand*. 74(1): 78.

180 Fearon A., Dahlstrom J.E., Twin J., Cook J. & Scott A. (**2014**). The Bonar score revisited: region of evaluation significantly influences the standardized assessment of tendon degeneration. *J Sci Med Sport*. 17(4): 346-350. DOI:10.1016/j.jsams.2013.07.008

181 Sano H., Ishii H., Yeadon A., Backman D.S., Brunet J.A. *et al.* (**2005**). Degeneration at the insertion weakens the tensile strength of the supraspinatus tendon: A comparative mechanical and histologic study of the bone‐tendon complex. *J Orthop Res*. 15(5): 719-726. DOI:10.1002/jor.1100150514

182 Carvalho Pde T., Silva I.S., Reis F.A., Belchior A.C., Aydos R.D. *et al.* (**2006**). Histological study of tendon healing in malnourished Wistar rats treated with ultrasound therapy. *Acta Cir Bras*. 21 Suppl 4(13-17. DOI:10.1590/s0102-86502006001000004

183 Murray M.M., Spindler K.P., Ballard P., Welch T.P., Zurakowski D. *et al.* (**2007**). Enhanced histologic repair in a central wound in the anterior cruciate ligament with a collagen-platelet-rich plasma scaffold. *J Orthop Res*. 25(8): 1007-1017. DOI:10.1002/jor.20367

184 Glazebrook M.A., Wright J.R., Jr., Langman M., Stanish W.D. & Lee J.M. (**2008**). Histological analysis of achilles tendons in an overuse rat model. *J Orthop Res*. 26(6): 840-846. DOI:10.1002/jor.20546

185 Schnabel L.V., Lynch M.E., van der Meulen M.C., Yeager A.E., Kornatowski M.A. *et al.* (**2009**). Mesenchymal stem cells and insulin-like growth factor-I gene-enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. *J Orthop Res*. 27(10): 1392-1398. DOI:10.1002/jor.20887

186 Peltz C.D., Hsu J.E., Zgonis M.H., Trasolini N.A., Glaser D.L. *et al.* (**2012**). Intra-articular changes precede extra-articular changes in the biceps tendon after rotator cuff tears in a rat model. *J Shoulder Elbow Surg*. 21(7): 873-881. DOI:10.1016/j.jse.2011.04.036

187 Maffulli N., Barrass V. & Ewen S.W. (**2000**). Light microscopic histology of achilles tendon ruptures. A comparison with unruptured tendons. *Am J Sports Med*. 28(6): 857-863. DOI:10.1177/03635465000280061401

188 Longo U.G., Franceschi F., Ruzzini L., Rabitti C., Morini S. *et al.* (**2009**). Characteristics at haematoxylin and eosin staining of ruptures of the long head of the biceps tendon. *Br J Sports Med*. 43(8): 603-607. DOI:10.1136/bjsm.2007.039016

189 Tallon C., Maffulli N. & Ewen S.W. (**2001**). Ruptured Achilles tendons are significantly more degenerated than tendinopathic tendons. *Med Sci Sports Exerc*. 33(12): 1983-1990. DOI:10.1097/00005768-200112000-00002

190 Longo U.G., Franceschi F., Ruzzini L., Rabitti C., Morini S. *et al.* (**2008**). Histopathology of the supraspinatus tendon in rotator cuff tears. *Am J Sports Med*. 36(3): 533-538. DOI:10.1177/0363546507308549

191 Maffulli N., Del Buono A., Spiezia F., Longo U.G. & Denaro V. (**2012**). Light microscopic histology of quadriceps tendon ruptures. *Int Orthop*. 36(11): 2367-2371. DOI:10.1007/s00264-012-1637-z

192 Maffulli N., Testa V., Capasso G., Ewen S.W., Sullo A. *et al.* (**2004**). Similar histopathological picture in males with Achilles and patellar tendinopathy. *Med Sci Sports Exerc*. 36(9): 1470-1475. DOI:10.1249/01.mss.0000139895.94846.8d

193 Burssens A., Forsyth R., Bongaerts W., Jagodzinski M., Mahieu N. *et al.* (**2013**). Arguments for an increasing differentiation towards fibrocartilaginous components in midportion Achilles tendinopathy. *Knee Surg Sports Traumatol Arthrosc*. 21(6): 1459-1467. DOI:10.1007/s00167-012-2203-3

194 Zabrzynski J., Paczesny L., Zabrzynska A., Grzanka D. & Lapaj L. (**2018**). Sonography in the instability of the long head of the biceps tendon confronted with histopathologic and arthroscopic findings. *Folia Morphol (Warsz)*. 77(3): 583-590. DOI:10.5603/FM.a2018.0012

195 Sethi P.M., Sheth C.D., Pauzenberger L., McCarthy M.B.R., Cote M.P. *et al.* (**2018**). Macroscopic Rotator Cuff Tendinopathy and Histopathology Do Not Predict Repair Outcomes of Rotator Cuff Tears. *Am J Sports Med*. 46(4): 779-785. DOI:10.1177/0363546517746986

196 Scott A., Cook J.L., Hart D.A., Walker D.C., Duronio V. *et al.* (**2007**). Tenocyte responses to mechanical loading in vivo: a role for local insulin-like growth factor 1 signaling in early tendinosis in rats. *Arthritis Rheum*. 56(3): 871-881. DOI:10.1002/art.22426

197 Dirks R.C., Richard J.S., Fearon A.M., Scott A., Koch L.G. *et al.* (**2013**). Uphill treadmill running does not induce histopathological changes in the rat Achilles tendon. *BMC Musculoskelet Disord*. 14(90. DOI:10.1186/1471-2474-14-90

198 Hapa O., Cakici H., Gideroğlu K., Ozturan K., Kükner A. *et al.* (**2009**). The effect of ethanol intake on tendon healing: a histological and biomechanical study in a rat model. *Arch Orthop Trauma Surg*. 129(12): 1721-1726. DOI:10.1007/s00402-009-0877-x

199 Tsang A.S., Dart A.J., Biasutti S.A., Jeffcott L.B., Smith M.M. *et al.* (**2019**). Effects of tendon injury on uninjured regional tendons in the distal limb: An in-vivo study using an ovine tendinopathy model. *PLoS One*. 14(4): e0215830. DOI:10.1371/journal.pone.0215830

200 Biasutti S., Dart A., Smith M., Blaker C., Clarke E. *et al.* (**2017**). Spatiotemporal variations in gene expression, histology and biomechanics in an ovine model of tendinopathy. *PLoS One*. 12(10): e0185282. DOI:10.1371/journal.pone.0185282

201 Jacobson E., Dart A.J., Mondori T., Horadogoda N., Jeffcott L.B. *et al.* (**2015**). Focal experimental injury leads to widespread gene expression and histologic changes in equine flexor tendons. *PLoS One*. 10(4): e0122220. DOI:10.1371/journal.pone.0122220

202 Maffulli N., Longo U.G., Franceschi F., Rabitti C. & Denaro V. (**2008**). Movin and Bonar scores assess the same characteristics of tendon histology. *Clin Orthop Relat Res*. 466(7): 1605-1611. DOI:10.1007/s11999-008-0261-0

203 Obeidat A.M., Kim S.Y., Burt K.G., Hu B., Li J. *et al.* (**2024**). A standardized approach to evaluation and reporting of synovial histopathology in two surgically induced murine models of osteoarthritis. *Osteoarthritis Cartilage*. 32(10): 1273-1282. DOI:10.1016/j.joca.2024.05.006

204 Delcroix G.J., Kaimrajh D.N., Baria D., Cooper S., Reiner T. *et al.* (**2013**). Histologic, biomechanical, and biological evaluation of fan-folded iliotibial band allografts for anterior cruciate ligament reconstruction. *Arthroscopy*. 29(4): 756-765. DOI:10.1016/j.arthro.2012.11.007

205 González J., Lopez C., Alvarez M., Perez Cardenas J. & Carmona J. (**2016**). Autologous leukocyte-reduced platelet-rich plasma therapy for Achilles tendinopathy induced by collagenase in a rabbit model. *Sci Rep*. 6(19623. DOI:10.1038/srep19623

206 Park Y.H., Kim T.J., Choi G.W. & Kim H.J. (**2019**). Achilles tendinosis does not always precede Achilles tendon rupture. *Knee Surg Sports Traumatol Arthrosc*. 27(10): 3297-3303. DOI:10.1007/s00167-018-5172-3

207 Ahlen M., Liden M., Movin T., Papadogiannakis N., Rostgard-Christensen L. *et al.* (**2014**). Histological Evaluation of Regenerated Semitendinosus Tendon a Minimum of 6 Years After Harvest for Anterior Cruciate Ligament Reconstruction. *Orthop J Sports Med*. 2(9): 2325967114550274. DOI:10.1177/2325967114550274

208 Bolam S.M., Satokar V.V., Konar S., Coleman B., Monk A.P. *et al.* (**2021**). A Maternal High Fat Diet Leads to Sex-Specific Programming of Mechanical Properties in Supraspinatus Tendons of Adult Rat Offspring. *Front Nutr*. 8(729427. DOI:10.3389/fnut.2021.729427

209 Hefferan S.A. (**2020**). *Histological Characteristics of Different Tendons in Humans of Advanced Age.* Bachelor of Medical Science (Honours) thesis, The University of Sydney.

210 Dahia C.L., Engiles J.B., Gullbrand S.E., Iatridis J.C., Lai A. *et al.* (**2021**). A perspective on the ORS Spine Section initiative to develop a multi-species JOR Spine histopathology series. *JOR Spine*. 4(2): e1165. DOI:10.1002/jsp2.1165

211 Gullbrand S.E., Ashinsky B.G., Lai A., Gansau J., Crowley J. *et al.* (**2021**). Development of a standardized histopathology scoring system for intervertebral disc degeneration and regeneration in rabbit models-An initiative of the ORSspine section. *JOR Spine*. 4(2): e1147. DOI:10.1002/jsp2.1147

212 Lai A., Gansau J., Gullbrand S.E., Crowley J., Cunha C. *et al.* (**2021**). Development of a standardized histopathology scoring system for intervertebral disc degeneration in rat models: An initiative of the ORS spine section. *JOR Spine*. 4(2): e1150. DOI:10.1002/jsp2.1150

213 Le Maitre C.L., Dahia C.L., Giers M., Illien-Junger S., Cicione C. *et al.* (**2021**). Development of a standardized histopathology scoring system for human intervertebral disc degeneration: an Orthopaedic Research Society Spine Section Initiative. *JOR Spine*. 4(2): e1167. DOI:10.1002/jsp2.1167

214 Melgoza I.P., Chenna S.S., Tessier S., Zhang Y., Tang S.Y. *et al.* (**2021**). Development of a standardized histopathology scoring system using machine learning algorithms for intervertebral disc degeneration in the mouse model-An ORS spine section initiative. *JOR Spine*. 4(2): e1164. DOI:10.1002/jsp2.1164

215 Landis J.R. & Koch G.G. (**1977**). The measurement of observer agreement for categorical data. *Biometrics*. 33(1): 159-174.

216 McHugh M.L. (**2012**). Interrater reliability: the kappa statistic. *Biochem Med (Zagreb)*. 22(3): 276-282.

217 Cicchetti D.V. (**1994**). Guidelines, Criteria, and Rules of Thumb for Evaluating Normed and Standardized Assessment Instruments in Psychology. *Psychol Assess*. 6(284-290.

218 Koo T.K. & Li M.Y. (**2016**). A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *J Chiropr Med*. 15(2): 155-163. DOI:10.1016/j.jcm.2016.02.012

219 Zec S., Soriani N., Comoretto R. & Baldi I. (**2017**). High Agreement and High Prevalence: The Paradox of Cohen's Kappa. *Open Nurs J*. 11(211-218. DOI:10.2174/1874434601711010211

220 Feinstein A.R. & Cicchetti D.V. (**1990**). High agreement but low kappa: I. The problems of two paradoxes. *J Clin Epidemiol*. 43(6): 543-549. DOI:10.1016/0895-4356(90)90158-l

221 Bexkens R., Claessen F.M., Kodde I.F., Oh L.S., Eygendaal D. *et al.* (**2018**). The kappa paradox. *Shoulder Elbow*. 10(4): 308. DOI:10.1177/1758573218791813

222 Cohen J. (**1960**). A Coefficient of Agreement for Nominal Scales. *Educ Psychol Meas*. 20(1): 37-46. DOI:10.1177/001316446002000104

223 Koch S. & Tillmann B. (**1995**). The distal tendon of the biceps brachii. *Annals of Anatomy - Anatomischer Anzeiger*. 177(5): 467-474. DOI:10.1016/s0940-9602(11)80155-x

224 Petersen W., Stein V. & Bobka T. (**2000**). Structure of the human tibialis anterior tendon. *J Anat*. 197 Pt 4(Pt 4): 617-625. DOI:10.1046/j.1469-7580.2000.19740617.x

225 Clegg P.D., Strassburg S. & Smith R.K. (**2007**). Cell phenotypic variation in normal and damaged tendons. *Int J Exp Pathol*. 88(4): 227-235. DOI:10.1111/j.1365-2613.2007.00549.x

226 Benjamin M., Qin S. & Ralphs J.R. (**1995**). Fibrocartilage associated with human tendons and their pulleys. *J Anat*. 187 (Pt 3)(Pt 3): 625-633.

227 Thorpe C.T., Riley G.P., Birch H.L., Clegg P.D. & Screen H.R.C. (**2016**). Fascicles and the interfascicular matrix show adaptation for fatigue resistance in energy storing tendons. *Acta Biomater*. 42(308-315. DOI:10.1016/j.actbio.2016.06.012

228 Thorpe C.T., Riley G.P., Birch H.L., Clegg P.D. & Screen H.R.C. (**2017**). Fascicles and the interfascicular matrix show decreased fatigue life with ageing in energy storing tendons. *Acta Biomater*. 56(58-64. DOI:10.1016/j.actbio.2017.03.024

229 Thorpe C.T., Riley G.P., Birch H.L., Clegg P.D. & Screen H.R. (**2014**). Fascicles from energy-storing tendons show an age-specific response to cyclic fatigue loading. *J R Soc Interface*. 11(92): 20131058. DOI:10.1098/rsif.2013.1058

230 Thorpe C.T., Godinho M.S.C., Riley G.P., Birch H.L., Clegg P.D. *et al.* (**2015**). The interfascicular matrix enables fascicle sliding and recovery in tendon, and behaves more elastically in energy storing tendons. *J Mech Behav Biomed Mater*. 52(85-94. DOI:10.1016/j.jmbbm.2015.04.009

231 Junqueira L.C., Cossermelli W. & Brentani R. (**1978**). Differential staining of collagens type I, II and III by Sirius Red and polarization microscopy. *Arch Histol Jpn*. 41(3): 267-274. DOI:10.1679/aohc1950.41.267

232 Rieppo L., Janssen L., Rahunen K., Lehenkari P., Finnila M.A.J. *et al.* (**2019**). Histochemical quantification of collagen content in articular cartilage. *PLoS One*. 14(11): e0224839. DOI:10.1371/journal.pone.0224839

233 Godinho M.S., Thorpe C.T., Greenwald S.E. & Screen H.R.C. (**2021**). Elastase treatment of tendon specifically impacts the mechanical properties of the interfascicular matrix. *Acta Biomater*. 123(187-196. DOI:10.1016/j.actbio.2021.01.030

234 Godinho M.S.C., Thorpe C.T., Greenwald S.E. & Screen H.R.C. (**2017**). Elastin is Localised to the Interfascicular Matrix of Energy Storing Tendons and Becomes Increasingly Disorganised With Ageing. *Sci Rep*. 7(1): 9713. DOI:10.1038/s41598-017-09995-4

235 Obuchowicz R., Ekiert M., Kohut P., Holak K., Ambrozinski L. *et al.* (**2019**). Interfascicular matrix-mediated transverse deformation and sliding of discontinuous tendon subcomponents control the viscoelasticity and failure of tendons. *J Mech Behav Biomed Mater*. 97(238-246. DOI:10.1016/j.jmbbm.2019.05.027

236 Kapit W. & Elson L.M. (**2014**). *The Anatomy Coloring Book*. (Pearson).

237 Mazzocca A.D., McCarthy M.B.R., Ledgard F.A., Chowaniec D.M., McKinnon W.J. *et al.* (**2013**). Histomorphologic Changes of the Long Head of the Biceps Tendon in Common Shoulder Pathologies. *Arthroscopy*. 29(6): 972-981. DOI:10.1016/j.arthro.2013.02.002

238 Tilley J.M., Murphy R.J., Chaudhury S., Czernuszka J.T. & Carr A.J. (**2014**). Effect of tear size, corticosteroids and subacromial decompression surgery on the hierarchical structural properties of torn supraspinatus tendons. *Bone Joint Res*. 3(8): 252-261. DOI:10.1302/2046-3758.38.2000251

239 Hinsley H., Ganderton C., Arden N.K. & Carr A.J. (**2022**). Prevalence of rotator cuff tendon tears and symptoms in a Chingford general population cohort, and the resultant impact on UK health services: a cross-sectional observational study. *BMJ Open*. 12(9): e059175. DOI:10.1136/bmjopen-2021-059175

240 Harwood K.L., Oganezova K., Orellana K.J., Ashe K., Williams B.A. *et al.* (**2023**). Rotator Cuff Injuries in the Pediatric Population: A Retrospective Review of Patient Characteristics and Treatment at a Single Center. *Sports Health: A Multidisciplinary Approach*. 16(3): 340-346. DOI:10.1177/19417381231174021

241 Birch H.L., Sinclair C., Goodship A.E. & Smith R.K.W. (**2014**). In: *Equine Sports Medicine and Surgery* 167-188.

242 Blevins F.T., Djurasovic M., Flatow E.L. & Vogel K.G. (**1997**). Biology of the rotator cuff tendon. *Orthop Clin North Am*. 28(1): 1-16. DOI:10.1016/s0030-5898(05)70260-1

243 Milz S., Putz R., Ralphs J.R. & Benjamin M. (**1999**). Fibrocartilage in the extensor tendons of the human metacarpophalangeal joints. *Anat Rec*. 256(2): 139-145. DOI:10.1002/(sici)1097-0185(19991001)256:2<139::Aid-ar4>3.0.Co;2-b

244 Thorpe C.T., Peffers M.J., Simpson D., Halliwell E., Screen H.R. *et al.* (**2016**). Anatomical heterogeneity of tendon: Fascicular and interfascicular tendon compartments have distinct proteomic composition. *Sci Rep*. 6(20455. DOI:10.1038/srep20455

245 Amirtharajah M. & Lattanza L. (**2015**). Open Extensor Tendon Injuries. *The Journal of Hand Surgery*. 40(2): 391-397. DOI:https://doi.org/10.1016/j.jhsa.2014.06.136

246 Colzani G., Tos P., Battiston B., Merolla G., Porcellini G. *et al.* (**2016**). Traumatic Extensor Tendon Injuries to the Hand: Clinical Anatomy, Biomechanics, and Surgical Procedure Review. *Journal of hand and microsurgery*. 8(1): 2-12. DOI:10.1055/s-0036-1572534

247 Wee T.C. & Simon N.G. (**2021**). Asymptomatic common extensor tendon pathology in patients with carpal tunnel syndrome. *Muscle Nerve*. 64(1): 64-69. DOI:10.1002/mus.27252

248 Clark J.M. & Harryman D.T.n. (**1992**). Tendons, ligaments, and capsule of the rotator cuff. Gross and microscopic anatomy. *JBJS*. 74(5): 713-725.

249 Nakajima T., Rokuuma N., Hamada K., Tomatsu T. & Fukuda H. (**1994**). Histologic and biomechanical characteristics of the supraspinatus tendon: Reference to rotator cuff tearing. *J Shoulder Elbow Surg*. 3(2): 79-87. DOI:10.1016/s1058-2746(09)80114-6

250 Tebaa E., Tantot J., Isaac-Pinet S. & Nove-Josserand L. (**2019**). Histologic characteristics of the subscapularis tendon from muscle to bone: reference to subscapularis lesions. *J Shoulder Elbow Surg*. 28(5): 959-965. DOI:10.1016/j.jse.2018.11.045

251 Maruvada S., Madrazo-Ibarra A. & Varacallo M. (**2024**). In: *StatPearls* (StatPearls Publishing, Treasure Island (FL)).

252 Arrillaga B., Miguel-Perez M., Moller I., Rubio L., Blasi J. *et al.* (**2024**). Human shoulder anatomy: new ultrasound, anatomical, and microscopic perspectives. *Anat Sci Int*. 99(3): 290-304. DOI:10.1007/s12565-024-00775-5

253 Terry G.C. & Chopp T.M. (**2000**). Functional anatomy of the shoulder. *J Athl Train*. 35(3): 248-255.

254 Teunis T., Lubberts B., Reilly B.T. & Ring D. (**2014**). A systematic review and pooled analysis of the prevalence of rotator cuff disease with increasing age. *J Shoulder Elbow Surg*. 23(12): 1913-1921. DOI:10.1016/j.jse.2014.08.001

255 Milgrom C., Schaffler M., Gilbert S. & van Holsbeeck M. (**1995**). Rotator-cuff changes in asymptomatic adults. The effect of age, hand dominance and gender. *J Bone Joint Surg Br*. 77(2): 296-298.

256 Glait S.A., Mahure S., Loomis C.A., Cammer M., Pham H. *et al.* (**2018**). Regional histologic differences in the long head of the biceps tendon following subpectoral biceps tenodesis in patients with rotator cuff tears and SLAP lesions. *Knee Surg Sports Traumatol Arthrosc*. 26(8): 2481-2489. DOI:10.1007/s00167-018-4839-0

257 Newport M.L., Blair W.F. & Steyers C.M. (**1990**). Long-term results of extensor tendon repair. *The Journal of Hand Surgery*. 15(6): 961-966. DOI:https://doi.org/10.1016/0363-5023(90)90024-L

258 Howard V. & Reed M.G. (**2005**). In: *Unbiased stereology : three-dimensional measurement in microscopy* (eds Howard V. & Reed M.G.) (QTP Publications / BIOS Scientific).

259 Ashton D.M., Blaker C.L., Hartnell N., Haubruck P., Hefferan S.A. *et al.* (**2023**). Challenging the Perceptions of Human Tendon Allografts: Influence of Donor Age, Sex, Height, and Tendon on Biomechanical Properties. *Am J Sports Med*. 51(3): 768-778. DOI:10.1177/03635465221143385

260 McCormick F., Nwachukwu B.U., Kim J. & Martin S.D. (**2013**). Autologous hamstring tendon used for revision of quadiceps tendon tears. *Orthopedics*. 36(4): e529-e532. DOI:10.3928/01477447-20130327-36

261 Sanders T.L., Maradit Kremers H., Bryan A.J., Larson D.R., Dahm D.L. *et al.* (**2016**). Incidence of Anterior Cruciate Ligament Tears and Reconstruction: A 21-Year Population-Based Study. *Am J Sports Med*. 44(6): 1502-1507. DOI:10.1177/0363546516629944

262 Buller L.T., Best M.J., Baraga M.G. & Kaplan L.D. (**2015**). Trends in Anterior Cruciate Ligament Reconstruction in the United States. *Orthop J Sports Med*. 3(1): 2325967114563664. DOI:10.1177/2325967114563664

263 Janssen K.W., Orchard J.W., Driscoll T.R. & van Mechelen W. (**2012**). High incidence and costs for anterior cruciate ligament reconstructions performed in Australia from 2003-2004 to 2007-2008: time for an anterior cruciate ligament register by Scandinavian model? *Scand J Med Sci Sports*. 22(4): 495-501. DOI:10.1111/j.1600-0838.2010.01253.x

264 Zbrojkiewicz D., Vertullo C. & Grayson J.E. (**2018**). Increasing rates of anterior cruciate ligament reconstruction in young Australians, 2000-2015. *Med J Aust*. 208(8): 354-358. DOI:10.5694/mja17.00974

265 Li F., Li B., Wang Q.M. & Wang J.H. (**2008**). Cell shape regulates collagen type I expression in human tendon fibroblasts. *Cell Motil Cytoskeleton*. 65(4): 332-341. DOI:10.1002/cm.20263

266 Thorpe C.T., Chaudhry S., Lei, II, Varone A., Riley G.P. *et al.* (**2015**). Tendon overload results in alterations in cell shape and increased markers of inflammation and matrix degradation. *Scand J Med Sci Sports*. 25(4): e381-391. DOI:10.1111/sms.12333

267 Weiss M., Unterhauser F.N. & Weiler A. (**2012**). Crimp frequency is strongly correlated to myofibroblast density in the human anterior cruciate ligament and its autologous tendon grafts. *Knee Surg Sports Traumatol Arthrosc*. 20(5): 889-895. DOI:10.1007/s00167-011-1644-4

268 Feo H.B., Biancalana A., Romero Nakagaki W., Aparecida de Aro A. & Gomes L. (**2019**). Morphological Alterations and Increased Gelatinase Activity in the Superficial Digital Flexor Tendon of Chickens During Growth and Maturation. *Anat Rec (Hoboken)*. 302(6): 964-972. DOI:10.1002/ar.24027

269 Hayashi K., Bhandal J., Kim S.Y., Walsh N., Entwistle R. *et al.* (**2019**). Comparative histomorphometric analysis of cellular phenotype in canine stifle ligaments and tendon. *Vet Surg*. 48(6): 1013-1018. DOI:10.1111/vsu.13227

270 Pardes A.M., Beach Z.M., Raja H., Rodriguez A.B., Freedman B.R. *et al.* (**2017**). Aging leads to inferior Achilles tendon mechanics and altered ankle function in rodents. *J Biomech*. 60(30-38. DOI:10.1016/j.jbiomech.2017.06.008

271 Dunkman A.A., Buckley M.R., Mienaltowski M.J., Adams S.M., Thomas S.J. *et al.* (**2013**). Decorin expression is important for age-related changes in tendon structure and mechanical properties. *Matrix Biol*. 32(1): 3-13. DOI:10.1016/j.matbio.2012.11.005

272 Thavarajah R., Mudimbaimannar V.K., Elizabeth J., Rao U.K. & Ranganathan K. (**2012**). Chemical and physical basics of routine formaldehyde fixation. *J Oral Maxillofac Pathol*. 16(3): 400-405. DOI:10.4103/0973-029X.102496

273 Taye N., Karoulias S.Z. & Hubmacher D. (**2020**). The "other" 15-40%: The Role of Non-Collagenous Extracellular Matrix Proteins and Minor Collagens in Tendon. *J Orthop Res*. 38(1): 23-35. DOI:10.1002/jor.24440

274 Ryan C.N., Sorushanova A., Lomas A.J., Mullen A.M., Pandit A. *et al.* (**2015**). Glycosaminoglycans in Tendon Physiology, Pathophysiology, and Therapy. *Bioconjug Chem*. 26(7): 1237-1251. DOI:10.1021/acs.bioconjchem.5b00091

275 Jung H.J., Fisher M.B. & Woo S.L. (**2009**). Role of biomechanics in the understanding of normal, injured, and healing ligaments and tendons. *Sports Med Arthrosc Rehabil Ther Technol*. 1(1): 9. DOI:10.1186/1758-2555-1-9

276 Bobzin L., Roberts R.R., Chen H.J., Crump J.G. & Merrill A.E. (**2021**). Development and maintenance of tendons and ligaments. *Development*. 148(8). DOI:10.1242/dev.186916

277 Yahia L.-H. & Drouin G. (**1989**). Microscopical investigation of canine anterior cruciate ligament and patellar tendon: Collagen fascicle morphology and architecture. *J Orthop Res*. 7(2): 243-251. DOI:https://doi.org/10.1002/jor.1100070212

278 O'Brien M. (**1992**). Functional Anatomy and Physiology of Tendons. *Clin Sports Med*. 11(3): 505-520. DOI:10.1016/s0278-5919(20)30505-6

279 Fratzl P. (**2008**). In: *Collagen - Structure and Mechanics* (ed Fratzl P.) 1-13 (Springer US).

280 Patel D., Zamboulis D.E., Spiesz E.M., Birch H.L., Clegg P.D. *et al.* (**2021**). Structure-function specialisation of the interfascicular matrix in the human achilles tendon. *Acta Biomater*. 131(381-390. DOI:10.1016/j.actbio.2021.07.019

281 Shah K., Solan M. & Dawe E. (**2020**). The gait cycle and its variations with disease and injury. *Orthopaedics and Trauma*. 34(3): 153-160. DOI:10.1016/j.mporth.2020.03.009

282 Falconiero R.P., DiStefano V.J. & Cook T.M. (**1998**). Revascularization and ligamentization of autogenous anterior cruciate ligament grafts in humans. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*. 14(2): 197-205. DOI:https://doi.org/10.1016/S0749-8063(98)70041-6

283 Sanchez M., Anitua E., Azofra J., Prado R., Muruzabal F. *et al.* (**2010**). Ligamentization of tendon grafts treated with an endogenous preparation rich in growth factors: gross morphology and histology. *Arthroscopy*. 26(4): 470-480. DOI:10.1016/j.arthro.2009.08.019

284 Lane J.G., McFadden P., Bowden K. & Amiel D. (**1993**). The ligamentization process: A 4 year case study following ACL reconstruction with a semitendinosis graft. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*. 9(2): 149-153. DOI:https://doi.org/10.1016/S0749-8063(05)80364-0

285 Stener S., Ejerhed L., Movin T., Sernert N., Papadogiannakis N. *et al.* (**2012**). The reharvested patellar tendon has the potential for ligamentization when used for anterior cruciate ligament revision surgery. *Knee Surg Sports Traumatol Arthrosc*. 20(6): 1168-1174. DOI:10.1007/s00167-012-1908-7

286 Claes S., Verdonk P., Forsyth R. & Bellemans J. (**2011**). The "ligamentization" process in anterior cruciate ligament reconstruction: what happens to the human graft? A systematic review of the literature. *Am J Sports Med*. 39(11): 2476-2483. DOI:10.1177/0363546511402662

287 Janssen R.P., van der Wijk J., Fiedler A., Schmidt T., Sala H.A. *et al.* (**2011**). Remodelling of human hamstring autografts after anterior cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthrosc*. 19(8): 1299-1306. DOI:10.1007/s00167-011-1419-y

288 Mayr H.O., Stoehr A., Dietrich M., von Eisenhart-Rothe R., Hube R. *et al.* (**2012**). Graft-dependent differences in the ligamentization process of anterior cruciate ligament grafts in a sheep trial. *Knee Surg Sports Traumatol Arthrosc*. 20(5): 947-956. DOI:10.1007/s00167-011-1678-7

289 Toumi H., Higashiyama I., Suzuki D., Kumai T., Bydder G. *et al.* (**2006**). Regional variations in human patellar trabecular architecture and the structure of the proximal patellar tendon enthesis. *J Anat*. 208(1): 47-57. DOI:10.1111/j.1469-7580.2006.00501.x

290 Theobald P., Bydder G., Dent C., Nokes L., Pugh N. *et al.* (**2006**). The functional anatomy of Kager's fat pad in relation to retrocalcaneal problems and other hindfoot disorders. *J Anat*. 208(1): 91-97. DOI:10.1111/j.1469-7580.2006.00510.x

291 Yuksel Y., Ergun T., Torun E., Unal M., Sonnow L. *et al.* (**2021**). The relationship between quadriceps fat pad syndrome and patellofemoral morphology: a case-control study. *J Orthop Traumatol*. 22(1): 17. DOI:10.1186/s10195-021-00580-0

292 Lee J.-H., Kim K.-J., Jeong Y.-G., Lee N.S., Han S.Y. *et al.* (**2014**). Pes anserinus and anserine bursa: anatomical study. *Anat Cell Biol*. 47(2). DOI:10.5115/acb.2014.47.2.127

293 Leese J. & Davies D.C. (**2020**). An investigation of the anatomy of the infrapatellar fat pad and its possible involvement in anterior pain syndrome: a cadaveric study. *J Anat*. 237(1): 20-28. DOI:10.1111/joa.13177

294 Benjamin M. (**2004**). Adipose tissue at entheses: the rheumatological implications of its distribution. A potential site of pain and stress dissipation? *Ann Rheum Dis*. 63(12): 1549-1555. DOI:10.1136/ard.2003.019182

295 Slane L.C., Dandois F., Bogaerts S., Vandenneucker H. & Scheys L. (**2018**). Non-uniformity in the healthy patellar tendon is greater in males and similar in different age groups. *J Biomech*. 80(16-22. DOI:10.1016/j.jbiomech.2018.08.021

296 Dan M., Parr W., Broe D., Cross M. & Walsh W.R. (**2018**). Biomechanics of the knee extensor mechanism and its relationship to patella tendinopathy: A review. *J Orthop Res*. 36(12): 3105-3112. DOI:10.1002/jor.24120

297 Mochizuki T., Akita K., Muneta T. & Sato T. (**2003**). Pes anserinus: Layered supportive structure on the medial side of the knee. *Clin Anat*. 17(1): 50-54. DOI:10.1002/ca.10142

298 Curtis B.R., Huang B.K., Pathria M.N., Resnick D.L. & Smitaman E. (**2019**). Pes Anserinus: Anatomy and Pathology of Native and Harvested Tendons. *AJR Am J Roentgenol*. 213(5): 1107-1116. DOI:10.2214/AJR.19.21315

299 Steinmann S., Pfeifer C.G., Brochhausen C. & Docheva D. (**2020**). Spectrum of Tendon Pathologies: Triggers, Trails and End-State. *Int J Mol Sci*. 21(3). DOI:10.3390/ijms21030844

300 Jarvinen M., Jozsa L., Kannus P., Jarvinen T.L., Kvist M. *et al.* (**1997**). Histopathological findings in chronic tendon disorders. *Scand J Med Sci Sports*. 7(2): 86-95. DOI:10.1111/j.1600-0838.1997.tb00124.x

301 Kannus P., Paavola M. & Józsa L. (**2005**). In: *Tendon Injuries: Basic Science and Clinical Medicine* (eds Maffulli N. *et al.*) 25-31 (Springer London).

302 Klatte-Schulz F., Minkwitz S., Schmock A., Bormann N., Kurtoglu A. *et al.* (**2018**). Different Achilles Tendon Pathologies Show Distinct Histological and Molecular Characteristics. *Int J Mol Sci*. 19(2). DOI:10.3390/ijms19020404

303 Fjordbakk C.T. & Marques-Smith P. (**2023**). The equine patellar ligaments and the infrapatellar fat pad - a microanatomical study. *BMC Vet Res*. 19(1): 20. DOI:10.1186/s12917-023-03579-3

304 Buckley M.R., Sarver J.J., Freedman B.R. & Soslowsky L.J. (**2013**). The dynamics of collagen uncrimping and lateral contraction in tendon and the effect of ionic concentration. *J Biomech*. 46(13): 2242-2249. DOI:10.1016/j.jbiomech.2013.06.029

305 Patterson-Kane J.C., Parry D.A., Goodship A.E. & Firth E.C. (**1997**). Exercise modifies the age-related change in crimp pattern in the core region of the equine superficial digital flexor tendon. *N Z Vet J*. 45(4): 135-139. DOI:10.1080/00480169.1997.36013

306 Miller K.S., Connizzo B.K., Feeney E., Tucker J.J. & Soslowsky L.J. (**2012**). Examining differences in local collagen fiber crimp frequency throughout mechanical testing in a developmental mouse supraspinatus tendon model. *J Biomech Eng*. 134(4): 041004. DOI:10.1115/1.4006538

307 Zhao L., Thambyah A. & Broom N. (**2015**). Crimp morphology in the ovine anterior cruciate ligament. *J Anat*. 226(3): 278-288. DOI:10.1111/joa.12276

308 Hefferan S.A., Blaker C.L., Ashton D.M., Clarke E.C. & Little C.B. (**2021**). In: *ORS 2021 Annual Meeting.* (Orthopaedic Research Scociety, 2021).

309 Bartolomei S., Grillone G., Di Michele R. & Cortesi M. (**2021**). A Comparison between Male and Female Athletes in Relative Strength and Power Performances. *J Funct Morphol Kinesiol*. 6(1). DOI:10.3390/jfmk6010017

310 Romanini E., D'Angelo F., De Masi S., Adriani E., Magaletti M. *et al.* (**2010**). Graft selection in arthroscopic anterior cruciate ligament reconstruction. *J Orthop Traumatol*. 11(4): 211-219. DOI:10.1007/s10195-010-0124-9

311 Lu H., Chen C., Xie S., Tang Y. & Qu J. (**2019**). Tendon Healing in Bone Tunnel after Human Anterior Cruciate Ligament Reconstruction: A Systematic Review of Histological Results. *J Knee Surg*. 32(5): 454-462. DOI:10.1055/s-0038-1653964

312 Borchers J.R., Pedroza A. & Kaeding C. (**2009**). Activity level and graft type as risk factors for anterior cruciate ligament graft failure: a case-control study. *Am J Sports Med*. 37(12): 2362-2367. DOI:10.1177/0363546509340633

313 Widner M., Dunleavy M. & Lynch S. (**2019**). Outcomes Following ACL Reconstruction Based on Graft Type: Are all Grafts Equivalent? *Curr Rev Musculoskelet Med*. 12(4): 460-465. DOI:10.1007/s12178-019-09588-w

314 Wiggins A.J., Grandhi R.K., Schneider D.K., Stanfield D., Webster K.E. *et al.* (**2016**). Risk of Secondary Injury in Younger Athletes After Anterior Cruciate Ligament Reconstruction: A Systematic Review and Meta-analysis. *Am J Sports Med*. 44(7): 1861-1876. DOI:10.1177/0363546515621554

315 Wipfler B., Donner S., Zechmann C.M., Springer J., Siebold R. *et al.* (**2011**). Anterior cruciate ligament reconstruction using patellar tendon versus hamstring tendon: a prospective comparative study with 9-year follow-up. *Arthroscopy*. 27(5): 653-665. DOI:10.1016/j.arthro.2011.01.015

316 Christino M.A., Tepolt F.A., Sugimoto D., Micheli L.J. & Kocher M.S. (**2020**). Revision ACL Reconstruction in Children and Adolescents. *J Pediatr Orthop*. 40(3): 129-134. DOI:10.1097/bpo.0000000000001155

317 Duchman K.R., Lynch T.S. & Spindler K.P. (**2017**). Graft Selection in Anterior Cruciate Ligament Surgery: Who gets What and Why? *Clin Sports Med*. 36(1): 25-33. DOI:10.1016/j.csm.2016.08.013

318 Marumo K., Saito M., Yamagishi T. & Fujii K. (**2005**). The "ligamentization" process in human anterior cruciate ligament reconstruction with autogenous patellar and hamstring tendons: a biochemical study. *Am J Sports Med*. 33(8): 1166-1173. DOI:10.1177/0363546504271973

319 Rosas S., Mesa C. & Mesa F. (**2017**). The Abductor Pollicis Longus Tendon as an Alternative Graft in Hand Surgery. *J Hand Surg Am*. 42(3): e205-e208. DOI:10.1016/j.jhsa.2016.12.006

320 Bora F.W., Jr., Osterman A.L., Thomas V.J., Maitin E.C. & Polineni S. (**1987**). The treatment of ruptures of multiple extensor tendons at wrist level by a free tendon graft in the rheumatoid patient. *J Hand Surg Am*. 12(6): 1038-1040. DOI:10.1016/s0363-5023(87)80107-7

321 Iwaasa T., Takahashi T., Tensho K., Koyama S., Takeshita K. *et al.* (**2023**). Suture Augmentation Does Not Change Biomechanical Properties and Histological Remodeling of Tendon Graft in Anterior Cruciate Ligament Reconstruction: A Study in a Porcine Model. *Arthroscopy*. 39(4): 1014-1024. DOI:10.1016/j.arthro.2022.10.049

322 Longo U.G., Lamberti A., Maffulli N. & Denaro V. (**2010**). Tendon augmentation grafts: a systematic review. *Br Med Bull*. 94(165-188. DOI:10.1093/bmb/ldp051

323 Lovati A.B., Bottagisio M. & Moretti M. (**2016**). Decellularized and Engineered Tendons as Biological Substitutes: A Critical Review. *Stem Cells Int*. 2016(7276150. DOI:10.1155/2016/7276150

324 Malige A., Baghdadi S., Hast M.W., Schmidt E.C., Shea K.G. *et al.* (**2022**). Biomechanical properties of common graft choices for anterior cruciate ligament reconstruction: A systematic review. *Clin Biomech (Bristol, Avon)*. 95(105636. DOI:10.1016/j.clinbiomech.2022.105636

325 Schmidt E.C., Chin M., Aoyama J.T., Ganley T.J., Shea K.G. *et al.* (**2019**). Mechanical and Microstructural Properties of Pediatric Anterior Cruciate Ligaments and Autograft Tendons Used for Reconstruction. *Orthop J Sports Med*. 7(1): 2325967118821667. DOI:10.1177/2325967118821667

326 Williams I.F., McCullagh K.G. & Silver I.A. (**1984**). The distribution of types I and III collagen and fibronectin in the healing equine tendon. *Connect Tissue Res*. 12(3-4): 211-227. DOI:10.3109/03008208409013684

327 Webbon P.M. (**1978**). A histological study of macroscopically normal equine digital flexor tendons. *Equine Vet J*. 10(4): 253-259. DOI:10.1111/j.2042-3306.1978.tb02275.x

328 Thorpe C.T., Udeze C.P., Birch H.L., Clegg P.D. & Screen H.R. (**2013**). Capacity for sliding between tendon fascicles decreases with ageing in injury prone equine tendons: a possible mechanism for age-related tendinopathy? *Eur Cell Mater*. 25(48-60. DOI:10.22203/ecm.v025a04

329 Birch H.L., Rutter G.A. & Goodship A.E. (**1997**). Oxidative energy metabolism in equine tendon cells. *Res Vet Sci*. 62(2): 93-97. DOI:https://doi.org/10.1016/S0034-5288(97)90127-2

330 Birch H.L., Bailey J.V.B., Bailey A.J. & Goodship A.E. (**1999**). Age‐related changes to the molecular and cellular components of equine flexor tendons. *Equine Vet J*. 31(5): 391-396. DOI:10.1111/j.2042-3306.1999.tb03838.x

331 Taqi S.A., Sami S.A., Sami L.B. & Zaki S.A. (**2018**). A review of artifacts in histopathology. *J Oral Maxillofac Pathol*. 22(2): 279. DOI:10.4103/jomfp.JOMFP_125_15

332 Choi R.K., Smith M.M., Martin J.H., Clarke J.L., Dart A.J. *et al.* (**2016**). Chondroitin sulphate glycosaminoglycans contribute to widespread inferior biomechanics in tendon after focal injury. *J Biomech*. 49(13): 2694-2701. DOI:10.1016/j.jbiomech.2016.06.006

333 Lee N.N., Salzer E., Bach F.C., Bonilla A.F., Cook J.L. *et al.* (**2021**). A comprehensive tool box for large animal studies of intervertebral disc degeneration. *JOR Spine*. 4(2): e1162. DOI:10.1002/jsp2.1162

334 Junqueira L.C., Montes G.S. & Sanchez E.M. (**1982**). The influence of tissue section thickness on the study of collagen by the Picrosirius-polarization method. *Histochemistry*. 74(1): 153-156. DOI:10.1007/bf00495061

335 Borges L.F., Gutierrez P.S., Marana H.R. & Taboga S.R. (**2007**). Picrosirius-polarization staining method as an efficient histopathological tool for collagenolysis detection in vesical prolapse lesions. *Micron*. 38(6): 580-583. DOI:10.1016/j.micron.2006.10.005

336 Frizziero A., Fini M., Salamanna F., Veicsteinas A., Maffulli N. *et al.* (**2011**). Effect of training and sudden detraining on the patellar tendon and its enthesis in rats. *BMC Musculoskelet Disord*. 12(20. DOI:10.1186/1471-2474-12-20

337 Fernandez-Sarmiento J.A., Dominguez J.M., Granados M.M., Morgaz J., Navarrete R. *et al.* (**2013**). Histological study of the influence of plasma rich in growth factors (PRGF) on the healing of divided Achilles tendons in sheep. *J Bone Joint Surg Am*. 95(3): 246-255. DOI:10.2106/JBJS.K.01659

338 Fritz T., Ducommun P., Pohlemann T., Calcagni M., Tschernig T. *et al.* (**2020**). Flexor tendon grafts for pulley reconstruction - Morphological aspects. *Ann Anat*. 231(151550. DOI:10.1016/j.aanat.2020.151550

339 Young M.M., Bullock J.B. & Lecy J.D. (**2019**). Artificial Discretion as a Tool of Governance: A Framework for Understanding the Impact of Artificial Intelligence on Public Administration. *Perspectives on Public Management and Governance*. DOI:10.1093/ppmgov/gvz014

340 Bullock J.B. (**2019**). Artificial Intelligence, Discretion, and Bureaucracy. *The American Review of Public Administration*. 49(7): 751-761. DOI:10.1177/0275074019856123

341 Greeley C., Holder L., Nilsson E.E. & Skinner M.K. (**2024**). Scalable deep learning artificial intelligence histopathology slide analysis and validation. *Sci Rep*. 14(1): 26748. DOI:10.1038/s41598-024-76807-x

342 Mannas M.P., Deng F.M., Ion-Margineanu A., Freudiger C., Jones D. *et al.* (**2025**). Intraoperative margin assessment with near real time pathology during partial gland ablation of prostate cancer: A feasibility study. *Urol Oncol*. 43(1): 64 e19-64 e25. DOI:10.1016/j.urolonc.2024.06.023

343 Faust K., Xie Q., Han D., Goyle K., Volynskaya Z. *et al.* (**2018**). Visualizing histopathologic deep learning classification and anomaly detection using nonlinear feature space dimensionality reduction. *BMC Bioinformatics*. 19(1): 173. DOI:10.1186/s12859-018-2184-4

344 Morozov A., Taratkin M., Bazarkin A., Rivas J.G., Puliatti S. *et al.* (**2023**). A systematic review and meta-analysis of artificial intelligence diagnostic accuracy in prostate cancer histology identification and grading. *Prostate Cancer Prostatic Dis*. 26(4): 681-692. DOI:10.1038/s41391-023-00673-3

345 Cannarozzi A.L., Massimino L., Latiano A., Parigi T.L., Giuliani F. *et al.* (**2024**). Artificial intelligence: A new tool in the pathologist's armamentarium for the diagnosis of IBD. *Comput Struct Biotechnol J*. 23(3407-3417. DOI:10.1016/j.csbj.2024.09.003

346 Arganda-Carreras I., Kaynig V., Rueden C., Eliceiri K.W., Schindelin J. *et al.* (**2017**). Trainable Weka Segmentation: a machine learning tool for microscopy pixel classification. *Bioinformatics*. 33(15): 2424-2426. DOI:10.1093/bioinformatics/btx180

347 Du Y. (**2023**). In: *2023 2nd International Conference on Data Analytics, Computing and Artificial Intelligence (ICDACAI)* 692-697 (

348 Hasan S.N.S., Jamil N.W. & Ahmat H. (**2023**). In: *WOMEN IN PHYSICS: 7th IUPAP International Conference on Women in Physics* (

349 Ayesha S., Hanif M.K. & Talib R. (**2020**). Overview and comparative study of dimensionality reduction techniques for high dimensional data. *Information Fusion*. 59(44-58. DOI:10.1016/j.inffus.2020.01.005

350 Ray P., Reddy S.S. & Banerjee T. (**2021**). Various dimension reduction techniques for high dimensional data analysis: a review. *Artificial Intelligence Review*. 54(5): 3473-3515. DOI:10.1007/s10462-020-09928-0

351 Hu W., Li H., Wang C., Gou S. & Fu L. (**2012**). Characterization of collagen fibers by means of texture analysis of second harmonic generation images using orientation-dependent gray level co-occurrence matrix method. *J Biomed Opt*. 17(2): 026007. DOI:10.1117/1.JBO.17.2.026007

352 Henninger H.B., Valdez W.R., Scott S.A. & Weiss J.A. (**2015**). Elastin governs the mechanical response of medial collateral ligament under shear and transverse tensile loading. *Acta Biomater*. 25(304-312. DOI:10.1016/j.actbio.2015.07.011

353 Zitnay J.L. & Weiss J.A. (**2018**). Load transfer, damage, and failure in ligaments and tendons. *J Orthop Res*. 36(12): 3093-3104. DOI:10.1002/jor.24134

354 Kalson N.S., Malone P.S., Bradley R.S., Withers P.J. & Lees V.C. (**2012**). Fibre bundles in the human extensor carpi ulnaris tendon are arranged in a spiral. *J Hand Surg Eur Vol*. 37(6): 550-554. DOI:10.1177/1753193411433228

355 Franchi M., Quaranta M., Macciocca M., De Pasquale V., Ottani V. *et al.* (**2009**). Structure relates to elastic recoil and functional role in quadriceps tendon and patellar ligament. *Micron*. 40(3): 370-377. DOI:10.1016/j.micron.2008.10.004

356 Slane L.C. & Thelen D.G. (**2015**). Achilles tendon displacement patterns during passive stretch and eccentric loading are altered in middle-aged adults. *Med Eng Phys*. 37(7): 712-716. DOI:10.1016/j.medengphy.2015.04.004

357 Stanley R.L., Goodship A.E., Edwards B., Firth E.C. & Patterson-Kane J.C. (**2008**). Effects of exercise on tenocyte cellularity and tenocyte nuclear morphology in immature and mature equine digital tendons. *Equine Vet J*. 40(2): 141-146. DOI:10.2746/042516408X266097

358 Ippolito E., Natali P.G., Postacchini F., Accinni L. & De Martino C. (**1980**). Morphological, immunochemical, and biochemical study of rabbit achilles tendon at various ages. *J Bone Joint Surg Am*. 62(4): 583-598.

359 Petersen W., Hohmann G., Pufe T., Tsokos M., Zantop T. *et al.* (**2004**). Structure of the human tibialis posterior tendon. *Arch Orthop Trauma Surg*. 124(4): 237-242. DOI:10.1007/s00402-003-0500-5

360 Riggin C.N., Morris T.R. & Soslowsky L.J. (**2015**). In: *Tendon Regeneration* (eds Gomes M.E. *et al.*) 149-183.

361 Riley G.P., Harrall R.L., Constant C.R., Chard M.D., Cawston T.E. *et al.* (**1994**). Glycosaminoglycans of human rotator cuff tendons: changes with age and in chronic rotator cuff tendinitis. *Ann Rheum Dis*. 53(6): 367-376. DOI:10.1136/ard.53.6.367

362 Takahashi N., Hirose T., Minaguchi J.A., Ueda H., Tangkawattana P. *et al.* (**2018**). Fibrillar architecture at three different sites of the bovine superficial digital flexor tendon. *J Vet Med Sci*. 80(3): 405-412. DOI:10.1292/jvms.17-0562

363 Chuen F.S., Chuk C.Y., Ping W.Y., Nar W.W., Kim H.L. *et al.* (**2004**). Immunohistochemical characterization of cells in adult human patellar tendons. *J Histochem Cytochem*. 52(9): 1151-1157. DOI:10.1369/jhc.3A6232.2004

364 Maffulli N., Cuozzo F., Migliorini F. & Oliva F. (**2023**). The tendon unit: biochemical, biomechanical, hormonal influences. *J Orthop Surg Res*. 18(1). DOI:10.1186/s13018-023-03796-4

365 Steffen D., Mienaltowski M. & Baar K. (**2023**). Spatial gene expression in the adult rat patellar tendon. *Matrix Biol Plus*. 19-20(100138. DOI:10.1016/j.mbplus.2023.100138

366 Fu W., Yang R. & Li J. (**2023**). Single-cell and spatial transcriptomics reveal changes in cell heterogeneity during progression of human tendinopathy. *BMC Biol*. 21(1): 132. DOI:10.1186/s12915-023-01613-2

367 Rees J., Wilson A. & Wolman R. (**2006**). Current concepts in the management of tendon disorders. *Rheumatology (Oxford)*. 45(508-521. DOI:10.1093/rheumatology/kel046

368 Woo S.L., Gomez M.A., Amiel D., Ritter M.A., Gelberman R.H. *et al.* (**1981**). The effects of exercise on the biomechanical and biochemical properties of swine digital flexor tendons. *J Biomech Eng*. 103(1): 51-56. DOI:10.1115/1.3138246

369 Murray M.M. & Spector M. (**1999**). Fibroblast distribution in the anteromedial bundle of the human anterior cruciate ligament: the presence of alpha-smooth muscle actin-positive cells. *J Orthop Res*. 17(1): 18-27. DOI:10.1002/jor.1100170105

370 Herchenhan A., Kalson N.S., Holmes D.F., Hill P., Kadler K.E. *et al.* (**2012**). Tenocyte contraction induces crimp formation in tendon-like tissue. *Biomech Model Mechanobiol*. 11(3-4): 449-459. DOI:10.1007/s10237-011-0324-0

371 Silver F.H., Freeman J.W. & Seehra G.P. (**2003**). Collagen self-assembly and the development of tendon mechanical properties. *J Biomech*. 36(10): 1529-1553. DOI:10.1016/s0021-9290(03)00135-0

372 Thorpe C.T., Klemt C., Riley G.P., Birch H.L., Clegg P.D. *et al.* (**2013**). Helical sub-structures in energy-storing tendons provide a possible mechanism for efficient energy storage and return. *Acta Biomater*. 9(8): 7948-7956. DOI:10.1016/j.actbio.2013.05.004

373 Lee A.H. & Elliott D.M. (**2019**). Comparative multi-scale hierarchical structure of the tail, plantaris, and Achilles tendons in the rat. *J Anat*. 234(2): 252-262. DOI:10.1111/joa.12913

374 Johnson J., von Stade D., Gadomski B., Regan D., Easley J. *et al.* (**2023**). Biomechanical and histological changes secondary to aging in the human rotator cuff: A preliminary analysis. *J Orthop Res*. 41(10): 2221-2231. DOI:10.1002/jor.25529

375 Freedman B.R., Sarver J.J., Buckley M.R., Voleti P.B. & Soslowsky L.J. (**2014**). Biomechanical and structural response of healing Achilles tendon to fatigue loading following acute injury. *J Biomech*. 47(9): 2028-2034. DOI:10.1016/j.jbiomech.2013.10.054

376 Loren G.J. & Lieber R.L. (**1995**). Tendon biomechanical properties enhance human wrist muscle specialization. *J Biomech*. 28(7): 791-799. DOI:https://doi.org/10.1016/0021-9290(94)00137-S

377 Noyes R.F., Butler L.D., Grood S.E., Zernicke F.R. & Hefzy S.M. (**1984**). Biomechanical analysis of human ligament grafts used in knee-ligament repairs and reconstructions. *The Journal of Bone & Joint Surgery*. 66(3): 344-352. DOI:10.2106/JBJS.F.00538

378 Zhu J., Zhang X., Ma Y., Zhou C. & Ao Y. (**2012**). Ultrastructural and morphological characteristics of human anterior cruciate ligament and hamstring tendons. *Anat Rec (Hoboken)*. 295(9): 1430-1436. DOI:10.1002/ar.22527

379 Orchard J.W., Engebretsen L. & Feller J.A. (**2018**). The rate of anterior cruciate ligament reconstruction in Australia is high: a national registry is needed. *Med J Aust*. 208(8): 341-342. DOI:10.5694/mja18.00095

380 Chao T., Yin K.L., Herbst E., Marx R.G. & Musahl V. (**2017**). In: *Controversies in the Technical Aspects of ACL Reconstruction: An Evidence-Based Medicine Approach* (eds Nakamura N. *et al.*) 325-331 (Springer Berlin Heidelberg).

381 Shaerf D.A., Pastides P.S., Sarraf K.M. & Willis-Owen C.A. (**2014**). Anterior cruciate ligament reconstruction best practice: A review of graft choice. *World J Orthop*. 5(1): 23-29. DOI:10.5312/wjo.v5.i1.23

382 Thorpe C.T., Riley G.P., Birch H.L., Clegg P.D. & Screen H.R. (**2014**). Effect of fatigue loading on structure and functional behaviour of fascicles from energy-storing tendons. *Acta Biomater*. 10(7): 3217-3224. DOI:10.1016/j.actbio.2014.04.008

383 Wolff J. (**1893**). Das Gesetz der Transformation der Knochen. *Dtsch Med Wochenschr*. 19(1222 - 1224.

384 Australian Bureau of Statistics A. (**2020**). *3101.0 - Australian Demographic Statistics, Hun 2019: Twenty years of population change*. available at: https://www.abs.gov.au/ausstats/abs@.nsf/0/1CD2B1952AFC5E7ACA257298000F2E76?OpenDocument

385 World Health Organisation W. (**2021**). *Ageing and Health*. available at: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health

386 Choi R., Smith M., Clarke E. & Little C. (**2018**). Cellular, matrix, and mechano-biological differences in load-bearing versus positional tendons throughout development and aging: a narrative review. *Connect Tissue Res*. 59(5): 483-494. DOI:10.1080/03008207.2018.1504929

387 Jozsa L., Kvist M., Balint B.J., Reffy A., Jarvinen M. *et al.* (**1989**). The role of recreational sport activity in Achilles tendon rupture. A clinical, pathoanatomical, and sociological study of 292 cases. *Am J Sports Med*. 17(3): 338-343. DOI:10.1177/036354658901700305

388 Irger M., Willinger L., Lacheta L., Pogorzelski J., Imhoff A.B. *et al.* (**2020**). Proximal hamstring tendon avulsion injuries occur predominately in middle-aged patients with distinct gender differences: epidemiologic analysis of 263 surgically treated cases. *Knee Surg Sports Traumatol Arthrosc*. 28(4): 1221-1229. DOI:10.1007/s00167-019-05717-7

389 Scott A., Grewal N. & Guy P. (**2014**). The seasonal variation of Achilles tendon ruptures in Vancouver, Canada: a retrospective study. *BMJ Open*. 4(2): e004320. DOI:10.1136/bmjopen-2013-004320

390 Mondini Trissino da Lodi C., Landini M.P., Asunis E. & Filardo G. (**2022**). Women Have Tendons... and Tendinopathy: Gender Bias is a "Gender Void" in Sports Medicine with a Lack of Women Data on Patellar Tendinopathy-A Systematic Review. *Sports Med Open*. 8(1): 74. DOI:10.1186/s40798-022-00455-6

391 Sarver D.C., Kharaz Y.A., Sugg K.B., Gumucio J.P., Comerford E. *et al.* (**2017**). Sex differences in tendon structure and function. *J Orthop Res*. 35(10): 2117-2126. DOI:10.1002/jor.23516

392 Stenroth L., Peltonen J., Cronin N.J., Sipila S. & Finni T. (**2012**). Age-related differences in Achilles tendon properties and triceps surae muscle architecture in vivo. *J Appl Physiol (1985)*. 113(10): 1537-1544. DOI:10.1152/japplphysiol.00782.2012

393 Hashemi J., Chandrashekar N., Mansouri H., Slauterbeck J.R. & Hardy D.M. (**2008**). The human anterior cruciate ligament: sex differences in ultrastructure and correlation with biomechanical properties. *J Orthop Res*. 26(7): 945-950. DOI:10.1002/jor.20621

394 Chandrashekar N., Mansouri H., Slauterbeck J. & Hashemi J. (**2006**). Sex-based differences in the tensile properties of the human anterior cruciate ligament. *J Biomech*. 39(16): 2943-2950. DOI:10.1016/j.jbiomech.2005.10.031

395 Lemoine J.K., Lee J.D. & Trappe T.A. (**2009**). Impact of sex and chronic resistance training on human patellar tendon dry mass, collagen content, and collagen cross-linking. *Am J Physiol Regul Integr Comp Physiol*. 296(1): R119-124. DOI:10.1152/ajpregu.90607.2008

396 Fratzl P. (**2008**). In: *Collagen: Structure and Mechanics* (ed Fratzl P.) 1-13 (Springer US).

397 Lin Y.L., Brama P.A., Kiers G.H., van Weeren P.R. & DeGroot J. (**2005**). Extracellular matrix composition of the equine superficial digital flexor tendon: relationship with age and anatomical site. *J Vet Med A Physiol Pathol Clin Med*. 52(7): 333-338. DOI:10.1111/j.1439-0442.2005.00742.x

398 Berenson M.C., Blevins F.T., Plaas A.H. & Vogel K.G. (**1996**). Proteoglycans of human rotator cuff tendons. *J Orthop Res*. 14(4): 518-525. DOI:10.1002/jor.1100140404

399 Zhang C., Couppé C., Scheijen J.L.J.M., Schalkwijk C.G., Kjaer M. *et al.* (**2020**). Regional collagen turnover and composition of the human patellar tendon. *J Appl Physiol (1985)*. 128(4): 884-891. DOI:10.1152/japplphysiol.00030.2020

400 Curwin S.L., Roy R.R. & Vailas A.C. (**1994**). Regional and age variations in growing tendon. *J Morphol*. 221(3): 309-320. DOI:10.1002/jmor.1052210306

401 Williams L.N., Elder S.H., Horstemeyer M.F. & Harbarger D. (**2008**). Variation of diameter distribution, number density, and area fraction of fibrils within five areas of the rabbit patellar tendon. *Ann Anat*. 190(5): 442-451. DOI:10.1016/j.aanat.2008.05.003

402 Slone H.S., Romine S.E., Premkumar A. & Xerogeanes J.W. (**2015**). Quadriceps tendon autograft for anterior cruciate ligament reconstruction: a comprehensive review of current literature and systematic review of clinical results. *Arthroscopy*. 31(3): 541-554. DOI:10.1016/j.arthro.2014.11.010

403 Bojsen-Moller J. & Magnusson S.P. (**2015**). Heterogeneous Loading of the Human Achilles Tendon In Vivo. *Exerc Sport Sci Rev*. 43(4): 190-197. DOI:10.1249/JES.0000000000000062

404 Wolfram S., Hodson-Tole E.F., Morse C.I., Winwood K.L. & McEwan I.M. (**2020**). Elongation differences between the sub-tendons of gastrocnemius medialis and lateralis during plantarflexion in different frontal plane position of the foot. *Gait Posture*. 75(149-154. DOI:10.1016/j.gaitpost.2019.10.020

405 Hansen P., Haraldsson B.T., Aagaard P., Kovanen V., Avery N.C. *et al.* (**2010**). Lower strength of the human posterior patellar tendon seems unrelated to mature collagen cross-linking and fibril morphology. *J Appl Physiol (1985)*. 108(1): 47-52. DOI:10.1152/japplphysiol.00944.2009

406 Khan K.M., Bonar F., Desmond P.M., Cook J.L., Young D.A. *et al.* (**1996**). Patellar tendinosis (jumper's knee): findings at histopathologic examination, US, and MR imaging. Victorian Institute of Sport Tendon Study Group. *Radiology*. 200(3): 821-827. DOI:10.1148/radiology.200.3.8756939

407 Kraus B.L., Kirker-Head C.A., Kraus K.H., Jakowski R.M. & Steckel R.R. (**1995**). Vascular supply of the tendon of the equine deep digital flexor muscle within the digital sheath. *Vet Surg*. 24(2): 102-111. DOI:10.1111/j.1532-950x.1995.tb01303.x

408 Birch H.L., Bailey A.J. & Goodship A.E. (**1998**). Macroscopic 'degeneration' of equine superficial digital flexor tendon is accompanied by a change in extracellular matrix composition. *Equine Vet J*. 30(6): 534-539. DOI:10.1111/j.2042-3306.1998.tb04530.x

409 Birch H.L., Wilson A.M. & Goodship A.E. (**1997**). The effect of exercise-induced localised hyperthermia on tendon cell survival. *J Exp Biol*. 200(Pt 11): 1703-1708. DOI:10.1242/jeb.200.11.1703

410 Wilson A.M. & Goodship A.E. (**1994**). Exercise-induced hyperthermia as a possible mechanism for tendon degeneration. *J Biomech*. 27(7): 899-905. DOI:https://doi.org/10.1016/0021-9290(94)90262-3

411 Patterson-Kane J.C., Firth E.C., Goodship A.E. & Parry D.A. (**1997**). Age-related differences in collagen crimp patterns in the superficial digital flexor tendon core region of untrained horses. *Aust Vet J*. 75(1): 39-44. DOI:10.1111/j.1751-0813.1997.tb13829.x

412 Gibson K.T., Burbidge H.M. & Robertson I.D. (**2002**). The effects of polyester (terylene) fibre implants on normal equine superficial digital flexor tendon. *N Z Vet J*. 50(5): 186-194. DOI:10.1080/00480169.2002.36309

413 Farfaras S., Ejerhed L.E., Hallström E.K., Hultenby K., Meknas K. *et al.* (**2018**). More histologic and ultrastructural degenerative signs in the subscapularis tendon and the joint capsule in male patients with shoulder impingement. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA*. 26(1): 79-87. DOI:10.1007/s00167-017-4442-9

414 Blaker C.L., Ashton D.M., Hartnell N., Little C.B. & Clarke E.C. (**2024**). Tendon biomechanical properties are altered by storage duration but not freeze-thaw temperatures or cycles. *J Orthop Res*. 42(6): 1180-1189. DOI:10.1002/jor.25783

415 Huang H., Zhang J., Sun K., Zhang X. & Tian S. (**2011**). Effects of repetitive multiple freeze-thaw cycles on the biomechanical properties of human flexor digitorum superficialis and flexor pollicis longus tendons. *Clin Biomech (Bristol)*. 26(4): 419-423. DOI:10.1016/j.clinbiomech.2010.12.006

416 Quirk N.P., Lopez De Padilla C., De La Vega R.E., Coenen M.J., Tovar A. *et al.* (**2018**). Effects of freeze-thaw on the biomechanical and structural properties of the rat Achilles tendon. *J Biomech*. 81(52-57. DOI:10.1016/j.jbiomech.2018.09.012

417 Shively S. & Miller W.R. (**2009**). The use of HMDS (hexamethyldisilazane) to Replace Critical Point Drying (CPD) in the Preparation of Tardigrades for SEM (Scanning Electron Microscope) Imaging. *Trans Kans Acad Sci*. 112(4): 198-200, 193.

418 Braet F., De Zanger R. & Wisse E. (**1997**). Drying cells for SEM, AFM and TEM by hexamethyldisilazane: a study on hepatic endothelial cells. *J Microsc*. 186(Pt 1): 84-87. DOI:10.1046/j.1365-2818.1997.1940755.x

419 Mehdizadeh Kashi A., Tahemanesh K., Chaichian S., Joghataei M.T., Moradi F. *et al.* (**2014**). How to Prepare Biological Samples and Live Tissues for Scanning Electron Microscopy (SEM). *Galen Medical Journal*. 3(2): 63-80. DOI:10.31661/gmj.v3i2.267

~ CHAPTER 9 ~

I'LL BE AT THE BACK

APPENDICES

"I think we can take them. Do you think we can take them?"

~ Batman, Superman/Batman: Public Enemies (2009) ~

9.1 Overview

The following chapter includes the appendix material referred to throughout this thesis.

9.2 Appendices for ~ Chapter 1 ~

9.2.1 Permissions for Image Inclusions (Figure 1.2.B)

The following section details the reprint permissions for the diagrams included in Figure 1.2.B:

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Image IX, Handsfield et al. (2016)[121]. License Number: 5894220174204. License date: Oct 23, 2024. Licensed Content Publisher: Elsevier.

9.3 Appendices for ~ Chapter 2 ~

9.3.1 Manuscript Draft

The following pages include the submitted version of the manuscript (pre amendments and proof) originally presented in *Chapter 2* of this thesis pre-proof. The manuscript has since been accepted (28th of January 2025), peer reviewed, revised and published (see *Chapter 2*).

Journal of Orthopaedic Research

Structural variations of tendons: A systematic search and narrative review of differences between tendons, regions within tendons, sex and age

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Keywords:	Ligament, Tendon, Collagen, Aging, Pathophysiology
Areas of Expertise:	Histology, Healthy/Normal, Tendon, Ligament, Tissue Architecture

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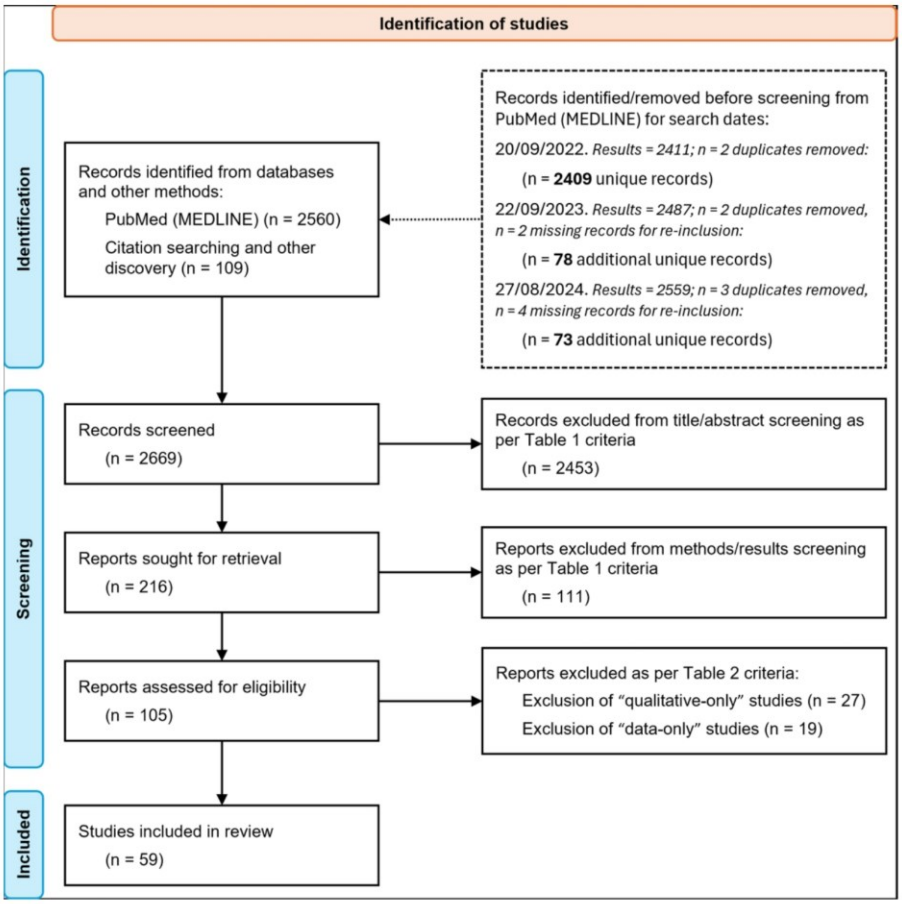

Figure 1. Flow diagram of literature inclusion and screening workflow. Diagram is modified from a readily accessible template[14].

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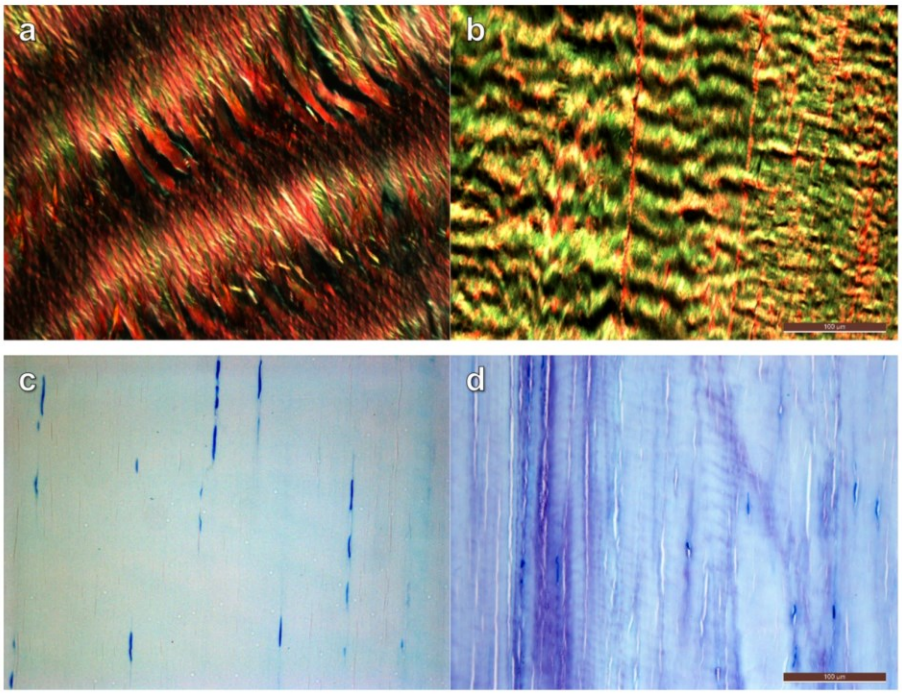

Figure 2. Light microscopy images showing variation in 'normal' human tendons. Histological samples are from cadaveric lower-leg tendons of female donors aged 71 (**a-b**) and 78 (**c-d**) years old. Polarised Picrosirius Red stained sections (**a-b**) depicting regional differences in crimp wavelength between matched proximal (**a**) and distal (**b**) regions of the Tibialis Anterior tendon. Toluidine Blue stained sections (**c-d**) depicting regional differences in proteoglycan content (purple staining) between matched proximal (**c**) and distal (**d**) regions of the Achilles tendon. *Longitudinally orientated, formalin-fixed, paraffin embedded histological sections at 4.5 µm thick. All images: 200x magnification; scale bars = 100µm.*

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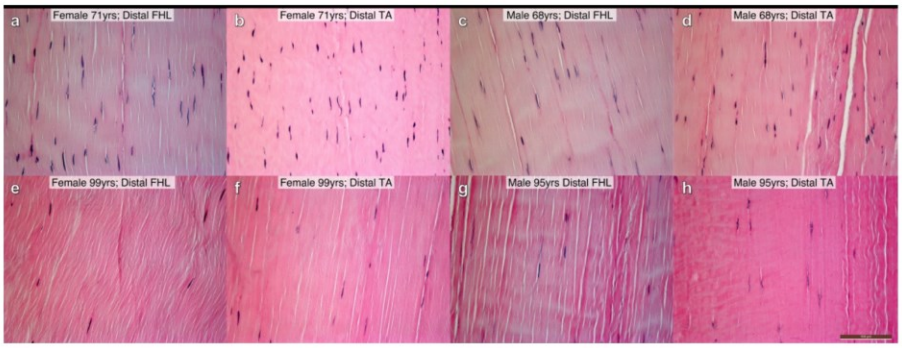

Figure 3. Light microscopy images showing cell density variation in 'normal' human tendons. Histological samples are from the distal region of cadaveric lower-leg Flexor Hallucis Longus (FHL; **a,c,e,g**) and Tibialis Anterior (TA; **b,d,f,h**) tendons. Female donors aged 71 (**a-b**) and 99 (**e-f**) years old, and male donors aged 68 (**c-d**) and 95 (**g-h**) years old. Note differences in relative cell density between younger (**a-d**) and older (**e-h**) donors. *Longitudinally orientated, formalin-fixed, paraffin embedded histological sections at 4.5 µm thick. All images: 200x magnification; scale bars = 100µm.*

599x226mm (300 x 300 DPI)

Textbox 1: Summary of knowledge gaps in the histological analysis of tendons.

Currently, there are limited studies which compare the multi-scale structural variations of normal (healthy) tendons. Further structural (e.g. histological, microscopy) investigations are needed in order to:

(1) Systematically compare variations between a wide range of different anatomical tendons,

(2) Characterize variations within tendons, e.g. within the longitudinal or cross-sectional planes, and to determine whether any such variations exist in a range of different tendons,

(3) Understand the effects of age on tendon structure, and the interaction between "normal" aging and pedantry lifestyle versus injury, pathology or disease.

(4) Compare the above outcomes between the sexes,

(5) Appreciate species-specific variations as well as other variables that may influence tendon structure, e.g. lifestyle, demographics, comorbidities etc.

(6) Understand how confounding factors might result in differences e.g. specific tendons exhibiting different regional, age or other variations.

(7) Appreciate how differing methodologies may influence research findings and translatability across the field.

Characteristics that have been identified as important and widely explored features of tendons, that should be considered for assessment in future tendon research include, but are not limited to:

(i) Cell populations (e.g. number/density, morphology etc.)

(ii) Compositional make-up (e.g. location, amount, spread etc. of different components)

(iii) Overall structural architecture (e.g. fibril/fiber/fascicle organization, collagen alignment, crimp patterns etc.)

Textbox 1. Summary of knowledge gaps in the histological analysis of tendons.

152x120mm (300 x 300 DPI)

Structural variations of tendons.

Structural variations of tendons: A systematic search and narrative review of differences between tendons, regions within tendons, sex and age

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Author contributions

The first author conducted the literature search described in this review. All authors contributed to the writing and preparation of this manuscript. There are no conflicts of interest to report. All authors have read and approved the final submitted manuscript.

Structural variations of tendons.

Abstract

Tendons are force-transmitting structures which facilitate musculoskeletal functioning. Characterising variations between different anatomical tendons, regions within tendons, as well as between the sexes and with age can improve understanding of tendon physiology and pathology. A systematic search of the literature was conducted to identify and summarise microscopic structural variations in normal/healthy tendons in relation to these variables (Tendon, Region, Age, Sex, and Other). Regional differences within individual tendons have been investigated in numerous studies, however investigations comparing structural variations between a range of different tendons are sparse, with most focusing on a few select tendons. When injured, ageing tendons typically have a greater degree of pathological changes than younger tendons, but few studies have documented variations in tendon histology throughout typical (un-injured) ageing or across large age spans. Similarly, sex-related observations of tendon structure are underreported. This review summarises studies on these topics and explores interactions between these variables, as well as the implications of these in the context of selecting control samples for studies of tendon pathology. Future studies should endeavour to improve knowledge of tendon structural variations – specifically focusing on normal tendons – to facilitate understanding of tendon structure-function relationships, physiological mechanisms involved in tendon damage/healing, and to aid clinical research and practice.

Keywords: histology; healthy/normal; tendon; ligament; tissue architecture

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Structural variations of tendons.

Introduction

Tendons are force transmitting units that facilitate muscular movement of bone and joints, playing an important role in biomechanical and locomotor function[1] contributing to musculoskeletal and overall well-being. Tendons are prone to injury, pathology and degenerative processes.[2-4] Despite the advent of different imaging modalities, histology remains a gold standard for assessing tissue health and disease.[5-11] Understanding normal tendon histological features is critical for gaining insight into the mechanisms of tendon damage, disease, degeneration, and repair.[12,13] While "healthy tendon" histology is often used as a baseline/control for studies of diseased tendons, knowledge about what constitutes "normal" histology and variations in multiscale aspects particularly of human tendons, are poorly explored. This review examines what is known and knowledge gaps on tendon structural characteristics assessed using microscopy modalities, with particular focus on variation between different tendons, tendon regions, ages, and sexes. A systematic literature search was conducted to identify published records which compared multiscale histological structural characteristics of normal/healthy tendons.

Systematic Literature Search: Structural variations of normal tendons

An electronic database search of PubMed (MEDLINE) was conducted with a focus on comparisons between 'variables of interest': *Tendon*; *Region*; *Age*; *Sex*; and *Other*. Keyword search terms were: (tendon OR tendons OR tend*) AND (histolog* OR histochem* OR microscop*) AND (normal OR healthy OR "non-pathological" OR "non pathological") AND (structu*) AND (compar* OR differen* OR variation OR variations). Supplementary Material A contains the full list of generated search terms and MeSH terms, indexed according to the terms

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list from the National Library of Medicine. The initial search (30th September 2022) found 2411 records, including 2 duplicate pairs (**2409** unique records). The search was re-run at two subsequent timepoints during manuscript preparation, using the full list of MeSH terms each time to ensure consistency: 22nd September 2023 (**78** new additional unique articles identified) and 27th August 2024 (**73** new additional unique articles identified). A final total of **2560** unique records were identified from the PubMed literature searches (Supplementary Table S-1, Figure 1). Cross-checking of citations/reference-lists and other publication discovery identifying an additional **109** records for review.

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Structural variations of tendons.

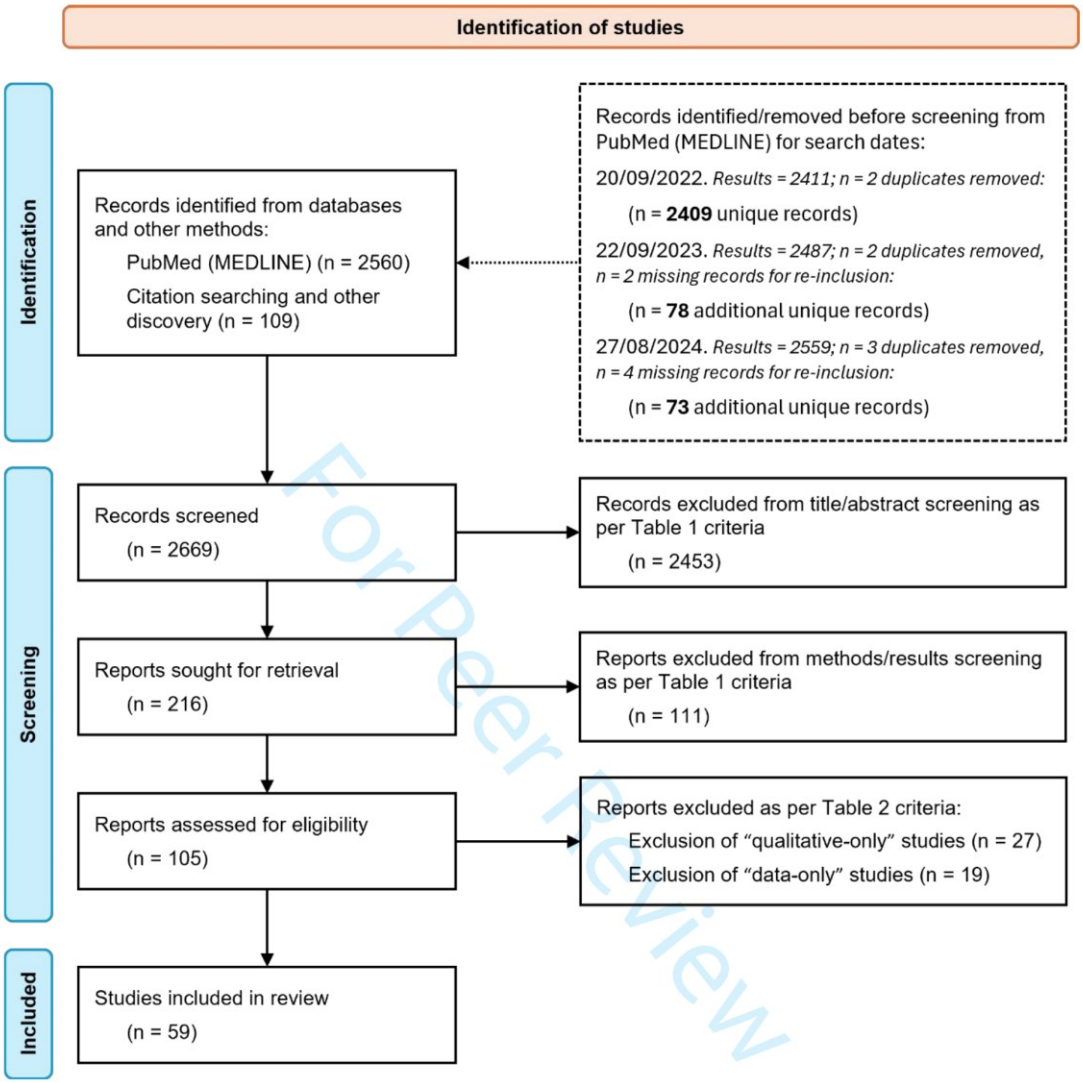

Figure 1. Flow diagram of literature inclusion and screening workflow. *Diagram is modified from a readily accessible template*[14].

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Structural variations of tendons.

All 2669 publications were screened by a single reviewer (S.H.) as per the workflow in Figure 1. Articles were first screened by title and abstract and excluded as per Table 1. The 216 articles passing title/abstract screening had the full text assessed for exclusion/eligibility as per Table 1 criteria. The 105 eligible papers underwent final review according to the criteria in Table 2 to remove publications with "qualitative-only" (n=27) or "data-only" (n=19) comparisons (these are recorded/summarised in Table S-1 for additional reference).

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Table 1: Eligibility and exclusion criteria for systematic search strategy (screening stages).

Exclusion: criterion for the exclusion of studies from review focus
Non-primary research (e.g. reviews, letters, editorials, etc.)
Full text not available through open access, institution subscriptions/resources or not written in English.
Studies *isolated* to any of the following: - Ligaments or ligament comparisons. - Tendon macro-structure characterisation (e.g. anatomical morphology, macroscopic exploration, etc.). - Characterisation of non-appendicular tendons (e.g. spinal, cranial, ocular, auditory, respiratory, etc.). - Enthesis (tendon-bone junction), musculotendinous junction, aponeurosis, or tendon sheath (epitenon) characterisation. - Utilisation of medical imaging techniques (e.g. MRI or ultrasound) for structural assessment.
Eligibility: criterion for considering studies for assessment
a) Types of studies
Primary research studies addressing the comparison of normal/healthy tendon micro-structure, including pathological/other studies offering comparative analysis of normal/healthy tendons utilised as control tissue.
Full manuscript text available through open access or institution resources and written in English.
b) Types of tissue explored
Tendons of the appendicular system of any species, sex or age (excluding pre-natal)
Tendon mid-substance (i.e. regions apart from enthesis (tendon-bone junction) or musculotendinous junction)
c) Types of outcome measures
Utilisation of microscopy techniques (e.g. variants of optical microscopy, electron microscopy, atomic force microscopy, etc.).
At least one of the following 'variables of interest' explored regarding structural comparisons of normal/healthy tendon mid-substance specimens: i- ***Tendon***: differences between tendons (or tendons and ligaments). ii- ***Region***: differences between tendon/ligament mid-substance regions. iii- ***Age***: differences in relation to age. iv- ***Sex***: differences between males and females. v- ***Other*****:** other identified differences comparing states of "normal" tendon, e.g. lifestyle differences (exercise, diet, etc.), species differences, etc.

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Table 2: Final inclusion criteria for publications to be reviewed. Excluded records ('noted exclusions') remain identified in Table S-1 as additional supplementary knowledge explored in the field.

*** Additional exclusion criterion A: exclusion of "qualitative-only" studies**
Studies which solely reported qualitative or descriptive comparisons for the 'variables of interest'. ** A list of these publications (n = 27) can be found in Table S-1 ('Noted Exclusions A').*
**** Additional exclusion criterion B: exclusions of "data-only" studies**
Studies which conducted objective measures in relation to any of the 'variables of interest' but did not explicitly report statistical comparisons for these variables. *** These publications (n = 19) are summarised and can be found in Table S-1 ('Noted Exclusions B').*
Inclusions: criterion for inclusion in summary Table 3
Studies which provided objective comparisons and reported statistical analysis for at least one of the variables of interest (Tendon, Region, Age, Sex, or Other).

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Structural variations of tendons.

Results

A total of 59 records representing studies which explicitly performed quantitative statistical comparisons for the *'variables of interest'* were included and summarised in Tables 3 and 4. Table 5 outlines acronyms referred to in this manuscript, Tables and Supplementary Tables.

Of the species utilised, 19 studies investigated human specimens, 23 large quadrupeds (15 equine, 4 bovine, 2 canine, 1 ovine, 1 caprine), 16 murine (12 rat, 4 mouse), and 3 other (2 avian, 1 rabbit). Only two studies included more than one species[15,16] (Table 3). Only Gsell et al.[15] compared species but even here direct comparability was limited as different anatomical tendons were utilised across species.

In terms of tendons/ligaments studied, 34 publications investigated a single tendon, 14 investigated two different tendons/ligaments, 5 investigated three, 3 investigated four, 2 investigated five, and 1 investigated six. Considering the *variables of interest* (Table 4), 20 studies compared different anatomical tendons/ligaments (Table S-2a), 22 studies compared within-tendon *Region* differences (Table S-2b), 30 *Age*-related differences (Table S-2c), 8 *Sex*-differences (Table S-2d), and 16 explored *Other* variables (Table S-2e).

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Structural variations of tendons.

Table 3. Summary of papers exploring statistical comparisons relating to *Tendon*, *Region*, *Age*, *Sex* or *Other 'variables of interest'*. Tendons/Ligaments included in the study but not statistically assessed for any of the variables are greyed-out. *Y = statistical analysis is included for the relevant variable. D = data/graphical representation of data is included for the relevant variable. Refer to Table 5 for acronym definitions.*

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Structural variations of tendons.

Paper (year)	Species	Tendons (T) | Ligaments (L)		Microscopy Modality	Tendon	Region	Age	Sex	Other
Ingelmark[17] (1948)	Murine (Rat)	1T	Achilles	TEM	-	-	Y	-	Y
Wilmink et al.[18] (1992)	Equine	1T	SDFT	OM	-	Y	Y	-	-
Patterson-Kane et al.[19] (1997a)	Equine	1T	SDFT	OM, TEM	-	Y	Y	-	-
Patterson-Kane et al.[20] (1997b)	Equine	1T	SDFT	OM	-	Y	D	-	D
Birch et al.[21] (1999)	Equine	2T	DDFT, SDFT	TEM	Y	Y	Y	-	-
Odetti et al.[22] (2000)	Murine (Rat)	1T	Tail	SFM	-	-	-	-	Y
Gibson et al.[23] (2002)	Equine	1T	SDFT	OM, TEM	-	Y	-	-	-
Cook et al.[24] (2004)	Human	1T	Patellar	OM	-	Y	Y	-	Y
Edwards et al.[25] (2005)	Equine	1T	CDET	TEM	-	-	Y	-	Y
Watanabe et al.[26] (2005)	Equine	1T	SDFT	TEM, hvEM	-	Y	-	-	-
Prado et al.[27] (2006)	Human	1T	Tibialis Posterior	OM	-	Y	Y	Y	Y
Hadjicostas et al.[28] (2007a)	Human	2T	Patellar, Quadriceps	OM, TEM	Y	-	Y	Y	-
Hadjicostas et al.[29] (2007b)	Human	3T	Gracilis, Semitendinosus, Patellar	OM, TEM	Y	-	Y	Y	-
Funakoshi et al.[30] (2008)	Caprine	2T	Infraspinatus, Patellar	OM	-	Y	-	-	Y
Hadjicostas et al.[31] (2008a)	Human	4T | 1L	Gracilis, Semitendinosus, Patellar, Quadriceps | ACL	OM, TEM	Y	-	Y	Y	-
Hadjicostas et al.[32] (2008b)	Human	2T	Gracilis, Semitendinosus	OM, TEM	Y	-	-	-	-
Stanley et al.[33] (2008)	Equine	2T	CDET, SDFT	OM	Y	Y	D	-	Y
Williams et al.[34] (2008)	Rabbit	1T	Patellar	TEM	-	Y	-	-	-
Franchi et al.[35] (2009)	Murine (Rat)	3T	Patellar, Rectus Femoris, Vastus Intermedius	OM	Y	Y	-	-	-
Hansen et al.[36] (2009)	Human	1T	Patellar	TEM	-	-	-	-	Y
Hansen et al.[37] (2010)	Human	1T	Patellar	TEM	-	Y	-	-	-
Hosaka et al.[38] (2010)	Equine	3T	CDET, DDFT, SDFT	OM	Y	-	-	-	-
Frizziero et al.[39] (2011)	Murine (Rat)	1T	Patellar	OM	-	-	-	-	Y
Hu et al.[40] (2012)	Murine (Rat)	1T	*Unnamed*	SHG	-	-	Y	-	-
Weiss et al.[41] (2012)	Human	5T | 1L	Gracilis, Semitendinosus, Patellar, Quadriceps, Tibialis Anterior | ACL	OM	Y	-	-	-	-
Zhu et al.[42] (2012)	Human	*1T | 1L	"Hamstring" (*Gracilis or Semitendinosus) | ACL	SEM, TEM	Y	-	-	-	-
Dunkman et al.[43] (2013)	Murine (Mice)	1T	Patellar	OM, TEM	-	-	Y	-	-
Lavagnino et al.[44] (2013)	Murine (rat)	1T	Tail	OM	-	-	Y	-	-
Mazzocca et al.[45] (2013)	Human	1T	LHBT	OM	-	Y	-	-	-
Thorpe et al.[46] (2013)	Equine	1T	SDFT	SEM	-	-	Y	-	-
Legerlotz et al.[47] (2014)	Murine (Mice)	1T	Tail	OM	-	-	Y	-	-
Tilley et al.[48] (2014)	Human	2T	Subscapularis, Supraspinatus	OM, AFM	Y	-	Y	-	-
Lenskjold et al.[49] (2015)	Human	1T	Achilles	TEM	-	-	-	-	Y
Herod et al.[50] (2016)	Bovine	2T	CDET, SDFT	SEM	Y	-	-	-	-
Pardes et al.[51] (2016)	Murine (Rat)	1T	Achilles	OM	-	-	-	Y	Y
Thorpe et al.[52] (2016a)	Equine	2T	CDET, SDFT	OM	Y	Y	-	-	-
Thorpe et al.[53] (2016b)	Equine	1T	SDFT	OM	-	Y	Y	-	-
Biasutti et al.[54] (2017)	Ovine	1T	SDFT	OM	-	Y	-	-	-
Godinho et al.[55] (2017)	Equine	2T	CDET, SDFT	OM	Y	D	Y	-	-
Marquez-Arabia et al.[56] (2017)	Human	1T	Gluteus Medius	OM	-	-	Y	-	-
Pardes et al.[57] (2017)	Murine (Rat)	1T	Achilles [37]	OM	-	-	Y	-	-
Sarver et al.[58] (2017)	Murine (Mice)	1T	Achilles	OM	-	-	-	Y	-
Gagliano et al.[59] (2018)	Human	2T	Gracilis, Semitendinosus	OM	-	-	Y	-	-
Kharaz et al.[60] (2018)	Canine	2T | 2L	LDET, SDFT | ACL, MCL	OM	Y	Y	-	-	-
Mazon et al.[61] (2018)	Murine (Rat)	1T	Calcaneal (Achilles)	OM	-	-	-	-	Y
Shu et al.[62] (2018)	Murine (Mice)	2T	Achilles, Tail	TEM	D	-	Y	-	-
Spiesz et al.[63] (2018)	Equine	2T	CDET, SDFT	OM	Y	Y	Y	-	-
Takahashi et al.[64] (2018)	Bovine	1T	SDFT	OM, TEM	-	Y	-	-	-
Feo et al.[65] (2019)	Avian (Pedrês chicken)	1T	SDFT	OM	-	Y	Y	-	-
Hayashi et al.[66] (2019)	Canine	1T | 3L	LDET | CaCL, CrCL, MCL	OM	Y	-	Y	Y	Y
Ristaniemi et al.[67] (2021)	Bovine	1T | 4L	Patellar | ACL, LCL, MCL, PCL	OM	Y	-	-	-	-
Asai et al.[68] (2022)	Human	1T	Semitendinosus	OM, TEM	-	-	Y	Y	Y
Bolam et al.[69] (2022)	Murine (Rat)	1T	Achilles	OM, TEM	-	-	-	-	Y
Naot et al.[70] (2022)	Human	1T	Semitendinosus	TEM/SEM	-	-	Y	-	-
Fjordbakk and Marques-Smith[71] (2023)	Equine	3T	Medial Patellar, Intermediate Patellar, Lateral Patellar	OM	Y	-	Y	-	-
Gsell et al.[15] (2023)	Bovine, Murine (Rat)	3T	LDET [bovine], SDFT [bovine], Tail [rat]	AFM	Y	-	-	-	Y
Johnson et al.[72] (2023)	Human	4T	Infraspinatus, Subscapularis, Supraspinatus, Teres Minor	OM	D	-	Y	-	-
Tinguely et al.[16] (2023)	Murine (Rat), Human	2T	Achilles [rat; human], Patellar [rat]	OM	D	-	Y	-	D
Zheng et al.[73] (2024)	Avian (Turkey)	1T	Gastrocnemius (Achilles)	sPHG	-	Y	-	-	-
N = 59			*Total variable (Y) count =*		20	22	30	8	16

Structural variations of tendons.

Table 4. Summary of papers by variable (statistical comparisons). *Refer to Table 5 for acronym definitions.*

Comparisons (variable)	Number of Papers	Number of Papers by Species										Tendons / Ligaments
		Human	Equine	Bovine	Canine	Ovine	Caprine	Rat	Mice	Avian	Rabbit	
Tendon See Table S-2a for details	**20**	7	7	3	2	-	-	2	-	-	-	**N = 17** CDET, DDFT, LDET, SDFT, Patellar, Rectus Femoris, Vastus Intermedius, Quadriceps, Gracilis, Semitendinosus, Tibialis Anterior, Subscapularis, Supraspinatus, Medial Patellar Ligament, Intermediate Patellar Ligament, Lateral Patellar Ligament, Tail
Region See Table S-2b for details	**22**	4	10	1	1	1	1	1	-	2	1	**N = 11** CDET, DDFT, LDET, SDFT, Patellar, Rectus Femoris, Vastus Intermedius, Tibialis Posterior, Infraspinatus, LHBT, Achilles/Gastrocnemius
Age See Table S-2c for details	**30**	12	9	-	1	-	-	5	3	1	-	**N = 19** CDET, DDFT, LDET, SDFT, Patellar, Medial Patellar Ligament, Intermediate Patellar Ligament, Lateral Patellar Ligament, Quadriceps, Gracilis, Semitendinosus, Tibialis Posterior, Gluteus Medius, Infraspinatus, Subscapularis, Supraspinatus, Teres Minor, Achilles, Tail
Sex See Table S-2d for details	**8**	5	-	-	1	-	-	1	1	-	-	**N = 7** LDET, Patellar, Quadriceps, Gracilis, Semitendinosus, Tibialis Posterior, Achilles
Other See Table S-2e for details	**16**	5	2	1	1	-	1	7	-	-	-	**N = 9** CDET, LDET, SDFT, Patellar, Semitendinosus, Tibialis Posterior, Infraspinatus, Achilles/Calcaneal, Tail

Structural variations of tendons.

Table 5. List and definition of acronyms included in manuscript and tables.

Tendon/Ligament Names	
CDET	Common Digital Extensor Tendon
DDFT	Deep Digital Flexor Tendon
LDET	Long Digital Extensor Tendon
SDFT	Superficial Digital Flexor Tendon
LHBT	Long Head of Biceps [brachii] Tendon
RFT	Rectus Femoris Tendon
VIT	Vastus Intermedius Tendon
TA	Tibialis Anterior
TP	Tibialis Posterior
FHL	Flexor Hallucis Longus
ACL	Anterior Cruciate Ligament
LCL	Lateral Collateral Ligament
MCL	Medial Collateral Ligament
PCL	Posterior Cruciate Ligament
CaCL	Caudal Cruciate Ligament
CrCL	Cranial Cruciate Ligament
Outcomes	
ECM	Extracellular matrix
GAG	Glycosaminoglycan
IFM	Interfascicular matrix
FM	Fascicular matrix
IHC	Immunohistochemistry
αSMA	Alpha-smooth muscle actin
CSA	Cross-sectional area
TB	Toluidine Blue
PSR	Picrosirius Red
Microscopy Modalities	
hvEM	High voltage electron microscopy
IHC	Immunohistochemistry
MLM	Multiphoton laser microscopy
OM	Optical microscopy (e.g. light, polarised, confocal, etc.)
pSHG	Polarisation-resolved second harmonic generation microscopy
SEM	Scanning electron microscopy
SFM	Scanning force microscopy
SHG	Second harmonic generation microscopy
TEM	Transmission electron microscopy

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Between-tendon differences

Direct comparison of tendons is limited. Two studies which passed initial eligibility but not second screening, included a large range of tendons but without any quantitative comparisons. Kannus and Jozsa[7] (Table S-1; *Noted Exclusions A*) investigated 16 'tendon-groups' (individual tendons not specified) and presented only descriptive findings, whilst another study comparing 24 different appendicular tendons[74] focussed on fibrocartilaginous presence and did not report statistical analyses regarding similarities/difference (Table S-1; *Noted Exclusions B*).

Most studies with quantitative analysis of normal tendon structure (34/59, Table 3) only investigated a single tendon. Where at least two tendons (or tendon/ligament) were included (n=25), only 20 explored specific *Tendon* differences (Table 4, Table S-2a). Of these, 3 papers incorporated only one tendon and compared this with ligament tissue(s).[42,66,67] Weiss et al.[41] compared five tendons and the anterior cruciate ligament (ACL) but did not explicitly perform direct tendon-vs-tendon statistical comparisons and instead reported similarities/differences in correlated variables (e.g. crimp-frequency versus cell-number). Of the 16 papers that made direct tendon-to-tendon statistical comparisons, the majority (10/16) compare only two tendons, 5 studies comparing three, and 1 study each comparing four/five tendons (Table S-2a).

Most studies evaluating between-tendon differences use animals (13/20: equine, bovine, canine, rat), and primarily focus on the comparison of energy-storing versus positional tendons. There are clear differences reported, with extensor/positional tendons generally having lower cell density, larger collagen fibres, larger crimp wavelength, and different protein makeup within the interfascicular and fascicular matrices (IFM and FM, respectively) in comparison to flexor/energy-storing tendons (Table S-2a). Compared with flexors, positional extensors also

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have overall, a more organised structure, containing relatively less IFM area,[55] less randomly distributed elastin fibres,[55] and larger collagen fibrils,[15,50] reflected in an overall greater total birefringence in picrosirius red (PSR) stained sections.[63]

Only 7/20 *Tendon*-comparison papers (Table S-2a) evaluated *human* tissues, with the majority (6/7) primarily focused on differences between tendons commonly used as ACL-reconstruction grafts and three making direct comparisons with the ACL. These studies identified notable differences (e.g. collagen type and fibril size, vascularity, cell density) between the Quadriceps and Patellar,[28,31,41] the hamstring and Patellar,[29,31,41] and the Gracilis and Semitendinosus tendons[31,32,41] (Table S-2a). Interestingly, across all studies and species, the ACL has unique characteristics, differing both from tendons[31,42,60,67] and other ligaments.[60,67] The seventh human study by Tilley et al.[48] investigating disease progression of rotator cuff tendons, included comparisons of the 'normal/control' Supraspinatus and Subscapularis tendons. However, different age cohorts (mean 23.3yrs for Subscapularis and 74.6yrs for Supraspinatus) confounds direct comparisons.

Current literature limitations

The limited number of studies and often low sample size (one to three donors[41,42,75]), reflect challenges in obtaining human specimens. A general limitation of current microscopy-based studies is the largely descriptive nature of the reports. For example, observations of collagenous structure commonly include descriptions such as "homogenous", "planar waveform", or "differentially orientated", while those relating to cell populations include terms like "slightly elongated", "plump", "acellular areas", and "regularly arranged".[7,9,11,42,76-81] This descriptive/qualitative approach allows for greater subjective interpretation, and limits capacity to standardise current knowledge and understanding of normal tendon structure, particularly

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differences between specific normal/healthy tendons. As an example, two investigations by the same group separately evaluated human Tibialis Anterior (TA)[80] and Tibialis Posterior (TP)[78] tendon structure. The descriptive "positive" Alcian-Blue staining reported, gives no indication of spread or intensity, and may inadvertently suggest similarities when comparing these two tendons. In contrast, quantitative evaluation of the thickness of the fibrocartilaginous layer at the gliding zone of the respective malleoli presents clearly defined differences: TA=30-50μm[78] and TP=100-200μm[78].

Within-tendon variations

A total of 22 publications assessed within-tendon *Regional* variations (Table 4, Table S2-b). Mostly these studies explore variations across the longitudinal and transverse (cross-sectional) planes of the tendon/ligament, although some evaluate differences between the IFM versus FM[52,53,63] or between individual bodies of tendons grouped together by a shared insertion point e.g. sub-tendons forming the Quadriceps.[35] Only 4/22 studies explored regional variations within human tendons.[24,27,37,45] No studies have reported a systematic investigation of regional variations across a range of normal tendons (human or animal) to determine whether regional patterns are consistent or different between tendons. Regional differences in a single anatomical tendon were evaluated in 15/22 papers (Table S2-b), and in those that evaluated more than one tendon-type, regional differences were typically described independently. Only three studies directly compared *Region* and *Tendon*: Stanley et al.[33] studied equine superficial digital flexor tendons (SDFT) and common digital extensor tendons (CDET), however central versus core regions were independently assessed only in SDFT (Table S2-a, Table S-2b); Thorpe et al.[52] and Spiesz et al.[63] compared IFM and FM structure within equine SDFT and CDET (Table S-2b) and also between tendons (Table S2-a).

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Transverse differences

Cross-sectional variations within tendons (e.g. central-versus-peripheral, palmar-versus-dorsal, medial-versus-lateral, etc.) likely result in response to and modulate the tissue's capacity to function under variable loading (tensile or compressive) conditions. A variety of cross-sectional differences have been reported (Table S-2b) including: greater posterior vs anterior fibril density in human Patellar tendons,[37] longer crimp wavelengths in superficial vs deep zones of caprine infraspinatus tendons,[30] and more ovoid cells in the central vs peripheral equine SDFT.[33] One of the most common cross-sectional regional comparisons were central (core) versus peripheral in the equine SDFT (Table S-2b), with findings often contradictory e.g. crimp angle and wavelength were variably greater or lesser in central regions.[18-20,23] Factors, such as *Age*[19,20] or *Lifestyle*[20,33] have been suggested to play a role in these disparate *Regional* structural variations. Cross-sectional differences may also vary with longitudinal location, e.g. Williams et al.[34] found medial/lateral fibril diameters and fibril-occupied areas varied between middle and distal rabbit Patellar tendon regions.

Longitudinal differences

Proximal-versus-distal differences could play an important role in appendicular tendon function as they traverse a joint or in the transition of tendon to muscle and bone, reflecting changes in loading and biomechanical properties in these areas. Differences identified in the literature include significantly greater GAG content in the bone- versus muscle-end of ovine SDFT[54] and human long head of biceps tendon (LHBT),[45] and greater GAG chain length in distal (bone-end) bovine SDFT[64] (Table S-2b). Mazzocca et al.[45] also found greater fibrillar organisation in the distal (muscle-end) compared to proximal (bone-end) LHBT. This is consistent with more unorganised collagenous structures closest to the bone in the human Patellar tendon,[24] suggesting

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commonality of less organised collagen at tendon-bone junctions. Conversely, vascular density was reported to increase from proximal to distal in human TA tendons,[27] while in ovine SDFT the middle portion had higher vascular density relative to proximal or distal regions.[54] Interestingly in canine Long Digital Extensor Tendon (LDET), SDFT, ACL and Medial Collateral Ligament (MCL), Kharaz et al.[60] noted no longitudinal variation for any histological measurements. Collectively these and other studies,[26,64] suggest tendon- and species-specific variability in longitudinal characteristics of normal tendons that require systematic exploration.

Hierarchy-related differences

Three studies investigated differences between hierarchical tendon structures, specifically IFM and FM (Table S-2b). IFM had higher cell density in equine SDFT,[53] while Spiesz et al.[63] found lower collagen content/organisation (birefringence) in IFM in both equine CDET and SDFT with no tendon-specific differences. Conversely, equine CDET and SDFT had tendon-specific IFM and FM protein compositions:[52] CDET IFM had significantly higher lumican immuno-staining than FM, while SDTF IFM had greater biglycan, lubricin and elastin staining than FM.

Future study design considerations

The aforementioned examples highlight that it is critical to explicitly state the region of sample collection and have region-matched controls to evaluate pathological change. As an example, Cetti et al.[82] compared calcaneal-insertional, rupture, and proximal (10 cm from calcaneus) regions in ruptured Achilles tendons but only a single control sample from the un-injured, contralateral tendon selected between 2.2–8.4 cm from the calcaneal insertion. The rupture site showed greater pathology (collagen degeneration, tenocyte necrosis, inflammation) than other regions in the injured tendon, and interestingly proximal-tendon had greater pathology than distal-tendon. However, as control samples were from various regions along the length of the un-

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injured tendon, no conclusions can be made about underlying regional differences that might alter injury susceptibility or changes post-rupture. Our own preliminary analysis of normal/healthy human cadaveric tendons,[83,84] identified clear longitudinal regional differences in crimp wavelength (Figure 2a,b) and proteoglycan content (Figure 2c,d), reaffirming the importance of region-matching control tissue samples.

In a series of studies in different animal models[12,54,85-87] we assessed topographical-response to injury highlighting the importance of region-matched controls. For example, Jacobson et al.[12] collected 12 proximal/distal and medial/lateral defined regional samples from each injured/pathological and contra-lateral/uninjured SDFT in horses, identifying distinct regionally-dependent histological differences. Cellularity, cell morphology, vascularity, cellular infiltration, collagen alignment and proteoglycan content scores were significantly increased in injured versus control samples in the most proximal medial (*except collagen alignment score) and lateral (*except proteoglycan score) regions, while in the most distal regions, injury only increased cell morphology, vascularity and proteoglycan scores medially, and cell morphology and proteoglycan scores laterally. Detailed analysis regarding variations between control sample regions were not reported, except for highlighting the expected increase in proteoglycan content near bony-attachment sites[12,86] as identified in other studies[45,54] (Table S-2b). A systematic study on normal (rather than pathological) regional histological characteristics in healthy tendons would increase understanding of how and why tendon response to injury or treatment differs.

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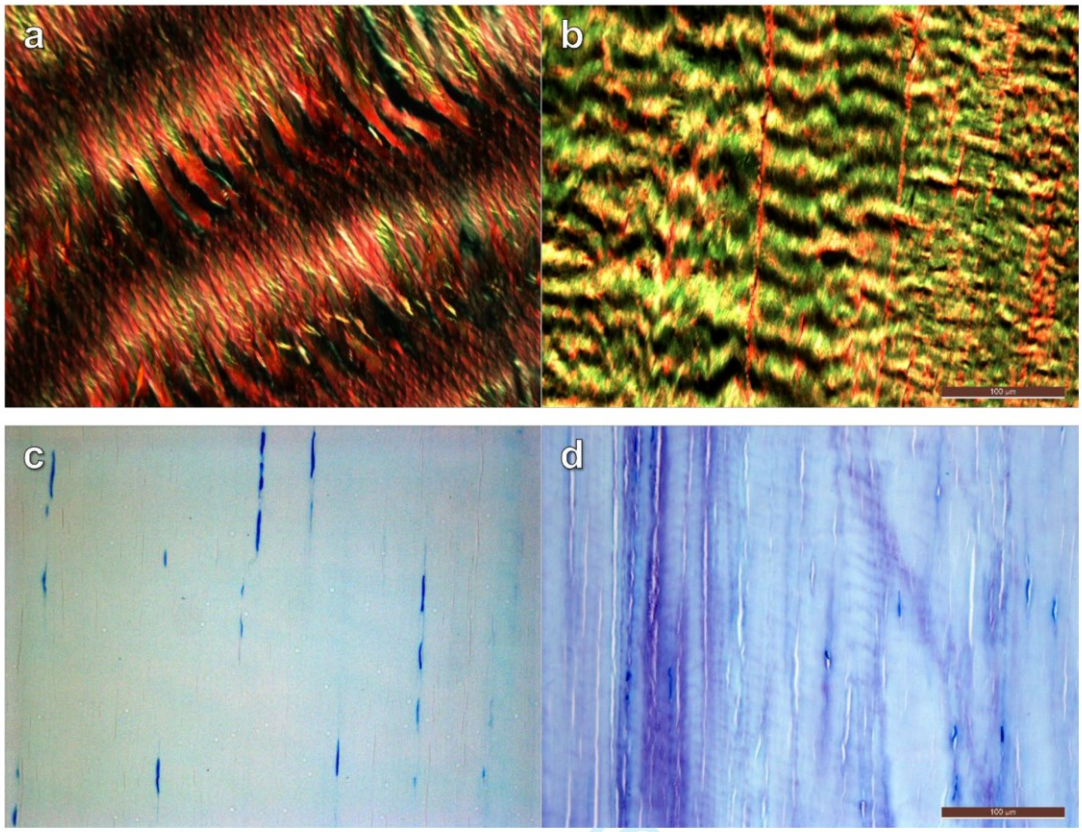

Figure 2. Light microscopy images showing variation in 'normal' human tendons. Histological samples are from cadaveric lower-leg tendons of female donors aged 71 (**a-b**) and 78 (**c-d**) years old. Polarised Picrosirius Red stained sections (**a-b**) depicting regional differences in crimp wavelength between matched proximal (**a**) and distal (**b**) regions of the Tibialis Anterior tendon. Toluidine Blue stained sections **(c-d)** depicting regional differences in proteoglycan content (purple staining) between matched proximal (**c**) and distal (**d**) regions of the Achilles tendon. *Longitudinally orientated, formalin-fixed, paraffin embedded histological sections at 4.5 µm thick. All images: 200x magnification; scale bars = 100µm.*

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Age-related differences

We identified 30 publications which statistically assessed *Age* effects, with 12 exploring human tissue (Table 4, Table S-2c). The largest specified age span in these human studies was 66yrs,[48] while most spanned 29-36yrs. In six of the ten human studies which specified ages (two did not specify), donor average age was >45yrs, while one investigated child and adolescent cohorts.[68] In consideration of the above discussion of tendon variables, 5/12 human studies utilised only one anatomical tendon, and 4/12 only two. Two studies with >2 tendons[29,31] analysed *Age*-effects for only 2 of the study outcomes (collagen fibril thickness and density; no correlations found). Lastly, Johnson et al.[72] statistically assessed total histopathological score but not individual structural parameters across four rotator cuff tendons.

Age-associated changes have been reported for tendon cellular content/features, structural characteristics, and compositional makeup (Table S-2c). Reduced cellularity[43,57,65,66,68] and increased cellular rounding[57,66] have been observed with increasing age in various animal tendons. We identified similar decreasing cell density with aging (Figure 3) in cadaveric human lower-leg tendons from male and female donors aged 68-99 years.[83,84] Importantly, changes with age may not be linear, for example Legerlotz et al.[47] found cell density drastically decreased after 1wk of age in mice, but then gradually increased again from 6wks to 6mths of age. Likewise, cell nuclear aspect ratios increased from 1 to 8 and 8 to 19mths in rats and then became more rounded between 19 and 28mths of age.[16] Furthermore, age-related changes may differ between tendons e.g. vascular density had a quadratic relationship across ages (18-68yrs) in human Gluteus Medius tendons,[56] while Prado et al.[27] reported no age:vascular-density correlation in human TA tendons.

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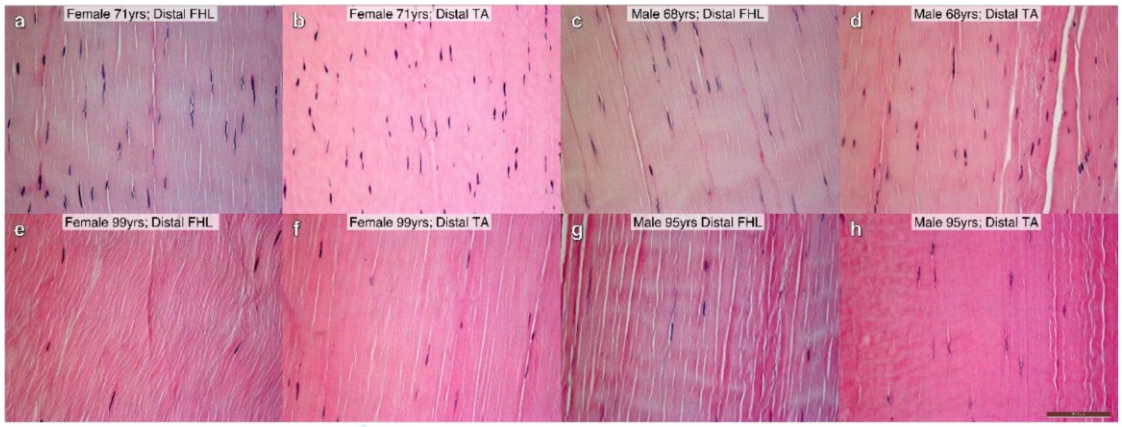

Figure 3. Light microscopy images showing cell density variation in 'normal' human tendons. Histological samples are from the distal region of cadaveric lower-leg Flexor Hallucis Longus (FHL; **a,c,e,g**) and Tibialis Anterior (TA; **b,d,f,h**) tendons. Female donors aged 71 (**a-b**) and 99 (**e-f**) years old, and male donors aged 68 (**c-d**) and 95 (**g-h**) years old. Note differences in relative cell density between younger (**a-d**) and older (**e-h**) donors. *Longitudinally orientated, formalin-fixed, paraffin embedded histological sections at 4.5 µm thick. All images: 200x magnification; scale bars = 100µm.*

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Many studies report no correlations/differences in fibril diameter with age.[19,21,28,29,31,70] However, Shu et al.[62] reported both Achilles and tail tendons have significantly larger fibril diameters in skeletally mature (12wk-old) compared to young (3wk-old) mice. Similarly, Asai et al.[68] found "adult" (average 35yrs) human Semitendinosus tendons had significantly larger fibril mean diameters compared to "immature" tendons (average 10yrs). Disparate crimp wavelength variations with age were reported in equine SDFT with shorter wavelength in older (average 11yrs) compared to younger (average 2.75yrs)[18] versus mouse tail tendon (shortest crimp in youngest 3wk-old compared to 6wk-, 9wk-, 12wk and 6mth-old cohorts).[47]

While these studies reveal some *Age*-associated cellular and structural differences, the heterogeneities reflect limitations in exploring these characteristics across different tendons and age brackets. Furthermore, direct comparison between studies is hampered by differing methodologies and potential species-specific differences in chronological- versus biological-age relationships. There is a major gap in knowledge of normal tendon aging throughout the human lifespan, particularly considering the narrow focus of specimens and outcome measures.

Age versus pathology

Histological research on aging of human tendons primarily focuses on injured/pathological samples with an unclear distinction between age-related versus injury/disease-driven changes. As an example of this poor division, the concept of "degeneration" is often used for both aging[88-90] and pathology[5,91,92] despite the potential for disparate mechanistic pathways in these two processes.[93,94] Furthermore, older age groups generally exhibit more severe pathological change in diseased/injured tendons than younger ages e.g. in rotator cuff tears,[92,95] Supraspinatus tendinitis[96] and Achilles tendinopathy.[97] While age was significantly correlated with histopathology-score in a study of 155 Achilles tendon biopsies from individuals with

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symptomatic tendinopathy, age was also inversely correlated to lifestyle activity levels.[97] Differences in older populations could indicate 'normal' physiological processes including those reflecting more sedentary lifestyles. It is important to consider differences between healthy or "normal" age-related changes or whether aging causes pathology, notwithstanding such a division remains difficult to define.

Intersection of age and anatomical tendon

While age evidently influences tendon properties, the interaction between age and different anatomical tendons requires consideration, as functionally distinct tendons can be affected differently. In horses for example, the energy-storing SDFT contains significantly more elastin (predominantly in the IFM) than the positional CDET, but IFM elastin content and FM elastin organisation decreased with age only in SDFTs, while FM elastin content increased significantly with age only in CDETs.[55] Age also led to the accumulation of partially degraded collagen[91] as well as increased IFM-stiffness[46] in SDFTs and not CDFTs (potentially explaining the age-related prevalence of injury in the SDFT). Unfortunately, less than half of the studies exploring *Age*-related differences (13/30; Table S-2c) assessed more than one tissue/tendon type. It is important for future histological studies on different tendons to consider age as a factor, particularly when existing knowledge for those specific tendons, species and ages is absent.

Study design and methodology considerations

The typically older age of human cadavers is a limiting factor for investigations utilising 'normal' controls or investigating normal tendon structure. When control specimens are taken from deceased patients,[11,98-104] it is not uncommon for the age difference from the often younger, more active and injury-prone cohort-of-interest to be in the order of twenty years.[98-100,103,104] For example, a histological study of quadriceps tendon ruptures compared biopsies from patients

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aged 28–81yrs (mean 51.3) to cadaveric controls aged 66-91yrs (mean 74.5).[100] Comparative patient biopsies may also be from different age cohorts due to circumstances/opportunities for tissue collection e.g. Zhu et al.[42] compared normal hamstring tendon grafts from patients undergoing surgery for ACL rupture (average age 25.3±7.3yrs) to "normal" ACL tissue from osteoarthritis patients undergoing total knee arthroplasty (average age 65.3±5.6yrs). It is critical that future studies better characterise 'normal' tendon across a wide age-range which will help overcome issues of age-matching control and experimental samples.

Another confounding factor for *age*-effects is the potential for *species*-related differences. Conflicting results have been reported for collagen fibril diameter, which was not correlated with age in many (but not all) human studies,[28,31,70] but was affected in equine,[25] rat[17] and mouse[62] tendons. Whether this reflects bona fide species-specific differences, the particular tendons evaluated, other environmental influences (e.g. activity[17,25]), or older chronological age in human studies is unclear. Study methodology may also affect outcomes as shown by Ingelmark[17] who used electron microscopy of fresh versus osmium tetroxide (OsO_4) impregnated samples to assess fibril diameters in old and young rats in response to different activity levels. OsO_4 treatment significantly reduced fibril diameters compared to fresh samples, however fresh samples showed no difference in fibril diameters between groups, while OsO_4 showed larger diameters for the old trained versus young untrained rats.[17] The complex age- and exercise-related differences in response to OsO_4 undoubtedly reflect changes in tendon biology but may not actually be indicative of altered fibril size *in vivo*. Likewise, tissue orientation affected significant findings of histological vascular density in the human Gluteus Medius tendon where correlations between density and age were observed in longitudinal but not transverse sections.[56]

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Sex-related differences

A noticeable gap in the literature is understanding of *sex*-related effects on tendon structure. Only 8 papers explored *Sex* as a variable microscopically, with over half (5/8) investigating human tendons (Table 4, Table S-2d). Of the 8 studies only one found a significant correlation between sex and structural characteristics: greater cellularity in the Achilles tendons of females compared to male mice.[58] No other studies reported sex-differences in cell density or morphology in various tendon/ligament models.[51,66,68] Pardes et al.[51] also found no differences between male, female or ovariectomized-female rat Achilles tendon proteoglycan staining. No correlations were found between sex and thickness or density of collagen fibrils in tendons commonly used for human grafts,[28,29,31] and Prado et al.[27] identified no sex-related differences in the vascular densities of human TP tendon.

The scope of research available on *Sex*-related differences in microscopic tendon structural properties is limited, with the major focus being on cellular features and very few studies reporting on these or other structural characteristics (Table S-2d). It is common for studies investigating animal tendons to omit reporting sex or to use mixed (pooled) populations,[9,19,52,60,105-107] often because it is unknown to the researcher (e.g. tendons from discarded limbs at an abattoir). Surprisingly, these issues are also observed in many human studies, where sex is either not disclosed[59,92,102,108,109] (potentially as a result of ethical/privacy issues), or *sex* as a variable is not included in the analyses due to limited sample availability.[5,24] While the few studies available suggest limited differences between sexes, it is important to continue to fill this gap to fully understand the effects that patient demographics have on tendon structure. From our own study exploring a range of histological outcomes in nine different cadaveric human lower-leg tendons (donors aged 68-99 years; five males, four females), females

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had significantly longer crimp wavelengths relative to males (manuscript in preparation). These types of structural differences may be implicated in reported sexually dimorphic tendon mechanical attributes,[20,110-112] which could influence the intervention approach or outcomes of tendon related injury. Large-scale studies exploring tendon variations and demographic differences including *Sex*-specific analyses are needed.

Other normal/healthy tendon variabilities

In addition to the primary *'variables of interest'*, 16 papers assessed *Other* factors such as species or lifestyle differences (e.g. exercise, diet, etc.) on tendon structure (Table 4, Table S-2e).

Species

Of the initial 105 eligible studies (Table S-1), only four incorporated more than one species, with three not conducting statistical *Species* comparisons. Aune et al.[113] used a rat model and human biopsies to study nerve regeneration following ACL reconstruction with Patellar tendon autografts but only observational between-species comparisons were made. Likewise, Tinguely et al.[16] reported only graphical data comparing rat and human aging and injury responses in tendons with no direct statistical comparisons. Sato et al.[114] compared the tendon healing of mice to that of Iberian ribbed newts, however control/normal tendons were not statistically compared. Gsell et al.[15] was the only paper specifically comparing tendons from different *Species*, exploring collagen fibril properties from bovine and rat tendons. However, the primary purpose of the study was investigating differences between positional (bovine LDET and rat Tail tendon) and energy storing (bovine SDFT) tendons. While differences were observed between the three tendon-types, species-specific comparisons were not a focus and use of different anatomical tendons limits interrogation of species-specific influences on functionally 'like-for-like' tendons.

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Other comparisons

Other variables' effects on normal/healthy tendon structural characteristics were explored in 15 papers (Table S-2e), with 6 of these studies having this as the only *'variable of interest'* assessed. Variables explored included: *Lifestyle* differences (e.g. level of physical activity/training/exercise,[17,24,25,33,39,49,61] or diet[69]); *Comorbidities*/general health status (e.g. diabetic versus non-diabetic conditions[22] or unrelated musculoskeletal injuries[24]); *Demographic* information (e.g. weight,[24,66,68] body mass index,[68] and height[24,68]); or *Other* correlations (e.g. hormonal status,[36,51] left versus right limbs,[27] or even specifics of individual subjects[30]). Unsurprisingly considering the load bearing role of tendons, exercise-related (*Lifestyle*) comparisons predominate the *Other* variables that significantly modulated tendon histological structural measures.

Conclusions

It is self-evident that knowledge of normal variations in tendon histo-anatomical structure is essential for conducting studies on tendon structure-function relationships, clinical pathology, and response to treatment; particularly when current practice in clinical and research settings utilises histopathological scoring systems which grade characteristics relative to a normal/control reference.[24,115,116] To date, no studies have systematically investigated structural variations between a range of different normal tendons using consistent methodologies. This is a major limiting factor in current tendon research and is critical foundational knowledge for future studies.

Tendons have a complex and varied structure, resulting in many considerations when it comes to understanding the structure-function relationships of the tissue. Although a considerable amount

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of literature is available on tendon structural/compositional variations, this knowledge is predominantly derived from studies which: 1) focus on pathological conditions/structural changes; 2) report only descriptive classifications; 3) pertain to only one or two distinct tendons and offer limited comparability to other investigations; or 4) assess limited outcomes, for example, only biochemical data with no other studies performed on the tissues for more comparative analysis. Accordingly, current knowledge on tendon structural characteristics and normal variations in response to variables such as the specific anatomical *Tendon*, *Region*, *Age* and *Sex* is limited. As a result, other areas of tendon research such as investigations into tendon physiological and pathophysiological mechanisms, biomechanical properties, or clinical outcomes, are inhibited by a lack of understanding of structural "baselines" and natural normal/healthy tissue variations for different tendons.

A structure must be known before a structure-function relationship can be fully understood. Similarly, normal must be known before an appreciation of abnormal can be achieved. This review has highlighted that further research is needed to quantitatively characterise normal tendon structure in greater detail and identify variations across a range of different tendons with consideration of influential variables. Textbox 1 identifies the key gaps in current knowledge on tendon structural variations that need to be addressed in future studies.

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Textbox 1: Summary of knowledge gaps in the histological analysis of tendons.

Currently, there are limited studies which compare the multi-scale structural variations of normal (healthy) tendons. Further structural (e.g. histological, microscopy) investigations are needed in order to:

(1) Systematically compare variations between a wide range of different anatomical tendons,

(2) Characterize variations within tendons, e.g. within the longitudinal or cross-sectional planes, and to determine whether any such variations exist in a range of different tendons,

(3) Understand the effects of age on tendon structure, and the interaction between "normal" aging and pedantry lifestyle versus injury, pathology or disease.

(4) Compare the above outcomes between the sexes,

(5) Appreciate species-specific variations as well as other variables that may influence tendon structure, e.g. lifestyle, demographics, comorbidities etc.

(6) Understand how confounding factors might result in differences e.g. specific tendons exhibiting different regional, age or other variations.

(7) Appreciate how differing methodologies may influence research findings and translatability across the field.

Characteristics that have been identified as important and widely explored features of tendons, that should be considered for assessment in future tendon research include, but are not limited to:

(i) Cell populations (e.g. number/density, morphology etc.)

(ii) Compositional make-up (e.g. location, amount, spread etc. of different components)

(iii) Overall structural architecture (e.g. fibril/fiber/fascicle organization, collagen alignment, crimp patterns etc.)

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Ethics

The histological images depicted in Figure 2 and Figure 3 were obtained as part of a project approved by Northern Sydney Local Health District Human Research Ethics Committee (NSLHD HREC, Sydney, Australia) in accordance with the National Health and Medical Research Council (NHMRC) Act 1992. Human ethics approval code: 2019/ETH08332.

References

1. Birch HL. 2007. Tendon matrix composition and turnover in relation to functional requirements. *Int J Exp Pathol* 88:241-248.
2. Clark ST, Zhu M, Gamble GD, et al. 2020. Epidemiology of tendon and ligament injuries in Aotearoa/New Zealand between 2010 and 2016. *Injury Epidemiology* 7.
3. Maffulli N, Wong J, Almekinders LC. 2003. Types and epidemiology of tendinopathy. *Clin Sports Med* 22:675-692.
4. Clayton RAE, Court-Brown CM. 2008. The epidemiology of musculoskeletal tendinous and ligamentous injuries. *Injury* 39:1338-1344.
5. Józsa L, Réffy A, Kannus P, et al. 1990. Pathological alterations in human tendons. *Arch Orthop Trauma Surg* 110:15-21.

For Peer Review

Structural variations of tendons.

6. Schmidt WJ. 1938. Neuere polarisationsoptische Arbeiten auf dem Gebiete der Biologie. *Protoplasma* 29:300-312.

7. Kannus P, Jozsa L. 1991. Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. *J Bone Joint Surg Am* 73:1507-1525.

8. Watkins JP, Auer JA, Gay S, et al. 1985. Healing of surgically created defects in the equine superficial digital flexor tendon: collagen-type transformation and tissue morphologic reorganization. *Am J Vet Res* 46:2091-2096.

9. Webbon PM. 1978. A histological study of macroscopically normal equine digital flexor tendons. *Equine Vet J* 10:253-259.

10. Farfaras S, Ejerhed LE, Hallström EK, et al. 2018. More histologic and ultrastructural degenerative signs in the subscapularis tendon and the joint capsule in male patients with shoulder impingement. *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA* 26:79-87.

11. Tebaa E, Tantot J, Isaac-Pinet S, et al. 2019. Histologic characteristics of the subscapularis tendon from muscle to bone: reference to subscapularis lesions. *J Shoulder Elbow Surg* 28:959-965.

12. Jacobson E, Dart AJ, Mondori T, et al. 2015. Focal experimental injury leads to widespread gene expression and histologic changes in equine flexor tendons. *PLoS One* 10:e0122220.

13. Arya S, Kulig K. 2010. Tendinopathy alters mechanical and material properties of the Achilles tendon. *J Appl Physiol (1985)* 108:670-675.

14. Haddaway NR, Page MJ, Pritchard CC, et al. 2022. PRISMA2020: An R package and Shiny app for producing PRISMA 2020-compliant flow diagrams, with interactivity for optimised digital transparency and Open Synthesis. *Campbell Systematic Reviews* 18:e1230.

For Peer Review

Structural variations of tendons.

15. Gsell KY, Veres SP, Kreplak L. 2023. Single collagen fibrils isolated from high stress and low stress tendons show differing susceptibility to enzymatic degradation by the interstitial collagenase matrix metalloproteinase-1 (MMP-1). *Matrix Biol Plus* 18:100129.

16. Tinguely Y, Shi V, Klatte-Schulz F, et al. 2023. Aging and injury affect nuclear shape heterogeneity in tendon. *J Orthop Res* 41:2186-2194.

17. Ingelmark BE. 1948. The Structure of Tendons at Various Ages and Under Different Functional Conditions. II. *Cells Tissues Organs* 6:193-225.

18. Wilmink J, Wilson AM, Goodship AE. 1992. Functional significance of the morphology and micromechanics of collagen fibres in relation to partial rupture of the superficial digital flexor tendon in racehorses. *Res Vet Sci* 53:354-359.

19. Patterson-Kane JC, Firth EC, Goodship AE, et al. 1997. Age-related differences in collagen crimp patterns in the superficial digital flexor tendon core region of untrained horses. *Aust Vet J* 75:39-44.

20. Patterson-Kane JC, Parry DA, Goodship AE, et al. 1997. Exercise modifies the age-related change in crimp pattern in the core region of the equine superficial digital flexor tendon. *N Z Vet J* 45:135-139.

21. Birch HL, Bailey JVB, Bailey AJ, et al. 1999. Age-related changes to the molecular and cellular components of equine flexor tendons. *Equine Vet J* 31:391-396.

22. Odetti P, Aragno I, Rolandi R, et al. 2000. Scanning force microscopy reveals structural alterations in diabetic rat collagen fibrils: role of protein glycation. *Diabetes Metab Res Rev* 16:74-81.

23. Gibson KT, Burbidge HM, Robertson ID. 2002. The effects of polyester (terylene) fibre implants on normal equine superficial digital flexor tendon. *N Z Vet J* 50:186-194.

For Peer Review

Structural variations of tendons.

24. Cook JL, Feller JA, Bonar SF, et al. 2004. Abnormal tenocyte morphology is more prevalent than collagen disruption in asymptomatic athletes' patellar tendons. *J Orthop Res* 22:334-338.

25. Edwards LJ, Goodship AE, Birch HL, et al. 2005. Effect of exercise on age-related changes in collagen fibril diameter distributions in the common digital extensor tendons of young horses. *Am J Vet Res* 66:564-568.

26. Watanabe T, Hosaka Y, Yamamoto E, et al. 2005. Control of the collagen fibril diameter in the equine superficial digital flexor tendon in horses by decorin. *J Vet Med Sci* 67:855-860.

27. Prado MP, de Carvalho AE, Jr., Rodrigues CJ, et al. 2006. Vascular density of the posterior tibial tendon: a cadaver study. *Foot Ankle Int* 27:628-631.

28. Hadjicostas PT, Soucacos PN, Berger I, et al. 2007. Comparative analysis of the morphologic structure of quadriceps and patellar tendon: a descriptive laboratory study. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association* 23:744-750.

29. Hadjicostas PT, Soucacos PN, Paessler HH, et al. 2007. Morphologic and histologic comparison between the patella and hamstring tendons grafts: a descriptive and anatomic study. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association* 23:751-756.

30. Funakoshi T, Schmid T, Hsu HP, et al. 2008. Lubricin distribution in the goat infraspinatus tendon: a basis for interfascicular lubrication. *J Bone Joint Surg Am* 90:803-814.

For Peer Review

Structural variations of tendons.

31. Hadjicostas PT, Soucacos PN, Koleganova N, et al. 2008. Comparative and morphological analysis of commonly used autografts for anterior cruciate ligament reconstruction with the native ACL: an electron, microscopic and morphologic study. *Knee Surg Sports Traumatol Arthrosc* 16:1099-1107.

32. Hadjicostas PT, Soucacos PN, Koleganova N, et al. 2008. Comparative analysis of the microstructure of the hamstring tendons: an electron microscopic, histologic, and morphologic study. *J Surg Orthop Adv* 17:153-158.

33. Stanley RL, Goodship AE, Edwards B, et al. 2008. Effects of exercise on tenocyte cellularity and tenocyte nuclear morphology in immature and mature equine digital tendons. *Equine Vet J* 40:141-146.

34. Williams LN, Elder SH, Horstemeyer MF, et al. 2008. Variation of diameter distribution, number density, and area fraction of fibrils within five areas of the rabbit patellar tendon. *Ann Anat* 190:442-451.

35. Franchi M, Quaranta M, Macciocca M, et al. 2009. Structure relates to elastic recoil and functional role in quadriceps tendon and patellar ligament. *Micron* 40:370-377.

36. Hansen M, Kongsgaard M, Holm L, et al. 2009. Effect of estrogen on tendon collagen synthesis, tendon structural characteristics, and biomechanical properties in postmenopausal women. *J Appl Physiol (1985)* 106:1385-1393.

37. Hansen P, Haraldsson BT, Aagaard P, et al. 2010. Lower strength of the human posterior patellar tendon seems unrelated to mature collagen cross-linking and fibril morphology. *J Appl Physiol (1985)* 108:47-52.

38. Hosaka YZ, Takahashi H, Uratsuji T, et al. 2010. Comparative study of the characteristics and properties of tendinocytes derived from three tendons in the equine forelimb. *Tissue Cell* 42:9-17.

For Peer Review

Structural variations of tendons.

39. Frizziero A, Fini M, Salamanna F, et al. 2011. Effect of training and sudden detraining on the patellar tendon and its enthesis in rats. *BMC Musculoskelet Disord* 12:20.

40. Hu W, Li H, Wang C, et al. 2012. Characterization of collagen fibers by means of texture analysis of second harmonic generation images using orientation-dependent gray level co-occurrence matrix method. *J Biomed Opt* 17:026007.

41. Weiss M, Unterhauser FN, Weiler A. 2012. Crimp frequency is strongly correlated to myofibroblast density in the human anterior cruciate ligament and its autologous tendon grafts. *Knee Surg Sports Traumatol Arthrosc* 20:889-895.

42. Zhu J, Zhang X, Ma Y, et al. 2012. Ultrastructural and morphological characteristics of human anterior cruciate ligament and hamstring tendons. *Anat Rec (Hoboken)* 295:1430-1436.

43. Dunkman AA, Buckley MR, Mienaltowski MJ, et al. 2013. Decorin expression is important for age-related changes in tendon structure and mechanical properties. *Matrix Biol* 32:3-13.

44. Lavagnino M, Gardner K, Arnoczky SP. 2013. Age-related changes in the cellular, mechanical, and contractile properties of rat tail tendons. *Connect Tissue Res* 54:70-75.

45. Mazzocca AD, McCarthy MBR, Ledgard FA, et al. 2013. Histomorphologic Changes of the Long Head of the Biceps Tendon in Common Shoulder Pathologies. *Arthroscopy* 29:972-981.

46. Thorpe CT, Udeze CP, Birch HL, et al. 2013. Capacity for sliding between tendon fascicles decreases with ageing in injury prone equine tendons: a possible mechanism for age-related tendinopathy? *Eur Cell Mater* 25:48-60.

47. Legerlotz K, Dorn J, Richter J, et al. 2014. Age-dependent regulation of tendon crimp structure, cell length and gap width with strain. *Acta Biomater* 10:4447-4455.

For Peer Review

Structural variations of tendons.

48. Tilley JM, Murphy RJ, Chaudhury S, et al. 2014. Effect of tear size, corticosteroids and subacromial decompression surgery on the hierarchical structural properties of torn supraspinatus tendons. *Bone Joint Res* 3:252-261.

49. Lenskjold A, Kongsgaard M, Larsen JO, et al. 2015. The influence of physical activity during youth on structural and functional properties of the Achilles tendon. *Scand J Med Sci Sports* 25:25-31.

50. Herod TW, Chambers NC, Veres SP. 2016. Collagen fibrils in functionally distinct tendons have differing structural responses to tendon rupture and fatigue loading. *Acta Biomater* 42:296-307.

51. Pardes AM, Freedman BR, Fryhofer GW, et al. 2016. Males have Inferior Achilles Tendon Material Properties Compared to Females in a Rodent Model. *Ann Biomed Eng* 44:2901-2910.

52. Thorpe CT, Karunaseelan KJ, Ng Chieng Hin J, et al. 2016. Distribution of proteins within different compartments of tendon varies according to tendon type. *J Anat* 229:450-458.

53. Thorpe CT, Peffers MJ, Simpson D, et al. 2016. Anatomical heterogeneity of tendon: Fascicular and interfascicular tendon compartments have distinct proteomic composition. *Sci Rep* 6:20455.

54. Biasutti S, Dart A, Smith M, et al. 2017. Spatiotemporal variations in gene expression, histology and biomechanics in an ovine model of tendinopathy. *PLoS One* 12:e0185282.

55. Godinho MSC, Thorpe CT, Greenwald SE, et al. 2017. Elastin is Localised to the Interfascicular Matrix of Energy Storing Tendons and Becomes Increasingly Disorganised With Ageing. *Sci Rep* 7:9713.

For Peer Review

Structural variations of tendons.

56. Marquez-Arabia WH, Gomez-Hoyos J, Gomez M, et al. 2017. Influence of Aging on Microvascular Supply of the Gluteus Medius Tendon: A Cadaveric and Histologic Study. *Arthroscopy* 33:1354-1360.

57. Pardes AM, Beach ZM, Raja H, et al. 2017. Aging leads to inferior Achilles tendon mechanics and altered ankle function in rodents. *J Biomech* 60:30-38.

58. Sarver DC, Kharaz YA, Sugg KB, et al. 2017. Sex differences in tendon structure and function. *J Orthop Res* 35:2117-2126.

59. Gagliano N, Menon A, Cabitza F, et al. 2018. Morphological and molecular characterization of human hamstrings shows that tendon features are not influenced by donor age. *Knee Surg Sports Traumatol Arthrosc* 26:343-352.

60. Kharaz YA, Canty-Laird EG, Tew SR, et al. 2018. Variations in internal structure, composition and protein distribution between intra- and extra-articular knee ligaments and tendons. *J Anat* 232:943-955.

61. Mazon J, de Aro AA, Simoes PW, et al. 2018. Effect of different resistance-training protocols on the extracellular matrix of the calcaneal tendon of rats. *Ann Anat* 216:75-81.

62. Shu CC, Smith MM, Appleyard RC, et al. 2018. Achilles and tail tendons of perlecan exon 3 null heparan sulphate deficient mice display surprising improvement in tendon tensile properties and altered collagen fibril organisation compared to C57BL/6 wild type mice. *PeerJ* 6:e5120.

63. Spiesz EM, Thorpe CT, Thurner PJ, et al. 2018. Structure and collagen crimp patterns of functionally distinct equine tendons, revealed by quantitative polarised light microscopy (qPLM). *Acta Biomater* 70:281-292.

64. Takahashi N, Hirose T, Minaguchi JA, et al. 2018. Fibrillar architecture at three different sites of the bovine superficial digital flexor tendon. *J Vet Med Sci* 80:405-412.

For Peer Review

Structural variations of tendons.

65. Feo HB, Biancalana A, Romero Nakagaki W, et al. 2019. Morphological Alterations and Increased Gelatinase Activity in the Superficial Digital Flexor Tendon of Chickens During Growth and Maturation. *Anat Rec (Hoboken)* 302:964-972.

66. Hayashi K, Bhandal J, Kim SY, et al. 2019. Comparative histomorphometric analysis of cellular phenotype in canine stifle ligaments and tendon. *Vet Surg* 48:1013-1018.

67. Ristaniemi A, Regmi D, Mondal D, et al. 2021. Structure, composition and fibril-reinforced poroviscoelastic properties of bovine knee ligaments and patellar tendon. *J R Soc Interface* 18:20200737.

68. Asai K, Nakase J, Ishikawa T, et al. 2022. Differences in cellular and microstructural properties of the semitendinosus muscle tendon between young and adult patients. *J Orthop Sci* 27:478-485.

69. Bolam SM, Konar S, Park YE, et al. 2022. A high-fat diet has negative effects on tendon resident cells in an in vivo rat model. *Int Orthop* 46:1181-1190.

70. Naot D, Choi AJ, Street M, et al. 2022. Age-related differences in hamstring tendon used as autograft in reconstructive anterior cruciate ligament surgery. *Int Orthop* 46:845-853.

71. Fjordbakk CT, Marques-Smith P. 2023. The equine patellar ligaments and the infrapatellar fat pad - a microanatomical study. *BMC Vet Res* 19:20.

72. Johnson J, von Stade D, Gadomski B, et al. 2023. Biomechanical and histological changes secondary to aging in the human rotator cuff: A preliminary analysis. *J Orthop Res* 41:2221-2231.

73. Zheng K, Zhong J, Hu J, et al. 2024. Effects of mineralization on the hierarchical organization of collagen-a synchrotron X-ray scattering and polarized second harmonic generation study. *Interface Focus* 14:20230046.

For Peer Review

Structural variations of tendons.

74. Benjamin M, Qin S, Ralphs JR. 1995. Fibrocartilage associated with human tendons and their pulleys. *J Anat* 187 (Pt 3):625-633.

75. Schmidt EC, Chin M, Aoyama JT, et al. 2019. Mechanical and Microstructural Properties of Pediatric Anterior Cruciate Ligaments and Autograft Tendons Used for Reconstruction. *Orthop J Sports Med* 7:2325967118821667.

76. Edwards DA. 1946. The blood supply and lymphatic drainage of tendons. *J Anat* 80:147-152.142.

77. Petersen W, Hohmann G. 2001. Collagenous fibril texture of the gliding zone of the human tibialis posterior tendon. *Foot Ankle Int* 22:126-132.

78. Petersen W, Hohmann G, Pufe T, et al. 2004. Structure of the human tibialis posterior tendon. *Arch Orthop Trauma Surg* 124:237-242.

79. Petersen W, Laprell H. 2000. Insertion of autologous tendon grafts to the bone: a histological and immunohistochemical study of hamstring and patellar tendon grafts. *Knee Surg Sports Traumatol Arthrosc* 8:26-31.

80. Petersen W, Stein V, Bobka T. 2000. Structure of the human tibialis anterior tendon. *J Anat* 197 Pt 4:617-625.

81. Delcroix GJ, Kaimrajh DN, Baria D, et al. 2013. Histologic, biomechanical, and biological evaluation of fan-folded iliotibial band allografts for anterior cruciate ligament reconstruction. *Arthroscopy* 29:756-765.

82. Cetti R, Junge J, Vyberg M. 2003. Spontaneous rupture of the Achilles tendon is preceded by widespread and bilateral tendon damage and ipsilateral inflammation. *Acta Orthop Scand* 74:78.

For Peer Review

Structural variations of tendons.

83. Hefferan SA, Blaker CL, Ashton DM, et al. 2021. Histological Variations in Aged Human Tendons. ORS 2021 Annual Meeting. Virtual All Access (online): Orthopaedic Research Scociety (ORS) p. 1.

84. Hefferan SA. 2020. Histological Characteristics of Different Tendons in Humans of Advanced Age. Honours Thesis, The University of Sydney.

85. Choi RK, Smith MM, Martin JH, et al. 2016. Chondroitin sulphate glycosaminoglycans contribute to widespread inferior biomechanics in tendon after focal injury. *J Biomech* 49:2694-2701.

86. Smith MM, Sakurai G, Smith SM, et al. 2008. Modulation of aggrecan and ADAMTS expression in ovine tendinopathy induced by altered strain. *Arthritis Rheum* 58:1055-1066.

87. Tsang AS, Dart AJ, Biasutti SA, et al. 2019. Effects of tendon injury on uninjured regional tendons in the distal limb: An in-vivo study using an ovine tendinopathy model. *PLoS One* 14:e0215830.

88. Ackerman JE, Bah I, Jonason JH, et al. 2017. Aging does not alter tendon mechanical properties during homeostasis, but does impair flexor tendon healing. *J Orthop Res* 35:2716-2724.

89. Kohler J, Popov C, Klotz B, et al. 2013. Uncovering the cellular and molecular changes in tendon stem/progenitor cells attributed to tendon aging and degeneration. *Aging Cell* 12:988-999.

90. Smith RKW, Birch HL, Goodman S, et al. 2002. The influence of ageing and exercise on tendon growth and degeneration—hypotheses for the initiation and prevention of strain-induced tendinopathies. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 133:1039-1050.

For Peer Review

Structural variations of tendons.

91. Thorpe CT, Streeter I, Pinchbeck GL, et al. 2010. Aspartic acid racemization and collagen degradation markers reveal an accumulation of damage in tendon collagen that is enhanced with aging. *J Biol Chem* 285:15674-15681.

92. Glait SA, Mahure S, Loomis CA, et al. 2018. Regional histologic differences in the long head of the biceps tendon following subpectoral biceps tenodesis in patients with rotator cuff tears and SLAP lesions. *Knee Surg Sports Traumatol Arthrosc* 26:2481-2489.

93. Magnusson SP, Kjaer M. 2019. The impact of loading, unloading, ageing and injury on the human tendon. *The Journal of physiology* 597:1283-1298.

94. Riley G. 2004. The pathogenesis of tendinopathy. A molecular perspective. *Rheumatology (Oxford)* 43:131-142.

95. Milgrom C, Schaffler M, Gilbert S, et al. 1995. Rotator-cuff changes in asymptomatic adults. The effect of age, hand dominance and gender. *J Bone Joint Surg Br* 77:296-298.

96. Riley GP, Goddard MJ, Hazleman BL. 2001. Histopathological assessment and pathological significance of matrix degeneration in supraspinatus tendons. *Rheumatology (Oxford)* 40:229-230.

97. Aström M, Rausing A. 1995. Chronic Achilles tendinopathy. A survey of surgical and histopathologic findings. *Clin Orthop Relat Res*:151.

98. Tallon C, Maffulli N, Ewen SW. 2001. Ruptured Achilles tendons are significantly more degenerated than tendinopathic tendons. *Med Sci Sports Exerc* 33:1983-1990.

99. Maffulli N, Barrass V, Ewen SW. 2000. Light microscopic histology of achilles tendon ruptures. A comparison with unruptured tendons. *Am J Sports Med* 28:857-863.

100. Maffulli N, Del Buono A, Spiezia F, et al. 2012. Light microscopic histology of quadriceps tendon ruptures. *Int Orthop* 36:2367-2371.

For Peer Review

Structural variations of tendons.

101. Longo UG, Franceschi F, Ruzzini L, et al. 2008. Histopathology of the supraspinatus tendon in rotator cuff tears. *Am J Sports Med* 36:533-538.

102. Harris NL, Smith DA, Lamoreaux L, et al. 1997. Central quadriceps tendon for anterior cruciate ligament reconstruction. Part I: Morphometric and biomechanical evaluation. *Am J Sports Med* 25:23-28.

103. Maffulli N, Testa V, Capasso G, et al. 2004. Similar histopathological picture in males with Achilles and patellar tendinopathy. *Med Sci Sports Exerc* 36:1470-1475.

104. Klatte-Schulz F, Minkwitz S, Schmock A, et al. 2018. Different Achilles Tendon Pathologies Show Distinct Histological and Molecular Characteristics. *Int J Mol Sci* 19.

105. Choi RK, Smith MM, Smith S, et al. 2019. Functionally distinct tendons have different biomechanical, biochemical and histological responses to in vitro unloading. *J Biomech* 95:109321.

106. Shadwick RE. 1990. Elastic energy storage in tendons: mechanical differences related to function and age. *J Appl Physiol (1985)* 68:1033-1040.

107. Robinson PS, Lin TW, Jawad AF, et al. 2004. Investigating tendon fascicle structure-function relationships in a transgenic-age mouse model using multiple regression models. *Ann Biomed Eng* 32:924-931.

108. Johnson GA, Tramaglini DM, Levine RE, et al. 1994. Tensile and viscoelastic properties of human patellar tendon. *J Orthop Res* 12:796-803.

109. Shani RH, Umpierez E, Nasert M, et al. 2016. Biomechanical Comparison of Quadriceps and Patellar Tendon Grafts in Anterior Cruciate Ligament Reconstruction. *Arthroscopy* 32:71-75.

For Peer Review

Structural variations of tendons.

110. Buckley MR, Sarver JJ, Freedman BR, et al. 2013. The dynamics of collagen uncrimping and lateral contraction in tendon and the effect of ionic concentration. *J Biomech* 46:2242-2249.

111. Miller KS, Connizzo BK, Feeney E, et al. 2012. Examining differences in local collagen fiber crimp frequency throughout mechanical testing in a developmental mouse supraspinatus tendon model. *J Biomech Eng* 134:041004.

112. Shearer T. 2015. A new strain energy function for modelling ligaments and tendons whose fascicles have a helical arrangement of fibrils. *J Biomech* 48:3017-3025.

113. Aune AK, Hukkanen M, Madsen JE, et al. 1996. Nerve regeneration during patellar tendon autograft remodelling after anterior cruciate ligament reconstruction: an experimental and clinical study. *J Orthop Res* 14:193-199.

114. Sato F, Masuda Y, Suzuki D, et al. 2024. Biomechanical analysis of tendon regeneration capacity of Iberian ribbed newts following transection injury: Comparison to a mouse model. *J Orthop Res* 42:607-617.

115. Loppini M, Longo UG, Niccoli G, et al. 2015. Histopathological scores for tissue-engineered, repaired and degenerated tendon: a systematic review of the literature. *Curr Stem Cell Res Ther* 10:43-55.

116. Movin T, Gad A, Reinholt FP, et al. 1997. Tendon pathology in long-standing achillodynia. Biopsy findings in 40 patients. *Acta Orthop Scand* 68:170-175.

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Structural variations of tendons.

Figure Legends

Figure 1. Flow diagram of literature inclusion and screening workflow. *Diagram is modified from a readily accessible template[14].*

Figure 2. Light microscopy images showing variation in 'normal' human tendons. Histological samples are from cadaveric lower-leg tendons of female donors aged 71 (**a-b**) and 78 (**c-d**) years old. Polarised Picrosirius Red stained sections (**a-b**) depicting regional differences in crimp wavelength between matched proximal (**a**) and distal (**b**) regions of the Tibialis Anterior tendon. Toluidine Blue stained sections **(c-d)** depicting regional differences in proteoglycan content (purple staining) between matched proximal (**c**) and distal (**d**) regions of the Achilles tendon. *Longitudinally orientated, formalin-fixed, paraffin embedded histological sections at 4.5 µm thick. All images: 200x magnification; scale bars = 100µm.*

Figure 3. Light microscopy images showing cell density variation in 'normal' human tendons. Histological samples are from the distal region of cadaveric lower-leg Flexor Hallucis Longus (FHL; **a,c,e,g**) and Tibialis Anterior (TA; **b,d,f,h**) tendons. Female donors aged 71 (**a-b**) and 99 (**e-f**) years old, and male donors aged 68 (**c-d**) and 95 (**g-h**) years old. Note differences in relative cell density between younger (**a-d**) and older (**e-h**) donors. *Longitudinally orientated, formalin-fixed, paraffin embedded histological sections at 4.5 µm thick. All images: 200x magnification; scale bars = 100µm.*

Textbox 1. Summary of knowledge gaps in the histological analysis of tendons.

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Structural variations of tendons.

Table Legends

Table 1: Eligibility and exclusion criteria for systematic search strategy (screening stages).

Table 2: Final inclusion criteria for publications to be reviewed. Excluded records ('noted exclusions') remain identified in Table S-1 as additional supplementary knowledge explored in the field.

Table 3. Summary of papers exploring statistical comparisons relating to *Tendon*, *Region*, *Age*, *Sex* or *Other 'variables of interest'*. Tendons/Ligaments included in the study but not statistically assessed for any of the variables are greyed-out. *Y = statistical analysis is included for the relevant variable. D = data/graphical representation of data is included for the relevant variable. Refer to Table 5 for acronym definitions.*

Table 4. Summary of papers by variable (statistical comparisons). *Refer to Table 5 for acronym definitions.*

Table 5. List and definition of acronyms included in manuscript and tables.

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9.4 Appendices for ~ Chapter 3 ~

9.4.1 Additional Methodology for the Human Tissue Atlas

During the research conducted for this thesis, a pilot study was initiated on the 'outcome 3' samples (refer to *Section 3.2.1* and *Figure 3.2.A* in *Chapter 3* for clarification) collected as part of the research laboratory's broader database of Human Musculoskeletal Tissue Characteristics (human tissue "atlas"). Utilising a subset of the tendons included in the human tissue atlas, the multiscale structural architecture of the tendons was explored via Scanning Electron Microscopy (SEM) and Differential Interference Contrast (DIC) microscopy techniques. The methods employed are listed below as reference to support images obtained from this pilot study which were included in *Chapter 7* of this thesis to support additional discussion of tendon/ligament structural characteristics.

9.4.1.1 *Multiscale Structure Pilot Study: Included Samples*

Specific details regarding donor and tissue procurement are outlined in *Chapter 3* of this thesis. Following retrieval (see *Section 3.2.2*), tendons/ligaments were immediately transected using a sharp scalpel and samples allocated to their respective outcome – for the electron microscopy study outcome (*Figure 3.2.A*), samples approximately 10mm long were collected and immediately fixed and stored in 10% neutral buffered formalin (room temperature) in labelled jars.

For this pilot study, 42 tissue samples in total were included: 7 different tendons from 6 donors (donors 1-6 as identified in *Table 3.2.a*). The tendons utilised consisted of: Achilles Tendon (AT), Tibialis Posterior (TP), Tibialis Anterior (TA), Flexor Hallucis Longus (FHL), Extensor Hallucis Longus (EHL), Flexor Digitorum Profundus of the 2nd Digit (FDP-2), and Extensor Digitorum Communis of the 2nd Digit (EDC-2).

9.4.1.2 *Tissue Preparation*

Tendon samples were removed from the 10% neutral buffered formalin jars and rinsed with tap water to clear excess formalin prior to cryo-sectioning on a Leica SM2000 R Sliding (Sledge) Microtome (Leica Microsystems, Wetzlar, Germany) with Personna 89-0043 Coated Injector Blades. For sectioning, a custom aluminium block was lined with Tissue-Tek® O.C.T. (optimal cutting temperature) Compound (Sakura Finetek, USA) and the tendon sample placed upright (cross-sectional faces to and away from block) and lowered into liquid nitrogen (N_{aq}) using a clamp until the O.C.T. fully set (opaque white in appearance). The block with mounted specimen was positioned and adjusted to be in plane with the blade.to cut cross-section slices. Rough trimming was conducted at 30 µm, and then once a clean face was obtained, sections were collected at 100 µm and stored in saline in labelled well-plates. The mounted specimen was maintained at cutting temperature during sectioning with N_{aq} (carefully poured over specimen and mount block as required). Approximately 4-5 sections were collected from each tendon sample to ensure adequate tissue availability for DIC and SEM studies. These were stored in saline solution in a commercial fridge (approximately 3°C) until further processing.

9.4.1.3 *DIC Microscopy*

Following sectioning, sections for DIC were imaged using a Nikon ECLIPSE Ni-E upright microscope with a Nikon DS-Ri2 digital camera (Nikon Instruments Inc., USA). First, a drop of saline was places on a standard glass slide and the tissue section carefully positioned and mounted (wet mounting) by covering with a glass coverslip. The microscope auto-focusing and scanning function was used to capture whole-section DIC images at 100x magnification (CFI Plan Apochromat Lambda D series 10x/0.45 objective for DIC observation) on NIS-Element Basic Research imaging software (Nikon Instruments Inc., USA).

9.4.1.4 *SEM Observation*

Sectioned tissue for SEM assessment were dehydrated in changes of ethanol (%v/v) over 3 hours using 2mL of each ethanol concentration in well plates: 15 minutes each of 50%, 60%, 80%, 90%, 100%, and 100%, and then changed to a third 100% for storage. Once all samples were dehydrated, sections were then dried in covered well plates containing 2mL of hexamethyldisilazane[417-419] (HMDS, conducted in a fume hood) for approximately 8 minutes before blotting dry on KimTech tissues to minimise fibre contamination.

Dehydrated and dried samples were then mounted on aluminium SEM studs using conductive carbon adhesive tape (Agar Scientific Ltd., UK) and sputter coated with platinum in vacuum (Quorum Q150R S Sputter Coater, Electron Microscopy Sciences, USA). Scans were then captured using a Philips XL 30 FEG Scanning Electron Microscope using a secondary electron (SE) Everhardt-Thornley detector (ETD) at various magnifications. Sections were scanned topographically and whole-section images stitched together using Adobe Illustrator.

9.4.1.5 *Project Acknowledgements*

Thank you to Prof. Ashvin Thambyah, Catherine Hobbis, and colleagues at the University of Auckland for hosting and mentoring this research project, and thank you to the Skipper and Jack Jacobs Charitable Trust Fund (North Foundation) for financial support.

9.5 Appendices for ~ Chapter 4 ~

9.5.1 Permissions for Image Inclusions (Figure 4.3.A)

The following section details the reprint permissions for the histological images included in Figure 4.3.A. (referred to as "Thesis Chapter 4 Figure 1" for reprint license orders).

Image I-III, Delcroix et al. (2013)[204]. License Number: 5892371341582. License date: Oct 19, 2024. Licensed Content Publisher: Elsevier.

Image IV Kartus et al. (2000)[171]. License Number: 5892380423745. License date: Oct 19, 2024. Licensed Content Publisher: Sage Publications.

Image V, Tsang et al. (2019)[199]. This is an open access article distributed under the terms of the Creative Commons Attribution License.

Image VI, González et al. (2016)[205]. This is an open access article distributed under the terms of the Creative Commons CC BY license.

Image VII, Park et al. (2019)[206]. License Number: 5892390203704. License date: Oct 19, 2024. Licensed Content Publisher: Springer Nature.

Image VIII, Ahlen et al. (2014)[207]. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 License.

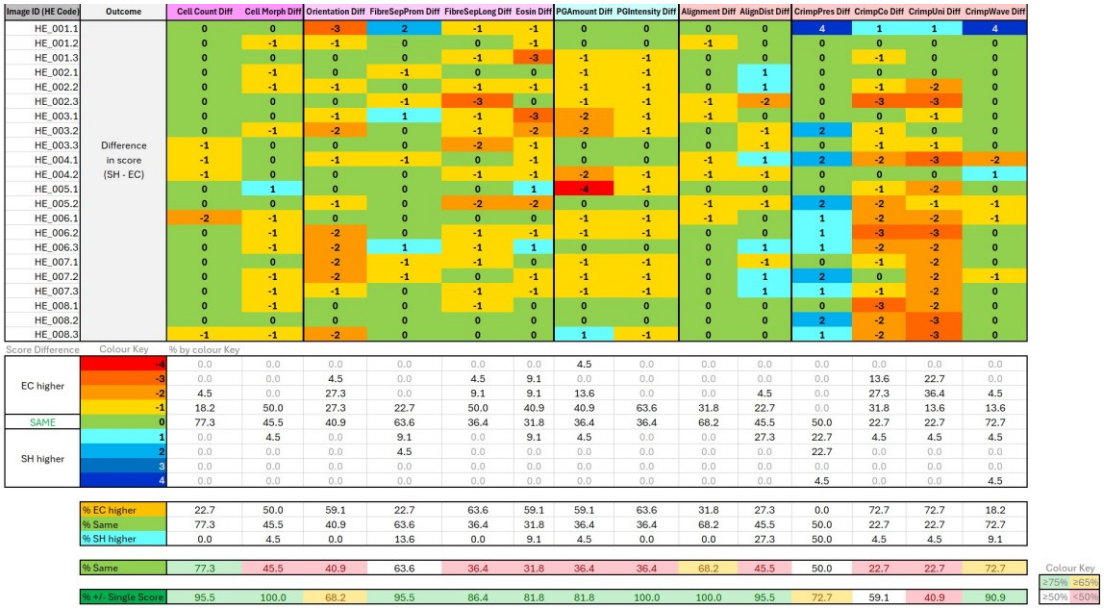
Image IX, Maffulli et al. (2008)[202]. License Number: 5892400646129. License date: Oct 19, 2024. Licensed Content Publisher: Wolters Kluwer Health, Inc.

Image X, Hapa et al. (2009)[198]. License Number: 5892400996759. License date: Oct 19, 2024. Licensed Content Publisher: Springer Nature.

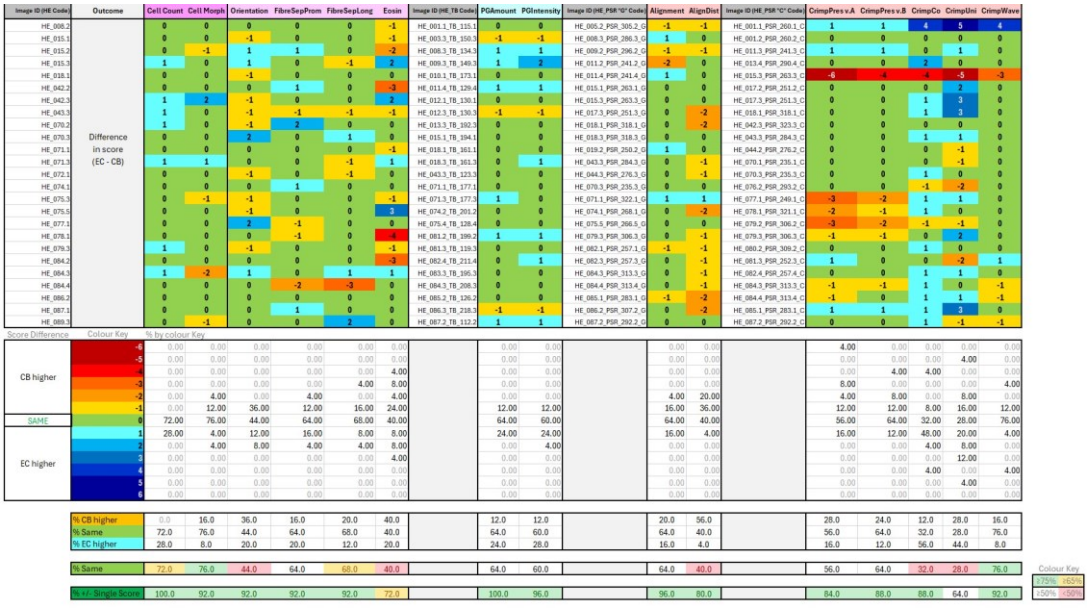
Image XI-XII, Bolam et al. (2021)[208]. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY).

9.5.2 Scoring System Version Tests

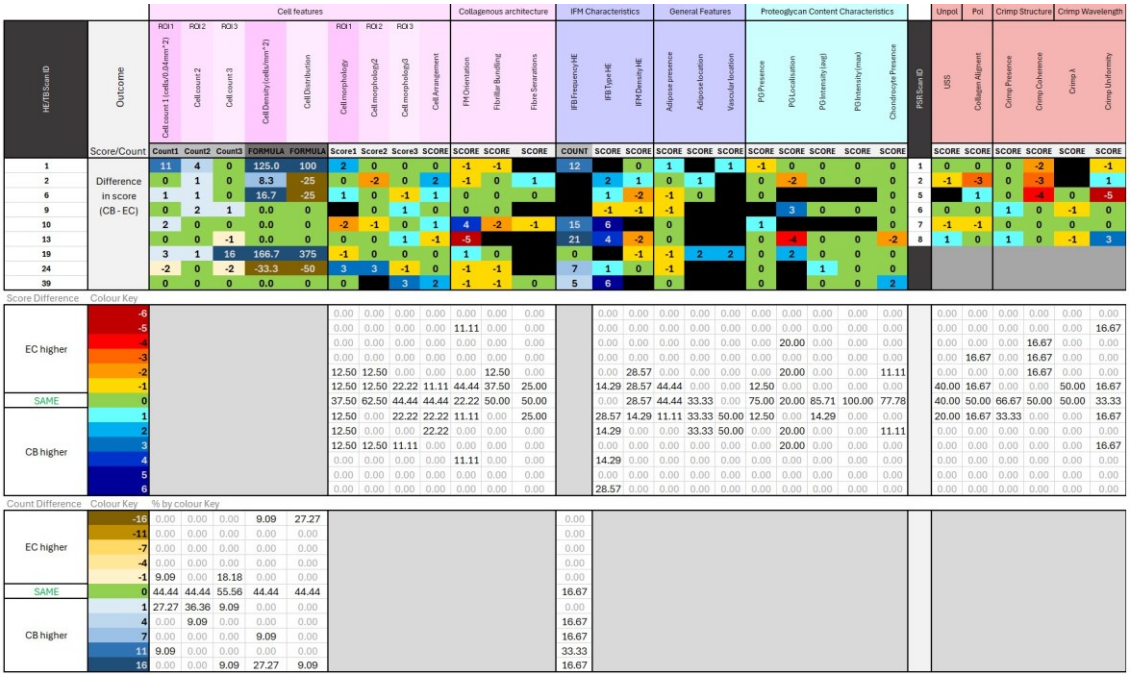
The following images depict agreement levels between raters for different versions of the scoring system tested during the development stages.



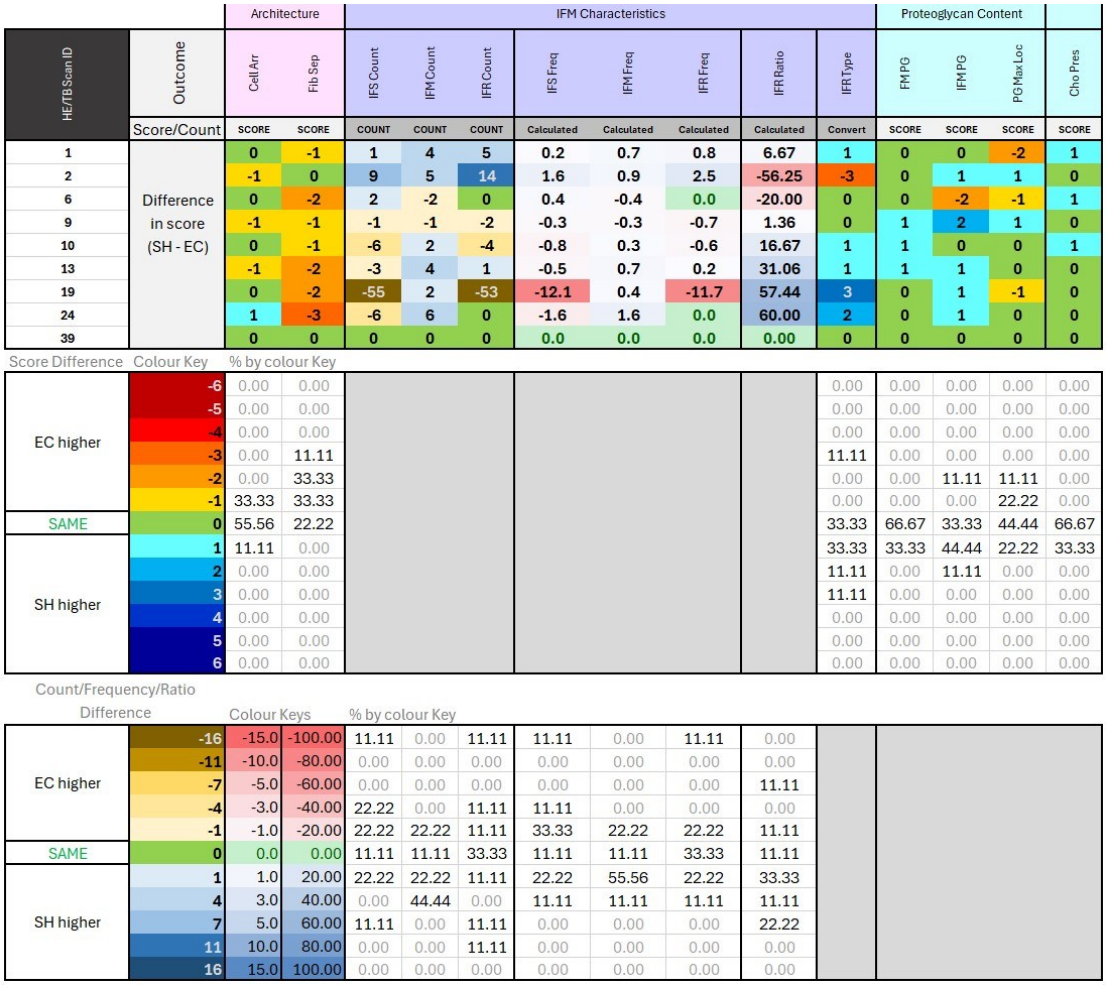
Supp. Figure 9.5.A Representation of scoring results of Round 1 inter-rater comparisons (SH vs EC) of SSS Version 2 test application to subset of 22 dataset images (advanced age human lower limb tendons).



Supp. Figure 9.5.B Representation of scoring results of Round 2 inter-rater comparisons (CB vs EC) of SSS Version 2 test application to subset of 22 dataset images (advanced age human lower limb tendons).



Supp. Figure 9.5.C Representation of scoring results of Round 1 inter-rater comparisons (CB vs EC) of SSS Version 3 test application to subset of 9 human tendon dataset images. Black cells indicate missing values where one or more raters were unable/unconfident in deciding a score.



		Architecture		IFM Characteristics								Proteoglycan Content			
HE/TB Scan ID	Outcome	Cell Arr	Fib Sep	IFS Count	IFM Count	IFR Count	IFS Freq	IFM Freq	IFR Freq	IFR Ratio	IFR Type	FM PG	IFM PG	PG Max Loc	Cho Pres
	Score/Count	SCORE	SCORE	COUNT	COUNT	COUNT	Calculated	Calculated	Calculated	Calculated	Convert	SCORE	SCORE	SCORE	SCORE
1	Difference in score (SH - EC)	0	-1	1	4	5	0.2	0.7	0.8	6.67	1	0	0	-2	1
2		-1	0	9	5	14	1.6	0.9	2.5	-56.25	-3	0	1	1	0
6		0	-2	2	-2	0	0.4	-0.4	0.0	-20.00	0	0	-2	-1	1
9		-1	-1	-1	-1	-2	-0.3	-0.3	-0.7	1.36	0	1	2	1	0
10		0	-1	-6	2	-4	-0.8	0.3	-0.6	16.67	1	1	0	0	1
13		-1	-2	-3	4	1	-0.5	0.7	0.2	31.06	1	1	1	0	0
19		0	-2	-55	2	-53	-12.1	0.4	-11.7	57.44	3	0	1	-1	0
24		1	-3	-6	6	0	-1.6	1.6	0.0	60.00	2	0	1	0	0
39		0	0	0	0	0	0.0	0.0	0.0	0.00	0	0	0	0	0

Score Difference	Colour Key	% by colour Key													
EC higher	-6	0.00	0.00								0.00	0.00	0.00	0.00	0.00
	-5	0.00	0.00								0.00	0.00	0.00	0.00	0.00
	-4	0.00	0.00								0.00	0.00	0.00	0.00	0.00
	-3	0.00	11.11								11.11	0.00	0.00	0.00	0.00
	-2	0.00	33.33								0.00	0.00	11.11	11.11	0.00
	-1	33.33	33.33								0.00	0.00	0.00	22.22	0.00
SAME	0	55.56	22.22								33.33	66.67	33.33	44.44	66.67
SH higher	1	11.11	0.00								33.33	33.33	44.44	22.22	33.33
	2	0.00	0.00								11.11	0.00	11.11	0.00	0.00
	3	0.00	0.00								11.11	0.00	0.00	0.00	0.00
	4	0.00	0.00								0.00	0.00	0.00	0.00	0.00
	5	0.00	0.00								0.00	0.00	0.00	0.00	0.00
	6	0.00	0.00								0.00	0.00	0.00	0.00	0.00

Count/Frequency/Ratio Difference		Colour Keys		% by colour Key								
EC higher	-16	-15.0	-100.00	11.11	0.00	11.11	11.11	0.00	11.11	0.00		
	-11	-10.0	-80.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	-7	-5.0	-60.00	0.00	0.00	0.00	0.00	0.00	0.00	11.11		
	-4	-3.0	-40.00	22.22	0.00	11.11	11.11	0.00	0.00	0.00		
	-1	-1.0	-20.00	22.22	22.22	11.11	33.33	22.22	22.22	11.11		
SAME	0	0.0	0.00	11.11	11.11	33.33	11.11	11.11	33.33	11.11		
SH higher	1	1.0	20.00	22.22	22.22	11.11	22.22	55.56	22.22	33.33		
	4	3.0	40.00	0.00	44.44	0.00	11.11	11.11	11.11	11.11		
	7	5.0	60.00	11.11	0.00	11.11	0.00	0.00	0.00	22.22		
	11	10.0	80.00	0.00	0.00	11.11	0.00	0.00	0.00	0.00		
	16	15.0	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		

Supp. Figure 9.5.D Scoring results of Round 2 inter-rater comparisons (SH vs EC) of SSS Version 3 test application to subset of 9 human tendon dataset images.

9.5.3 Version 3.2 of the Scoring System

The following tables/figures outline the scoring system (v3.2) as applied by SH and CBL in evaluating inter- and intra-rater agreement of the outcome measures as detailed in *Section 4.4.4*.

Supp. Table 9.5.a Scoring matrices from version 3.2 for assessing characteristics of cell populations from H&E-stained sections.

Tenocyte Population
Haematoxylin & Eosin
The structure of the tendon-proper is typically comprised of two primary sub-regions (excluding paratenon): 1. **The fascicular matrix (FM):** is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure. 2. **The interfascicular region (IFR):** the regions between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance. ***Note: The following scoring matrices refer to the tenocyte population within the FM only (i.e. excluding IFR cells).***

Outcome – Cell Density **Method:** Count the number of cells within a region of interest (ROI) in the FM area, and average per mm^2.	
__	cells/mm^2
Cell Density Score *– Cell density can be scored using the below scoring matrix.*	
0	No cells present.
1	1-100 cells/mm^2
2	101-250 cells/mm^2
3	251-500 cells/mm^2
4	501-750 cells/mm^2
5	751-1000 cells/mm^2
6	1001-1500 cells/mm^2
7	> 1500 cells/mm^2

Outcome – Cell Distribution **NOTE:** Cell density must be assessed across multiple ROIs to determine Cell Distribution. **Method:** Determine the difference in cell density between ROIs (calculate as *Maximum minus Minimum* Cell Density).	
__	cells/mm^2 difference

Outcome – Cell Morphology **Method:** Assess the morphology (shape) of the tenocyte nuclei (defined below), and score the predominant shape. Cell (nuclear) morphologies are categorised into three shapes based on their aspect ratio (width:length): **Spindle-shaped nuclei** – (fusiform), long, skinny, streak-like. Aspect ratio >1:5 **Cigar-shaped nuclei** – (ovoid), elongated but plump. Aspect ratio between 1:3 and 1:5 **Spherical nuclei** – (spheroid), rounded, egg-shaped, very plump. Aspect ratio < 1:3 Illustrations of the different cell (nuclei) morphologies and their discerning aspect ratios can be seen in Figure "M".	
NS	N/A – No cells present to score *(Cell Density = 0)*
1	Mixed morphology of cells (spindle-shaped, cigar-shaped and spherical all <=50% each)
2	**> 50%** of cells are **spindle-shaped**
3	**> 50%** of cells are **cigar-shaped**
4	**> 50%** of cells are **spherical**

Outcome – Cell Arrangement **Method:** Assess and score the arrangement of tenocytes relative to one-another (defined below). Cells are categorised into two major arrangement types: **Solitary** – cells show no particular arrangement relative to neighbouring cells; isolated/independent of each other. **Chains** – cells **(≥ 3)** are arranged longitudinally in-series within their inter-fibre space and are located within 2 (average) nuclei-lengths apart. Examples of the presence of tenocytes arranged in chains can be seen in Figure "C."	
NS	N/A – No cells present to score *(Cell Density = 0)*
1	Cells are solitary; only occasional (up to 5% of) cells arranged in chains.
2	**< 1/3** of cells arranged in **chains**.
3	**1/3 – 2/3** of cells arranged in **chains**.
4	**> 2/3** of cells arranged in **chains**.

Figure "M". Visual guide for the three different cell (nuclei) morphologies as determined by their aspect ratio (width:length).

Figure "C." Reference example of tenocytes arranged in chains. Black boxes indicate tenocyte chains as per the defined criteria.

Supp. Table 9.5.b Scoring matrices from version 3.2 for assessing characteristics of collagenous architecture from H&E-stained sections.

Collagenous Architecture

Haematoxylin & Eosin

The structure of the tendon-proper is typically comprised of two primary sub-regions (excluding paratenon):

1. **The fascicular matrix (FM):** is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure. The collagenous FM is hierarchically structured as follows:
   - The *collagen molecule* (tropocollagen) – primarily fibrillar collagen type-I
   - The *microfibril* – aggregates of collagen molecules
   - The *fibril* – formed by microfibrils; fibrils typically range from 50-500 nm in diameter
   - The *fibre* – bundles of fibrils, typically around 10 µm in diameter
   - The *fascicle* – bundles of fibres; the major hierarchical unit second to the *tendon* itself.
2. **The interfascicular region (IFR):** the regions between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance:
   - **Interfascicular matrix (IFM)** comprises the *presence* of connective tissue between/dividing fascicle units.
   - **Interfascicular space (IFS)** comprises the *absence* of connective tissue between/dividing fascicle units; NOTE – includes directly adjacent fascicles with no visible white space (or matrix). Interfascicular cells may be present.

Outcome – Fascicular Matrix (FM) Organisation

Method:

Score the proportion of FM area associated with organised vs disorganised FM structure (defined below).

FM organisation is categorised into two major definitions based on the alignment/orientation of the collagenous structure:

Organised = Highly unified/parallel collagenous FM structure aligned along the primary tendon axis.

Disorganised = Variable or negligible alignment of the collagenous FM structure with the primary tendon axis; collagenous FM structure alignment is haphazard, swirling, squashed, in disarray etc.

0	All (100%) of the collagenous FM structure is **organised**	None (0%) of the collagenous FM structure is **disorganised**.
1	>95% of the FM structure is **organised**	<5% of the FM structure is **disorganised**
2	76-95% of the FM structure is **organised**	5-24% of the FM structure is **disorganised**
3	51-75% of the FM structure is **organised**	25-49% of the FM structure is **disorganised**
4	26-50% of the FM structure is **organised**	50-74% of the FM structure is **disorganised**
5	5-25% of the FM structure is **organised**	75-95% of the FM structure is **disorganised**
6	<5% of the FM structure is **organised**	>95% of the FM structure is **disorganised**

Outcome – Fibrillar Bundling ^

^ Refers to ***intra-fibre*** spaces, where spaces/separations are *between fibrils,* seen as visible spacing ***within*** *the observable fibres.* ***Fibrils*** typically range from 50-500 nm in diameter. ***Fibres*** are bundles of fibrils, typically around 10 µm in diameter.

Method:

Score the proportion of FM area associated with bundled vs loose packing (defined below) of the fibrillar structure.

Best assessed at 200x magnification.

Arrangement of collagen ***fibrils*** is categorised into two major definitions based on their packing density:

Bundled = neighbouring fibrils are all tightly adhered (highly dense), uniformly aligned, and form/integrate into distinct fibre structures.

Loose = neighbouring fibrils are dispersed / show spreading from one another, segregating out from the apparent fibre structure or preventing clear identification of distinct fibre-level structures within the tendon hierarchical organisation.

Examples of bundled versus loose fibrillar structure can be seen in Figure "F."

1	>95% collagen fibrils are **bundled** tightly	<5% of collagen fibrils are **loose**/dispersed
2	76-95% collagen fibrils are **bundled** tightly	5-24% of collagen fibrils are **loose**/dispersed
3	51-75% collagen fibrils are **bundled** tightly	25-49% of collagen fibrils are **loose**/dispersed
4	26-50% collagen fibrils are **bundled** tightly	50-74% of collagen fibrils are **loose**/dispersed
5	5-25% collagen fibrils are **bundled** tightly	75-95% of collagen fibrils are **loose**/dispersed
6	<5% collagen fibrils are **bundled** tightly	>95% collagen fibrils are **loose**/dispersed

Outcome – Fibre Separations^ Frequency

NOTE:

'*Fibre Separations'* can only be assessed in areas with tightly bundled fibrils covering >25% tendon area ('*Fibrillar Bundling'* ≤ 4).

^ Refers to ***inter-fibre*** separations, which are the visible boundaries/spacing *between* neighbouring fibres. They exclude IFR boundaries at fascicle edges. ***Fibrils*** typically range from 50-500 nm in diameter. ***Fibres*** are bundles of fibrils, typically around 10 µm in diameter.

Method:

Along a transverse line*, score the frequency of separations between fibres intersecting the line.

Best assessed at 200x magnification.

** The same line is used for outcomes 'Fibre Separations Frequency' and 'Fibre Separations Type'.*

The placement of transverse line should cover areas of tightly bundled fibrils; all scorers to use the same region (length and position; selection needs to be pre-determined and documented).

NS	*Intra- versus Inter-fibre separations are indistinguishable. All fibrils are loose/dispersed within sample. (Fibrillar Bundling score ≥ 5)*	
0	All (100%) of collagen fibres exhibit *no separations (neighbouring fibres are adjoined).*	No (0%) of collagen fibres exhibit visible separations.
1	>95% of collagen fibres exhibit no separations.	<5% of collagen fibres exhibit visible separations.
2	76-95% of collagen fibres exhibit *no separations.*	5-24% of collagen fibres exhibit visible separations.
3	51-75% of collagen fibres exhibit *no separations.*	25-49% of collagen fibres exhibit visible separations.
4	26-50% of collagen fibres exhibit *no separations.*	50-74% of collagen fibres exhibit visible separations.
5	5-25% of collagen fibres exhibit *no separations*.	75-95% of collagen fibres exhibit visible separations.
6	<5% of collagen fibres exhibit *no separations*.	>95% collagen fibres exhibit visible 5separations.

Outcome – Fibre Separations^ Type

NOTE:

'*Fibre Separations'* can only be assessed in areas with tightly bundled fibrils covering >25% tendon area ('*Fibrillar Bundling'* < 5).

^ Refers to ***inter-fibre*** separations, which are the visible boundaries/spacing *between* neighbouring fibres. They exclude IFR boundaries at fascicle edges. ***Fibrils*** typically range from 50-500 nm in diameter. ***Fibres*** are bundles of fibrils, typically around 10 µm in diameter.

Method:

Along a transverse line*, assess the most common (predominant) separation type between neighbouring fibres (where present).

Best assessed at 200x magnification.

** The same line is used for outcomes 'Fibre Separations Frequency' and 'Fibre Separations Type'.*

The placement of transverse line should cover areas of tightly bundled fibrils; all scorers to use the same region (length and position; selection needs to be pre-determined and documented).

NS	*Intra- versus Inter-fibre separations are indistinguishable. All fibrils are loose/dispersed within sample. (Fibrillar Bundling score ≥ 5)*
NS	*No fibre separations to assess. (Fibre Separations Frequency score = 0)*
1	Majority (> 50%) of fibres have separations that are predominantly "short", averaging <50 µm in length.
2	Majority (> 50%) of fibres have separations that are predominantly "medium", averaging 50-100 µm in length.
3	Majority (> 50%) of fibres have separations that are predominantly "long", averaging 100-150 µm in length.
4	Majority (> 50%) of fibres have separations that are predominantly "very long", averaging >150 µm in length.

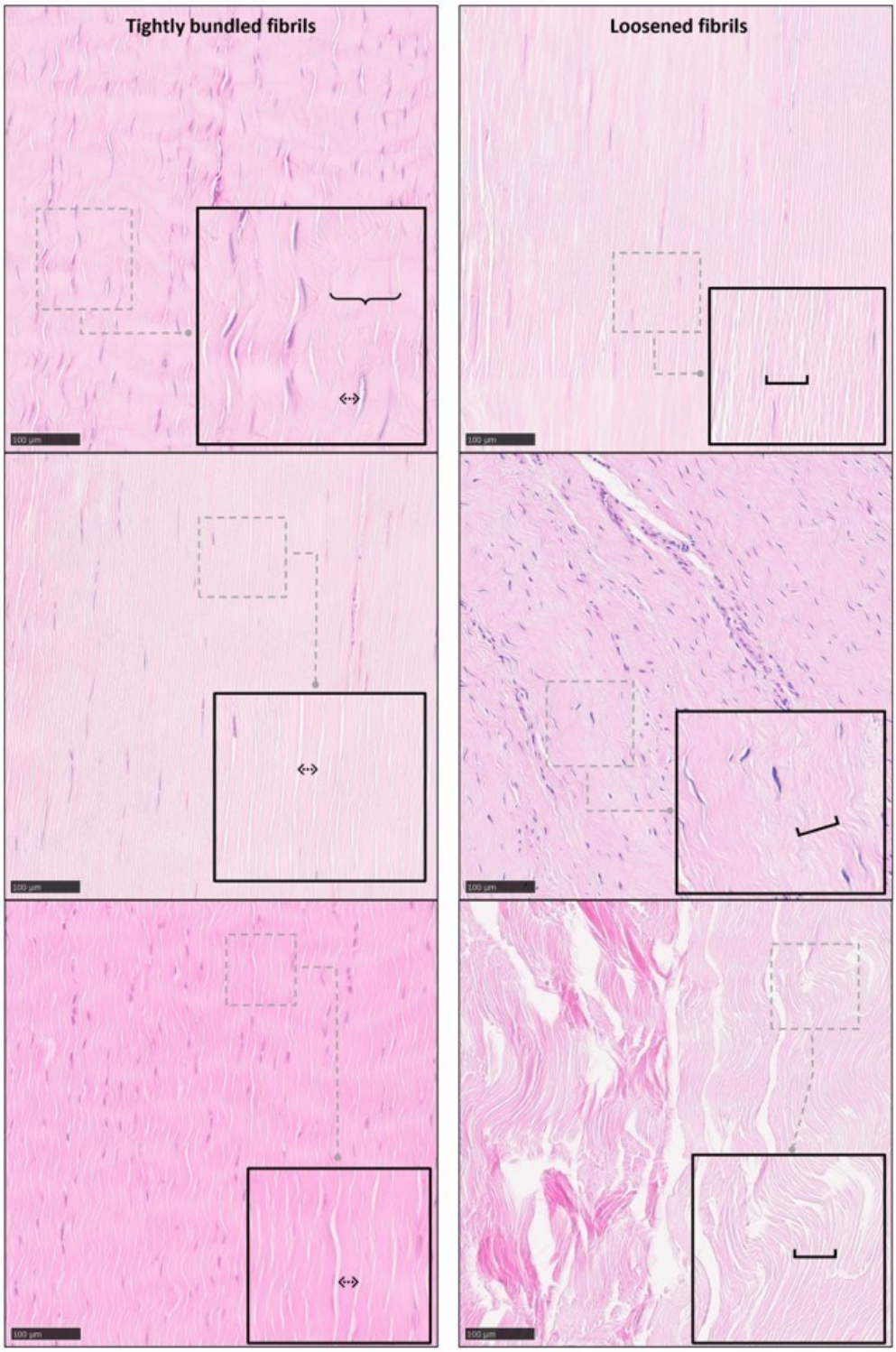


Figure "F." Reference examples of tightly bundled (left frames) versus loose fibrillar structure (right frames). **Left frames:** Tightly bundled fibrils create distinct fibre structures between *inter*-fibre spaces (fibres identified by dotted lines with arrow heads) or homogenous, dense fibre groups (within braces). **Right frames:** Loosened fibrils display *intra*-fibre spacing (within square brackets), diminishing identification of fibre boundaries.

Supp. Table 9.5.c Scoring matrices from version 3.2 for assessing characteristics of the tendon interfascicular region (IFR) from suitably stained sections that identify IFR features. **IFR characteristics can be differentiated from different standard histochemical stains. Selection of staining technique can be chosen as available to the laboratory. Recommendation immunohistochemical staining identification should be enlisted where available.*

IFR Characteristics
(suitable staining technique for identification of IFR features)
The **interfascicular region (IFR)**[#] is the region(s) between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance, as follows: • **Interfascicular matrix (IFM)** comprises the *presence* of connective tissue between/dividing fascicle units. • **Interfascicular space (IFS)** comprises the *absence* of connective tissue between/dividing fascicle units; NOTE – includes directly adjacent fascicles with no visible white space (or matrix). Interfascicular cells may be present. [#]*excluding paratenon*

Outcome – Interfascicular Matrix (IFM) Frequency	
Method: Across a transverse line, count the number of IFMs intersecting the line* and take an average per 100 µm. **Assess each respective IFR within ± 150 µm of the transverse line; count only if* ***≥ 50%*** *of the IFR within the ROI is IFM.* Examples of interfascicular regions (IFRs) classified as interfascicular matrix (IFM) can be seen in Figure "I."	
__	IFM regions / 100 µm

Outcome – Interfascicular Space (IFS) Frequency	
Method: Across a transverse line, count the number of IFSs intersecting the line* and take an average per 100 µm. **Assess each respective IFR within ± 150 µm of the transverse line; count only if* ***> 50%*** *of the IFR within the ROI is IFS.* Examples of interfascicular regions (IFRs) classified as interfascicular space (IFS) can be seen in Figure "I."	
__	IFS regions / 100µm

Outcome – Interfascicular Region (IFR) Frequency	
NOTE: IFR Frequency is determined by adding the IFS and IFM frequencies. Alternatively, it can be independently counted instead of the individual IFS and IFM frequencies (using the method below). **Method:** Across a transverse line, count the number of IFRs intersecting the line and take an average per 100 µm. Examples of interfascicular regions (IFR) can be seen in Figure "I."	
__	IFRs / 100µm

Outcome – Interfascicular Region (IFR) Type Proportions

Method:

Across a transverse line*, determine the proportion of IFRs represented by IFM versus IFS.

(Assessed as *Percent of IFRs representing IFM, or scored according to the matrix below*).

Assess each respective IFR within ± 150 µm of the transverse line; count as IFM if **≥50% of ROI or IFS if **>50%** of ROI.*

__	% of IFRs are ***IFM***.

IFR Proportion Score *– IFR proportion can alternatively be scored using the below scoring matrix.*

NS	N/A – no apparent IFRs to score *(IFR Frequency Count = 0).*	
0	None (0%) of interfascicular regions are IFM	All (100%) of interfascicular regions are IFS
1	<10% of interfascicular regions are IFM.	>90% of interfascicular regions are IFS
2	10-40% of interfascicular regions are IFM.	60-90% of interfascicular regions are IFS
3	41-60% of interfascicular regions are IFM.	40-59% of interfascicular regions are IFS
4	61-90% of interfascicular regions are IFM.	10-39% of interfascicular regions are IFS
5	>90% of interfascicular regions are IFM.	<10% of interfascicular regions are IFS
6	All (100%) of interfascicular regions are IFM.	None (0%) of interfascicular regions are IFS

Outcome – Interfascicular Matrix (IFM) Density

Method:

Across a transverse line*, assess the matrix density of IFMs present, and score the most frequent (predominant) type (defined below).

Assess each respective **IFM within ± 150 µm of the transverse line; categorise based on the appearance of the **majority of the matrix present** within the ROI.*

Regarding the matrix-containing region, IFM densities are categorised into three generalised types based on how compact the matrix material/substance is, regardless of the amount of matrix or number of cells between the fascicles:

Low density – loosely connected (dispersed, wispy) matrix substance present between neighbouring fascicles.

Moderate density – moderately connected matrix substance present between neighbouring fascicles.

High density – tightly connected (compact, concentrated) matrix substance present between neighbouring fascicles.

Examples of the different IFM density categories can be seen in Figure "I."

NS	N/A – no apparent IFRs to score (*IFR Frequency Count = 0*).
NS	N/A – all IFRs present are IFS (no IFMs to score, *IFR Type %/Score = 0)*
1	IFMs are of mixed densities (no majority density type).
2	The density in majority of IFMs is **low**.
3	The density in majority of IFMs is **moderate**.
4	The density in majority of IFMs is **high**.

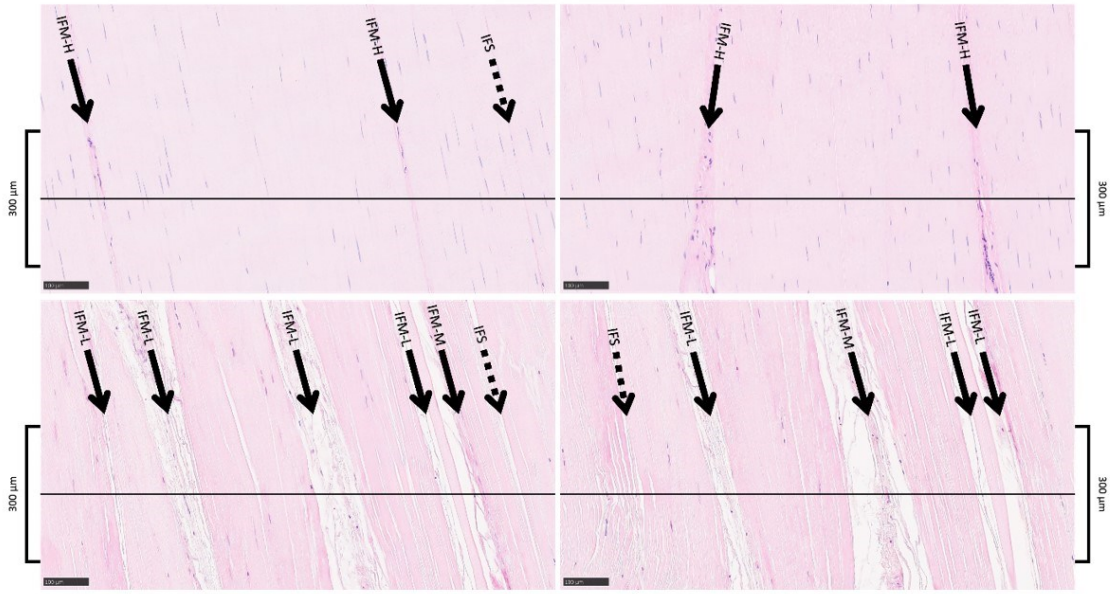


Figure "I." Reference examples of interfascicular regions (IFRs) classified as interfascicular space (IFS, dashed arrow), or interfascicular matrix (IFM, solid arrow) with low (-L), medium (-M) or high (-H) matrix density. IFRs are classified using definitions/methods as per *Table C*. Respective IFRs are assessed within a 300 µm region of interest (ROI; ± 150 µm of a transverse line) and classified as IFM if **≥50%** of ROI contains interfascicular connective tissue or IFS if **>50%** of ROI contains no discernible interfascicular connective tissue. Within images, unmarked spacings are determined to be *intra*-fascicular and thus not identified as IFRs. Scale bars = 100µm.

Supp. Table 9.5.d Scoring matrices from version 3.2 for assessing general characteristics of tendon from suitably stained sections that identify adipocytes and vasculature. **Characteristics can be differentiated from different standard histochemical stains. Selection of staining technique can be chosen as available to the laboratory. Recommendation immunohistochemical staining identification should be enlisted where available.*

General Tissue Characteristics *(suitable staining technique for identification of adipocytes and vasculature)*	
Outcome – Adipocyte Presence **Method:** Score the proportion of tendon area containing adipocytes.	
0	Adipocytes absent from tendon area (0%).
1	Adipocytes are present in <5% of tendon area.
2	Adipocytes are present in 5-25% of tendon area.
3	Adipocytes are present in 26-50% of tendon area.
4	Adipocytes are present in 51-75% of tendon area.
5	Adipocytes are present in 76-95% of tendon area.
6	Adipocytes are present in >95% of tendon area.

Outcome – Adipocyte Localisation **Method:** Score the proportion of adipocytes that are within the IFR and/or FM areas. The structure of the tendon-proper is typically comprised of two primary sub-regions (excluding paratenon): 1. **The fascicular matrix (FM):** is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure. 2. **The interfascicular region (IFR):** the regions between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance.		
NS	N/A – no adipocytes to score (*Adipose Presence Score = 0*).	
0	All (100%) adipocytes are located within the IFR	No (0%) adipocytes are located within the FM area.
1	>90% of adipocytes are located within the IFR	<10% of adipocytes are located within the FM area.
2	61-90% of adipocytes are located within the IFR	10-39% of adipocytes are located within the FM area.
3	41-60% of adipocytes are located within the IFR	40-59% of adipocytes are located within the FM area.
4	10-40% of adipocytes are located within the IFR	60-90% of adipocytes are located within the FM area.
5	<10% of adipocytes are located within the IFR	>90% of adipocytes are located within the FM area.
6	No (0%) adipocytes are located within the IFR	All (100%) adipocytes are located within the FM area.

Outcome – Vasculature Presence

Method:

Segment the tendon area into ten* even regions, count the number of regions positive for vascular presence, and calculate the percentage of positive regions.

** The number and placement of regions may be adjusted for the specific study, but all scorers must use the same regions (selection needs to be pre-determined and documented).*

__	% of regions positive for vasculature.

Outcome – Vasculature Localisation

Method:

Score the proportion of vasculature that are within the IFR and/or FM areas.

The structure of the tendon-proper is typically comprised of two primary sub-regions (excluding paratenon):

1. **The fascicular matrix (FM):** is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure.
2. **The interfascicular region (IFR):** the regions between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance.

NS	N/A – no vasculature to score. *(Vascular Presence = 0)*	
0	All (100%) vasculature is located within the IFR	No (0%) vasculature is located within the FM area.
1	>90% of vasculature is located within the IFR	<10% of vasculature is located within the FM area.
2	61-90% of vasculature is located within the IFR	10-39% of vasculature is located within the FM area.
3	41-60% of vasculature is located within the IFR	40-59% of vasculature is located within the FM area.
4	10-40% of vasculature is located within the IFR	60-90% of vasculature is located within the FM area.
5	<10% of vasculature is located within the IFR	>90% of vasculature is located within the FM area.
6	No(0%) vasculature is located within the IFR	All (100%) vasculature is located within the FM area.

Supp. Table 9.5.e Scoring matrices from version 3.2 for assessing proteoglycan characteristics and chondrocyte-like cell transformation of tendon from TB-stained sections.

Proteoglycan (PG) Content and Chondrocyte-Like Cells	
Toluidine Blue	
Outcome – Fascicular Matrix (FM) Proteoglycan Presence **Method:** Score the proportion of FM area with proteoglycan (purple) staining present. The **fascicular matrix (FM)**[#] is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure. [#]*excluding paratenon*	
0	None (0%) of the FM area contains proteoglycan staining
1	<5% of FM area contains proteoglycan staining.
2	5-25% of FM area contains proteoglycan staining.
3	26-50% of FM area contains proteoglycan staining.
4	51-75% of FM area contains proteoglycan staining.
5	76-95% of FM area contains proteoglycan staining.
6	>95% of FM area contains proteoglycan staining.
Outcome – Interfascicular Region (IFR) Proteoglycan Presence **Method:** Score the proportion of IFR area with proteoglycan (purple) staining present. The **interfascicular region (IFR)**[#] is the region(s) between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance. [#]*excluding paratenon*	
NS	*No IFR area to score. (Interfascicular Region (IFR) Frequency = 0)*
0	None (0%) of the IFR contains proteoglycan staining.
1	<5% of IFR area contains proteoglycan staining.
2	5-25% of IFR area contains proteoglycan staining.
3	26-50% of IFR area contains proteoglycan staining.
4	51-75% of IFR area contains proteoglycan staining.
5	76-95% of IFR area contains proteoglycan staining.
6	>95% of IFR area contains proteoglycan staining.
Outcome – Proteoglycan (PG) Staining Intensity (Average) **Method:** Score the ***average (predominant)*** intensity of the proteoglycan (purple) staining within the tendon area (including IFR and FM). *Note: disregard small (approximately cell-sized) staining artefacts.* Examples of differing levels of proteoglycan (purple) staining intensities can be seen in Figure "P."	
NS	N/A – no proteoglycan staining to score. (*FM and IFR Proteoglycan Presence scores = 0*).
1	**Pale** purple staining.
2	**Moderate** purple staining.
3	**Deep** purple staining.
Outcome – Proteoglycan (PG) Staining Intensity (Maximal) **Method:** Score the ***maximal intensity*** of the proteoglycan (purple) staining within the tendon area (including IFR and FM). *Note: disregard small (approximately cell-sized) staining artefacts.* Examples of differing levels of proteoglycan (purple) staining intensities can be seen in Figure "P."	
NS	N/A – no proteoglycan staining to score. *(FM and IFR Proteoglycan Presence scores = 0).*
1	**Pale** purple staining.
2	**Moderate** purple staining.
3	**Deep** purple staining.

Outcome – Maximal Proteoglycan (PG) Staining Location

Method:

Score the location of ***maximal intensity*** proteoglycan (purple) staining as being in IFR and/or FM.

The structure of the tendon-proper is typically comprised of two primary sub-regions (excluding paratenon):

1. **The fascicular matrix (FM):** is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure.
2. **The interfascicular region (IFR):** the regions between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance.

NS	N/A – no proteoglycan staining to score. *(FM and IFR Proteoglycan Presence scores = 0).*
1	**Maximal** purple staining is in the IFR.
2	**Maximal** purple staining is in both the IFR and FM.
3	**Maximal** purple staining is in the FM.

Outcome – Chondrocyte-like Cell Presence

Method:

Score the presence of chondrocyte-like cells (defined below) within the tendon area.

Chondrocyte-like cells are defined as those with ***at least two*** of the following:

- cigar/spherical nuclei
- nuclei surrounded by a lacunae/"halo"
- pericellular proteoglycan (purple) staining

NS	N/A – no cells to score. *(Cell Density = 0)*
0	No chondrocyte-like cells present.
1	**Very few** chondrocyte-like cells present.
2	**Moderate number** of chondrocyte-like cells present
3	**Numerous** chondrocyte-like cells present

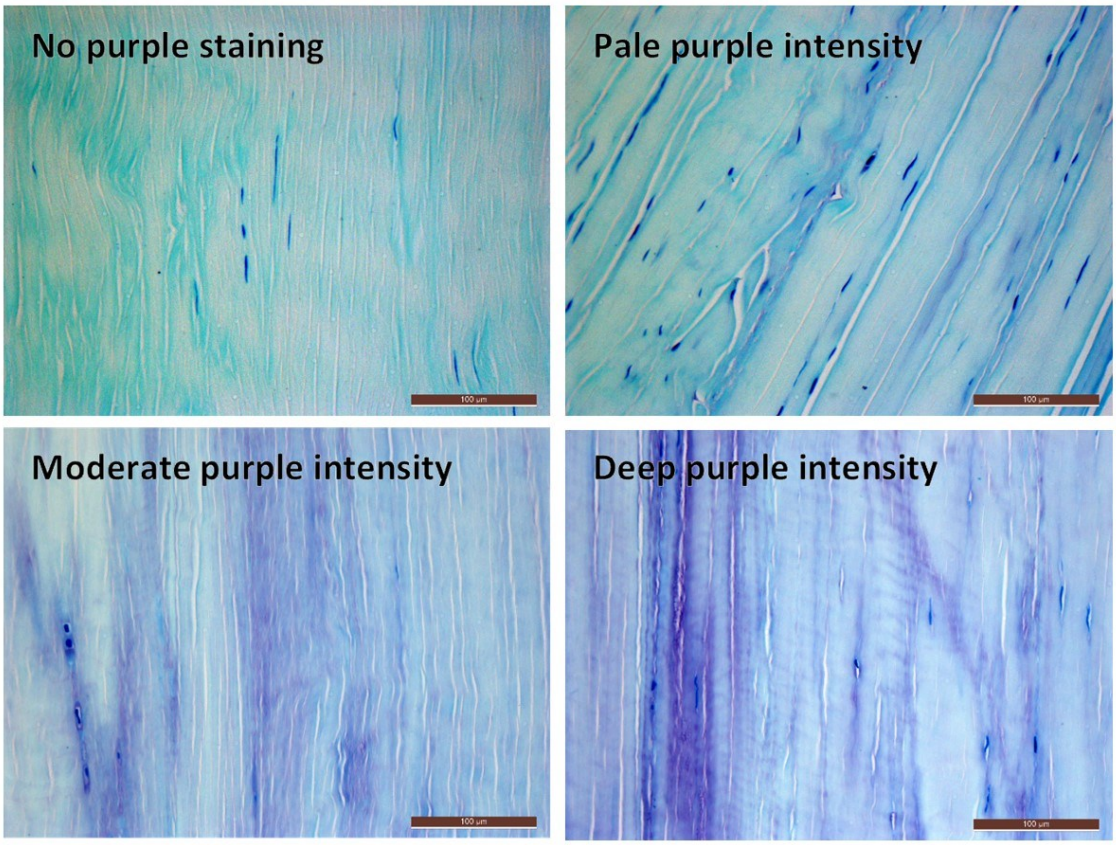


Figure "P." Visual representations of differing intensity levels of the maximal proteoglycan (purple) staining.

Supp. Table 9.5.f Scoring matrices from version 3.2 for assessing characteristics of tendon collagenous alignment from PSR- stained sections under non-polarised and polarised light.

Collagenous Alignment
Picrosirius Red; non-polarised and polarised light
Picrosirius Red histochemical staining reflects two characteristic colours of staining when viewed under ***non-polarised*** light: 1. **Yellow-Orange** – reflective of aligned fibrillar structure. 2. **Red** – reflective of unaligned fibrillar structure. Picrosirius Red histochemical staining reflects two characteristic colours when viewed under ***polarised*** light: 1. **Green-Yellow** – reflective of aligned fibrillar structure. 2. **Red-Orange** – reflective of unaligned fibrillar structure. ***Note: The following scoring matrices refer to the FM area only (i.e. excluding IFR characteristics and paratenon). Cut ends of sections as well as certain artefacts (e.g. tissue fraying/tearing artefacts) should be excluded/ignored where possible.*** The structure of the tendon-proper is typically comprised of two primary sub-regions (excluding paratenon): 1. **The fascicular matrix (FM):** is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure. 2. **The interfascicular region (IFR):** the regions between neighbouring fascicle units – including those both with and without the presence of distinct matrix substance.

Outcome – Collagen Alignment (Non-Polarised)

Method:

Score the proportion of FM area that is yellow-orange vs red.

0	None (0%) of the FM area is red	All (100%) of the FM area is yellow-orange
1	< 5% of the FM area is red	> 95% of the FM area is yellow-orange
2	5-25% of FM area is red	75-95% of the FM area is yellow-orange
3	26-50% of the FM area is red	50-74% of the FM area is yellow-orange
4	51-75% of the FM area is red	25-49% of the FM area is yellow-orange
5	76-95% of the FM area is red	5-24% of the FM area is yellow-orange
6	> 95% of the FM area is red	< 5% of the FM area is yellow-orange
7	All (100%) of the FM area is red	None (0%) of the FM area is yellow-orange

Outcome – Collagen Alignment (Polarised^)

^ For a given region of interest, rotate specimen through polariser range to observe maximal area of achievable green-yellow coverage, *minimising black/'shadowed' areas.*

Method:

Score the proportion of FM area that is green-yellow vs red-orange.

0	None (0%) of the FM area is red-orange	All (100%) of the FM area is green-yellow
1	< 5% of the FM area is red-orange	> 95% of the FM area is green-yellow
2	5-25% of the FM area is red-orange	75-95% of the FM area is green-yellow
3	26-50% of the FM area is red-orange	50-74% of the FM area is green-yellow
4	51-75% of the FM area is red-orange	25-49% of the FM area is green-yellow
5	76-95% of the FM area is red-orange	5-24% of the FM area is green-yellow
6	> 95% of the FM area is red-orange	< 5% of the FM area is green-yellow
7	All (100%) of the FM area is red-orange	None (0%) of the FM area is green-yellow

Supp. Table 9.5.g Scoring matrices from version 3.2 for assessing tendon crimp characteristics from PSR- stained sections under polarised light.

Crimp Structure
Picrosirius Red; polarised light
Scored using polarised light^ *^For a given region of interest, rotate specimen through polariser range to observe greatest definition of crimp waveform.* **The fascicular matrix (FM)#** is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure. *#excluding paratenon*

Outcome – Crimp Presence **Method:** Score the proportion of FM area associated with the presence of crimp (waveform pattern).	
0	No apparent crimp within FM area
1	Crimp present in <10% of FM area
2	10-40% of FM area contains apparent crimp
3	41-60% of FM area contains apparent crimp
4	61-90% of FM area contains apparent crimp
5	Crimp present in >90% of FM area

Outcome – Crimp Alignment **Method:** Assess the alignment of crimp pattern between neighbouring fibres (within respective fascicles), and score the proportion of total ***crimped*** FM area that is aligned versus unaligned (in-phase versus out-of-phase; defined below). Alignment of crimp is defined into two main categories: **Aligned** –neighbouring fibres are 'in-phase' and follow the same waving/corrugated plane. **Unaligned** – neighbouring fibres are 'out-of-phase' and exhibit sporadic/disconnected crimping planes.		
NS	N/A – no apparent crimp within FM area *(Crimp Presence Score = 0).*	
1	<10% of crimp area is unaligned	>90% of crimp area is aligned
2	10-40% of crimp area is unaligned	60-90% of crimp area is aligned
3	41-60% of crimp area is unaligned	40-59% of crimp area is aligned
4	61-90% of crimp area is unaligned	10-39% of crimp area is aligned
5	>90% of crimp area is unaligned	<10% of crimp area is aligned

Supp. Table 9.5.h Scoring matrices from version 3.2 for assessing tendon crimp wavelength characteristics from PSR-stained sections under polarised light.

Crimp Wavelength
Picrosirius Red; polarised light
Scored using polarised light^ *^For a given region of interest, rotate specimen through polariser range to observe greatest definition of crimp waveform.* **The fascicular matrix (FM)#** is comprised of fascicle units which are hierarchically organised, highly dense connective tissue making up the load-bearing tendon structure. *#excluding paratenon*

Outcome – Crimp Wavelength (Predominant) **Method:** Measure and score the ***predominant*** crimp wavelength (occupying the greatest FM area).	
NS	N/A – no apparent crimp within FM area *(Crimp Presence score 0).*
1	Crimp wavelength < 25 µm
2	Crimp wavelength 26-50 µm
3	Crimp wavelength 51-75 µm
4	Crimp wavelength 76-100 µm
5	Crimp wavelength 101-125 µm
6	Crimp wavelength 126-150 µm
7	Crimp wavelength 151-200 µm
8	Crimp wavelength 201-250 µm
9	Crimp wavelength 251-300 µm
10	Crimp wavelength 301-350 µm
11	Crimp wavelength 351-400 µm
12	Crimp wavelength 401-500 µm
13	Crimp wavelength 501-600 µm
14	Crimp wavelength 601-700 µm
15	Crimp wavelength >700 µm

Outcome – Crimp Wavelength Area **Method:** Score the proportion of crimped FM area associated with the ***predominant*** crimp wavelength (scored above; *Outcome - Crimp Wavelength (Predominant)).*	
NS	N/A – no apparent crimp within FM area *(Crimp Presence score 0).*
0	*Predominant* crimp wavelength constitutes all (100%) of visible crimp area.
1	*Predominant* crimp wavelength constitutes >90% of visible crimp area.
2	*Predominant* crimp wavelength constitutes 61-90% of visible crimp area.
3	*Predominant* crimp wavelength constitutes 41-60% of visible crimp area.
4	*Predominant* crimp wavelength constitutes 10-40% of visible crimp area.
5	*Predominant* crimp wavelength constitutes <10% of visible crimp area.

9.6 Appendices for ~ Chapter 5 ~

9.6.1 Upper Limb Pairwise Comparisons

The following pages contain the multiple pairwise comparison tests for the human upper limb tendons included in this project. Differences between tendons for nominal and ordinal outcomes were analysed using non-parametric Kruskal-Wallis tests followed by Dunn's test with a Bonferroni correction for multiple pairwise comparisons (GraphPad Prism). Differences between tendons for continuous (counted) outcomes were analysed using mixed-effects linear regression models with specific pairwise tendon comparisons evaluated using Sidak's adjustment for multiple comparisons (Stata). Statistical significance was taken as an adjusted $p < 0.05$.

Supp. Table 9.6.a Pairwise comparisons for upper limb tendon cell characteristics. *Green cells indicate significant p-values (< 0.05).*

Tendon Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangement		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
1 vs. 2	SSP vs. IS	-263.101	<0.0001	-427.585	-98.617	-65.375	>0.9999	-267.796	137.046	27.88	>0.9999	-31.13	>0.9999	7.13	>0.9999
1 vs. 3	SSP vs. SSC	-201.793	0.0010	-366.277	-37.309	-94.000	>0.9999	-296.421	108.421	-15.38	>0.9999	-20.75	>0.9999	33.00	>0.9999
1 vs. 4	SSP vs. LHBT	-268.310	<0.0001	-432.793	-103.826	-171.625	0.3847	-374.046	30.796	13.38	>0.9999	-105.90	0.8183	46.50	>0.9999
1 vs. 5	SSP vs. DBT	-300.601	<0.0001	-465.085	-136.117	-165.375	0.5212	-367.796	37.046	-16.63	>0.9999	-41.50	>0.9999	113.60	0.1923
1 vs. 6	SSP vs. BrR	-357.893	<0.0001	-522.377	-193.409	-240.375	0.0020	-442.796	-37.954	-16.63	>0.9999	-31.13	>0.9999	157.90	0.0004
1 vs. 7	SSP vs. DTT	-361.018	<0.0001	-525.502	-196.534	-134.125	0.9923	-336.546	68.296	-16.63	>0.9999	-50.94	>0.9999	88.31	>0.9999
1 vs. 8	SSP vs. FCR	-391.226	<0.0001	-555.710	-226.742	-206.000	0.0379	-408.421	-3.579	-16.63	>0.9999	-65.38	>0.9999	146.10	0.0026
1 vs. 9	SSP vs. PL	-358.935	<0.0001	-523.418	-194.451	-243.500	0.0015	-445.921	-41.079	-16.63	>0.9999	-61.31	>0.9999	157.90	0.0004
1 vs. 10	SSP vs. FCU	-427.685	<0.0001	-592.168	-263.201	-268.500	0.0001	-470.921	-66.079	-16.63	>0.9999	-61.31	>0.9999	157.90	0.0004
1 vs. 11	SSP vs. ECRL	-399.560	<0.0001	-564.043	-235.076	-252.875	0.0006	-455.296	-50.454	-16.63	>0.9999	-50.94	>0.9999	134.30	0.0140
1 vs. 12	SSP vs. ECRB	-362.060	<0.0001	-526.543	-197.576	-196.625	0.0771	-399.046	5.796	-16.63	>0.9999	-106.90	0.7445	140.80	0.0056
1 vs. 13	SSP vs. ECU	-332.893	<0.0001	-497.377	-168.409	-181.000	0.2240	-383.421	21.421	-16.63	>0.9999	-110.40	0.5198	117.20	0.1258
1 vs. 14	SSP vs. FDS-2	-286.018	<0.0001	-450.502	-121.534	-165.375	0.5212	-367.796	37.046	12.38	>0.9999	-159.10	0.0012	84.75	>0.9999
1 vs. 15	SSP vs. FDS-3	-321.435	<0.0001	-485.918	-156.951	-221.625	0.0106	-424.046	-19.204	-16.63	>0.9999	-164.10	0.0006	78.19	>0.9999
1 vs. 16	SSP vs. FDS-4	-290.185	<0.0001	-454.668	-125.701	-181.000	0.2240	-383.421	21.421	-2.13	>0.9999	-133.90	0.0359	69.94	>0.9999
1 vs. 17	SSP vs. FDS-5	-286.018	<0.0001	-450.502	-121.534	-168.500	0.4507	-370.921	33.921	-2.13	>0.9999	-144.60	0.0091	71.25	>0.9999
1 vs. 18	SSP vs. FDP-2	-294.351	<0.0001	-458.835	-129.867	-215.375	0.0179	-417.796	-12.954	-2.13	>0.9999	-135.20	0.0305	88.31	>0.9999
1 vs. 19	SSP vs. FDP-3	-278.726	<0.0001	-443.210	-114.242	-106.000	>0.9999	-308.421	96.421	-15.63	>0.9999	-140.20	0.0162	90.00	>0.9999
1 vs. 20	SSP vs. FDP-4	-257.893	<0.0001	-422.377	-93.409	-131.000	0.9969	-333.421	71.421	-30.13	>0.9999	-125.80	0.0952	55.88	>0.9999
1 vs. 21	SSP vs. FDP-5	-282.893	<0.0001	-447.377	-118.409	-152.875	0.8039	-355.296	49.546	-30.13	>0.9999	-110.40	0.5198	83.06	>0.9999
1 vs. 22	SSP vs. EIP	-255.810	<0.0001	-420.293	-91.326	-187.250	0.1493	-389.671	15.171	-16.63	>0.9999	-100.90	>0.9999	146.10	0.0026
1 vs. 23	SSP vs. EDC-2	-249.560	<0.0001	-414.043	-85.076	-243.500	0.0015	-445.921	-41.079	-16.63	>0.9999	-61.31	>0.9999	140.80	0.0056
1 vs. 24	SSP vs. EDC-3	-379.768	<0.0001	-544.252	-215.284	-196.625	0.0771	-399.046	5.796	-16.63	>0.9999	-51.88	>0.9999	157.90	0.0004
1 vs. 25	SSP vs. EDC-4	-355.810	<0.0001	-520.293	-191.326	-218.500	0.0138	-420.921	-16.079	-2.13	>0.9999	-61.31	>0.9999	146.10	0.0026
1 vs. 26	SSP vs. EDC-5	-278.783	<0.0001	-456.790	-100.775	-195.514	0.2274	-414.464	23.436	-34.63	>0.9999	-94.33	>0.9999	135.10	0.0470
1 vs. 27	SSP vs. EDM	-311.018	<0.0001	-475.502	-146.534	-184.125	0.1836	-386.546	18.296	-2.13	>0.9999	-60.38	>0.9999	146.10	0.0026
1 vs. 28	SSP vs. FPL	-309.976	<0.0001	-474.460	-145.492	-181.000	0.2240	-383.421	21.421	-2.13	>0.9999	-130.80	0.0526	75.94	>0.9999
1 vs. 29	SSP vs. APL	-294.351	<0.0001	-458.835	-129.867	-227.875	0.0062	-430.296	-25.454	-16.63	>0.9999	-61.31	>0.9999	140.80	0.0056
1 vs. 30	SSP vs. EPB	-270.393	<0.0001	-434.877	-105.909	-174.750	0.3245	-377.171	27.671	-44.50	>0.9999	-74.81	>0.9999	101.80	0.7231
1 vs. 31	SSP vs. EPL	-242.268	<0.0001	-406.752	-77.784	-140.375	0.9673	-342.796	62.046	26.88	>0.9999	-120.40	0.1762	84.75	>0.9999
2 vs. 3	IS vs. SSC	61.308	>0.9999	-103.176	225.792	-28.625	>0.9999	-231.046	173.796	-43.25	>0.9999	10.38	>0.9999	25.88	>0.9999
2 vs. 4	IS vs. LHBT	-5.208	>0.9999	-169.692	159.276	-106.250	>0.9999	-308.671	96.171	-14.50	>0.9999	-74.81	>0.9999	39.38	>0.9999
2 vs. 5	IS vs. DBT	-37.500	>0.9999	-201.984	126.984	-100.000	>0.9999	-302.421	102.421	-44.50	>0.9999	-10.38	>0.9999	106.50	0.4340
2 vs. 6	IS vs. BrR	-94.792	>0.9999	-259.276	69.692	-175.000	0.3200	-377.421	27.421	-44.50	>0.9999	0.00	>0.9999	150.80	0.0013
2 vs. 7	IS vs. DTT	-97.917	>0.9999	-262.401	66.567	-68.750	>0.9999	-271.171	133.671	-44.50	>0.9999	-19.81	>0.9999	81.19	>0.9999
2 vs. 8	IS vs. FCR	-128.125	0.7013	-292.609	36.359	-140.625	0.9657	-343.046	61.796	-44.50	>0.9999	-34.25	>0.9999	138.90	0.0073
2 vs. 9	IS vs. PL	-95.833	>0.9999	-260.317	68.651	-178.125	0.2669	-380.546	24.296	-44.50	>0.9999	-30.19	>0.9999	150.80	0.0013
2 vs. 10	IS vs. FCU	-164.583	0.0495	-329.067	-0.099	-203.125	0.0474	-405.546	-0.704	-44.50	>0.9999	-30.19	>0.9999	150.80	0.0013
2 vs. 11	IS vs. ECRL	-136.458	0.4632	-300.942	28.026	-187.500	0.1468	-389.921	14.921	-44.50	>0.9999	-19.81	>0.9999	127.10	0.0362
2 vs. 12	IS vs. ECRB	-98.958	0.9999	-263.442	65.526	-131.250	0.9966	-333.671	71.171	-44.50	>0.9999	-75.75	>0.9999	133.70	0.0151
2 vs. 13	IS vs. ECU	-69.792	>0.9999	-234.276	94.692	-115.625	>0.9999	-318.046	86.796	-44.50	>0.9999	-79.25	>0.9999	110.10	0.2905
2 vs. 14	IS vs. FDS-2	-22.917	>0.9999	-187.401	141.567	-100.000	>0.9999	-302.421	102.421	-15.50	>0.9999	-127.90	0.0736	77.63	>0.9999
2 vs. 15	IS vs. FDS-3	-58.333	>0.9999	-222.817	106.151	-156.250	0.7333	-358.671	46.171	-44.50	>0.9999	-132.90	0.0403	71.06	>0.9999
2 vs. 16	IS vs. FDS-4	-27.083	>0.9999	-191.567	137.401	-115.625	>0.9999	-318.046	86.796	-30.00	>0.9999	-102.80	>0.9999	62.81	>0.9999
2 vs. 17	IS vs. FDS-5	-22.917	>0.9999	-187.401	141.567	-103.125	>0.9999	-305.546	99.296	-30.00	>0.9999	-113.50	0.3739	64.13	>0.9999
2 vs. 18	IS vs. FDP-2	-31.250	>0.9999	-195.734	133.234	-150.000	0.8564	-352.421	52.421	-30.00	>0.9999	-104.10	0.9865	81.19	>0.9999
2 vs. 19	IS vs. FDP-3	-15.625	>0.9999	-180.109	148.859	-40.625	>0.9999	-243.046	161.796	-43.50	>0.9999	-109.10	0.5954	82.88	>0.9999
2 vs. 20	IS vs. FDP-4	5.208	>0.9999	-159.276	169.692	-65.625	>0.9999	-268.046	136.796	-58.00	>0.9999	-94.63	>0.9999	48.75	>0.9999
2 vs. 21	IS vs. FDP-5	-19.792	>0.9999	-184.276	144.692	-87.500	>0.9999	-289.921	114.921	-58.00	>0.9999	-79.25	>0.9999	75.94	>0.9999

Tendon Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangement		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
2 vs. 22	IS vs. EIP	7.292	>0.9999	-157.192	171.776	-121.875	0.9999	-324.296	80.546	-44.50	>0.9999	-69.81	>0.9999	138.90	0.0073
2 vs. 23	IS vs. EDC-2	13.542	>0.9999	-150.942	178.026	-178.125	0.2669	-380.546	24.296	-44.50	>0.9999	-30.19	>0.9999	133.70	0.0151
2 vs. 24	IS vs. EDC-3	-116.667	0.9417	-281.151	47.817	-131.250	0.9966	-333.671	71.171	-44.50	>0.9999	-20.75	>0.9999	150.80	0.0013
2 vs. 25	IS vs. EDC-4	-92.708	>0.9999	-257.192	71.776	-153.125	0.7989	-355.546	49.296	-30.00	>0.9999	-30.19	>0.9999	138.90	0.0073
2 vs. 26	IS vs. EDC-5	-15.681	>0.9999	-193.689	162.326	-130.139	>0.9999	-349.089	88.811	-62.50	>0.9999	-63.21	>0.9999	128.00	0.1072
2 vs. 27	IS vs. EDM	-47.917	>0.9999	-212.401	116.567	-118.750	>0.9999	-321.171	83.671	-30.00	>0.9999	-29.25	>0.9999	138.90	0.0073
2 vs. 28	IS vs. FPL	-46.875	>0.9999	-211.359	117.609	-115.625	>0.9999	-318.046	86.796	-30.00	>0.9999	-99.63	>0.9999	68.81	>0.9999
2 vs. 29	IS vs. APL	-31.250	>0.9999	-195.734	133.234	-162.500	0.5886	-364.921	39.921	-44.50	>0.9999	-30.19	>0.9999	133.70	0.0151
2 vs. 30	IS vs. EPB	-7.292	>0.9999	-171.776	157.192	-109.375	>0.9999	-311.796	93.046	-72.38	0.7066	-43.69	>0.9999	94.69	>0.9999
2 vs. 31	IS vs. EPL	20.833	>0.9999	-143.651	185.317	-75.000	>0.9999	-277.421	127.421	-1.00	>0.9999	-89.25	>0.9999	77.63	>0.9999
3 vs. 4	SSC vs. LHBT	-66.516	>0.9999	-231.000	97.968	-77.625	>0.9999	-280.046	124.796	28.75	>0.9999	-85.19	>0.9999	13.50	>0.9999
3 vs. 5	SSC vs. DBT	-98.808	0.9999	-263.292	65.676	-71.375	>0.9999	-273.796	131.046	-1.25	>0.9999	-20.75	>0.9999	80.63	>0.9999
3 vs. 6	SSC vs. BrR	-156.100	0.1068	-320.584	8.384	-146.375	0.9103	-348.796	56.046	-1.25	>0.9999	-10.38	>0.9999	124.90	0.0484
3 vs. 7	SSC vs. DTT	-159.225	0.0811	-323.709	5.259	-40.125	>0.9999	-242.546	162.296	-1.25	>0.9999	-30.19	>0.9999	55.31	>0.9999
3 vs. 8	SSC vs. FCR	-189.433	0.0039	-353.917	-24.949	-112.000	>0.9999	-314.421	90.421	-1.25	>0.9999	-44.63	>0.9999	113.10	0.2054
3 vs. 9	SSC vs. PL	-157.141	0.0975	-321.625	7.343	-149.500	0.8647	-351.921	52.921	-1.25	>0.9999	-40.56	>0.9999	124.90	0.0484
3 vs. 10	SSC vs. FCU	-225.891	0.0001	-390.375	-61.407	-174.500	0.3291	-376.921	27.921	-1.25	>0.9999	-40.56	>0.9999	124.90	0.0484
3 vs. 11	SSC vs. ECRL	-197.766	0.0015	-362.250	-33.282	-158.875	0.6738	-361.296	43.546	-1.25	>0.9999	-30.19	>0.9999	101.30	0.7677
3 vs. 12	SSC vs. ECRB	-160.266	0.0738	-324.750	4.218	-102.625	>0.9999	-305.046	99.796	-1.25	>0.9999	-86.13	>0.9999	107.80	0.3748
3 vs. 13	SSC vs. ECU	-131.100	0.6161	-295.584	33.384	-87.000	>0.9999	-289.421	115.421	-1.25	>0.9999	-89.63	>0.9999	84.19	>0.9999
3 vs. 14	SSC vs. FDS-2	-84.225	>0.9999	-248.709	80.259	-71.375	>0.9999	-273.796	131.046	27.75	>0.9999	-138.30	0.0206	51.75	>0.9999
3 vs. 15	SSC vs. FDS-3	-119.641	0.8988	-284.125	44.843	-127.625	0.9990	-330.046	74.796	-1.25	>0.9999	-143.30	0.0108	45.19	>0.9999
3 vs. 16	SSC vs. FDS-4	-88.391	>0.9999	-252.875	76.093	-87.000	>0.9999	-289.421	115.421	13.25	>0.9999	-113.10	0.3892	36.94	>0.9999
3 vs. 17	SSC vs. FDS-5	-84.225	>0.9999	-248.709	80.259	-74.500	>0.9999	-276.921	127.921	13.25	>0.9999	-123.90	0.1183	38.25	>0.9999
3 vs. 18	SSC vs. FDP-2	-92.558	>0.9999	-257.042	71.926	-121.375	0.9999	-323.796	81.046	13.25	>0.9999	-114.40	0.3383	55.31	>0.9999
3 vs. 19	SSC vs. FDP-3	-76.933	>0.9999	-241.417	87.551	-12.000	>0.9999	-214.421	190.421	-0.25	>0.9999	-119.40	0.1957	57.00	>0.9999
3 vs. 20	SSC vs. FDP-4	-56.100	>0.9999	-220.584	108.384	-37.000	>0.9999	-239.421	165.421	-14.75	>0.9999	-105.00	0.8988	22.88	>0.9999
3 vs. 21	SSC vs. FDP-5	-81.100	>0.9999	-245.584	83.384	-58.875	>0.9999	-261.296	143.546	-14.75	>0.9999	-89.63	>0.9999	50.06	>0.9999
3 vs. 22	SSC vs. EIP	-54.016	>0.9999	-218.500	110.468	-93.250	>0.9999	-295.671	109.171	-1.25	>0.9999	-80.19	>0.9999	113.10	0.2054
3 vs. 23	SSC vs. EDC-2	-47.766	>0.9999	-212.250	116.718	-149.500	0.8647	-351.921	52.921	-1.25	>0.9999	-40.56	>0.9999	107.80	0.3748
3 vs. 24	SSC vs. EDC-3	-177.975	0.0132	-342.459	-13.491	-102.625	>0.9999	-305.046	99.796	-1.25	>0.9999	-31.13	>0.9999	124.90	0.0484
3 vs. 25	SSC vs. EDC-4	-154.016	0.1277	-318.500	10.468	-124.500	0.9997	-326.921	77.921	13.25	>0.9999	-40.56	>0.9999	113.10	0.2054
3 vs. 26	SSC vs. EDC-5	-76.989	>0.9999	-254.997	101.018	-101.514	>0.9999	-320.464	117.436	-19.25	>0.9999	-73.58	>0.9999	102.10	>0.9999
3 vs. 27	SSC vs. EDM	-109.225	0.9916	-273.709	55.259	-90.125	>0.9999	-292.546	112.296	13.25	>0.9999	-39.63	>0.9999	113.10	0.2054
3 vs. 28	SSC vs. FPL	-108.183	0.9941	-272.667	56.301	-87.000	>0.9999	-289.421	115.421	13.25	>0.9999	-110.00	0.5404	42.94	>0.9999
3 vs. 29	SSC vs. APL	-92.558	>0.9999	-257.042	71.926	-133.875	0.9928	-336.296	68.546	-1.25	>0.9999	-40.56	>0.9999	107.80	0.3748
3 vs. 30	SSC vs. EPB	-68.600	>0.9999	-233.084	95.884	-80.750	>0.9999	-283.171	121.671	-29.13	>0.9999	-54.06	>0.9999	68.81	>0.9999
3 vs. 31	SSC vs. EPL	-40.475	>0.9999	-204.959	124.009	-46.375	>0.9999	-248.796	156.046	42.25	>0.9999	-99.63	>0.9999	51.75	>0.9999
4 vs. 5	LHBT vs. DBT	-32.292	>0.9999	-196.776	132.192	6.250	>0.9999	-196.171	208.671	-30.00	>0.9999	64.44	>0.9999	67.13	>0.9999
4 vs. 6	LHBT vs. BrR	-89.583	>0.9999	-254.067	74.901	-68.750	>0.9999	-271.171	133.671	-30.00	>0.9999	74.81	>0.9999	111.40	0.2499
4 vs. 7	LHBT vs. DTT	-92.708	>0.9999	-257.192	71.776	37.500	>0.9999	-164.921	239.921	-30.00	>0.9999	55.00	>0.9999	41.81	>0.9999
4 vs. 8	LHBT vs. FCR	-122.917	0.8343	-287.401	41.567	-34.375	>0.9999	-236.796	168.046	-30.00	>0.9999	40.56	>0.9999	99.56	0.9174
4 vs. 9	LHBT vs. PL	-90.625	>0.9999	-255.109	73.859	-71.875	>0.9999	-274.296	130.546	-30.00	>0.9999	44.63	>0.9999	111.40	0.2499
4 vs. 10	LHBT vs. FCU	-159.375	0.0800	-323.859	5.109	-96.875	>0.9999	-299.296	105.546	-30.00	>0.9999	44.63	>0.9999	111.40	0.2499
4 vs. 11	LHBT vs. ECRL	-131.250	0.6117	-295.734	33.234	-81.250	>0.9999	-283.671	121.171	-30.00	>0.9999	55.00	>0.9999	87.75	>0.9999
4 vs. 12	LHBT vs. ECRB	-93.750	>0.9999	-258.234	70.734	-25.000	>0.9999	-227.421	177.421	-30.00	>0.9999	-0.94	>0.9999	94.31	>0.9999
4 vs. 13	LHBT vs. ECU	-64.583	>0.9999	-229.067	99.901	-9.375	>0.9999	-211.796	193.046	-30.00	>0.9999	-4.44	>0.9999	70.69	>0.9999
4 vs. 14	LHBT vs. FDS-2	-17.708	>0.9999	-182.192	146.776	6.250	>0.9999	-196.171	208.671	-1.00	>0.9999	-53.13	>0.9999	38.25	>0.9999
4 vs. 15	LHBT vs. FDS-3	-53.125	>0.9999	-217.609	111.359	-50.000	>0.9999	-252.421	152.421	-30.00	>0.9999	-58.13	>0.9999	31.69	>0.9999
4 vs. 16	LHBT vs. FDS-4	-21.875	>0.9999	-186.359	142.609	-9.375	>0.9999	-211.796	193.046	-15.50	>0.9999	-27.94	>0.9999	23.44	>0.9999
4 vs. 17	LHBT vs. FDS-5	-17.708	>0.9999	-182.192	146.776	3.125	>0.9999	-199.296	205.546	-15.50	>0.9999	-38.69	>0.9999	24.75	>0.9999
4 vs. 18	LHBT vs. FDP-2	-26.042	>0.9999	-190.526	138.442	-43.750	>0.9999	-246.171	158.671	-15.50	>0.9999	-29.25	>0.9999	41.81	>0.9999

Tendon Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangement		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
4 vs. 19	LHBT vs. FDP-3	-10.417	>0.9999	-174.901	154.067	65.625	>0.9999	-136.796	268.046	-29.00	>0.9999	-34.25	>0.9999	43.50	>0.9999
4 vs. 20	LHBT vs. FDP-4	10.417	>0.9999	-154.067	174.901	40.625	>0.9999	-161.796	243.046	-43.50	>0.9999	-19.81	>0.9999	9.38	>0.9999
4 vs. 21	LHBT vs. FDP-5	-14.583	>0.9999	-179.067	149.901	18.750	>0.9999	-183.671	221.171	-43.50	>0.9999	-4.44	>0.9999	36.56	>0.9999
4 vs. 22	LHBT vs. EIP	12.500	>0.9999	-151.984	176.984	-15.625	>0.9999	-218.046	186.796	-30.00	>0.9999	5.00	>0.9999	99.56	0.9174
4 vs. 23	LHBT vs. EDC-2	18.750	>0.9999	-145.734	183.234	-71.875	>0.9999	-274.296	130.546	-30.00	>0.9999	44.63	>0.9999	94.31	>0.9999
4 vs. 24	LHBT vs. EDC-3	-111.458	0.9835	-275.942	53.026	-25.000	>0.9999	-227.421	177.421	-30.00	>0.9999	54.06	>0.9999	111.40	0.2499
4 vs. 25	LHBT vs. EDC-4	-87.500	>0.9999	-251.984	76.984	-46.875	>0.9999	-249.296	155.546	-15.50	>0.9999	44.63	>0.9999	99.56	0.9174
4 vs. 26	LHBT vs. EDC-5	-10.473	>0.9999	-188.481	167.535	-23.889	>0.9999	-242.839	195.061	-48.00	>0.9999	11.60	>0.9999	88.63	>0.9999
4 vs. 27	LHBT vs. EDM	-42.708	>0.9999	-207.192	121.776	-12.500	>0.9999	-214.921	189.921	-15.50	>0.9999	45.56	>0.9999	99.56	0.9174
4 vs. 28	LHBT vs. FPL	-41.667	>0.9999	-206.151	122.817	-9.375	>0.9999	-211.796	193.046	-15.50	>0.9999	-24.81	>0.9999	29.44	>0.9999
4 vs. 29	LHBT vs. APL	-26.042	>0.9999	-190.526	138.442	-56.250	>0.9999	-258.671	146.171	-30.00	>0.9999	44.63	>0.9999	94.31	>0.9999
4 vs. 30	LHBT vs. EPB	-2.083	>0.9999	-166.567	162.401	-3.125	>0.9999	-205.546	199.296	-57.88	>0.9999	31.13	>0.9999	55.31	>0.9999
4 vs. 31	LHBT vs. EPL	26.042	>0.9999	-138.442	190.526	31.250	>0.9999	-171.171	233.671	13.50	>0.9999	-14.44	>0.9999	38.25	>0.9999
5 vs. 6	DBT vs. BrR	-57.292	>0.9999	-221.776	107.192	-75.000	>0.9999	-277.421	127.421	0.00	>0.9999	10.38	>0.9999	44.25	>0.9999
5 vs. 7	DBT vs. DTT	-60.417	>0.9999	-224.901	104.067	31.250	>0.9999	-171.171	233.671	0.00	>0.9999	-9.44	>0.9999	-25.31	>0.9999
5 vs. 8	DBT vs. FCR	-90.625	>0.9999	-255.109	73.859	-40.625	>0.9999	-243.046	161.796	0.00	>0.9999	-23.88	>0.9999	32.44	>0.9999
5 vs. 9	DBT vs. PL	-58.333	>0.9999	-222.817	106.151	-78.125	>0.9999	-280.546	124.296	0.00	>0.9999	-19.81	>0.9999	44.25	>0.9999
5 vs. 10	DBT vs. FCU	-127.083	0.7301	-291.567	37.401	-103.125	>0.9999	-305.546	99.296	0.00	>0.9999	-19.81	>0.9999	44.25	>0.9999
5 vs. 11	DBT vs. ECRL	-98.958	0.9999	-263.442	65.526	-87.500	>0.9999	-289.921	114.921	0.00	>0.9999	-9.44	>0.9999	20.63	>0.9999
5 vs. 12	DBT vs. ECRB	-61.458	>0.9999	-225.942	103.026	-31.250	>0.9999	-233.671	171.171	0.00	>0.9999	-65.38	>0.9999	27.19	>0.9999
5 vs. 13	DBT vs. ECU	-32.292	>0.9999	-196.776	132.192	-15.625	>0.9999	-218.046	186.796	0.00	>0.9999	-68.88	>0.9999	3.56	>0.9999
5 vs. 14	DBT vs. FDS-2	14.583	>0.9999	-149.901	179.067	0.000	>0.9999	-202.421	202.421	29.00	>0.9999	-117.60	0.2408	-28.88	>0.9999
5 vs. 15	DBT vs. FDS-3	-20.833	>0.9999	-185.317	143.651	-56.250	>0.9999	-258.671	146.171	0.00	>0.9999	-122.60	0.1376	-35.44	>0.9999
5 vs. 16	DBT vs. FDS-4	10.417	>0.9999	-154.067	174.901	-15.625	>0.9999	-218.046	186.796	14.50	>0.9999	-92.38	>0.9999	-43.69	>0.9999
5 vs. 17	DBT vs. FDS-5	14.583	>0.9999	-149.901	179.067	-3.125	>0.9999	-205.546	199.296	14.50	>0.9999	-103.10	>0.9999	-42.38	>0.9999
5 vs. 18	DBT vs. FDP-2	6.250	>0.9999	-158.234	170.734	-50.000	>0.9999	-252.421	152.421	14.50	>0.9999	-93.69	>0.9999	-25.31	>0.9999
5 vs. 19	DBT vs. FDP-3	21.875	>0.9999	-142.609	186.359	59.375	>0.9999	-143.046	261.796	1.00	>0.9999	-98.69	>0.9999	-23.63	>0.9999
5 vs. 20	DBT vs. FDP-4	42.708	>0.9999	-121.776	207.192	34.375	>0.9999	-168.046	236.796	-13.50	>0.9999	-84.25	>0.9999	-57.75	>0.9999
5 vs. 21	DBT vs. FDP-5	17.708	>0.9999	-146.776	182.192	12.500	>0.9999	-189.921	214.921	-13.50	>0.9999	-68.88	>0.9999	-30.56	>0.9999
5 vs. 22	DBT vs. EIP	44.792	>0.9999	-119.692	209.276	-21.875	>0.9999	-224.296	180.546	0.00	>0.9999	-59.44	>0.9999	32.44	>0.9999
5 vs. 23	DBT vs. EDC-2	51.042	>0.9999	-113.442	215.526	-78.125	>0.9999	-280.546	124.296	0.00	>0.9999	-19.81	>0.9999	27.19	>0.9999
5 vs. 24	DBT vs. EDC-3	-79.167	>0.9999	-243.651	85.317	-31.250	>0.9999	-233.671	171.171	0.00	>0.9999	-10.38	>0.9999	44.25	>0.9999
5 vs. 25	DBT vs. EDC-4	-55.208	>0.9999	-219.692	109.276	-53.125	>0.9999	-255.546	149.296	14.50	>0.9999	-19.81	>0.9999	32.44	>0.9999
5 vs. 26	DBT vs. EDC-5	21.819	>0.9999	-156.189	199.826	-30.139	>0.9999	-249.089	188.811	-18.00	>0.9999	-52.83	>0.9999	21.50	>0.9999
5 vs. 27	DBT vs. EDM	-10.417	>0.9999	-174.901	154.067	-18.750	>0.9999	-221.171	183.671	14.50	>0.9999	-18.88	>0.9999	32.44	>0.9999
5 vs. 28	DBT vs. FPL	-9.375	>0.9999	-173.859	155.109	-15.625	>0.9999	-218.046	186.796	14.50	>0.9999	-89.25	>0.9999	-37.69	>0.9999
5 vs. 29	DBT vs. APL	6.250	>0.9999	-158.234	170.734	-62.500	>0.9999	-264.921	139.921	0.00	>0.9999	-19.81	>0.9999	27.19	>0.9999
5 vs. 30	DBT vs. EPB	30.208	>0.9999	-134.276	194.692	-9.375	>0.9999	-211.796	193.046	-27.88	>0.9999	-33.31	>0.9999	-11.81	>0.9999
5 vs. 31	DBT vs. EPL	58.333	>0.9999	-106.151	222.817	25.000	>0.9999	-177.421	227.421	43.50	>0.9999	-78.88	>0.9999	-28.88	>0.9999
6 vs. 7	BrR vs. DTT	-3.125	>0.9999	-167.609	161.359	106.250	>0.9999	-96.171	308.671	0.00	>0.9999	-19.81	>0.9999	-69.56	>0.9999
6 vs. 8	BrR vs. FCR	-33.333	>0.9999	-197.817	131.151	34.375	>0.9999	-168.046	236.796	0.00	>0.9999	-34.25	>0.9999	-11.81	>0.9999
6 vs. 9	BrR vs. PL	-1.042	>0.9999	-165.526	163.442	-3.125	>0.9999	-205.546	199.296	0.00	>0.9999	-30.19	>0.9999	0.00	>0.9999
6 vs. 10	BrR vs. FCU	-69.792	>0.9999	-234.276	94.692	-28.125	>0.9999	-230.546	174.296	0.00	>0.9999	-30.19	>0.9999	0.00	>0.9999
6 vs. 11	BrR vs. ECRL	-41.667	>0.9999	-206.151	122.817	-12.500	>0.9999	-214.921	189.921	0.00	>0.9999	-19.81	>0.9999	-23.63	>0.9999
6 vs. 12	BrR vs. ECRB	-4.167	>0.9999	-168.651	160.317	43.750	>0.9999	-158.671	246.171	0.00	>0.9999	-75.75	>0.9999	-17.06	>0.9999
6 vs. 13	BrR vs. ECU	25.000	>0.9999	-139.484	189.484	59.375	>0.9999	-143.046	261.796	0.00	>0.9999	-79.25	>0.9999	-40.69	>0.9999
6 vs. 14	BrR vs. FDS-2	71.875	>0.9999	-92.609	236.359	75.000	>0.9999	-127.421	277.421	29.00	>0.9999	-127.90	0.0736	-73.13	>0.9999
6 vs. 15	BrR vs. FDS-3	36.458	>0.9999	-128.026	200.942	18.750	>0.9999	-183.671	221.171	0.00	>0.9999	-132.90	0.0403	-79.69	>0.9999
6 vs. 16	BrR vs. FDS-4	67.708	>0.9999	-96.776	232.192	59.375	>0.9999	-143.046	261.796	14.50	>0.9999	-102.80	>0.9999	-87.94	>0.9999
6 vs. 17	BrR vs. FDS-5	71.875	>0.9999	-92.609	236.359	71.875	>0.9999	-130.546	274.296	14.50	>0.9999	-113.50	0.3739	-86.63	>0.9999
6 vs. 18	BrR vs. FDP-2	63.542	>0.9999	-100.942	228.026	25.000	>0.9999	-177.421	227.421	14.50	>0.9999	-104.10	0.9865	-69.56	>0.9999
6 vs. 19	BrR vs. FDP-3	79.167	>0.9999	-85.317	243.651	134.375	0.9917	-68.046	336.796	1.00	>0.9999	-109.10	0.5954	-67.88	>0.9999

Tendon Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangement		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
6 vs. 20	BrR vs. FDP-4	100.000	0.9998	-64.484	264.484	109.375	>0.9999	-93.046	311.796	-13.50	>0.9999	-94.63	>0.9999	-102.00	0.7087
6 vs. 21	BrR vs. FDP-5	75.000	>0.9999	-89.484	239.484	87.500	>0.9999	-114.921	289.921	-13.50	>0.9999	-79.25	>0.9999	-74.81	>0.9999
6 vs. 22	BrR vs. EIP	102.083	0.9995	-62.401	266.567	53.125	>0.9999	-149.296	255.546	0.00	>0.9999	-69.81	>0.9999	-11.81	>0.9999
6 vs. 23	BrR vs. EDC-2	108.333	0.9938	-56.151	272.817	-3.125	>0.9999	-205.546	199.296	0.00	>0.9999	-30.19	>0.9999	-17.06	>0.9999
6 vs. 24	BrR vs. EDC-3	-21.875	>0.9999	-186.359	142.609	43.750	>0.9999	-158.671	246.171	0.00	>0.9999	-20.75	>0.9999	0.00	>0.9999
6 vs. 25	BrR vs. EDC-4	2.083	>0.9999	-162.401	166.567	21.875	>0.9999	-180.546	224.296	14.50	>0.9999	-30.19	>0.9999	-11.81	>0.9999
6 vs. 26	BrR vs. EDC-5	79.110	>0.9999	-98.897	257.118	44.861	>0.9999	-174.089	263.811	-18.00	>0.9999	-63.21	>0.9999	-22.75	>0.9999
6 vs. 27	BrR vs. EDM	46.875	>0.9999	-117.609	211.359	56.250	>0.9999	-146.171	258.671	14.50	>0.9999	-29.25	>0.9999	-11.81	>0.9999
6 vs. 28	BrR vs. FPL	47.917	>0.9999	-116.567	212.401	59.375	>0.9999	-143.046	261.796	14.50	>0.9999	-99.63	>0.9999	-81.94	>0.9999
6 vs. 29	BrR vs. APL	63.542	>0.9999	-100.942	228.026	12.500	>0.9999	-189.921	214.921	0.00	>0.9999	-30.19	>0.9999	-17.06	>0.9999
6 vs. 30	BrR vs. EPB	87.500	>0.9999	-76.984	251.984	65.625	>0.9999	-136.796	268.046	-27.88	>0.9999	-43.69	>0.9999	-56.06	>0.9999
6 vs. 31	BrR vs. EPL	115.625	0.9532	-48.859	280.109	100.000	>0.9999	-102.421	302.421	43.50	>0.9999	-89.25	>0.9999	-73.13	>0.9999
7 vs. 8	DTT vs. FCR	-30.208	>0.9999	-194.692	134.276	-71.875	>0.9999	-274.296	130.546	0.00	>0.9999	-14.44	>0.9999	57.75	>0.9999
7 vs. 9	DTT vs. PL	2.083	>0.9999	-162.401	166.567	-109.375	>0.9999	-311.796	93.046	0.00	>0.9999	-10.38	>0.9999	69.56	>0.9999
7 vs. 10	DTT vs. FCU	-66.667	>0.9999	-231.151	97.817	-134.375	0.9917	-336.796	68.046	0.00	>0.9999	-10.38	>0.9999	69.56	>0.9999
7 vs. 11	DTT vs. ECRL	-38.542	>0.9999	-203.026	125.942	-118.750	>0.9999	-321.171	83.671	0.00	>0.9999	0.00	>0.9999	45.94	>0.9999
7 vs. 12	DTT vs. ECRB	-1.042	>0.9999	-165.526	163.442	-62.500	>0.9999	-264.921	139.921	0.00	>0.9999	-55.94	>0.9999	52.50	>0.9999
7 vs. 13	DTT vs. ECU	28.125	>0.9999	-136.359	192.609	-46.875	>0.9999	-249.296	155.546	0.00	>0.9999	-59.44	>0.9999	28.88	>0.9999
7 vs. 14	DTT vs. FDS-2	75.000	>0.9999	-89.484	239.484	-31.250	>0.9999	-233.671	171.171	29.00	>0.9999	-108.10	0.6556	-3.56	>0.9999
7 vs. 15	DTT vs. FDS-3	39.583	>0.9999	-124.901	204.067	-87.500	>0.9999	-289.921	114.921	0.00	>0.9999	-113.10	0.3892	-10.13	>0.9999
7 vs. 16	DTT vs. FDS-4	70.833	>0.9999	-93.651	235.317	-46.875	>0.9999	-249.296	155.546	14.50	>0.9999	-82.94	>0.9999	-18.38	>0.9999
7 vs. 17	DTT vs. FDS-5	75.000	>0.9999	-89.484	239.484	-34.375	>0.9999	-236.796	168.046	14.50	>0.9999	-93.69	>0.9999	-17.06	>0.9999
7 vs. 18	DTT vs. FDP-2	66.667	>0.9999	-97.817	231.151	-81.250	>0.9999	-283.671	121.171	14.50	>0.9999	-84.25	>0.9999	0.00	>0.9999
7 vs. 19	DTT vs. FDP-3	82.292	>0.9999	-82.192	246.776	28.125	>0.9999	-174.296	230.546	1.00	>0.9999	-89.25	>0.9999	1.69	>0.9999
7 vs. 20	DTT vs. FDP-4	103.125	0.9992	-61.359	267.609	3.125	>0.9999	-199.296	205.546	-13.50	>0.9999	-74.81	>0.9999	-32.44	>0.9999
7 vs. 21	DTT vs. FDP-5	78.125	>0.9999	-86.359	242.609	-18.750	>0.9999	-221.171	183.671	-13.50	>0.9999	-59.44	>0.9999	-5.25	>0.9999
7 vs. 22	DTT vs. EIP	105.208	0.9981	-59.276	269.692	-53.125	>0.9999	-255.546	149.296	0.00	>0.9999	-50.00	>0.9999	57.75	>0.9999
7 vs. 23	DTT vs. EDC-2	111.458	0.9835	-53.026	275.942	-109.375	>0.9999	-311.796	93.046	0.00	>0.9999	-10.38	>0.9999	52.50	>0.9999
7 vs. 24	DTT vs. EDC-3	-18.750	>0.9999	-183.234	145.734	-62.500	>0.9999	-264.921	139.921	0.00	>0.9999	-0.94	>0.9999	69.56	>0.9999
7 vs. 25	DTT vs. EDC-4	5.208	>0.9999	-159.276	169.692	-84.375	>0.9999	-286.796	118.046	14.50	>0.9999	-10.38	>0.9999	57.75	>0.9999
7 vs. 26	DTT vs. EDC-5	82.235	>0.9999	-95.772	260.243	-61.389	>0.9999	-280.339	157.561	-18.00	>0.9999	-43.40	>0.9999	46.81	>0.9999
7 vs. 27	DTT vs. EDM	50.000	>0.9999	-114.484	214.484	-50.000	>0.9999	-252.421	152.421	14.50	>0.9999	-9.44	>0.9999	57.75	>0.9999
7 vs. 28	DTT vs. FPL	51.042	>0.9999	-113.442	215.526	-46.875	>0.9999	-249.296	155.546	14.50	>0.9999	-79.81	>0.9999	-12.38	>0.9999
7 vs. 29	DTT vs. APL	66.667	>0.9999	-97.817	231.151	-93.750	>0.9999	-296.171	108.671	0.00	>0.9999	-10.38	>0.9999	52.50	>0.9999
7 vs. 30	DTT vs. EPB	90.625	>0.9999	-73.859	255.109	-40.625	>0.9999	-243.046	161.796	-27.88	>0.9999	-23.88	>0.9999	13.50	>0.9999
7 vs. 31	DTT vs. EPL	118.750	0.9133	-45.734	283.234	-6.250	>0.9999	-208.671	196.171	43.50	>0.9999	-69.44	>0.9999	-3.56	>0.9999
8 vs. 9	FCR vs. PL	32.292	>0.9999	-132.192	196.776	-37.500	>0.9999	-239.921	164.921	0.00	>0.9999	4.06	>0.9999	11.81	>0.9999
8 vs. 10	FCR vs. FCU	-36.458	>0.9999	-200.942	128.026	-62.500	>0.9999	-264.921	139.921	0.00	>0.9999	4.06	>0.9999	11.81	>0.9999
8 vs. 11	FCR vs. ECRL	-8.333	>0.9999	-172.817	156.151	-46.875	>0.9999	-249.296	155.546	0.00	>0.9999	14.44	>0.9999	-11.81	>0.9999
8 vs. 12	FCR vs. ECRB	29.167	>0.9999	-135.317	193.651	9.375	>0.9999	-193.046	211.796	0.00	>0.9999	-41.50	>0.9999	-5.25	>0.9999
8 vs. 13	FCR vs. ECU	58.333	>0.9999	-106.151	222.817	25.000	>0.9999	-177.421	227.421	0.00	>0.9999	-45.00	>0.9999	-28.88	>0.9999
8 vs. 14	FCR vs. FDS-2	105.208	0.9981	-59.276	269.692	40.625	>0.9999	-161.796	243.046	29.00	>0.9999	-93.69	>0.9999	-61.31	>0.9999
8 vs. 15	FCR vs. FDS-3	69.792	>0.9999	-94.692	234.276	-15.625	>0.9999	-218.046	186.796	0.00	>0.9999	-98.69	>0.9999	-67.88	>0.9999
8 vs. 16	FCR vs. FDS-4	101.042	0.9997	-63.442	265.526	25.000	>0.9999	-177.421	227.421	14.50	>0.9999	-68.50	>0.9999	-76.13	>0.9999
8 vs. 17	FCR vs. FDS-5	105.208	0.9981	-59.276	269.692	37.500	>0.9999	-164.921	239.921	14.50	>0.9999	-79.25	>0.9999	-74.81	>0.9999
8 vs. 18	FCR vs. FDP-2	96.875	>0.9999	-67.609	261.359	-9.375	>0.9999	-211.796	193.046	14.50	>0.9999	-69.81	>0.9999	-57.75	>0.9999
8 vs. 19	FCR vs. FDP-3	112.500	0.9780	-51.984	276.984	100.000	>0.9999	-102.421	302.421	1.00	>0.9999	-74.81	>0.9999	-56.06	>0.9999
8 vs. 20	FCR vs. FDP-4	133.333	0.5513	-31.151	297.817	75.000	>0.9999	-127.421	277.421	-13.50	>0.9999	-60.38	>0.9999	-90.19	>0.9999
8 vs. 21	FCR vs. FDP-5	108.333	0.9938	-56.151	272.817	53.125	>0.9999	-149.296	255.546	-13.50	>0.9999	-45.00	>0.9999	-63.00	>0.9999
8 vs. 22	FCR vs. EIP	135.417	0.4920	-29.067	299.901	18.750	>0.9999	-183.671	221.171	0.00	>0.9999	-35.56	>0.9999	0.00	>0.9999
8 vs. 23	FCR vs. EDC-2	141.667	0.3320	-22.817	306.151	-37.500	>0.9999	-239.921	164.921	0.00	>0.9999	4.06	>0.9999	-5.25	>0.9999
8 vs. 24	FCR vs. EDC-3	11.458	>0.9999	-153.026	175.942	9.375	>0.9999	-193.046	211.796	0.00	>0.9999	13.50	>0.9999	11.81	>0.9999

Tendon Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangement		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
8 vs. 25	FCR vs. EDC-4	35.417	>0.9999	-129.067	199.901	-12.500	>0.9999	-214.921	189.921	14.50	>0.9999	4.06	>0.9999	0.00	>0.9999
8 vs. 26	FCR vs. EDC-5	112.444	0.9989	-65.564	290.451	10.486	>0.9999	-208.464	229.436	-18.00	>0.9999	-28.96	>0.9999	-10.94	>0.9999
8 vs. 27	FCR vs. EDM	80.208	>0.9999	-84.276	244.692	21.875	>0.9999	-180.546	224.296	14.50	>0.9999	5.00	>0.9999	0.00	>0.9999
8 vs. 28	FCR vs. FPL	81.250	>0.9999	-83.234	245.734	25.000	>0.9999	-177.421	227.421	14.50	>0.9999	-65.38	>0.9999	-70.13	>0.9999
8 vs. 29	FCR vs. APL	96.875	>0.9999	-67.609	261.359	-21.875	>0.9999	-224.296	180.546	0.00	>0.9999	4.06	>0.9999	-5.25	>0.9999
8 vs. 30	FCR vs. EPB	120.833	0.8774	-43.651	285.317	31.250	>0.9999	-171.171	233.671	-27.88	>0.9999	-9.44	>0.9999	-44.25	>0.9999
8 vs. 31	FCR vs. EPL	148.958	0.1934	-15.526	313.442	65.625	>0.9999	-136.796	268.046	43.50	>0.9999	-55.00	>0.9999	-61.31	>0.9999
9 vs. 10	PL vs. FCU	-68.750	>0.9999	-233.234	95.734	-25.000	>0.9999	-227.421	177.421	0.00	>0.9999	0.00	>0.9999	0.00	>0.9999
9 vs. 11	PL vs. ECRL	-40.625	>0.9999	-205.109	123.859	-9.375	>0.9999	-211.796	193.046	0.00	>0.9999	10.38	>0.9999	-23.63	>0.9999
9 vs. 12	PL vs. ECRB	-3.125	>0.9999	-167.609	161.359	46.875	>0.9999	-155.546	249.296	0.00	>0.9999	-45.56	>0.9999	-17.06	>0.9999
9 vs. 13	PL vs. ECU	26.042	>0.9999	-138.442	190.526	62.500	>0.9999	-139.921	264.921	0.00	>0.9999	-49.06	>0.9999	-40.69	>0.9999
9 vs. 14	PL vs. FDS-2	72.917	>0.9999	-91.567	237.401	78.125	>0.9999	-124.296	280.546	29.00	>0.9999	-97.75	>0.9999	-73.13	>0.9999
9 vs. 15	PL vs. FDS-3	37.500	>0.9999	-126.984	201.984	21.875	>0.9999	-180.546	224.296	0.00	>0.9999	-102.80	>0.9999	-79.69	>0.9999
9 vs. 16	PL vs. FDS-4	68.750	>0.9999	-95.734	233.234	62.500	>0.9999	-139.921	264.921	14.50	>0.9999	-72.56	>0.9999	-87.94	>0.9999
9 vs. 17	PL vs. FDS-5	72.917	>0.9999	-91.567	237.401	75.000	>0.9999	-127.421	277.421	14.50	>0.9999	-83.31	>0.9999	-86.63	>0.9999
9 vs. 18	PL vs. FDP-2	64.583	>0.9999	-99.901	229.067	28.125	>0.9999	-174.296	230.546	14.50	>0.9999	-73.88	>0.9999	-69.56	>0.9999
9 vs. 19	PL vs. FDP-3	80.208	>0.9999	-84.276	244.692	137.500	0.9822	-64.921	339.921	1.00	>0.9999	-78.88	>0.9999	-67.88	>0.9999
9 vs. 20	PL vs. FDP-4	101.042	0.9997	-63.442	265.526	112.500	>0.9999	-89.921	314.921	-13.50	>0.9999	-64.44	>0.9999	-102.00	0.7087
9 vs. 21	PL vs. FDP-5	76.042	>0.9999	-88.442	240.526	90.625	>0.9999	-111.796	293.046	-13.50	>0.9999	-49.06	>0.9999	-74.81	>0.9999
9 vs. 22	PL vs. EIP	103.125	0.9992	-61.359	267.609	56.250	>0.9999	-146.171	258.671	0.00	>0.9999	-39.63	>0.9999	-11.81	>0.9999
9 vs. 23	PL vs. EDC-2	109.375	0.9912	-55.109	273.859	0.000	>0.9999	-202.421	202.421	0.00	>0.9999	0.00	>0.9999	-17.06	>0.9999
9 vs. 24	PL vs. EDC-3	-20.833	>0.9999	-185.317	143.651	46.875	>0.9999	-155.546	249.296	0.00	>0.9999	9.44	>0.9999	0.00	>0.9999
9 vs. 25	PL vs. EDC-4	3.125	>0.9999	-161.359	167.609	25.000	>0.9999	-177.421	227.421	14.50	>0.9999	0.00	>0.9999	-11.81	>0.9999
9 vs. 26	PL vs. EDC-5	80.152	>0.9999	-97.856	258.160	47.986	>0.9999	-170.964	266.936	-18.00	>0.9999	-33.02	>0.9999	-22.75	>0.9999
9 vs. 27	PL vs. EDM	47.917	>0.9999	-116.567	212.401	59.375	>0.9999	-143.046	261.796	14.50	>0.9999	0.94	>0.9999	-11.81	>0.9999
9 vs. 28	PL vs. FPL	48.958	>0.9999	-115.526	213.442	62.500	>0.9999	-139.921	264.921	14.50	>0.9999	-69.44	>0.9999	-81.94	>0.9999
9 vs. 29	PL vs. APL	64.583	>0.9999	-99.901	229.067	15.625	>0.9999	-186.796	218.046	0.00	>0.9999	0.00	>0.9999	-17.06	>0.9999
9 vs. 30	PL vs. EPB	88.542	>0.9999	-75.942	253.026	68.750	>0.9999	-133.671	271.171	-27.88	>0.9999	-13.50	>0.9999	-56.06	>0.9999
9 vs. 31	PL vs. EPL	116.667	0.9417	-47.817	281.151	103.125	>0.9999	-99.296	305.546	43.50	>0.9999	-59.06	>0.9999	-73.13	>0.9999
10 vs. 11	FCU vs. ECRL	28.125	>0.9999	-136.359	192.609	15.625	>0.9999	-186.796	218.046	0.00	>0.9999	10.38	>0.9999	-23.63	>0.9999
10 vs. 12	FCU vs. ECRB	65.625	>0.9999	-98.859	230.109	71.875	>0.9999	-130.546	274.296	0.00	>0.9999	-45.56	>0.9999	-17.06	>0.9999
10 vs. 13	FCU vs. ECU	94.792	>0.9999	-69.692	259.276	87.500	>0.9999	-114.921	289.921	0.00	>0.9999	-49.06	>0.9999	-40.69	>0.9999
10 vs. 14	FCU vs. FDS-2	141.667	0.3320	-22.817	306.151	103.125	>0.9999	-99.296	305.546	29.00	>0.9999	-97.75	>0.9999	-73.13	>0.9999
10 vs. 15	FCU vs. FDS-3	106.250	0.9971	-58.234	270.734	46.875	>0.9999	-155.546	249.296	0.00	>0.9999	-102.80	>0.9999	-79.69	>0.9999
10 vs. 16	FCU vs. FDS-4	137.500	0.4351	-26.984	301.984	87.500	>0.9999	-114.921	289.921	14.50	>0.9999	-72.56	>0.9999	-87.94	>0.9999
10 vs. 17	FCU vs. FDS-5	141.667	0.3320	-22.817	306.151	100.000	>0.9999	-102.421	302.421	14.50	>0.9999	-83.31	>0.9999	-86.63	>0.9999
10 vs. 18	FCU vs. FDP-2	133.333	0.5513	-31.151	297.817	53.125	>0.9999	-149.296	255.546	14.50	>0.9999	-73.88	>0.9999	-69.56	>0.9999
10 vs. 19	FCU vs. FDP-3	148.958	0.1934	-15.526	313.442	162.500	0.5886	-39.921	364.921	1.00	>0.9999	-78.88	>0.9999	-67.88	>0.9999
10 vs. 20	FCU vs. FDP-4	169.792	0.0301	5.308	334.276	137.500	0.9822	-64.921	339.921	-13.50	>0.9999	-64.44	>0.9999	-102.00	0.7087
10 vs. 21	FCU vs. FDP-5	144.792	0.2659	-19.692	309.276	115.625	>0.9999	-86.796	318.046	-13.50	>0.9999	-49.06	>0.9999	-74.81	>0.9999
10 vs. 22	FCU vs. EIP	171.875	0.0245	7.391	336.359	81.250	>0.9999	-121.171	283.671	0.00	>0.9999	-39.63	>0.9999	-11.81	>0.9999
10 vs. 23	FCU vs. EDC-2	178.125	0.0130	13.641	342.609	25.000	>0.9999	-177.421	227.421	0.00	>0.9999	0.00	>0.9999	-17.06	>0.9999
10 vs. 24	FCU vs. EDC-3	47.917	>0.9999	-116.567	212.401	71.875	>0.9999	-130.546	274.296	0.00	>0.9999	9.44	>0.9999	0.00	>0.9999
10 vs. 25	FCU vs. EDC-4	71.875	>0.9999	-92.609	236.359	50.000	>0.9999	-152.421	252.421	14.50	>0.9999	0.00	>0.9999	-11.81	>0.9999
10 vs. 26	FCU vs. EDC-5	148.902	0.4328	-29.106	326.910	72.986	>0.9999	-145.964	291.936	-18.00	>0.9999	-33.02	>0.9999	-22.75	>0.9999
10 vs. 27	FCU vs. EDM	116.667	0.9417	-47.817	281.151	84.375	>0.9999	-118.046	286.796	14.50	>0.9999	0.94	>0.9999	-11.81	>0.9999
10 vs. 28	FCU vs. FPL	117.708	0.9284	-46.776	282.192	87.500	>0.9999	-114.921	289.921	14.50	>0.9999	-69.44	>0.9999	-81.94	>0.9999
10 vs. 29	FCU vs. APL	133.333	0.5513	-31.151	297.817	40.625	>0.9999	-161.796	243.046	0.00	>0.9999	0.00	>0.9999	-17.06	>0.9999
10 vs. 30	FCU vs. EPB	157.292	0.0962	-7.192	321.776	93.750	>0.9999	-108.671	296.171	-27.88	>0.9999	-13.50	>0.9999	-56.06	>0.9999
10 vs. 31	FCU vs. EPL	185.417	0.0061	20.933	349.901	128.125	0.9988	-74.296	330.546	43.50	>0.9999	-59.06	>0.9999	-73.13	>0.9999
11 vs. 12	ECRL vs. ECRB	37.500	>0.9999	-126.984	201.984	56.250	>0.9999	-146.171	258.671	0.00	>0.9999	-55.94	>0.9999	6.56	>0.9999
11 vs. 13	ECRL vs. ECU	66.667	>0.9999	-97.817	231.151	71.875	>0.9999	-130.546	274.296	0.00	>0.9999	-59.44	>0.9999	-17.06	>0.9999

Tendon Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangement		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
11 vs. 14	ECRL vs. FDS-2	113.542	0.9713	-50.942	278.026	87.500	>0.9999	-114.921	289.921	29.00	>0.9999	-108.10	0.6556	-49.50	>0.9999
11 vs. 15	ECRL vs. FDS-3	78.125	>0.9999	-86.359	242.609	31.250	>0.9999	-171.171	233.671	0.00	>0.9999	-113.10	0.3892	-56.06	>0.9999
11 vs. 16	ECRL vs. FDS-4	109.375	0.9912	-55.109	273.859	71.875	>0.9999	-130.546	274.296	14.50	>0.9999	-82.94	>0.9999	-64.31	>0.9999
11 vs. 17	ECRL vs. FDS-5	113.542	0.9713	-50.942	278.026	84.375	>0.9999	-118.046	286.796	14.50	>0.9999	-93.69	>0.9999	-63.00	>0.9999
11 vs. 18	ECRL vs. FDP-2	105.208	0.9981	-59.276	269.692	37.500	>0.9999	-164.921	239.921	14.50	>0.9999	-84.25	>0.9999	-45.94	>0.9999
11 vs. 19	ECRL vs. FDP-3	120.833	0.8774	-43.651	285.317	146.875	0.9037	-55.546	349.296	1.00	>0.9999	-89.25	>0.9999	-44.25	>0.9999
11 vs. 20	ECRL vs. FDP-4	141.667	0.3320	-22.817	306.151	121.875	0.9999	-80.546	324.296	-13.50	>0.9999	-74.81	>0.9999	-78.38	>0.9999
11 vs. 21	ECRL vs. FDP-5	116.667	0.9417	-47.817	281.151	100.000	>0.9999	-102.421	302.421	-13.50	>0.9999	-59.44	>0.9999	-51.19	>0.9999
11 vs. 22	ECRL vs. EIP	143.750	0.2868	-20.734	308.234	65.625	>0.9999	-136.796	268.046	0.00	>0.9999	-50.00	>0.9999	11.81	>0.9999
11 vs. 23	ECRL vs. EDC-2	150.000	0.1780	-14.484	314.484	9.375	>0.9999	-193.046	211.796	0.00	>0.9999	-10.38	>0.9999	6.56	>0.9999
11 vs. 24	ECRL vs. EDC-3	19.792	>0.9999	-144.692	184.276	56.250	>0.9999	-146.171	258.671	0.00	>0.9999	-0.94	>0.9999	23.63	>0.9999
11 vs. 25	ECRL vs. EDC-4	43.750	>0.9999	-120.734	208.234	34.375	>0.9999	-168.046	236.796	14.50	>0.9999	-10.38	>0.9999	11.81	>0.9999
11 vs. 26	ECRL vs. EDC-5	120.777	0.9828	-57.231	298.785	57.361	>0.9999	-161.589	276.311	-18.00	>0.9999	-43.40	>0.9999	0.88	>0.9999
11 vs. 27	ECRL vs. EDM	88.542	>0.9999	-75.942	253.026	68.750	>0.9999	-133.671	271.171	14.50	>0.9999	-9.44	>0.9999	11.81	>0.9999
11 vs. 28	ECRL vs. FPL	89.583	>0.9999	-74.901	254.067	71.875	>0.9999	-130.546	274.296	14.50	>0.9999	-79.81	>0.9999	-58.31	>0.9999
11 vs. 29	ECRL vs. APL	105.208	0.9981	-59.276	269.692	25.000	>0.9999	-177.421	227.421	0.00	>0.9999	-10.38	>0.9999	6.56	>0.9999
11 vs. 30	ECRL vs. EPB	129.167	0.6719	-35.317	293.651	78.125	>0.9999	-124.296	280.546	-27.88	>0.9999	-23.88	>0.9999	-32.44	>0.9999
11 vs. 31	ECRL vs. EPL	157.292	0.0962	-7.192	321.776	112.500	>0.9999	-89.921	314.921	43.50	>0.9999	-69.44	>0.9999	-49.50	>0.9999
12 vs. 13	ECRB vs. ECU	29.167	>0.9999	-135.317	193.651	15.625	>0.9999	-186.796	218.046	0.00	>0.9999	-3.50	>0.9999	-23.63	>0.9999
12 vs. 14	ECRB vs. FDS-2	76.042	>0.9999	-88.442	240.526	31.250	>0.9999	-171.171	233.671	29.00	>0.9999	-52.19	>0.9999	-56.06	>0.9999
12 vs. 15	ECRB vs. FDS-3	40.625	>0.9999	-123.859	205.109	-25.000	>0.9999	-227.421	177.421	0.00	>0.9999	-57.19	>0.9999	-62.63	>0.9999
12 vs. 16	ECRB vs. FDS-4	71.875	>0.9999	-92.609	236.359	15.625	>0.9999	-186.796	218.046	14.50	>0.9999	-27.00	>0.9999	-70.88	>0.9999
12 vs. 17	ECRB vs. FDS-5	76.042	>0.9999	-88.442	240.526	28.125	>0.9999	-174.296	230.546	14.50	>0.9999	-37.75	>0.9999	-69.56	>0.9999
12 vs. 18	ECRB vs. FDP-2	67.708	>0.9999	-96.776	232.192	-18.750	>0.9999	-221.171	183.671	14.50	>0.9999	-28.31	>0.9999	-52.50	>0.9999
12 vs. 19	ECRB vs. FDP-3	83.333	>0.9999	-81.151	247.817	90.625	>0.9999	-111.796	293.046	1.00	>0.9999	-33.31	>0.9999	-50.81	>0.9999
12 vs. 20	ECRB vs. FDP-4	104.167	0.9988	-60.317	268.651	65.625	>0.9999	-136.796	268.046	-13.50	>0.9999	-18.88	>0.9999	-84.94	>0.9999
12 vs. 21	ECRB vs. FDP-5	79.167	>0.9999	-85.317	243.651	43.750	>0.9999	-158.671	246.171	-13.50	>0.9999	-3.50	>0.9999	-57.75	>0.9999
12 vs. 22	ECRB vs. EIP	106.250	0.9971	-58.234	270.734	9.375	>0.9999	-193.046	211.796	0.00	>0.9999	5.94	>0.9999	5.25	>0.9999
12 vs. 23	ECRB vs. EDC-2	112.500	0.9780	-51.984	276.984	-46.875	>0.9999	-249.296	155.546	0.00	>0.9999	45.56	>0.9999	0.00	>0.9999
12 vs. 24	ECRB vs. EDC-3	-17.708	>0.9999	-182.192	146.776	0.000	>0.9999	-202.421	202.421	0.00	>0.9999	55.00	>0.9999	17.06	>0.9999
12 vs. 25	ECRB vs. EDC-4	6.250	>0.9999	-158.234	170.734	-21.875	>0.9999	-224.296	180.546	14.50	>0.9999	45.56	>0.9999	5.25	>0.9999
12 vs. 26	ECRB vs. EDC-5	83.277	>0.9999	-94.731	261.285	1.111	>0.9999	-217.839	220.061	-18.00	>0.9999	12.54	>0.9999	-5.69	>0.9999
12 vs. 27	ECRB vs. EDM	51.042	>0.9999	-113.442	215.526	12.500	>0.9999	-189.921	214.921	14.50	>0.9999	46.50	>0.9999	5.25	>0.9999
12 vs. 28	ECRB vs. FPL	52.083	>0.9999	-112.401	216.567	15.625	>0.9999	-186.796	218.046	14.50	>0.9999	-23.88	>0.9999	-64.88	>0.9999
12 vs. 29	ECRB vs. APL	67.708	>0.9999	-96.776	232.192	-31.250	>0.9999	-233.671	171.171	0.00	>0.9999	45.56	>0.9999	0.00	>0.9999
12 vs. 30	ECRB vs. EPB	91.667	>0.9999	-72.817	256.151	21.875	>0.9999	-180.546	224.296	-27.88	>0.9999	32.06	>0.9999	-39.00	>0.9999
12 vs. 31	ECRB vs. EPL	119.792	0.8963	-44.692	284.276	56.250	>0.9999	-146.171	258.671	43.50	>0.9999	-13.50	>0.9999	-56.06	>0.9999
13 vs. 14	ECU vs. FDS-2	46.875	>0.9999	-117.609	211.359	15.625	>0.9999	-186.796	218.046	29.00	>0.9999	-48.69	>0.9999	-32.44	>0.9999
13 vs. 15	ECU vs. FDS-3	11.458	>0.9999	-153.026	175.942	-40.625	>0.9999	-243.046	161.796	0.00	>0.9999	-53.69	>0.9999	-39.00	>0.9999
13 vs. 16	ECU vs. FDS-4	42.708	>0.9999	-121.776	207.192	0.000	>0.9999	-202.421	202.421	14.50	>0.9999	-23.50	>0.9999	-47.25	>0.9999
13 vs. 17	ECU vs. FDS-5	46.875	>0.9999	-117.609	211.359	12.500	>0.9999	-189.921	214.921	14.50	>0.9999	-34.25	>0.9999	-45.94	>0.9999
13 vs. 18	ECU vs. FDP-2	38.542	>0.9999	-125.942	203.026	-34.375	>0.9999	-236.796	168.046	14.50	>0.9999	-24.81	>0.9999	-28.88	>0.9999
13 vs. 19	ECU vs. FDP-3	54.167	>0.9999	-110.317	218.651	75.000	>0.9999	-127.421	277.421	1.00	>0.9999	-29.81	>0.9999	-27.19	>0.9999
13 vs. 20	ECU vs. FDP-4	75.000	>0.9999	-89.484	239.484	50.000	>0.9999	-152.421	252.421	-13.50	>0.9999	-15.38	>0.9999	-61.31	>0.9999
13 vs. 21	ECU vs. FDP-5	50.000	>0.9999	-114.484	214.484	28.125	>0.9999	-174.296	230.546	-13.50	>0.9999	0.00	>0.9999	-34.13	>0.9999
13 vs. 22	ECU vs. EIP	77.083	>0.9999	-87.401	241.567	-6.250	>0.9999	-208.671	196.171	0.00	>0.9999	9.44	>0.9999	28.88	>0.9999
13 vs. 23	ECU vs. EDC-2	83.333	>0.9999	-81.151	247.817	-62.500	>0.9999	-264.921	139.921	0.00	>0.9999	49.06	>0.9999	23.63	>0.9999
13 vs. 24	ECU vs. EDC-3	-46.875	>0.9999	-211.359	117.609	-15.625	>0.9999	-218.046	186.796	0.00	>0.9999	58.50	>0.9999	40.69	>0.9999
13 vs. 25	ECU vs. EDC-4	-22.917	>0.9999	-187.401	141.567	-37.500	>0.9999	-239.921	164.921	14.50	>0.9999	49.06	>0.9999	28.88	>0.9999
13 vs. 26	ECU vs. EDC-5	54.110	>0.9999	-123.897	232.118	-14.514	>0.9999	-233.464	204.436	-18.00	>0.9999	16.04	>0.9999	17.94	>0.9999
13 vs. 27	ECU vs. EDM	21.875	>0.9999	-142.609	186.359	-3.125	>0.9999	-205.546	199.296	14.50	>0.9999	50.00	>0.9999	28.88	>0.9999
13 vs. 28	ECU vs. FPL	22.917	>0.9999	-141.567	187.401	0.000	>0.9999	-202.421	202.421	14.50	>0.9999	-20.38	>0.9999	-41.25	>0.9999

Tendon Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangement		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
13 vs. 29	ECU vs. APL	38.542	>0.9999	-125.942	203.026	-46.875	>0.9999	-249.296	155.546	0.00	>0.9999	49.06	>0.9999	23.63	>0.9999
13 vs. 30	ECU vs. EPB	62.500	>0.9999	-101.984	226.984	6.250	>0.9999	-196.171	208.671	-27.88	>0.9999	35.56	>0.9999	-15.38	>0.9999
13 vs. 31	ECU vs. EPL	90.625	>0.9999	-73.859	255.109	40.625	>0.9999	-161.796	243.046	43.50	>0.9999	-10.00	>0.9999	-32.44	>0.9999
14 vs. 15	FDS-2 vs. FDS-3	-35.417	>0.9999	-199.901	129.067	-56.250	>0.9999	-258.671	146.171	-29.00	>0.9999	-5.00	>0.9999	-6.56	>0.9999
14 vs. 16	FDS-2 vs. FDS-4	-4.167	>0.9999	-168.651	160.317	-15.625	>0.9999	-218.046	186.796	-14.50	>0.9999	25.19	>0.9999	-14.81	>0.9999
14 vs. 17	FDS-2 vs. FDS-5	0.000	>0.9999	-164.484	164.484	-3.125	>0.9999	-205.546	199.296	-14.50	>0.9999	14.44	>0.9999	-13.50	>0.9999
14 vs. 18	FDS-2 vs. FDP-2	-8.333	>0.9999	-172.817	156.151	-50.000	>0.9999	-252.421	152.421	-14.50	>0.9999	23.88	>0.9999	3.56	>0.9999
14 vs. 19	FDS-2 vs. FDP-3	7.292	>0.9999	-157.192	171.776	59.375	>0.9999	-143.046	261.796	-28.00	>0.9999	18.88	>0.9999	5.25	>0.9999
14 vs. 20	FDS-2 vs. FDP-4	28.125	>0.9999	-136.359	192.609	34.375	>0.9999	-168.046	236.796	-42.50	>0.9999	33.31	>0.9999	-28.88	>0.9999
14 vs. 21	FDS-2 vs. FDP-5	3.125	>0.9999	-161.359	167.609	12.500	>0.9999	-189.921	214.921	-42.50	>0.9999	48.69	>0.9999	-1.69	>0.9999
14 vs. 22	FDS-2 vs. EIP	30.208	>0.9999	-134.276	194.692	-21.875	>0.9999	-224.296	180.546	-29.00	>0.9999	58.13	>0.9999	61.31	>0.9999
14 vs. 23	FDS-2 vs. EDC-2	36.458	>0.9999	-128.026	200.942	-78.125	>0.9999	-280.546	124.296	-29.00	>0.9999	97.75	>0.9999	56.06	>0.9999
14 vs. 24	FDS-2 vs. EDC-3	-93.750	>0.9999	-258.234	70.734	-31.250	>0.9999	-233.671	171.171	-29.00	>0.9999	107.20	0.7212	73.13	>0.9999
14 vs. 25	FDS-2 vs. EDC-4	-69.792	>0.9999	-234.276	94.692	-53.125	>0.9999	-255.546	149.296	-14.50	>0.9999	97.75	>0.9999	61.31	>0.9999
14 vs. 26	FDS-2 vs. EDC-5	7.235	>0.9999	-170.772	185.243	-30.139	>0.9999	-249.089	188.811	-47.00	>0.9999	64.73	>0.9999	50.38	>0.9999
14 vs. 27	FDS-2 vs. EDM	-25.000	>0.9999	-189.484	139.484	-18.750	>0.9999	-221.171	183.671	-14.50	>0.9999	98.69	>0.9999	61.31	>0.9999
14 vs. 28	FDS-2 vs. FPL	-23.958	>0.9999	-188.442	140.526	-15.625	>0.9999	-218.046	186.796	-14.50	>0.9999	28.31	>0.9999	-8.81	>0.9999
14 vs. 29	FDS-2 vs. APL	-8.333	>0.9999	-172.817	156.151	-62.500	>0.9999	-264.921	139.921	-29.00	>0.9999	97.75	>0.9999	56.06	>0.9999
14 vs. 30	FDS-2 vs. EPB	15.625	>0.9999	-148.859	180.109	-9.375	>0.9999	-211.796	193.046	-56.88	>0.9999	84.25	>0.9999	17.06	>0.9999
14 vs. 31	FDS-2 vs. EPL	43.750	>0.9999	-120.734	208.234	25.000	>0.9999	-177.421	227.421	14.50	>0.9999	38.69	>0.9999	0.00	>0.9999
15 vs. 16	FDS-3 vs. FDS-4	31.250	>0.9999	-133.234	195.734	40.625	>0.9999	-161.796	243.046	14.50	>0.9999	30.19	>0.9999	-8.25	>0.9999
15 vs. 17	FDS-3 vs. FDS-5	35.417	>0.9999	-129.067	199.901	53.125	>0.9999	-149.296	255.546	14.50	>0.9999	19.44	>0.9999	-6.94	>0.9999
15 vs. 18	FDS-3 vs. FDP-2	27.083	>0.9999	-137.401	191.567	6.250	>0.9999	-196.171	208.671	14.50	>0.9999	28.88	>0.9999	10.13	>0.9999
15 vs. 19	FDS-3 vs. FDP-3	42.708	>0.9999	-121.776	207.192	115.625	>0.9999	-86.796	318.046	1.00	>0.9999	23.88	>0.9999	11.81	>0.9999
15 vs. 20	FDS-3 vs. FDP-4	63.542	>0.9999	-100.942	228.026	90.625	>0.9999	-111.796	293.046	-13.50	>0.9999	38.31	>0.9999	-22.31	>0.9999
15 vs. 21	FDS-3 vs. FDP-5	38.542	>0.9999	-125.942	203.026	68.750	>0.9999	-133.671	271.171	-13.50	>0.9999	53.69	>0.9999	4.88	>0.9999
15 vs. 22	FDS-3 vs. EIP	65.625	>0.9999	-98.859	230.109	34.375	>0.9999	-168.046	236.796	0.00	>0.9999	63.13	>0.9999	67.88	>0.9999
15 vs. 23	FDS-3 vs. EDC-2	71.875	>0.9999	-92.609	236.359	-21.875	>0.9999	-224.296	180.546	0.00	>0.9999	102.80	>0.9999	62.63	>0.9999
15 vs. 24	FDS-3 vs. EDC-3	-58.333	>0.9999	-222.817	106.151	25.000	>0.9999	-177.421	227.421	0.00	>0.9999	112.20	0.4298	79.69	>0.9999
15 vs. 25	FDS-3 vs. EDC-4	-34.375	>0.9999	-198.859	130.109	3.125	>0.9999	-199.296	205.546	14.50	>0.9999	102.80	>0.9999	67.88	>0.9999
15 vs. 26	FDS-3 vs. EDC-5	42.652	>0.9999	-135.356	220.660	26.111	>0.9999	-192.839	245.061	-18.00	>0.9999	69.73	>0.9999	56.94	>0.9999
15 vs. 27	FDS-3 vs. EDM	10.417	>0.9999	-154.067	174.901	37.500	>0.9999	-164.921	239.921	14.50	>0.9999	103.70	>0.9999	67.88	>0.9999
15 vs. 28	FDS-3 vs. FPL	11.458	>0.9999	-153.026	175.942	40.625	>0.9999	-161.796	243.046	14.50	>0.9999	33.31	>0.9999	-2.25	>0.9999
15 vs. 29	FDS-3 vs. APL	27.083	>0.9999	-137.401	191.567	-6.250	>0.9999	-208.671	196.171	0.00	>0.9999	102.80	>0.9999	62.63	>0.9999
15 vs. 30	FDS-3 vs. EPB	51.042	>0.9999	-113.442	215.526	46.875	>0.9999	-155.546	249.296	-27.88	>0.9999	89.25	>0.9999	23.63	>0.9999
15 vs. 31	FDS-3 vs. EPL	79.167	>0.9999	-85.317	243.651	81.250	>0.9999	-121.171	283.671	43.50	>0.9999	43.69	>0.9999	6.56	>0.9999
16 vs. 17	FDS-4 vs. FDS-5	4.167	>0.9999	-160.317	168.651	12.500	>0.9999	-189.921	214.921	0.00	>0.9999	-10.75	>0.9999	1.31	>0.9999
16 vs. 18	FDS-4 vs. FDP-2	-4.167	>0.9999	-168.651	160.317	-34.375	>0.9999	-236.796	168.046	0.00	>0.9999	-1.31	>0.9999	18.38	>0.9999
16 vs. 19	FDS-4 vs. FDP-3	11.458	>0.9999	-153.026	175.942	75.000	>0.9999	-127.421	277.421	-13.50	>0.9999	-6.31	>0.9999	20.06	>0.9999
16 vs. 20	FDS-4 vs. FDP-4	32.292	>0.9999	-132.192	196.776	50.000	>0.9999	-152.421	252.421	-28.00	>0.9999	8.13	>0.9999	-14.06	>0.9999
16 vs. 21	FDS-4 vs. FDP-5	7.292	>0.9999	-157.192	171.776	28.125	>0.9999	-174.296	230.546	-28.00	>0.9999	23.50	>0.9999	13.13	>0.9999
16 vs. 22	FDS-4 vs. EIP	34.375	>0.9999	-130.109	198.859	-6.250	>0.9999	-208.671	196.171	-14.50	>0.9999	32.94	>0.9999	76.13	>0.9999
16 vs. 23	FDS-4 vs. EDC-2	40.625	>0.9999	-123.859	205.109	-62.500	>0.9999	-264.921	139.921	-14.50	>0.9999	72.56	>0.9999	70.88	>0.9999
16 vs. 24	FDS-4 vs. EDC-3	-89.583	>0.9999	-254.067	74.901	-15.625	>0.9999	-218.046	186.796	-14.50	>0.9999	82.00	>0.9999	87.94	>0.9999
16 vs. 25	FDS-4 vs. EDC-4	-65.625	>0.9999	-230.109	98.859	-37.500	>0.9999	-239.921	164.921	0.00	>0.9999	72.56	>0.9999	76.13	>0.9999
16 vs. 26	FDS-4 vs. EDC-5	11.402	>0.9999	-166.606	189.410	-14.514	>0.9999	-233.464	204.436	-32.50	>0.9999	39.54	>0.9999	65.19	>0.9999
16 vs. 27	FDS-4 vs. EDM	-20.833	>0.9999	-185.317	143.651	-3.125	>0.9999	-205.546	199.296	0.00	>0.9999	73.50	>0.9999	76.13	>0.9999
16 vs. 28	FDS-4 vs. FPL	-19.792	>0.9999	-184.276	144.692	0.000	>0.9999	-202.421	202.421	0.00	>0.9999	3.13	>0.9999	6.00	>0.9999
16 vs. 29	FDS-4 vs. APL	-4.167	>0.9999	-168.651	160.317	-46.875	>0.9999	-249.296	155.546	-14.50	>0.9999	72.56	>0.9999	70.88	>0.9999
16 vs. 30	FDS-4 vs. EPB	19.792	>0.9999	-144.692	184.276	6.250	>0.9999	-196.171	208.671	-42.38	>0.9999	59.06	>0.9999	31.88	>0.9999
16 vs. 31	FDS-4 vs. EPL	47.917	>0.9999	-116.567	212.401	40.625	>0.9999	-161.796	243.046	29.00	>0.9999	13.50	>0.9999	14.81	>0.9999
17 vs. 18	FDS-5 vs. FDP-2	-8.333	>0.9999	-172.817	156.151	-46.875	>0.9999	-249.296	155.546	0.00	>0.9999	9.44	>0.9999	17.06	>0.9999

Tendon Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangement		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
17 vs. 19	FDS-5 vs. FDP-3	7.292	>0.9999	-157.192	171.776	62.500	>0.9999	-139.921	264.921	-13.50	>0.9999	4.44	>0.9999	18.75	>0.9999
17 vs. 20	FDS-5 vs. FDP-4	28.125	>0.9999	-136.359	192.609	37.500	>0.9999	-164.921	239.921	-28.00	>0.9999	18.88	>0.9999	-15.38	>0.9999
17 vs. 21	FDS-5 vs. FDP-5	3.125	>0.9999	-161.359	167.609	15.625	>0.9999	-186.796	218.046	-28.00	>0.9999	34.25	>0.9999	11.81	>0.9999
17 vs. 22	FDS-5 vs. EIP	30.208	>0.9999	-134.276	194.692	-18.750	>0.9999	-221.171	183.671	-14.50	>0.9999	43.69	>0.9999	74.81	>0.9999
17 vs. 23	FDS-5 vs. EDC-2	36.458	>0.9999	-128.026	200.942	-75.000	>0.9999	-277.421	127.421	-14.50	>0.9999	83.31	>0.9999	69.56	>0.9999
17 vs. 24	FDS-5 vs. EDC-3	-93.750	>0.9999	-258.234	70.734	-28.125	>0.9999	-230.546	174.296	-14.50	>0.9999	92.75	>0.9999	86.63	>0.9999
17 vs. 25	FDS-5 vs. EDC-4	-69.792	>0.9999	-234.276	94.692	-50.000	>0.9999	-252.421	152.421	0.00	>0.9999	83.31	>0.9999	74.81	>0.9999
17 vs. 26	FDS-5 vs. EDC-5	7.235	>0.9999	-170.772	185.243	-27.014	>0.9999	-245.964	191.936	-32.50	>0.9999	50.29	>0.9999	63.88	>0.9999
17 vs. 27	FDS-5 vs. EDM	-25.000	>0.9999	-189.484	139.484	-15.625	>0.9999	-218.046	186.796	0.00	>0.9999	84.25	>0.9999	74.81	>0.9999
17 vs. 28	FDS-5 vs. FPL	-23.958	>0.9999	-188.442	140.526	-12.500	>0.9999	-214.921	189.921	0.00	>0.9999	13.88	>0.9999	4.69	>0.9999
17 vs. 29	FDS-5 vs. APL	-8.333	>0.9999	-172.817	156.151	-59.375	>0.9999	-261.796	143.046	-14.50	>0.9999	83.31	>0.9999	69.56	>0.9999
17 vs. 30	FDS-5 vs. EPB	15.625	>0.9999	-148.859	180.109	-6.250	>0.9999	-208.671	196.171	-42.38	>0.9999	69.81	>0.9999	30.56	>0.9999
17 vs. 31	FDS-5 vs. EPL	43.750	>0.9999	-120.734	208.234	28.125	>0.9999	-174.296	230.546	29.00	>0.9999	24.25	>0.9999	13.50	>0.9999
18 vs. 19	FDP-2 vs. FDP-3	15.625	>0.9999	-148.859	180.109	109.375	>0.9999	-93.046	311.796	-13.50	>0.9999	-5.00	>0.9999	1.69	>0.9999
18 vs. 20	FDP-2 vs. FDP-4	36.458	>0.9999	-128.026	200.942	84.375	>0.9999	-118.046	286.796	-28.00	>0.9999	9.44	>0.9999	-32.44	>0.9999
18 vs. 21	FDP-2 vs. FDP-5	11.458	>0.9999	-153.026	175.942	62.500	>0.9999	-139.921	264.921	-28.00	>0.9999	24.81	>0.9999	-5.25	>0.9999
18 vs. 22	FDP-2 vs. EIP	38.542	>0.9999	-125.942	203.026	28.125	>0.9999	-174.296	230.546	-14.50	>0.9999	34.25	>0.9999	57.75	>0.9999
18 vs. 23	FDP-2 vs. EDC-2	44.792	>0.9999	-119.692	209.276	-28.125	>0.9999	-230.546	174.296	-14.50	>0.9999	73.88	>0.9999	52.50	>0.9999
18 vs. 24	FDP-2 vs. EDC-3	-85.417	>0.9999	-249.901	79.067	18.750	>0.9999	-183.671	221.171	-14.50	>0.9999	83.31	>0.9999	69.56	>0.9999
18 vs. 25	FDP-2 vs. EDC-4	-61.458	>0.9999	-225.942	103.026	-3.125	>0.9999	-205.546	199.296	0.00	>0.9999	73.88	>0.9999	57.75	>0.9999
18 vs. 26	FDP-2 vs. EDC-5	15.569	>0.9999	-162.439	193.576	19.861	>0.9999	-199.089	238.811	-32.50	>0.9999	40.85	>0.9999	46.81	>0.9999
18 vs. 27	FDP-2 vs. EDM	-16.667	>0.9999	-181.151	147.817	31.250	>0.9999	-171.171	233.671	0.00	>0.9999	74.81	>0.9999	57.75	>0.9999
18 vs. 28	FDP-2 vs. FPL	-15.625	>0.9999	-180.109	148.859	34.375	>0.9999	-168.046	236.796	0.00	>0.9999	4.44	>0.9999	-12.38	>0.9999
18 vs. 29	FDP-2 vs. APL	0.000	>0.9999	-164.484	164.484	-12.500	>0.9999	-214.921	189.921	-14.50	>0.9999	73.88	>0.9999	52.50	>0.9999
18 vs. 30	FDP-2 vs. EPB	23.958	>0.9999	-140.526	188.442	40.625	>0.9999	-161.796	243.046	-42.38	>0.9999	60.38	>0.9999	13.50	>0.9999
18 vs. 31	FDP-2 vs. EPL	52.083	>0.9999	-112.401	216.567	75.000	>0.9999	-127.421	277.421	29.00	>0.9999	14.81	>0.9999	-3.56	>0.9999
19 vs. 20	FDP-3 vs. FDP-4	20.833	>0.9999	-143.651	185.317	-25.000	>0.9999	-227.421	177.421	-14.50	>0.9999	14.44	>0.9999	-34.13	>0.9999
19 vs. 21	FDP-3 vs. FDP-5	-4.167	>0.9999	-168.651	160.317	-46.875	>0.9999	-249.296	155.546	-14.50	>0.9999	29.81	>0.9999	-6.94	>0.9999
19 vs. 22	FDP-3 vs. EIP	22.917	>0.9999	-141.567	187.401	-81.250	>0.9999	-283.671	121.171	-1.00	>0.9999	39.25	>0.9999	56.06	>0.9999
19 vs. 23	FDP-3 vs. EDC-2	29.167	>0.9999	-135.317	193.651	-137.500	0.9822	-339.921	64.921	-1.00	>0.9999	78.88	>0.9999	50.81	>0.9999
19 vs. 24	FDP-3 vs. EDC-3	-101.042	0.9997	-265.526	63.442	-90.625	>0.9999	-293.046	111.796	-1.00	>0.9999	88.31	>0.9999	67.88	>0.9999
19 vs. 25	FDP-3 vs. EDC-4	-77.083	>0.9999	-241.567	87.401	-112.500	>0.9999	-314.921	89.921	13.50	>0.9999	78.88	>0.9999	56.06	>0.9999
19 vs. 26	FDP-3 vs. EDC-5	-0.056	>0.9999	-178.064	177.951	-89.514	>0.9999	-308.464	129.436	-19.00	>0.9999	45.85	>0.9999	45.13	>0.9999
19 vs. 27	FDP-3 vs. EDM	-32.292	>0.9999	-196.776	132.192	-78.125	>0.9999	-280.546	124.296	13.50	>0.9999	79.81	>0.9999	56.06	>0.9999
19 vs. 28	FDP-3 vs. FPL	-31.250	>0.9999	-195.734	133.234	-75.000	>0.9999	-277.421	127.421	13.50	>0.9999	9.44	>0.9999	-14.06	>0.9999
19 vs. 29	FDP-3 vs. APL	-15.625	>0.9999	-180.109	148.859	-121.875	0.9999	-324.296	80.546	-1.00	>0.9999	78.88	>0.9999	50.81	>0.9999
19 vs. 30	FDP-3 vs. EPB	8.333	>0.9999	-156.151	172.817	-68.750	>0.9999	-271.171	133.671	-28.88	>0.9999	65.38	>0.9999	11.81	>0.9999
19 vs. 31	FDP-3 vs. EPL	36.458	>0.9999	-128.026	200.942	-34.375	>0.9999	-236.796	168.046	42.50	>0.9999	19.81	>0.9999	-5.25	>0.9999
20 vs. 21	FDP-4 vs. FDP-5	-25.000	>0.9999	-189.484	139.484	-21.875	>0.9999	-224.296	180.546	0.00	>0.9999	15.38	>0.9999	27.19	>0.9999
20 vs. 22	FDP-4 vs. EIP	2.083	>0.9999	-162.401	166.567	-56.250	>0.9999	-258.671	146.171	13.50	>0.9999	24.81	>0.9999	90.19	>0.9999
20 vs. 23	FDP-4 vs. EDC-2	8.333	>0.9999	-156.151	172.817	-112.500	>0.9999	-314.921	89.921	13.50	>0.9999	64.44	>0.9999	84.94	>0.9999
20 vs. 24	FDP-4 vs. EDC-3	-121.875	0.8567	-286.359	42.609	-65.625	>0.9999	-268.046	136.796	13.50	>0.9999	73.88	>0.9999	102.00	0.7087
20 vs. 25	FDP-4 vs. EDC-4	-97.917	>0.9999	-262.401	66.567	-87.500	>0.9999	-289.921	114.921	28.00	>0.9999	64.44	>0.9999	90.19	>0.9999
20 vs. 26	FDP-4 vs. EDC-5	-20.890	>0.9999	-198.897	157.118	-64.514	>0.9999	-283.464	154.436	-4.50	>0.9999	31.42	>0.9999	79.25	>0.9999
20 vs. 27	FDP-4 vs. EDM	-53.125	>0.9999	-217.609	111.359	-53.125	>0.9999	-255.546	149.296	28.00	>0.9999	65.38	>0.9999	90.19	>0.9999
20 vs. 28	FDP-4 vs. FPL	-52.083	>0.9999	-216.567	112.401	-50.000	>0.9999	-252.421	152.421	28.00	>0.9999	-5.00	>0.9999	20.06	>0.9999
20 vs. 29	FDP-4 vs. APL	-36.458	>0.9999	-200.942	128.026	-96.875	>0.9999	-299.296	105.546	13.50	>0.9999	64.44	>0.9999	84.94	>0.9999
20 vs. 30	FDP-4 vs. EPB	-12.500	>0.9999	-176.984	151.984	-43.750	>0.9999	-246.171	158.671	-14.38	>0.9999	50.94	>0.9999	45.94	>0.9999
20 vs. 31	FDP-4 vs. EPL	15.625	>0.9999	-148.859	180.109	-9.375	>0.9999	-211.796	193.046	57.00	>0.9999	5.38	>0.9999	28.88	>0.9999
21 vs. 22	FDP-5 vs. EIP	27.083	>0.9999	-137.401	191.567	-34.375	>0.9999	-236.796	168.046	13.50	>0.9999	9.44	>0.9999	63.00	>0.9999
21 vs. 23	FDP-5 vs. EDC-2	33.333	>0.9999	-131.151	197.817	-90.625	>0.9999	-293.046	111.796	13.50	>0.9999	49.06	>0.9999	57.75	>0.9999
21 vs. 24	FDP-5 vs. EDC-3	-96.875	>0.9999	-261.359	67.609	-43.750	>0.9999	-246.171	158.671	13.50	>0.9999	58.50	>0.9999	74.81	>0.9999

Tendon Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangement		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
21 vs. 25	FDP-5 vs. EDC-4	-72.917	>0.9999	-237.401	91.567	-65.625	>0.9999	-268.046	136.796	28.00	>0.9999	49.06	>0.9999	63.00	>0.9999
21 vs. 26	FDP-5 vs. EDC-5	4.110	>0.9999	-173.897	182.118	-42.639	>0.9999	-261.589	176.311	-4.50	>0.9999	16.04	>0.9999	52.06	>0.9999
21 vs. 27	FDP-5 vs. EDM	-28.125	>0.9999	-192.609	136.359	-31.250	>0.9999	-233.671	171.171	28.00	>0.9999	50.00	>0.9999	63.00	>0.9999
21 vs. 28	FDP-5 vs. FPL	-27.083	>0.9999	-191.567	137.401	-28.125	>0.9999	-230.546	174.296	28.00	>0.9999	-20.38	>0.9999	-7.13	>0.9999
21 vs. 29	FDP-5 vs. APL	-11.458	>0.9999	-175.942	153.026	-75.000	>0.9999	-277.421	127.421	13.50	>0.9999	49.06	>0.9999	57.75	>0.9999
21 vs. 30	FDP-5 vs. EPB	12.500	>0.9999	-151.984	176.984	-21.875	>0.9999	-224.296	180.546	-14.38	>0.9999	35.56	>0.9999	18.75	>0.9999
21 vs. 31	FDP-5 vs. EPL	40.625	>0.9999	-123.859	205.109	12.500	>0.9999	-189.921	214.921	57.00	>0.9999	-10.00	>0.9999	1.69	>0.9999
22 vs. 23	EIP vs. EDC-2	6.250	>0.9999	-158.234	170.734	-56.250	>0.9999	-258.671	146.171	0.00	>0.9999	39.63	>0.9999	-5.25	>0.9999
22 vs. 24	EIP vs. EDC-3	-123.958	0.8102	-288.442	40.526	-9.375	>0.9999	-211.796	193.046	0.00	>0.9999	49.06	>0.9999	11.81	>0.9999
22 vs. 25	EIP vs. EDC-4	-100.000	0.9998	-264.484	64.484	-31.250	>0.9999	-233.671	171.171	14.50	>0.9999	39.63	>0.9999	0.00	>0.9999
22 vs. 26	EIP vs. EDC-5	-22.973	>0.9999	-200.981	155.035	-8.264	>0.9999	-227.214	210.686	-18.00	>0.9999	6.60	>0.9999	-10.94	>0.9999
22 vs. 27	EIP vs. EDM	-55.208	>0.9999	-219.692	109.276	3.125	>0.9999	-199.296	205.546	14.50	>0.9999	40.56	>0.9999	0.00	>0.9999
22 vs. 28	EIP vs. FPL	-54.167	>0.9999	-218.651	110.317	6.250	>0.9999	-196.171	208.671	14.50	>0.9999	-29.81	>0.9999	-70.13	>0.9999
22 vs. 29	EIP vs. APL	-38.542	>0.9999	-203.026	125.942	-40.625	>0.9999	-243.046	161.796	0.00	>0.9999	39.63	>0.9999	-5.25	>0.9999
22 vs. 30	EIP vs. EPB	-14.583	>0.9999	-179.067	149.901	12.500	>0.9999	-189.921	214.921	-27.88	>0.9999	26.13	>0.9999	-44.25	>0.9999
22 vs. 31	EIP vs. EPL	13.542	>0.9999	-150.942	178.026	46.875	>0.9999	-155.546	249.296	43.50	>0.9999	-19.44	>0.9999	-61.31	>0.9999
23 vs. 24	EDC-2 vs. EDC-3	-130.208	0.6419	-294.692	34.276	46.875	>0.9999	-155.546	249.296	0.00	>0.9999	9.44	>0.9999	17.06	>0.9999
23 vs. 25	EDC-2 vs. EDC-4	-106.250	0.9971	-270.734	58.234	25.000	>0.9999	-177.421	227.421	14.50	>0.9999	0.00	>0.9999	5.25	>0.9999
23 vs. 26	EDC-2 vs. EDC-5	-29.223	>0.9999	-207.231	148.785	47.986	>0.9999	-170.964	266.936	-18.00	>0.9999	-33.02	>0.9999	-5.69	>0.9999
23 vs. 27	EDC-2 vs. EDM	-61.458	>0.9999	-225.942	103.026	59.375	>0.9999	-143.046	261.796	14.50	>0.9999	0.94	>0.9999	5.25	>0.9999
23 vs. 28	EDC-2 vs. FPL	-60.417	>0.9999	-224.901	104.067	62.500	>0.9999	-139.921	264.921	14.50	>0.9999	-69.44	>0.9999	-64.88	>0.9999
23 vs. 29	EDC-2 vs. APL	-44.792	>0.9999	-209.276	119.692	15.625	>0.9999	-186.796	218.046	0.00	>0.9999	0.00	>0.9999	0.00	>0.9999
23 vs. 30	EDC-2 vs. EPB	-20.833	>0.9999	-185.317	143.651	68.750	>0.9999	-133.671	271.171	-27.88	>0.9999	-13.50	>0.9999	-39.00	>0.9999
23 vs. 31	EDC-2 vs. EPL	7.292	>0.9999	-157.192	171.776	103.125	>0.9999	-99.296	305.546	43.50	>0.9999	-59.06	>0.9999	-56.06	>0.9999
24 vs. 25	EDC-3 vs. EDC-4	23.958	>0.9999	-140.526	188.442	-21.875	>0.9999	-224.296	180.546	14.50	>0.9999	-9.44	>0.9999	-11.81	>0.9999
24 vs. 26	EDC-3 vs. EDC-5	100.985	>0.9999	-77.022	278.993	1.111	>0.9999	-217.839	220.061	-18.00	>0.9999	-42.46	>0.9999	-22.75	>0.9999
24 vs. 27	EDC-3 vs. EDM	68.750	>0.9999	-95.734	233.234	12.500	>0.9999	-189.921	214.921	14.50	>0.9999	-8.50	>0.9999	-11.81	>0.9999
24 vs. 28	EDC-3 vs. FPL	69.792	>0.9999	-94.692	234.276	15.625	>0.9999	-186.796	218.046	14.50	>0.9999	-78.88	>0.9999	-81.94	>0.9999
24 vs. 29	EDC-3 vs. APL	85.417	>0.9999	-79.067	249.901	-31.250	>0.9999	-233.671	171.171	0.00	>0.9999	-9.44	>0.9999	-17.06	>0.9999
24 vs. 30	EDC-3 vs. EPB	109.375	0.9912	-55.109	273.859	21.875	>0.9999	-180.546	224.296	-27.88	>0.9999	-22.94	>0.9999	-56.06	>0.9999
24 vs. 31	EDC-3 vs. EPL	137.500	0.4351	-26.984	301.984	56.250	>0.9999	-146.171	258.671	43.50	>0.9999	-68.50	>0.9999	-73.13	>0.9999
25 vs. 26	EDC-4 vs. EDC-5	77.027	>0.9999	-100.981	255.035	22.986	>0.9999	-195.964	241.936	-32.50	>0.9999	-33.02	>0.9999	-10.94	>0.9999
25 vs. 27	EDC-4 vs. EDM	44.792	>0.9999	-119.692	209.276	34.375	>0.9999	-168.046	236.796	0.00	>0.9999	0.94	>0.9999	0.00	>0.9999
25 vs. 28	EDC-4 vs. FPL	45.833	>0.9999	-118.651	210.317	37.500	>0.9999	-164.921	239.921	0.00	>0.9999	-69.44	>0.9999	-70.13	>0.9999
25 vs. 29	EDC-4 vs. APL	61.458	>0.9999	-103.026	225.942	-9.375	>0.9999	-211.796	193.046	-14.50	>0.9999	0.00	>0.9999	-5.25	>0.9999
25 vs. 30	EDC-4 vs. EPB	85.417	>0.9999	-79.067	249.901	43.750	>0.9999	-158.671	246.171	-42.38	>0.9999	-13.50	>0.9999	-44.25	>0.9999
25 vs. 31	EDC-4 vs. EPL	113.542	0.9713	-50.942	278.026	78.125	>0.9999	-124.296	280.546	29.00	>0.9999	-59.06	>0.9999	-61.31	>0.9999
26 vs. 27	EDC-5 vs. EDM	-32.235	>0.9999	-210.243	145.772	11.389	>0.9999	-207.561	230.339	32.50	>0.9999	33.96	>0.9999	10.94	>0.9999
26 vs. 28	EDC-5 vs. FPL	-31.194	>0.9999	-209.201	146.814	14.514	>0.9999	-204.436	233.464	32.50	>0.9999	-36.42	>0.9999	-59.19	>0.9999
26 vs. 29	EDC-5 vs. APL	-15.569	>0.9999	-193.576	162.439	-32.361	>0.9999	-251.311	186.589	18.00	>0.9999	33.02	>0.9999	5.69	>0.9999
26 vs. 30	EDC-5 vs. EPB	8.390	>0.9999	-169.618	186.397	20.764	>0.9999	-198.186	239.714	-9.88	>0.9999	19.52	>0.9999	-33.31	>0.9999
26 vs. 31	EDC-5 vs. EPL	36.515	>0.9999	-141.493	214.522	55.139	>0.9999	-163.811	274.089	61.50	>0.9999	-26.04	>0.9999	-50.38	>0.9999
27 vs. 28	EDM vs. FPL	1.042	>0.9999	-163.442	165.526	3.125	>0.9999	-199.296	205.546	0.00	>0.9999	-70.38	>0.9999	-70.13	>0.9999
27 vs. 29	EDM vs. APL	16.667	>0.9999	-147.817	181.151	-43.750	>0.9999	-246.171	158.671	-14.50	>0.9999	-0.94	>0.9999	-5.25	>0.9999
27 vs. 30	EDM vs. EPB	40.625	>0.9999	-123.859	205.109	9.375	>0.9999	-193.046	211.796	-42.38	>0.9999	-14.44	>0.9999	-44.25	>0.9999
27 vs. 31	EDM vs. EPL	68.750	>0.9999	-95.734	233.234	43.750	>0.9999	-158.671	246.171	29.00	>0.9999	-60.00	>0.9999	-61.31	>0.9999
28 vs. 29	FPL vs. APL	15.625	>0.9999	-148.859	180.109	-46.875	>0.9999	-249.296	155.546	-14.50	>0.9999	69.44	>0.9999	64.88	>0.9999
28 vs. 30	FPL vs. EPB	39.583	>0.9999	-124.901	204.067	6.250	>0.9999	-196.171	208.671	-42.38	>0.9999	55.94	>0.9999	25.88	>0.9999
28 vs. 31	FPL vs. EPL	67.708	>0.9999	-96.776	232.192	40.625	>0.9999	-161.796	243.046	29.00	>0.9999	10.38	>0.9999	8.81	>0.9999
29 vs. 30	APL vs. EPB	23.958	>0.9999	-140.526	188.442	53.125	>0.9999	-149.296	255.546	-27.88	>0.9999	-13.50	>0.9999	-39.00	>0.9999
29 vs. 31	APL vs. EPL	52.083	>0.9999	-112.401	216.567	87.500	>0.9999	-114.921	289.921	43.50	>0.9999	-59.06	>0.9999	-56.06	>0.9999
30 vs. 31	EPB vs. EPL	28.125	>0.9999	-136.359	192.609	34.375	>0.9999	-168.046	236.796	71.38	0.8209	-45.56	>0.9999	-17.06	>0.9999

Supp. Table 9.6.b Pairwise comparisons for upper limb tendon proteoglycan/GAG characteristics. *Green cells indicate significant p-values (< 0.05).*

Tendon Comparison		Proteoglycan (PG) Presence		PG Staining Intensity [Average]		PG Staining Intensity [Maximum]	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
1 vs. 2	SSP vs. IS	1.25	>0.9999	0.00	>0.9999	2.63	>0.9999
1 vs. 3	SSP vs. SSC	36.00	>0.9999	23.54	>0.9999	35.07	>0.9999
1 vs. 4	SSP vs. LHBT	27.31	>0.9999	30.19	0.6564	19.79	>0.9999
1 vs. 5	SSP vs. DBT	113.90	0.1144	30.19	>0.9999	53.50	0.4224
1 vs. 6	SSP vs. BrR	134.40	0.0070	30.19	>0.9999	53.50	>0.9999
1 vs. 7	SSP vs. DTT	87.63	>0.9999	20.89	>0.9999	40.70	>0.9999
1 vs. 8	SSP vs. FCR	146.60	0.0011	30.19	>0.9999	53.50	>0.9999
1 vs. 9	SSP vs. PL	158.80	0.0001				
1 vs. 10	SSP vs. FCU	146.60	0.0011	30.19	>0.9999	53.50	>0.9999
1 vs. 11	SSP vs. ECRL	158.80	0.0001				
1 vs. 12	SSP vs. ECRB	110.10	0.1835	18.56	>0.9999	32.00	>0.9999
1 vs. 13	SSP vs. ECU	101.70	0.4930	18.56	>0.9999	21.25	>0.9999
1 vs. 14	SSP vs. FDS-2	124.20	0.0296	30.19	>0.9999	10.50	>0.9999
1 vs. 15	SSP vs. FDS-3	113.90	0.1144	30.19	>0.9999	53.50	0.4224
1 vs. 16	SSP vs. FDS-4	118.10	0.0669	14.69	>0.9999	39.17	>0.9999
1 vs. 17	SSP vs. FDS-5	146.60	0.0011	30.19	>0.9999	53.50	>0.9999
1 vs. 18	SSP vs. FDP-2	101.70	0.4930	30.19	>0.9999	53.50	0.1207
1 vs. 19	SSP vs. FDP-3	122.30	0.0385	14.69	>0.9999	39.17	>0.9999
1 vs. 20	SSP vs. FDP-4	104.00	0.3779	30.19	>0.9999	53.50	0.1207
1 vs. 21	SSP vs. FDP-5	118.10	0.0669	30.19	>0.9999	53.50	0.4224
1 vs. 22	SSP vs. EIP	146.60	0.0011	30.19	>0.9999	53.50	>0.9999
1 vs. 23	SSP vs. EDC-2	118.10	0.0669	30.19	>0.9999	24.83	>0.9999
1 vs. 24	SSP vs. EDC-3	130.30	0.0128	30.19	>0.9999	53.50	>0.9999
1 vs. 25	SSP vs. EDC-4	142.40	0.0021	30.19	>0.9999	10.50	>0.9999
1 vs. 26	SSP vs. EDC-5	126.30	0.0774	30.19	>0.9999	53.50	>0.9999
1 vs. 27	SSP vs. EDM	118.10	0.0669	30.19	>0.9999	39.17	>0.9999
1 vs. 28	SSP vs. FPL	85.31	>0.9999	20.89	>0.9999	27.70	>0.9999
1 vs. 29	SSP vs. APL	158.80	0.0001				
1 vs. 30	SSP vs. EPB	97.88	0.7559	20.89	>0.9999	44.90	0.4387
1 vs. 31	SSP vs. EPL	158.80	0.0001				
2 vs. 3	IS vs. SSC	34.75	>0.9999	23.54	>0.9999	32.45	>0.9999
2 vs. 4	IS vs. LHBT	26.06	>0.9999	30.19	0.6564	17.16	>0.9999
2 vs. 5	IS vs. DBT	112.60	0.1338	30.19	>0.9999	50.88	0.7285
2 vs. 6	IS vs. BrR	133.20	0.0084	30.19	>0.9999	50.88	>0.9999
2 vs. 7	IS vs. DTT	86.38	>0.9999	20.89	>0.9999	38.08	>0.9999
2 vs. 8	IS vs. FCR	145.40	0.0013	30.19	>0.9999	50.88	>0.9999
2 vs. 9	IS vs. PL	157.60	0.0002				
2 vs. 10	IS vs. FCU	145.40	0.0013	30.19	>0.9999	50.88	>0.9999
2 vs. 11	IS vs. ECRL	157.60	0.0002				
2 vs. 12	IS vs. ECRB	108.80	0.2136	18.56	>0.9999	29.38	>0.9999
2 vs. 13	IS vs. ECU	100.40	0.5681	18.56	>0.9999	18.63	>0.9999
2 vs. 14	IS vs. FDS-2	122.90	0.0351	30.19	>0.9999	7.88	>0.9999
2 vs. 15	IS vs. FDS-3	112.60	0.1338	30.19	>0.9999	50.88	0.7285
2 vs. 16	IS vs. FDS-4	116.80	0.0787	14.69	>0.9999	36.54	>0.9999
2 vs. 17	IS vs. FDS-5	145.40	0.0013	30.19	>0.9999	50.88	>0.9999
2 vs. 18	IS vs. FDP-2	100.40	0.5681	30.19	>0.9999	50.88	0.2329
2 vs. 19	IS vs. FDP-3	121.00	0.0455	14.69	>0.9999	36.54	>0.9999
2 vs. 20	IS vs. FDP-4	102.80	0.4366	30.19	>0.9999	50.88	0.2329
2 vs. 21	IS vs. FDP-5	116.80	0.0787	30.19	>0.9999	50.88	0.7285
2 vs. 22	IS vs. EIP	145.40	0.0013	30.19	>0.9999	50.88	>0.9999
2 vs. 23	IS vs. EDC-2	116.80	0.0787	30.19	>0.9999	22.21	>0.9999
2 vs. 24	IS vs. EDC-3	129.00	0.0152	30.19	>0.9999	50.88	>0.9999
2 vs. 25	IS vs. EDC-4	141.20	0.0025	30.19	>0.9999	7.88	>0.9999
2 vs. 26	IS vs. EDC-5	125.10	0.0898	30.19	>0.9999	50.88	>0.9999
2 vs. 27	IS vs. EDM	116.80	0.0787	30.19	>0.9999	36.54	>0.9999
2 vs. 28	IS vs. FPL	84.06	>0.9999	20.89	>0.9999	25.08	>0.9999
2 vs. 29	IS vs. APL	157.60	0.0002				
2 vs. 30	IS vs. EPB	96.63	0.8670	20.89	>0.9999	42.28	0.8344
2 vs. 31	IS vs. EPL	157.60	0.0002				
3 vs. 4	SSC vs. LHBT	-8.69	>0.9999	6.64	>0.9999	-15.29	>0.9999
3 vs. 5	SSC vs. DBT	77.88	>0.9999	6.64	>0.9999	18.43	>0.9999
3 vs. 6	SSC vs. BrR	98.44	0.7104	6.64	>0.9999	18.43	>0.9999
3 vs. 7	SSC vs. DTT	51.63	>0.9999	-2.66	>0.9999	5.63	>0.9999
3 vs. 8	SSC vs. FCR	110.60	0.1713	6.64	>0.9999	18.43	>0.9999
3 vs. 9	SSC vs. PL	122.80	0.0357				
3 vs. 10	SSC vs. FCU	110.60	0.1713	6.64	>0.9999	18.43	>0.9999
3 vs. 11	SSC vs. ECRL	122.80	0.0357				
3 vs. 12	SSC vs. ECRB	74.06	>0.9999	-4.98	>0.9999	-3.07	>0.9999
3 vs. 13	SSC vs. ECU	65.69	>0.9999	-4.98	>0.9999	-13.82	>0.9999
3 vs. 14	SSC vs. FDS-2	88.19	>0.9999	6.64	>0.9999	-24.57	>0.9999
3 vs. 15	SSC vs. FDS-3	77.88	>0.9999	6.64	>0.9999	18.43	>0.9999
3 vs. 16	SSC vs. FDS-4	82.06	>0.9999	-8.86	>0.9999	4.10	>0.9999
3 vs. 17	SSC vs. FDS-5	110.60	0.1713	6.64	>0.9999	18.43	>0.9999
3 vs. 18	SSC vs. FDP-2	65.69	>0.9999	6.64	>0.9999	18.43	>0.9999
3 vs. 19	SSC vs. FDP-3	86.25	>0.9999	-8.86	>0.9999	4.10	>0.9999
3 vs. 20	SSC vs. FDP-4	68.00	>0.9999	6.64	>0.9999	18.43	>0.9999
3 vs. 21	SSC vs. FDP-5	82.06	>0.9999	6.64	>0.9999	18.43	>0.9999
3 vs. 22	SSC vs. EIP	110.60	0.1713	6.64	>0.9999	18.43	>0.9999
3 vs. 23	SSC vs. EDC-2	82.06	>0.9999	6.64	>0.9999	-10.24	>0.9999
3 vs. 24	SSC vs. EDC-3	94.25	>0.9999	6.64	>0.9999	18.43	>0.9999
3 vs. 25	SSC vs. EDC-4	106.40	0.2838	6.64	>0.9999	-24.57	>0.9999
3 vs. 26	SSC vs. EDC-5	90.31	>0.9999	6.64	>0.9999	18.43	>0.9999

Tendon Comparison		Proteoglycan (PG) Presence		PG Staining Intensity [Average]		PG Staining Intensity [Maximum]	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
3 vs. 27	SSC vs. EDM	82.06	>0.9999	6.64	>0.9999	4.10	>0.9999
3 vs. 28	SSC vs. FPL	49.31	>0.9999	-2.66	>0.9999	-7.37	>0.9999
3 vs. 29	SSC vs. APL	122.80	0.0357				
3 vs. 30	SSC vs. EPB	61.88	>0.9999	-2.66	>0.9999	9.83	>0.9999
3 vs. 31	SSC vs. EPL	122.80	0.0357				
4 vs. 5	LHBT vs. DBT	86.56	>0.9999	0.00	>0.9999	33.71	>0.9999
4 vs. 6	LHBT vs. BrR	107.10	0.2616	0.00	>0.9999	33.71	>0.9999
4 vs. 7	LHBT vs. DTT	60.31	>0.9999	-9.30	>0.9999	20.91	>0.9999
4 vs. 8	LHBT vs. FCR	119.30	0.0569	0.00	>0.9999	33.71	>0.9999
4 vs. 9	LHBT vs. PL	131.50	0.0107				
4 vs. 10	LHBT vs. FCU	119.30	0.0569	0.00	>0.9999	33.71	>0.9999
4 vs. 11	LHBT vs. ECRL	131.50	0.0107				
4 vs. 12	LHBT vs. ECRB	82.75	>0.9999	-11.63	>0.9999	12.21	>0.9999
4 vs. 13	LHBT vs. ECU	74.38	>0.9999	-11.63	>0.9999	1.46	>0.9999
4 vs. 14	LHBT vs. FDS-2	96.88	0.8436	0.00	>0.9999	-9.29	>0.9999
4 vs. 15	LHBT vs. FDS-3	86.56	>0.9999	0.00	>0.9999	33.71	>0.9999
4 vs. 16	LHBT vs. FDS-4	90.75	>0.9999	-15.50	>0.9999	19.38	>0.9999
4 vs. 17	LHBT vs. FDS-5	119.30	0.0569	0.00	>0.9999	33.71	>0.9999
4 vs. 18	LHBT vs. FDP-2	74.38	>0.9999	0.00	>0.9999	33.71	>0.9999
4 vs. 19	LHBT vs. FDP-3	94.94	>0.9999	-15.50	>0.9999	19.38	>0.9999
4 vs. 20	LHBT vs. FDP-4	76.69	>0.9999	0.00	>0.9999	33.71	>0.9999
4 vs. 21	LHBT vs. FDP-5	90.75	>0.9999	0.00	>0.9999	33.71	>0.9999
4 vs. 22	LHBT vs. EIP	119.30	0.0569	0.00	>0.9999	33.71	>0.9999
4 vs. 23	LHBT vs. EDC-2	90.75	>0.9999	0.00	>0.9999	5.05	>0.9999
4 vs. 24	LHBT vs. EDC-3	102.90	0.4273	0.00	>0.9999	33.71	>0.9999
4 vs. 25	LHBT vs. EDC-4	115.10	0.0977	0.00	>0.9999	-9.29	>0.9999
4 vs. 26	LHBT vs. EDC-5	99.00	>0.9999	0.00	>0.9999	33.71	>0.9999
4 vs. 27	LHBT vs. EDM	90.75	>0.9999	0.00	>0.9999	19.38	>0.9999
4 vs. 28	LHBT vs. FPL	58.00	>0.9999	-9.30	>0.9999	7.91	>0.9999
4 vs. 29	LHBT vs. APL	131.50	0.0107				
4 vs. 30	LHBT vs. EPB	70.56	>0.9999	-9.30	>0.9999	25.11	>0.9999
4 vs. 31	LHBT vs. EPL	131.50	0.0107				
5 vs. 6	DBT vs. BrR	20.56	>0.9999	0.00	>0.9999	0.00	>0.9999
5 vs. 7	DBT vs. DTT	-26.25	>0.9999	-9.30	>0.9999	-12.80	>0.9999
5 vs. 8	DBT vs. FCR	32.75	>0.9999	0.00	>0.9999	0.00	>0.9999
5 vs. 9	DBT vs. PL	44.94	>0.9999				
5 vs. 10	DBT vs. FCU	32.75	>0.9999	0.00	>0.9999	0.00	>0.9999
5 vs. 11	DBT vs. ECRL	44.94	>0.9999				
5 vs. 12	DBT vs. ECRB	-3.81	>0.9999	-11.63	>0.9999	-21.50	>0.9999
5 vs. 13	DBT vs. ECU	-12.19	>0.9999	-11.63	>0.9999	-32.25	>0.9999
5 vs. 14	DBT vs. FDS-2	10.31	>0.9999	0.00	>0.9999	-43.00	>0.9999
5 vs. 15	DBT vs. FDS-3	0.00	>0.9999	0.00	>0.9999	0.00	>0.9999
5 vs. 16	DBT vs. FDS-4	4.19	>0.9999	-15.50	>0.9999	-14.33	>0.9999
5 vs. 17	DBT vs. FDS-5	32.75	>0.9999	0.00	>0.9999	0.00	>0.9999
5 vs. 18	DBT vs. FDP-2	-12.19	>0.9999	0.00	>0.9999	0.00	>0.9999
5 vs. 19	DBT vs. FDP-3	8.38	>0.9999	-15.50	>0.9999	-14.33	>0.9999
5 vs. 20	DBT vs. FDP-4	-9.88	>0.9999	0.00	>0.9999	0.00	>0.9999
5 vs. 21	DBT vs. FDP-5	4.19	>0.9999	0.00	>0.9999	0.00	>0.9999
5 vs. 22	DBT vs. EIP	32.75	>0.9999	0.00	>0.9999	0.00	>0.9999
5 vs. 23	DBT vs. EDC-2	4.19	>0.9999	0.00	>0.9999	-28.67	>0.9999
5 vs. 24	DBT vs. EDC-3	16.38	>0.9999	0.00	>0.9999	0.00	>0.9999
5 vs. 25	DBT vs. EDC-4	28.56	>0.9999	0.00	>0.9999	-43.00	>0.9999
5 vs. 26	DBT vs. EDC-5	12.44	>0.9999	0.00	>0.9999	0.00	>0.9999
5 vs. 27	DBT vs. EDM	4.19	>0.9999	0.00	>0.9999	-14.33	>0.9999
5 vs. 28	DBT vs. FPL	-28.56	>0.9999	-9.30	>0.9999	-25.80	>0.9999
5 vs. 29	DBT vs. APL	44.94	>0.9999				
5 vs. 30	DBT vs. EPB	-16.00	>0.9999	-9.30	>0.9999	-8.60	>0.9999
5 vs. 31	DBT vs. EPL	44.94	>0.9999				
6 vs. 7	BrR vs. DTT	-46.81	>0.9999	-9.30	>0.9999	-12.80	>0.9999
6 vs. 8	BrR vs. FCR	12.19	>0.9999	0.00	>0.9999	0.00	>0.9999
6 vs. 9	BrR vs. PL	24.38	>0.9999				
6 vs. 10	BrR vs. FCU	12.19	>0.9999	0.00	>0.9999	0.00	>0.9999
6 vs. 11	BrR vs. ECRL	24.38	>0.9999				
6 vs. 12	BrR vs. ECRB	-24.38	>0.9999	-11.63	>0.9999	-21.50	>0.9999
6 vs. 13	BrR vs. ECU	-32.75	>0.9999	-11.63	>0.9999	-32.25	>0.9999
6 vs. 14	BrR vs. FDS-2	-10.25	>0.9999	0.00	>0.9999	-43.00	>0.9999
6 vs. 15	BrR vs. FDS-3	-20.56	>0.9999	0.00	>0.9999	0.00	>0.9999
6 vs. 16	BrR vs. FDS-4	-16.38	>0.9999	-15.50	>0.9999	-14.33	>0.9999
6 vs. 17	BrR vs. FDS-5	12.19	>0.9999	0.00	>0.9999	0.00	>0.9999
6 vs. 18	BrR vs. FDP-2	-32.75	>0.9999	0.00	>0.9999	0.00	>0.9999
6 vs. 19	BrR vs. FDP-3	-12.19	>0.9999	-15.50	>0.9999	-14.33	>0.9999
6 vs. 20	BrR vs. FDP-4	-30.44	>0.9999	0.00	>0.9999	0.00	>0.9999
6 vs. 21	BrR vs. FDP-5	-16.38	>0.9999	0.00	>0.9999	0.00	>0.9999
6 vs. 22	BrR vs. EIP	12.19	>0.9999	0.00	>0.9999	0.00	>0.9999
6 vs. 23	BrR vs. EDC-2	-16.38	>0.9999	0.00	>0.9999	-28.67	>0.9999
6 vs. 24	BrR vs. EDC-3	-4.19	>0.9999	0.00	>0.9999	0.00	>0.9999
6 vs. 25	BrR vs. EDC-4	8.00	>0.9999	0.00	>0.9999	-43.00	>0.9999
6 vs. 26	BrR vs. EDC-5	-8.13	>0.9999	0.00	>0.9999	0.00	>0.9999
6 vs. 27	BrR vs. EDM	-16.38	>0.9999	0.00	>0.9999	-14.33	>0.9999
6 vs. 28	BrR vs. FPL	-49.13	>0.9999	-9.30	>0.9999	-25.80	>0.9999
6 vs. 29	BrR vs. APL	24.38	>0.9999				
6 vs. 30	BrR vs. EPB	-36.56	>0.9999	-9.30	>0.9999	-8.60	>0.9999
6 vs. 31	BrR vs. EPL	24.38	>0.9999				
7 vs. 8	DTT vs. FCR	59.00	>0.9999	9.30	>0.9999	12.80	>0.9999
7 vs. 9	DTT vs. PL	71.19	>0.9999				
7 vs. 10	DTT vs. FCU	59.00	>0.9999	9.30	>0.9999	12.80	>0.9999
7 vs. 11	DTT vs. ECRL	71.19	>0.9999				

Tendon Comparison		Proteoglycan (PG) Presence		PG Staining Intensity [Average]		PG Staining Intensity [Maximum]	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
7 vs. 12	DTT vs. ECRB	22.44	>0.9999	-2.33	>0.9999	-8.70	>0.9999
7 vs. 13	DTT vs. ECU	14.06	>0.9999	-2.33	>0.9999	-19.45	>0.9999
7 vs. 14	DTT vs. FDS-2	36.56	>0.9999	9.30	>0.9999	-30.20	>0.9999
7 vs. 15	DTT vs. FDS-3	26.25	>0.9999	9.30	>0.9999	12.80	>0.9999
7 vs. 16	DTT vs. FDS-4	30.44	>0.9999	-6.20	>0.9999	-1.53	>0.9999
7 vs. 17	DTT vs. FDS-5	59.00	>0.9999	9.30	>0.9999	12.80	>0.9999
7 vs. 18	DTT vs. FDP-2	14.06	>0.9999	9.30	>0.9999	12.80	>0.9999
7 vs. 19	DTT vs. FDP-3	34.63	>0.9999	-6.20	>0.9999	-1.53	>0.9999
7 vs. 20	DTT vs. FDP-4	16.38	>0.9999	9.30	>0.9999	12.80	>0.9999
7 vs. 21	DTT vs. FDP-5	30.44	>0.9999	9.30	>0.9999	12.80	>0.9999
7 vs. 22	DTT vs. EIP	59.00	>0.9999	9.30	>0.9999	12.80	>0.9999
7 vs. 23	DTT vs. EDC-2	30.44	>0.9999	9.30	>0.9999	-15.87	>0.9999
7 vs. 24	DTT vs. EDC-3	42.63	>0.9999	9.30	>0.9999	12.80	>0.9999
7 vs. 25	DTT vs. EDC-4	54.81	>0.9999	9.30	>0.9999	-30.20	>0.9999
7 vs. 26	DTT vs. EDC-5	38.69	>0.9999	9.30	>0.9999	12.80	>0.9999
7 vs. 27	DTT vs. EDM	30.44	>0.9999	9.30	>0.9999	-1.53	>0.9999
7 vs. 28	DTT vs. FPL	-2.31	>0.9999	0.00	>0.9999	-13.00	>0.9999
7 vs. 29	DTT vs. APL	71.19	>0.9999				
7 vs. 30	DTT vs. EPB	10.25	>0.9999	0.00	>0.9999	4.20	>0.9999
7 vs. 31	DTT vs. EPL	71.19	>0.9999				
8 vs. 9	FCR vs. PL	12.19	>0.9999				
8 vs. 10	FCR vs. FCU	0.00	>0.9999	0.00	>0.9999	0.00	>0.9999
8 vs. 11	FCR vs. ECRL	12.19	>0.9999				
8 vs. 12	FCR vs. ECRB	-36.56	>0.9999	-11.63	>0.9999	-21.50	>0.9999
8 vs. 13	FCR vs. ECU	-44.94	>0.9999	-11.63	>0.9999	-32.25	>0.9999
8 vs. 14	FCR vs. FDS-2	-22.44	>0.9999	0.00	>0.9999	-43.00	>0.9999
8 vs. 15	FCR vs. FDS-3	-32.75	>0.9999	0.00	>0.9999	0.00	>0.9999
8 vs. 16	FCR vs. FDS-4	-28.56	>0.9999	-15.50	>0.9999	-14.33	>0.9999
8 vs. 17	FCR vs. FDS-5	0.00	>0.9999	0.00	>0.9999	0.00	>0.9999
8 vs. 18	FCR vs. FDP-2	-44.94	>0.9999	0.00	>0.9999	0.00	>0.9999
8 vs. 19	FCR vs. FDP-3	-24.38	>0.9999	-15.50	>0.9999	-14.33	>0.9999
8 vs. 20	FCR vs. FDP-4	-42.63	>0.9999	0.00	>0.9999	0.00	>0.9999
8 vs. 21	FCR vs. FDP-5	-28.56	>0.9999	0.00	>0.9999	0.00	>0.9999
8 vs. 22	FCR vs. EIP	0.00	>0.9999	0.00	>0.9999	0.00	>0.9999
8 vs. 23	FCR vs. EDC-2	-28.56	>0.9999	0.00	>0.9999	-28.67	>0.9999
8 vs. 24	FCR vs. EDC-3	-16.38	>0.9999	0.00	>0.9999	0.00	>0.9999
8 vs. 25	FCR vs. EDC-4	-4.19	>0.9999	0.00	>0.9999	-43.00	>0.9999
8 vs. 26	FCR vs. EDC-5	-20.31	>0.9999	0.00	>0.9999	0.00	>0.9999
8 vs. 27	FCR vs. EDM	-28.56	>0.9999	0.00	>0.9999	-14.33	>0.9999
8 vs. 28	FCR vs. FPL	-61.31	>0.9999	-9.30	>0.9999	-25.80	>0.9999
8 vs. 29	FCR vs. APL	12.19	>0.9999				
8 vs. 30	FCR vs. EPB	-48.75	>0.9999	-9.30	>0.9999	-8.60	>0.9999
8 vs. 31	FCR vs. EPL	12.19	>0.9999				
9 vs. 10	PL vs. FCU	-12.19	>0.9999				
9 vs. 11	PL vs. ECRL	0.00	>0.9999				
9 vs. 12	PL vs. ECRB	-48.75	>0.9999				
9 vs. 13	PL vs. ECU	-57.13	>0.9999				
9 vs. 14	PL vs. FDS-2	-34.63	>0.9999				
9 vs. 15	PL vs. FDS-3	-44.94	>0.9999				
9 vs. 16	PL vs. FDS-4	-40.75	>0.9999				
9 vs. 17	PL vs. FDS-5	-12.19	>0.9999				
9 vs. 18	PL vs. FDP-2	-57.13	>0.9999				
9 vs. 19	PL vs. FDP-3	-36.56	>0.9999				
9 vs. 20	PL vs. FDP-4	-54.81	>0.9999				
9 vs. 21	PL vs. FDP-5	-40.75	>0.9999				
9 vs. 22	PL vs. EIP	-12.19	>0.9999				
9 vs. 23	PL vs. EDC-2	-40.75	>0.9999				
9 vs. 24	PL vs. EDC-3	-28.56	>0.9999				
9 vs. 25	PL vs. EDC-4	-16.38	>0.9999				
9 vs. 26	PL vs. EDC-5	-32.50	>0.9999				
9 vs. 27	PL vs. EDM	-40.75	>0.9999				
9 vs. 28	PL vs. FPL	-73.50	>0.9999				
9 vs. 29	PL vs. APL	0.00	>0.9999				
9 vs. 30	PL vs. EPB	-60.94	>0.9999				
9 vs. 31	PL vs. EPL	0.00	>0.9999				
10 vs. 11	FCU vs. ECRL	12.19	>0.9999				
10 vs. 12	FCU vs. ECRB	-36.56	>0.9999	-11.63	>0.9999	-21.50	>0.9999
10 vs. 13	FCU vs. ECU	-44.94	>0.9999	-11.63	>0.9999	-32.25	>0.9999
10 vs. 14	FCU vs. FDS-2	-22.44	>0.9999	0.00	>0.9999	-43.00	>0.9999
10 vs. 15	FCU vs. FDS-3	-32.75	>0.9999	0.00	>0.9999	0.00	>0.9999
10 vs. 16	FCU vs. FDS-4	-28.56	>0.9999	-15.50	>0.9999	-14.33	>0.9999
10 vs. 17	FCU vs. FDS-5	0.00	>0.9999	0.00	>0.9999	0.00	>0.9999
10 vs. 18	FCU vs. FDP-2	-44.94	>0.9999	0.00	>0.9999	0.00	>0.9999
10 vs. 19	FCU vs. FDP-3	-24.38	>0.9999	-15.50	>0.9999	-14.33	>0.9999
10 vs. 20	FCU vs. FDP-4	-42.63	>0.9999	0.00	>0.9999	0.00	>0.9999
10 vs. 21	FCU vs. FDP-5	-28.56	>0.9999	0.00	>0.9999	0.00	>0.9999
10 vs. 22	FCU vs. EIP	0.00	>0.9999	0.00	>0.9999	0.00	>0.9999
10 vs. 23	FCU vs. EDC-2	-28.56	>0.9999	0.00	>0.9999	-28.67	>0.9999
10 vs. 24	FCU vs. EDC-3	-16.38	>0.9999	0.00	>0.9999	0.00	>0.9999
10 vs. 25	FCU vs. EDC-4	-4.19	>0.9999	0.00	>0.9999	-43.00	>0.9999
10 vs. 26	FCU vs. EDC-5	-20.31	>0.9999	0.00	>0.9999	0.00	>0.9999
10 vs. 27	FCU vs. EDM	-28.56	>0.9999	0.00	>0.9999	-14.33	>0.9999
10 vs. 28	FCU vs. FPL	-61.31	>0.9999	-9.30	>0.9999	-25.80	>0.9999
10 vs. 29	FCU vs. APL	12.19	>0.9999				
10 vs. 30	FCU vs. EPB	-48.75	>0.9999	-9.30	>0.9999	-8.60	>0.9999
10 vs. 31	FCU vs. EPL	12.19	>0.9999				
11 vs. 12	ECRL vs. ECRB	-48.75	>0.9999				

Tendon Comparison		Proteoglycan (PG) Presence		PG Staining Intensity [Average]		PG Staining Intensity [Maximum]	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
11 vs. 13	ECRL vs. ECU	-57.13	>0.9999				
11 vs. 14	ECRL vs. FDS-2	-34.63	>0.9999				
11 vs. 15	ECRL vs. FDS-3	-44.94	>0.9999				
11 vs. 16	ECRL vs. FDS-4	-40.75	>0.9999				
11 vs. 17	ECRL vs. FDS-5	-12.19	>0.9999				
11 vs. 18	ECRL vs. FDP-2	-57.13	>0.9999				
11 vs. 19	ECRL vs. FDP-3	-36.56	>0.9999				
11 vs. 20	ECRL vs. FDP-4	-54.81	>0.9999				
11 vs. 21	ECRL vs. FDP-5	-40.75	>0.9999				
11 vs. 22	ECRL vs. EIP	-12.19	>0.9999				
11 vs. 23	ECRL vs. EDC-2	-40.75	>0.9999				
11 vs. 24	ECRL vs. EDC-3	-28.56	>0.9999				
11 vs. 25	ECRL vs. EDC-4	-16.38	>0.9999				
11 vs. 26	ECRL vs. EDC-5	-32.50	>0.9999				
11 vs. 27	ECRL vs. EDM	-40.75	>0.9999				
11 vs. 28	ECRL vs. FPL	-73.50	>0.9999				
11 vs. 29	ECRL vs. APL	0.00	>0.9999				
11 vs. 30	ECRL vs. EPB	-60.94	>0.9999				
11 vs. 31	ECRL vs. EPL	0.00	>0.9999				
12 vs. 13	ECRB vs. ECU	-8.38	>0.9999	0.00	>0.9999	-10.75	>0.9999
12 vs. 14	ECRB vs. FDS-2	14.13	>0.9999	11.63	>0.9999	-21.50	>0.9999
12 vs. 15	ECRB vs. FDS-3	3.81	>0.9999	11.63	>0.9999	21.50	>0.9999
12 vs. 16	ECRB vs. FDS-4	8.00	>0.9999	-3.88	>0.9999	7.17	>0.9999
12 vs. 17	ECRB vs. FDS-5	36.56	>0.9999	11.63	>0.9999	21.50	>0.9999
12 vs. 18	ECRB vs. FDP-2	-8.38	>0.9999	11.63	>0.9999	21.50	>0.9999
12 vs. 19	ECRB vs. FDP-3	12.19	>0.9999	-3.88	>0.9999	7.17	>0.9999
12 vs. 20	ECRB vs. FDP-4	-6.06	>0.9999	11.63	>0.9999	21.50	>0.9999
12 vs. 21	ECRB vs. FDP-5	8.00	>0.9999	11.63	>0.9999	21.50	>0.9999
12 vs. 22	ECRB vs. EIP	36.56	>0.9999	11.63	>0.9999	21.50	>0.9999
12 vs. 23	ECRB vs. EDC-2	8.00	>0.9999	11.63	>0.9999	-7.17	>0.9999
12 vs. 24	ECRB vs. EDC-3	20.19	>0.9999	11.63	>0.9999	21.50	>0.9999
12 vs. 25	ECRB vs. EDC-4	32.38	>0.9999	11.63	>0.9999	-21.50	>0.9999
12 vs. 26	ECRB vs. EDC-5	16.25	>0.9999	11.63	>0.9999	21.50	>0.9999
12 vs. 27	ECRB vs. EDM	8.00	>0.9999	11.63	>0.9999	7.17	>0.9999
12 vs. 28	ECRB vs. FPL	-24.75	>0.9999	2.33	>0.9999	-4.30	>0.9999
12 vs. 29	ECRB vs. APL	48.75	>0.9999				
12 vs. 30	ECRB vs. EPB	-12.19	>0.9999	2.33	>0.9999	12.90	>0.9999
12 vs. 31	ECRB vs. EPL	48.75	>0.9999				
13 vs. 14	ECU vs. FDS-2	22.50	>0.9999	11.63	>0.9999	-10.75	>0.9999
13 vs. 15	ECU vs. FDS-3	12.19	>0.9999	11.63	>0.9999	32.25	>0.9999
13 vs. 16	ECU vs. FDS-4	16.38	>0.9999	-3.88	>0.9999	17.92	>0.9999
13 vs. 17	ECU vs. FDS-5	44.94	>0.9999	11.63	>0.9999	32.25	>0.9999
13 vs. 18	ECU vs. FDP-2	0.00	>0.9999	11.63	>0.9999	32.25	>0.9999
13 vs. 19	ECU vs. FDP-3	20.56	>0.9999	-3.88	>0.9999	17.92	>0.9999
13 vs. 20	ECU vs. FDP-4	2.31	>0.9999	11.63	>0.9999	32.25	>0.9999
13 vs. 21	ECU vs. FDP-5	16.38	>0.9999	11.63	>0.9999	32.25	>0.9999
13 vs. 22	ECU vs. EIP	44.94	>0.9999	11.63	>0.9999	32.25	>0.9999
13 vs. 23	ECU vs. EDC-2	16.38	>0.9999	11.63	>0.9999	3.58	>0.9999
13 vs. 24	ECU vs. EDC-3	28.56	>0.9999	11.63	>0.9999	32.25	>0.9999
13 vs. 25	ECU vs. EDC-4	40.75	>0.9999	11.63	>0.9999	-10.75	>0.9999
13 vs. 26	ECU vs. EDC-5	24.63	>0.9999	11.63	>0.9999	32.25	>0.9999
13 vs. 27	ECU vs. EDM	16.38	>0.9999	11.63	>0.9999	17.92	>0.9999
13 vs. 28	ECU vs. FPL	-16.38	>0.9999	2.33	>0.9999	6.45	>0.9999
13 vs. 29	ECU vs. APL	57.13	>0.9999				
13 vs. 30	ECU vs. EPB	-3.81	>0.9999	2.33	>0.9999	23.65	>0.9999
13 vs. 31	ECU vs. EPL	57.13	>0.9999				
14 vs. 15	FDS-2 vs. FDS-3	-10.31	>0.9999	0.00	>0.9999	43.00	>0.9999
14 vs. 16	FDS-2 vs. FDS-4	-6.13	>0.9999	-15.50	>0.9999	28.67	>0.9999
14 vs. 17	FDS-2 vs. FDS-5	22.44	>0.9999	0.00	>0.9999	43.00	>0.9999
14 vs. 18	FDS-2 vs. FDP-2	-22.50	>0.9999	0.00	>0.9999	43.00	>0.9999
14 vs. 19	FDS-2 vs. FDP-3	-1.94	>0.9999	-15.50	>0.9999	28.67	>0.9999
14 vs. 20	FDS-2 vs. FDP-4	-20.19	>0.9999	0.00	>0.9999	43.00	>0.9999
14 vs. 21	FDS-2 vs. FDP-5	-6.13	>0.9999	0.00	>0.9999	43.00	>0.9999
14 vs. 22	FDS-2 vs. EIP	22.44	>0.9999	0.00	>0.9999	43.00	>0.9999
14 vs. 23	FDS-2 vs. EDC-2	-6.13	>0.9999	0.00	>0.9999	14.33	>0.9999
14 vs. 24	FDS-2 vs. EDC-3	6.06	>0.9999	0.00	>0.9999	43.00	>0.9999
14 vs. 25	FDS-2 vs. EDC-4	18.25	>0.9999	0.00	>0.9999	0.00	>0.9999
14 vs. 26	FDS-2 vs. EDC-5	2.13	>0.9999	0.00	>0.9999	43.00	>0.9999
14 vs. 27	FDS-2 vs. EDM	-6.13	>0.9999	0.00	>0.9999	28.67	>0.9999
14 vs. 28	FDS-2 vs. FPL	-38.88	>0.9999	-9.30	>0.9999	17.20	>0.9999
14 vs. 29	FDS-2 vs. APL	34.63	>0.9999				
14 vs. 30	FDS-2 vs. EPB	-26.31	>0.9999	-9.30	>0.9999	34.40	>0.9999
14 vs. 31	FDS-2 vs. EPL	34.63	>0.9999				
15 vs. 16	FDS-3 vs. FDS-4	4.19	>0.9999	-15.50	>0.9999	-14.33	>0.9999
15 vs. 17	FDS-3 vs. FDS-5	32.75	>0.9999	0.00	>0.9999	0.00	>0.9999
15 vs. 18	FDS-3 vs. FDP-2	-12.19	>0.9999	0.00	>0.9999	0.00	>0.9999
15 vs. 19	FDS-3 vs. FDP-3	8.38	>0.9999	-15.50	>0.9999	-14.33	>0.9999
15 vs. 20	FDS-3 vs. FDP-4	-9.88	>0.9999	0.00	>0.9999	0.00	>0.9999
15 vs. 21	FDS-3 vs. FDP-5	4.19	>0.9999	0.00	>0.9999	0.00	>0.9999
15 vs. 22	FDS-3 vs. EIP	32.75	>0.9999	0.00	>0.9999	0.00	>0.9999
15 vs. 23	FDS-3 vs. EDC-2	4.19	>0.9999	0.00	>0.9999	-28.67	>0.9999
15 vs. 24	FDS-3 vs. EDC-3	16.38	>0.9999	0.00	>0.9999	0.00	>0.9999
15 vs. 25	FDS-3 vs. EDC-4	28.56	>0.9999	0.00	>0.9999	-43.00	>0.9999
15 vs. 26	FDS-3 vs. EDC-5	12.44	>0.9999	0.00	>0.9999	0.00	>0.9999
15 vs. 27	FDS-3 vs. EDM	4.19	>0.9999	0.00	>0.9999	-14.33	>0.9999
15 vs. 28	FDS-3 vs. FPL	-28.56	>0.9999	-9.30	>0.9999	-25.80	>0.9999
15 vs. 29	FDS-3 vs. APL	44.94	>0.9999				

Tendon Comparison		Proteoglycan (PG) Presence		PG Staining Intensity [Average]		PG Staining Intensity [Maximum]	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
15 vs. 30	FDS-3 vs. EPB	-16.00	>0.9999	-9.30	>0.9999	-8.60	>0.9999
15 vs. 31	FDS-3 vs. EPL	44.94	>0.9999				
16 vs. 17	FDS-4 vs. FDS-5	28.56	>0.9999	15.50	>0.9999	14.33	>0.9999
16 vs. 18	FDS-4 vs. FDP-2	-16.38	>0.9999	15.50	>0.9999	14.33	>0.9999
16 vs. 19	FDS-4 vs. FDP-3	4.19	>0.9999	0.00	>0.9999	0.00	>0.9999
16 vs. 20	FDS-4 vs. FDP-4	-14.06	>0.9999	15.50	>0.9999	14.33	>0.9999
16 vs. 21	FDS-4 vs. FDP-5	0.00	>0.9999	15.50	>0.9999	14.33	>0.9999
16 vs. 22	FDS-4 vs. EIP	28.56	>0.9999	15.50	>0.9999	14.33	>0.9999
16 vs. 23	FDS-4 vs. EDC-2	0.00	>0.9999	15.50	>0.9999	-14.33	>0.9999
16 vs. 24	FDS-4 vs. EDC-3	12.19	>0.9999	15.50	>0.9999	14.33	>0.9999
16 vs. 25	FDS-4 vs. EDC-4	24.38	>0.9999	15.50	>0.9999	-28.67	>0.9999
16 vs. 26	FDS-4 vs. EDC-5	8.25	>0.9999	15.50	>0.9999	14.33	>0.9999
16 vs. 27	FDS-4 vs. EDM	0.00	>0.9999	15.50	>0.9999	0.00	>0.9999
16 vs. 28	FDS-4 vs. FPL	-32.75	>0.9999	6.20	>0.9999	-11.47	>0.9999
16 vs. 29	FDS-4 vs. APL	40.75	>0.9999				
16 vs. 30	FDS-4 vs. EPB	-20.19	>0.9999	6.20	>0.9999	5.73	>0.9999
16 vs. 31	FDS-4 vs. EPL	40.75	>0.9999				
17 vs. 18	FDS-5 vs. FDP-2	-44.94	>0.9999	0.00	>0.9999	0.00	>0.9999
17 vs. 19	FDS-5 vs. FDP-3	-24.38	>0.9999	-15.50	>0.9999	-14.33	>0.9999
17 vs. 20	FDS-5 vs. FDP-4	-42.63	>0.9999	0.00	>0.9999	0.00	>0.9999
17 vs. 21	FDS-5 vs. FDP-5	-28.56	>0.9999	0.00	>0.9999	0.00	>0.9999
17 vs. 22	FDS-5 vs. EIP	0.00	>0.9999	0.00	>0.9999	0.00	>0.9999
17 vs. 23	FDS-5 vs. EDC-2	-28.56	>0.9999	0.00	>0.9999	-28.67	>0.9999
17 vs. 24	FDS-5 vs. EDC-3	-16.38	>0.9999	0.00	>0.9999	0.00	>0.9999
17 vs. 25	FDS-5 vs. EDC-4	-4.19	>0.9999	0.00	>0.9999	-43.00	>0.9999
17 vs. 26	FDS-5 vs. EDC-5	-20.31	>0.9999	0.00	>0.9999	0.00	>0.9999
17 vs. 27	FDS-5 vs. EDM	-28.56	>0.9999	0.00	>0.9999	-14.33	>0.9999
17 vs. 28	FDS-5 vs. FPL	-61.31	>0.9999	-9.30	>0.9999	-25.80	>0.9999
17 vs. 29	FDS-5 vs. APL	12.19	>0.9999				
17 vs. 30	FDS-5 vs. EPB	-48.75	>0.9999	-9.30	>0.9999	-8.60	>0.9999
17 vs. 31	FDS-5 vs. EPL	12.19	>0.9999				
18 vs. 19	FDP-2 vs. FDP-3	20.56	>0.9999	-15.50	>0.9999	-14.33	>0.9999
18 vs. 20	FDP-2 vs. FDP-4	2.31	>0.9999	0.00	>0.9999	0.00	>0.9999
18 vs. 21	FDP-2 vs. FDP-5	16.38	>0.9999	0.00	>0.9999	0.00	>0.9999
18 vs. 22	FDP-2 vs. EIP	44.94	>0.9999	0.00	>0.9999	0.00	>0.9999
18 vs. 23	FDP-2 vs. EDC-2	16.38	>0.9999	0.00	>0.9999	-28.67	>0.9999
18 vs. 24	FDP-2 vs. EDC-3	28.56	>0.9999	0.00	>0.9999	0.00	>0.9999
18 vs. 25	FDP-2 vs. EDC-4	40.75	>0.9999	0.00	>0.9999	-43.00	>0.9999
18 vs. 26	FDP-2 vs. EDC-5	24.63	>0.9999	0.00	>0.9999	0.00	>0.9999
18 vs. 27	FDP-2 vs. EDM	16.38	>0.9999	0.00	>0.9999	-14.33	>0.9999
18 vs. 28	FDP-2 vs. FPL	-16.38	>0.9999	-9.30	>0.9999	-25.80	>0.9999
18 vs. 29	FDP-2 vs. APL	57.13	>0.9999				
18 vs. 30	FDP-2 vs. EPB	-3.81	>0.9999	-9.30	>0.9999	-8.60	>0.9999
18 vs. 31	FDP-2 vs. EPL	57.13	>0.9999				
19 vs. 20	FDP-3 vs. FDP-4	-18.25	>0.9999	15.50	>0.9999	14.33	>0.9999
19 vs. 21	FDP-3 vs. FDP-5	-4.19	>0.9999	15.50	>0.9999	14.33	>0.9999
19 vs. 22	FDP-3 vs. EIP	24.38	>0.9999	15.50	>0.9999	14.33	>0.9999
19 vs. 23	FDP-3 vs. EDC-2	-4.19	>0.9999	15.50	>0.9999	-14.33	>0.9999
19 vs. 24	FDP-3 vs. EDC-3	8.00	>0.9999	15.50	>0.9999	14.33	>0.9999
19 vs. 25	FDP-3 vs. EDC-4	20.19	>0.9999	15.50	>0.9999	-28.67	>0.9999
19 vs. 26	FDP-3 vs. EDC-5	4.06	>0.9999	15.50	>0.9999	14.33	>0.9999
19 vs. 27	FDP-3 vs. EDM	-4.19	>0.9999	15.50	>0.9999	0.00	>0.9999
19 vs. 28	FDP-3 vs. FPL	-36.94	>0.9999	6.20	>0.9999	-11.47	>0.9999
19 vs. 29	FDP-3 vs. APL	36.56	>0.9999				
19 vs. 30	FDP-3 vs. EPB	-24.38	>0.9999	6.20	>0.9999	5.73	>0.9999
19 vs. 31	FDP-3 vs. EPL	36.56	>0.9999				
20 vs. 21	FDP-4 vs. FDP-5	14.06	>0.9999	0.00	>0.9999	0.00	>0.9999
20 vs. 22	FDP-4 vs. EIP	42.63	>0.9999	0.00	>0.9999	0.00	>0.9999
20 vs. 23	FDP-4 vs. EDC-2	14.06	>0.9999	0.00	>0.9999	-28.67	>0.9999
20 vs. 24	FDP-4 vs. EDC-3	26.25	>0.9999	0.00	>0.9999	0.00	>0.9999
20 vs. 25	FDP-4 vs. EDC-4	38.44	>0.9999	0.00	>0.9999	-43.00	>0.9999
20 vs. 26	FDP-4 vs. EDC-5	22.31	>0.9999	0.00	>0.9999	0.00	>0.9999
20 vs. 27	FDP-4 vs. EDM	14.06	>0.9999	0.00	>0.9999	-14.33	>0.9999
20 vs. 28	FDP-4 vs. FPL	-18.69	>0.9999	-9.30	>0.9999	-25.80	>0.9999
20 vs. 29	FDP-4 vs. APL	54.81	>0.9999				
20 vs. 30	FDP-4 vs. EPB	-6.13	>0.9999	-9.30	>0.9999	-8.60	>0.9999
20 vs. 31	FDP-4 vs. EPL	54.81	>0.9999				
21 vs. 22	FDP-5 vs. EIP	28.56	>0.9999	0.00	>0.9999	0.00	>0.9999
21 vs. 23	FDP-5 vs. EDC-2	0.00	>0.9999	0.00	>0.9999	-28.67	>0.9999
21 vs. 24	FDP-5 vs. EDC-3	12.19	>0.9999	0.00	>0.9999	0.00	>0.9999
21 vs. 25	FDP-5 vs. EDC-4	24.38	>0.9999	0.00	>0.9999	-43.00	>0.9999
21 vs. 26	FDP-5 vs. EDC-5	8.25	>0.9999	0.00	>0.9999	0.00	>0.9999
21 vs. 27	FDP-5 vs. EDM	0.00	>0.9999	0.00	>0.9999	-14.33	>0.9999
21 vs. 28	FDP-5 vs. FPL	-32.75	>0.9999	-9.30	>0.9999	-25.80	>0.9999
21 vs. 29	FDP-5 vs. APL	40.75	>0.9999				
21 vs. 30	FDP-5 vs. EPB	-20.19	>0.9999	-9.30	>0.9999	-8.60	>0.9999
21 vs. 31	FDP-5 vs. EPL	40.75	>0.9999				
22 vs. 23	EIP vs. EDC-2	-28.56	>0.9999	0.00	>0.9999	-28.67	>0.9999
22 vs. 24	EIP vs. EDC-3	-16.38	>0.9999	0.00	>0.9999	0.00	>0.9999
22 vs. 25	EIP vs. EDC-4	-4.19	>0.9999	0.00	>0.9999	-43.00	>0.9999
22 vs. 26	EIP vs. EDC-5	-20.31	>0.9999	0.00	>0.9999	0.00	>0.9999
22 vs. 27	EIP vs. EDM	-28.56	>0.9999	0.00	>0.9999	-14.33	>0.9999
22 vs. 28	EIP vs. FPL	-61.31	>0.9999	-9.30	>0.9999	-25.80	>0.9999
22 vs. 29	EIP vs. APL	12.19	>0.9999				
22 vs. 30	EIP vs. EPB	-48.75	>0.9999	-9.30	>0.9999	-8.60	>0.9999
22 vs. 31	EIP vs. EPL	12.19	>0.9999				
23 vs. 24	EDC-2 vs. EDC-3	12.19	>0.9999	0.00	>0.9999	28.67	>0.9999

Tendon Comparison		Proteoglycan (PG) Presence		PG Staining Intensity [Average]		PG Staining Intensity [Maximum]	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
23 vs. 25	EDC-2 vs. EDC-4	24.38	>0.9999	0.00	>0.9999	-14.33	>0.9999
23 vs. 26	EDC-2 vs. EDC-5	8.25	>0.9999	0.00	>0.9999	28.67	>0.9999
23 vs. 27	EDC-2 vs. EDM	0.00	>0.9999	0.00	>0.9999	14.33	>0.9999
23 vs. 28	EDC-2 vs. FPL	-32.75	>0.9999	-9.30	>0.9999	2.87	>0.9999
23 vs. 29	EDC-2 vs. APL	40.75	>0.9999				
23 vs. 30	EDC-2 vs. EPB	-20.19	>0.9999	-9.30	>0.9999	20.07	>0.9999
23 vs. 31	EDC-2 vs. EPL	40.75	>0.9999				
24 vs. 25	EDC-3 vs. EDC-4	12.19	>0.9999	0.00	>0.9999	-43.00	>0.9999
24 vs. 26	EDC-3 vs. EDC-5	-3.94	>0.9999	0.00	>0.9999	0.00	>0.9999
24 vs. 27	EDC-3 vs. EDM	-12.19	>0.9999	0.00	>0.9999	-14.33	>0.9999
24 vs. 28	EDC-3 vs. FPL	-44.94	>0.9999	-9.30	>0.9999	-25.80	>0.9999
24 vs. 29	EDC-3 vs. APL	28.56	>0.9999				
24 vs. 30	EDC-3 vs. EPB	-32.38	>0.9999	-9.30	>0.9999	-8.60	>0.9999
24 vs. 31	EDC-3 vs. EPL	28.56	>0.9999				
25 vs. 26	EDC-4 vs. EDC-5	-16.13	>0.9999	0.00	>0.9999	43.00	>0.9999
25 vs. 27	EDC-4 vs. EDM	-24.38	>0.9999	0.00	>0.9999	28.67	>0.9999
25 vs. 28	EDC-4 vs. FPL	-57.13	>0.9999	-9.30	>0.9999	17.20	>0.9999
25 vs. 29	EDC-4 vs. APL	16.38	>0.9999				
25 vs. 30	EDC-4 vs. EPB	-44.56	>0.9999	-9.30	>0.9999	34.40	>0.9999
25 vs. 31	EDC-4 vs. EPL	16.38	>0.9999				
26 vs. 27	EDC-5 vs. EDM	-8.25	>0.9999	0.00	>0.9999	-14.33	>0.9999
26 vs. 28	EDC-5 vs. FPL	-41.00	>0.9999	-9.30	>0.9999	-25.80	>0.9999
26 vs. 29	EDC-5 vs. APL	32.50	>0.9999				
26 vs. 30	EDC-5 vs. EPB	-28.44	>0.9999	-9.30	>0.9999	-8.60	>0.9999
26 vs. 31	EDC-5 vs. EPL	32.50	>0.9999				
27 vs. 28	EDM vs. FPL	-32.75	>0.9999	-9.30	>0.9999	-11.47	>0.9999
27 vs. 29	EDM vs. APL	40.75	>0.9999				
27 vs. 30	EDM vs. EPB	-20.19	>0.9999	-9.30	>0.9999	5.73	>0.9999
27 vs. 31	EDM vs. EPL	40.75	>0.9999				
28 vs. 29	FPL vs. APL	73.50	>0.9999				
28 vs. 30	FPL vs. EPB	12.56	>0.9999	0.00	>0.9999	17.20	>0.9999
28 vs. 31	FPL vs. EPL	73.50	>0.9999				
29 vs. 30	APL vs. EPB	-60.94	>0.9999				
29 vs. 31	APL vs. EPL	0.00	>0.9999				
30 vs. 31	EPB vs. EPL	60.94	>0.9999				

Supp. Table 9.6.c Pairwise comparisons for upper limb tendon architectural characteristics. *Green cells indicate significant p-values (< 0.05).*

Tendon Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value
1 vs. 2	SSP vs. IS	14.13	>0.9999	88.06	0.8752	-5.06	>0.9999	26.00	>0.9999	40.25	>0.9999	15.13	>0.9999
1 vs. 3	SSP vs. SSC	9.13	>0.9999	74.19	>0.9999	-34.81	>0.9999	95.75	0.6136	8.00	>0.9999	-7.63	>0.9999
1 vs. 4	SSP vs. LHBT	96.56	>0.9999	124.40	0.0053	-33.44	>0.9999	39.95	>0.9999	139.80	0.0168	79.31	>0.9999
1 vs. 5	SSP vs. DBT	84.56	>0.9999	88.06	0.8752	-115.30	>0.9999	95.75	0.5231	86.50	>0.9999	-17.25	>0.9999
1 vs. 6	SSP vs. BrR	196.40	<0.0001	142.80	0.0002	-43.25	>0.9999	80.15	>0.9999	184.80	<0.0001	92.50	0.5746
1 vs. 7	SSP vs. DTT	84.38	>0.9999	115.40	0.0214	-20.19	>0.9999	79.00	>0.9999	94.13	>0.9999	-5.19	>0.9999
1 vs. 8	SSP vs. FCR	173.00	0.0002	109.80	0.0494	-27.11	>0.9999	95.75	>0.9999	124.90	0.1046	62.31	>0.9999
1 vs. 9	SSP vs. PL	204.90	<0.0001	115.40	0.0214	-108.10	>0.9999	86.00	>0.9999	209.80	<0.0001	88.69	0.9080
1 vs. 10	SSP vs. FCU	170.70	0.0003	129.10	0.0024	-59.00	>0.9999	64.55	>0.9999	121.30	0.1573	75.50	>0.9999
1 vs. 11	SSP vs. ECRL	196.40	<0.0001	129.10	0.0024	-16.44	>0.9999	95.75	>0.9999	178.40	<0.0001	79.31	>0.9999
1 vs. 12	SSP vs. ECRB	170.90	0.0003	142.80	0.0002	-2.56	>0.9999	62.25	>0.9999	149.60	0.0046	41.50	>0.9999
1 vs. 13	SSP vs. ECU	127.80	0.0889	142.80	0.0002	-12.63	>0.9999	75.65	>0.9999	158.40	0.0013	49.13	>0.9999
1 vs. 14	SSP vs. FDS-2	105.60	0.9538	101.80	0.1530	-78.81	>0.9999	69.75	>0.9999	77.75	>0.9999	105.70	0.1038
1 vs. 15	SSP vs. FDS-3	93.44	>0.9999	52.44	>0.9999	-118.30	>0.9999	95.75	0.5231	48.25	>0.9999	79.31	>0.9999
1 vs. 16	SSP vs. FDS-4	110.60	0.5784	79.81	>0.9999	-92.69	>0.9999	84.61	>0.9999	65.63	>0.9999	92.50	0.5746
1 vs. 17	SSP vs. FDS-5	102.10	>0.9999	142.80	0.0002	-59.06	>0.9999	42.89	>0.9999	111.50	0.4590	105.70	0.1038
1 vs. 18	SSP vs. FDP-2	67.38	>0.9999	66.13	>0.9999	-119.10	>0.9999	76.25	>0.9999	59.00	>0.9999	66.13	>0.9999
1 vs. 19	SSP vs. FDP-3	79.56	>0.9999	63.88	>0.9999	-110.70	>0.9999	73.46	>0.9999	64.63	>0.9999	62.31	>0.9999
1 vs. 20	SSP vs. FDP-4	51.81	>0.9999	45.44	>0.9999	-117.00	>0.9999	60.46	>0.9999	52.75	>0.9999	66.13	>0.9999
1 vs. 21	SSP vs. FDP-5	93.44	>0.9999	98.25	0.2440	-111.60	>0.9999	86.00	>0.9999	107.00	0.7305	105.70	0.1038
1 vs. 22	SSP vs. EIP	153.70	0.0034	142.80	0.0002	-5.06	>0.9999	28.38	>0.9999	158.40	0.0013	105.70	0.1038
1 vs. 23	SSP vs. EDC-2	145.20	0.0105	129.10	0.0024	-86.88	>0.9999	81.39	>0.9999	120.30	0.1761	92.50	0.5746
1 vs. 24	SSP vs. EDC-3	187.90	<0.0001	129.10	0.0024	-66.06	>0.9999	80.15	>0.9999	138.90	0.0190	92.50	0.5746
1 vs. 25	SSP vs. EDC-4	170.50	0.0003	129.10	0.0024	-65.56	>0.9999	95.75	0.6136	138.90	0.0190	88.69	0.9080
1 vs. 26	SSP vs. EDC-5	159.30	0.0078	124.60	0.0218	-106.50	>0.9999	50.83	>0.9999	121.80	0.4036	105.70	0.2941
1 vs. 27	SSP vs. EDM	136.30	0.0323	98.25	0.2440	-67.25	>0.9999	84.61	>0.9999	130.10	0.0566	69.75	>0.9999
1 vs. 28	SSP vs. FPL	93.25	>0.9999	129.10	0.0024	-63.31	>0.9999	95.75	0.6136	99.38	>0.9999	105.70	0.1038
1 vs. 29	SSP vs. APL	128.00	0.0870	142.80	0.0002	-5.06	>0.9999	95.75	>0.9999	130.10	0.0566	75.50	>0.9999
1 vs. 30	SSP vs. EPB	136.50	0.0315	110.70	0.0435	-12.00	>0.9999	45.50	>0.9999	125.60	0.0965	75.50	>0.9999
1 vs. 31	SSP vs. EPL	136.50	0.0315	142.80	0.0002	8.06	>0.9999	95.75	>0.9999	175.10	0.0001	105.70	0.1038
2 vs. 3	IS vs. SSC	-5.00	>0.9999	-13.88	>0.9999	-29.75	>0.9999	69.75	>0.9999	-32.25	>0.9999	-22.75	>0.9999
2 vs. 4	IS vs. LHBT	82.44	>0.9999	36.31	>0.9999	-28.38	>0.9999	13.95	>0.9999	99.56	>0.9999	64.19	>0.9999
2 vs. 5	IS vs. DBT	70.44	>0.9999	0.00	>0.9999	-110.20	0.5791	69.75	>0.9999	46.25	>0.9999	-32.38	>0.9999
2 vs. 6	IS vs. BrR	182.30	<0.0001	54.75	>0.9999	-38.19	>0.9999	54.15	>0.9999	144.60	0.0090	77.38	>0.9999
2 vs. 7	IS vs. DTT	70.25	>0.9999	27.38	>0.9999	-15.13	>0.9999	53.00	>0.9999	53.88	>0.9999	-20.31	>0.9999
2 vs. 8	IS vs. FCR	158.90	0.0016	21.75	>0.9999	-22.04	>0.9999	69.75	>0.9999	84.63	>0.9999	47.19	>0.9999
2 vs. 9	IS vs. PL	190.80	<0.0001	27.38	>0.9999	-103.00	>0.9999	60.00	>0.9999	169.50	0.0003	73.56	>0.9999
2 vs. 10	IS vs. FCU	156.60	0.0023	41.06	>0.9999	-53.94	>0.9999	38.55	>0.9999	81.06	>0.9999	60.38	>0.9999
2 vs. 11	IS vs. ECRL	182.30	<0.0001	41.06	>0.9999	-11.38	>0.9999	69.75	>0.9999	138.20	0.0207	64.19	>0.9999
2 vs. 12	IS vs. ECRB	156.80	0.0022	54.75	>0.9999	2.50	>0.9999	36.25	>0.9999	109.30	0.5765	26.38	>0.9999
2 vs. 13	IS vs. ECU	113.70	0.4215	54.75	>0.9999	-7.56	>0.9999	49.65	>0.9999	118.10	0.2247	34.00	>0.9999
2 vs. 14	IS vs. FDS-2	91.50	>0.9999	13.69	>0.9999	-73.75	>0.9999	43.75	>0.9999	37.50	>0.9999	90.56	0.7265
2 vs. 15	IS vs. FDS-3	79.31	>0.9999	-35.63	>0.9999	-113.20	0.4244	69.75	>0.9999	8.00	>0.9999	64.19	>0.9999
2 vs. 16	IS vs. FDS-4	96.50	>0.9999	-8.25	>0.9999	-87.63	>0.9999	58.61	>0.9999	25.38	>0.9999	77.38	>0.9999
2 vs. 17	IS vs. FDS-5	88.00	>0.9999	54.75	>0.9999	-54.00	>0.9999	16.89	>0.9999	71.25	>0.9999	90.56	0.7265
2 vs. 18	IS vs. FDP-2	53.25	>0.9999	-21.94	>0.9999	-114.10	0.3871	50.25	>0.9999	18.75	>0.9999	51.00	>0.9999
2 vs. 19	IS vs. FDP-3	65.44	>0.9999	-24.19	>0.9999	-105.60	0.9164	47.46	>0.9999	24.38	>0.9999	47.19	>0.9999
2 vs. 20	IS vs. FDP-4	37.69	>0.9999	-42.63	>0.9999	-111.90	0.4835	34.46	>0.9999	12.50	>0.9999	51.00	>0.9999
2 vs. 21	IS vs. FDP-5	79.31	>0.9999	10.19	>0.9999	-106.60	0.8351	60.00	>0.9999	66.75	>0.9999	90.56	0.7265

Tendon Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value
2 vs. 22	IS vs. EIP	139.60	0.0216	54.75	>0.9999	0.00	>0.9999	2.38	>0.9999	118.10	0.2247	90.56	0.7265
2 vs. 23	IS vs. EDC-2	131.10	0.0608	41.06	>0.9999	-81.81	>0.9999	55.39	>0.9999	80.06	>0.9999	77.38	>0.9999
2 vs. 24	IS vs. EDC-3	173.80	0.0002	41.06	>0.9999	-61.00	>0.9999	54.15	>0.9999	98.63	>0.9999	77.38	>0.9999
2 vs. 25	IS vs. EDC-4	156.40	0.0023	41.06	>0.9999	-60.50	>0.9999	69.75	>0.9999	98.63	>0.9999	73.56	>0.9999
2 vs. 26	IS vs. EDC-5	145.20	0.0408	36.50	>0.9999	-101.40	>0.9999	24.83	>0.9999	81.50	>0.9999	90.56	>0.9999
2 vs. 27	IS vs. EDM	122.20	0.1685	10.19	>0.9999	-62.19	>0.9999	58.61	>0.9999	89.81	>0.9999	54.63	>0.9999
2 vs. 28	IS vs. FPL	79.13	>0.9999	41.06	>0.9999	-58.25	>0.9999	69.75	>0.9999	59.13	>0.9999	90.56	0.7265
2 vs. 29	IS vs. APL	113.90	0.4134	54.75	>0.9999	0.00	>0.9999	69.75	>0.9999	89.81	>0.9999	60.38	>0.9999
2 vs. 30	IS vs. EPB	122.40	0.1650	22.63	>0.9999	-6.94	>0.9999	19.50	>0.9999	85.31	>0.9999	60.38	>0.9999
2 vs. 31	IS vs. EPL	122.40	0.1650	54.75	>0.9999	13.13	>0.9999	69.75	>0.9999	134.80	0.0317	90.56	0.7265
3 vs. 4	SSC vs. LHBT	87.44	>0.9999	50.19	>0.9999	1.38	>0.9999	-55.80	>0.9999	131.80	0.0458	86.94	>0.9999
3 vs. 5	SSC vs. DBT	75.44	>0.9999	13.88	>0.9999	-80.44	>0.9999	0.00	>0.9999	78.50	>0.9999	-9.63	>0.9999
3 vs. 6	SSC vs. BrR	187.30	<0.0001	68.63	>0.9999	-8.44	>0.9999	-15.60	>0.9999	176.80	<0.0001	100.10	0.2190
3 vs. 7	SSC vs. DTT	75.25	>0.9999	41.25	>0.9999	14.63	>0.9999	-16.75	>0.9999	86.13	>0.9999	2.44	>0.9999
3 vs. 8	SSC vs. FCR	163.90	0.0008	35.63	>0.9999	7.71	>0.9999	0.00	>0.9999	116.90	0.2578	69.94	>0.9999
3 vs. 9	SSC vs. PL	195.80	<0.0001	41.25	>0.9999	-73.25	>0.9999	-9.75	>0.9999	201.80	<0.0001	96.31	0.3577
3 vs. 10	SSC vs. FCU	161.60	0.0011	54.94	>0.9999	-24.19	>0.9999	-31.20	>0.9999	113.30	0.3789	83.13	>0.9999
3 vs. 11	SSC vs. ECRL	187.30	<0.0001	54.94	>0.9999	18.38	>0.9999	0.00	>0.9999	170.40	0.0002	86.94	>0.9999
3 vs. 12	SSC vs. ECRB	161.80	0.0011	68.63	>0.9999	32.25	>0.9999	-33.50	>0.9999	141.60	0.0134	49.13	>0.9999
3 vs. 13	SSC vs. ECU	118.70	0.2475	68.63	>0.9999	22.19	>0.9999	-20.10	>0.9999	150.40	0.0041	56.75	>0.9999
3 vs. 14	SSC vs. FDS-2	96.50	>0.9999	27.56	>0.9999	-44.00	>0.9999	-26.00	>0.9999	69.75	>0.9999	113.30	0.0352
3 vs. 15	SSC vs. FDS-3	84.31	>0.9999	-21.75	>0.9999	-83.44	>0.9999	0.00	>0.9999	40.25	>0.9999	86.94	>0.9999
3 vs. 16	SSC vs. FDS-4	101.50	>0.9999	5.63	>0.9999	-57.88	>0.9999	-11.14	>0.9999	57.63	>0.9999	100.10	0.2190
3 vs. 17	SSC vs. FDS-5	93.00	>0.9999	68.63	>0.9999	-24.25	>0.9999	-52.86	>0.9999	103.50	>0.9999	113.30	0.0352
3 vs. 18	SSC vs. FDP-2	58.25	>0.9999	-8.06	>0.9999	-84.31	>0.9999	-19.50	>0.9999	51.00	>0.9999	73.75	>0.9999
3 vs. 19	SSC vs. FDP-3	70.44	>0.9999	-10.31	>0.9999	-75.88	>0.9999	-22.29	>0.9999	56.63	>0.9999	69.94	>0.9999
3 vs. 20	SSC vs. FDP-4	42.69	>0.9999	-28.75	>0.9999	-82.19	>0.9999	-35.29	>0.9999	44.75	>0.9999	73.75	>0.9999
3 vs. 21	SSC vs. FDP-5	84.31	>0.9999	24.06	>0.9999	-76.81	>0.9999	-9.75	>0.9999	99.00	>0.9999	113.30	0.0352
3 vs. 22	SSC vs. EIP	144.60	0.0114	68.63	>0.9999	29.75	>0.9999	-67.38	>0.9999	150.40	0.0041	113.30	0.0352
3 vs. 23	SSC vs. EDC-2	136.10	0.0333	54.94	>0.9999	-52.06	>0.9999	-14.36	>0.9999	112.30	0.4213	100.10	0.2190
3 vs. 24	SSC vs. EDC-3	178.80	<0.0001	54.94	>0.9999	-31.25	>0.9999	-15.60	>0.9999	130.90	0.0513	100.10	0.2190
3 vs. 25	SSC vs. EDC-4	161.40	0.0012	54.94	>0.9999	-30.75	>0.9999	0.00	>0.9999	130.90	0.0513	96.31	0.3577
3 vs. 26	SSC vs. EDC-5	150.20	0.0231	50.38	>0.9999	-71.69	>0.9999	-44.92	>0.9999	113.80	0.8660	113.30	0.1156
3 vs. 27	SSC vs. EDM	127.20	0.0956	24.06	>0.9999	-32.44	>0.9999	-11.14	>0.9999	122.10	0.1445	77.38	>0.9999
3 vs. 28	SSC vs. FPL	84.13	>0.9999	54.94	>0.9999	-28.50	>0.9999	0.00	>0.9999	91.38	>0.9999	113.30	0.0352
3 vs. 29	SSC vs. APL	118.90	0.2425	68.63	>0.9999	29.75	>0.9999	0.00	>0.9999	122.10	0.1445	83.13	>0.9999
3 vs. 30	SSC vs. EPB	127.40	0.0936	36.50	>0.9999	22.81	>0.9999	-50.25	>0.9999	117.60	0.2391	83.13	>0.9999
3 vs. 31	SSC vs. EPL	127.40	0.0936	68.63	>0.9999	42.88	>0.9999	0.00	>0.9999	167.10	0.0004	113.30	0.0352
4 vs. 5	LHBT vs. DBT	-12.00	>0.9999	-36.31	>0.9999	-81.81	>0.9999	55.80	>0.9999	-53.31	>0.9999	-96.56	0.3465
4 vs. 6	LHBT vs. BrR	99.81	>0.9999	18.44	>0.9999	-9.81	>0.9999	40.20	>0.9999	45.00	>0.9999	13.19	>0.9999
4 vs. 7	LHBT vs. DTT	-12.19	>0.9999	-8.94	>0.9999	13.25	>0.9999	39.05	>0.9999	-45.69	>0.9999	-84.50	>0.9999
4 vs. 8	LHBT vs. FCR	76.44	>0.9999	-14.56	>0.9999	6.33	>0.9999	55.80	>0.9999	-14.94	>0.9999	-17.00	>0.9999
4 vs. 9	LHBT vs. PL	108.30	0.7307	-8.94	>0.9999	-74.63	>0.9999	46.05	>0.9999	69.94	>0.9999	9.38	>0.9999
4 vs. 10	LHBT vs. FCU	74.13	>0.9999	4.75	>0.9999	-25.56	>0.9999	24.60	>0.9999	-18.50	>0.9999	-3.81	>0.9999
4 vs. 11	LHBT vs. ECRL	99.81	>0.9999	4.75	>0.9999	17.00	>0.9999	55.80	>0.9999	38.63	>0.9999	0.00	>0.9999
4 vs. 12	LHBT vs. ECRB	74.31	>0.9999	18.44	>0.9999	30.88	>0.9999	22.30	>0.9999	9.75	>0.9999	-37.81	>0.9999
4 vs. 13	LHBT vs. ECU	31.25	>0.9999	18.44	>0.9999	20.81	>0.9999	35.70	>0.9999	18.56	>0.9999	-30.19	>0.9999
4 vs. 14	LHBT vs. FDS-2	9.06	>0.9999	-22.63	>0.9999	-45.38	>0.9999	29.80	>0.9999	-62.06	>0.9999	26.38	>0.9999
4 vs. 15	LHBT vs. FDS-3	-3.13	>0.9999	-71.94	>0.9999	-84.81	>0.9999	55.80	>0.9999	-91.56	>0.9999	0.00	>0.9999
4 vs. 16	LHBT vs. FDS-4	14.06	>0.9999	-44.56	>0.9999	-59.25	>0.9999	44.66	>0.9999	-74.19	>0.9999	13.19	>0.9999
4 vs. 17	LHBT vs. FDS-5	5.56	>0.9999	18.44	>0.9999	-25.63	>0.9999	2.94	>0.9999	-28.31	>0.9999	26.38	>0.9999
4 vs. 18	LHBT vs. FDP-2	-29.19	>0.9999	-58.25	>0.9999	-85.69	>0.9999	36.30	>0.9999	-80.81	>0.9999	-13.19	>0.9999

Tendon Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value
4 vs. 19	LHBT vs. FDP-3	-17.00	>0.9999	-60.50	>0.9999	-77.25	>0.9999	33.51	>0.9999	-75.19	>0.9999	-17.00	>0.9999
4 vs. 20	LHBT vs. FDP-4	-44.75	>0.9999	-78.94	>0.9999	-83.56	>0.9999	20.51	>0.9999	-87.06	>0.9999	-13.19	>0.9999
4 vs. 21	LHBT vs. FDP-5	-3.13	>0.9999	-26.13	>0.9999	-78.19	>0.9999	46.05	>0.9999	-32.81	>0.9999	26.38	>0.9999
4 vs. 22	LHBT vs. EIP	57.13	>0.9999	18.44	>0.9999	28.38	>0.9999	-11.58	>0.9999	18.56	>0.9999	26.38	>0.9999
4 vs. 23	LHBT vs. EDC-2	48.63	>0.9999	4.75	>0.9999	-53.44	>0.9999	41.44	>0.9999	-19.50	>0.9999	13.19	>0.9999
4 vs. 24	LHBT vs. EDC-3	91.31	>0.9999	4.75	>0.9999	-32.63	>0.9999	40.20	>0.9999	-0.94	>0.9999	13.19	>0.9999
4 vs. 25	LHBT vs. EDC-4	73.94	>0.9999	4.75	>0.9999	-32.13	>0.9999	55.80	>0.9999	-0.94	>0.9999	9.38	>0.9999
4 vs. 26	LHBT vs. EDC-5	62.73	>0.9999	0.19	>0.9999	-73.06	>0.9999	10.88	>0.9999	-18.06	>0.9999	26.38	>0.9999
4 vs. 27	LHBT vs. EDM	39.75	>0.9999	-26.13	>0.9999	-33.81	>0.9999	44.66	>0.9999	-9.75	>0.9999	-9.56	>0.9999
4 vs. 28	LHBT vs. FPL	-3.31	>0.9999	4.75	>0.9999	-29.88	>0.9999	55.80	>0.9999	-40.44	>0.9999	26.38	>0.9999
4 vs. 29	LHBT vs. APL	31.44	>0.9999	18.44	>0.9999	28.38	>0.9999	55.80	>0.9999	-9.75	>0.9999	-3.81	>0.9999
4 vs. 30	LHBT vs. EPB	39.94	>0.9999	-13.69	>0.9999	21.44	>0.9999	5.55	>0.9999	-14.25	>0.9999	-3.81	>0.9999
4 vs. 31	LHBT vs. EPL	39.94	>0.9999	18.44	>0.9999	41.50	>0.9999	55.80	>0.9999	35.25	>0.9999	26.38	>0.9999
5 vs. 6	DBT vs. BrR	111.80	0.5121	54.75	>0.9999	72.00	>0.9999	-15.60	>0.9999	98.31	>0.9999	109.80	0.0589
5 vs. 7	DBT vs. DTT	-0.19	>0.9999	27.38	>0.9999	95.06	>0.9999	-16.75	>0.9999	7.63	>0.9999	12.06	>0.9999
5 vs. 8	DBT vs. FCR	88.44	>0.9999	21.75	>0.9999	88.14	>0.9999	0.00	>0.9999	38.38	>0.9999	79.56	>0.9999
5 vs. 9	DBT vs. PL	120.30	0.2073	27.38	>0.9999	7.19	>0.9999	-9.75	>0.9999	123.30	0.1261	105.90	0.1003
5 vs. 10	DBT vs. FCU	86.13	>0.9999	41.06	>0.9999	56.25	>0.9999	-31.20	>0.9999	34.81	>0.9999	92.75	0.5573
5 vs. 11	DBT vs. ECRL	111.80	0.5121	41.06	>0.9999	98.81	>0.9999	0.00	>0.9999	91.94	>0.9999	96.56	0.3465
5 vs. 12	DBT vs. ECRB	86.31	>0.9999	54.75	>0.9999	112.70	0.4472	-33.50	>0.9999	63.06	>0.9999	58.75	>0.9999
5 vs. 13	DBT vs. ECU	43.25	>0.9999	54.75	>0.9999	102.60	>0.9999	-20.10	>0.9999	71.88	>0.9999	66.38	>0.9999
5 vs. 14	DBT vs. FDS-2	21.06	>0.9999	13.69	>0.9999	36.44	>0.9999	-26.00	>0.9999	-8.75	>0.9999	122.90	0.0082
5 vs. 15	DBT vs. FDS-3	8.88	>0.9999	-35.63	>0.9999	-3.00	>0.9999	0.00	>0.9999	-38.25	>0.9999	96.56	0.3465
5 vs. 16	DBT vs. FDS-4	26.06	>0.9999	-8.25	>0.9999	22.56	>0.9999	-11.14	>0.9999	-20.88	>0.9999	109.80	0.0589
5 vs. 17	DBT vs. FDS-5	17.56	>0.9999	54.75	>0.9999	56.19	>0.9999	-52.86	>0.9999	25.00	>0.9999	122.90	0.0082
5 vs. 18	DBT vs. FDP-2	-17.19	>0.9999	-21.94	>0.9999	-3.88	>0.9999	-19.50	>0.9999	-27.50	>0.9999	83.38	>0.9999
5 vs. 19	DBT vs. FDP-3	-5.00	>0.9999	-24.19	>0.9999	4.56	>0.9999	-22.29	>0.9999	-21.88	>0.9999	79.56	>0.9999
5 vs. 20	DBT vs. FDP-4	-32.75	>0.9999	-42.63	>0.9999	-1.75	>0.9999	-35.29	>0.9999	-33.75	>0.9999	83.38	>0.9999
5 vs. 21	DBT vs. FDP-5	8.88	>0.9999	10.19	>0.9999	3.63	>0.9999	-9.75	>0.9999	20.50	>0.9999	122.90	0.0082
5 vs. 22	DBT vs. EIP	69.13	>0.9999	54.75	>0.9999	110.20	0.5791	-67.38	>0.9999	71.88	>0.9999	122.90	0.0082
5 vs. 23	DBT vs. EDC-2	60.63	>0.9999	41.06	>0.9999	28.38	>0.9999	-14.36	>0.9999	33.81	>0.9999	109.80	0.0589
5 vs. 24	DBT vs. EDC-3	103.30	>0.9999	41.06	>0.9999	49.19	>0.9999	-15.60	>0.9999	52.38	>0.9999	109.80	0.0589
5 vs. 25	DBT vs. EDC-4	85.94	>0.9999	41.06	>0.9999	49.69	>0.9999	0.00	>0.9999	52.38	>0.9999	105.90	0.1003
5 vs. 26	DBT vs. EDC-5	74.73	>0.9999	36.50	>0.9999	8.75	>0.9999	-44.92	>0.9999	35.25	>0.9999	122.90	0.0327
5 vs. 27	DBT vs. EDM	51.75	>0.9999	10.19	>0.9999	48.00	>0.9999	-11.14	>0.9999	43.56	>0.9999	87.00	>0.9999
5 vs. 28	DBT vs. FPL	8.69	>0.9999	41.06	>0.9999	51.94	>0.9999	0.00	>0.9999	12.88	>0.9999	122.90	0.0082
5 vs. 29	DBT vs. APL	43.44	>0.9999	54.75	>0.9999	110.20	0.5791	0.00	>0.9999	43.56	>0.9999	92.75	0.5573
5 vs. 30	DBT vs. EPB	51.94	>0.9999	22.63	>0.9999	103.30	>0.9999	-50.25	>0.9999	39.06	>0.9999	92.75	0.5573
5 vs. 31	DBT vs. EPL	51.94	>0.9999	54.75	>0.9999	123.30	0.1409	0.00	>0.9999	88.56	>0.9999	122.90	0.0082
6 vs. 7	BrR vs. DTT	-112.00	0.5023	-27.38	>0.9999	23.06	>0.9999	-1.15	>0.9999	-90.69	>0.9999	-97.69	0.3002
6 vs. 8	BrR vs. FCR	-23.38	>0.9999	-33.00	>0.9999	16.14	>0.9999	15.60	>0.9999	-59.94	>0.9999	-30.19	>0.9999
6 vs. 9	BrR vs. PL	8.50	>0.9999	-27.38	>0.9999	-64.81	>0.9999	5.85	>0.9999	24.94	>0.9999	-3.81	>0.9999
6 vs. 10	BrR vs. FCU	-25.69	>0.9999	-13.69	>0.9999	-15.75	>0.9999	-15.60	>0.9999	-63.50	>0.9999	-17.00	>0.9999
6 vs. 11	BrR vs. ECRL	0.00	>0.9999	-13.69	>0.9999	26.81	>0.9999	15.60	>0.9999	-6.38	>0.9999	-13.19	>0.9999
6 vs. 12	BrR vs. ECRB	-25.50	>0.9999	0.00	>0.9999	40.69	>0.9999	-17.90	>0.9999	-35.25	>0.9999	-51.00	>0.9999
6 vs. 13	BrR vs. ECU	-68.56	>0.9999	0.00	>0.9999	30.63	>0.9999	-4.50	>0.9999	-26.44	>0.9999	-43.38	>0.9999
6 vs. 14	BrR vs. FDS-2	-90.75	>0.9999	-41.06	>0.9999	-35.56	>0.9999	-10.40	>0.9999	-107.10	0.7259	13.19	>0.9999
6 vs. 15	BrR vs. FDS-3	-102.90	>0.9999	-90.38	0.6620	-75.00	>0.9999	15.60	>0.9999	-136.60	0.0254	-13.19	>0.9999
6 vs. 16	BrR vs. FDS-4	-85.75	>0.9999	-63.00	>0.9999	-49.44	>0.9999	4.46	>0.9999	-119.20	0.1997	0.00	>0.9999
6 vs. 17	BrR vs. FDS-5	-94.25	>0.9999	0.00	>0.9999	-15.81	>0.9999	-37.26	>0.9999	-73.31	>0.9999	13.19	>0.9999
6 vs. 18	BrR vs. FDP-2	-129.00	0.0775	-76.69	>0.9999	-75.88	>0.9999	-3.90	>0.9999	-125.80	0.0937	-26.38	>0.9999
6 vs. 19	BrR vs. FDP-3	-116.80	0.3029	-78.94	>0.9999	-67.44	>0.9999	-6.69	>0.9999	-120.20	0.1786	-30.19	>0.9999

Tendon Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value
6 vs. 20	BrR vs. FDP-4	-144.60	0.0114	-97.38	0.2736	-73.75	>0.9999	-19.69	>0.9999	-132.10	0.0444	-26.38	>0.9999
6 vs. 21	BrR vs. FDP-5	-102.90	>0.9999	-44.56	>0.9999	-68.38	>0.9999	5.85	>0.9999	-77.81	>0.9999	13.19	>0.9999
6 vs. 22	BrR vs. EIP	-42.69	>0.9999	0.00	>0.9999	38.19	>0.9999	-51.78	>0.9999	-26.44	>0.9999	13.19	>0.9999
6 vs. 23	BrR vs. EDC-2	-51.19	>0.9999	-13.69	>0.9999	-43.63	>0.9999	1.24	>0.9999	-64.50	>0.9999	0.00	>0.9999
6 vs. 24	BrR vs. EDC-3	-8.50	>0.9999	-13.69	>0.9999	-22.81	>0.9999	0.00	>0.9999	-45.94	>0.9999	0.00	>0.9999
6 vs. 25	BrR vs. EDC-4	-25.88	>0.9999	-13.69	>0.9999	-22.31	>0.9999	15.60	>0.9999	-45.94	>0.9999	-3.81	>0.9999
6 vs. 26	BrR vs. EDC-5	-37.08	>0.9999	-18.25	>0.9999	-63.25	>0.9999	-29.32	>0.9999	-63.06	>0.9999	13.19	>0.9999
6 vs. 27	BrR vs. EDM	-60.06	>0.9999	-44.56	>0.9999	-24.00	>0.9999	4.46	>0.9999	-54.75	>0.9999	-22.75	>0.9999
6 vs. 28	BrR vs. FPL	-103.10	>0.9999	-13.69	>0.9999	-20.06	>0.9999	15.60	>0.9999	-85.44	>0.9999	13.19	>0.9999
6 vs. 29	BrR vs. APL	-68.38	>0.9999	0.00	>0.9999	38.19	>0.9999	15.60	>0.9999	-54.75	>0.9999	-17.00	>0.9999
6 vs. 30	BrR vs. EPB	-59.88	>0.9999	-32.13	>0.9999	31.25	>0.9999	-34.65	>0.9999	-59.25	>0.9999	-17.00	>0.9999
6 vs. 31	BrR vs. EPL	-59.88	>0.9999	0.00	>0.9999	51.31	>0.9999	15.60	>0.9999	-9.75	>0.9999	13.19	>0.9999
7 vs. 8	DTT vs. FCR	88.63	>0.9999	-5.63	>0.9999	-6.92	>0.9999	16.75	>0.9999	30.75	>0.9999	67.50	>0.9999
7 vs. 9	DTT vs. PL	120.50	0.2031	0.00	>0.9999	-87.88	>0.9999	7.00	>0.9999	115.60	0.2954	93.88	0.4852
7 vs. 10	DTT vs. FCU	86.31	>0.9999	13.69	>0.9999	-38.81	>0.9999	-14.45	>0.9999	27.19	>0.9999	80.69	>0.9999
7 vs. 11	DTT vs. ECRL	112.00	0.5023	13.69	>0.9999	3.75	>0.9999	16.75	>0.9999	84.31	>0.9999	84.50	>0.9999
7 vs. 12	DTT vs. ECRB	86.50	>0.9999	27.38	>0.9999	17.63	>0.9999	-16.75	>0.9999	55.44	>0.9999	46.69	>0.9999
7 vs. 13	DTT vs. ECU	43.44	>0.9999	27.38	>0.9999	7.56	>0.9999	-3.35	>0.9999	64.25	>0.9999	54.31	>0.9999
7 vs. 14	DTT vs. FDS-2	21.25	>0.9999	-13.69	>0.9999	-58.63	>0.9999	-9.25	>0.9999	-16.38	>0.9999	110.90	0.0501
7 vs. 15	DTT vs. FDS-3	9.06	>0.9999	-63.00	>0.9999	-98.06	>0.9999	16.75	>0.9999	-45.88	>0.9999	84.50	>0.9999
7 vs. 16	DTT vs. FDS-4	26.25	>0.9999	-35.63	>0.9999	-72.50	>0.9999	5.61	>0.9999	-28.50	>0.9999	97.69	0.3002
7 vs. 17	DTT vs. FDS-5	17.75	>0.9999	27.38	>0.9999	-38.88	>0.9999	-36.11	>0.9999	17.38	>0.9999	110.90	0.0501
7 vs. 18	DTT vs. FDP-2	-17.00	>0.9999	-49.31	>0.9999	-98.94	>0.9999	-2.75	>0.9999	-35.13	>0.9999	71.31	>0.9999
7 vs. 19	DTT vs. FDP-3	-4.81	>0.9999	-51.56	>0.9999	-90.50	>0.9999	-5.54	>0.9999	-29.50	>0.9999	67.50	>0.9999
7 vs. 20	DTT vs. FDP-4	-32.56	>0.9999	-70.00	>0.9999	-96.81	>0.9999	-18.54	>0.9999	-41.38	>0.9999	71.31	>0.9999
7 vs. 21	DTT vs. FDP-5	9.06	>0.9999	-17.19	>0.9999	-91.44	>0.9999	7.00	>0.9999	12.88	>0.9999	110.90	0.0501
7 vs. 22	DTT vs. EIP	69.31	>0.9999	27.38	>0.9999	15.13	>0.9999	-50.63	>0.9999	64.25	>0.9999	110.90	0.0501
7 vs. 23	DTT vs. EDC-2	60.81	>0.9999	13.69	>0.9999	-66.69	>0.9999	2.39	>0.9999	26.19	>0.9999	97.69	0.3002
7 vs. 24	DTT vs. EDC-3	103.50	>0.9999	13.69	>0.9999	-45.88	>0.9999	1.15	>0.9999	44.75	>0.9999	97.69	0.3002
7 vs. 25	DTT vs. EDC-4	86.13	>0.9999	13.69	>0.9999	-45.38	>0.9999	16.75	>0.9999	44.75	>0.9999	93.88	0.4852
7 vs. 26	DTT vs. EDC-5	74.92	>0.9999	9.13	>0.9999	-86.31	>0.9999	-28.17	>0.9999	27.63	>0.9999	110.90	0.1567
7 vs. 27	DTT vs. EDM	51.94	>0.9999	-17.19	>0.9999	-47.06	>0.9999	5.61	>0.9999	35.94	>0.9999	74.94	>0.9999
7 vs. 28	DTT vs. FPL	8.88	>0.9999	13.69	>0.9999	-43.13	>0.9999	16.75	>0.9999	5.25	>0.9999	110.90	0.0501
7 vs. 29	DTT vs. APL	43.63	>0.9999	27.38	>0.9999	15.13	>0.9999	16.75	>0.9999	35.94	>0.9999	80.69	>0.9999
7 vs. 30	DTT vs. EPB	52.13	>0.9999	-4.75	>0.9999	8.19	>0.9999	-33.50	>0.9999	31.44	>0.9999	80.69	>0.9999
7 vs. 31	DTT vs. EPL	52.13	>0.9999	27.38	>0.9999	28.25	>0.9999	16.75	>0.9999	80.94	>0.9999	110.90	0.0501
8 vs. 9	FCR vs. PL	31.88	>0.9999	5.63	>0.9999	-80.96	>0.9999	-9.75	>0.9999	84.88	>0.9999	26.38	>0.9999
8 vs. 10	FCR vs. FCU	-2.31	>0.9999	19.31	>0.9999	-31.89	>0.9999	-31.20	>0.9999	-3.56	>0.9999	13.19	>0.9999
8 vs. 11	FCR vs. ECRL	23.38	>0.9999	19.31	>0.9999	10.67	>0.9999	0.00	>0.9999	53.56	>0.9999	17.00	>0.9999
8 vs. 12	FCR vs. ECRB	-2.13	>0.9999	33.00	>0.9999	24.54	>0.9999	-33.50	>0.9999	24.69	>0.9999	-20.81	>0.9999
8 vs. 13	FCR vs. ECU	-45.19	>0.9999	33.00	>0.9999	14.48	>0.9999	-20.10	>0.9999	33.50	>0.9999	-13.19	>0.9999
8 vs. 14	FCR vs. FDS-2	-67.38	>0.9999	-8.06	>0.9999	-51.71	>0.9999	-26.00	>0.9999	-47.13	>0.9999	43.38	>0.9999
8 vs. 15	FCR vs. FDS-3	-79.56	>0.9999	-57.38	>0.9999	-91.14	>0.9999	0.00	>0.9999	-76.63	>0.9999	17.00	>0.9999
8 vs. 16	FCR vs. FDS-4	-62.38	>0.9999	-30.00	>0.9999	-65.58	>0.9999	-11.14	>0.9999	-59.25	>0.9999	30.19	>0.9999
8 vs. 17	FCR vs. FDS-5	-70.88	>0.9999	33.00	>0.9999	-31.96	>0.9999	-52.86	>0.9999	-13.38	>0.9999	43.38	>0.9999
8 vs. 18	FCR vs. FDP-2	-105.60	0.9538	-43.69	>0.9999	-92.02	>0.9999	-19.50	>0.9999	-65.88	>0.9999	3.81	>0.9999
8 vs. 19	FCR vs. FDP-3	-93.44	>0.9999	-45.94	>0.9999	-83.58	>0.9999	-22.29	>0.9999	-60.25	>0.9999	0.00	>0.9999
8 vs. 20	FCR vs. FDP-4	-121.20	0.1882	-64.38	>0.9999	-89.89	>0.9999	-35.29	>0.9999	-72.13	>0.9999	3.81	>0.9999
8 vs. 21	FCR vs. FDP-5	-79.56	>0.9999	-11.56	>0.9999	-84.52	>0.9999	-9.75	>0.9999	-17.88	>0.9999	43.38	>0.9999
8 vs. 22	FCR vs. EIP	-19.31	>0.9999	33.00	>0.9999	22.04	>0.9999	-67.38	>0.9999	33.50	>0.9999	43.38	>0.9999
8 vs. 23	FCR vs. EDC-2	-27.81	>0.9999	19.31	>0.9999	-59.77	>0.9999	-14.36	>0.9999	-4.56	>0.9999	30.19	>0.9999
8 vs. 24	FCR vs. EDC-3	14.88	>0.9999	19.31	>0.9999	-38.96	>0.9999	-15.60	>0.9999	14.00	>0.9999	30.19	>0.9999

Tendon Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value
8 vs. 25	FCR vs. EDC-4	-2.50	>0.9999	19.31	>0.9999	-38.46	>0.9999	0.00	>0.9999	14.00	>0.9999	26.38	>0.9999
8 vs. 26	FCR vs. EDC-5	-13.71	>0.9999	14.75	>0.9999	-79.39	>0.9999	-44.92	>0.9999	-3.13	>0.9999	43.38	>0.9999
8 vs. 27	FCR vs. EDM	-36.69	>0.9999	-11.56	>0.9999	-40.14	>0.9999	-11.14	>0.9999	5.19	>0.9999	7.44	>0.9999
8 vs. 28	FCR vs. FPL	-79.75	>0.9999	19.31	>0.9999	-36.21	>0.9999	0.00	>0.9999	-25.50	>0.9999	43.38	>0.9999
8 vs. 29	FCR vs. APL	-45.00	>0.9999	33.00	>0.9999	22.04	>0.9999	0.00	>0.9999	5.19	>0.9999	13.19	>0.9999
8 vs. 30	FCR vs. EPB	-36.50	>0.9999	0.88	>0.9999	15.11	>0.9999	-50.25	>0.9999	0.69	>0.9999	13.19	>0.9999
8 vs. 31	FCR vs. EPL	-36.50	>0.9999	33.00	>0.9999	35.17	>0.9999	0.00	>0.9999	50.19	>0.9999	43.38	>0.9999
9 vs. 10	PL vs. FCU	-34.19	>0.9999	13.69	>0.9999	49.06	>0.9999	-21.45	>0.9999	-88.44	>0.9999	-13.19	>0.9999
9 vs. 11	PL vs. ECRL	-8.50	>0.9999	13.69	>0.9999	91.63	>0.9999	9.75	>0.9999	-31.31	>0.9999	-9.38	>0.9999
9 vs. 12	PL vs. ECRB	-34.00	>0.9999	27.38	>0.9999	105.50	0.9278	-23.75	>0.9999	-60.19	>0.9999	-47.19	>0.9999
9 vs. 13	PL vs. ECU	-77.06	>0.9999	27.38	>0.9999	95.44	>0.9999	-10.35	>0.9999	-51.38	>0.9999	-39.56	>0.9999
9 vs. 14	PL vs. FDS-2	-99.25	>0.9999	-13.69	>0.9999	29.25	>0.9999	-16.25	>0.9999	-132.00	0.0448	17.00	>0.9999
9 vs. 15	PL vs. FDS-3	-111.40	0.5322	-63.00	>0.9999	-10.19	>0.9999	9.75	>0.9999	-161.50	0.0009	-9.38	>0.9999
9 vs. 16	PL vs. FDS-4	-94.25	>0.9999	-35.63	>0.9999	15.38	>0.9999	-1.39	>0.9999	-144.10	0.0096	3.81	>0.9999
9 vs. 17	PL vs. FDS-5	-102.80	>0.9999	27.38	>0.9999	49.00	>0.9999	-43.11	>0.9999	-98.25	>0.9999	17.00	>0.9999
9 vs. 18	PL vs. FDP-2	-137.50	0.0279	-49.31	>0.9999	-11.06	>0.9999	-9.75	>0.9999	-150.80	0.0039	-22.56	>0.9999
9 vs. 19	PL vs. FDP-3	-125.30	0.1185	-51.56	>0.9999	-2.63	>0.9999	-12.54	>0.9999	-145.10	0.0084	-26.38	>0.9999
9 vs. 20	PL vs. FDP-4	-153.10	0.0037	-70.00	>0.9999	-8.94	>0.9999	-25.54	>0.9999	-157.00	0.0016	-22.56	>0.9999
9 vs. 21	PL vs. FDP-5	-111.40	0.5322	-17.19	>0.9999	-3.56	>0.9999	0.00	>0.9999	-102.80	>0.9999	17.00	>0.9999
9 vs. 22	PL vs. EIP	-51.19	>0.9999	27.38	>0.9999	103.00	>0.9999	-57.63	>0.9999	-51.38	>0.9999	17.00	>0.9999
9 vs. 23	PL vs. EDC-2	-59.69	>0.9999	13.69	>0.9999	21.19	>0.9999	-4.61	>0.9999	-89.44	>0.9999	3.81	>0.9999
9 vs. 24	PL vs. EDC-3	-17.00	>0.9999	13.69	>0.9999	42.00	>0.9999	-5.85	>0.9999	-70.88	>0.9999	3.81	>0.9999
9 vs. 25	PL vs. EDC-4	-34.38	>0.9999	13.69	>0.9999	42.50	>0.9999	9.75	>0.9999	-70.88	>0.9999	0.00	>0.9999
9 vs. 26	PL vs. EDC-5	-45.58	>0.9999	9.13	>0.9999	1.56	>0.9999	-35.17	>0.9999	-88.00	>0.9999	17.00	>0.9999
9 vs. 27	PL vs. EDM	-68.56	>0.9999	-17.19	>0.9999	40.81	>0.9999	-1.39	>0.9999	-79.69	>0.9999	-18.94	>0.9999
9 vs. 28	PL vs. FPL	-111.60	0.5220	13.69	>0.9999	44.75	>0.9999	9.75	>0.9999	-110.40	0.5164	17.00	>0.9999
9 vs. 29	PL vs. APL	-76.88	>0.9999	27.38	>0.9999	103.00	>0.9999	9.75	>0.9999	-79.69	>0.9999	-13.19	>0.9999
9 vs. 30	PL vs. EPB	-68.38	>0.9999	-4.75	>0.9999	96.06	>0.9999	-40.50	>0.9999	-84.19	>0.9999	-13.19	>0.9999
9 vs. 31	PL vs. EPL	-68.38	>0.9999	27.38	>0.9999	116.10	0.3108	9.75	>0.9999	-34.69	>0.9999	17.00	>0.9999
10 vs. 11	FCU vs. ECRL	25.69	>0.9999	0.00	>0.9999	42.56	>0.9999	31.20	>0.9999	57.13	>0.9999	3.81	>0.9999
10 vs. 12	FCU vs. ECRB	0.19	>0.9999	13.69	>0.9999	56.44	>0.9999	-2.30	>0.9999	28.25	>0.9999	-34.00	>0.9999
10 vs. 13	FCU vs. ECU	-42.88	>0.9999	13.69	>0.9999	46.38	>0.9999	11.10	>0.9999	37.06	>0.9999	-26.38	>0.9999
10 vs. 14	FCU vs. FDS-2	-65.06	>0.9999	-27.38	>0.9999	-19.81	>0.9999	5.20	>0.9999	-43.56	>0.9999	30.19	>0.9999
10 vs. 15	FCU vs. FDS-3	-77.25	>0.9999	-76.69	>0.9999	-59.25	>0.9999	31.20	>0.9999	-73.06	>0.9999	3.81	>0.9999
10 vs. 16	FCU vs. FDS-4	-60.06	>0.9999	-49.31	>0.9999	-33.69	>0.9999	20.06	>0.9999	-55.69	>0.9999	17.00	>0.9999
10 vs. 17	FCU vs. FDS-5	-68.56	>0.9999	13.69	>0.9999	-0.06	>0.9999	-21.66	>0.9999	-9.81	>0.9999	30.19	>0.9999
10 vs. 18	FCU vs. FDP-2	-103.30	>0.9999	-63.00	>0.9999	-60.13	>0.9999	11.70	>0.9999	-62.31	>0.9999	-9.38	>0.9999
10 vs. 19	FCU vs. FDP-3	-91.13	>0.9999	-65.25	>0.9999	-51.69	>0.9999	8.91	>0.9999	-56.69	>0.9999	-13.19	>0.9999
10 vs. 20	FCU vs. FDP-4	-118.90	0.2425	-83.69	>0.9999	-58.00	>0.9999	-4.09	>0.9999	-68.56	>0.9999	-9.38	>0.9999
10 vs. 21	FCU vs. FDP-5	-77.25	>0.9999	-30.88	>0.9999	-52.63	>0.9999	21.45	>0.9999	-14.31	>0.9999	30.19	>0.9999
10 vs. 22	FCU vs. EIP	-17.00	>0.9999	13.69	>0.9999	53.94	>0.9999	-36.18	>0.9999	37.06	>0.9999	30.19	>0.9999
10 vs. 23	FCU vs. EDC-2	-25.50	>0.9999	0.00	>0.9999	-27.88	>0.9999	16.84	>0.9999	-1.00	>0.9999	17.00	>0.9999
10 vs. 24	FCU vs. EDC-3	17.19	>0.9999	0.00	>0.9999	-7.06	>0.9999	15.60	>0.9999	17.56	>0.9999	17.00	>0.9999
10 vs. 25	FCU vs. EDC-4	-0.19	>0.9999	0.00	>0.9999	-6.56	>0.9999	31.20	>0.9999	17.56	>0.9999	13.19	>0.9999
10 vs. 26	FCU vs. EDC-5	-11.40	>0.9999	-4.56	>0.9999	-47.50	>0.9999	-13.72	>0.9999	0.44	>0.9999	30.19	>0.9999
10 vs. 27	FCU vs. EDM	-34.38	>0.9999	-30.88	>0.9999	-8.25	>0.9999	20.06	>0.9999	8.75	>0.9999	-5.75	>0.9999
10 vs. 28	FCU vs. FPL	-77.44	>0.9999	0.00	>0.9999	-4.31	>0.9999	31.20	>0.9999	-21.94	>0.9999	30.19	>0.9999
10 vs. 29	FCU vs. APL	-42.69	>0.9999	13.69	>0.9999	53.94	>0.9999	31.20	>0.9999	8.75	>0.9999	0.00	>0.9999
10 vs. 30	FCU vs. EPB	-34.19	>0.9999	-18.44	>0.9999	47.00	>0.9999	-19.05	>0.9999	4.25	>0.9999	0.00	>0.9999
10 vs. 31	FCU vs. EPL	-34.19	>0.9999	13.69	>0.9999	67.06	>0.9999	31.20	>0.9999	53.75	>0.9999	30.19	>0.9999
11 vs. 12	ECRL vs. ECRB	-25.50	>0.9999	13.69	>0.9999	13.88	>0.9999	-33.50	>0.9999	-28.88	>0.9999	-37.81	>0.9999
11 vs. 13	ECRL vs. ECU	-68.56	>0.9999	13.69	>0.9999	3.81	>0.9999	-20.10	>0.9999	-20.06	>0.9999	-30.19	>0.9999

Tendon Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value
11 vs. 14	ECRL vs. FDS-2	-90.75	>0.9999	-27.38	>0.9999	-62.38	>0.9999	-26.00	>0.9999	-100.70	>0.9999	26.38	>0.9999
11 vs. 15	ECRL vs. FDS-3	-102.90	>0.9999	-76.69	>0.9999	-101.80	>0.9999	0.00	>0.9999	-130.20	0.0558	0.00	>0.9999
11 vs. 16	ECRL vs. FDS-4	-85.75	>0.9999	-49.31	>0.9999	-76.25	>0.9999	-11.14	>0.9999	-112.80	0.3996	13.19	>0.9999
11 vs. 17	ECRL vs. FDS-5	-94.25	>0.9999	13.69	>0.9999	-42.63	>0.9999	-52.86	>0.9999	-66.94	>0.9999	26.38	>0.9999
11 vs. 18	ECRL vs. FDP-2	-129.00	0.0775	-63.00	>0.9999	-102.70	>0.9999	-19.50	>0.9999	-119.40	0.1942	-13.19	>0.9999
11 vs. 19	ECRL vs. FDP-3	-116.80	0.3029	-65.25	>0.9999	-94.25	>0.9999	-22.29	>0.9999	-113.80	0.3592	-17.00	>0.9999
11 vs. 20	ECRL vs. FDP-4	-144.60	0.0114	-83.69	>0.9999	-100.60	>0.9999	-35.29	>0.9999	-125.70	0.0951	-13.19	>0.9999
11 vs. 21	ECRL vs. FDP-5	-102.90	>0.9999	-30.88	>0.9999	-95.19	>0.9999	-9.75	>0.9999	-71.44	>0.9999	26.38	>0.9999
11 vs. 22	ECRL vs. EIP	-42.69	>0.9999	13.69	>0.9999	11.38	>0.9999	-67.38	>0.9999	-20.06	>0.9999	26.38	>0.9999
11 vs. 23	ECRL vs. EDC-2	-51.19	>0.9999	0.00	>0.9999	-70.44	>0.9999	-14.36	>0.9999	-58.13	>0.9999	13.19	>0.9999
11 vs. 24	ECRL vs. EDC-3	-8.50	>0.9999	0.00	>0.9999	-49.63	>0.9999	-15.60	>0.9999	-39.56	>0.9999	13.19	>0.9999
11 vs. 25	ECRL vs. EDC-4	-25.88	>0.9999	0.00	>0.9999	-49.13	>0.9999	0.00	>0.9999	-39.56	>0.9999	9.38	>0.9999
11 vs. 26	ECRL vs. EDC-5	-37.08	>0.9999	-4.56	>0.9999	-90.06	>0.9999	-44.92	>0.9999	-56.69	>0.9999	26.38	>0.9999
11 vs. 27	ECRL vs. EDM	-60.06	>0.9999	-30.88	>0.9999	-50.81	>0.9999	-11.14	>0.9999	-48.38	>0.9999	-9.56	>0.9999
11 vs. 28	ECRL vs. FPL	-103.10	>0.9999	0.00	>0.9999	-46.88	>0.9999	0.00	>0.9999	-79.06	>0.9999	26.38	>0.9999
11 vs. 29	ECRL vs. APL	-68.38	>0.9999	13.69	>0.9999	11.38	>0.9999	0.00	>0.9999	-48.38	>0.9999	-3.81	>0.9999
11 vs. 30	ECRL vs. EPB	-59.88	>0.9999	-18.44	>0.9999	4.44	>0.9999	-50.25	>0.9999	-52.88	>0.9999	-3.81	>0.9999
11 vs. 31	ECRL vs. EPL	-59.88	>0.9999	13.69	>0.9999	24.50	>0.9999	0.00	>0.9999	-3.38	>0.9999	26.38	>0.9999
12 vs. 13	ECRB vs. ECU	-43.06	>0.9999	0.00	>0.9999	-10.06	>0.9999	13.40	>0.9999	8.81	>0.9999	7.63	>0.9999
12 vs. 14	ECRB vs. FDS-2	-65.25	>0.9999	-41.06	>0.9999	-76.25	>0.9999	7.50	>0.9999	-71.81	>0.9999	64.19	>0.9999
12 vs. 15	ECRB vs. FDS-3	-77.44	>0.9999	-90.38	0.6620	-115.70	0.3257	33.50	>0.9999	-101.30	>0.9999	37.81	>0.9999
12 vs. 16	ECRB vs. FDS-4	-60.25	>0.9999	-63.00	>0.9999	-90.13	>0.9999	22.36	>0.9999	-83.94	>0.9999	51.00	>0.9999
12 vs. 17	ECRB vs. FDS-5	-68.75	>0.9999	0.00	>0.9999	-56.50	>0.9999	-19.36	>0.9999	-38.06	>0.9999	64.19	>0.9999
12 vs. 18	ECRB vs. FDP-2	-103.50	>0.9999	-76.69	>0.9999	-116.60	0.2965	14.00	>0.9999	-90.56	>0.9999	24.63	>0.9999
12 vs. 19	ECRB vs. FDP-3	-91.31	>0.9999	-78.94	>0.9999	-108.10	0.7141	11.21	>0.9999	-84.94	>0.9999	20.81	>0.9999
12 vs. 20	ECRB vs. FDP-4	-119.10	0.2376	-97.38	0.2736	-114.40	0.3720	-1.79	>0.9999	-96.81	>0.9999	24.63	>0.9999
12 vs. 21	ECRB vs. FDP-5	-77.44	>0.9999	-44.56	>0.9999	-109.10	0.6495	23.75	>0.9999	-42.56	>0.9999	64.19	>0.9999
12 vs. 22	ECRB vs. EIP	-17.19	>0.9999	0.00	>0.9999	-2.50	>0.9999	-33.88	>0.9999	8.81	>0.9999	64.19	>0.9999
12 vs. 23	ECRB vs. EDC-2	-25.69	>0.9999	-13.69	>0.9999	-84.31	>0.9999	19.14	>0.9999	-29.25	>0.9999	51.00	>0.9999
12 vs. 24	ECRB vs. EDC-3	17.00	>0.9999	-13.69	>0.9999	-63.50	>0.9999	17.90	>0.9999	-10.69	>0.9999	51.00	>0.9999
12 vs. 25	ECRB vs. EDC-4	-0.38	>0.9999	-13.69	>0.9999	-63.00	>0.9999	33.50	>0.9999	-10.69	>0.9999	47.19	>0.9999
12 vs. 26	ECRB vs. EDC-5	-11.58	>0.9999	-18.25	>0.9999	-103.90	>0.9999	-11.42	>0.9999	-27.81	>0.9999	64.19	>0.9999
12 vs. 27	ECRB vs. EDM	-34.56	>0.9999	-44.56	>0.9999	-64.69	>0.9999	22.36	>0.9999	-19.50	>0.9999	28.25	>0.9999
12 vs. 28	ECRB vs. FPL	-77.63	>0.9999	-13.69	>0.9999	-60.75	>0.9999	33.50	>0.9999	-50.19	>0.9999	64.19	>0.9999
12 vs. 29	ECRB vs. APL	-42.88	>0.9999	0.00	>0.9999	-2.50	>0.9999	33.50	>0.9999	-19.50	>0.9999	34.00	>0.9999
12 vs. 30	ECRB vs. EPB	-34.38	>0.9999	-32.13	>0.9999	-9.44	>0.9999	-16.75	>0.9999	-24.00	>0.9999	34.00	>0.9999
12 vs. 31	ECRB vs. EPL	-34.38	>0.9999	0.00	>0.9999	10.63	>0.9999	33.50	>0.9999	25.50	>0.9999	64.19	>0.9999
13 vs. 14	ECU vs. FDS-2	-22.19	>0.9999	-41.06	>0.9999	-66.19	>0.9999	-5.90	>0.9999	-80.63	>0.9999	56.56	>0.9999
13 vs. 15	ECU vs. FDS-3	-34.38	>0.9999	-90.38	0.6620	-105.60	0.9164	20.10	>0.9999	-110.10	0.5300	30.19	>0.9999
13 vs. 16	ECU vs. FDS-4	-17.19	>0.9999	-63.00	>0.9999	-80.06	>0.9999	8.96	>0.9999	-92.75	>0.9999	43.38	>0.9999
13 vs. 17	ECU vs. FDS-5	-25.69	>0.9999	0.00	>0.9999	-46.44	>0.9999	-32.76	>0.9999	-46.88	>0.9999	56.56	>0.9999
13 vs. 18	ECU vs. FDP-2	-60.44	>0.9999	-76.69	>0.9999	-106.50	0.8403	0.60	>0.9999	-99.38	>0.9999	17.00	>0.9999
13 vs. 19	ECU vs. FDP-3	-48.25	>0.9999	-78.94	>0.9999	-98.06	>0.9999	-2.19	>0.9999	-93.75	>0.9999	13.19	>0.9999
13 vs. 20	ECU vs. FDP-4	-76.00	>0.9999	-97.38	0.2736	-104.40	>0.9999	-15.19	>0.9999	-105.60	0.8392	17.00	>0.9999
13 vs. 21	ECU vs. FDP-5	-34.38	>0.9999	-44.56	>0.9999	-99.00	>0.9999	10.35	>0.9999	-51.38	>0.9999	56.56	>0.9999
13 vs. 22	ECU vs. EIP	25.88	>0.9999	0.00	>0.9999	7.56	>0.9999	-47.28	>0.9999	0.00	>0.9999	56.56	>0.9999
13 vs. 23	ECU vs. EDC-2	17.38	>0.9999	-13.69	>0.9999	-74.25	>0.9999	5.74	>0.9999	-38.06	>0.9999	43.38	>0.9999
13 vs. 24	ECU vs. EDC-3	60.06	>0.9999	-13.69	>0.9999	-53.44	>0.9999	4.50	>0.9999	-19.50	>0.9999	43.38	>0.9999
13 vs. 25	ECU vs. EDC-4	42.69	>0.9999	-13.69	>0.9999	-52.94	>0.9999	20.10	>0.9999	-19.50	>0.9999	39.56	>0.9999
13 vs. 26	ECU vs. EDC-5	31.48	>0.9999	-18.25	>0.9999	-93.88	>0.9999	-24.82	>0.9999	-36.63	>0.9999	56.56	>0.9999
13 vs. 27	ECU vs. EDM	8.50	>0.9999	-44.56	>0.9999	-54.63	>0.9999	8.96	>0.9999	-28.31	>0.9999	20.63	>0.9999
13 vs. 28	ECU vs. FPL	-34.56	>0.9999	-13.69	>0.9999	-50.69	>0.9999	20.10	>0.9999	-59.00	>0.9999	56.56	>0.9999

Tendon Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value
13 vs. 29	ECU vs. APL	0.19	>0.9999	0.00	>0.9999	7.56	>0.9999	20.10	>0.9999	-28.31	>0.9999	26.38	>0.9999
13 vs. 30	ECU vs. EPB	8.69	>0.9999	-32.13	>0.9999	0.63	>0.9999	-30.15	>0.9999	-32.81	>0.9999	26.38	>0.9999
13 vs. 31	ECU vs. EPL	8.69	>0.9999	0.00	>0.9999	20.69	>0.9999	20.10	>0.9999	16.69	>0.9999	56.56	>0.9999
14 vs. 15	FDS-2 vs. FDS-3	-12.19	>0.9999	-49.31	>0.9999	-39.44	>0.9999	26.00	>0.9999	-29.50	>0.9999	-26.38	>0.9999
14 vs. 16	FDS-2 vs. FDS-4	5.00	>0.9999	-21.94	>0.9999	-13.88	>0.9999	14.86	>0.9999	-12.13	>0.9999	-13.19	>0.9999
14 vs. 17	FDS-2 vs. FDS-5	-3.50	>0.9999	41.06	>0.9999	19.75	>0.9999	-26.86	>0.9999	33.75	>0.9999	0.00	>0.9999
14 vs. 18	FDS-2 vs. FDP-2	-38.25	>0.9999	-35.63	>0.9999	-40.31	>0.9999	6.50	>0.9999	-18.75	>0.9999	-39.56	>0.9999
14 vs. 19	FDS-2 vs. FDP-3	-26.06	>0.9999	-37.88	>0.9999	-31.88	>0.9999	3.71	>0.9999	-13.13	>0.9999	-43.38	>0.9999
14 vs. 20	FDS-2 vs. FDP-4	-53.81	>0.9999	-56.31	>0.9999	-38.19	>0.9999	-9.29	>0.9999	-25.00	>0.9999	-39.56	>0.9999
14 vs. 21	FDS-2 vs. FDP-5	-12.19	>0.9999	-3.50	>0.9999	-32.81	>0.9999	16.25	>0.9999	29.25	>0.9999	0.00	>0.9999
14 vs. 22	FDS-2 vs. EIP	48.06	>0.9999	41.06	>0.9999	73.75	>0.9999	-41.38	>0.9999	80.63	>0.9999	0.00	>0.9999
14 vs. 23	FDS-2 vs. EDC-2	39.56	>0.9999	27.38	>0.9999	-8.06	>0.9999	11.64	>0.9999	42.56	>0.9999	-13.19	>0.9999
14 vs. 24	FDS-2 vs. EDC-3	82.25	>0.9999	27.38	>0.9999	12.75	>0.9999	10.40	>0.9999	61.13	>0.9999	-13.19	>0.9999
14 vs. 25	FDS-2 vs. EDC-4	64.88	>0.9999	27.38	>0.9999	13.25	>0.9999	26.00	>0.9999	61.13	>0.9999	-17.00	>0.9999
14 vs. 26	FDS-2 vs. EDC-5	53.67	>0.9999	22.81	>0.9999	-27.69	>0.9999	-18.92	>0.9999	44.00	>0.9999	0.00	>0.9999
14 vs. 27	FDS-2 vs. EDM	30.69	>0.9999	-3.50	>0.9999	11.56	>0.9999	14.86	>0.9999	52.31	>0.9999	-35.94	>0.9999
14 vs. 28	FDS-2 vs. FPL	-12.38	>0.9999	27.38	>0.9999	15.50	>0.9999	26.00	>0.9999	21.63	>0.9999	0.00	>0.9999
14 vs. 29	FDS-2 vs. APL	22.38	>0.9999	41.06	>0.9999	73.75	>0.9999	26.00	>0.9999	52.31	>0.9999	-30.19	>0.9999
14 vs. 30	FDS-2 vs. EPB	30.88	>0.9999	8.94	>0.9999	66.81	>0.9999	-24.25	>0.9999	47.81	>0.9999	-30.19	>0.9999
14 vs. 31	FDS-2 vs. EPL	30.88	>0.9999	41.06	>0.9999	86.88	>0.9999	26.00	>0.9999	97.31	>0.9999	0.00	>0.9999
15 vs. 16	FDS-3 vs. FDS-4	17.19	>0.9999	27.38	>0.9999	25.56	>0.9999	-11.14	>0.9999	17.38	>0.9999	13.19	>0.9999
15 vs. 17	FDS-3 vs. FDS-5	8.69	>0.9999	90.38	0.6620	59.19	>0.9999	-52.86	>0.9999	63.25	>0.9999	26.38	>0.9999
15 vs. 18	FDS-3 vs. FDP-2	-26.06	>0.9999	13.69	>0.9999	-0.88	>0.9999	-19.50	>0.9999	10.75	>0.9999	-13.19	>0.9999
15 vs. 19	FDS-3 vs. FDP-3	-13.88	>0.9999	11.44	>0.9999	7.56	>0.9999	-22.29	>0.9999	16.38	>0.9999	-17.00	>0.9999
15 vs. 20	FDS-3 vs. FDP-4	-41.63	>0.9999	-7.00	>0.9999	1.25	>0.9999	-35.29	>0.9999	4.50	>0.9999	-13.19	>0.9999
15 vs. 21	FDS-3 vs. FDP-5	0.00	>0.9999	45.81	>0.9999	6.63	>0.9999	-9.75	>0.9999	58.75	>0.9999	26.38	>0.9999
15 vs. 22	FDS-3 vs. EIP	60.25	>0.9999	90.38	0.6620	113.20	0.4244	-67.38	>0.9999	110.10	0.5300	26.38	>0.9999
15 vs. 23	FDS-3 vs. EDC-2	51.75	>0.9999	76.69	>0.9999	31.38	>0.9999	-14.36	>0.9999	72.06	>0.9999	13.19	>0.9999
15 vs. 24	FDS-3 vs. EDC-3	94.44	>0.9999	76.69	>0.9999	52.19	>0.9999	-15.60	>0.9999	90.63	>0.9999	13.19	>0.9999
15 vs. 25	FDS-3 vs. EDC-4	77.06	>0.9999	76.69	>0.9999	52.69	>0.9999	0.00	>0.9999	90.63	>0.9999	9.38	>0.9999
15 vs. 26	FDS-3 vs. EDC-5	65.85	>0.9999	72.13	>0.9999	11.75	>0.9999	-44.92	>0.9999	73.50	>0.9999	26.38	>0.9999
15 vs. 27	FDS-3 vs. EDM	42.88	>0.9999	45.81	>0.9999	51.00	>0.9999	-11.14	>0.9999	81.81	>0.9999	-9.56	>0.9999
15 vs. 28	FDS-3 vs. FPL	-0.19	>0.9999	76.69	>0.9999	54.94	>0.9999	0.00	>0.9999	51.13	>0.9999	26.38	>0.9999
15 vs. 29	FDS-3 vs. APL	34.56	>0.9999	90.38	0.6620	113.20	0.4244	0.00	>0.9999	81.81	>0.9999	-3.81	>0.9999
15 vs. 30	FDS-3 vs. EPB	43.06	>0.9999	58.25	>0.9999	106.30	0.8614	-50.25	>0.9999	77.31	>0.9999	-3.81	>0.9999
15 vs. 31	FDS-3 vs. EPL	43.06	>0.9999	90.38	0.6620	126.30	0.1000	0.00	>0.9999	126.80	0.0834	26.38	>0.9999
16 vs. 17	FDS-4 vs. FDS-5	-8.50	>0.9999	63.00	>0.9999	33.63	>0.9999	-41.71	>0.9999	45.88	>0.9999	13.19	>0.9999
16 vs. 18	FDS-4 vs. FDP-2	-43.25	>0.9999	-13.69	>0.9999	-26.44	>0.9999	-8.36	>0.9999	-6.63	>0.9999	-26.38	>0.9999
16 vs. 19	FDS-4 vs. FDP-3	-31.06	>0.9999	-15.94	>0.9999	-18.00	>0.9999	-11.14	>0.9999	-1.00	>0.9999	-30.19	>0.9999
16 vs. 20	FDS-4 vs. FDP-4	-58.81	>0.9999	-34.38	>0.9999	-24.31	>0.9999	-24.14	>0.9999	-12.88	>0.9999	-26.38	>0.9999
16 vs. 21	FDS-4 vs. FDP-5	-17.19	>0.9999	18.44	>0.9999	-18.94	>0.9999	1.39	>0.9999	41.38	>0.9999	13.19	>0.9999
16 vs. 22	FDS-4 vs. EIP	43.06	>0.9999	63.00	>0.9999	87.63	>0.9999	-56.23	>0.9999	92.75	>0.9999	13.19	>0.9999
16 vs. 23	FDS-4 vs. EDC-2	34.56	>0.9999	49.31	>0.9999	5.81	>0.9999	-3.21	>0.9999	54.69	>0.9999	0.00	>0.9999
16 vs. 24	FDS-4 vs. EDC-3	77.25	>0.9999	49.31	>0.9999	26.63	>0.9999	-4.46	>0.9999	73.25	>0.9999	0.00	>0.9999
16 vs. 25	FDS-4 vs. EDC-4	59.88	>0.9999	49.31	>0.9999	27.13	>0.9999	11.14	>0.9999	73.25	>0.9999	-3.81	>0.9999
16 vs. 26	FDS-4 vs. EDC-5	48.67	>0.9999	44.75	>0.9999	-13.81	>0.9999	-33.77	>0.9999	56.13	>0.9999	13.19	>0.9999
16 vs. 27	FDS-4 vs. EDM	25.69	>0.9999	18.44	>0.9999	25.44	>0.9999	0.00	>0.9999	64.44	>0.9999	-22.75	>0.9999
16 vs. 28	FDS-4 vs. FPL	-17.38	>0.9999	49.31	>0.9999	29.38	>0.9999	11.14	>0.9999	33.75	>0.9999	13.19	>0.9999
16 vs. 29	FDS-4 vs. APL	17.38	>0.9999	63.00	>0.9999	87.63	>0.9999	11.14	>0.9999	64.44	>0.9999	-17.00	>0.9999
16 vs. 30	FDS-4 vs. EPB	25.88	>0.9999	30.88	>0.9999	80.69	>0.9999	-39.11	>0.9999	59.94	>0.9999	-17.00	>0.9999
16 vs. 31	FDS-4 vs. EPL	25.88	>0.9999	63.00	>0.9999	100.80	>0.9999	11.14	>0.9999	109.40	0.5691	13.19	>0.9999
17 vs. 18	FDS-5 vs. FDP-2	-34.75	>0.9999	-76.69	>0.9999	-60.06	>0.9999	33.36	>0.9999	-52.50	>0.9999	-39.56	>0.9999

Tendon Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value
17 vs. 19	FDS-5 vs. FDP-3	-22.56	>0.9999	-78.94	>0.9999	-51.63	>0.9999	30.57	>0.9999	-46.88	>0.9999	-43.38	>0.9999
17 vs. 20	FDS-5 vs. FDP-4	-50.31	>0.9999	-97.38	0.2736	-57.94	>0.9999	17.57	>0.9999	-58.75	>0.9999	-39.56	>0.9999
17 vs. 21	FDS-5 vs. FDP-5	-8.69	>0.9999	-44.56	>0.9999	-52.56	>0.9999	43.11	>0.9999	-4.50	>0.9999	0.00	>0.9999
17 vs. 22	FDS-5 vs. EIP	51.56	>0.9999	0.00	>0.9999	54.00	>0.9999	-14.52	>0.9999	46.88	>0.9999	0.00	>0.9999
17 vs. 23	FDS-5 vs. EDC-2	43.06	>0.9999	-13.69	>0.9999	-27.81	>0.9999	38.50	>0.9999	8.81	>0.9999	-13.19	>0.9999
17 vs. 24	FDS-5 vs. EDC-3	85.75	>0.9999	-13.69	>0.9999	-7.00	>0.9999	37.26	>0.9999	27.38	>0.9999	-13.19	>0.9999
17 vs. 25	FDS-5 vs. EDC-4	68.38	>0.9999	-13.69	>0.9999	-6.50	>0.9999	52.86	>0.9999	27.38	>0.9999	-17.00	>0.9999
17 vs. 26	FDS-5 vs. EDC-5	57.17	>0.9999	-18.25	>0.9999	-47.44	>0.9999	7.94	>0.9999	10.25	>0.9999	0.00	>0.9999
17 vs. 27	FDS-5 vs. EDM	34.19	>0.9999	-44.56	>0.9999	-8.19	>0.9999	41.71	>0.9999	18.56	>0.9999	-35.94	>0.9999
17 vs. 28	FDS-5 vs. FPL	-8.88	>0.9999	-13.69	>0.9999	-4.25	>0.9999	52.86	>0.9999	-12.13	>0.9999	0.00	>0.9999
17 vs. 29	FDS-5 vs. APL	25.88	>0.9999	0.00	>0.9999	54.00	>0.9999	52.86	>0.9999	18.56	>0.9999	-30.19	>0.9999
17 vs. 30	FDS-5 vs. EPB	34.38	>0.9999	-32.13	>0.9999	47.06	>0.9999	2.61	>0.9999	14.06	>0.9999	-30.19	>0.9999
17 vs. 31	FDS-5 vs. EPL	34.38	>0.9999	0.00	>0.9999	67.13	>0.9999	52.86	>0.9999	63.56	>0.9999	0.00	>0.9999
18 vs. 19	FDP-2 vs. FDP-3	12.19	>0.9999	-2.25	>0.9999	8.44	>0.9999	-2.79	>0.9999	5.63	>0.9999	-3.81	>0.9999
18 vs. 20	FDP-2 vs. FDP-4	-15.56	>0.9999	-20.69	>0.9999	2.13	>0.9999	-15.79	>0.9999	-6.25	>0.9999	0.00	>0.9999
18 vs. 21	FDP-2 vs. FDP-5	26.06	>0.9999	32.13	>0.9999	7.50	>0.9999	9.75	>0.9999	48.00	>0.9999	39.56	>0.9999
18 vs. 22	FDP-2 vs. EIP	86.31	>0.9999	76.69	>0.9999	114.10	0.3871	-47.88	>0.9999	99.38	>0.9999	39.56	>0.9999
18 vs. 23	FDP-2 vs. EDC-2	77.81	>0.9999	63.00	>0.9999	32.25	>0.9999	5.14	>0.9999	61.31	>0.9999	26.38	>0.9999
18 vs. 24	FDP-2 vs. EDC-3	120.50	0.2031	63.00	>0.9999	53.06	>0.9999	3.90	>0.9999	79.88	>0.9999	26.38	>0.9999
18 vs. 25	FDP-2 vs. EDC-4	103.10	>0.9999	63.00	>0.9999	53.56	>0.9999	19.50	>0.9999	79.88	>0.9999	22.56	>0.9999
18 vs. 26	FDP-2 vs. EDC-5	91.92	>0.9999	58.44	>0.9999	12.63	>0.9999	-25.42	>0.9999	62.75	>0.9999	39.56	>0.9999
18 vs. 27	FDP-2 vs. EDM	68.94	>0.9999	32.13	>0.9999	51.88	>0.9999	8.36	>0.9999	71.06	>0.9999	3.63	>0.9999
18 vs. 28	FDP-2 vs. FPL	25.88	>0.9999	63.00	>0.9999	55.81	>0.9999	19.50	>0.9999	40.38	>0.9999	39.56	>0.9999
18 vs. 29	FDP-2 vs. APL	60.63	>0.9999	76.69	>0.9999	114.10	0.3871	19.50	>0.9999	71.06	>0.9999	9.38	>0.9999
18 vs. 30	FDP-2 vs. EPB	69.13	>0.9999	44.56	>0.9999	107.10	0.7895	-30.75	>0.9999	66.56	>0.9999	9.38	>0.9999
18 vs. 31	FDP-2 vs. EPL	69.13	>0.9999	76.69	>0.9999	127.20	0.0903	19.50	>0.9999	116.10	0.2817	39.56	>0.9999
19 vs. 20	FDP-3 vs. FDP-4	-27.75	>0.9999	-18.44	>0.9999	-6.31	>0.9999	-13.00	>0.9999	-11.88	>0.9999	3.81	>0.9999
19 vs. 21	FDP-3 vs. FDP-5	13.88	>0.9999	34.38	>0.9999	-0.94	>0.9999	12.54	>0.9999	42.38	>0.9999	43.38	>0.9999
19 vs. 22	FDP-3 vs. EIP	74.13	>0.9999	78.94	>0.9999	105.60	0.9164	-45.09	>0.9999	93.75	>0.9999	43.38	>0.9999
19 vs. 23	FDP-3 vs. EDC-2	65.63	>0.9999	65.25	>0.9999	23.81	>0.9999	7.93	>0.9999	55.69	>0.9999	30.19	>0.9999
19 vs. 24	FDP-3 vs. EDC-3	108.30	0.7307	65.25	>0.9999	44.63	>0.9999	6.69	>0.9999	74.25	>0.9999	30.19	>0.9999
19 vs. 25	FDP-3 vs. EDC-4	90.94	>0.9999	65.25	>0.9999	45.13	>0.9999	22.29	>0.9999	74.25	>0.9999	26.38	>0.9999
19 vs. 26	FDP-3 vs. EDC-5	79.73	>0.9999	60.69	>0.9999	4.19	>0.9999	-22.63	>0.9999	57.13	>0.9999	43.38	>0.9999
19 vs. 27	FDP-3 vs. EDM	56.75	>0.9999	34.38	>0.9999	43.44	>0.9999	11.14	>0.9999	65.44	>0.9999	7.44	>0.9999
19 vs. 28	FDP-3 vs. FPL	13.69	>0.9999	65.25	>0.9999	47.38	>0.9999	22.29	>0.9999	34.75	>0.9999	43.38	>0.9999
19 vs. 29	FDP-3 vs. APL	48.44	>0.9999	78.94	>0.9999	105.60	0.9164	22.29	>0.9999	65.44	>0.9999	13.19	>0.9999
19 vs. 30	FDP-3 vs. EPB	56.94	>0.9999	46.81	>0.9999	98.69	>0.9999	-27.96	>0.9999	60.94	>0.9999	13.19	>0.9999
19 vs. 31	FDP-3 vs. EPL	56.94	>0.9999	78.94	>0.9999	118.80	0.2339	22.29	>0.9999	110.40	0.5130	43.38	>0.9999
20 vs. 21	FDP-4 vs. FDP-5	41.63	>0.9999	52.81	>0.9999	5.38	>0.9999	25.54	>0.9999	54.25	>0.9999	39.56	>0.9999
20 vs. 22	FDP-4 vs. EIP	101.90	>0.9999	97.38	0.2736	111.90	0.4835	-32.09	>0.9999	105.60	0.8392	39.56	>0.9999
20 vs. 23	FDP-4 vs. EDC-2	93.38	>0.9999	83.69	>0.9999	30.13	>0.9999	20.93	>0.9999	67.56	>0.9999	26.38	>0.9999
20 vs. 24	FDP-4 vs. EDC-3	136.10	0.0333	83.69	>0.9999	50.94	>0.9999	19.69	>0.9999	86.13	>0.9999	26.38	>0.9999
20 vs. 25	FDP-4 vs. EDC-4	118.70	0.2475	83.69	>0.9999	51.44	>0.9999	35.29	>0.9999	86.13	>0.9999	22.56	>0.9999
20 vs. 26	FDP-4 vs. EDC-5	107.50	>0.9999	79.13	>0.9999	10.50	>0.9999	-9.63	>0.9999	69.00	>0.9999	39.56	>0.9999
20 vs. 27	FDP-4 vs. EDM	84.50	>0.9999	52.81	>0.9999	49.75	>0.9999	24.14	>0.9999	77.31	>0.9999	3.63	>0.9999
20 vs. 28	FDP-4 vs. FPL	41.44	>0.9999	83.69	>0.9999	53.69	>0.9999	35.29	>0.9999	46.63	>0.9999	39.56	>0.9999
20 vs. 29	FDP-4 vs. APL	76.19	>0.9999	97.38	0.2736	111.90	0.4835	35.29	>0.9999	77.31	>0.9999	9.38	>0.9999
20 vs. 30	FDP-4 vs. EPB	84.69	>0.9999	65.25	>0.9999	105.00	0.9746	-14.96	>0.9999	72.81	>0.9999	9.38	>0.9999
20 vs. 31	FDP-4 vs. EPL	84.69	>0.9999	97.38	0.2736	125.10	0.1154	35.29	>0.9999	122.30	0.1404	39.56	>0.9999
21 vs. 22	FDP-5 vs. EIP	60.25	>0.9999	44.56	>0.9999	106.60	0.8351	-57.63	>0.9999	51.38	>0.9999	0.00	>0.9999
21 vs. 23	FDP-5 vs. EDC-2	51.75	>0.9999	30.88	>0.9999	24.75	>0.9999	-4.61	>0.9999	13.31	>0.9999	-13.19	>0.9999
21 vs. 24	FDP-5 vs. EDC-3	94.44	>0.9999	30.88	>0.9999	45.56	>0.9999	-5.85	>0.9999	31.88	>0.9999	-13.19	>0.9999

Tendon Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value	Mean rank diff.	Adjusted P value
21 vs. 25	FDP-5 vs. EDC-4	77.06	>0.9999	30.88	>0.9999	46.06	>0.9999	9.75	>0.9999	31.88	>0.9999	-17.00	>0.9999
21 vs. 26	FDP-5 vs. EDC-5	65.85	>0.9999	26.31	>0.9999	5.13	>0.9999	-35.17	>0.9999	14.75	>0.9999	0.00	>0.9999
21 vs. 27	FDP-5 vs. EDM	42.88	>0.9999	0.00	>0.9999	44.38	>0.9999	-1.39	>0.9999	23.06	>0.9999	-35.94	>0.9999
21 vs. 28	FDP-5 vs. FPL	-0.19	>0.9999	30.88	>0.9999	48.31	>0.9999	9.75	>0.9999	-7.63	>0.9999	0.00	>0.9999
21 vs. 29	FDP-5 vs. APL	34.56	>0.9999	44.56	>0.9999	106.60	0.8351	9.75	>0.9999	23.06	>0.9999	-30.19	>0.9999
21 vs. 30	FDP-5 vs. EPB	43.06	>0.9999	12.44	>0.9999	99.63	>0.9999	-40.50	>0.9999	18.56	>0.9999	-30.19	>0.9999
21 vs. 31	FDP-5 vs. EPL	43.06	>0.9999	44.56	>0.9999	119.70	0.2111	9.75	>0.9999	68.06	>0.9999	0.00	>0.9999
22 vs. 23	EIP vs. EDC-2	-8.50	>0.9999	-13.69	>0.9999	-81.81	>0.9999	53.02	>0.9999	-38.06	>0.9999	-13.19	>0.9999
22 vs. 24	EIP vs. EDC-3	34.19	>0.9999	-13.69	>0.9999	-61.00	>0.9999	51.78	>0.9999	-19.50	>0.9999	-13.19	>0.9999
22 vs. 25	EIP vs. EDC-4	16.81	>0.9999	-13.69	>0.9999	-60.50	>0.9999	67.38	>0.9999	-19.50	>0.9999	-17.00	>0.9999
22 vs. 26	EIP vs. EDC-5	5.60	>0.9999	-18.25	>0.9999	-101.40	>0.9999	22.46	>0.9999	-36.63	>0.9999	0.00	>0.9999
22 vs. 27	EIP vs. EDM	-17.38	>0.9999	-44.56	>0.9999	-62.19	>0.9999	56.23	>0.9999	-28.31	>0.9999	-35.94	>0.9999
22 vs. 28	EIP vs. FPL	-60.44	>0.9999	-13.69	>0.9999	-58.25	>0.9999	67.38	>0.9999	-59.00	>0.9999	0.00	>0.9999
22 vs. 29	EIP vs. APL	-25.69	>0.9999	0.00	>0.9999	0.00	>0.9999	67.38	>0.9999	-28.31	>0.9999	-30.19	>0.9999
22 vs. 30	EIP vs. EPB	-17.19	>0.9999	-32.13	>0.9999	-6.94	>0.9999	17.13	>0.9999	-32.81	>0.9999	-30.19	>0.9999
22 vs. 31	EIP vs. EPL	-17.19	>0.9999	0.00	>0.9999	13.13	>0.9999	67.38	>0.9999	16.69	>0.9999	0.00	>0.9999
23 vs. 24	EDC-2 vs. EDC-3	42.69	>0.9999	0.00	>0.9999	20.81	>0.9999	-1.24	>0.9999	18.56	>0.9999	0.00	>0.9999
23 vs. 25	EDC-2 vs. EDC-4	25.31	>0.9999	0.00	>0.9999	21.31	>0.9999	14.36	>0.9999	18.56	>0.9999	-3.81	>0.9999
23 vs. 26	EDC-2 vs. EDC-5	14.10	>0.9999	-4.56	>0.9999	-19.63	>0.9999	-30.56	>0.9999	1.44	>0.9999	13.19	>0.9999
23 vs. 27	EDC-2 vs. EDM	-8.88	>0.9999	-30.88	>0.9999	19.63	>0.9999	3.21	>0.9999	9.75	>0.9999	-22.75	>0.9999
23 vs. 28	EDC-2 vs. FPL	-51.94	>0.9999	0.00	>0.9999	23.56	>0.9999	14.36	>0.9999	-20.94	>0.9999	13.19	>0.9999
23 vs. 29	EDC-2 vs. APL	-17.19	>0.9999	13.69	>0.9999	81.81	>0.9999	14.36	>0.9999	9.75	>0.9999	-17.00	>0.9999
23 vs. 30	EDC-2 vs. EPB	-8.69	>0.9999	-18.44	>0.9999	74.88	>0.9999	-35.89	>0.9999	5.25	>0.9999	-17.00	>0.9999
23 vs. 31	EDC-2 vs. EPL	-8.69	>0.9999	13.69	>0.9999	94.94	>0.9999	14.36	>0.9999	54.75	>0.9999	13.19	>0.9999
24 vs. 25	EDC-3 vs. EDC-4	-17.38	>0.9999	0.00	>0.9999	0.50	>0.9999	15.60	>0.9999	0.00	>0.9999	-3.81	>0.9999
24 vs. 26	EDC-3 vs. EDC-5	-28.58	>0.9999	-4.56	>0.9999	-40.44	>0.9999	-29.32	>0.9999	-17.13	>0.9999	13.19	>0.9999
24 vs. 27	EDC-3 vs. EDM	-51.56	>0.9999	-30.88	>0.9999	-1.19	>0.9999	4.46	>0.9999	-8.81	>0.9999	-22.75	>0.9999
24 vs. 28	EDC-3 vs. FPL	-94.63	>0.9999	0.00	>0.9999	2.75	>0.9999	15.60	>0.9999	-39.50	>0.9999	13.19	>0.9999
24 vs. 29	EDC-3 vs. APL	-59.88	>0.9999	13.69	>0.9999	61.00	>0.9999	15.60	>0.9999	-8.81	>0.9999	-17.00	>0.9999
24 vs. 30	EDC-3 vs. EPB	-51.38	>0.9999	-18.44	>0.9999	54.06	>0.9999	-34.65	>0.9999	-13.31	>0.9999	-17.00	>0.9999
24 vs. 31	EDC-3 vs. EPL	-51.38	>0.9999	13.69	>0.9999	74.13	>0.9999	15.60	>0.9999	36.19	>0.9999	13.19	>0.9999
25 vs. 26	EDC-4 vs. EDC-5	-11.21	>0.9999	-4.56	>0.9999	-40.94	>0.9999	-44.92	>0.9999	-17.13	>0.9999	17.00	>0.9999
25 vs. 27	EDC-4 vs. EDM	-34.19	>0.9999	-30.88	>0.9999	-1.69	>0.9999	-11.14	>0.9999	-8.81	>0.9999	-18.94	>0.9999
25 vs. 28	EDC-4 vs. FPL	-77.25	>0.9999	0.00	>0.9999	2.25	>0.9999	0.00	>0.9999	-39.50	>0.9999	17.00	>0.9999
25 vs. 29	EDC-4 vs. APL	-42.50	>0.9999	13.69	>0.9999	60.50	>0.9999	0.00	>0.9999	-8.81	>0.9999	-13.19	>0.9999
25 vs. 30	EDC-4 vs. EPB	-34.00	>0.9999	-18.44	>0.9999	53.56	>0.9999	-50.25	>0.9999	-13.31	>0.9999	-13.19	>0.9999
25 vs. 31	EDC-4 vs. EPL	-34.00	>0.9999	13.69	>0.9999	73.63	>0.9999	0.00	>0.9999	36.19	>0.9999	17.00	>0.9999
26 vs. 27	EDC-5 vs. EDM	-22.98	>0.9999	-26.31	>0.9999	39.25	>0.9999	33.77	>0.9999	8.31	>0.9999	-35.94	>0.9999
26 vs. 28	EDC-5 vs. FPL	-66.04	>0.9999	4.56	>0.9999	43.19	>0.9999	44.92	>0.9999	-22.38	>0.9999	0.00	>0.9999
26 vs. 29	EDC-5 vs. APL	-31.29	>0.9999	18.25	>0.9999	101.40	>0.9999	44.92	>0.9999	8.31	>0.9999	-30.19	>0.9999
26 vs. 30	EDC-5 vs. EPB	-22.79	>0.9999	-13.88	>0.9999	94.50	>0.9999	-5.33	>0.9999	3.81	>0.9999	-30.19	>0.9999
26 vs. 31	EDC-5 vs. EPL	-22.79	>0.9999	18.25	>0.9999	114.60	0.8775	44.92	>0.9999	53.31	>0.9999	0.00	>0.9999
27 vs. 28	EDM vs. FPL	-43.06	>0.9999	30.88	>0.9999	3.94	>0.9999	11.14	>0.9999	-30.69	>0.9999	35.94	>0.9999
27 vs. 29	EDM vs. APL	-8.31	>0.9999	44.56	>0.9999	62.19	>0.9999	11.14	>0.9999	0.00	>0.9999	5.75	>0.9999
27 vs. 30	EDM vs. EPB	0.19	>0.9999	12.44	>0.9999	55.25	>0.9999	-39.11	>0.9999	-4.50	>0.9999	5.75	>0.9999
27 vs. 31	EDM vs. EPL	0.19	>0.9999	44.56	>0.9999	75.31	>0.9999	11.14	>0.9999	45.00	>0.9999	35.94	>0.9999
28 vs. 29	FPL vs. APL	34.75	>0.9999	13.69	>0.9999	58.25	>0.9999	0.00	>0.9999	30.69	>0.9999	-30.19	>0.9999
28 vs. 30	FPL vs. EPB	43.25	>0.9999	-18.44	>0.9999	51.31	>0.9999	-50.25	>0.9999	26.19	>0.9999	-30.19	>0.9999
28 vs. 31	FPL vs. EPL	43.25	>0.9999	13.69	>0.9999	71.38	>0.9999	0.00	>0.9999	75.69	>0.9999	0.00	>0.9999
29 vs. 30	APL vs. EPB	8.50	>0.9999	-32.13	>0.9999	-6.94	>0.9999	-50.25	>0.9999	-4.50	>0.9999	0.00	>0.9999
29 vs. 31	APL vs. EPL	8.50	>0.9999	0.00	>0.9999	13.13	>0.9999	0.00	>0.9999	45.00	>0.9999	30.19	>0.9999
30 vs. 31	EPB vs. EPL	0.00	>0.9999	32.13	>0.9999	20.06	>0.9999	50.25	>0.9999	49.50	>0.9999	30.19	>0.9999

Supp. Table 9.6.d Pairwise comparisons for upper limb tendon interfascicular region (IFR) characteristics. *Green cells indicate significant p-values (< 0.05).*

Tendon Comparison		Interfascicular Region (IFR) Frequency				Interfascicular Region (IFR) Type Proportion			
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval	
1 vs. 2	SSP vs. IS	-3.783	0.9999	-10.063	2.497	-15.875	>0.9999	-61.248	29.498
1 vs. 3	SSP vs. SSC	-2.686	>0.9999	-8.966	3.594	-6.950	>0.9999	-52.323	38.422
1 vs. 4	SSP vs. LHBT	-3.382	>0.9999	-9.661	2.898	-26.452	>0.9999	-71.825	18.920
1 vs. 5	SSP vs. DBT	-5.599	0.2312	-11.879	0.680	-23.441	>0.9999	-68.814	21.932
1 vs. 6	SSP vs. BrR	-8.397	0.0001	-14.677	-2.117	-31.234	0.9991	-80.822	18.354
1 vs. 7	SSP vs. DTT	-6.491	0.0295	-12.771	-0.211	-39.590	0.2916	-84.963	5.783
1 vs. 8	SSP vs. FCR	-7.152	0.0049	-13.432	-0.872	-44.868	0.0593	-90.240	0.505
1 vs. 9	SSP vs. PL	-9.304	<0.0001	-15.584	-3.024	-85.631	0.0001	-149.775	-21.487
1 vs. 10	SSP vs. FCU	-7.949	0.0005	-14.229	-1.669	-61.246	0.0001	-106.619	-15.873
1 vs. 11	SSP vs. ECRL	-8.406	0.0001	-14.686	-2.126	-46.605	0.0326	-91.978	-1.232
1 vs. 12	SSP vs. ECRB	-6.465	0.0314	-12.745	-0.185	-30.311	0.9897	-75.684	15.062
1 vs. 13	SSP vs. ECU	-4.235	0.9859	-10.514	2.045	-44.484	0.0674	-89.857	0.889
1 vs. 14	SSP vs. FDS-2	-4.236	0.9857	-10.516	2.044	-15.358	>0.9999	-60.731	30.014
1 vs. 15	SSP vs. FDS-3	-4.766	0.7890	-11.046	1.514	-9.466	>0.9999	-54.838	35.907
1 vs. 16	SSP vs. FDS-4	-5.019	0.6053	-11.299	1.260	-19.742	>0.9999	-65.115	25.630
1 vs. 17	SSP vs. FDS-5	0.697	>0.9999	-5.583	6.977	-28.147	0.9996	-73.520	17.225
1 vs. 18	SSP vs. FDP-2	-3.380	>0.9999	-9.660	2.900	-8.023	>0.9999	-53.396	37.350
1 vs. 19	SSP vs. FDP-3	-5.276	0.4171	-11.556	1.004	-6.327	>0.9999	-51.700	39.046
1 vs. 20	SSP vs. FDP-4	-4.651	0.8580	-10.931	1.629	-11.711	>0.9999	-57.084	33.662
1 vs. 21	SSP vs. FDP-5	-2.480	>0.9999	-8.760	3.800	-15.545	>0.9999	-60.918	29.828
1 vs. 22	SSP vs. EIP	-3.038	>0.9999	-9.318	3.242	-43.127	0.1046	-88.499	2.246
1 vs. 23	SSP vs. EDC-2	-1.469	>0.9999	-7.749	4.810	-30.463	0.9878	-75.836	14.910
1 vs. 24	SSP vs. EDC-3	-7.859	0.0006	-14.139	-1.579	-39.957	0.2648	-85.330	5.416
1 vs. 25	SSP vs. EDC-4	-6.133	0.0713	-12.413	0.147	-46.699	0.0758	-94.714	1.317
1 vs. 26	SSP vs. EDC-5	-2.762	>0.9999	-9.385	3.862	-39.983	0.5791	-89.666	9.699
1 vs. 27	SSP vs. EDM	-2.006	>0.9999	-8.286	4.274	-30.412	0.9885	-75.784	14.961
1 vs. 28	SSP vs. FPL	-1.401	>0.9999	-7.681	4.878	-18.053	>0.9999	-63.426	27.320
1 vs. 29	SSP vs. APL	-5.632	0.2168	-11.911	0.648	-20.489	>0.9999	-65.862	24.884
1 vs. 30	SSP vs. EPB	-0.378	>0.9999	-6.658	5.902	-28.851	0.9985	-74.224	16.522
1 vs. 31	SSP vs. EPL	-1.281	>0.9999	-7.561	4.999	-40.772	0.2117	-86.145	4.601
2 vs. 3	IS vs. SSC	1.097	>0.9999	-4.004	6.197	8.924	>0.9999	-27.913	45.762
2 vs. 4	IS vs. LHBT	0.401	>0.9999	-4.699	5.502	-10.578	>0.9999	-47.415	26.260
2 vs. 5	IS vs. DBT	-1.817	>0.9999	-6.917	3.284	-7.567	>0.9999	-44.404	29.271
2 vs. 6	IS vs. BrR	-4.614	0.1959	-9.714	0.486	-15.359	>0.9999	-57.468	26.750
2 vs. 7	IS vs. DTT	-2.708	>0.9999	-7.808	2.393	-23.715	0.9975	-60.553	13.122
2 vs. 8	IS vs. FCR	-3.369	0.9931	-8.469	1.731	-28.993	0.6636	-65.831	7.845
2 vs. 9	IS vs. PL	-5.521	0.0131	-10.622	-0.421	-69.756	0.0021	-128.548	-10.965
2 vs. 10	IS vs. FCU	-4.166	0.5221	-9.266	0.934	-45.372	0.0009	-82.209	-8.534
2 vs. 11	IS vs. ECRL	-4.623	0.1913	-9.724	0.477	-30.731	0.4427	-67.568	6.107
2 vs. 12	IS vs. ECRB	-2.682	>0.9999	-7.783	2.418	-14.436	>0.9999	-51.274	22.401
2 vs. 13	IS vs. ECU	-0.452	>0.9999	-5.552	4.649	-28.609	0.7119	-65.447	8.228
2 vs. 14	IS vs. FDS-2	-0.453	>0.9999	-5.554	4.647	0.516	>0.9999	-36.321	37.354
2 vs. 15	IS vs. FDS-3	-0.983	>0.9999	-6.083	4.117	6.409	>0.9999	-30.428	43.247
2 vs. 16	IS vs. FDS-4	-1.237	>0.9999	-6.337	3.864	-3.868	>0.9999	-40.705	32.970
2 vs. 17	IS vs. FDS-5	4.480	0.2720	-0.620	9.580	-12.273	>0.9999	-49.110	24.565
2 vs. 18	IS vs. FDP-2	0.403	>0.9999	-4.698	5.503	7.851	>0.9999	-28.986	44.689
2 vs. 19	IS vs. FDP-3	-1.493	>0.9999	-6.593	3.607	9.548	>0.9999	-27.290	46.385
2 vs. 20	IS vs. FDP-4	-0.868	>0.9999	-5.968	4.233	4.164	>0.9999	-32.674	41.001
2 vs. 21	IS vs. FDP-5	1.302	>0.9999	-3.798	6.403	0.330	>0.9999	-36.508	37.167
2 vs. 22	IS vs. EIP	0.745	>0.9999	-4.355	5.845	-27.252	0.8606	-64.090	9.586
2 vs. 23	IS vs. EDC-2	2.313	>0.9999	-2.787	7.414	-14.588	>0.9999	-51.426	22.250
2 vs. 24	IS vs. EDC-3	-4.076	0.6057	-9.177	1.024	-24.082	0.9953	-60.920	12.755
2 vs. 25	IS vs. EDC-4	-2.350	>0.9999	-7.451	2.750	-30.824	0.7253	-70.664	9.017
2 vs. 26	IS vs. EDC-5	1.021	>0.9999	-4.496	6.538	-24.109	>0.9999	-66.275	18.058
2 vs. 27	IS vs. EDM	1.776	>0.9999	-3.324	6.877	-14.537	>0.9999	-51.375	22.301
2 vs. 28	IS vs. FPL	2.381	>0.9999	-2.719	7.482	-2.178	>0.9999	-39.016	34.660
2 vs. 29	IS vs. APL	-1.849	>0.9999	-6.949	3.252	-4.615	>0.9999	-41.452	32.223
2 vs. 30	IS vs. EPB	3.405	0.9899	-1.695	8.505	-12.976	>0.9999	-49.814	23.862
2 vs. 31	IS vs. EPL	2.502	>0.9999	-2.599	7.602	-24.897	0.9848	-61.735	11.941
3 vs. 4	SSC vs. LHBT	-0.696	>0.9999	-5.796	4.405	-19.502	>0.9999	-56.340	17.336
3 vs. 5	SSC vs. DBT	-2.913	>0.9999	-8.014	2.187	-16.491	>0.9999	-53.328	20.347
3 vs. 6	SSC vs. BrR	-5.711	0.0069	-10.811	-0.610	-24.284	>0.9999	-66.393	17.825
3 vs. 7	SSC vs. DTT	-3.805	0.8392	-8.905	1.296	-32.640	0.2479	-69.477	4.198
3 vs. 8	SSC vs. FCR	-4.466	0.2813	-9.566	0.635	-37.917	0.0315	-74.755	-1.080
3 vs. 9	SSC vs. PL	-6.618	0.0002	-11.718	-1.518	-78.681	0.0001	-137.472	-19.890
3 vs. 10	SSC vs. FCU	-5.263	0.0303	-10.363	-0.162	-54.296	<0.0001	-91.133	-17.458
3 vs. 11	SSC vs. ECRL	-5.720	0.0067	-10.820	-0.620	-39.655	0.0145	-76.492	-2.817
3 vs. 12	SSC vs. ECRB	-3.779	0.8568	-8.879	1.321	-23.361	0.9987	-60.198	13.477
3 vs. 13	SSC vs. ECU	-1.549	>0.9999	-6.649	3.552	-37.534	0.0372	-74.371	-0.696
3 vs. 14	SSC vs. FDS-2	-1.550	>0.9999	-6.650	3.550	-8.408	>0.9999	-45.246	28.430
3 vs. 15	SSC vs. FDS-3	-2.080	>0.9999	-7.180	3.021	-2.515	>0.9999	-39.353	34.323
3 vs. 16	SSC vs. FDS-4	-2.333	>0.9999	-7.434	2.767	-12.792	>0.9999	-49.630	24.046
3 vs. 17	SSC vs. FDS-5	3.383	0.9919	-1.717	8.484	-21.197	>0.9999	-58.035	15.641
3 vs. 18	SSC vs. FDP-2	-0.694	>0.9999	-5.794	4.406	-1.073	>0.9999	-37.910	35.765
3 vs. 19	SSC vs. FDP-3	-2.590	>0.9999	-7.690	2.511	0.623	>0.9999	-36.214	37.461
3 vs. 20	SSC vs. FDP-4	-1.965	>0.9999	-7.065	3.136	-4.761	>0.9999	-41.598	32.077
3 vs. 21	SSC vs. FDP-5	0.206	>0.9999	-4.895	5.306	-8.595	>0.9999	-45.432	28.243
3 vs. 22	SSC vs. EIP	-0.352	>0.9999	-5.452	4.749	-36.176	0.0658	-73.014	0.662
3 vs. 23	SSC vs. EDC-2	1.217	>0.9999	-3.884	6.317	-23.512	0.9983	-60.350	13.325
3 vs. 24	SSC vs. EDC-3	-5.173	0.0401	-10.273	-0.073	-33.006	0.2189	-69.844	3.831
3 vs. 25	SSC vs. EDC-4	-3.447	0.9848	-8.547	1.653	-39.748	0.0518	-79.589	0.092
3 vs. 26	SSC vs. EDC-5	-0.076	>0.9999	-5.593	5.442	-33.033	0.6807	-75.199	9.133

Tendon Comparison		Interfascicular Region (IFR) Frequency				Interfascicular Region (IFR) Type Proportion			
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval	
3 vs. 27	SSC vs. EDM	0.680	>0.9999	-4.421	5.780	-23.461	0.9984	-60.299	13.377
3 vs. 28	SSC vs. FPL	1.285	>0.9999	-3.816	6.385	-11.102	>0.9999	-47.940	25.735
3 vs. 29	SSC vs. APL	-2.945	>0.9999	-8.046	2.155	-13.539	>0.9999	-50.376	23.299
3 vs. 30	SSC vs. EPB	2.308	>0.9999	-2.792	7.409	-21.900	>0.9999	-58.738	14.937
3 vs. 31	SSC vs. EPL	1.405	>0.9999	-3.696	6.505	-33.821	0.1640	-70.659	3.016
4 vs. 5	LHBT vs. DBT	-2.218	>0.9999	-7.318	2.883	3.011	>0.9999	-33.826	39.849
4 vs. 6	LHBT vs. BrR	-5.015	0.0645	-10.116	0.085	-4.782	>0.9999	-46.891	37.327
4 vs. 7	LHBT vs. DTT	-3.109	0.9998	-8.209	1.991	-13.138	>0.9999	-49.975	23.700
4 vs. 8	LHBT vs. FCR	-3.770	0.8625	-8.871	1.330	-18.415	>0.9999	-55.253	18.422
4 vs. 9	LHBT vs. PL	-5.923	0.0033	-11.023	-0.822	-59.179	0.0451	-117.970	-0.388
4 vs. 10	LHBT vs. FCU	-4.567	0.2204	-9.668	0.533	-34.794	0.1139	-71.631	2.044
4 vs. 11	LHBT vs. ECRL	-5.025	0.0628	-10.125	0.076	-20.153	>0.9999	-56.990	16.685
4 vs. 12	LHBT vs. ECRB	-3.084	0.9999	-8.184	2.017	-3.859	>0.9999	-40.696	32.979
4 vs. 13	LHBT vs. ECU	-0.853	>0.9999	-5.953	4.247	-18.032	>0.9999	-54.869	18.806
4 vs. 14	LHBT vs. FDS-2	-0.855	>0.9999	-5.955	4.246	11.094	>0.9999	-25.744	47.932
4 vs. 15	LHBT vs. FDS-3	-1.384	>0.9999	-6.485	3.716	16.987	>0.9999	-19.851	53.825
4 vs. 16	LHBT vs. FDS-4	-1.638	>0.9999	-6.738	3.463	6.710	>0.9999	-30.128	43.548
4 vs. 17	LHBT vs. FDS-5	4.079	0.6032	-1.021	9.179	-1.695	>0.9999	-38.533	35.143
4 vs. 18	LHBT vs. FDP-2	0.002	>0.9999	-5.099	5.102	18.429	>0.9999	-18.408	55.267
4 vs. 19	LHBT vs. FDP-3	-1.894	>0.9999	-6.995	3.206	20.125	>0.9999	-16.712	56.963
4 vs. 20	LHBT vs. FDP-4	-1.269	>0.9999	-6.369	3.831	14.741	>0.9999	-22.096	51.579
4 vs. 21	LHBT vs. FDP-5	0.901	>0.9999	-4.199	6.002	10.907	>0.9999	-25.930	47.745
4 vs. 22	LHBT vs. EIP	0.344	>0.9999	-4.757	5.444	-16.674	>0.9999	-53.512	20.164
4 vs. 23	LHBT vs. EDC-2	1.912	>0.9999	-3.188	7.013	-4.010	>0.9999	-40.848	32.827
4 vs. 24	LHBT vs. EDC-3	-4.477	0.2738	-9.578	0.623	-13.504	>0.9999	-50.342	23.333
4 vs. 25	LHBT vs. EDC-4	-2.751	>0.9999	-7.852	2.349	-20.246	>0.9999	-60.087	19.594
4 vs. 26	LHBT vs. EDC-5	0.620	>0.9999	-4.897	6.137	-13.531	>0.9999	-55.697	28.635
4 vs. 27	LHBT vs. EDM	1.375	>0.9999	-3.725	6.476	-3.959	>0.9999	-40.797	32.878
4 vs. 28	LHBT vs. FPL	1.980	>0.9999	-3.120	7.080	8.400	>0.9999	-28.438	45.237
4 vs. 29	LHBT vs. APL	-2.250	>0.9999	-7.350	2.850	5.963	>0.9999	-30.874	42.801
4 vs. 30	LHBT vs. EPB	3.004	>0.9999	-2.096	8.104	-2.398	>0.9999	-39.236	34.439
4 vs. 31	LHBT vs. EPL	2.100	>0.9999	-3.000	7.201	-14.319	>0.9999	-51.157	22.518
5 vs. 6	DBT vs. BrR	-2.797	>0.9999	-7.898	2.303	-7.793	>0.9999	-49.902	34.316
5 vs. 7	DBT vs. DTT	-0.891	>0.9999	-5.992	4.209	-16.149	>0.9999	-52.986	20.689
5 vs. 8	DBT vs. FCR	-1.552	>0.9999	-6.653	3.548	-21.426	>0.9999	-58.264	15.411
5 vs. 9	DBT vs. PL	-3.705	0.9016	-8.805	1.396	-62.190	0.0198	-120.981	-3.399
5 vs. 10	DBT vs. FCU	-2.349	>0.9999	-7.450	2.751	-37.805	0.0331	-74.643	-0.967
5 vs. 11	DBT vs. ECRL	-2.807	>0.9999	-7.907	2.294	-23.164	0.9991	-60.002	13.674
5 vs. 12	DBT vs. ECRB	-0.866	>0.9999	-5.966	4.235	-6.870	>0.9999	-43.707	29.968
5 vs. 13	DBT vs. ECU	1.365	>0.9999	-3.736	6.465	-21.043	>0.9999	-57.880	15.795
5 vs. 14	DBT vs. FDS-2	1.363	>0.9999	-3.737	6.464	8.083	>0.9999	-28.755	44.921
5 vs. 15	DBT vs. FDS-3	0.834	>0.9999	-4.267	5.934	13.976	>0.9999	-22.862	50.813
5 vs. 16	DBT vs. FDS-4	0.580	>0.9999	-4.520	5.680	3.699	>0.9999	-33.139	40.537
5 vs. 17	DBT vs. FDS-5	6.297	0.0008	1.196	11.397	-4.706	>0.9999	-41.544	32.131
5 vs. 18	DBT vs. FDP-2	2.219	>0.9999	-2.881	7.320	15.418	>0.9999	-21.420	52.256
5 vs. 19	DBT vs. FDP-3	0.324	>0.9999	-4.777	5.424	17.114	>0.9999	-19.723	53.952
5 vs. 20	DBT vs. FDP-4	0.949	>0.9999	-4.152	6.049	11.730	>0.9999	-25.107	48.568
5 vs. 21	DBT vs. FDP-5	3.119	0.9998	-1.981	8.219	7.896	>0.9999	-28.942	44.734
5 vs. 22	DBT vs. EIP	2.562	>0.9999	-2.539	7.662	-19.685	>0.9999	-56.523	17.152
5 vs. 23	DBT vs. EDC-2	4.130	0.5554	-0.970	9.230	-7.021	>0.9999	-43.859	29.816
5 vs. 24	DBT vs. EDC-3	-2.260	>0.9999	-7.360	2.841	-16.516	>0.9999	-53.353	20.322
5 vs. 25	DBT vs. EDC-4	-0.534	>0.9999	-5.634	4.567	-23.257	>0.9999	-63.098	16.583
5 vs. 26	DBT vs. EDC-5	2.838	>0.9999	-2.680	8.355	-16.542	>0.9999	-58.709	25.624
5 vs. 27	DBT vs. EDM	3.593	0.9506	-1.507	8.693	-6.970	>0.9999	-43.808	29.867
5 vs. 28	DBT vs. FPL	4.198	0.4930	-0.902	9.298	5.388	>0.9999	-31.449	42.226
5 vs. 29	DBT vs. APL	-0.032	>0.9999	-5.132	5.068	2.952	>0.9999	-33.886	39.790
5 vs. 30	DBT vs. EPB	5.222	0.0344	0.121	10.322	-5.409	>0.9999	-42.247	31.428
5 vs. 31	DBT vs. EPL	4.318	0.3897	-0.782	9.419	-17.330	>0.9999	-54.168	19.507
6 vs. 7	BrR vs. DTT	1.906	>0.9999	-3.194	7.007	-8.356	>0.9999	-50.465	33.753
6 vs. 8	BrR vs. FCR	1.245	>0.9999	-3.855	6.345	-13.634	>0.9999	-55.743	28.475
6 vs. 9	BrR vs. PL	-0.907	>0.9999	-6.008	4.193	-54.397	0.2853	-116.607	7.813
6 vs. 10	BrR vs. FCU	0.448	>0.9999	-4.652	5.548	-30.012	0.9347	-72.121	12.097
6 vs. 11	BrR vs. ECRL	-0.009	>0.9999	-5.110	5.091	-15.371	>0.9999	-57.480	26.738
6 vs. 12	BrR vs. ECRB	1.932	>0.9999	-3.169	7.032	0.923	>0.9999	-41.186	43.032
6 vs. 13	BrR vs. ECU	4.162	0.5256	-0.938	9.263	-13.250	>0.9999	-55.359	28.859
6 vs. 14	BrR vs. FDS-2	4.161	0.5271	-0.940	9.261	15.876	>0.9999	-26.233	57.985
6 vs. 15	BrR vs. FDS-3	3.631	0.9365	-1.469	8.731	21.768	>0.9999	-20.341	63.878
6 vs. 16	BrR vs. FDS-4	3.377	0.9925	-1.723	8.478	11.492	>0.9999	-30.617	53.601
6 vs. 17	BrR vs. FDS-5	9.094	<0.0001	3.994	14.194	3.087	>0.9999	-39.022	45.196
6 vs. 18	BrR vs. FDP-2	5.017	0.0642	-0.084	10.117	23.211	>0.9999	-18.898	65.320
6 vs. 19	BrR vs. FDP-3	3.121	0.9998	-1.979	8.221	24.907	>0.9999	-17.202	67.016
6 vs. 20	BrR vs. FDP-4	3.746	0.8778	-1.354	8.846	19.523	>0.9999	-22.586	61.632
6 vs. 21	BrR vs. FDP-5	5.916	0.0034	0.816	11.017	15.689	>0.9999	-26.420	57.798
6 vs. 22	BrR vs. EIP	5.359	0.0223	0.259	10.459	-11.893	>0.9999	-54.002	30.216
6 vs. 23	BrR vs. EDC-2	6.927	0.0001	1.827	12.028	0.771	>0.9999	-41.338	42.880
6 vs. 24	BrR vs. EDC-3	0.538	>0.9999	-4.563	5.638	-8.723	>0.9999	-50.832	33.386
6 vs. 25	BrR vs. EDC-4	2.264	>0.9999	-2.837	7.364	-15.465	>0.9999	-60.332	29.403
6 vs. 26	BrR vs. EDC-5	5.635	0.0358	0.118	11.152	-8.749	>0.9999	-55.449	37.950
6 vs. 27	BrR vs. EDM	6.390	0.0006	1.290	11.491	0.822	>0.9999	-41.287	42.931
6 vs. 28	BrR vs. FPL	6.995	0.0001	1.895	12.096	13.181	>0.9999	-28.928	55.290
6 vs. 29	BrR vs. APL	2.765	>0.9999	-2.335	7.866	10.745	>0.9999	-31.364	52.854
6 vs. 30	BrR vs. EPB	8.019	<0.0001	2.919	13.119	2.383	>0.9999	-39.726	44.492
6 vs. 31	BrR vs. EPL	7.115	<0.0001	2.015	12.216	-9.538	>0.9999	-51.647	32.571
7 vs. 8	DTT vs. FCR	-0.661	>0.9999	-5.762	4.439	-5.278	>0.9999	-42.115	31.560
7 vs. 9	DTT vs. PL	-2.814	>0.9999	-7.914	2.287	-46.041	0.6819	-104.832	12.750
7 vs. 10	DTT vs. FCU	-1.458	>0.9999	-6.559	3.642	-21.656	>0.9999	-58.494	15.181
7 vs. 11	DTT vs. ECRL	-1.916	>0.9999	-7.016	3.185	-7.015	>0.9999	-43.853	29.822

Tendon Comparison		Interfascicular Region (IFR) Frequency				Interfascicular Region (IFR) Type Proportion			
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval	
7 vs. 12	DTT vs. ECRB	0.026	>0.9999	-5.075	5.126	9.279	>0.9999	-27.559	46.117
7 vs. 13	DTT vs. ECU	2.256	>0.9999	-2.844	7.356	-4.894	>0.9999	-41.732	31.944
7 vs. 14	DTT vs. FDS-2	2.254	>0.9999	-2.846	7.355	24.232	0.9941	-12.606	61.069
7 vs. 15	DTT vs. FDS-3	1.725	>0.9999	-3.376	6.825	30.125	0.5176	-6.713	66.962
7 vs. 16	DTT vs. FDS-4	1.471	>0.9999	-3.629	6.572	19.848	>0.9999	-16.990	56.685
7 vs. 17	DTT vs. FDS-5	7.188	<0.0001	2.088	12.288	11.443	>0.9999	-25.395	48.280
7 vs. 18	DTT vs. FDP-2	3.111	0.9998	-1.990	8.211	31.567	0.3486	-5.271	68.404
7 vs. 19	DTT vs. FDP-3	1.215	>0.9999	-3.886	6.315	33.263	0.2002	-3.575	70.101
7 vs. 20	DTT vs. FDP-4	1.840	>0.9999	-3.260	6.940	27.879	0.7976	-8.959	64.717
7 vs. 21	DTT vs. FDP-5	4.010	0.6672	-1.090	9.111	24.045	0.9956	-12.793	60.883
7 vs. 22	DTT vs. EIP	3.453	0.9840	-1.647	8.553	-3.537	>0.9999	-40.374	33.301
7 vs. 23	DTT vs. EDC-2	5.021	0.0634	-0.079	10.122	9.127	>0.9999	-27.710	45.965
7 vs. 24	DTT vs. EDC-3	-1.368	>0.9999	-6.469	3.732	-0.367	>0.9999	-37.205	36.471
7 vs. 25	DTT vs. EDC-4	0.358	>0.9999	-4.743	5.458	-7.109	>0.9999	-46.949	32.732
7 vs. 26	DTT vs. EDC-5	3.729	0.9848	-1.788	9.246	-0.393	>0.9999	-42.560	41.773
7 vs. 27	DTT vs. EDM	4.484	0.2693	-0.616	9.585	9.178	>0.9999	-27.659	46.016
7 vs. 28	DTT vs. FPL	5.089	0.0517	-0.011	10.190	21.537	>0.9999	-15.300	58.375
7 vs. 29	DTT vs. APL	0.859	>0.9999	-4.241	5.959	19.101	>0.9999	-17.737	55.938
7 vs. 30	DTT vs. EPB	6.113	0.0017	1.013	11.213	10.739	>0.9999	-26.098	47.577
7 vs. 31	DTT vs. EPL	5.209	0.0358	0.109	10.310	-1.182	>0.9999	-38.019	35.656
8 vs. 9	FCR vs. PL	-2.152	>0.9999	-7.253	2.948	-40.764	0.9675	-99.555	18.028
8 vs. 10	FCR vs. FCU	-0.797	>0.9999	-5.897	4.303	-16.379	>0.9999	-53.216	20.459
8 vs. 11	FCR vs. ECRL	-1.254	>0.9999	-6.355	3.846	-1.738	>0.9999	-38.575	35.100
8 vs. 12	FCR vs. ECRB	0.687	>0.9999	-4.414	5.787	14.557	>0.9999	-22.281	51.394
8 vs. 13	FCR vs. ECU	2.917	>0.9999	-2.183	8.018	0.384	>0.9999	-36.454	37.221
8 vs. 14	FCR vs. FDS-2	2.916	>0.9999	-2.185	8.016	29.509	0.5968	-7.328	66.347
8 vs. 15	FCR vs. FDS-3	2.386	>0.9999	-2.714	7.486	35.402	0.0898	-1.436	72.240
8 vs. 16	FCR vs. FDS-4	2.132	>0.9999	-2.968	7.233	25.125	0.9798	-11.712	61.963
8 vs. 17	FCR vs. FDS-5	7.849	<0.0001	2.749	12.950	16.720	>0.9999	-20.118	53.558
8 vs. 18	FCR vs. FDP-2	3.772	0.8615	-1.329	8.872	36.844	0.0499	0.007	73.682
8 vs. 19	FCR vs. FDP-3	1.876	>0.9999	-3.224	6.976	38.541	0.0240	1.703	75.378
8 vs. 20	FCR vs. FDP-4	2.501	>0.9999	-2.599	7.602	33.157	0.2078	-3.681	69.994
8 vs. 21	FCR vs. FDP-5	4.672	0.1689	-0.429	9.772	29.322	0.6211	-7.515	66.160
8 vs. 22	FCR vs. EIP	4.114	0.5702	-0.986	9.214	1.741	>0.9999	-35.097	38.579
8 vs. 23	FCR vs. EDC-2	5.682	0.0076	0.582	10.783	14.405	>0.9999	-22.433	51.243
8 vs. 24	FCR vs. EDC-3	-0.707	>0.9999	-5.808	4.393	4.911	>0.9999	-31.927	41.748
8 vs. 25	FCR vs. EDC-4	1.019	>0.9999	-4.082	6.119	-1.831	>0.9999	-41.671	38.009
8 vs. 26	FCR vs. EDC-5	4.390	0.6225	-1.127	9.908	4.884	>0.9999	-37.282	47.050
8 vs. 27	FCR vs. EDM	5.146	0.0436	0.045	10.246	14.456	>0.9999	-22.382	51.294
8 vs. 28	FCR vs. FPL	5.750	0.0060	0.650	10.851	26.815	0.8973	-10.023	63.652
8 vs. 29	FCR vs. APL	1.520	>0.9999	-3.580	6.621	24.378	0.9926	-12.459	61.216
8 vs. 30	FCR vs. EPB	6.774	0.0001	1.674	11.875	16.017	>0.9999	-20.821	52.855
8 vs. 31	FCR vs. EPL	5.871	0.0040	0.770	10.971	4.096	>0.9999	-32.742	40.934
9 vs. 10	PL vs. FCU	1.355	>0.9999	-3.745	6.456	24.385	>0.9999	-34.406	83.176
9 vs. 11	PL vs. ECRL	0.898	>0.9999	-4.202	5.998	39.026	0.9918	-19.765	97.817
9 vs. 12	PL vs. ECRB	2.839	>0.9999	-2.261	7.939	55.320	0.1198	-3.471	114.111
9 vs. 13	PL vs. ECU	5.070	0.0549	-0.031	10.170	41.147	0.9582	-17.644	99.938
9 vs. 14	PL vs. FDS-2	5.068	0.0551	-0.032	10.168	70.273	0.0018	11.482	129.064
9 vs. 15	PL vs. FDS-3	4.538	0.2366	-0.562	9.639	76.166	0.0003	17.375	134.957
9 vs. 16	PL vs. FDS-4	4.285	0.4172	-0.816	9.385	65.889	0.0068	7.098	124.680
9 vs. 17	PL vs. FDS-5	10.001	<0.0001	4.901	15.102	57.484	0.0701	-1.307	116.275
9 vs. 18	PL vs. FDP-2	5.924	0.0033	0.824	11.024	77.608	0.0002	18.817	136.399
9 vs. 19	PL vs. FDP-3	4.028	0.6504	-1.072	9.129	79.304	0.0001	20.513	138.095
9 vs. 20	PL vs. FDP-4	4.654	0.1770	-0.447	9.754	73.920	0.0005	15.129	132.711
9 vs. 21	PL vs. FDP-5	6.824	0.0001	1.724	11.924	70.086	0.0019	11.295	128.877
9 vs. 22	PL vs. EIP	6.266	0.0009	1.166	11.367	42.505	0.9107	-16.286	101.296
9 vs. 23	PL vs. EDC-2	7.835	<0.0001	2.734	12.935	55.168	0.1242	-3.623	113.959
9 vs. 24	PL vs. EDC-3	1.445	>0.9999	-3.655	6.546	45.674	0.7108	-13.117	104.465
9 vs. 25	PL vs. EDC-4	3.171	0.9995	-1.929	8.272	38.933	0.9981	-21.920	99.785
9 vs. 26	PL vs. EDC-5	6.542	0.0021	1.025	12.060	45.648	0.8726	-16.361	107.657
9 vs. 27	PL vs. EDM	7.298	<0.0001	2.198	12.398	55.220	0.1227	-3.571	114.011
9 vs. 28	PL vs. FPL	7.903	<0.0001	2.802	13.003	67.578	0.0041	8.787	126.369
9 vs. 29	PL vs. APL	3.673	0.9180	-1.428	8.773	65.142	0.0085	6.351	123.933
9 vs. 30	PL vs. EPB	8.926	<0.0001	3.826	14.027	56.780	0.0838	-2.011	115.572
9 vs. 31	PL vs. EPL	8.023	<0.0001	2.923	13.123	44.859	0.7719	-13.932	103.650
10 vs. 11	FCU vs. ECRL	-0.457	>0.9999	-5.558	4.643	14.641	>0.9999	-22.197	51.479
10 vs. 12	FCU vs. ECRB	1.484	>0.9999	-3.617	6.584	30.935	0.4186	-5.902	67.773
10 vs. 13	FCU vs. ECU	3.714	0.8965	-1.386	8.815	16.762	>0.9999	-20.076	53.600
10 vs. 14	FCU vs. FDS-2	3.713	0.8973	-1.388	8.813	45.888	0.0007	9.050	82.726
10 vs. 15	FCU vs. FDS-3	3.183	0.9994	-1.917	8.283	51.781	<0.0001	14.943	88.618
10 vs. 16	FCU vs. FDS-4	2.929	>0.9999	-2.171	8.030	41.504	0.0061	4.666	78.342
10 vs. 17	FCU vs. FDS-5	8.646	<0.0001	3.546	13.746	33.099	0.2120	-3.739	69.936
10 vs. 18	FCU vs. FDP-2	4.569	0.2195	-0.532	9.669	53.223	<0.0001	16.385	90.061
10 vs. 19	FCU vs. FDP-3	2.673	>0.9999	-2.427	7.773	54.919	<0.0001	18.082	91.757
10 vs. 20	FCU vs. FDP-4	3.298	0.9970	-1.802	8.399	49.535	0.0001	12.698	86.373
10 vs. 21	FCU vs. FDP-5	5.468	0.0156	0.368	10.569	45.701	0.0007	8.863	82.539
10 vs. 22	FCU vs. EIP	4.911	0.0874	-0.189	10.011	18.120	>0.9999	-18.718	54.957
10 vs. 23	FCU vs. EDC-2	6.479	0.0004	1.379	11.580	30.783	0.4364	-6.054	67.621
10 vs. 24	FCU vs. EDC-3	0.090	>0.9999	-5.011	5.190	21.289	>0.9999	-15.548	58.127
10 vs. 25	FCU vs. EDC-4	1.816	>0.9999	-3.285	6.916	14.548	>0.9999	-25.293	54.388
10 vs. 26	FCU vs. EDC-5	5.187	0.1212	-0.330	10.704	21.263	>0.9999	-20.904	63.429
10 vs. 27	FCU vs. EDM	5.942	0.0031	0.842	11.043	30.835	0.4304	-6.003	67.672
10 vs. 28	FCU vs. FPL	6.547	0.0003	1.447	11.648	43.193	0.0027	6.356	80.031
10 vs. 29	FCU vs. APL	2.317	>0.9999	-2.783	7.418	40.757	0.0087	3.919	77.595
10 vs. 30	FCU vs. EPB	7.571	<0.0001	2.471	12.671	32.396	0.2687	-4.442	69.233
10 vs. 31	FCU vs. EPL	6.668	0.0002	1.567	11.768	20.474	>0.9999	-16.363	57.312
11 vs. 12	ECRL vs. ECRB	1.941	>0.9999	-3.159	7.041	16.294	>0.9999	-20.543	53.132

Tendon Comparison		Interfascicular Region (IFR) Frequency				Interfascicular Region (IFR) Type Proportion			
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval	
11 vs. 13	ECRL vs. ECU	4.172	0.5170	-0.929	9.272	2.121	>0.9999	-34.716	38.959
11 vs. 14	ECRL vs. FDS-2	4.170	0.5184	-0.930	9.270	31.247	0.3832	-5.591	68.085
11 vs. 15	ECRL vs. FDS-3	3.640	0.9326	-1.460	8.741	37.140	0.0440	0.302	73.977
11 vs. 16	ECRL vs. FDS-4	3.387	0.9917	-1.714	8.487	26.863	0.8936	-9.975	63.701
11 vs. 17	ECRL vs. FDS-5	9.103	<0.0001	4.003	14.204	18.458	>0.9999	-18.380	55.295
11 vs. 18	ECRL vs. FDP-2	5.026	0.0625	-0.074	10.126	38.582	0.0236	1.744	75.420
11 vs. 19	ECRL vs. FDP-3	3.130	0.9997	-1.970	8.231	40.278	0.0109	3.441	77.116
11 vs. 20	ECRL vs. FDP-4	3.755	0.8720	-1.345	8.856	34.894	0.1096	-1.943	71.732
11 vs. 21	ECRL vs. FDP-5	5.926	0.0033	0.825	11.026	31.060	0.4042	-5.778	67.898
11 vs. 22	ECRL vs. EIP	5.368	0.0216	0.268	10.469	3.479	>0.9999	-33.359	40.316
11 vs. 23	ECRL vs. EDC-2	6.937	0.0001	1.836	12.037	16.143	>0.9999	-20.695	52.980
11 vs. 24	ECRL vs. EDC-3	0.547	>0.9999	-4.553	5.647	6.648	>0.9999	-30.189	43.486
11 vs. 25	ECRL vs. EDC-4	2.273	>0.9999	-2.827	7.373	-0.093	>0.9999	-39.934	39.747
11 vs. 26	ECRL vs. EDC-5	5.644	0.0349	0.127	11.162	6.622	>0.9999	-35.545	48.788
11 vs. 27	ECRL vs. EDM	6.400	0.0006	1.299	11.500	16.194	>0.9999	-20.644	53.031
11 vs. 28	ECRL vs. FPL	7.005	0.0001	1.904	12.105	28.552	0.7190	-8.285	65.390
11 vs. 29	ECRL vs. APL	2.775	>0.9999	-2.326	7.875	26.116	0.9424	-10.722	62.954
11 vs. 30	ECRL vs. EPB	8.028	<0.0001	2.928	13.129	17.755	>0.9999	-19.083	54.592
11 vs. 31	ECRL vs. EPL	7.125	<0.0001	2.024	12.225	5.834	>0.9999	-31.004	42.671
12 vs. 13	ECRB vs. ECU	2.231	>0.9999	-2.870	7.331	-14.173	>0.9999	-51.011	22.665
12 vs. 14	ECRB vs. FDS-2	2.229	>0.9999	-2.871	7.329	14.953	>0.9999	-21.885	51.790
12 vs. 15	ECRB vs. FDS-3	1.699	>0.9999	-3.401	6.800	20.846	>0.9999	-15.992	57.683
12 vs. 16	ECRB vs. FDS-4	1.446	>0.9999	-3.655	6.546	10.569	>0.9999	-26.269	47.406
12 vs. 17	ECRB vs. FDS-5	7.162	<0.0001	2.062	12.263	2.164	>0.9999	-34.674	39.001
12 vs. 18	ECRB vs. FDP-2	3.085	0.9999	-2.015	8.185	22.288	0.9999	-14.550	59.125
12 vs. 19	ECRB vs. FDP-3	1.189	>0.9999	-3.911	6.290	23.984	0.9960	-12.854	60.822
12 vs. 20	ECRB vs. FDP-4	1.814	>0.9999	-3.286	6.915	18.600	>0.9999	-18.238	55.438
12 vs. 21	ECRB vs. FDP-5	3.985	0.6906	-1.116	9.085	14.766	>0.9999	-22.072	51.604
12 vs. 22	ECRB vs. EIP	3.427	0.9874	-1.673	8.528	-12.816	>0.9999	-49.653	24.022
12 vs. 23	ECRB vs. EDC-2	4.996	0.0684	-0.105	10.096	-0.152	>0.9999	-36.989	36.686
12 vs. 24	ECRB vs. EDC-3	-1.394	>0.9999	-6.494	3.706	-9.646	>0.9999	-46.484	27.192
12 vs. 25	ECRB vs. EDC-4	0.332	>0.9999	-4.768	5.432	-16.388	>0.9999	-56.228	23.453
12 vs. 26	ECRB vs. EDC-5	3.703	0.9879	-1.814	9.221	-9.672	>0.9999	-51.839	32.494
12 vs. 27	ECRB vs. EDM	4.459	0.2859	-0.642	9.559	-0.101	>0.9999	-36.938	36.737
12 vs. 28	ECRB vs. FPL	5.064	0.0559	-0.037	10.164	12.258	>0.9999	-24.579	49.096
12 vs. 29	ECRB vs. APL	0.834	>0.9999	-4.267	5.934	9.822	>0.9999	-27.016	46.659
12 vs. 30	ECRB vs. EPB	6.087	0.0018	0.987	11.188	1.460	>0.9999	-35.377	38.298
12 vs. 31	ECRB vs. EPL	5.184	0.0387	0.083	10.284	-10.461	>0.9999	-47.298	26.377
13 vs. 14	ECU vs. FDS-2	-0.002	>0.9999	-5.102	5.099	29.126	0.6465	-7.712	65.963
13 vs. 15	ECU vs. FDS-3	-0.531	>0.9999	-5.632	4.569	35.019	0.1044	-1.819	71.856
13 vs. 16	ECU vs. FDS-4	-0.785	>0.9999	-5.885	4.316	24.742	0.9876	-12.096	61.579
13 vs. 17	ECU vs. FDS-5	4.932	0.0823	-0.168	10.032	16.337	>0.9999	-20.501	53.174
13 vs. 18	ECU vs. FDP-2	0.855	>0.9999	-4.246	5.955	36.461	0.0585	-0.377	73.299
13 vs. 19	ECU vs. FDP-3	-1.041	>0.9999	-6.142	4.059	38.157	0.0284	1.319	74.995
13 vs. 20	ECU vs. FDP-4	-0.416	>0.9999	-5.516	4.684	32.773	0.2370	-4.065	69.611
13 vs. 21	ECU vs. FDP-5	1.754	>0.9999	-3.346	6.855	28.939	0.6705	-7.899	65.777
13 vs. 22	ECU vs. EIP	1.197	>0.9999	-3.904	6.297	1.358	>0.9999	-35.480	38.195
13 vs. 23	ECU vs. EDC-2	2.765	>0.9999	-2.335	7.866	14.021	>0.9999	-22.816	50.859
13 vs. 24	ECU vs. EDC-3	-3.624	0.9391	-8.725	1.476	4.527	>0.9999	-32.310	41.365
13 vs. 25	ECU vs. EDC-4	-1.898	>0.9999	-6.999	3.202	-2.215	>0.9999	-42.055	37.626
13 vs. 26	ECU vs. EDC-5	1.473	>0.9999	-4.044	6.990	4.501	>0.9999	-37.666	46.667
13 vs. 27	ECU vs. EDM	2.228	>0.9999	-2.872	7.329	14.072	>0.9999	-22.765	50.910
13 vs. 28	ECU vs. FPL	2.833	>0.9999	-2.267	7.933	26.431	0.9241	-10.406	63.269
13 vs. 29	ECU vs. APL	-1.397	>0.9999	-6.497	3.703	23.995	0.9960	-12.843	60.833
13 vs. 30	ECU vs. EPB	3.857	0.8000	-1.243	8.957	15.633	>0.9999	-21.204	52.471
13 vs. 31	ECU vs. EPL	2.953	>0.9999	-2.147	8.054	3.712	>0.9999	-33.125	40.550
14 vs. 15	FDS-2 vs. FDS-3	-0.530	>0.9999	-5.630	4.571	5.893	>0.9999	-30.945	42.731
14 vs. 16	FDS-2 vs. FDS-4	-0.783	>0.9999	-5.884	4.317	-4.384	>0.9999	-41.222	32.454
14 vs. 17	FDS-2 vs. FDS-5	4.933	0.0820	-0.167	10.034	-12.789	>0.9999	-49.627	24.049
14 vs. 18	FDS-2 vs. FDP-2	0.856	>0.9999	-4.244	5.956	7.335	>0.9999	-29.502	44.173
14 vs. 19	FDS-2 vs. FDP-3	-1.040	>0.9999	-6.140	4.061	9.031	>0.9999	-27.806	45.869
14 vs. 20	FDS-2 vs. FDP-4	-0.414	>0.9999	-5.515	4.686	3.647	>0.9999	-33.190	40.485
14 vs. 21	FDS-2 vs. FDP-5	1.756	>0.9999	-3.345	6.856	-0.187	>0.9999	-37.024	36.651
14 vs. 22	FDS-2 vs. EIP	1.198	>0.9999	-3.902	6.299	-27.768	0.8095	-64.606	9.070
14 vs. 23	FDS-2 vs. EDC-2	2.767	>0.9999	-2.334	7.867	-15.104	>0.9999	-51.942	21.733
14 vs. 24	FDS-2 vs. EDC-3	-3.623	0.9397	-8.723	1.478	-24.599	0.9898	-61.436	12.239
14 vs. 25	FDS-2 vs. EDC-4	-1.897	>0.9999	-6.997	3.204	-31.340	0.6655	-71.181	8.500
14 vs. 26	FDS-2 vs. EDC-5	1.474	>0.9999	-4.043	6.992	-24.625	>0.9999	-66.791	17.541
14 vs. 27	FDS-2 vs. EDM	2.230	>0.9999	-2.871	7.330	-15.053	>0.9999	-51.891	21.784
14 vs. 28	FDS-2 vs. FPL	2.835	>0.9999	-2.266	7.935	-2.694	>0.9999	-39.532	34.143
14 vs. 29	FDS-2 vs. APL	-1.395	>0.9999	-6.496	3.705	-5.131	>0.9999	-41.968	31.707
14 vs. 30	FDS-2 vs. EPB	3.858	0.7988	-1.242	8.959	-13.492	>0.9999	-50.330	23.345
14 vs. 31	FDS-2 vs. EPL	2.955	>0.9999	-2.145	8.055	-25.413	0.9717	-62.251	11.424
15 vs. 16	FDS-3 vs. FDS-4	-0.254	>0.9999	-5.354	4.847	-10.277	>0.9999	-47.114	26.561
15 vs. 17	FDS-3 vs. FDS-5	5.463	0.0159	0.363	10.564	-18.682	>0.9999	-55.520	18.156
15 vs. 18	FDS-3 vs. FDP-2	1.386	>0.9999	-3.715	6.486	1.442	>0.9999	-35.395	38.280
15 vs. 19	FDS-3 vs. FDP-3	-0.510	>0.9999	-5.610	4.590	3.138	>0.9999	-33.699	39.976
15 vs. 20	FDS-3 vs. FDP-4	0.115	>0.9999	-4.985	5.216	-2.245	>0.9999	-39.083	34.592
15 vs. 21	FDS-3 vs. FDP-5	2.286	>0.9999	-2.815	7.386	-6.080	>0.9999	-42.917	30.758
15 vs. 22	FDS-3 vs. EIP	1.728	>0.9999	-3.372	6.828	-33.661	0.1738	-70.499	3.177
15 vs. 23	FDS-3 vs. EDC-2	3.296	0.9970	-1.804	8.397	-20.997	>0.9999	-57.835	15.840
15 vs. 24	FDS-3 vs. EDC-3	-3.093	0.9999	-8.193	2.007	-30.491	0.4717	-67.329	6.346
15 vs. 25	FDS-3 vs. EDC-4	-1.367	>0.9999	-6.467	3.733	-37.233	0.1310	-77.074	2.607
15 vs. 26	FDS-3 vs. EDC-5	2.004	>0.9999	-3.513	7.522	-30.518	0.9087	-72.684	11.648
15 vs. 27	FDS-3 vs. EDM	2.760	>0.9999	-2.341	7.860	-20.946	>0.9999	-57.784	15.892
15 vs. 28	FDS-3 vs. FPL	3.364	0.9934	-1.736	8.465	-8.587	>0.9999	-45.425	28.250
15 vs. 29	FDS-3 vs. APL	-0.866	>0.9999	-5.966	4.235	-11.024	>0.9999	-47.861	25.814

Tendon Comparison		Interfascicular Region (IFR) Frequency				Interfascicular Region (IFR) Type Proportion			
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval	
15 vs. 30	FDS-3 vs. EPB	4.388	0.3355	-0.712	9.489	-19.385	>0.9999	-56.223	17.452
15 vs. 31	FDS-3 vs. EPL	3.485	0.9787	-1.616	8.585	-31.306	0.3766	-68.144	5.531
16 vs. 17	FDS-4 vs. FDS-5	5.717	0.0068	0.616	10.817	-8.405	>0.9999	-45.243	28.433
16 vs. 18	FDS-4 vs. FDP-2	1.639	>0.9999	-3.461	6.740	11.719	>0.9999	-25.119	48.557
16 vs. 19	FDS-4 vs. FDP-3	-0.256	>0.9999	-5.357	4.844	13.415	>0.9999	-23.422	50.253
16 vs. 20	FDS-4 vs. FDP-4	0.369	>0.9999	-4.732	5.469	8.031	>0.9999	-28.806	44.869
16 vs. 21	FDS-4 vs. FDP-5	2.539	>0.9999	-2.561	7.639	4.197	>0.9999	-32.641	41.035
16 vs. 22	FDS-4 vs. EIP	1.982	>0.9999	-3.119	7.082	-23.384	0.9986	-60.222	13.453
16 vs. 23	FDS-4 vs. EDC-2	3.550	0.9639	-1.550	8.650	-10.720	>0.9999	-47.558	26.117
16 vs. 24	FDS-4 vs. EDC-3	-2.840	>0.9999	-7.940	2.261	-20.215	>0.9999	-57.052	16.623
16 vs. 25	FDS-4 vs. EDC-4	-1.114	>0.9999	-6.214	3.987	-26.956	0.9843	-66.797	12.884
16 vs. 26	FDS-4 vs. EDC-5	2.258	>0.9999	-3.260	7.775	-20.241	>0.9999	-62.407	21.925
16 vs. 27	FDS-4 vs. EDM	3.013	>0.9999	-2.087	8.113	-10.669	>0.9999	-47.507	26.168
16 vs. 28	FDS-4 vs. FPL	3.618	0.9416	-1.482	8.718	1.690	>0.9999	-35.148	38.527
16 vs. 29	FDS-4 vs. APL	-0.612	>0.9999	-5.712	4.488	-0.747	>0.9999	-37.585	36.091
16 vs. 30	FDS-4 vs. EPB	4.642	0.1825	-0.459	9.742	-9.108	>0.9999	-45.946	27.729
16 vs. 31	FDS-4 vs. EPL	3.738	0.8826	-1.362	8.839	-21.029	>0.9999	-57.867	15.808
17 vs. 18	FDS-5 vs. FDP-2	-4.077	0.6046	-9.178	1.023	20.124	>0.9999	-16.713	56.962
17 vs. 19	FDS-5 vs. FDP-3	-5.973	0.0028	-11.073	-0.873	21.820	>0.9999	-15.017	58.658
17 vs. 20	FDS-5 vs. FDP-4	-5.348	0.0231	-10.448	-0.248	16.436	>0.9999	-20.401	53.274
17 vs. 21	FDS-5 vs. FDP-5	-3.178	0.9994	-8.278	1.923	12.602	>0.9999	-24.235	49.440
17 vs. 22	FDS-5 vs. EIP	-3.735	0.8845	-8.835	1.365	-14.979	>0.9999	-51.817	21.859
17 vs. 23	FDS-5 vs. EDC-2	-2.167	>0.9999	-7.267	2.934	-2.315	>0.9999	-39.153	34.522
17 vs. 24	FDS-5 vs. EDC-3	-8.556	<0.0001	-13.657	-3.456	-11.809	>0.9999	-48.647	25.028
17 vs. 25	FDS-5 vs. EDC-4	-6.830	0.0001	-11.931	-1.730	-18.551	>0.9999	-58.392	21.289
17 vs. 26	FDS-5 vs. EDC-5	-3.459	0.9992	-8.976	2.058	-11.836	>0.9999	-54.002	30.330
17 vs. 27	FDS-5 vs. EDM	-2.704	>0.9999	-7.804	2.397	-2.264	>0.9999	-39.102	34.574
17 vs. 28	FDS-5 vs. FPL	-2.099	>0.9999	-7.199	3.002	10.095	>0.9999	-26.743	46.932
17 vs. 29	FDS-5 vs. APL	-6.329	0.0007	-11.429	-1.228	7.658	>0.9999	-29.179	44.496
17 vs. 30	FDS-5 vs. EPB	-1.075	>0.9999	-6.175	4.025	-0.703	>0.9999	-37.541	36.134
17 vs. 31	FDS-5 vs. EPL	-1.979	>0.9999	-7.079	3.122	-12.624	>0.9999	-49.462	24.213
18 vs. 19	FDP-2 vs. FDP-3	-1.896	>0.9999	-6.996	3.205	1.696	>0.9999	-35.142	38.534
18 vs. 20	FDP-2 vs. FDP-4	-1.271	>0.9999	-6.371	3.830	-3.688	>0.9999	-40.525	33.150
18 vs. 21	FDP-2 vs. FDP-5	0.900	>0.9999	-4.201	6.000	-7.522	>0.9999	-44.360	29.316
18 vs. 22	FDP-2 vs. EIP	0.342	>0.9999	-4.758	5.443	-35.103	0.1010	-71.941	1.734
18 vs. 23	FDP-2 vs. EDC-2	1.911	>0.9999	-3.190	7.011	-22.440	0.9998	-59.277	14.398
18 vs. 24	FDP-2 vs. EDC-3	-4.479	0.2728	-9.579	0.621	-31.934	0.3115	-68.771	4.904
18 vs. 25	FDP-2 vs. EDC-4	-2.753	>0.9999	-7.853	2.347	-38.675	0.0778	-78.516	1.165
18 vs. 26	FDP-2 vs. EDC-5	0.618	>0.9999	-4.899	6.136	-31.960	0.7929	-74.127	10.206
18 vs. 27	FDP-2 vs. EDM	1.374	>0.9999	-3.727	6.474	-22.388	0.9999	-59.226	14.449
18 vs. 28	FDP-2 vs. FPL	1.979	>0.9999	-3.122	7.079	-10.030	>0.9999	-46.867	26.808
18 vs. 29	FDP-2 vs. APL	-2.251	>0.9999	-7.352	2.849	-12.466	>0.9999	-49.304	24.372
18 vs. 30	FDP-2 vs. EPB	3.002	>0.9999	-2.098	8.103	-20.827	>0.9999	-57.665	16.010
18 vs. 31	FDP-2 vs. EPL	2.099	>0.9999	-3.002	7.199	-32.749	0.2390	-69.586	4.089
19 vs. 20	FDP-3 vs. FDP-4	0.625	>0.9999	-4.475	5.726	-5.384	>0.9999	-42.222	31.454
19 vs. 21	FDP-3 vs. FDP-5	2.795	>0.9999	-2.305	7.896	-9.218	>0.9999	-46.056	27.620
19 vs. 22	FDP-3 vs. EIP	2.238	>0.9999	-2.862	7.338	-36.799	0.0508	-73.637	0.038
19 vs. 23	FDP-3 vs. EDC-2	3.806	0.8379	-1.294	8.907	-24.136	0.9949	-60.973	12.702
19 vs. 24	FDP-3 vs. EDC-3	-2.583	>0.9999	-7.684	2.517	-33.630	0.1757	-70.467	3.208
19 vs. 25	FDP-3 vs. EDC-4	-0.857	>0.9999	-5.958	4.243	-40.372	0.0406	-80.212	-0.531
19 vs. 26	FDP-3 vs. EDC-5	2.514	>0.9999	-3.003	8.031	-33.656	0.6106	-75.823	8.510
19 vs. 27	FDP-3 vs. EDM	3.269	0.9979	-1.831	8.370	-24.085	0.9953	-60.922	12.753
19 vs. 28	FDP-3 vs. FPL	3.874	0.7861	-1.226	8.975	-11.726	>0.9999	-48.563	25.112
19 vs. 29	FDP-3 vs. APL	-0.356	>0.9999	-5.456	4.745	-14.162	>0.9999	-51.000	22.675
19 vs. 30	FDP-3 vs. EPB	4.898	0.0907	-0.202	9.998	-22.524	0.9998	-59.361	14.314
19 vs. 31	FDP-3 vs. EPL	3.995	0.6817	-1.106	9.095	-34.445	0.1301	-71.282	2.393
20 vs. 21	FDP-4 vs. FDP-5	2.170	>0.9999	-2.930	7.271	-3.834	>0.9999	-40.672	33.004
20 vs. 22	FDP-4 vs. EIP	1.613	>0.9999	-3.487	6.713	-31.416	0.3647	-68.253	5.422
20 vs. 23	FDP-4 vs. EDC-2	3.181	0.9994	-1.919	8.282	-18.752	>0.9999	-55.589	18.086
20 vs. 24	FDP-4 vs. EDC-3	-3.208	0.9991	-8.309	1.892	-28.246	0.7559	-65.084	8.592
20 vs. 25	FDP-4 vs. EDC-4	-1.482	>0.9999	-6.583	3.618	-34.988	0.2727	-74.828	4.853
20 vs. 26	FDP-4 vs. EDC-5	1.889	>0.9999	-3.628	7.406	-28.272	0.9883	-70.439	13.894
20 vs. 27	FDP-4 vs. EDM	2.644	>0.9999	-2.456	7.745	-18.701	>0.9999	-55.538	18.137
20 vs. 28	FDP-4 vs. FPL	3.249	0.9984	-1.851	8.350	-6.342	>0.9999	-43.179	30.496
20 vs. 29	FDP-4 vs. APL	-0.981	>0.9999	-6.081	4.119	-8.778	>0.9999	-45.616	28.059
20 vs. 30	FDP-4 vs. EPB	4.273	0.4272	-0.827	9.373	-17.140	>0.9999	-53.977	19.698
20 vs. 31	FDP-4 vs. EPL	3.369	0.9931	-1.731	8.470	-29.061	0.6549	-65.898	7.777
21 vs. 22	FDP-5 vs. EIP	-0.557	>0.9999	-5.658	4.543	-27.581	0.8289	-64.419	9.256
21 vs. 23	FDP-5 vs. EDC-2	1.011	>0.9999	-4.089	6.111	-14.918	>0.9999	-51.755	21.920
21 vs. 24	FDP-5 vs. EDC-3	-5.379	0.0209	-10.479	-0.278	-24.412	0.9922	-61.249	12.426
21 vs. 25	FDP-5 vs. EDC-4	-3.653	0.9272	-8.753	1.448	-31.153	0.6874	-70.994	8.687
21 vs. 26	FDP-5 vs. EDC-5	-0.281	>0.9999	-5.799	5.236	-24.438	>0.9999	-66.605	17.728
21 vs. 27	FDP-5 vs. EDM	0.474	>0.9999	-4.626	5.574	-14.866	>0.9999	-51.704	21.971
21 vs. 28	FDP-5 vs. FPL	1.079	>0.9999	-4.022	6.179	-2.508	>0.9999	-39.345	34.330
21 vs. 29	FDP-5 vs. APL	-3.151	0.9996	-8.252	1.949	-4.944	>0.9999	-41.782	31.894
21 vs. 30	FDP-5 vs. EPB	2.103	>0.9999	-2.998	7.203	-13.306	>0.9999	-50.143	23.532
21 vs. 31	FDP-5 vs. EPL	1.199	>0.9999	-3.901	6.299	-25.227	0.9772	-62.064	11.611
22 vs. 23	EIP vs. EDC-2	1.568	>0.9999	-3.532	6.669	12.664	>0.9999	-24.174	49.501
22 vs. 24	EIP vs. EDC-3	-4.821	0.1127	-9.922	0.279	3.170	>0.9999	-33.668	40.007
22 vs. 25	EIP vs. EDC-4	-3.095	0.9999	-8.196	2.005	-3.572	>0.9999	-43.413	36.268
22 vs. 26	EIP vs. EDC-5	0.276	>0.9999	-5.241	5.793	3.143	>0.9999	-39.023	45.309
22 vs. 27	EIP vs. EDM	1.031	>0.9999	-4.069	6.132	12.715	>0.9999	-24.123	49.553
22 vs. 28	EIP vs. FPL	1.636	>0.9999	-3.464	6.737	25.074	0.9810	-11.764	61.911
22 vs. 29	EIP vs. APL	-2.594	>0.9999	-7.694	2.507	22.637	0.9997	-14.200	59.475
22 vs. 30	EIP vs. EPB	2.660	>0.9999	-2.440	7.760	14.276	>0.9999	-22.562	51.113
22 vs. 31	EIP vs. EPL	1.756	>0.9999	-3.344	6.857	2.355	>0.9999	-34.483	39.192
23 vs. 24	EDC-2 vs. EDC-3	-6.390	0.0006	-11.490	-1.289	-9.494	>0.9999	-46.332	27.344

Tendon Comparison		Interfascicular Region (IFR) Frequency				Interfascicular Region (IFR) Type Proportion			
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval	
23 vs. 25	EDC-2 vs. EDC-4	-4.664	0.1724	-9.764	0.437	-16.236	>0.9999	-56.076	23.605
23 vs. 26	EDC-2 vs. EDC-5	-1.292	>0.9999	-6.810	4.225	-9.521	>0.9999	-51.687	32.646
23 vs. 27	EDC-2 vs. EDM	-0.537	>0.9999	-5.637	4.563	0.051	>0.9999	-36.787	36.889
23 vs. 28	EDC-2 vs. FPL	0.068	>0.9999	-5.032	5.168	12.410	>0.9999	-24.428	49.248
23 vs. 29	EDC-2 vs. APL	-4.162	0.5257	-9.262	0.938	9.974	>0.9999	-26.864	46.811
23 vs. 30	EDC-2 vs. EPB	1.092	>0.9999	-4.009	6.192	1.612	>0.9999	-35.226	38.450
23 vs. 31	EDC-2 vs. EPL	0.188	>0.9999	-4.912	5.289	-10.309	>0.9999	-47.147	26.529
24 vs. 25	EDC-3 vs. EDC-4	1.726	>0.9999	-3.374	6.826	-6.742	>0.9999	-46.582	33.099
24 vs. 26	EDC-3 vs. EDC-5	5.097	0.1517	-0.420	10.615	-0.027	>0.9999	-42.193	42.140
24 vs. 27	EDC-3 vs. EDM	5.853	0.0042	0.752	10.953	9.545	>0.9999	-27.292	46.383
24 vs. 28	EDC-3 vs. FPL	6.458	0.0005	1.357	11.558	21.904	>0.9999	-14.934	58.742
24 vs. 29	EDC-3 vs. APL	2.228	>0.9999	-2.873	7.328	19.468	>0.9999	-17.370	56.305
24 vs. 30	EDC-3 vs. EPB	7.481	<0.0001	2.381	12.582	11.106	>0.9999	-25.731	47.944
24 vs. 31	EDC-3 vs. EPL	6.578	0.0003	1.477	11.678	-0.815	>0.9999	-37.653	36.023
25 vs. 26	EDC-4 vs. EDC-5	3.371	0.9998	-2.146	8.889	6.715	>0.9999	-38.178	51.608
25 vs. 27	EDC-4 vs. EDM	4.127	0.5585	-0.974	9.227	16.287	>0.9999	-23.553	56.127
25 vs. 28	EDC-4 vs. FPL	4.732	0.1440	-0.369	9.832	28.646	0.9206	-11.195	68.486
25 vs. 29	EDC-4 vs. APL	0.502	>0.9999	-4.599	5.602	26.209	0.9941	-13.631	66.050
25 vs. 30	EDC-4 vs. EPB	5.755	0.0059	0.655	10.856	17.848	>0.9999	-21.993	57.688
25 vs. 31	EDC-4 vs. EPL	4.852	0.1035	-0.249	9.952	5.927	>0.9999	-33.914	45.767
26 vs. 27	EDC-5 vs. EDM	0.755	>0.9999	-4.762	6.273	9.572	>0.9999	-32.594	51.738
26 vs. 28	EDC-5 vs. FPL	1.360	>0.9999	-4.157	6.878	21.931	>0.9999	-20.236	64.097
26 vs. 29	EDC-5 vs. APL	-2.870	>0.9999	-8.387	2.648	19.494	>0.9999	-22.672	61.661
26 vs. 30	EDC-5 vs. EPB	2.384	>0.9999	-3.133	7.901	11.133	>0.9999	-31.034	53.299
26 vs. 31	EDC-5 vs. EPL	1.480	>0.9999	-4.037	6.998	-0.788	>0.9999	-42.955	41.378
27 vs. 28	EDM vs. FPL	0.605	>0.9999	-4.496	5.705	12.359	>0.9999	-24.479	49.196
27 vs. 29	EDM vs. APL	-3.625	0.9388	-8.726	1.475	9.922	>0.9999	-26.915	46.760
27 vs. 30	EDM vs. EPB	1.629	>0.9999	-3.472	6.729	1.561	>0.9999	-35.277	38.399
27 vs. 31	EDM vs. EPL	0.725	>0.9999	-4.375	5.825	-10.360	>0.9999	-47.198	26.478
28 vs. 29	FPL vs. APL	-4.230	0.4644	-9.330	0.870	-2.436	>0.9999	-39.274	34.401
28 vs. 30	FPL vs. EPB	1.024	>0.9999	-4.077	6.124	-10.798	>0.9999	-47.636	26.040
28 vs. 31	FPL vs. EPL	0.120	>0.9999	-4.980	5.221	-22.719	0.9997	-59.557	14.119
29 vs. 30	APL vs. EPB	5.254	0.0311	0.153	10.354	-8.361	>0.9999	-45.199	28.476
29 vs. 31	APL vs. EPL	4.350	0.3643	-0.750	9.451	-20.283	>0.9999	-57.120	16.555
30 vs. 31	EPB vs. EPL	-0.904	>0.9999	-6.004	4.197	-11.921	>0.9999	-48.759	24.917

Supp. Table 9.6.e Pairwise comparisons for upper limb tendon crimp characteristics. *Green cells indicate significant p-values (< 0.05).*

Tendon Comparison		Crimp Absence		Crimp Wavelength		Crimp Wavelength Variation	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
1 vs. 2	SSP vs. IS	12.13	>0.9999	-22.44	>0.9999	42.88	>0.9999
1 vs. 3	SSP vs. SSC	8.38	>0.9999	-42.88	>0.9999	21.75	>0.9999
1 vs. 4	SSP vs. LHBT	77.13	>0.9999	-35.44	>0.9999	46.94	>0.9999
1 vs. 5	SSP vs. DBT	132.10	0.0004	-162.80	0.0010	119.80	0.1650
1 vs. 6	SSP vs. BrR	145.90	<0.0001	-140.40	0.0210	96.50	>0.9999
1 vs. 7	SSP vs. DTT	104.60	0.0425	-127.40	0.0988	91.31	>0.9999
1 vs. 8	SSP vs. FCR	127.80	0.0008	-140.40	0.0210	119.80	0.1650
1 vs. 9	SSP vs. PL	145.90	<0.0001	-128.50	0.0874	152.40	0.0026
1 vs. 10	SSP vs. FCU	145.90	<0.0001	-168.70	0.0004	81.81	>0.9999
1 vs. 11	SSP vs. ECRL	145.90	<0.0001	-179.50	<0.0001	99.75	>0.9999
1 vs. 12	SSP vs. ECRB	145.90	<0.0001	-136.40	0.0341	68.06	>0.9999
1 vs. 13	SSP vs. ECU	145.90	<0.0001	-57.75	>0.9999	81.13	>0.9999
1 vs. 14	SSP vs. FDS-2	118.40	0.0044	-165.20	0.0007	126.80	0.0728
1 vs. 15	SSP vs. FDS-3	132.10	0.0004	-215.10	<0.0001	175.60	<0.0001
1 vs. 16	SSP vs. FDS-4	145.90	<0.0001	-206.80	<0.0001	119.80	0.1650
1 vs. 17	SSP vs. FDS-5	145.90	<0.0001	-117.60	0.2947	112.80	0.3587
1 vs. 18	SSP vs. FDP-2	77.13	>0.9999	-125.00	0.1303	96.50	>0.9999
1 vs. 19	SSP vs. FDP-3	90.88	0.3153	-146.30	0.0098	88.81	>0.9999
1 vs. 20	SSP vs. FDP-4	57.38	>0.9999	-127.00	0.1038	112.10	0.3863
1 vs. 21	SSP vs. FDP-5	118.40	0.0044	-102.20	>0.9999	108.10	0.5860
1 vs. 22	SSP vs. EIP	145.90	<0.0001	-77.88	>0.9999	131.40	0.0415
1 vs. 23	SSP vs. EDC-2	145.90	<0.0001	-75.88	>0.9999	93.44	>0.9999
1 vs. 24	SSP vs. EDC-3	145.90	<0.0001	-162.80	0.0010	96.50	>0.9999
1 vs. 25	SSP vs. EDC-4	132.10	0.0004	-129.00	0.0825	64.31	>0.9999
1 vs. 26	SSP vs. EDC-5	127.50	0.0047	-97.00	>0.9999	101.10	>0.9999
1 vs. 27	SSP vs. EDM	145.90	<0.0001	-84.06	>0.9999	88.81	>0.9999
1 vs. 28	SSP vs. FPL	104.60	0.0425	-135.40	0.0388	96.50	>0.9999
1 vs. 29	SSP vs. APL	145.90	<0.0001	-146.30	0.0098	105.10	0.8030
1 vs. 30	SSP vs. EPB	145.90	<0.0001	-80.56	>0.9999	64.31	>0.9999
1 vs. 31	SSP vs. EPL	132.10	0.0004	-47.06	>0.9999	100.40	>0.9999
2 vs. 3	IS vs. SSC	-3.75	>0.9999	-20.44	>0.9999	-21.13	>0.9999
2 vs. 4	IS vs. LHBT	65.00	>0.9999	-13.00	>0.9999	4.06	>0.9999
2 vs. 5	IS vs. DBT	120.00	0.0034	-140.30	0.0211	76.88	>0.9999
2 vs. 6	IS vs. BrR	133.80	0.0003	-117.90	0.2831	53.63	>0.9999
2 vs. 7	IS vs. DTT	92.50	0.2520	-105.00	>0.9999	48.44	>0.9999
2 vs. 8	IS vs. FCR	115.60	0.0071	-117.90	0.2831	76.88	>0.9999
2 vs. 9	IS vs. PL	133.80	0.0003	-106.10	0.9544	109.50	0.5074
2 vs. 10	IS vs. FCU	133.80	0.0003	-146.30	0.0099	38.94	>0.9999
2 vs. 11	IS vs. ECRL	133.80	0.0003	-157.10	0.0023	56.88	>0.9999
2 vs. 12	IS vs. ECRB	133.80	0.0003	-114.00	0.4288	25.19	>0.9999
2 vs. 13	IS vs. ECU	133.80	0.0003	-35.31	>0.9999	38.25	>0.9999
2 vs. 14	IS vs. FDS-2	106.30	0.0330	-142.80	0.0155	83.88	>0.9999
2 vs. 15	IS vs. FDS-3	120.00	0.0034	-192.70	<0.0001	132.80	0.0349
2 vs. 16	IS vs. FDS-4	133.80	0.0003	-184.30	<0.0001	76.88	>0.9999
2 vs. 17	IS vs. FDS-5	133.80	0.0003	-95.13	>0.9999	69.88	>0.9999
2 vs. 18	IS vs. FDP-2	65.00	>0.9999	-102.60	>0.9999	53.63	>0.9999
2 vs. 19	IS vs. FDP-3	78.75	>0.9999	-123.90	0.1478	45.94	>0.9999
2 vs. 20	IS vs. FDP-4	45.25	>0.9999	-104.60	>0.9999	69.19	>0.9999
2 vs. 21	IS vs. FDP-5	106.30	0.0330	-79.75	>0.9999	65.25	>0.9999
2 vs. 22	IS vs. EIP	133.80	0.0003	-55.44	>0.9999	88.50	>0.9999
2 vs. 23	IS vs. EDC-2	133.80	0.0003	-53.44	>0.9999	50.56	>0.9999
2 vs. 24	IS vs. EDC-3	133.80	0.0003	-140.30	0.0211	53.63	>0.9999
2 vs. 25	IS vs. EDC-4	120.00	0.0034	-106.60	0.9088	21.44	>0.9999
2 vs. 26	IS vs. EDC-5	115.40	0.0300	-74.56	>0.9999	58.25	>0.9999
2 vs. 27	IS vs. EDM	133.80	0.0003	-61.63	>0.9999	45.94	>0.9999
2 vs. 28	IS vs. FPL	92.50	0.2520	-112.90	0.4787	53.63	>0.9999
2 vs. 29	IS vs. APL	133.80	0.0003	-123.90	0.1478	62.19	>0.9999
2 vs. 30	IS vs. EPB	133.80	0.0003	-58.13	>0.9999	21.44	>0.9999
2 vs. 31	IS vs. EPL	120.00	0.0034	-24.63	>0.9999	57.56	>0.9999
3 vs. 4	SSC vs. LHBT	68.75	>0.9999	7.44	>0.9999	25.19	>0.9999
3 vs. 5	SSC vs. DBT	123.80	0.0017	-119.90	0.2297	98.00	>0.9999
3 vs. 6	SSC vs. BrR	137.50	0.0001	-97.50	>0.9999	74.75	>0.9999
3 vs. 7	SSC vs. DTT	96.25	0.1484	-84.56	>0.9999	69.56	>0.9999
3 vs. 8	SSC vs. FCR	119.40	0.0037	-97.50	>0.9999	98.00	>0.9999
3 vs. 9	SSC vs. PL	137.50	0.0001	-85.63	>0.9999	130.60	0.0455
3 vs. 10	SSC vs. FCU	137.50	0.0001	-125.80	0.1189	60.06	>0.9999
3 vs. 11	SSC vs. ECRL	137.50	0.0001	-136.60	0.0333	78.00	>0.9999
3 vs. 12	SSC vs. ECRB	137.50	0.0001	-93.56	>0.9999	46.31	>0.9999
3 vs. 13	SSC vs. ECU	137.50	0.0001	-14.88	>0.9999	59.38	>0.9999
3 vs. 14	SSC vs. FDS-2	110.00	0.0181	-122.30	0.1759	105.00	0.8082
3 vs. 15	SSC vs. FDS-3	123.80	0.0017	-172.30	0.0003	153.90	0.0021
3 vs. 16	SSC vs. FDS-4	137.50	0.0001	-163.90	0.0009	98.00	>0.9999
3 vs. 17	SSC vs. FDS-5	137.50	0.0001	-74.69	>0.9999	91.00	>0.9999
3 vs. 18	SSC vs. FDP-2	68.75	>0.9999	-82.13	>0.9999	74.75	>0.9999
3 vs. 19	SSC vs. FDP-3	82.50	0.9461	-103.40	>0.9999	67.06	>0.9999
3 vs. 20	SSC vs. FDP-4	49.00	>0.9999	-84.13	>0.9999	90.31	>0.9999
3 vs. 21	SSC vs. FDP-5	110.00	0.0181	-59.31	>0.9999	86.38	>0.9999
3 vs. 22	SSC vs. EIP	137.50	0.0001	-35.00	>0.9999	109.60	0.5007
3 vs. 23	SSC vs. EDC-2	137.50	0.0001	-33.00	>0.9999	71.69	>0.9999
3 vs. 24	SSC vs. EDC-3	137.50	0.0001	-119.90	0.2297	74.75	>0.9999
3 vs. 25	SSC vs. EDC-4	123.80	0.0017	-86.13	>0.9999	42.56	>0.9999
3 vs. 26	SSC vs. EDC-5	119.20	0.0172	-54.13	>0.9999	79.38	>0.9999

3 vs. 27	SSC vs. EDM	137.50	0.0001	-41.19	>0.9999	67.06	>0.9999
3 vs. 28	SSC vs. FPL	96.25	0.1484	-92.50	>0.9999	74.75	>0.9999
3 vs. 29	SSC vs. APL	137.50	0.0001	-103.40	>0.9999	83.31	>0.9999
3 vs. 30	SSC vs. EPB	137.50	0.0001	-37.69	>0.9999	42.56	>0.9999
3 vs. 31	SSC vs. EPL	123.80	0.0017	-4.19	>0.9999	78.69	>0.9999
4 vs. 5	LHBT vs. DBT	55.00	>0.9999	-127.30	0.1002	72.81	>0.9999
4 vs. 6	LHBT vs. BrR	68.75	>0.9999	-104.90	>0.9999	49.56	>0.9999
4 vs. 7	LHBT vs. DTT	27.50	>0.9999	-92.00	>0.9999	44.38	>0.9999
4 vs. 8	LHBT vs. FCR	50.63	>0.9999	-104.90	>0.9999	72.81	>0.9999
4 vs. 9	LHBT vs. PL	68.75	>0.9999	-93.06	>0.9999	105.40	0.7730
4 vs. 10	LHBT vs. FCU	68.75	>0.9999	-133.30	0.0500	34.88	>0.9999
4 vs. 11	LHBT vs. ECRL	68.75	>0.9999	-144.10	0.0131	52.81	>0.9999
4 vs. 12	LHBT vs. ECRB	68.75	>0.9999	-101.00	>0.9999	21.13	>0.9999
4 vs. 13	LHBT vs. ECU	68.75	>0.9999	-22.31	>0.9999	34.19	>0.9999
4 vs. 14	LHBT vs. FDS-2	41.25	>0.9999	-129.80	0.0756	79.81	>0.9999
4 vs. 15	LHBT vs. FDS-3	55.00	>0.9999	-179.70	<0.0001	128.70	0.0576
4 vs. 16	LHBT vs. FDS-4	68.75	>0.9999	-171.30	0.0003	72.81	>0.9999
4 vs. 17	LHBT vs. FDS-5	68.75	>0.9999	-82.13	>0.9999	65.81	>0.9999
4 vs. 18	LHBT vs. FDP-2	0.00	>0.9999	-89.56	>0.9999	49.56	>0.9999
4 vs. 19	LHBT vs. FDP-3	13.75	>0.9999	-110.90	0.5911	41.88	>0.9999
4 vs. 20	LHBT vs. FDP-4	-19.75	>0.9999	-91.56	>0.9999	65.13	>0.9999
4 vs. 21	LHBT vs. FDP-5	41.25	>0.9999	-66.75	>0.9999	61.19	>0.9999
4 vs. 22	LHBT vs. EIP	68.75	>0.9999	-42.44	>0.9999	84.44	>0.9999
4 vs. 23	LHBT vs. EDC-2	68.75	>0.9999	-40.44	>0.9999	46.50	>0.9999
4 vs. 24	LHBT vs. EDC-3	68.75	>0.9999	-127.30	0.1002	49.56	>0.9999
4 vs. 25	LHBT vs. EDC-4	55.00	>0.9999	-93.56	>0.9999	17.38	>0.9999
4 vs. 26	LHBT vs. EDC-5	50.42	>0.9999	-61.56	>0.9999	54.19	>0.9999
4 vs. 27	LHBT vs. EDM	68.75	>0.9999	-48.63	>0.9999	41.88	>0.9999
4 vs. 28	LHBT vs. FPL	27.50	>0.9999	-99.94	>0.9999	49.56	>0.9999
4 vs. 29	LHBT vs. APL	68.75	>0.9999	-110.90	0.5911	58.13	>0.9999
4 vs. 30	LHBT vs. EPB	68.75	>0.9999	-45.13	>0.9999	17.38	>0.9999
4 vs. 31	LHBT vs. EPL	55.00	>0.9999	-11.63	>0.9999	53.50	>0.9999
5 vs. 6	DBT vs. BrR	13.75	>0.9999	22.38	>0.9999	-23.25	>0.9999
5 vs. 7	DBT vs. DTT	-27.50	>0.9999	35.31	>0.9999	-28.44	>0.9999
5 vs. 8	DBT vs. FCR	-4.38	>0.9999	22.38	>0.9999	0.00	>0.9999
5 vs. 9	DBT vs. PL	13.75	>0.9999	34.25	>0.9999	32.63	>0.9999
5 vs. 10	DBT vs. FCU	13.75	>0.9999	-5.94	>0.9999	-37.94	>0.9999
5 vs. 11	DBT vs. ECRL	13.75	>0.9999	-16.75	>0.9999	-20.00	>0.9999
5 vs. 12	DBT vs. ECRB	13.75	>0.9999	26.31	>0.9999	-51.69	>0.9999
5 vs. 13	DBT vs. ECU	13.75	>0.9999	105.00	>0.9999	-38.63	>0.9999
5 vs. 14	DBT vs. FDS-2	-13.75	>0.9999	-2.44	>0.9999	7.00	>0.9999
5 vs. 15	DBT vs. FDS-3	0.00	>0.9999	-52.38	>0.9999	55.88	>0.9999
5 vs. 16	DBT vs. FDS-4	13.75	>0.9999	-44.00	>0.9999	0.00	>0.9999
5 vs. 17	DBT vs. FDS-5	13.75	>0.9999	45.19	>0.9999	-7.00	>0.9999
5 vs. 18	DBT vs. FDP-2	-55.00	>0.9999	37.75	>0.9999	-23.25	>0.9999
5 vs. 19	DBT vs. FDP-3	-41.25	>0.9999	16.44	>0.9999	-30.94	>0.9999
5 vs. 20	DBT vs. FDP-4	-74.75	>0.9999	35.75	>0.9999	-7.69	>0.9999
5 vs. 21	DBT vs. FDP-5	-13.75	>0.9999	60.56	>0.9999	-11.63	>0.9999
5 vs. 22	DBT vs. EIP	13.75	>0.9999	84.88	>0.9999	11.63	>0.9999
5 vs. 23	DBT vs. EDC-2	13.75	>0.9999	86.88	>0.9999	-26.31	>0.9999
5 vs. 24	DBT vs. EDC-3	13.75	>0.9999	0.00	>0.9999	-23.25	>0.9999
5 vs. 25	DBT vs. EDC-4	0.00	>0.9999	33.75	>0.9999	-55.44	>0.9999
5 vs. 26	DBT vs. EDC-5	-4.58	>0.9999	65.75	>0.9999	-18.63	>0.9999
5 vs. 27	DBT vs. EDM	13.75	>0.9999	78.69	>0.9999	-30.94	>0.9999
5 vs. 28	DBT vs. FPL	-27.50	>0.9999	27.38	>0.9999	-23.25	>0.9999
5 vs. 29	DBT vs. APL	13.75	>0.9999	16.44	>0.9999	-14.69	>0.9999
5 vs. 30	DBT vs. EPB	13.75	>0.9999	82.19	>0.9999	-55.44	>0.9999
5 vs. 31	DBT vs. EPL	0.00	>0.9999	115.70	0.3595	-19.31	>0.9999
6 vs. 7	BrR vs. DTT	-41.25	>0.9999	12.94	>0.9999	-5.19	>0.9999
6 vs. 8	BrR vs. FCR	-18.13	>0.9999	0.00	>0.9999	23.25	>0.9999
6 vs. 9	BrR vs. PL	0.00	>0.9999	11.88	>0.9999	55.88	>0.9999
6 vs. 10	BrR vs. FCU	0.00	>0.9999	-28.31	>0.9999	-14.69	>0.9999
6 vs. 11	BrR vs. ECRL	0.00	>0.9999	-39.13	>0.9999	3.25	>0.9999
6 vs. 12	BrR vs. ECRB	0.00	>0.9999	3.94	>0.9999	-28.44	>0.9999
6 vs. 13	BrR vs. ECU	0.00	>0.9999	82.63	>0.9999	-15.38	>0.9999
6 vs. 14	BrR vs. FDS-2	-27.50	>0.9999	-24.81	>0.9999	30.25	>0.9999
6 vs. 15	BrR vs. FDS-3	-13.75	>0.9999	-74.75	>0.9999	79.13	>0.9999
6 vs. 16	BrR vs. FDS-4	0.00	>0.9999	-66.38	>0.9999	23.25	>0.9999
6 vs. 17	BrR vs. FDS-5	0.00	>0.9999	22.81	>0.9999	16.25	>0.9999
6 vs. 18	BrR vs. FDP-2	-68.75	>0.9999	15.38	>0.9999	0.00	>0.9999
6 vs. 19	BrR vs. FDP-3	-55.00	>0.9999	-5.94	>0.9999	-7.69	>0.9999
6 vs. 20	BrR vs. FDP-4	-88.50	0.4346	13.38	>0.9999	15.56	>0.9999
6 vs. 21	BrR vs. FDP-5	-27.50	>0.9999	38.19	>0.9999	11.63	>0.9999
6 vs. 22	BrR vs. EIP	0.00	>0.9999	62.50	>0.9999	34.88	>0.9999
6 vs. 23	BrR vs. EDC-2	0.00	>0.9999	64.50	>0.9999	-3.06	>0.9999
6 vs. 24	BrR vs. EDC-3	0.00	>0.9999	-22.38	>0.9999	0.00	>0.9999
6 vs. 25	BrR vs. EDC-4	-13.75	>0.9999	11.38	>0.9999	-32.19	>0.9999
6 vs. 26	BrR vs. EDC-5	-18.33	>0.9999	43.38	>0.9999	4.63	>0.9999
6 vs. 27	BrR vs. EDM	0.00	>0.9999	56.31	>0.9999	-7.69	>0.9999
6 vs. 28	BrR vs. FPL	-41.25	>0.9999	5.00	>0.9999	0.00	>0.9999
6 vs. 29	BrR vs. APL	0.00	>0.9999	-5.94	>0.9999	8.56	>0.9999
6 vs. 30	BrR vs. EPB	0.00	>0.9999	59.81	>0.9999	-32.19	>0.9999
6 vs. 31	BrR vs. EPL	-13.75	>0.9999	93.31	>0.9999	3.94	>0.9999
7 vs. 8	DTT vs. FCR	23.13	>0.9999	-12.94	>0.9999	28.44	>0.9999
7 vs. 9	DTT vs. PL	41.25	>0.9999	-1.06	>0.9999	61.06	>0.9999
7 vs. 10	DTT vs. FCU	41.25	>0.9999	-41.25	>0.9999	-9.50	>0.9999
7 vs. 11	DTT vs. ECRL	41.25	>0.9999	-52.06	>0.9999	8.44	>0.9999
7 vs. 12	DTT vs. ECRB	41.25	>0.9999	-9.00	>0.9999	-23.25	>0.9999
7 vs. 13	DTT vs. ECU	41.25	>0.9999	69.69	>0.9999	-10.19	>0.9999

7 vs. 14	DTT vs. FDS-2	13.75	>0.9999	-37.75	>0.9999	35.44	>0.9999
7 vs. 15	DTT vs. FDS-3	27.50	>0.9999	-87.69	>0.9999	84.31	>0.9999
7 vs. 16	DTT vs. FDS-4	41.25	>0.9999	-79.31	>0.9999	28.44	>0.9999
7 vs. 17	DTT vs. FDS-5	41.25	>0.9999	9.88	>0.9999	21.44	>0.9999
7 vs. 18	DTT vs. FDP-2	-27.50	>0.9999	2.44	>0.9999	5.19	>0.9999
7 vs. 19	DTT vs. FDP-3	-13.75	>0.9999	-18.88	>0.9999	-2.50	>0.9999
7 vs. 20	DTT vs. FDP-4	-47.25	>0.9999	0.44	>0.9999	20.75	>0.9999
7 vs. 21	DTT vs. FDP-5	13.75	>0.9999	25.25	>0.9999	16.81	>0.9999
7 vs. 22	DTT vs. EIP	41.25	>0.9999	49.56	>0.9999	40.06	>0.9999
7 vs. 23	DTT vs. EDC-2	41.25	>0.9999	51.56	>0.9999	2.13	>0.9999
7 vs. 24	DTT vs. EDC-3	41.25	>0.9999	-35.31	>0.9999	5.19	>0.9999
7 vs. 25	DTT vs. EDC-4	27.50	>0.9999	-1.56	>0.9999	-27.00	>0.9999
7 vs. 26	DTT vs. EDC-5	22.92	>0.9999	30.44	>0.9999	9.81	>0.9999
7 vs. 27	DTT vs. EDM	41.25	>0.9999	43.38	>0.9999	-2.50	>0.9999
7 vs. 28	DTT vs. FPL	0.00	>0.9999	-7.94	>0.9999	5.19	>0.9999
7 vs. 29	DTT vs. APL	41.25	>0.9999	-18.88	>0.9999	13.75	>0.9999
7 vs. 30	DTT vs. EPB	41.25	>0.9999	46.88	>0.9999	-27.00	>0.9999
7 vs. 31	DTT vs. EPL	27.50	>0.9999	80.38	>0.9999	9.13	>0.9999
8 vs. 9	FCR vs. PL	18.13	>0.9999	11.88	>0.9999	32.63	>0.9999
8 vs. 10	FCR vs. FCU	18.13	>0.9999	-28.31	>0.9999	-37.94	>0.9999
8 vs. 11	FCR vs. ECRL	18.13	>0.9999	-39.13	>0.9999	-20.00	>0.9999
8 vs. 12	FCR vs. ECRB	18.13	>0.9999	3.94	>0.9999	-51.69	>0.9999
8 vs. 13	FCR vs. ECU	18.13	>0.9999	82.63	>0.9999	-38.63	>0.9999
8 vs. 14	FCR vs. FDS-2	-9.38	>0.9999	-24.81	>0.9999	7.00	>0.9999
8 vs. 15	FCR vs. FDS-3	4.38	>0.9999	-74.75	>0.9999	55.88	>0.9999
8 vs. 16	FCR vs. FDS-4	18.13	>0.9999	-66.38	>0.9999	0.00	>0.9999
8 vs. 17	FCR vs. FDS-5	18.13	>0.9999	22.81	>0.9999	-7.00	>0.9999
8 vs. 18	FCR vs. FDP-2	-50.63	>0.9999	15.38	>0.9999	-23.25	>0.9999
8 vs. 19	FCR vs. FDP-3	-36.88	>0.9999	-5.94	>0.9999	-30.94	>0.9999
8 vs. 20	FCR vs. FDP-4	-70.38	>0.9999	13.38	>0.9999	-7.69	>0.9999
8 vs. 21	FCR vs. FDP-5	-9.38	>0.9999	38.19	>0.9999	-11.63	>0.9999
8 vs. 22	FCR vs. EIP	18.13	>0.9999	62.50	>0.9999	11.63	>0.9999
8 vs. 23	FCR vs. EDC-2	18.13	>0.9999	64.50	>0.9999	-26.31	>0.9999
8 vs. 24	FCR vs. EDC-3	18.13	>0.9999	-22.38	>0.9999	-23.25	>0.9999
8 vs. 25	FCR vs. EDC-4	4.38	>0.9999	11.38	>0.9999	-55.44	>0.9999
8 vs. 26	FCR vs. EDC-5	-0.21	>0.9999	43.38	>0.9999	-18.63	>0.9999
8 vs. 27	FCR vs. EDM	18.13	>0.9999	56.31	>0.9999	-30.94	>0.9999
8 vs. 28	FCR vs. FPL	-23.13	>0.9999	5.00	>0.9999	-23.25	>0.9999
8 vs. 29	FCR vs. APL	18.13	>0.9999	-5.94	>0.9999	-14.69	>0.9999
8 vs. 30	FCR vs. EPB	18.13	>0.9999	59.81	>0.9999	-55.44	>0.9999
8 vs. 31	FCR vs. EPL	4.38	>0.9999	93.31	>0.9999	-19.31	>0.9999
9 vs. 10	PL vs. FCU	0.00	>0.9999	-40.19	>0.9999	-70.56	>0.9999
9 vs. 11	PL vs. ECRL	0.00	>0.9999	-51.00	>0.9999	-52.63	>0.9999
9 vs. 12	PL vs. ECRB	0.00	>0.9999	-7.94	>0.9999	-84.31	>0.9999
9 vs. 13	PL vs. ECU	0.00	>0.9999	70.75	>0.9999	-71.25	>0.9999
9 vs. 14	PL vs. FDS-2	-27.50	>0.9999	-36.69	>0.9999	-25.63	>0.9999
9 vs. 15	PL vs. FDS-3	-13.75	>0.9999	-86.63	>0.9999	23.25	>0.9999
9 vs. 16	PL vs. FDS-4	0.00	>0.9999	-78.25	>0.9999	-32.63	>0.9999
9 vs. 17	PL vs. FDS-5	0.00	>0.9999	10.94	>0.9999	-39.63	>0.9999
9 vs. 18	PL vs. FDP-2	-68.75	>0.9999	3.50	>0.9999	-55.88	>0.9999
9 vs. 19	PL vs. FDP-3	-55.00	>0.9999	-17.81	>0.9999	-63.56	>0.9999
9 vs. 20	PL vs. FDP-4	-88.50	0.4346	1.50	>0.9999	-40.31	>0.9999
9 vs. 21	PL vs. FDP-5	-27.50	>0.9999	26.31	>0.9999	-44.25	>0.9999
9 vs. 22	PL vs. EIP	0.00	>0.9999	50.63	>0.9999	-21.00	>0.9999
9 vs. 23	PL vs. EDC-2	0.00	>0.9999	52.63	>0.9999	-58.94	>0.9999
9 vs. 24	PL vs. EDC-3	0.00	>0.9999	-34.25	>0.9999	-55.88	>0.9999
9 vs. 25	PL vs. EDC-4	-13.75	>0.9999	-0.50	>0.9999	-88.06	>0.9999
9 vs. 26	PL vs. EDC-5	-18.33	>0.9999	31.50	>0.9999	-51.25	>0.9999
9 vs. 27	PL vs. EDM	0.00	>0.9999	44.44	>0.9999	-63.56	>0.9999
9 vs. 28	PL vs. FPL	-41.25	>0.9999	-6.88	>0.9999	-55.88	>0.9999
9 vs. 29	PL vs. APL	0.00	>0.9999	-17.81	>0.9999	-47.31	>0.9999
9 vs. 30	PL vs. EPB	0.00	>0.9999	47.94	>0.9999	-88.06	>0.9999
9 vs. 31	PL vs. EPL	-13.75	>0.9999	81.44	>0.9999	-51.94	>0.9999
10 vs. 11	FCU vs. ECRL	0.00	>0.9999	-10.81	>0.9999	17.94	>0.9999
10 vs. 12	FCU vs. ECRB	0.00	>0.9999	32.25	>0.9999	-13.75	>0.9999
10 vs. 13	FCU vs. ECU	0.00	>0.9999	110.90	0.5874	-0.69	>0.9999
10 vs. 14	FCU vs. FDS-2	-27.50	>0.9999	3.50	>0.9999	44.94	>0.9999
10 vs. 15	FCU vs. FDS-3	-13.75	>0.9999	-46.44	>0.9999	93.81	>0.9999
10 vs. 16	FCU vs. FDS-4	0.00	>0.9999	-38.06	>0.9999	37.94	>0.9999
10 vs. 17	FCU vs. FDS-5	0.00	>0.9999	51.13	>0.9999	30.94	>0.9999
10 vs. 18	FCU vs. FDP-2	-68.75	>0.9999	43.69	>0.9999	14.69	>0.9999
10 vs. 19	FCU vs. FDP-3	-55.00	>0.9999	22.38	>0.9999	7.00	>0.9999
10 vs. 20	FCU vs. FDP-4	-88.50	0.4346	41.69	>0.9999	30.25	>0.9999
10 vs. 21	FCU vs. FDP-5	-27.50	>0.9999	66.50	>0.9999	26.31	>0.9999
10 vs. 22	FCU vs. EIP	0.00	>0.9999	90.81	>0.9999	49.56	>0.9999
10 vs. 23	FCU vs. EDC-2	0.00	>0.9999	92.81	>0.9999	11.63	>0.9999
10 vs. 24	FCU vs. EDC-3	0.00	>0.9999	5.94	>0.9999	14.69	>0.9999
10 vs. 25	FCU vs. EDC-4	-13.75	>0.9999	39.69	>0.9999	-17.50	>0.9999
10 vs. 26	FCU vs. EDC-5	-18.33	>0.9999	71.69	>0.9999	19.31	>0.9999
10 vs. 27	FCU vs. EDM	0.00	>0.9999	84.63	>0.9999	7.00	>0.9999
10 vs. 28	FCU vs. FPL	-41.25	>0.9999	33.31	>0.9999	14.69	>0.9999
10 vs. 29	FCU vs. APL	0.00	>0.9999	22.38	>0.9999	23.25	>0.9999
10 vs. 30	FCU vs. EPB	0.00	>0.9999	88.13	>0.9999	-17.50	>0.9999
10 vs. 31	FCU vs. EPL	-13.75	>0.9999	121.60	0.1897	18.63	>0.9999
11 vs. 12	ECRL vs. ECRB	0.00	>0.9999	43.06	>0.9999	-31.69	>0.9999
11 vs. 13	ECRL vs. ECU	0.00	>0.9999	121.80	0.1871	-18.63	>0.9999
11 vs. 14	ECRL vs. FDS-2	-27.50	>0.9999	14.31	>0.9999	27.00	>0.9999
11 vs. 15	ECRL vs. FDS-3	-13.75	>0.9999	-35.63	>0.9999	75.88	>0.9999
11 vs. 16	ECRL vs. FDS-4	0.00	>0.9999	-27.25	>0.9999	20.00	>0.9999

11 vs. 17	ECRL vs. FDS-5	0.00	>0.9999	61.94	>0.9999	13.00	>0.9999
11 vs. 18	ECRL vs. FDP-2	-68.75	>0.9999	54.50	>0.9999	-3.25	>0.9999
11 vs. 19	ECRL vs. FDP-3	-55.00	>0.9999	33.19	>0.9999	-10.94	>0.9999
11 vs. 20	ECRL vs. FDP-4	-88.50	0.4346	52.50	>0.9999	12.31	>0.9999
11 vs. 21	ECRL vs. FDP-5	-27.50	>0.9999	77.31	>0.9999	8.38	>0.9999
11 vs. 22	ECRL vs. EIP	0.00	>0.9999	101.60	>0.9999	31.63	>0.9999
11 vs. 23	ECRL vs. EDC-2	0.00	>0.9999	103.60	>0.9999	-6.31	>0.9999
11 vs. 24	ECRL vs. EDC-3	0.00	>0.9999	16.75	>0.9999	-3.25	>0.9999
11 vs. 25	ECRL vs. EDC-4	-13.75	>0.9999	50.50	>0.9999	-35.44	>0.9999
11 vs. 26	ECRL vs. EDC-5	-18.33	>0.9999	82.50	>0.9999	1.38	>0.9999
11 vs. 27	ECRL vs. EDM	0.00	>0.9999	95.44	>0.9999	-10.94	>0.9999
11 vs. 28	ECRL vs. FPL	-41.25	>0.9999	44.13	>0.9999	-3.25	>0.9999
11 vs. 29	ECRL vs. APL	0.00	>0.9999	33.19	>0.9999	5.31	>0.9999
11 vs. 30	ECRL vs. EPB	0.00	>0.9999	98.94	>0.9999	-35.44	>0.9999
11 vs. 31	ECRL vs. EPL	-13.75	>0.9999	132.40	0.0551	0.69	>0.9999
12 vs. 13	ECRB vs. ECU	0.00	>0.9999	78.69	>0.9999	13.06	>0.9999
12 vs. 14	ECRB vs. FDS-2	-27.50	>0.9999	-28.75	>0.9999	58.69	>0.9999
12 vs. 15	ECRB vs. FDS-3	-13.75	>0.9999	-78.69	>0.9999	107.60	0.6212
12 vs. 16	ECRB vs. FDS-4	0.00	>0.9999	-70.31	>0.9999	51.69	>0.9999
12 vs. 17	ECRB vs. FDS-5	0.00	>0.9999	18.88	>0.9999	44.69	>0.9999
12 vs. 18	ECRB vs. FDP-2	-68.75	>0.9999	11.44	>0.9999	28.44	>0.9999
12 vs. 19	ECRB vs. FDP-3	-55.00	>0.9999	-9.88	>0.9999	20.75	>0.9999
12 vs. 20	ECRB vs. FDP-4	-88.50	0.4346	9.44	>0.9999	44.00	>0.9999
12 vs. 21	ECRB vs. FDP-5	-27.50	>0.9999	34.25	>0.9999	40.06	>0.9999
12 vs. 22	ECRB vs. EIP	0.00	>0.9999	58.56	>0.9999	63.31	>0.9999
12 vs. 23	ECRB vs. EDC-2	0.00	>0.9999	60.56	>0.9999	25.38	>0.9999
12 vs. 24	ECRB vs. EDC-3	0.00	>0.9999	-26.31	>0.9999	28.44	>0.9999
12 vs. 25	ECRB vs. EDC-4	-13.75	>0.9999	7.44	>0.9999	-3.75	>0.9999
12 vs. 26	ECRB vs. EDC-5	-18.33	>0.9999	39.44	>0.9999	33.06	>0.9999
12 vs. 27	ECRB vs. EDM	0.00	>0.9999	52.38	>0.9999	20.75	>0.9999
12 vs. 28	ECRB vs. FPL	-41.25	>0.9999	1.06	>0.9999	28.44	>0.9999
12 vs. 29	ECRB vs. APL	0.00	>0.9999	-9.88	>0.9999	37.00	>0.9999
12 vs. 30	ECRB vs. EPB	0.00	>0.9999	55.88	>0.9999	-3.75	>0.9999
12 vs. 31	ECRB vs. EPL	-13.75	>0.9999	89.38	>0.9999	32.38	>0.9999
13 vs. 14	ECU vs. FDS-2	-27.50	>0.9999	-107.40	0.8338	45.63	>0.9999
13 vs. 15	ECU vs. FDS-3	-13.75	>0.9999	-157.40	0.0022	94.50	>0.9999
13 vs. 16	ECU vs. FDS-4	0.00	>0.9999	-149.00	0.0069	38.63	>0.9999
13 vs. 17	ECU vs. FDS-5	0.00	>0.9999	-59.81	>0.9999	31.63	>0.9999
13 vs. 18	ECU vs. FDP-2	-68.75	>0.9999	-67.25	>0.9999	15.38	>0.9999
13 vs. 19	ECU vs. FDP-3	-55.00	>0.9999	-88.56	>0.9999	7.69	>0.9999
13 vs. 20	ECU vs. FDP-4	-88.50	0.4346	-69.25	>0.9999	30.94	>0.9999
13 vs. 21	ECU vs. FDP-5	-27.50	>0.9999	-44.44	>0.9999	27.00	>0.9999
13 vs. 22	ECU vs. EIP	0.00	>0.9999	-20.13	>0.9999	50.25	>0.9999
13 vs. 23	ECU vs. EDC-2	0.00	>0.9999	-18.13	>0.9999	12.31	>0.9999
13 vs. 24	ECU vs. EDC-3	0.00	>0.9999	-105.00	>0.9999	15.38	>0.9999
13 vs. 25	ECU vs. EDC-4	-13.75	>0.9999	-71.25	>0.9999	-16.81	>0.9999
13 vs. 26	ECU vs. EDC-5	-18.33	>0.9999	-39.25	>0.9999	20.00	>0.9999
13 vs. 27	ECU vs. EDM	0.00	>0.9999	-26.31	>0.9999	7.69	>0.9999
13 vs. 28	ECU vs. FPL	-41.25	>0.9999	-77.63	>0.9999	15.38	>0.9999
13 vs. 29	ECU vs. APL	0.00	>0.9999	-88.56	>0.9999	23.94	>0.9999
13 vs. 30	ECU vs. EPB	0.00	>0.9999	-22.81	>0.9999	-16.81	>0.9999
13 vs. 31	ECU vs. EPL	-13.75	>0.9999	10.69	>0.9999	19.31	>0.9999
14 vs. 15	FDS-2 vs. FDS-3	13.75	>0.9999	-49.94	>0.9999	48.88	>0.9999
14 vs. 16	FDS-2 vs. FDS-4	27.50	>0.9999	-41.56	>0.9999	-7.00	>0.9999
14 vs. 17	FDS-2 vs. FDS-5	27.50	>0.9999	47.63	>0.9999	-14.00	>0.9999
14 vs. 18	FDS-2 vs. FDP-2	-41.25	>0.9999	40.19	>0.9999	-30.25	>0.9999
14 vs. 19	FDS-2 vs. FDP-3	-27.50	>0.9999	18.88	>0.9999	-37.94	>0.9999
14 vs. 20	FDS-2 vs. FDP-4	-61.00	>0.9999	38.19	>0.9999	-14.69	>0.9999
14 vs. 21	FDS-2 vs. FDP-5	0.00	>0.9999	63.00	>0.9999	-18.63	>0.9999
14 vs. 22	FDS-2 vs. EIP	27.50	>0.9999	87.31	>0.9999	4.63	>0.9999
14 vs. 23	FDS-2 vs. EDC-2	27.50	>0.9999	89.31	>0.9999	-33.31	>0.9999
14 vs. 24	FDS-2 vs. EDC-3	27.50	>0.9999	2.44	>0.9999	-30.25	>0.9999
14 vs. 25	FDS-2 vs. EDC-4	13.75	>0.9999	36.19	>0.9999	-62.44	>0.9999
14 vs. 26	FDS-2 vs. EDC-5	9.17	>0.9999	68.19	>0.9999	-25.63	>0.9999
14 vs. 27	FDS-2 vs. EDM	27.50	>0.9999	81.13	>0.9999	-37.94	>0.9999
14 vs. 28	FDS-2 vs. FPL	-13.75	>0.9999	29.81	>0.9999	-30.25	>0.9999
14 vs. 29	FDS-2 vs. APL	27.50	>0.9999	18.88	>0.9999	-21.69	>0.9999
14 vs. 30	FDS-2 vs. EPB	27.50	>0.9999	84.63	>0.9999	-62.44	>0.9999
14 vs. 31	FDS-2 vs. EPL	13.75	>0.9999	118.10	0.2775	-26.31	>0.9999
15 vs. 16	FDS-3 vs. FDS-4	13.75	>0.9999	8.38	>0.9999	-55.88	>0.9999
15 vs. 17	FDS-3 vs. FDS-5	13.75	>0.9999	97.56	>0.9999	-62.88	>0.9999
15 vs. 18	FDS-3 vs. FDP-2	-55.00	>0.9999	90.13	>0.9999	-79.13	>0.9999
15 vs. 19	FDS-3 vs. FDP-3	-41.25	>0.9999	68.81	>0.9999	-86.81	>0.9999
15 vs. 20	FDS-3 vs. FDP-4	-74.75	>0.9999	88.13	>0.9999	-63.56	>0.9999
15 vs. 21	FDS-3 vs. FDP-5	-13.75	>0.9999	112.90	0.4787	-67.50	>0.9999
15 vs. 22	FDS-3 vs. EIP	13.75	>0.9999	137.30	0.0309	-44.25	>0.9999
15 vs. 23	FDS-3 vs. EDC-2	13.75	>0.9999	139.30	0.0241	-82.19	>0.9999
15 vs. 24	FDS-3 vs. EDC-3	13.75	>0.9999	52.38	>0.9999	-79.13	>0.9999
15 vs. 25	FDS-3 vs. EDC-4	0.00	>0.9999	86.13	>0.9999	-111.30	0.4186
15 vs. 26	FDS-3 vs. EDC-5	-4.58	>0.9999	118.10	0.6885	-74.50	>0.9999
15 vs. 27	FDS-3 vs. EDM	13.75	>0.9999	131.10	0.0648	-86.81	>0.9999
15 vs. 28	FDS-3 vs. FPL	-27.50	>0.9999	79.75	>0.9999	-79.13	>0.9999
15 vs. 29	FDS-3 vs. APL	13.75	>0.9999	68.81	>0.9999	-70.56	>0.9999
15 vs. 30	FDS-3 vs. EPB	13.75	>0.9999	134.60	0.0428	-111.30	0.4186
15 vs. 31	FDS-3 vs. EPL	0.00	>0.9999	168.10	0.0005	-75.19	>0.9999
16 vs. 17	FDS-4 vs. FDS-5	0.00	>0.9999	89.19	>0.9999	-7.00	>0.9999
16 vs. 18	FDS-4 vs. FDP-2	-68.75	>0.9999	81.75	>0.9999	-23.25	>0.9999
16 vs. 19	FDS-4 vs. FDP-3	-55.00	>0.9999	60.44	>0.9999	-30.94	>0.9999
16 vs. 20	FDS-4 vs. FDP-4	-88.50	0.4346	79.75	>0.9999	-7.69	>0.9999

16 vs. 21	FDS-4 vs. FDP-5	-27.50	>0.9999	104.60	>0.9999	-11.63	>0.9999
16 vs. 22	FDS-4 vs. EIP	0.00	>0.9999	128.90	0.0837	11.63	>0.9999
16 vs. 23	FDS-4 vs. EDC-2	0.00	>0.9999	130.90	0.0663	-26.31	>0.9999
16 vs. 24	FDS-4 vs. EDC-3	0.00	>0.9999	44.00	>0.9999	-23.25	>0.9999
16 vs. 25	FDS-4 vs. EDC-4	-13.75	>0.9999	77.75	>0.9999	-55.44	>0.9999
16 vs. 26	FDS-4 vs. EDC-5	-18.33	>0.9999	109.80	>0.9999	-18.63	>0.9999
16 vs. 27	FDS-4 vs. EDM	0.00	>0.9999	122.70	0.1687	-30.94	>0.9999
16 vs. 28	FDS-4 vs. FPL	-41.25	>0.9999	71.38	>0.9999	-23.25	>0.9999
16 vs. 29	FDS-4 vs. APL	0.00	>0.9999	60.44	>0.9999	-14.69	>0.9999
16 vs. 30	FDS-4 vs. EPB	0.00	>0.9999	126.20	0.1139	-55.44	>0.9999
16 vs. 31	FDS-4 vs. EPL	-13.75	>0.9999	159.70	0.0016	-19.31	>0.9999
17 vs. 18	FDS-5 vs. FDP-2	-68.75	>0.9999	-7.44	>0.9999	-16.25	>0.9999
17 vs. 19	FDS-5 vs. FDP-3	-55.00	>0.9999	-28.75	>0.9999	-23.94	>0.9999
17 vs. 20	FDS-5 vs. FDP-4	-88.50	0.4346	-9.44	>0.9999	-0.69	>0.9999
17 vs. 21	FDS-5 vs. FDP-5	-27.50	>0.9999	15.38	>0.9999	-4.63	>0.9999
17 vs. 22	FDS-5 vs. EIP	0.00	>0.9999	39.69	>0.9999	18.63	>0.9999
17 vs. 23	FDS-5 vs. EDC-2	0.00	>0.9999	41.69	>0.9999	-19.31	>0.9999
17 vs. 24	FDS-5 vs. EDC-3	0.00	>0.9999	-45.19	>0.9999	-16.25	>0.9999
17 vs. 25	FDS-5 vs. EDC-4	-13.75	>0.9999	-11.44	>0.9999	-48.44	>0.9999
17 vs. 26	FDS-5 vs. EDC-5	-18.33	>0.9999	20.56	>0.9999	-11.63	>0.9999
17 vs. 27	FDS-5 vs. EDM	0.00	>0.9999	33.50	>0.9999	-23.94	>0.9999
17 vs. 28	FDS-5 vs. FPL	-41.25	>0.9999	-17.81	>0.9999	-16.25	>0.9999
17 vs. 29	FDS-5 vs. APL	0.00	>0.9999	-28.75	>0.9999	-7.69	>0.9999
17 vs. 30	FDS-5 vs. EPB	0.00	>0.9999	37.00	>0.9999	-48.44	>0.9999
17 vs. 31	FDS-5 vs. EPL	-13.75	>0.9999	70.50	>0.9999	-12.31	>0.9999
18 vs. 19	FDP-2 vs. FDP-3	13.75	>0.9999	-21.31	>0.9999	-7.69	>0.9999
18 vs. 20	FDP-2 vs. FDP-4	-19.75	>0.9999	-2.00	>0.9999	15.56	>0.9999
18 vs. 21	FDP-2 vs. FDP-5	41.25	>0.9999	22.81	>0.9999	11.63	>0.9999
18 vs. 22	FDP-2 vs. EIP	68.75	>0.9999	47.13	>0.9999	34.88	>0.9999
18 vs. 23	FDP-2 vs. EDC-2	68.75	>0.9999	49.13	>0.9999	-3.06	>0.9999
18 vs. 24	FDP-2 vs. EDC-3	68.75	>0.9999	-37.75	>0.9999	0.00	>0.9999
18 vs. 25	FDP-2 vs. EDC-4	55.00	>0.9999	-4.00	>0.9999	-32.19	>0.9999
18 vs. 26	FDP-2 vs. EDC-5	50.42	>0.9999	28.00	>0.9999	4.63	>0.9999
18 vs. 27	FDP-2 vs. EDM	68.75	>0.9999	40.94	>0.9999	-7.69	>0.9999
18 vs. 28	FDP-2 vs. FPL	27.50	>0.9999	-10.38	>0.9999	0.00	>0.9999
18 vs. 29	FDP-2 vs. APL	68.75	>0.9999	-21.31	>0.9999	8.56	>0.9999
18 vs. 30	FDP-2 vs. EPB	68.75	>0.9999	44.44	>0.9999	-32.19	>0.9999
18 vs. 31	FDP-2 vs. EPL	55.00	>0.9999	77.94	>0.9999	3.94	>0.9999
19 vs. 20	FDP-3 vs. FDP-4	-33.50	>0.9999	19.31	>0.9999	23.25	>0.9999
19 vs. 21	FDP-3 vs. FDP-5	27.50	>0.9999	44.13	>0.9999	19.31	>0.9999
19 vs. 22	FDP-3 vs. EIP	55.00	>0.9999	68.44	>0.9999	42.56	>0.9999
19 vs. 23	FDP-3 vs. EDC-2	55.00	>0.9999	70.44	>0.9999	4.63	>0.9999
19 vs. 24	FDP-3 vs. EDC-3	55.00	>0.9999	-16.44	>0.9999	7.69	>0.9999
19 vs. 25	FDP-3 vs. EDC-4	41.25	>0.9999	17.31	>0.9999	-24.50	>0.9999
19 vs. 26	FDP-3 vs. EDC-5	36.67	>0.9999	49.31	>0.9999	12.31	>0.9999
19 vs. 27	FDP-3 vs. EDM	55.00	>0.9999	62.25	>0.9999	0.00	>0.9999
19 vs. 28	FDP-3 vs. FPL	13.75	>0.9999	10.94	>0.9999	7.69	>0.9999
19 vs. 29	FDP-3 vs. APL	55.00	>0.9999	0.00	>0.9999	16.25	>0.9999
19 vs. 30	FDP-3 vs. EPB	55.00	>0.9999	65.75	>0.9999	-24.50	>0.9999
19 vs. 31	FDP-3 vs. EPL	41.25	>0.9999	99.25	>0.9999	11.63	>0.9999
20 vs. 21	FDP-4 vs. FDP-5	61.00	>0.9999	24.81	>0.9999	-3.94	>0.9999
20 vs. 22	FDP-4 vs. EIP	88.50	0.4346	49.13	>0.9999	19.31	>0.9999
20 vs. 23	FDP-4 vs. EDC-2	88.50	0.4346	51.13	>0.9999	-18.63	>0.9999
20 vs. 24	FDP-4 vs. EDC-3	88.50	0.4346	-35.75	>0.9999	-15.56	>0.9999
20 vs. 25	FDP-4 vs. EDC-4	74.75	>0.9999	-2.00	>0.9999	-47.75	>0.9999
20 vs. 26	FDP-4 vs. EDC-5	70.17	>0.9999	30.00	>0.9999	-10.94	>0.9999
20 vs. 27	FDP-4 vs. EDM	88.50	0.4346	42.94	>0.9999	-23.25	>0.9999
20 vs. 28	FDP-4 vs. FPL	47.25	>0.9999	-8.38	>0.9999	-15.56	>0.9999
20 vs. 29	FDP-4 vs. APL	88.50	0.4346	-19.31	>0.9999	-7.00	>0.9999
20 vs. 30	FDP-4 vs. EPB	88.50	0.4346	46.44	>0.9999	-47.75	>0.9999
20 vs. 31	FDP-4 vs. EPL	74.75	>0.9999	79.94	>0.9999	-11.63	>0.9999
21 vs. 22	FDP-5 vs. EIP	27.50	>0.9999	24.31	>0.9999	23.25	>0.9999
21 vs. 23	FDP-5 vs. EDC-2	27.50	>0.9999	26.31	>0.9999	-14.69	>0.9999
21 vs. 24	FDP-5 vs. EDC-3	27.50	>0.9999	-60.56	>0.9999	-11.63	>0.9999
21 vs. 25	FDP-5 vs. EDC-4	13.75	>0.9999	-26.81	>0.9999	-43.81	>0.9999
21 vs. 26	FDP-5 vs. EDC-5	9.17	>0.9999	5.19	>0.9999	-7.00	>0.9999
21 vs. 27	FDP-5 vs. EDM	27.50	>0.9999	18.13	>0.9999	-19.31	>0.9999
21 vs. 28	FDP-5 vs. FPL	-13.75	>0.9999	-33.19	>0.9999	-11.63	>0.9999
21 vs. 29	FDP-5 vs. APL	27.50	>0.9999	-44.13	>0.9999	-3.06	>0.9999
21 vs. 30	FDP-5 vs. EPB	27.50	>0.9999	21.63	>0.9999	-43.81	>0.9999
21 vs. 31	FDP-5 vs. EPL	13.75	>0.9999	55.13	>0.9999	-7.69	>0.9999
22 vs. 23	EIP vs. EDC-2	0.00	>0.9999	2.00	>0.9999	-37.94	>0.9999
22 vs. 24	EIP vs. EDC-3	0.00	>0.9999	-84.88	>0.9999	-34.88	>0.9999
22 vs. 25	EIP vs. EDC-4	-13.75	>0.9999	-51.13	>0.9999	-67.06	>0.9999
22 vs. 26	EIP vs. EDC-5	-18.33	>0.9999	-19.13	>0.9999	-30.25	>0.9999
22 vs. 27	EIP vs. EDM	0.00	>0.9999	-6.19	>0.9999	-42.56	>0.9999
22 vs. 28	EIP vs. FPL	-41.25	>0.9999	-57.50	>0.9999	-34.88	>0.9999
22 vs. 29	EIP vs. APL	0.00	>0.9999	-68.44	>0.9999	-26.31	>0.9999
22 vs. 30	EIP vs. EPB	0.00	>0.9999	-2.69	>0.9999	-67.06	>0.9999
22 vs. 31	EIP vs. EPL	-13.75	>0.9999	30.81	>0.9999	-30.94	>0.9999
23 vs. 24	EDC-2 vs. EDC-3	0.00	>0.9999	-86.88	>0.9999	3.06	>0.9999
23 vs. 25	EDC-2 vs. EDC-4	-13.75	>0.9999	-53.13	>0.9999	-29.13	>0.9999
23 vs. 26	EDC-2 vs. EDC-5	-18.33	>0.9999	-21.13	>0.9999	7.69	>0.9999
23 vs. 27	EDC-2 vs. EDM	0.00	>0.9999	-8.19	>0.9999	-4.63	>0.9999
23 vs. 28	EDC-2 vs. FPL	-41.25	>0.9999	-59.50	>0.9999	3.06	>0.9999
23 vs. 29	EDC-2 vs. APL	0.00	>0.9999	-70.44	>0.9999	11.63	>0.9999
23 vs. 30	EDC-2 vs. EPB	0.00	>0.9999	-4.69	>0.9999	-29.13	>0.9999
23 vs. 31	EDC-2 vs. EPL	-13.75	>0.9999	28.81	>0.9999	7.00	>0.9999
24 vs. 25	EDC-3 vs. EDC-4	-13.75	>0.9999	33.75	>0.9999	-32.19	>0.9999

24 vs. 26	EDC-3 vs. EDC-5	-18.33	>0.9999	65.75	>0.9999	4.63	>0.9999
24 vs. 27	EDC-3 vs. EDM	0.00	>0.9999	78.69	>0.9999	-7.69	>0.9999
24 vs. 28	EDC-3 vs. FPL	-41.25	>0.9999	27.38	>0.9999	0.00	>0.9999
24 vs. 29	EDC-3 vs. APL	0.00	>0.9999	16.44	>0.9999	8.56	>0.9999
24 vs. 30	EDC-3 vs. EPB	0.00	>0.9999	82.19	>0.9999	-32.19	>0.9999
24 vs. 31	EDC-3 vs. EPL	-13.75	>0.9999	115.70	0.3595	3.94	>0.9999
25 vs. 26	EDC-4 vs. EDC-5	-4.58	>0.9999	32.00	>0.9999	36.81	>0.9999
25 vs. 27	EDC-4 vs. EDM	13.75	>0.9999	44.94	>0.9999	24.50	>0.9999
25 vs. 28	EDC-4 vs. FPL	-27.50	>0.9999	-6.38	>0.9999	32.19	>0.9999
25 vs. 29	EDC-4 vs. APL	13.75	>0.9999	-17.31	>0.9999	40.75	>0.9999
25 vs. 30	EDC-4 vs. EPB	13.75	>0.9999	48.44	>0.9999	0.00	>0.9999
25 vs. 31	EDC-4 vs. EPL	0.00	>0.9999	81.94	>0.9999	36.13	>0.9999
26 vs. 27	EDC-5 vs. EDM	18.33	>0.9999	12.94	>0.9999	-12.31	>0.9999
26 vs. 28	EDC-5 vs. FPL	-22.92	>0.9999	-38.38	>0.9999	-4.63	>0.9999
26 vs. 29	EDC-5 vs. APL	18.33	>0.9999	-49.31	>0.9999	3.94	>0.9999
26 vs. 30	EDC-5 vs. EPB	18.33	>0.9999	16.44	>0.9999	-36.81	>0.9999
26 vs. 31	EDC-5 vs. EPL	4.58	>0.9999	49.94	>0.9999	-0.69	>0.9999
27 vs. 28	EDM vs. FPL	-41.25	>0.9999	-51.31	>0.9999	7.69	>0.9999
27 vs. 29	EDM vs. APL	0.00	>0.9999	-62.25	>0.9999	16.25	>0.9999
27 vs. 30	EDM vs. EPB	0.00	>0.9999	3.50	>0.9999	-24.50	>0.9999
27 vs. 31	EDM vs. EPL	-13.75	>0.9999	37.00	>0.9999	11.63	>0.9999
28 vs. 29	FPL vs. APL	41.25	>0.9999	-10.94	>0.9999	8.56	>0.9999
28 vs. 30	FPL vs. EPB	41.25	>0.9999	54.81	>0.9999	-32.19	>0.9999
28 vs. 31	FPL vs. EPL	27.50	>0.9999	88.31	>0.9999	3.94	>0.9999
29 vs. 30	APL vs. EPB	0.00	>0.9999	65.75	>0.9999	-40.75	>0.9999
29 vs. 31	APL vs. EPL	-13.75	>0.9999	99.25	>0.9999	-4.63	>0.9999
30 vs. 31	EPB vs. EPL	-13.75	>0.9999	33.50	>0.9999	36.13	>0.9999

9.7 Appendices for ~ Chapter 6 ~

9.7.1 Lower Limb Pairwise Comparisons

The following pages contain the multiple pairwise comparison tests for the lower upper limb tendons and ligaments included in this project. Differences between tendons for nominal and ordinal outcomes were analysed using non-parametric Kruskal-Wallis tests followed by Dunn's test with a Bonferroni correction for multiple pairwise comparisons (GraphPad Prism). Differences between tendons for continuous (counted) outcomes were analysed using mixed-effects linear regression models with specific pairwise tendon comparisons evaluated using Sidak's adjustment for multiple comparisons (Stata). Statistical significance was taken as an adjusted $p < 0.05$.

Supp. Table 9.7.a Pairwise comparisons for lower limb tendon/ligament cell characteristics. *Green cells indicate significant p-values (< 0.05).*

Tissue Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangment		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
1 vs. 2	AT vs. Plt	-58.953	>0.9999	-298.742	180.835	10.874	>0.9999	-364.042	385.789	0.00	>0.9999	-16.92	>0.9999	53.19	0.9002
1 vs. 3	AT vs. TP	-47.917	>0.9999	-269.037	173.204	-12.500	>0.9999	-358.228	333.228	7.50	>0.9999	18.56	>0.9999	10.63	>0.9999
1 vs. 4	AT vs. TA	-101.042	>0.9999	-322.162	120.079	-3.125	>0.9999	-348.853	342.603	15.00	>0.9999	35.56	>0.9999	47.75	>0.9999
1 vs. 5	AT vs. FL	-13.542	>0.9999	-234.662	207.579	9.375	>0.9999	-336.353	355.103	0.00	>0.9999	39.94	>0.9999	44.63	>0.9999
1 vs. 6	AT vs. FB	-62.500	>0.9999	-283.621	158.621	-50.000	>0.9999	-395.728	295.728	-14.75	>0.9999	11.25	>0.9999	21.75	>0.9999
1 vs. 7	AT vs. FDL	-59.375	>0.9999	-280.496	161.746	-65.625	>0.9999	-411.353	280.103	0.00	>0.9999	27.56	>0.9999	31.44	>0.9999
1 vs. 8	AT vs. FHL	-29.167	>0.9999	-250.287	191.954	87.500	>0.9999	-258.228	433.228	7.50	>0.9999	-9.44	>0.9999	5.75	>0.9999
1 vs. 9	AT vs. EHL	-48.958	>0.9999	-270.079	172.162	18.750	>0.9999	-326.978	364.478	0.00	>0.9999	12.38	>0.9999	47.75	>0.9999
1 vs. 10	AT vs. ST	-88.542	>0.9999	-309.662	132.579	-21.875	>0.9999	-367.603	323.853	7.50	>0.9999	-6.19	>0.9999	36.06	>0.9999
1 vs. 11	AT vs. GT	-48.958	>0.9999	-270.079	172.162	31.250	>0.9999	-314.478	376.978	7.50	>0.9999	16.31	>0.9999	42.31	>0.9999
1 vs. 12	AT vs. QT	11.458	>0.9999	-209.662	232.579	56.250	>0.9999	-289.478	401.978	7.50	>0.9999	33.75	>0.9999	-5.69	>0.9999
1 vs. 13	AT vs. PT	115.625	0.9999	-105.496	336.746	259.375	0.6477	-86.353	605.103	-6.56	>0.9999	27.56	>0.9999	33.75	>0.9999
1 vs. 14	AT vs. ACL	93.750	>0.9999	-127.371	314.871	206.250	0.9908	-139.478	551.978	30.25	>0.9999	1.81	>0.9999	-26.31	>0.9999
1 vs. 15	AT vs. PCL	88.542	>0.9999	-132.579	309.662	62.500	>0.9999	-283.228	408.228	-19.44	>0.9999	-21.81	>0.9999	-29.69	>0.9999
1 vs. 16	AT vs. MCL	73.958	>0.9999	-147.162	295.079	106.250	>0.9999	-239.478	451.978	9.38	>0.9999	8.69	>0.9999	-9.38	>0.9999
1 vs. 17	AT vs. LCL	212.333	0.1253	-16.794	441.459	157.151	>0.9999	-201.094	515.395	17.29	>0.9999	-14.68	>0.9999	-5.60	>0.9999
2 vs. 3	Plt vs. TP	11.037	>0.9999	-228.752	250.825	-23.374	>0.9999	-398.289	351.542	7.50	>0.9999	35.48	>0.9999	-42.56	>0.9999
2 vs. 4	Plt vs. TA	-42.088	>0.9999	-281.877	197.700	-13.999	>0.9999	-388.914	360.917	15.00	>0.9999	52.48	0.9872	-5.44	>0.9999
2 vs. 5	Plt vs. FL	45.412	>0.9999	-194.377	285.200	-1.499	>0.9999	-376.414	373.417	0.00	>0.9999	56.85	0.4938	-8.56	>0.9999
2 vs. 6	Plt vs. FB	-3.547	>0.9999	-243.335	236.242	-60.874	>0.9999	-435.789	314.042	-14.75	>0.9999	28.17	>0.9999	-31.44	>0.9999
2 vs. 7	Plt vs. FDL	-0.422	>0.9999	-240.210	239.367	-76.499	>0.9999	-451.414	298.417	0.00	>0.9999	44.48	>0.9999	-21.75	>0.9999
2 vs. 8	Plt vs. FHL	29.787	>0.9999	-210.002	269.575	76.626	>0.9999	-298.289	451.542	7.50	>0.9999	7.48	>0.9999	-47.44	>0.9999
2 vs. 9	Plt vs. EHL	9.995	>0.9999	-229.793	249.783	7.876	>0.9999	-367.039	382.792	0.00	>0.9999	29.29	>0.9999	-5.44	>0.9999
2 vs. 10	Plt vs. ST	-29.588	>0.9999	-269.377	210.200	-32.749	>0.9999	-407.664	342.167	7.50	>0.9999	10.73	>0.9999	-17.13	>0.9999
2 vs. 11	Plt vs. GT	9.995	>0.9999	-229.793	249.783	20.376	>0.9999	-354.539	395.292	7.50	>0.9999	33.23	>0.9999	-10.88	>0.9999
2 vs. 12	Plt vs. QT	70.412	>0.9999	-169.377	310.200	45.376	>0.9999	-329.539	420.292	7.50	>0.9999	50.67	>0.9999	-58.88	0.3603
2 vs. 13	Plt vs. PT	174.578	0.7320	-65.210	414.367	248.501	0.9204	-126.414	623.417	-6.56	>0.9999	44.48	>0.9999	-19.44	>0.9999
2 vs. 14	Plt vs. ACL	152.703	0.9609	-87.085	392.492	195.376	0.9999	-179.539	570.292	30.25	>0.9999	18.73	>0.9999	-79.50	0.0067
2 vs. 15	Plt vs. PCL	147.495	0.9810	-92.293	387.283	51.626	>0.9999	-323.289	426.542	-19.44	>0.9999	-4.90	>0.9999	-82.88	0.0032
2 vs. 16	Plt vs. MCL	132.912	0.9989	-106.877	372.700	95.376	>0.9999	-279.539	470.292	9.38	>0.9999	25.60	>0.9999	-62.56	0.1908
2 vs. 17	Plt vs. LCL	271.286	0.0132	23.663	518.909	146.277	>0.9999	-240.889	533.442	17.29	>0.9999	2.24	>0.9999	-58.79	0.4863
3 vs. 4	TP vs. TA	-53.125	>0.9999	-274.246	167.996	9.375	>0.9999	-336.353	355.103	7.50	>0.9999	17.00	>0.9999	37.13	>0.9999
3 vs. 5	TP vs. FL	34.375	>0.9999	-186.746	255.496	21.875	>0.9999	-323.853	367.603	-7.50	>0.9999	21.38	>0.9999	34.00	>0.9999
3 vs. 6	TP vs. FB	-14.583	>0.9999	-235.704	206.537	-37.500	>0.9999	-383.228	308.228	-22.25	>0.9999	-7.31	>0.9999	11.13	>0.9999
3 vs. 7	TP vs. FDL	-11.458	>0.9999	-232.579	209.662	-53.125	>0.9999	-398.853	292.603	-7.50	>0.9999	9.00	>0.9999	20.81	>0.9999
3 vs. 8	TP vs. FHL	18.750	>0.9999	-202.371	239.871	100.000	>0.9999	-245.728	445.728	0.00	>0.9999	-28.00	>0.9999	-4.88	>0.9999
3 vs. 9	TP vs. EHL	-1.042	>0.9999	-222.162	220.079	31.250	>0.9999	-314.478	376.978	-7.50	>0.9999	-6.19	>0.9999	37.13	>0.9999
3 vs. 10	TP vs. ST	-40.625	>0.9999	-261.746	180.496	-9.375	>0.9999	-355.103	336.353	0.00	>0.9999	-24.75	>0.9999	25.44	>0.9999
3 vs. 11	TP vs. GT	-1.042	>0.9999	-222.162	220.079	43.750	>0.9999	-301.978	389.478	0.00	>0.9999	-2.25	>0.9999	31.69	>0.9999
3 vs. 12	TP vs. QT	59.375	>0.9999	-161.746	280.496	68.750	>0.9999	-276.978	414.478	0.00	>0.9999	15.19	>0.9999	-16.31	>0.9999
3 vs. 13	TP vs. PT	163.542	0.6887	-57.579	384.662	271.875	0.5060	-73.853	617.603	-14.06	>0.9999	9.00	>0.9999	23.13	>0.9999
3 vs. 14	TP vs. ACL	141.667	0.9561	-79.454	362.787	218.750	0.9656	-126.978	564.478	22.75	>0.9999	-16.75	>0.9999	-36.94	>0.9999
3 vs. 15	TP vs. PCL	136.458	0.9796	-84.662	357.579	75.000	>0.9999	-270.728	420.728	-26.94	>0.9999	-40.38	>0.9999	-40.31	>0.9999
3 vs. 16	TP vs. MCL	121.875	0.9991	-99.245	342.996	118.750	>0.9999	-226.978	464.478	1.88	>0.9999	-9.88	>0.9999	-20.00	>0.9999
3 vs. 17	TP vs. LCL	260.249	0.0073	31.123	489.376	169.651	>0.9999	-188.594	527.895	9.79	>0.9999	-33.24	>0.9999	-16.22	>0.9999
4 vs. 5	TA vs. FL	87.500	>0.9999	-133.621	308.621	12.500	>0.9999	-333.228	358.228	-15.00	>0.9999	4.38	>0.9999	-3.13	>0.9999
4 vs. 6	TA vs. FB	38.542	>0.9999	-182.579	259.662	-46.875	>0.9999	-392.603	298.853	-29.75	>0.9999	-24.31	>0.9999	-26.00	>0.9999
4 vs. 7	TA vs. FDL	41.667	>0.9999	-179.454	262.787	-62.500	>0.9999	-408.228	283.228	-15.00	>0.9999	-8.00	>0.9999	-16.31	>0.9999
4 vs. 8	TA vs. FHL	71.875	>0.9999	-149.246	292.996	90.625	>0.9999	-255.103	436.353	-7.50	>0.9999	-45.00	>0.9999	-42.00	>0.9999

Tissue Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangment		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
4 vs. 9	TA vs. EHL	52.083	>0.9999	-169.037	273.204	21.875	>0.9999	-323.853	367.603	-15.00	>0.9999	-23.19	>0.9999	0.00	>0.9999
4 vs. 10	TA vs. ST	12.500	>0.9999	-208.621	233.621	-18.750	>0.9999	-364.478	326.978	-7.50	>0.9999	-41.75	>0.9999	-11.69	>0.9999
4 vs. 11	TA vs. GT	52.083	>0.9999	-169.037	273.204	34.375	>0.9999	-311.353	380.103	-7.50	>0.9999	-19.25	>0.9999	-5.44	>0.9999
4 vs. 12	TA vs. QT	112.500	>0.9999	-108.621	333.621	59.375	>0.9999	-286.353	405.103	-7.50	>0.9999	-1.81	>0.9999	-53.44	0.4367
4 vs. 13	TA vs. PT	216.667	0.0650	-4.454	437.787	262.500	0.6122	-83.228	608.228	-21.56	>0.9999	-8.00	>0.9999	-14.00	>0.9999
4 vs. 14	TA vs. ACL	194.792	0.2103	-26.329	415.912	209.375	0.9868	-136.353	555.103	15.25	>0.9999	-33.75	>0.9999	-74.06	0.0060
4 vs. 15	TA vs. PCL	189.583	0.2689	-31.537	410.704	65.625	>0.9999	-280.103	411.353	-34.44	>0.9999	-57.38	0.2071	-77.44	0.0027
4 vs. 16	TA vs. MCL	175.000	0.4868	-46.120	396.121	109.375	>0.9999	-236.353	455.103	-5.63	>0.9999	-26.88	>0.9999	-57.13	0.2220
4 vs. 17	TA vs. LCL	313.374	0.0002	84.248	542.501	160.276	>0.9999	-197.969	518.520	2.29	>0.9999	-50.24	0.9952	-53.35	0.6095
5 vs. 6	FL vs. FB	-48.958	>0.9999	-270.079	172.162	-59.375	>0.9999	-405.103	286.353	-14.75	>0.9999	-28.69	>0.9999	-22.88	>0.9999
5 vs. 7	FL vs. FDL	-45.833	>0.9999	-266.954	175.287	-75.000	>0.9999	-420.728	270.728	0.00	>0.9999	-12.38	>0.9999	-13.19	>0.9999
5 vs. 8	FL vs. FHL	-15.625	>0.9999	-236.746	205.496	78.125	>0.9999	-267.603	423.853	7.50	>0.9999	-49.38	0.8658	-38.88	>0.9999
5 vs. 9	FL vs. EHL	-35.417	>0.9999	-256.537	185.704	9.375	>0.9999	-336.353	355.103	0.00	>0.9999	-27.56	>0.9999	3.13	>0.9999
5 vs. 10	FL vs. ST	-75.000	>0.9999	-296.121	146.121	-31.250	>0.9999	-376.978	314.478	7.50	>0.9999	-46.13	>0.9999	-8.56	>0.9999
5 vs. 11	FL vs. GT	-35.417	>0.9999	-256.537	185.704	21.875	>0.9999	-323.853	367.603	7.50	>0.9999	-23.63	>0.9999	-2.31	>0.9999
5 vs. 12	FL vs. QT	25.000	>0.9999	-196.121	246.121	46.875	>0.9999	-298.853	392.603	7.50	>0.9999	-6.19	>0.9999	-50.31	0.7520
5 vs. 13	FL vs. PT	129.167	0.9947	-91.954	350.287	250.000	0.7498	-95.728	595.728	-6.56	>0.9999	-12.38	>0.9999	-10.88	>0.9999
5 vs. 14	FL vs. ACL	107.292	>0.9999	-113.829	328.412	196.875	0.9974	-148.853	542.603	30.25	>0.9999	-38.13	>0.9999	-70.94	0.0125
5 vs. 15	FL vs. PCL	102.083	>0.9999	-119.037	323.204	53.125	>0.9999	-292.603	398.853	-19.44	>0.9999	-61.75	0.0877	-74.31	0.0057
5 vs. 16	FL vs. MCL	87.500	>0.9999	-133.621	308.621	96.875	>0.9999	-248.853	442.603	9.38	>0.9999	-31.25	>0.9999	-54.00	0.3949
5 vs. 17	FL vs. LCL	225.874	0.0602	-3.252	455.001	147.776	>0.9999	-210.469	506.020	17.29	>0.9999	-54.62	0.4828	-50.22	>0.9999
6 vs. 7	FB vs. FDL	3.125	>0.9999	-217.996	224.246	-15.625	>0.9999	-361.353	330.103	14.75	>0.9999	16.31	>0.9999	9.69	>0.9999
6 vs. 8	FB vs. FHL	33.333	>0.9999	-187.787	254.454	137.500	>0.9999	-208.228	483.228	22.25	>0.9999	-20.69	>0.9999	-16.00	>0.9999
6 vs. 9	FB vs. EHL	13.542	>0.9999	-207.579	234.662	68.750	>0.9999	-276.978	414.478	14.75	>0.9999	1.13	>0.9999	26.00	>0.9999
6 vs. 10	FB vs. ST	-26.042	>0.9999	-247.162	195.079	28.125	>0.9999	-317.603	373.853	22.25	>0.9999	-17.44	>0.9999	14.31	>0.9999
6 vs. 11	FB vs. GT	13.542	>0.9999	-207.579	234.662	81.250	>0.9999	-264.478	426.978	22.25	>0.9999	5.06	>0.9999	20.56	>0.9999
6 vs. 12	FB vs. QT	73.958	>0.9999	-147.162	295.079	106.250	>0.9999	-239.478	451.978	22.25	>0.9999	22.50	>0.9999	-27.44	>0.9999
6 vs. 13	FB vs. PT	178.125	0.4343	-42.995	399.246	309.375	0.1806	-36.353	655.103	8.19	>0.9999	16.31	>0.9999	12.00	>0.9999
6 vs. 14	FB vs. ACL	156.250	0.8061	-64.870	377.371	256.250	0.6827	-89.478	601.978	45.00	0.3036	-9.44	>0.9999	-48.06	>0.9999
6 vs. 15	FB vs. PCL	151.042	0.8746	-70.079	372.162	112.500	>0.9999	-233.228	458.228	-4.69	>0.9999	-33.06	>0.9999	-51.44	0.6204
6 vs. 16	FB vs. MCL	136.458	0.9796	-84.662	357.579	156.250	>0.9999	-189.478	501.978	24.13	>0.9999	-2.56	>0.9999	-31.13	>0.9999
6 vs. 17	FB vs. LCL	274.833	0.0027	45.706	503.959	207.151	0.9960	-151.094	565.395	32.04	>0.9999	-25.93	>0.9999	-27.35	>0.9999
7 vs. 8	FDL vs. FHL	30.208	>0.9999	-190.912	251.329	153.125	>0.9999	-192.603	498.853	7.50	>0.9999	-37.00	>0.9999	-25.69	>0.9999
7 vs. 9	FDL vs. EHL	10.417	>0.9999	-210.704	231.537	84.375	>0.9999	-261.353	430.103	0.00	>0.9999	-15.19	>0.9999	16.31	>0.9999
7 vs. 10	FDL vs. ST	-29.167	>0.9999	-250.287	191.954	43.750	>0.9999	-301.978	389.478	7.50	>0.9999	-33.75	>0.9999	4.63	>0.9999
7 vs. 11	FDL vs. GT	10.417	>0.9999	-210.704	231.537	96.875	>0.9999	-248.853	442.603	7.50	>0.9999	-11.25	>0.9999	10.88	>0.9999
7 vs. 12	FDL vs. QT	70.833	>0.9999	-150.287	291.954	121.875	>0.9999	-223.853	467.603	7.50	>0.9999	6.19	>0.9999	-37.13	>0.9999
7 vs. 13	FDL vs. PT	175.000	0.4868	-46.120	396.121	325.000	0.1068	-20.728	670.728	-6.56	>0.9999	0.00	>0.9999	2.31	>0.9999
7 vs. 14	FDL vs. ACL	153.125	0.8490	-67.995	374.246	271.875	0.5060	-73.853	617.603	30.25	>0.9999	-25.75	>0.9999	-57.75	0.1972
7 vs. 15	FDL vs. PCL	147.917	0.9079	-73.204	369.037	128.125	>0.9999	-217.603	473.853	-19.44	>0.9999	-49.38	0.8658	-61.13	0.1020
7 vs. 16	FDL vs. MCL	133.333	0.9881	-87.787	354.454	171.875	>0.9999	-173.853	517.603	9.38	>0.9999	-18.88	>0.9999	-40.81	>0.9999
7 vs. 17	FDL vs. LCL	271.708	0.0034	42.581	500.834	222.776	0.9759	-135.469	581.020	17.29	>0.9999	-42.24	>0.9999	-37.04	>0.9999
8 vs. 9	FHL vs. EHL	-19.792	>0.9999	-240.912	201.329	-68.750	>0.9999	-414.478	276.978	-7.50	>0.9999	21.81	>0.9999	42.00	>0.9999
8 vs. 10	FHL vs. ST	-59.375	>0.9999	-280.496	161.746	-109.375	>0.9999	-455.103	236.353	0.00	>0.9999	3.25	>0.9999	30.31	>0.9999
8 vs. 11	FHL vs. GT	-19.792	>0.9999	-240.912	201.329	-56.250	>0.9999	-401.978	289.478	0.00	>0.9999	25.75	>0.9999	36.56	>0.9999
8 vs. 12	FHL vs. QT	40.625	>0.9999	-180.496	261.746	-31.250	>0.9999	-376.978	314.478	0.00	>0.9999	43.19	>0.9999	-11.44	>0.9999
8 vs. 13	FHL vs. PT	144.792	0.9350	-76.329	365.912	171.875	>0.9999	-173.853	517.603	-14.06	>0.9999	37.00	>0.9999	28.00	>0.9999
8 vs. 14	FHL vs. ACL	122.917	0.9988	-98.204	344.037	118.750	>0.9999	-226.978	464.478	22.75	>0.9999	11.25	>0.9999	-32.06	>0.9999
8 vs. 15	FHL vs. PCL	117.708	0.9997	-103.412	338.829	-25.000	>0.9999	-370.728	320.728	-26.94	>0.9999	-12.38	>0.9999	-35.44	>0.9999
8 vs. 16	FHL vs. MCL	103.125	>0.9999	-117.996	324.246	18.750	>0.9999	-326.978	364.478	1.88	>0.9999	18.13	>0.9999	-15.13	>0.9999
8 vs. 17	FHL vs. LCL	241.499	0.0240	12.373	470.626	69.651	>0.9999	-288.594	427.895	9.79	>0.9999	-5.24	>0.9999	-11.35	>0.9999
9 vs. 10	EHL vs. ST	-39.583	>0.9999	-260.704	181.537	-40.625	>0.9999	-386.353	305.103	7.50	>0.9999	-18.56	>0.9999	-11.69	>0.9999

Tissue Comparison		Cell Density				Cell Distribution				Cell Morphology		Cell Arrangment		Atypical-Phenotype Cell Presence	
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval		Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
9 vs. 11	EHL vs. GT	0.000	>0.9999	-221.121	221.121	12.500	>0.9999	-333.228	358.228	7.50	>0.9999	3.94	>0.9999	-5.44	>0.9999
9 vs. 12	EHL vs. QT	60.417	>0.9999	-160.704	281.537	37.500	>0.9999	-308.228	383.228	7.50	>0.9999	21.38	>0.9999	-53.44	0.4367
9 vs. 13	EHL vs. PT	164.583	0.6706	-56.537	385.704	240.625	0.8389	-105.103	586.353	-6.56	>0.9999	15.19	>0.9999	-14.00	>0.9999
9 vs. 14	EHL vs. ACL	142.708	0.9497	-78.412	363.829	187.500	0.9995	-158.228	533.228	30.25	>0.9999	-10.56	>0.9999	-74.06	0.0060
9 vs. 15	EHL vs. PCL	137.500	0.9759	-83.620	358.621	43.750	>0.9999	-301.978	389.478	-19.44	>0.9999	-34.19	>0.9999	-77.44	0.0027
9 vs. 16	EHL vs. MCL	122.917	0.9988	-98.204	344.037	87.500	>0.9999	-258.228	433.228	9.38	>0.9999	-3.69	>0.9999	-57.13	0.2220
9 vs. 17	EHL vs. LCL	261.291	0.0068	32.165	490.417	138.401	>0.9999	-219.844	496.645	17.29	>0.9999	-27.05	>0.9999	-53.35	0.6095
10 vs. 11	ST vs. GT	39.583	>0.9999	-181.537	260.704	53.125	>0.9999	-292.603	398.853	0.00	>0.9999	22.50	>0.9999	6.25	>0.9999
10 vs. 12	ST vs. QT	100.000	>0.9999	-121.121	321.121	78.125	>0.9999	-267.603	423.853	0.00	>0.9999	39.94	>0.9999	-41.75	>0.9999
10 vs. 13	ST vs. PT	204.167	0.1304	-16.954	425.287	281.250	0.4059	-64.478	626.978	-14.06	>0.9999	33.75	>0.9999	-2.31	>0.9999
10 vs. 14	ST vs. ACL	182.292	0.3687	-38.829	403.412	228.125	0.9261	-117.603	573.853	22.75	>0.9999	8.00	>0.9999	-62.38	0.0792
10 vs. 15	ST vs. PCL	177.083	0.4515	-44.037	398.204	84.375	>0.9999	-261.353	430.103	-26.94	>0.9999	-15.63	>0.9999	-65.75	0.0392
10 vs. 16	ST vs. MCL	162.500	0.7066	-58.620	383.621	128.125	>0.9999	-217.603	473.853	1.88	>0.9999	14.88	>0.9999	-45.44	>0.9999
10 vs. 17	ST vs. LCL	300.874	0.0004	71.748	530.001	179.026	>0.9999	-179.219	537.270	9.79	>0.9999	-8.49	>0.9999	-41.66	>0.9999
11 vs. 12	GT vs. QT	60.417	>0.9999	-160.704	281.537	25.000	>0.9999	-320.728	370.728	0.00	>0.9999	17.44	>0.9999	-48.00	>0.9999
11 vs. 13	GT vs. PT	164.583	0.6706	-56.537	385.704	228.125	0.9261	-117.603	573.853	-14.06	>0.9999	11.25	>0.9999	-8.56	>0.9999
11 vs. 14	GT vs. ACL	142.708	0.9497	-78.412	363.829	175.000	>0.9999	-170.728	520.728	22.75	>0.9999	-14.50	>0.9999	-68.63	0.0210
11 vs. 15	GT vs. PCL	137.500	0.9759	-83.620	358.621	31.250	>0.9999	-314.478	376.978	-26.94	>0.9999	-38.13	>0.9999	-72.00	0.0098
11 vs. 16	GT vs. MCL	122.917	0.9988	-98.204	344.037	75.000	>0.9999	-270.728	420.728	1.88	>0.9999	-7.63	>0.9999	-51.69	0.5941
11 vs. 17	GT vs. LCL	261.291	0.0068	32.165	490.417	125.901	>0.9999	-232.344	484.145	9.79	>0.9999	-30.99	>0.9999	-47.91	>0.9999
12 vs. 13	QT vs. PT	104.167	>0.9999	-116.954	325.287	203.125	0.9938	-142.603	548.853	-14.06	>0.9999	-6.19	>0.9999	39.44	>0.9999
12 vs. 14	QT vs. ACL	82.292	>0.9999	-138.829	303.412	150.000	>0.9999	-195.728	495.728	22.75	>0.9999	-31.94	>0.9999	-20.63	>0.9999
12 vs. 15	QT vs. PCL	77.083	>0.9999	-144.037	298.204	6.250	>0.9999	-339.478	351.978	-26.94	>0.9999	-55.56	0.2909	-24.00	>0.9999
12 vs. 16	QT vs. MCL	62.500	>0.9999	-158.621	283.621	50.000	>0.9999	-295.728	395.728	1.88	>0.9999	-25.06	>0.9999	-3.69	>0.9999
12 vs. 17	QT vs. LCL	200.874	0.2201	-28.252	430.001	100.901	>0.9999	-257.344	459.145	9.79	>0.9999	-48.43	>0.9999	0.09	>0.9999
13 vs. 14	PT vs. ACL	-21.875	>0.9999	-242.996	199.246	-53.125	>0.9999	-398.853	292.603	36.81	>0.9999	-25.75	>0.9999	-60.06	0.1259
13 vs. 15	PT vs. PCL	-27.083	>0.9999	-248.204	194.037	-196.875	0.9974	-542.603	148.853	-12.88	>0.9999	-49.38	0.8658	-63.44	0.0637
13 vs. 16	PT vs. MCL	-41.667	>0.9999	-262.787	179.454	-153.125	>0.9999	-498.853	192.603	15.94	>0.9999	-18.88	>0.9999	-43.13	>0.9999
13 vs. 17	PT vs. LCL	96.708	>0.9999	-132.419	325.834	-102.225	>0.9999	-460.469	256.020	23.85	>0.9999	-42.24	>0.9999	-39.35	>0.9999
14 vs. 15	ACL vs. PCL	-5.208	>0.9999	-226.329	215.912	-143.750	>0.9999	-489.478	201.978	-49.69	0.1000	-23.63	>0.9999	-3.38	>0.9999
14 vs. 16	ACL vs. MCL	-19.792	>0.9999	-240.912	201.329	-100.000	>0.9999	-445.728	245.728	-20.88	>0.9999	6.88	>0.9999	16.94	>0.9999
14 vs. 17	ACL vs. LCL	118.583	0.9999	-110.544	347.709	-49.099	>0.9999	-407.344	309.145	-12.96	>0.9999	-16.49	>0.9999	20.71	>0.9999
15 vs. 16	PCL vs. MCL	-14.583	>0.9999	-235.704	206.537	43.750	>0.9999	-301.978	389.478	28.81	>0.9999	30.50	>0.9999	20.31	>0.9999
15 vs. 17	PCL vs. LCL	123.791	0.9995	-105.335	352.917	94.651	>0.9999	-263.594	452.895	36.72	>0.9999	7.13	>0.9999	24.09	>0.9999
16 vs. 17	MCL vs. LCL	138.374	0.9876	-90.752	367.501	50.901	>0.9999	-307.344	409.145	7.91	>0.9999	-23.37	>0.9999	3.78	>0.9999

Supp. Table 9.7.b Pairwise comparisons for lower limb tendon/ligament proteoglycan/GAG characteristics. *Green cells indicate significant p-values (< 0.05).*

Tissue Comparison		Proteoglycan Presence		Proteoglycan Staining Intensity [Average]		Proteoglycan Staining Intensity [Maximum]	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
1 vs. 2	AT vs. Plt	45.96	>0.9999	16.08	>0.9999	23.75	>0.9999
1 vs. 3	AT vs. TP	5.81	>0.9999	16.08	>0.9999	12.04	>0.9999
1 vs. 4	AT vs. TA	37.06	>0.9999	16.08	>0.9999	10.08	>0.9999
1 vs. 5	AT vs. FL	24.19	>0.9999	16.08	>0.9999	15.55	>0.9999
1 vs. 6	AT vs. FB	14.19	>0.9999	8.75	>0.9999	0.00	>0.9999
1 vs. 7	AT vs. FDL	40.75	>0.9999	16.08	>0.9999	3.25	>0.9999
1 vs. 8	AT vs. FHL	13.13	>0.9999	16.08	>0.9999	16.92	>0.9999
1 vs. 9	AT vs. EHL	51.75	0.7885	16.08	>0.9999	23.75	>0.9999
1 vs. 10	AT vs. ST	20.81	>0.9999	16.08	>0.9999	23.75	>0.9999
1 vs. 11	AT vs. GT	42.50	>0.9999	16.08	>0.9999	23.75	>0.9999
1 vs. 12	AT vs. QT	-11.94	>0.9999	10.58	>0.9999	-6.75	>0.9999
1 vs. 13	AT vs. PT	5.88	>0.9999	16.08	>0.9999	6.18	>0.9999
1 vs. 14	AT vs. ACL	-37.69	>0.9999	-11.42	>0.9999	-19.69	>0.9999
1 vs. 15	AT vs. PCL	-32.81	>0.9999	-0.42	>0.9999	-1.88	>0.9999
1 vs. 16	AT vs. MCL	-17.44	>0.9999	-2.77	>0.9999	-2.46	>0.9999
1 vs. 17	AT vs. LCL	-1.77	>0.9999	-3.22	>0.9999	-0.45	>0.9999
2 vs. 3	Plt vs. TP	-40.15	>0.9999	0.00	>0.9999	-11.71	>0.9999
2 vs. 4	Plt vs. TA	-8.90	>0.9999	0.00	>0.9999	-13.67	>0.9999
2 vs. 5	Plt vs. FL	-21.77	>0.9999	0.00	>0.9999	-8.20	>0.9999
2 vs. 6	Plt vs. FB	-31.77	>0.9999	-7.33	>0.9999	-23.75	>0.9999
2 vs. 7	Plt vs. FDL	-5.21	>0.9999	0.00	>0.9999	-20.50	>0.9999
2 vs. 8	Plt vs. FHL	-32.83	>0.9999	0.00	>0.9999	-6.83	>0.9999
2 vs. 9	Plt vs. EHL	5.79	>0.9999	0.00	>0.9999	0.00	>0.9999
2 vs. 10	Plt vs. ST	-25.15	>0.9999	0.00	>0.9999	0.00	>0.9999
2 vs. 11	Plt vs. GT	-3.46	>0.9999	0.00	>0.9999	0.00	>0.9999
2 vs. 12	Plt vs. QT	-57.90	0.5804	-5.50	>0.9999	-30.50	>0.9999
2 vs. 13	Plt vs. PT	-40.08	>0.9999	0.00	>0.9999	-17.57	>0.9999
2 vs. 14	Plt vs. ACL	-83.65	0.0050	-27.50	>0.9999	-43.44	>0.9999
2 vs. 15	Plt vs. PCL	-78.77	0.0137	-16.50	>0.9999	-25.63	>0.9999
2 vs. 16	Plt vs. MCL	-63.40	0.2384	-18.86	>0.9999	-26.21	>0.9999
2 vs. 17	Plt vs. LCL	-47.73	>0.9999	-19.30	>0.9999	-24.20	>0.9999
3 vs. 4	TP vs. TA	31.25	>0.9999	0.00	>0.9999	-1.95	>0.9999
3 vs. 5	TP vs. FL	18.38	>0.9999	0.00	>0.9999	3.51	>0.9999
3 vs. 6	TP vs. FB	8.38	>0.9999	-7.33	>0.9999	-12.04	>0.9999
3 vs. 7	TP vs. FDL	34.94	>0.9999	0.00	>0.9999	-8.79	>0.9999
3 vs. 8	TP vs. FHL	7.31	>0.9999	0.00	>0.9999	4.88	>0.9999
3 vs. 9	TP vs. EHL	45.94	>0.9999	0.00	>0.9999	11.71	>0.9999
3 vs. 10	TP vs. ST	15.00	>0.9999	0.00	>0.9999	11.71	>0.9999
3 vs. 11	TP vs. GT	36.69	>0.9999	0.00	>0.9999	11.71	>0.9999
3 vs. 12	TP vs. QT	-17.75	>0.9999	-5.50	>0.9999	-18.79	>0.9999
3 vs. 13	TP vs. PT	0.06	>0.9999	0.00	>0.9999	-5.86	>0.9999
3 vs. 14	TP vs. ACL	-43.50	>0.9999	-27.50	0.3757	-31.72	>0.9999
3 vs. 15	TP vs. PCL	-38.63	>0.9999	-16.50	>0.9999	-13.91	>0.9999
3 vs. 16	TP vs. MCL	-23.25	>0.9999	-18.86	>0.9999	-14.50	>0.9999
3 vs. 17	TP vs. LCL	-7.58	>0.9999	-19.30	>0.9999	-12.49	>0.9999
4 vs. 5	TA vs. FL	-12.88	>0.9999	0.00	>0.9999	5.47	>0.9999
4 vs. 6	TA vs. FB	-22.88	>0.9999	-7.33	>0.9999	-10.08	>0.9999
4 vs. 7	TA vs. FDL	3.69	>0.9999	0.00	>0.9999	-6.83	>0.9999
4 vs. 8	TA vs. FHL	-23.94	>0.9999	0.00	>0.9999	6.83	>0.9999
4 vs. 9	TA vs. EHL	14.69	>0.9999	0.00	>0.9999	13.67	>0.9999
4 vs. 10	TA vs. ST	-16.25	>0.9999	0.00	>0.9999	13.67	>0.9999
4 vs. 11	TA vs. GT	5.44	>0.9999	0.00	>0.9999	13.67	>0.9999
4 vs. 12	TA vs. QT	-49.00	>0.9999	-5.50	>0.9999	-16.83	>0.9999
4 vs. 13	TA vs. PT	-31.19	>0.9999	0.00	>0.9999	-3.91	>0.9999
4 vs. 14	TA vs. ACL	-74.75	0.0092	-27.50	>0.9999	-29.77	>0.9999
4 vs. 15	TA vs. PCL	-69.88	0.0265	-16.50	>0.9999	-11.96	>0.9999
4 vs. 16	TA vs. MCL	-54.50	0.4985	-18.86	>0.9999	-12.55	>0.9999
4 vs. 17	TA vs. LCL	-38.83	>0.9999	-19.30	>0.9999	-10.53	>0.9999
5 vs. 6	FL vs. FB	-10.00	>0.9999	-7.33	>0.9999	-15.55	>0.9999
5 vs. 7	FL vs. FDL	16.56	>0.9999	0.00	>0.9999	-12.30	>0.9999
5 vs. 8	FL vs. FHL	-11.06	>0.9999	0.00	>0.9999	1.37	>0.9999
5 vs. 9	FL vs. EHL	27.56	>0.9999	0.00	>0.9999	8.20	>0.9999
5 vs. 10	FL vs. ST	-3.38	>0.9999	0.00	>0.9999	8.20	>0.9999
5 vs. 11	FL vs. GT	18.31	>0.9999	0.00	>0.9999	8.20	>0.9999
5 vs. 12	FL vs. QT	-36.13	>0.9999	-5.50	>0.9999	-22.30	>0.9999
5 vs. 13	FL vs. PT	-18.31	>0.9999	0.00	>0.9999	-9.37	>0.9999
5 vs. 14	FL vs. ACL	-61.88	0.1321	-27.50	0.8954	-35.24	0.9868
5 vs. 15	FL vs. PCL	-57.00	0.3229	-16.50	>0.9999	-17.43	>0.9999
5 vs. 16	FL vs. MCL	-41.63	>0.9999	-18.86	>0.9999	-18.01	>0.9999
5 vs. 17	FL vs. LCL	-25.96	>0.9999	-19.30	>0.9999	-16.00	>0.9999
6 vs. 7	FB vs. FDL	26.56	>0.9999	7.33	>0.9999	3.25	>0.9999
6 vs. 8	FB vs. FHL	-1.06	>0.9999	7.33	>0.9999	16.92	>0.9999
6 vs. 9	FB vs. EHL	37.56	>0.9999	7.33	>0.9999	23.75	>0.9999
6 vs. 10	FB vs. ST	6.63	>0.9999	7.33	>0.9999	23.75	>0.9999
6 vs. 11	FB vs. GT	28.31	>0.9999	7.33	>0.9999	23.75	>0.9999
6 vs. 12	FB vs. QT	-26.13	>0.9999	1.83	>0.9999	-6.75	>0.9999
6 vs. 13	FB vs. PT	-8.31	>0.9999	7.33	>0.9999	6.18	>0.9999
6 vs. 14	FB vs. ACL	-51.88	0.7726	-20.17	>0.9999	-19.69	>0.9999
6 vs. 15	FB vs. PCL	-47.00	>0.9999	-9.17	>0.9999	-1.88	>0.9999
6 vs. 16	FB vs. MCL	-31.63	>0.9999	-11.52	>0.9999	-2.46	>0.9999
6 vs. 17	FB vs. LCL	-15.96	>0.9999	-11.97	>0.9999	-0.45	>0.9999
7 vs. 8	FDL vs. FHL	-27.63	>0.9999	0.00	>0.9999	13.67	>0.9999

Tissue Comparison		Proteoglycan Presence		Proteoglycan Staining Intensity [Average]		Proteoglycan Staining Intensity [Maximum]	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
7 vs. 9	FDL vs. EHL	11.00	>0.9999	0.00	>0.9999	20.50	>0.9999
7 vs. 10	FDL vs. ST	-19.94	>0.9999	0.00	>0.9999	20.50	>0.9999
7 vs. 11	FDL vs. GT	1.75	>0.9999	0.00	>0.9999	20.50	>0.9999
7 vs. 12	FDL vs. QT	-52.69	0.6759	-5.50	>0.9999	-10.00	>0.9999
7 vs. 13	FDL vs. PT	-34.88	>0.9999	0.00	>0.9999	2.93	>0.9999
7 vs. 14	FDL vs. ACL	-78.44	0.0039	-27.50	>0.9999	-22.94	>0.9999
7 vs. 15	FDL vs. PCL	-73.56	0.0119	-16.50	>0.9999	-5.13	>0.9999
7 vs. 16	FDL vs. MCL	-58.19	0.2612	-18.86	>0.9999	-5.71	>0.9999
7 vs. 17	FDL vs. LCL	-42.52	>0.9999	-19.30	>0.9999	-3.70	>0.9999
8 vs. 9	FHL vs. EHL	38.63	>0.9999	0.00	>0.9999	6.83	>0.9999
8 vs. 10	FHL vs. ST	7.69	>0.9999	0.00	>0.9999	6.83	>0.9999
8 vs. 11	FHL vs. GT	29.38	>0.9999	0.00	>0.9999	6.83	>0.9999
8 vs. 12	FHL vs. QT	-25.06	>0.9999	-5.50	>0.9999	-23.67	>0.9999
8 vs. 13	FHL vs. PT	-7.25	>0.9999	0.00	>0.9999	-10.74	>0.9999
8 vs. 14	FHL vs. ACL	-50.81	0.9178	-27.50	0.5613	-36.60	0.4406
8 vs. 15	FHL vs. PCL	-45.94	>0.9999	-16.50	>0.9999	-18.79	>0.9999
8 vs. 16	FHL vs. MCL	-30.56	>0.9999	-18.86	>0.9999	-19.38	>0.9999
8 vs. 17	FHL vs. LCL	-14.89	>0.9999	-19.30	>0.9999	-17.37	>0.9999
9 vs. 10	EHL vs. ST	-30.94	>0.9999	0.00	>0.9999	0.00	>0.9999
9 vs. 11	EHL vs. GT	-9.25	>0.9999	0.00	>0.9999	0.00	>0.9999
9 vs. 12	EHL vs. QT	-63.69	0.0932	-5.50	>0.9999	-30.50	>0.9999
9 vs. 13	EHL vs. PT	-45.88	>0.9999	0.00	>0.9999	-17.57	>0.9999
9 vs. 14	EHL vs. ACL	-89.44	0.0003	-27.50	>0.9999	-43.44	>0.9999
9 vs. 15	EHL vs. PCL	-84.56	0.0009	-16.50	>0.9999	-25.63	>0.9999
9 vs. 16	EHL vs. MCL	-69.19	0.0307	-18.86	>0.9999	-26.21	>0.9999
9 vs. 17	EHL vs. LCL	-53.52	0.7945	-19.30	>0.9999	-24.20	>0.9999
10 vs. 11	ST vs. GT	21.69	>0.9999	0.00	>0.9999	0.00	>0.9999
10 vs. 12	ST vs. QT	-32.75	>0.9999	-5.50	>0.9999	-30.50	>0.9999
10 vs. 13	ST vs. PT	-14.94	>0.9999	0.00	>0.9999	-17.57	>0.9999
10 vs. 14	ST vs. ACL	-58.50	0.2469	-27.50	0.3757	-43.44	0.0363
10 vs. 15	ST vs. PCL	-53.63	0.5780	-16.50	>0.9999	-25.63	>0.9999
10 vs. 16	ST vs. MCL	-38.25	>0.9999	-18.86	>0.9999	-26.21	>0.9999
10 vs. 17	ST vs. LCL	-22.58	>0.9999	-19.30	>0.9999	-24.20	>0.9999
11 vs. 12	GT vs. QT	-54.44	0.5038	-5.50	>0.9999	-30.50	>0.9999
11 vs. 13	GT vs. PT	-36.63	>0.9999	0.00	>0.9999	-17.57	>0.9999
11 vs. 14	GT vs. ACL	-80.19	0.0026	-27.50	>0.9999	-43.44	0.7235
11 vs. 15	GT vs. PCL	-75.31	0.0081	-16.50	>0.9999	-25.63	>0.9999
11 vs. 16	GT vs. MCL	-59.94	0.1898	-18.86	>0.9999	-26.21	>0.9999
11 vs. 17	GT vs. LCL	-44.27	>0.9999	-19.30	>0.9999	-24.20	>0.9999
12 vs. 13	QT vs. PT	17.81	>0.9999	5.50	>0.9999	12.93	>0.9999
12 vs. 14	QT vs. ACL	-25.75	>0.9999	-22.00	>0.9999	-12.94	>0.9999
12 vs. 15	QT vs. PCL	-20.88	>0.9999	-11.00	>0.9999	4.88	>0.9999
12 vs. 16	QT vs. MCL	-5.50	>0.9999	-13.36	>0.9999	4.29	>0.9999
12 vs. 17	QT vs. LCL	10.17	>0.9999	-13.80	>0.9999	6.30	>0.9999
13 vs. 14	PT vs. ACL	-43.56	>0.9999	-27.50	0.3757	-25.87	>0.9999
13 vs. 15	PT vs. PCL	-38.69	>0.9999	-16.50	>0.9999	-8.05	>0.9999
13 vs. 16	PT vs. MCL	-23.31	>0.9999	-18.86	>0.9999	-8.64	>0.9999
13 vs. 17	PT vs. LCL	-7.64	>0.9999	-19.30	>0.9999	-6.63	>0.9999
14 vs. 15	ACL vs. PCL	4.88	>0.9999	11.00	>0.9999	17.81	>0.9999
14 vs. 16	ACL vs. MCL	20.25	>0.9999	8.64	>0.9999	17.22	>0.9999
14 vs. 17	ACL vs. LCL	35.92	>0.9999	8.20	>0.9999	19.24	>0.9999
15 vs. 16	PCL vs. MCL	15.38	>0.9999	-2.36	>0.9999	-0.59	>0.9999
15 vs. 17	PCL vs. LCL	31.04	>0.9999	-2.80	>0.9999	1.43	>0.9999
16 vs. 17	MCL vs. LCL	15.67	>0.9999	-0.44	>0.9999	2.01	>0.9999

Supp. Table 9.7.c Pairwise comparisons for lower limb tendon/ligament architectural characteristics. *Green cells indicate significant p-values (< 0.05).*

Tissue Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
1 vs. 2	AT vs. Plt	35.63	>0.9999	19.88	>0.9999	-38.42	>0.9999	-29.45	>0.9999	49.75	>0.9999	32.75	>0.9999
1 vs. 3	AT vs. TP	45.00	>0.9999	6.63	>0.9999	4.31	>0.9999	-17.16	>0.9999	39.00	>0.9999	-0.06	>0.9999
1 vs. 4	AT vs. TA	40.63	>0.9999	-15.13	>0.9999	-24.25	>0.9999	13.80	>0.9999	35.44	>0.9999	9.81	>0.9999
1 vs. 5	AT vs. FL	45.00	>0.9999	-5.75	>0.9999	-21.88	>0.9999	12.61	>0.9999	40.44	>0.9999	6.50	>0.9999
1 vs. 6	AT vs. FB	-5.56	>0.9999	-5.75	>0.9999	-16.81	>0.9999	-1.36	>0.9999	16.31	>0.9999	13.06	>0.9999
1 vs. 7	AT vs. FDL	39.69	>0.9999	2.94	>0.9999	20.75	>0.9999	19.71	>0.9999	41.81	>0.9999	13.06	>0.9999
1 vs. 8	AT vs. FHL	19.38	>0.9999	19.88	>0.9999	-12.63	>0.9999	-5.64	>0.9999	32.63	>0.9999	19.63	>0.9999
1 vs. 9	AT vs. EHL	50.31	0.8831	13.25	>0.9999	0.44	>0.9999	19.71	>0.9999	58.31	0.1592	22.94	>0.9999
1 vs. 10	AT vs. ST	30.00	>0.9999	-26.44	>0.9999	-48.75	>0.9999	19.71	>0.9999	-17.06	>0.9999	-22.00	>0.9999
1 vs. 11	AT vs. GT	39.69	>0.9999	2.94	>0.9999	-3.75	>0.9999	19.71	>0.9999	31.31	>0.9999	6.56	>0.9999
1 vs. 12	AT vs. QT	-23.56	>0.9999	-4.81	>0.9999	3.44	>0.9999	1.57	>0.9999	19.13	>0.9999	-40.06	>0.9999
1 vs. 13	AT vs. PT	-2.56	>0.9999	-25.63	>0.9999	-21.75	>0.9999	-0.57	>0.9999	7.13	>0.9999	-22.75	>0.9999
1 vs. 14	AT vs. ACL	-29.56	>0.9999	-23.56	>0.9999	39.25	>0.9999			34.06	>0.9999	-6.63	>0.9999
1 vs. 15	AT vs. PCL	-37.00	>0.9999	-51.00	0.3575	0.61	>0.9999	-17.16	>0.9999	-22.00	>0.9999	-22.75	>0.9999
1 vs. 16	AT vs. MCL	-30.56	>0.9999	-23.38	>0.9999	-4.25	>0.9999	-25.37	>0.9999	16.38	>0.9999	-13.69	>0.9999
1 vs. 17	AT vs. LCL	-6.88	>0.9999	12.30	>0.9999	28.68	>0.9999	-3.54	>0.9999	56.68	0.3134	-0.75	>0.9999
2 vs. 3	Plt vs. TP	9.38	>0.9999	-13.25	>0.9999	42.73	>0.9999	12.29	>0.9999	-10.75	>0.9999	-32.81	>0.9999
2 vs. 4	Plt vs. TA	5.00	>0.9999	-35.00	>0.9999	14.17	>0.9999	43.25	0.1529	-14.31	>0.9999	-22.94	>0.9999
2 vs. 5	Plt vs. FL	9.38	>0.9999	-25.63	>0.9999	16.54	>0.9999	42.07	0.3372	-9.31	>0.9999	-26.25	>0.9999
2 vs. 6	Plt vs. FB	-41.19	>0.9999	-25.63	>0.9999	21.60	>0.9999	28.10	>0.9999	-33.44	>0.9999	-19.69	>0.9999
2 vs. 7	Plt vs. FDL	4.06	>0.9999	-16.94	>0.9999	59.17	0.3824	49.17	0.1264	-7.94	>0.9999	-19.69	>0.9999
2 vs. 8	Plt vs. FHL	-16.25	>0.9999	0.00	>0.9999	25.79	>0.9999	23.81	>0.9999	-17.13	>0.9999	-13.13	>0.9999
2 vs. 9	Plt vs. EHL	14.69	>0.9999	-6.63	>0.9999	38.85	>0.9999	49.17	0.0575	8.56	>0.9999	-9.81	>0.9999
2 vs. 10	Plt vs. ST	-5.63	>0.9999	-46.31	>0.9999	-10.33	>0.9999	49.17	0.0108	-66.81	0.0782	-54.75	0.5932
2 vs. 11	Plt vs. GT	4.06	>0.9999	-16.94	>0.9999	34.67	>0.9999	49.17	0.0575	-18.44	>0.9999	-26.19	>0.9999
2 vs. 12	Plt vs. QT	-59.19	0.4125	-24.69	>0.9999	41.85	>0.9999	31.02	>0.9999	-30.63	>0.9999	-72.81	0.0204
2 vs. 13	Plt vs. PT	-38.19	>0.9999	-45.50	>0.9999	16.67	>0.9999	28.88	>0.9999	-42.63	>0.9999	-55.50	0.5243
2 vs. 14	Plt vs. ACL	-65.19	0.1489	-43.44	>0.9999	77.67	0.0191			-15.69	>0.9999	-39.38	>0.9999
2 vs. 15	Plt vs. PCL	-72.63	0.0375	-70.88	0.0148	39.02	>0.9999	12.29	>0.9999	-71.75	0.0296	-55.50	0.5243
2 vs. 16	Plt vs. MCL	-66.19	0.1247	-43.25	>0.9999	34.17	>0.9999	4.08	>0.9999	-33.38	>0.9999	-46.44	>0.9999
2 vs. 17	Plt vs. LCL	-42.50	>0.9999	-7.57	>0.9999	67.10	0.1369	25.92	>0.9999	6.93	>0.9999	-33.50	>0.9999
3 vs. 4	TP vs. TA	-4.38	>0.9999	-21.75	>0.9999	-28.56	>0.9999	30.96	>0.9999	-3.56	>0.9999	9.88	>0.9999
3 vs. 5	TP vs. FL	0.00	>0.9999	-12.38	>0.9999	-26.19	>0.9999	29.78	>0.9999	1.44	>0.9999	6.56	>0.9999
3 vs. 6	TP vs. FB	-50.56	0.8477	-12.38	>0.9999	-21.13	>0.9999	15.80	>0.9999	-22.69	>0.9999	13.13	>0.9999
3 vs. 7	TP vs. FDL	-5.31	>0.9999	-3.69	>0.9999	16.44	>0.9999	36.88	>0.9999	2.81	>0.9999	13.13	>0.9999
3 vs. 8	TP vs. FHL	-25.63	>0.9999	13.25	>0.9999	-16.94	>0.9999	11.52	>0.9999	-6.38	>0.9999	19.69	>0.9999
3 vs. 9	TP vs. EHL	5.31	>0.9999	6.63	>0.9999	-3.88	>0.9999	36.88	>0.9999	19.31	>0.9999	23.00	>0.9999
3 vs. 10	TP vs. ST	-15.00	>0.9999	-33.06	>0.9999	-53.06	0.5172	36.88	>0.9999	-56.06	0.2454	-21.94	>0.9999
3 vs. 11	TP vs. GT	-5.31	>0.9999	-3.69	>0.9999	-8.06	>0.9999	36.88	>0.9999	-7.69	>0.9999	6.63	>0.9999
3 vs. 12	TP vs. QT	-68.56	0.0283	-11.44	>0.9999	-0.88	>0.9999	18.73	>0.9999	-19.88	>0.9999	-40.00	>0.9999
3 vs. 13	TP vs. PT	-47.56	>0.9999	-32.25	>0.9999	-26.06	>0.9999	16.59	>0.9999	-31.88	>0.9999	-22.69	>0.9999
3 vs. 14	TP vs. ACL	-74.56	0.0075	-30.19	>0.9999	34.94	>0.9999			-4.94	>0.9999	-6.56	>0.9999
3 vs. 15	TP vs. PCL	-82.00	0.0012	-57.63	0.0920	-3.71	>0.9999	0.00	>0.9999	-61.00	0.0932	-22.69	>0.9999
3 vs. 16	TP vs. MCL	-75.56	0.0059	-30.00	>0.9999	-8.56	>0.9999	-8.21	>0.9999	-22.63	>0.9999	-13.63	>0.9999
3 vs. 17	TP vs. LCL	-51.88	0.9120	5.68	>0.9999	24.37	>0.9999	13.63	>0.9999	17.68	>0.9999	-0.69	>0.9999
4 vs. 5	TA vs. FL	4.38	>0.9999	9.38	>0.9999	2.38	>0.9999	-1.18	>0.9999	5.00	>0.9999	-3.31	>0.9999
4 vs. 6	TA vs. FB	-46.19	>0.9999	9.38	>0.9999	7.44	>0.9999	-15.15	>0.9999	-19.13	>0.9999	3.25	>0.9999
4 vs. 7	TA vs. FDL	-0.94	>0.9999	18.06	>0.9999	45.00	>0.9999	5.92	>0.9999	6.38	>0.9999	3.25	>0.9999
4 vs. 8	TA vs. FHL	-21.25	>0.9999	35.00	>0.9999	11.63	>0.9999	-19.44	>0.9999	-2.81	>0.9999	9.81	>0.9999

Tissue Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
4 vs. 9	TA vs. EHL	9.69	>0.9999	28.38	>0.9999	24.69	>0.9999	5.92	>0.9999	22.88	>0.9999	13.13	>0.9999
4 vs. 10	TA vs. ST	-10.63	>0.9999	-11.31	>0.9999	-24.50	>0.9999	5.92	>0.9999	-52.50	0.4725	-31.81	>0.9999
4 vs. 11	TA vs. GT	-0.94	>0.9999	18.06	>0.9999	20.50	>0.9999	5.92	>0.9999	-4.13	>0.9999	-3.25	>0.9999
4 vs. 12	TA vs. QT	-64.19	0.0702	10.31	>0.9999	27.69	>0.9999	-12.23	>0.9999	-16.31	>0.9999	-49.88	0.6843
4 vs. 13	TA vs. PT	-43.19	>0.9999	-10.50	>0.9999	2.50	>0.9999	-14.37	>0.9999	-28.31	>0.9999	-32.56	>0.9999
4 vs. 14	TA vs. ACL	-70.19	0.0199	-8.44	>0.9999	63.50	0.1115			-1.38	>0.9999	-16.44	>0.9999
4 vs. 15	TA vs. PCL	-77.63	0.0036	-35.88	>0.9999	24.86	>0.9999	-30.96	>0.9999	-57.44	0.1887	-32.56	>0.9999
4 vs. 16	TA vs. MCL	-71.19	0.0160	-8.25	>0.9999	20.00	>0.9999	-39.17	0.4233	-19.06	>0.9999	-23.50	>0.9999
4 vs. 17	TA vs. LCL	-47.50	>0.9999	27.43	>0.9999	52.93	0.7193	-17.33	>0.9999	21.24	>0.9999	-10.56	>0.9999
5 vs. 6	FL vs. FB	-50.56	0.8477	0.00	>0.9999	5.06	>0.9999	-13.97	>0.9999	-24.13	>0.9999	6.56	>0.9999
5 vs. 7	FL vs. FDL	-5.31	>0.9999	8.69	>0.9999	42.63	>0.9999	7.10	>0.9999	1.38	>0.9999	6.56	>0.9999
5 vs. 8	FL vs. FHL	-25.63	>0.9999	25.63	>0.9999	9.25	>0.9999	-18.26	>0.9999	-7.81	>0.9999	13.13	>0.9999
5 vs. 9	FL vs. EHL	5.31	>0.9999	19.00	>0.9999	22.31	>0.9999	7.10	>0.9999	17.88	>0.9999	16.44	>0.9999
5 vs. 10	FL vs. ST	-15.00	>0.9999	-20.69	>0.9999	-26.88	>0.9999	7.10	>0.9999	-57.50	0.1865	-28.50	>0.9999
5 vs. 11	FL vs. GT	-5.31	>0.9999	8.69	>0.9999	18.13	>0.9999	7.10	>0.9999	-9.13	>0.9999	0.06	>0.9999
5 vs. 12	FL vs. QT	-68.56	0.0283	0.94	>0.9999	25.31	>0.9999	-11.04	>0.9999	-21.31	>0.9999	-46.56	>0.9999
5 vs. 13	FL vs. PT	-47.56	>0.9999	-19.88	>0.9999	0.13	>0.9999	-13.19	>0.9999	-33.31	>0.9999	-29.25	>0.9999
5 vs. 14	FL vs. ACL	-74.56	0.0075	-17.81	>0.9999	61.13	0.1739			-6.38	>0.9999	-13.13	>0.9999
5 vs. 15	FL vs. PCL	-82.00	0.0012	-45.25	>0.9999	22.48	>0.9999	-29.78	>0.9999	-62.44	0.0694	-29.25	>0.9999
5 vs. 16	FL vs. MCL	-75.56	0.0059	-17.63	>0.9999	17.63	>0.9999	-37.98	0.8377	-24.06	>0.9999	-20.19	>0.9999
5 vs. 17	FL vs. LCL	-51.88	0.9120	18.05	>0.9999	50.55	>0.9999	-16.15	>0.9999	16.24	>0.9999	-7.25	>0.9999
6 vs. 7	FB vs. FDL	45.25	>0.9999	8.69	>0.9999	37.56	>0.9999	21.07	>0.9999	25.50	>0.9999	0.00	>0.9999
6 vs. 8	FB vs. FHL	24.94	>0.9999	25.63	>0.9999	4.19	>0.9999	-4.29	>0.9999	16.31	>0.9999	6.56	>0.9999
6 vs. 9	FB vs. EHL	55.88	0.3408	19.00	>0.9999	17.25	>0.9999	21.07	>0.9999	42.00	>0.9999	9.88	>0.9999
6 vs. 10	FB vs. ST	35.56	>0.9999	-20.69	>0.9999	-31.94	>0.9999	21.07	>0.9999	-33.38	>0.9999	-35.06	>0.9999
6 vs. 11	FB vs. GT	45.25	>0.9999	8.69	>0.9999	13.06	>0.9999	21.07	>0.9999	15.00	>0.9999	-6.50	>0.9999
6 vs. 12	FB vs. QT	-18.00	>0.9999	0.94	>0.9999	20.25	>0.9999	2.93	>0.9999	2.81	>0.9999	-53.13	0.3822
6 vs. 13	FB vs. PT	3.00	>0.9999	-19.88	>0.9999	-4.94	>0.9999	0.79	>0.9999	-9.19	>0.9999	-35.81	>0.9999
6 vs. 14	FB vs. ACL	-24.00	>0.9999	-17.81	>0.9999	56.06	0.4266			17.75	>0.9999	-19.69	>0.9999
6 vs. 15	FB vs. PCL	-31.44	>0.9999	-45.25	>0.9999	17.42	>0.9999	-15.80	>0.9999	-38.31	>0.9999	-35.81	>0.9999
6 vs. 16	FB vs. MCL	-25.00	>0.9999	-17.63	>0.9999	12.56	>0.9999	-24.01	>0.9999	0.06	>0.9999	-26.75	>0.9999
6 vs. 17	FB vs. LCL	-1.31	>0.9999	18.05	>0.9999	45.49	>0.9999	-2.18	>0.9999	40.37	>0.9999	-13.81	>0.9999
7 vs. 8	FDL vs. FHL	-20.31	>0.9999	16.94	>0.9999	-33.38	>0.9999	-25.36	>0.9999	-9.19	>0.9999	6.56	>0.9999
7 vs. 9	FDL vs. EHL	10.63	>0.9999	10.31	>0.9999	-20.31	>0.9999	0.00	>0.9999	16.50	>0.9999	9.88	>0.9999
7 vs. 10	FDL vs. ST	-9.69	>0.9999	-29.38	>0.9999	-69.50	0.0204	0.00	>0.9999	-58.88	0.1426	-35.06	>0.9999
7 vs. 11	FDL vs. GT	0.00	>0.9999	0.00	>0.9999	-24.50	>0.9999	0.00	>0.9999	-10.50	>0.9999	-6.50	>0.9999
7 vs. 12	FDL vs. QT	-63.25	0.0847	-7.75	>0.9999	-17.31	>0.9999	-18.14	>0.9999	-22.69	>0.9999	-53.13	0.3822
7 vs. 13	FDL vs. PT	-42.25	>0.9999	-28.56	>0.9999	-42.50	>0.9999	-20.29	>0.9999	-34.69	>0.9999	-35.81	>0.9999
7 vs. 14	FDL vs. ACL	-69.25	0.0244	-26.50	>0.9999	18.50	>0.9999			-7.75	>0.9999	-19.69	>0.9999
7 vs. 15	FDL vs. PCL	-76.69	0.0046	-53.94	0.1993	-20.14	>0.9999	-36.88	>0.9999	-63.81	0.0520	-35.81	>0.9999
7 vs. 16	FDL vs. MCL	-70.25	0.0197	-26.31	>0.9999	-25.00	>0.9999	-45.08	0.3200	-25.44	>0.9999	-26.75	>0.9999
7 vs. 17	FDL vs. LCL	-46.56	>0.9999	9.37	>0.9999	7.93	>0.9999	-23.25	>0.9999	14.87	>0.9999	-13.81	>0.9999
8 vs. 9	FHL vs. EHL	30.94	>0.9999	-6.63	>0.9999	13.06	>0.9999	25.36	>0.9999	25.69	>0.9999	3.31	>0.9999
8 vs. 10	FHL vs. ST	10.63	>0.9999	-46.31	0.8570	-36.13	>0.9999	25.36	>0.9999	-49.69	0.7723	-41.63	>0.9999
8 vs. 11	FHL vs. GT	20.31	>0.9999	-16.94	>0.9999	8.88	>0.9999	25.36	>0.9999	-1.31	>0.9999	-13.06	>0.9999
8 vs. 12	FHL vs. QT	-42.94	>0.9999	-24.69	>0.9999	16.06	>0.9999	7.21	>0.9999	-13.50	>0.9999	-59.69	0.1072
8 vs. 13	FHL vs. PT	-21.94	>0.9999	-45.50	0.9901	-9.13	>0.9999	5.07	>0.9999	-25.50	>0.9999	-42.38	>0.9999
8 vs. 14	FHL vs. ACL	-48.94	>0.9999	-43.44	>0.9999	51.88	0.8526			1.44	>0.9999	-26.25	>0.9999
8 vs. 15	FHL vs. PCL	-56.38	0.3115	-70.88	0.0040	13.23	>0.9999	-11.52	>0.9999	-54.63	0.3211	-42.38	>0.9999
8 vs. 16	FHL vs. MCL	-49.94	0.9389	-43.25	>0.9999	8.38	>0.9999	-19.73	>0.9999	-16.25	>0.9999	-33.31	>0.9999
8 vs. 17	FHL vs. LCL	-26.25	>0.9999	-7.57	>0.9999	41.30	>0.9999	2.11	>0.9999	24.05	>0.9999	-20.38	>0.9999
9 vs. 10	EHL vs. ST	-20.31	>0.9999	-39.69	>0.9999	-49.19	0.9932	0.00	>0.9999	-75.38	0.0037	-44.94	>0.9999

Tissue Comparison		Collagenous Organisation		Fibrillar Loosening		Fibre Separations [Frequency]		Fibre Separations [Type]		Collagen Alignment [Non-Polarised]		Adipose Presence	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
9 vs. 11	EHL vs. GT	-10.63	>0.9999	-10.31	>0.9999	-4.19	>0.9999	0.00	>0.9999	-27.00	>0.9999	-16.38	>0.9999
9 vs. 12	EHL vs. QT	-73.88	0.0087	-18.06	>0.9999	3.00	>0.9999	-18.14	>0.9999	-39.19	>0.9999	-63.00	0.0538
9 vs. 13	EHL vs. PT	-52.88	0.5755	-38.88	>0.9999	-22.19	>0.9999	-20.29	>0.9999	-51.19	0.5960	-45.69	>0.9999
9 vs. 14	EHL vs. ACL	-79.88	0.0021	-36.81	>0.9999	38.81	>0.9999			-24.25	>0.9999	-29.56	>0.9999
9 vs. 15	EHL vs. PCL	-87.31	0.0003	-64.25	0.0205	0.17	>0.9999	-36.88	>0.9999	-80.31	0.0011	-45.69	>0.9999
9 vs. 16	EHL vs. MCL	-80.88	0.0017	-36.63	>0.9999	-4.69	>0.9999	-45.08	0.1638	-41.94	>0.9999	-36.63	>0.9999
9 vs. 17	EHL vs. LCL	-57.19	0.3809	-0.95	>0.9999	28.24	>0.9999	-23.25	>0.9999	-1.63	>0.9999	-23.69	>0.9999
10 vs. 11	ST vs. GT	9.69	>0.9999	29.38	>0.9999	45.00	>0.9999	0.00	>0.9999	48.38	0.9639	28.56	>0.9999
10 vs. 12	ST vs. QT	-53.56	0.5115	21.63	>0.9999	52.19	0.6015	-18.14	>0.9999	36.19	>0.9999	-18.06	>0.9999
10 vs. 13	ST vs. PT	-32.56	>0.9999	0.81	>0.9999	27.00	>0.9999	-20.29	>0.9999	24.19	>0.9999	-0.75	>0.9999
10 vs. 14	ST vs. ACL	-59.56	0.1730	2.88	>0.9999	88.00	0.0005			51.13	0.6025	15.38	>0.9999
10 vs. 15	ST vs. PCL	-67.00	0.0394	-24.56	>0.9999	49.36	>0.9999	-36.88	>0.9999	-4.94	>0.9999	-0.75	>0.9999
10 vs. 16	ST vs. MCL	-60.56	0.1431	3.06	>0.9999	44.50	>0.9999	-45.08	0.0397	33.44	>0.9999	8.31	>0.9999
10 vs. 17	ST vs. LCL	-36.88	>0.9999	38.74	>0.9999	77.43	0.0061	-23.25	>0.9999	73.74	0.0100	21.25	>0.9999
11 vs. 12	GT vs. QT	-63.25	0.0847	-7.75	>0.9999	7.19	>0.9999	-18.14	>0.9999	-12.19	>0.9999	-46.63	>0.9999
11 vs. 13	GT vs. PT	-42.25	>0.9999	-28.56	>0.9999	-18.00	>0.9999	-20.29	>0.9999	-24.19	>0.9999	-29.31	>0.9999
11 vs. 14	GT vs. ACL	-69.25	0.0244	-26.50	>0.9999	43.00	>0.9999			2.75	>0.9999	-13.19	>0.9999
11 vs. 15	GT vs. PCL	-76.69	0.0046	-53.94	0.1993	4.36	>0.9999	-36.88	>0.9999	-53.31	0.4082	-29.31	>0.9999
11 vs. 16	GT vs. MCL	-70.25	0.0197	-26.31	>0.9999	-0.50	>0.9999	-45.08	0.1638	-14.94	>0.9999	-20.25	>0.9999
11 vs. 17	GT vs. LCL	-46.56	>0.9999	9.37	>0.9999	32.43	>0.9999	-23.25	>0.9999	25.37	>0.9999	-7.31	>0.9999
12 vs. 13	QT vs. PT	21.00	>0.9999	-20.81	>0.9999	-25.19	>0.9999	-2.14	>0.9999	-12.00	>0.9999	17.31	>0.9999
12 vs. 14	QT vs. ACL	-6.00	>0.9999	-18.75	>0.9999	35.81	>0.9999			14.94	>0.9999	33.44	>0.9999
12 vs. 15	QT vs. PCL	-13.44	>0.9999	-46.19	0.8764	-2.83	>0.9999	-18.73	>0.9999	-41.13	>0.9999	17.31	>0.9999
12 vs. 16	QT vs. MCL	-7.00	>0.9999	-18.56	>0.9999	-7.69	>0.9999	-26.94	>0.9999	-2.75	>0.9999	26.38	>0.9999
12 vs. 17	QT vs. LCL	16.69	>0.9999	17.12	>0.9999	25.24	>0.9999	-5.11	>0.9999	37.55	>0.9999	39.31	>0.9999
13 vs. 14	PT vs. ACL	-27.00	>0.9999	2.06	>0.9999	61.00	0.1779			26.94	>0.9999	16.13	>0.9999
13 vs. 15	PT vs. PCL	-34.44	>0.9999	-25.38	>0.9999	22.36	>0.9999	-16.59	>0.9999	-29.13	>0.9999	0.00	>0.9999
13 vs. 16	PT vs. MCL	-28.00	>0.9999	2.25	>0.9999	17.50	>0.9999	-24.80	>0.9999	9.25	>0.9999	9.06	>0.9999
13 vs. 17	PT vs. LCL	-4.31	>0.9999	37.93	>0.9999	50.43	>0.9999	-2.96	>0.9999	49.55	>0.9999	22.00	>0.9999
14 vs. 15	ACL vs. PCL	-7.44	>0.9999	-27.44	>0.9999	-38.64	>0.9999			-56.06	0.2454	-16.13	>0.9999
14 vs. 16	ACL vs. MCL	-1.00	>0.9999	0.19	>0.9999	-43.50	>0.9999			-17.69	>0.9999	-7.06	>0.9999
14 vs. 17	ACL vs. LCL	22.69	>0.9999	35.87	>0.9999	-10.57	>0.9999			22.62	>0.9999	5.88	>0.9999
15 vs. 16	PCL vs. MCL	6.44	>0.9999	27.63	>0.9999	-4.86	>0.9999	-8.21	>0.9999	38.38	>0.9999	9.06	>0.9999
15 vs. 17	PCL vs. LCL	30.13	>0.9999	63.30	0.0421	28.07	>0.9999	13.63	>0.9999	78.68	0.0032	22.00	>0.9999
16 vs. 17	MCL vs. LCL	23.69	>0.9999	35.68	>0.9999	32.93	>0.9999	21.83	>0.9999	40.30	>0.9999	12.94	>0.9999

Supp. Table 9.7.d Pairwise comparisons for lower limb tendon/ligament interfascicular region (IFR) characteristics. *Green cells indicate significant p-values (< 0.05).*

Tissue Comparison		Interfascicular Region Frewuency				Interfascicular Region (IFR) Type Proportion			
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval	
1 vs. 2	AT vs. Plt	-2.675	0.9996	-7.660	2.309	15.310	>0.9999	-42.255	72.876
1 vs. 3	AT vs. TP	0.458	>0.9999	-4.138	5.055	-9.647	>0.9999	-45.816	26.522
1 vs. 4	AT vs. TA	-1.602	>0.9999	-6.198	2.995	-27.338	0.6256	-63.507	8.830
1 vs. 5	AT vs. FL	-1.034	>0.9999	-5.630	3.563	-17.677	>0.9999	-53.845	18.492
1 vs. 6	AT vs. FB	3.417	0.6744	-1.180	8.014	6.363	>0.9999	-29.806	42.532
1 vs. 7	AT vs. FDL	0.807	>0.9999	-3.790	5.403	-7.018	>0.9999	-43.187	29.151
1 vs. 8	AT vs. FHL	3.372	0.7114	-1.224	7.969	7.148	>0.9999	-29.020	43.317
1 vs. 9	AT vs. EHL	0.757	>0.9999	-3.840	5.354	-26.124	0.7528	-62.293	10.044
1 vs. 10	AT vs. ST	0.903	>0.9999	-3.694	5.499	-3.028	>0.9999	-39.197	33.141
1 vs. 11	AT vs. GT	0.805	>0.9999	-3.792	5.402	8.246	>0.9999	-27.923	44.415
1 vs. 12	AT vs. QT	2.358	0.9999	-2.238	6.955	18.029	>0.9999	-18.140	54.197
1 vs. 13	AT vs. PT	2.204	>0.9999	-2.392	6.801	-4.379	>0.9999	-40.548	31.789
1 vs. 14	AT vs. ACL	2.927	0.9609	-1.669	7.524	10.065	>0.9999	-26.104	46.233
1 vs. 15	AT vs. PCL	5.372	0.0044	0.775	9.968	17.444	>0.9999	-18.725	53.613
1 vs. 16	AT vs. MCL	8.792	<0.0001	4.195	13.389	12.782	>0.9999	-23.387	48.951
1 vs. 17	AT vs. LCL	3.517	0.6931	-1.246	8.281	-0.162	>0.9999	-37.642	37.317
2 vs. 3	Plt vs. TP	3.134	0.9698	-1.851	8.119	-24.957	>0.9999	-82.523	32.608
2 vs. 4	Plt vs. TA	1.074	>0.9999	-3.911	6.059	-42.648	0.6839	-100.214	14.917
2 vs. 5	Plt vs. FL	1.642	>0.9999	-3.343	6.627	-32.987	0.9969	-90.552	24.579
2 vs. 6	Plt vs. FB	6.092	0.0019	1.107	11.077	-8.947	>0.9999	-66.513	48.618
2 vs. 7	Plt vs. FDL	3.482	0.8314	-1.503	8.467	-22.328	>0.9999	-79.894	35.237
2 vs. 8	Plt vs. FHL	6.048	0.0022	1.063	11.032	-8.162	>0.9999	-65.728	49.404
2 vs. 9	Plt vs. EHL	3.432	0.8601	-1.553	8.417	-41.435	0.7617	-99.000	16.131
2 vs. 10	Plt vs. ST	3.578	0.7687	-1.407	8.563	-18.338	>0.9999	-75.904	39.227
2 vs. 11	Plt vs. GT	3.480	0.8325	-1.505	8.465	-7.064	>0.9999	-64.630	50.501
2 vs. 12	Plt vs. QT	5.034	0.0439	0.049	10.019	2.719	>0.9999	-54.847	60.284
2 vs. 13	Plt vs. PT	4.880	0.0658	-0.105	9.865	-19.690	>0.9999	-77.255	37.876
2 vs. 14	Plt vs. ACL	5.603	0.0087	0.618	10.588	-5.246	>0.9999	-62.811	52.320
2 vs. 15	Plt vs. PCL	8.047	<0.0001	3.062	13.032	2.134	>0.9999	-55.432	59.700
2 vs. 16	Plt vs. MCL	11.467	<0.0001	6.483	16.452	-2.528	>0.9999	-60.094	55.038
2 vs. 17	Plt vs. LCL	6.193	0.0026	1.045	11.340	-15.472	>0.9999	-73.866	42.922
3 vs. 4	TP vs. TA	-2.060	>0.9999	-6.657	2.537	-17.691	>0.9999	-53.860	18.478
3 vs. 5	TP vs. FL	-1.492	>0.9999	-6.089	3.105	-8.029	>0.9999	-44.198	28.139
3 vs. 6	TP vs. FB	2.958	0.9522	-1.638	7.555	16.010	>0.9999	-20.159	52.179
3 vs. 7	TP vs. FDL	0.348	>0.9999	-4.249	4.945	2.629	>0.9999	-33.540	38.798
3 vs. 8	TP vs. FHL	2.914	0.9643	-1.683	7.511	16.795	>0.9999	-19.373	52.964
3 vs. 9	TP vs. EHL	0.298	>0.9999	-4.298	4.895	-16.477	>0.9999	-52.646	19.691
3 vs. 10	TP vs. ST	0.444	>0.9999	-4.153	5.041	6.619	>0.9999	-29.550	42.788
3 vs. 11	TP vs. GT	0.346	>0.9999	-4.250	4.943	17.893	>0.9999	-18.276	54.062
3 vs. 12	TP vs. QT	1.900	>0.9999	-2.697	6.497	27.676	0.5889	-8.493	63.845
3 vs. 13	TP vs. PT	1.746	>0.9999	-2.851	6.343	5.268	>0.9999	-30.901	41.437
3 vs. 14	TP vs. ACL	2.469	0.9996	-2.128	7.066	19.712	0.9994	-16.457	55.881
3 vs. 15	TP vs. PCL	4.913	0.0195	0.316	9.510	27.091	0.6524	-9.077	63.260
3 vs. 16	TP vs. MCL	8.334	<0.0001	3.737	12.930	22.429	0.9773	-13.740	58.598
3 vs. 17	TP vs. LCL	3.059	0.9541	-1.704	7.822	9.485	>0.9999	-27.995	46.965
4 vs. 5	TA vs. FL	0.568	>0.9999	-4.029	5.165	9.662	>0.9999	-26.507	45.830
4 vs. 6	TA vs. FB	5.018	0.0140	0.422	9.615	33.701	0.1180	-2.467	69.870
4 vs. 7	TA vs. FDL	2.408	0.9998	-2.189	7.005	20.320	0.9983	-15.848	56.489
4 vs. 8	TA vs. FHL	4.974	0.0161	0.377	9.571	34.487	0.0906	-1.682	70.655
4 vs. 9	TA vs. EHL	2.359	0.9999	-2.238	6.955	1.214	>0.9999	-34.955	37.383
4 vs. 10	TA vs. ST	2.504	0.9994	-2.093	7.101	24.310	0.9009	-11.858	60.479
4 vs. 11	TA vs. GT	2.406	0.9999	-2.190	7.003	35.584	0.0617	-0.585	71.753
4 vs. 12	TA vs. QT	3.960	0.2580	-0.637	8.557	45.367	0.0011	9.198	81.536
4 vs. 13	TA vs. PT	3.806	0.3569	-0.791	8.403	22.959	0.9633	-13.210	59.128
4 vs. 14	TA vs. ACL	4.529	0.0606	-0.068	9.126	37.403	0.0316	1.234	73.572
4 vs. 15	TA vs. PCL	6.973	<0.0001	2.376	11.570	44.782	0.0015	8.614	80.951
4 vs. 16	TA vs. MCL	10.394	<0.0001	5.797	14.990	40.120	0.0108	3.952	76.289
4 vs. 17	TA vs. LCL	5.119	0.0179	0.356	9.882	27.176	0.7427	-10.303	64.656
5 vs. 6	FL vs. FB	4.451	0.0754	-0.146	9.047	24.040	0.9168	-12.129	60.208
5 vs. 7	FL vs. FDL	1.840	>0.9999	-2.757	6.437	10.658	>0.9999	-25.510	46.827
5 vs. 8	FL vs. FHL	4.406	0.0852	-0.191	9.003	24.825	0.8660	-11.344	60.994
5 vs. 9	FL vs. EHL	1.791	>0.9999	-2.806	6.387	-8.448	>0.9999	-44.617	27.721
5 vs. 10	FL vs. ST	1.936	>0.9999	-2.661	6.533	14.649	>0.9999	-21.520	50.817
5 vs. 11	FL vs. GT	1.839	>0.9999	-2.758	6.435	25.922	0.7724	-10.246	62.091
5 vs. 12	FL vs. QT	3.392	0.6952	-1.205	7.989	35.705	0.0591	-0.463	71.874
5 vs. 13	FL vs. PT	3.238	0.8131	-1.359	7.835	13.297	>0.9999	-22.872	49.466
5 vs. 14	FL vs. ACL	3.961	0.2573	-0.636	8.558	27.741	0.5817	-8.428	63.910
5 vs. 15	FL vs. PCL	6.405	0.0001	1.809	11.002	35.121	0.0727	-1.048	71.289
5 vs. 16	FL vs. MCL	9.826	<0.0001	5.229	14.423	30.459	0.3125	-5.710	66.627
5 vs. 17	FL vs. LCL	4.551	0.0884	-0.212	9.314	17.514	>0.9999	-19.965	54.994
6 vs. 7	FB vs. FDL	-2.610	0.9976	-7.207	1.986	-13.381	>0.9999	-49.550	22.788
6 vs. 8	FB vs. FHL	-0.045	>0.9999	-4.641	4.552	0.785	>0.9999	-35.384	36.954
6 vs. 9	FB vs. EHL	-2.660	0.9959	-7.257	1.937	-32.487	0.1740	-68.656	3.681
6 vs. 10	FB vs. ST	-2.514	0.9993	-7.111	2.082	-9.391	>0.9999	-45.560	26.778
6 vs. 11	FB vs. GT	-2.612	0.9976	-7.209	1.985	1.883	>0.9999	-34.286	38.052
6 vs. 12	FB vs. QT	-1.059	>0.9999	-5.655	3.538	11.666	>0.9999	-24.503	47.834
6 vs. 13	FB vs. PT	-1.213	>0.9999	-5.809	3.384	-10.742	>0.9999	-46.911	25.426
6 vs. 14	FB vs. ACL	-0.489	>0.9999	-5.086	4.107	3.702	>0.9999	-32.467	39.870
6 vs. 15	FB vs. PCL	1.955	>0.9999	-2.642	6.551	11.081	>0.9999	-25.088	47.250
6 vs. 16	FB vs. MCL	5.375	0.0044	0.778	9.972	6.419	>0.9999	-29.750	42.588
6 vs. 17	FB vs. LCL	0.100	>0.9999	-4.663	4.864	-6.525	>0.9999	-44.005	30.954
7 vs. 8	FDL vs. FHL	2.566	0.9986	-2.031	7.162	14.166	>0.9999	-22.002	50.335

Tissue Comparison		Interfascicular Region Frewuency				Interfascicular Region (IFR) Type Proportion			
Graph position	Abbreviation	Contrast	Adjust. P value	95% Confidence Interval		Contrast	Adjust. P value	95% Confidence Interval	
7 vs. 9	FDL vs. EHL	-0.050	>0.9999	-4.646	4.547	-19.106	0.9998	-55.275	17.062
7 vs. 10	FDL vs. ST	0.096	>0.9999	-4.501	4.693	3.990	>0.9999	-32.179	40.159
7 vs. 11	FDL vs. GT	-0.002	>0.9999	-4.598	4.595	15.264	>0.9999	-20.905	51.433
7 vs. 12	FDL vs. QT	1.552	>0.9999	-3.045	6.149	25.047	0.8490	-11.122	61.216
7 vs. 13	FDL vs. PT	1.398	>0.9999	-3.199	5.995	2.639	>0.9999	-33.530	38.807
7 vs. 14	FDL vs. ACL	2.121	>0.9999	-2.476	6.718	17.083	>0.9999	-19.086	53.251
7 vs. 15	FDL vs. PCL	4.565	0.0547	-0.032	9.162	24.462	0.8912	-11.707	60.631
7 vs. 16	FDL vs. MCL	7.986	<0.0001	3.389	12.582	19.800	0.9993	-16.369	55.969
7 vs. 17	FDL vs. LCL	2.711	0.9975	-2.052	7.474	6.856	>0.9999	-30.624	44.335
8 vs. 9	FHL vs. EHL	-2.615	0.9975	-7.212	1.981	-33.273	0.1357	-69.441	2.896
8 vs. 10	FHL vs. ST	-2.470	0.9996	-7.066	2.127	-10.176	>0.9999	-46.345	25.992
8 vs. 11	FHL vs. GT	-2.567	0.9986	-7.164	2.029	1.098	>0.9999	-35.071	37.266
8 vs. 12	FHL vs. QT	-1.014	>0.9999	-5.611	3.583	10.880	>0.9999	-25.288	47.049
8 vs. 13	FHL vs. PT	-1.168	>0.9999	-5.765	3.429	-11.528	>0.9999	-47.696	24.641
8 vs. 14	FHL vs. ACL	-0.445	>0.9999	-5.042	4.152	2.916	>0.9999	-33.252	39.085
8 vs. 15	FHL vs. PCL	1.999	>0.9999	-2.597	6.596	10.296	>0.9999	-25.873	46.465
8 vs. 16	FHL vs. MCL	5.420	0.0037	0.823	10.017	5.634	>0.9999	-30.535	41.803
8 vs. 17	FHL vs. LCL	0.145	>0.9999	-4.618	4.908	-7.310	>0.9999	-44.790	30.169
9 vs. 10	EHL vs. ST	0.146	>0.9999	-4.451	4.742	23.096	0.9588	-13.072	59.265
9 vs. 11	EHL vs. GT	0.048	>0.9999	-4.549	4.645	34.370	0.0943	-1.798	70.539
9 vs. 12	EHL vs. QT	1.601	>0.9999	-2.995	6.198	44.153	0.0019	7.984	80.322
9 vs. 13	EHL vs. PT	1.447	>0.9999	-3.149	6.044	21.745	0.9889	-14.424	57.914
9 vs. 14	EHL vs. ACL	2.170	>0.9999	-2.426	6.767	36.189	0.0496	0.020	72.358
9 vs. 15	EHL vs. PCL	4.615	0.0475	0.018	9.211	43.569	0.0025	7.400	79.737
9 vs. 16	EHL vs. MCL	8.035	<0.0001	3.438	12.632	38.906	0.0176	2.738	75.075
9 vs. 17	EHL vs. LCL	2.760	0.9957	-2.003	7.524	25.962	0.8485	-11.517	63.442
10 vs. 11	ST vs. GT	-0.098	>0.9999	-4.694	4.499	11.274	>0.9999	-24.895	47.443
10 vs. 12	ST vs. QT	1.456	>0.9999	-3.141	6.053	21.057	0.9952	-15.112	57.225
10 vs. 13	ST vs. PT	1.302	>0.9999	-3.295	5.899	-1.351	>0.9999	-37.520	34.817
10 vs. 14	ST vs. ACL	2.025	>0.9999	-2.572	6.622	13.093	>0.9999	-23.076	49.261
10 vs. 15	ST vs. PCL	4.469	0.0717	-0.128	9.066	20.472	0.9979	-15.697	56.641
10 vs. 16	ST vs. MCL	7.890	<0.0001	3.293	12.486	15.810	>0.9999	-20.359	51.979
10 vs. 17	ST vs. LCL	2.615	0.9992	-2.148	7.378	2.866	>0.9999	-34.614	40.345
11 vs. 12	GT vs. QT	1.553	>0.9999	-3.043	6.150	9.783	>0.9999	-26.386	45.952
11 vs. 13	GT vs. PT	1.400	>0.9999	-3.197	5.996	-12.625	>0.9999	-48.794	23.544
11 vs. 14	GT vs. ACL	2.123	>0.9999	-2.474	6.719	1.819	>0.9999	-34.350	37.987
11 vs. 15	GT vs. PCL	4.567	0.0545	-0.030	9.164	9.198	>0.9999	-26.970	45.367
11 vs. 16	GT vs. MCL	7.987	<0.0001	3.390	12.584	4.536	>0.9999	-31.633	40.705
11 vs. 17	GT vs. LCL	2.712	0.9974	-2.051	7.476	-8.408	>0.9999	-45.888	29.072
12 vs. 13	QT vs. PT	-0.154	>0.9999	-4.751	4.443	-22.408	0.9778	-58.577	13.761
12 vs. 14	QT vs. ACL	0.569	>0.9999	-4.028	5.166	-7.964	>0.9999	-44.133	28.205
12 vs. 15	QT vs. PCL	3.013	0.9338	-1.583	7.610	-0.585	>0.9999	-36.753	35.584
12 vs. 16	QT vs. MCL	6.434	0.0001	1.837	11.031	-5.247	>0.9999	-41.415	30.922
12 vs. 17	QT vs. LCL	1.159	>0.9999	-3.604	5.922	-18.191	>0.9999	-55.670	19.289
13 vs. 14	PT vs. ACL	0.723	>0.9999	-3.874	5.320	14.444	>0.9999	-21.725	50.613
13 vs. 15	PT vs. PCL	3.167	0.8588	-1.430	7.764	21.823	0.9879	-14.345	57.992
13 vs. 16	PT vs. MCL	6.588	<0.0001	1.991	11.184	17.161	>0.9999	-19.007	53.330
13 vs. 17	PT vs. LCL	1.313	>0.9999	-3.450	6.076	4.217	>0.9999	-33.262	41.697
14 vs. 15	ACL vs. PCL	2.444	0.9997	-2.153	7.041	7.379	>0.9999	-28.789	43.548
14 vs. 16	ACL vs. MCL	5.865	0.0008	1.268	10.461	2.717	>0.9999	-33.451	38.886
14 vs. 17	ACL vs. LCL	0.590	>0.9999	-4.173	5.353	-10.227	>0.9999	-47.706	27.253
15 vs. 16	PCL vs. MCL	3.420	0.6714	-1.176	8.017	-4.662	>0.9999	-40.831	31.507
15 vs. 17	PCL vs. LCL	-1.854	>0.9999	-6.617	2.909	-17.606	>0.9999	-55.086	19.873
16 vs. 17	MCL vs. LCL	-5.275	0.0111	-10.038	-0.512	-12.944	>0.9999	-50.424	24.535

Supp. Table 9.7.e Pairwise comparisons for lower limb tendon/ligament crimp characteristics. *Green cells indicate significant p-values (< 0.05).*

Tissue Comparison		Crimp Absence		Crimp Wavelength		Crimp Wavelength Variation	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
1 vs. 2	AT vs. Plt	0.00	>0.9999	-42.58	>0.9999	42.08	>0.9999
1 vs. 3	AT vs. TP	-16.25	>0.9999	-7.06	>0.9999	51.75	0.7205
1 vs. 4	AT vs. TA	0.00	>0.9999	-51.19	0.8606	44.50	>0.9999
1 vs. 5	AT vs. FL	-8.13	>0.9999	-24.50	>0.9999	46.63	>0.9999
1 vs. 6	AT vs. FB	-24.38	>0.9999	7.50	>0.9999	32.50	>0.9999
1 vs. 7	AT vs. FDL	-8.13	>0.9999	-52.00	0.7539	25.38	>0.9999
1 vs. 8	AT vs. FHL	-16.25	>0.9999	-19.69	>0.9999	61.13	0.1346
1 vs. 9	AT vs. EHL	0.00	>0.9999	-32.75	>0.9999	51.25	0.7828
1 vs. 10	AT vs. ST	-8.13	>0.9999	-57.75	0.2812	65.75	0.0539
1 vs. 11	AT vs. GT	0.00	>0.9999	-44.63	>0.9999	59.25	0.1919
1 vs. 12	AT vs. QT	-58.88	0.0053	-16.06	>0.9999	13.50	>0.9999
1 vs. 13	AT vs. PT	0.00	>0.9999	-37.25	>0.9999	15.63	>0.9999
1 vs. 14	AT vs. ACL	-24.38	>0.9999	29.75	>0.9999	4.50	>0.9999
1 vs. 15	AT vs. PCL	-40.63	0.6166	29.75	>0.9999	7.13	>0.9999
1 vs. 16	AT vs. MCL	-44.63	0.2477	23.75	>0.9999	-0.50	>0.9999
1 vs. 17	AT vs. LCL	-18.57	>0.9999	34.89	>0.9999	10.96	>0.9999
2 vs. 3	Plt vs. TP	-16.25	>0.9999	35.52	>0.9999	9.67	>0.9999
2 vs. 4	Plt vs. TA	0.00	>0.9999	-8.60	>0.9999	2.42	>0.9999
2 vs. 5	Plt vs. FL	-8.13	>0.9999	18.08	>0.9999	4.54	>0.9999
2 vs. 6	Plt vs. FB	-24.38	>0.9999	50.08	>0.9999	-9.58	>0.9999
2 vs. 7	Plt vs. FDL	-8.13	>0.9999	-9.42	>0.9999	-16.71	>0.9999
2 vs. 8	Plt vs. FHL	-16.25	>0.9999	22.90	>0.9999	19.04	>0.9999
2 vs. 9	Plt vs. EHL	0.00	>0.9999	9.83	>0.9999	9.17	>0.9999
2 vs. 10	Plt vs. ST	-8.13	>0.9999	-15.17	>0.9999	23.67	>0.9999
2 vs. 11	Plt vs. GT	0.00	>0.9999	-2.04	>0.9999	17.17	>0.9999
2 vs. 12	Plt vs. QT	-58.88	0.0190	26.52	>0.9999	-28.58	>0.9999
2 vs. 13	Plt vs. PT	0.00	>0.9999	5.33	>0.9999	-26.46	>0.9999
2 vs. 14	Plt vs. ACL	-24.38	>0.9999	72.33	0.0482	-37.58	>0.9999
2 vs. 15	Plt vs. PCL	-40.63	>0.9999	72.33	0.0482	-34.96	>0.9999
2 vs. 16	Plt vs. MCL	-44.63	0.5296	66.33	0.1434	-42.58	>0.9999
2 vs. 17	Plt vs. LCL	-18.57	>0.9999	77.48	0.0278	-31.12	>0.9999
3 vs. 4	TP vs. TA	16.25	>0.9999	-44.13	>0.9999	-7.25	>0.9999
3 vs. 5	TP vs. FL	8.13	>0.9999	-17.44	>0.9999	-5.13	>0.9999
3 vs. 6	TP vs. FB	-8.13	>0.9999	14.56	>0.9999	-19.25	>0.9999
3 vs. 7	TP vs. FDL	8.13	>0.9999	-44.94	>0.9999	-26.38	>0.9999
3 vs. 8	TP vs. FHL	0.00	>0.9999	-12.63	>0.9999	9.38	>0.9999
3 vs. 9	TP vs. EHL	16.25	>0.9999	-25.69	>0.9999	-0.50	>0.9999
3 vs. 10	TP vs. ST	8.13	>0.9999	-50.69	0.9328	14.00	>0.9999
3 vs. 11	TP vs. GT	16.25	>0.9999	-37.56	>0.9999	7.50	>0.9999
3 vs. 12	TP vs. QT	-42.63	0.3944	-9.00	>0.9999	-38.25	>0.9999
3 vs. 13	TP vs. PT	16.25	>0.9999	-30.19	>0.9999	-36.13	>0.9999
3 vs. 14	TP vs. ACL	-8.13	>0.9999	36.81	>0.9999	-47.25	>0.9999
3 vs. 15	TP vs. PCL	-24.38	>0.9999	36.81	>0.9999	-44.63	>0.9999
3 vs. 16	TP vs. MCL	-28.38	>0.9999	30.81	>0.9999	-52.25	0.6628
3 vs. 17	TP vs. LCL	-2.32	>0.9999	41.96	>0.9999	-40.79	>0.9999
4 vs. 5	TA vs. FL	-8.13	>0.9999	26.69	>0.9999	2.13	>0.9999
4 vs. 6	TA vs. FB	-24.38	>0.9999	58.69	0.2375	-12.00	>0.9999
4 vs. 7	TA vs. FDL	-8.13	>0.9999	-0.81	>0.9999	-19.13	>0.9999
4 vs. 8	TA vs. FHL	-16.25	>0.9999	31.50	>0.9999	16.63	>0.9999
4 vs. 9	TA vs. EHL	0.00	>0.9999	18.44	>0.9999	6.75	>0.9999
4 vs. 10	TA vs. ST	-8.13	>0.9999	-6.56	>0.9999	21.25	>0.9999
4 vs. 11	TA vs. GT	0.00	>0.9999	6.56	>0.9999	14.75	>0.9999
4 vs. 12	TA vs. QT	-58.88	0.0053	35.13	>0.9999	-31.00	>0.9999
4 vs. 13	TA vs. PT	0.00	>0.9999	13.94	>0.9999	-28.88	>0.9999
4 vs. 14	TA vs. ACL	-24.38	>0.9999	80.94	0.0022	-40.00	>0.9999
4 vs. 15	TA vs. PCL	-40.63	0.6166	80.94	0.0022	-37.38	>0.9999
4 vs. 16	TA vs. MCL	-44.63	0.2477	74.94	0.0087	-45.00	>0.9999
4 vs. 17	TA vs. LCL	-18.57	>0.9999	86.08	0.0012	-33.54	>0.9999
5 vs. 6	FL vs. FB	-16.25	>0.9999	32.00	>0.9999	-14.13	>0.9999
5 vs. 7	FL vs. FDL	0.00	>0.9999	-27.50	>0.9999	-21.25	>0.9999
5 vs. 8	FL vs. FHL	-8.13	>0.9999	4.81	>0.9999	14.50	>0.9999
5 vs. 9	FL vs. EHL	8.13	>0.9999	-8.25	>0.9999	4.63	>0.9999
5 vs. 10	FL vs. ST	0.00	>0.9999	-33.25	>0.9999	19.13	>0.9999
5 vs. 11	FL vs. GT	8.13	>0.9999	-20.13	>0.9999	12.63	>0.9999
5 vs. 12	FL vs. QT	-50.75	0.0532	8.44	>0.9999	-33.13	>0.9999
5 vs. 13	FL vs. PT	8.13	>0.9999	-12.75	>0.9999	-31.00	>0.9999
5 vs. 14	FL vs. ACL	-16.25	>0.9999	54.25	0.5179	-42.13	>0.9999
5 vs. 15	FL vs. PCL	-32.50	>0.9999	54.25	0.5179	-39.50	>0.9999
5 vs. 16	FL vs. MCL	-36.50	>0.9999	48.25	>0.9999	-47.13	>0.9999
5 vs. 17	FL vs. LCL	-10.45	>0.9999	59.39	0.3005	-35.66	>0.9999
6 vs. 7	FB vs. FDL	16.25	>0.9999	-59.50	0.2047	-7.13	>0.9999
6 vs. 8	FB vs. FHL	8.13	>0.9999	-27.19	>0.9999	28.63	>0.9999
6 vs. 9	FB vs. EHL	24.38	>0.9999	-40.25	>0.9999	18.75	>0.9999
6 vs. 10	FB vs. ST	16.25	>0.9999	-65.25	0.0681	33.25	>0.9999
6 vs. 11	FB vs. GT	24.38	>0.9999	-52.13	0.7386	26.75	>0.9999
6 vs. 12	FB vs. QT	-34.50	>0.9999	-23.56	>0.9999	-19.00	>0.9999
6 vs. 13	FB vs. PT	24.38	>0.9999	-44.75	>0.9999	-16.88	>0.9999
6 vs. 14	FB vs. ACL	0.00	>0.9999	22.25	>0.9999	-28.00	>0.9999
6 vs. 15	FB vs. PCL	-16.25	>0.9999	22.25	>0.9999	-25.38	>0.9999
6 vs. 16	FB vs. MCL	-20.25	>0.9999	16.25	>0.9999	-33.00	>0.9999
6 vs. 17	FB vs. LCL	5.80	>0.9999	27.39	>0.9999	-21.54	>0.9999
7 vs. 8	FDL vs. FHL	-8.13	>0.9999	32.31	>0.9999	35.75	>0.9999

Tissue Comparison		Crimp Absence		Crimp Wavelength		Crimp Wavelength Variation	
Graph position	Abbreviation	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value	Mean rank diff.	Adjust. P value
7 vs. 9	FDL vs. EHL	8.13	>0.9999	19.25	>0.9999	25.88	>0.9999
7 vs. 10	FDL vs. ST	0.00	>0.9999	-5.75	>0.9999	40.38	>0.9999
7 vs. 11	FDL vs. GT	8.13	>0.9999	7.38	>0.9999	33.88	>0.9999
7 vs. 12	FDL vs. QT	-50.75	0.0532	35.94	>0.9999	-11.88	>0.9999
7 vs. 13	FDL vs. PT	8.13	>0.9999	14.75	>0.9999	-9.75	>0.9999
7 vs. 14	FDL vs. ACL	-16.25	>0.9999	81.75	0.0018	-20.88	>0.9999
7 vs. 15	FDL vs. PCL	-32.50	>0.9999	81.75	0.0018	-18.25	>0.9999
7 vs. 16	FDL vs. MCL	-36.50	>0.9999	75.75	0.0073	-25.88	>0.9999
7 vs. 17	FDL vs. LCL	-10.45	>0.9999	86.89	0.0010	-14.41	>0.9999
8 vs. 9	FHL vs. EHL	16.25	>0.9999	-13.06	>0.9999	-9.88	>0.9999
8 vs. 10	FHL vs. ST	8.13	>0.9999	-38.06	>0.9999	4.63	>0.9999
8 vs. 11	FHL vs. GT	16.25	>0.9999	-24.94	>0.9999	-1.88	>0.9999
8 vs. 12	FHL vs. QT	-42.63	0.3944	3.63	>0.9999	-47.63	>0.9999
8 vs. 13	FHL vs. PT	16.25	>0.9999	-17.56	>0.9999	-45.50	>0.9999
8 vs. 14	FHL vs. ACL	-8.13	>0.9999	49.44	>0.9999	-56.63	0.3102
8 vs. 15	FHL vs. PCL	-24.38	>0.9999	49.44	>0.9999	-54.00	0.4922
8 vs. 16	FHL vs. MCL	-28.38	>0.9999	43.44	>0.9999	-61.63	0.1222
8 vs. 17	FHL vs. LCL	-2.32	>0.9999	54.58	0.6685	-50.16	>0.9999
9 vs. 10	EHL vs. ST	-8.13	>0.9999	-25.00	>0.9999	14.50	>0.9999
9 vs. 11	EHL vs. GT	0.00	>0.9999	-11.88	>0.9999	8.00	>0.9999
9 vs. 12	EHL vs. QT	-58.88	0.0053	16.69	>0.9999	-37.75	>0.9999
9 vs. 13	EHL vs. PT	0.00	>0.9999	-4.50	>0.9999	-35.63	>0.9999
9 vs. 14	EHL vs. ACL	-24.38	>0.9999	62.50	0.1166	-46.75	>0.9999
9 vs. 15	EHL vs. PCL	-40.63	0.6166	62.50	0.1166	-44.13	>0.9999
9 vs. 16	EHL vs. MCL	-44.63	0.2477	56.50	0.3511	-51.75	0.7205
9 vs. 17	EHL vs. LCL	-18.57	>0.9999	67.64	0.0668	-40.29	>0.9999
10 vs. 11	ST vs. GT	8.13	>0.9999	13.13	>0.9999	-6.50	>0.9999
10 vs. 12	ST vs. QT	-50.75	0.0532	41.69	>0.9999	-52.25	0.6628
10 vs. 13	ST vs. PT	8.13	>0.9999	20.50	>0.9999	-50.13	0.9408
10 vs. 14	ST vs. ACL	-16.25	>0.9999	87.50	0.0004	-61.25	0.1314
10 vs. 15	ST vs. PCL	-32.50	>0.9999	87.50	0.0004	-58.63	0.2155
10 vs. 16	ST vs. MCL	-36.50	>0.9999	81.50	0.0019	-66.25	0.0486
10 vs. 17	ST vs. LCL	-10.45	>0.9999	92.64	0.0002	-54.79	0.5912
11 vs. 12	GT vs. QT	-58.88	0.0053	28.56	>0.9999	-45.75	>0.9999
11 vs. 13	GT vs. PT	0.00	>0.9999	7.38	>0.9999	-43.63	>0.9999
11 vs. 14	GT vs. ACL	-24.38	>0.9999	74.38	0.0099	-54.75	0.4322
11 vs. 15	GT vs. PCL	-40.63	0.6166	74.38	0.0099	-52.13	0.6768
11 vs. 16	GT vs. MCL	-44.63	0.2477	68.38	0.0361	-59.75	0.1747
11 vs. 17	GT vs. LCL	-18.57	>0.9999	79.52	0.0057	-48.29	>0.9999
12 vs. 13	QT vs. PT	58.88	0.0053	-21.19	>0.9999	2.13	>0.9999
12 vs. 14	QT vs. ACL	34.50	>0.9999	45.81	>0.9999	-9.00	>0.9999
12 vs. 15	QT vs. PCL	18.25	>0.9999	45.81	>0.9999	-6.38	>0.9999
12 vs. 16	QT vs. MCL	14.25	>0.9999	39.81	>0.9999	-14.00	>0.9999
12 vs. 17	QT vs. LCL	40.30	0.8865	50.96	>0.9999	-2.54	>0.9999
13 vs. 14	PT vs. ACL	-24.38	>0.9999	67.00	0.0479	-11.13	>0.9999
13 vs. 15	PT vs. PCL	-40.63	0.6166	67.00	0.0479	-8.50	>0.9999
13 vs. 16	PT vs. MCL	-44.63	0.2477	61.00	0.1549	-16.13	>0.9999
13 vs. 17	PT vs. LCL	-18.57	>0.9999	72.14	0.0274	-4.66	>0.9999
14 vs. 15	ACL vs. PCL	-16.25	>0.9999	0.00	>0.9999	2.63	>0.9999
14 vs. 16	ACL vs. MCL	-20.25	>0.9999	-6.00	>0.9999	-5.00	>0.9999
14 vs. 17	ACL vs. LCL	5.80	>0.9999	5.14	>0.9999	6.46	>0.9999
15 vs. 16	PCL vs. MCL	-4.00	>0.9999	-6.00	>0.9999	-7.63	>0.9999
15 vs. 17	PCL vs. LCL	22.05	>0.9999	5.14	>0.9999	3.84	>0.9999
16 vs. 17	MCL vs. LCL	26.05	>0.9999	11.14	>0.9999	11.46	>0.9999

THE END

"Wowee Batsam, that was an adventure!"

~ (2024) ~

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