Skeletal Muscle c-Jun N-terminal Kinase (JNK) Activity after Acute Resistive Exercise in Elder Adults with Type 2 Diabetes: Metabolic and Clinical Correlates

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This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Applied Science (Exercise and Sports Science), for the University of Sydney, NSW Australia

Discipline of Exercise and Sport Science, Faculty of Health Sciences, University of Sydney. August, 2010
Supervisor’s Declaration

This is to certify that the thesis entitled “Skeletal Muscle c-Jun N-terminal Kinase (JNK) Activity after Acute Resistive Exercise in Elder Adults with Type 2”, submitted by Qianyu Guo in fulfillment of the degree of Master of Applied Science (Exercise and Sport Science), is ready for submission.

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August 31st, 2010

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August 31st, 2010
Author Declaration

I hereby declare that this thesis is entirely my own work. This thesis does not, to the best of my knowledge, contain any material from any other source, except where due reference is made. This thesis was written completely and solely for the degree of Master of Applied Science, and has not been submitted for a higher degree or diploma at any other academic institution.

Qianyu Guo

August 31st, 2010
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CHAPTER 1
Introduction
Diabetes mellitus (DM) is a disorder primarily defined by the level of hyperglycaemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy)\(^1\). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life\(^1\).

**The Epidemiology of Type 2 Diabetes**

According to its etiology, DM can be classified into type 1 diabetes, type 2 diabetes (T2D), gestational diabetes and other specific categories\(^2\). Type 1 diabetes is a result of autoimmune destruction of pancreatic \(\beta\)-cells, which leads to absolute insulin insufficiency\(^3\). In comparison, T2D is the end of a series of metabolic disturbances, starting with insulin resistance (IR), in which the body can’t use insulin efficiently, then proceeding to hyperglycemia, and finally T2D\(^4\). Approximately 90% of diagnosed diabetes is T2D and approximately half subjects remain un-diagnosed\(^4-5\). The total number of T2D subjects is expected to grow exponentially in the future due to physical inactivity and obesity\(^4-8\).

Most T2D is accompanied by metabolic syndrome, which includes the following disorders: abdominal obesity; atherogenic dyslipidemia; elevated blood pressure; insulin resistance or glucose intolerance; prothrombotic state; proinflammatory state\(^9\). People with the metabolic syndrome are at increased risk of T2D\(^9\). The dominant underlying risk factors for this syndrome are abdominal obesity and insulin resistance\(^9\). This is why
the metabolic syndrome is also called the insulin resistance syndrome\textsuperscript{9}. Type 2 diabetes were primarily diagnosed in older adults in the past. However, the proportion of overweight and obesity in children and young adults in the population worldwide is increasing rapidly; hence a growing number of children and young adolescence are now being diagnosed with T2D\textsuperscript{10} It is estimated that 285 million people around the world have diagnosed diabetes currently. This number is still increasing: 7 million additional people are diagnosed with T2D each year. It is projected that T2D will affect 438 million people globally in 2029\textsuperscript{11}. Thus, efforts to better understand and prevent the rapid expansion of this syndrome are critically needed.

\textit{Exercise & Type 2 Diabetes}

For decades, exercise has been considered as the cornerstone of diabetes management, along with medication and diet. Increased physical activity can reduce the incidence of metabolic syndrome and T2D\textsuperscript{7}. Additionally, lifestyle modification including regular physical activity with or without medication and dietary changes have proven efficacy in T2D\textsuperscript{7}. The benefits of exercise in the management of T2D include improved glycaemic control and insulin sensitivity, decreased body fat content (especially visceral fat content), improved blood pressure control and composition of blood lipids, and reduced chronic inflammation\textsuperscript{12-14}.

\textit{c-Jun N-terminal Kinase (JNK), Insulin Resistance & Type 2 Diabetes}

c-Jun N-terminal kinase (JNK) is regarded as a critical stress kinase involving in the progression of insulin resistance/chronic inflammation/T2D\textsuperscript{15}. Exercise has been shown
to be of benefit in improving insulin sensitivity and body composition\textsuperscript{7,16} and controlling blood glucose and lipids\textsuperscript{7}. It is possible that some of the benefits of exercise for T2D subjects could be mediated by alterations in JNK. Below is a brief description of the known associations between JNK and major factors involving in the progression of T2D as well as how these signaling molecules response to exercise.

**Mechanisms of Insulin Resistance in Skeletal Muscles**

Skeletal muscle is the largest insulin-responsive organ in humans, accounting for 80% insulin-stimulated glucose disposal\textsuperscript{17}. It is the most influential tissue determining whole-body glucose homeostasis\textsuperscript{17}. Insulin binds to insulin receptor (IR), which localizes on the skeletal muscle cell surface, thereby triggering IR’s intrinsic tyrosine kinase activity and leading to subsequent recruitment of insulin receptor substrates (IRS) from cytoplasm\textsuperscript{18}. As a consequence, the PI-3K/Akt signaling pathway is activated and Akt 160kDa substrate (AS160) is phosphorylated\textsuperscript{4,18}. In basal state, AS160 retains glucose transporter 4 (GLUT4) on the membrane of intracellular vesicles\textsuperscript{19-20}. Upon the stimulus of insulin or muscle contraction, phospho-AS160 releases GLUT4 to allow its translocation from intracellular vesicles to cell surface\textsuperscript{4,19-20}. Increasing the amount of GLUT4 on the cell surface promotes skeletal muscle uptake of glucose\textsuperscript{4}.

Insulin resistance in skeletal muscle is considered a major precursor of T2D\textsuperscript{21}. Due to intensive study over the past few decades, mechanisms of insulin resistance in skeletal muscle have been partially elucidated. Disturbance of the balance between serine and tyrosine phosphorylations of IRS is regarded as a critical molecular “switcher”: IRS
tyrosine phosphorylation results in IRS activation, while serine phosphorylation leads to suppression of its activity\textsuperscript{18}. Phosphorylation of serine negatively impacts on IRS activity by rendering its tyrosine residues poor substrates for activated IR, therefore insulin signaling is hampered\textsuperscript{18}. Inhibition the serine phosphorylation of IRS by JNK has been highlighted as a key molecular event in insulin resistance\textsuperscript{22-23}. Thus, a primary consequence of JNK activation, IRS dependent PI-3K/Akt activation is impaired\textsuperscript{22}. Several factors are known to activate JNK, such as saturated free fatty acids (FFA), tumor necrosis factor α (TNFα) and C-reactive protein (CRP)\textsuperscript{23-24}.

**Exercise-dependent Improvement of Insulin Sensitivity**

How exercise improves insulin sensitivity is not fully clarified. Some studies indicate that exercise can decrease serine phosphorylation and increase tyrosine phosphorylation\textsuperscript{25-27}. The reversal of IRS serine phosphorylation is accompanied by a reduction of JNK activity in skeletal muscle after exercise\textsuperscript{26}. However, the detailed pathways linking the regulation of JNK activity and the exercise induced amelioration of insulin resistance is not yet fully elucidated. Further studies are required to define to the role of JNK in the progression of insulin resistance/T2D, its adaptations to different modalities and doses of exercise, and its interactions with key mediators of insulin signaling pathways.

Several major downstream effectors of IRS participate in the exercise-dependent increase of insulin signaling: acute exercise tends to elevate PI-3K/Akt/AS160 activities without change their protein levels\textsuperscript{25-26,28}; while chronic training has been shown to increase both
their phosphorylated and total protein levels\textsuperscript{29}. The final results of the above changes is to promote GLUT4 translocation to the cell surface. Additionally, exercise directly enhances GLUT4 mRNA and protein levels and therefore further increases the capacity for glucose uptake\textsuperscript{29-30}.

**Transcriptional Regulation of Glucose and Lipid Metabolic Genes**

Proliferator-activated receptors (PPARs) are a group of nuclear hormone receptors which are closely related to the development of metabolic syndrome, and are also linked to JNK. They were originally identified as nuclear hormone receptor transcriptional factors which regulate the expression of mitochondria-localized proteins\textsuperscript{31}. Recent evidence has reported that PPARs also regulates glucose and lipids metabolic genes, including GLUT4, HMG-CoA synthase, apo-AI and apo-AII\textsuperscript{31-33}. The expression of HMG-CoA synthase, the rate-limited enzyme in cholesterol, is inhibited by PPARs\textsuperscript{31}. The expression of apo-AI and apo-AII, two major HDL apolipoproteins, are enhanced by PPARs\textsuperscript{31}. Therefore, the activation of PPARs decreases synthesis of cholesterol and increases glucose uptake and HDL formation.

Severe reduction of protein levels and activities of PPARs has been correlated to insulin resistance and and other components of metabolic syndrome in T2D patients\textsuperscript{21}. In T2D, activated JNK can phosphorylate PPARs hence inhibit their transcriptional activities\textsuperscript{34-35}. This may partially explain the mechanisms underlying decreased activity of PPARs in this condition.
Cytokines, Chronic inflammation & Insulin Resistance

Low-grade chronic inflammation has been implicated as a link between obesity and progression of insulin resistance/T2D\textsuperscript{36}, and is also related to JNK activation and subsequent changes to insulin signaling described above. Several inflammatory factors, such as tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP), are dramatically elevated in obese population\textsuperscript{36}. As a major contributor of impaired IRS-mediated signaling, chronic inflammation activates certain serine/threonine kinases which are capable of phosphorylating IRS serine residues and JNK is one of those kinases\textsuperscript{22}.

(Figure 1.1) Theoretically, exercise offers protection against low-grade chronic inflammation, by down-regulating pro-inflammatory cytokines and up-regulating anti-inflammatory cytokines\textsuperscript{37}.

C-reactive protein (CRP) is a sensitive physiological markers of subclinical systemic inflammation produced primarily in the liver in response to IL-6 and TNFα released from visceral adipocytes\textsuperscript{38-39}. Modest elevation of CRP is associated with low-grade chronic inflammation, insulin resistance, and T2D\textsuperscript{39}. Additionally, increasing circulating CRP is a risk factor for T2D as well as cardiovascular disease and mortality\textsuperscript{39-40}. The effects of exercise on CRP are not fully elucidated yet: some authors have reported a reduction of CRP after chronic exercise training\textsuperscript{14,40}, whereas the others have not found any change in CRP level\textsuperscript{41}.

TNFα is an inflammatory marker associated with obesity/T2D. It has a higher concentration in patients with T2D and correlates with fasting glucose and insulin levels.
in obese individuals. TNFα increases JNK phosphorylation, which might partially explain the observation that TNFα is directly related to IRS serine phosphorylation and thus the reduction of insulin signaling. The influence of exercise on TNFα levels is still controversial: Some authors have observed that TNFα production is attenuated by aerobic exercise, while others have reported that exercise training does not have a significant effect on expression of TNFα.

The role of IL-6 in the progression of insulin resistance and the benefits of exercise for T2D treatment remains unclear, but again it is linked to JNK activity. Most studies regard muscle-derived IL-6 as an anti-inflammatory cytokine and its post-exercise elevation protects the body against insulin resistance. Other investigators describe a dual role of IL-6: It is a mediator of impaired insulin action in obesity and a facilitator of increased fuel metabolism during exercise. Some authors have found that exercise training does not have a significant effect on IL-6. Post-exercise elevation of IL-6 is accompanied by JNK activation. Even though the effect of JNK activation on IL-6 gene transcription in skeletal muscle is not fully described, it is reported that JNK regulates IL-6 gene expression via activation of c-Jun.

Finally, adiponectin is a unique adipokine which is primarily secreted by adipose tissue. Its relationship to JNK has not been described in the literature to date. Epidemiological studies indicate that circulating adiponectin is reduced in patients with diabetes and central obesity. Low adiponectin concentrations are significantly correlated with insulin resistance. Interventions to reduce inflammation associated with
obesity have been shown to elevate adiponectin concentrations in some cases. For example, adiponectin has been shown to be increased in response to dietinduced weight loss but it appears that weight loss must be >10% of body weight\(^57\-\)\(^58\). The effects of exercise on plasma adiponectin are controversial at this time, because exercise programs do not consistently affect adiponectin\(^59\). However, when combined with dietary weight loss interventions, it appears that effects are more consistent, particularly with aerobic and resistance training programs that are of adequate intensity and duration to promote significant fat loss\(^59\).

**Aims of our study**

The overall study was a randomized double-blind, sham-exercise controlled trial designed to assess the efficacy of power training on older adults with type 2 diabetes. Our sub-study concerned selected baseline characteristics of the whole cohort.

The aims of this substudy were:

- To summarize current knowledge as to how JNK adapts to exercise in human via a systematic review of all published human studies to date.

- To describe the baseline characteristics of older adults with T2D enrolled in a randomized double-blind, sham exercise controlled trial designed to assess the efficacy of power training in T2D.

- To investigate baseline characteristics which were potentially related to skeletal muscle JNK after exercise exposure in our older T2D cohort.

- To investigate characteristics which were potentially correlated to activation level of skeletal muscle JNK after acute exercise exposure in this cohort.
Overview of the Following Chapters

Chapter 2: Adaptations of c-Jun N-terminal Kinase (JNK) to Exercise in human cohorts: A Systematic Review

Chapter Two will provide a systematic review of how human JNK adapts to exercise exposure in human cohorts. The influence of exercise on JNK activity in human beings is of great interest because a growing numbers of disorders/syndromes, including neuronal degenerative diseases, chronic inflammation/obesity/insulin resistance/diabetes, cardiovascular diseases and cancers, are associated with abnormal JNK activity. Therefore, the benefits of exercise on these disorders might be related in part to a change of JNK activity. In addition to the role of JNK in T2D introduced above, other examples of the significance of JNK in these other chronic conditions are reviewed briefly in the paragraphs below.

JNK phosphorylates pro-apoptotic transcriptional factors, such as c-Jun and activates transcription factor 2 (ATF-2), and pro-apoptotic Bcl-2 family members, to promote activation of caspase apoptotic cascades. JNK inhibits expression of chaperones, such as heat shock proteins (HSPs) and activates endoplasmic reticulum stress (ERS), hence the protein folding and secretion are disturbed. Irreversible ERS finally promotes cell apoptosis as well. These JNK-dependent cascades play a critical role in excessive cell apoptosis-related disorders, such as neuron loss in neurodegenerative diseases and pancreatic β cell loss in diabetes.

JNK also involves in disturbance of the cell cycle by crosstalkling with many cyclin-dependent kinases (CDKs). Additionally, JNK phosphorylates cell adhesive proteins such
as paxillin to promote the cancer metastasis\textsuperscript{64}. Therefore, it is highly related to cancer cell proliferation and metastasis\textsuperscript{65}.

Inflammatory factors such as TNF\textgreek{a} activates JNK to promote oxidative stress. JNK is also related to the disturbance of blood lipids balance via its effect on PPARs as mentioned in the previous paragraphs\textsuperscript{66}. These abnormalities are related to metabolic disorders such as hypertension, coronary heart disease and stroke\textsuperscript{66}.

Exercise has been shown to be of benefit in prevention of many of these pathological conditions, such as heart disease, stroke, diabetes, some cancers, depression and dementia, as well as in the treatment of established risk factors or diseases, for example improving insulin sensitivity and body composition\textsuperscript{7,16}, controlling blood glucose and lipids\textsuperscript{7}, relieving the symptoms of cognitive impairment and depression\textsuperscript{67-68}, helping the recovery after stroke\textsuperscript{69}, improving the immunological function in cancer patients\textsuperscript{70}. It is possible that some of these wide-ranging preventive and therapeutic benefits of exercise could be mediated in part by alterations in JNK.

Pharmacological inhibition of JNK via certain chemicals has been tested as treatment in some of the above diseases, for example SP600125\textsuperscript{15,63}. However, exercise has an adjutative or even alternative role to play in most of these chronic diseases, often with fewer side effects and broader range of benefits than pharmacotherapy alone. Therefore, it is important to understand the effects of acute and chronic exercise on JNK, to better determine the optimal exercise prescription for inhibition of JNK which may lead to
clinical benefits ultimately. Thus, the published studies of the influence of exercise exposure (acute and chronic) on JNK is summarized in the systematic review.

Chapter 3: Skeletal Muscle c-Jun N-terminal Kinase (JNK) Activity after Acute Resistive Exercise in Older Adults with Type 2 Diabetes: Methods of Investigating Metabolic and Clinical Correlates

According to the results of the systematic review, there’s no human data describing the adaptations of JNK to exercise and its potential correlations to the markers of chronic inflammation/insulin resistance in T2D subjects\textsuperscript{71-79}. Hence our studies will fill in the gaps in this area.

Chapter Three described the methodology of baseline substudy of the randomized double-blind, sham-exercise controlled trial designed to assess the efficacy of power training on older adults with type 2 diabetes. This chapter included: an overview of the study design; methods of how to analyze health status, body composition, glucose homeostasis and insulin resistance, blood lipids and cytokines; procedures of muscle and adipose tissue biopsy; methods of investigating the factors in muscle and adipose tissues we hypothesized would be correlated to insulin sensitivity and exercise adaptations. All the protocols were based on published articles\textsuperscript{80-93}. We also present the reliability of the Western Blot assay method, and compare to published literature for adequacy\textsuperscript{94}. 

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Chapter 4: Skeletal Muscle c-Jun N-terminal Kinase (JNK) Activity after Acute Resistive Exercise in Older Adults with Type 2 Diabetes: Results of Metabolic and Clinical Correlates

Chapter Four describes the results of this baseline substudy. The baseline characteristics, such as health status, body composition, physical activity level, glucose homeostasis, insulin resistance, serum cytokines and lipids, myokines, adipokines and selected components in insulin signaling pathways in skeletal muscle and adipose, and potential characteristics or factors which were correlated to skeletal muscle JNK, were analyzed in this chapter. Health status and burdens of diseases of our cohort are compared to corresponding cohorts in other studies or Australian T2D cohort. Factors correlated with skeletal muscle are described and possible mechanisms leading to these correlations were provided.

Chapter 5: Discussion

Chapter 5 retrospects this baseline sub-study and discusses the novelties and limitations of this baseline sub-study. The novelties included that JNK had expected relationships to metabolic and general health status in most cases, and novel associations were found with habitual physical activity level.
REFERENCES


CHAPTER 2

Adaptations of c-Jun N-terminal Kinase (JNK) to Exercise in Human Cohorts:

A Systematic Review
AUTHOR DECLARATIONS

I hereby acknowledge that my contribution to the manuscript titled:

Adaptations of c-Jun N-terminal Kinase (JNK) to Exercise in Human Cohorts:

A Systematic Review

is accurately described, and I give permission for Qianyu Guo to submit this manuscript as part of her thesis for the fulfillment of the award of M.App.Sc

Author Contributions

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Signed: Prof. Maria Fiatarone Singh Date: 28/08/2010
ABSTRACT

Background

It is known that c-Jun N-terminal kinase (JNK), a stress-responsive kinase, is involved in numerous disorders such as obesity, insulin resistance, type 2 diabetes, neurodegenerative disorders, depression, and cardiovascular disease. Exercise has been reported to alter JNK expression in skeletal muscle. Therefore, some of the benefits of exercise in these conditions could be mediated in part by alterations in JNK.

Objective

The aim of this investigation was to review the published literature reporting adaptations of JNK to acute or chronic exercise exposure in human cohorts.

Methods

A comprehensive, systematic search for manuscripts was performed from 1966 to June 2010 using computerized databases, including Medline, Premedline, CINAHL, AMED, EMBASE and SportDiscus. Eligible articles included studies testing JNK in any human cohort with acute or chronic exercise as an intervention. Three reviewers (QG, DS and MFS) independently assessed studies for potential inclusion. All data on quality, study design and adaptations were extracted by QG.
Results

A total of 9 exercise studies were selected for review, including 2 randomized controlled trials and 7 uncontrolled trials. The quality of the literature was modest, with notable limitations including lack of controls and small sample sizes.

There were 7 studies of acute exercise exposure (single bout), reporting mixed results for JNK adaptation: 4 increased and 3 decreased. There were 2 chronic exercise trials reporting mixed results: 1 in which JNK increased with training and 1 in which JNK levels were similar after weight loss diet or weight loss diet plus exercise. All studies included only healthy young subjects with the exception of 2 trials of overweight/obese subjects, one trial of healthy older adults, but no studies of clinical cohorts.

Conclusion

The influence of acute and chronic exercise exposure on JNK remains controversial, due to the modest quantity and quality of published literature to date. Only 7 studies with acute exercise response were found and only one of these was randomized. Within the 7 studies with acute exercise intervention, all subjects were healthy except one study included overweight young adults. Only 2 trials of chronic exercise training were found. Within these 2 chronic exercise studies, one was randomized in obese young adults and the other was uncontrolled in healthy young adults. Only one acute exposure uncontrolled trial of healthy older adults was found. Therefore, more trials, especially robustly designed chronic exercise training studies with clinical and older cohorts are
needed to understand how JNK adapts to acute and chronic exercise and how it is related to metabolic outcomes.
INTRODUCTION

As a member of the mitogen-activated protein kinase (MAPKs) family, c-Jun N-terminal kinase (JNK) was originally identified as a stress-responsive kinase. JNK participates in many intracellular pathways, such as cell proliferation, differentiation, transformation, apoptosis, migration, and cytoskeletal integrity. Certain disorders/syndromes, including neuronal degenerative diseases, chronic inflammation/obesity/insulin resistance/diabetes, cardiovascular diseases and cancers, are associated with abnormal JNK activity. JNK may contribute to the pathology of these conditions by promoting neuronal apoptosis, worsening insulin resistance, chronic inflammation and lipid toxicity, or disturbing the cell cycle.

Exercise has been shown to be of benefit in many of these pathological conditions. For example, it may improve insulin sensitivity and body composition, improve blood glucose and lipids, prevent or treat cognitive impairment and depression, assist with the recovery after stroke, and improve the immunological function in cancer patients. It is possible that some of the benefits of exercise may be mediated in part by alterations in JNK.

Existing animal studies in this field are summarized in Table 2. Two studies with obese/diabetic rats reported a decrease of JNK activity after exercise exposure, one study reported post-exercise JNK remained unaltered after exercise, and the other 7 studies reported JNK activity was increased.
However, the evidence provided by this small and heterogeneous animal literature must be verified in humans. Therefore, it is important to summarize the available data concerning the effects of exercise on JNK in humans, to identify the state of current knowledge and gaps in the literature.
METHODS

Literature Search Strategy

A literature review was conducted from 1966 to 2010 using computerized databases including Medline (1967-2010), Premedline (most recently published), CINAHL (1982-2010), AMED (1985-2010), EMBASE (1966-2010) and SportDiscus (1967-2010), with the last search being conducted in July, 2010.

First of all, two categorical searches were conducted using the following search terms in all fields: (1) exercise, physical activity, training, aerobic, resistance, weight lifting, endurance, fitness, active or sedentary. and (2) JNK or c-Jun N-terminal kinase. The search terms within each category were combined with “OR”. Secondly, category 1 and 2 results were combined using “AND” and duplicate results were removed. (Refer to Appendix 1 for an example of step-by-step search in Medline (Ovid). This search was performed in the same way in all other databases. All titles resulting from the combined searches were reviewed for potential inclusion, and retrieved studies were read in full.

In addition to the database search, bibliographies of all eligible papers and reviews identified from the electronic search were manually searched.

Inclusion and Exclusion Criteria

Articles were selected from the initial search on the basis of following criteria: Any types of study design was acceptable; the full-length article was published in a peer-reviewed journal; if there were multiple interventions, the influence of exercise was able to be separated from the other components. Subjects were humans of any age or health status.
The exercise intervention could be any modality of structured exercise, whether supervised or not. Aerobic exercise involves exercise performed for extended periods with large muscle activity involving hundreds or thousands of consecutive repetitions that challenge the delivery of oxygen to the active muscles. Resistance exercise involves weight lifting/lowering or the use of high-resistance machines with exercise that is limited to a few repetitions (generally less than 20) before exhaustion. Both single exposure (an acute bout of exercise) and chronic training studies were included. Chronic training for the purposes of this review was defined as more than 1 bout of exercise. Articles that measured the adaptations of any of following molecules to exercise were all regarded as measuring “JNK”: JNK mRNA, total JNK, phospho-JNK or phospho-JNK/total-JNK ratio. Phosphorylation of JNK represents the activated form of the protein, which may be expressed on its own, or as the ratio of active to total JNK expression.

The following types of articles were excluded: studies with unstructured physical activity; non-English language or unpublished papers or theses; reviews or abstracts; studies of animals. Studies where exercise could not be isolated from other intervention components.

**Study Selection and Data Extraction**

A systematic review of all published literature, regardless of study design, investigating adaptations of JNK to any exercise exposure in any human cohort was conducted. One of the authors (QYG) conducted the search and extracted data. After eliminating
duplications, all papers found were screened by the author, first by title and then by abstract, using the eligibility criteria listed above. Articles selected for evaluation were further screened by two authors (DS and MFS) for eligibility and accuracy of data extraction onto pre-designed forms. Disagreements were resolved by consensus.

Study Quality Assessment

The quality of selected articles were assessed according to a modified criteria based on the Delphi list and the Physiotherapy Evidence Database (PEDro) scale\textsuperscript{30-31}. Additional quality variables considered were supervision of exercise and compliance, which were considered as significant components of reporting for exercise studies\textsuperscript{32}.

Statistical analysis

Mean±standard deviation (SD) reported in all characteristics and results were extracted from the articles or estimated from graphs if necessary. All the standard errors (SE) reported were transformed into SD according to formula 1.

Formula 1: \( \text{SD} = \sqrt{n - 1} \times \text{SE} \) (n= sample size)

Relative effect size (ES) adjusted via Hedges bias-corrected ES for small sample sizes were calculated based mean±SD. For an uncontrolled trial, ES was calculated according to formula 2.

Formula 2: \( \text{ES} = \frac{\text{Post Treatment Value} - \text{Pre Treatment Value}}{\text{Baseline SD}} \)

For a randomized controlled trial, ES was calculated according to formula 3.

Formula 3: \( \text{ES} = \frac{\Delta \text{Treatment} - \Delta \text{Control}}{\text{Pooled SD}} \), \( \text{Pooled SD} = \sqrt{\frac{(n_t-1)SD_t^2 + (n_c-1)SD_c^2}{n_t-n_c}} \)
ES were interpreted according to the following criteria: trivial (ES<0.20), small (0.20≤ES<0.50), moderate (0.50≤ES<0.80), large (ES≥0.80). A quantitative data synthesis (meta-analysis) was not performed because of the heterogeneity of study designs and interventions, which made pooling of data across trials inappropriate.
RESULTS

Search results

The study selection flow chart is presented in Figure 2.1. There were 7 uncontrolled trials\textsuperscript{33-39} and 2 randomized controlled trials\textsuperscript{40-41}. Among 69 articles fully assessed for inclusion in this review, 62 were eliminated for the following reasons: 6 articles didn’t include exercise exposure; 7 articles didn’t analyze JNK; 22 articles were reviews; 13 were animal studies.

Study quality assessment

An assessment of the study quality is presented in Table 2.2. In general, the study quality was not robust, and no study met all the quality criteria. Only 2 of the 9 studies were randomized controlled trials (RCTs). Treatment allocation concealment and supervision of exercise were not reported in both RCTs. All studies specified their inclusion criteria, and reported groups or subjects were similar at baseline. None of the studies reported the supervision of exercise, although the acute bout studies were interpreted as supervised.

Intervention characteristics

The modality, dose, intensity and volume of exercise exposure or training utilized in these studies are presented in Table 2.3.1 and Table 2.3.2. Regarding duration, seven studies described the response to a single bout of acute exercise only\textsuperscript{33,35-39,41} while 2 studies used chronic exercise training for 3-4 weeks\textsuperscript{40} and 10 sessions\textsuperscript{34}, respectively as the intervention\textsuperscript{34,40}.
As for the type of training, four studies used aerobic training\textsuperscript{35,39-41}, 3 used resistance training\textsuperscript{34,36,38} and 2 used both modalities\textsuperscript{33,37}. All the studies consisted of either moderate or high intensity training. The intensity was high in all the aerobic training studies\textsuperscript{35,39-41}. Two of the 3 resistance training studies used high intensity exercise\textsuperscript{34,39} and 1 used moderate intensity\textsuperscript{38}. In the 2 studies reporting both aerobic and resistance training arms, both were high intensity\textsuperscript{33,37}. The acute exposure studies ranged from 30 min to 3:35±0:11 (marathon run) of aerobic exercise, and 1 to 6 repetitions of resistive exercise.

**Biopsy details**

The target tissue was skeletal muscle in all studies. As the potential changes of JNK activity may be transient, we regarded the time of muscle biopsy as a critical factor in the interpretation of the results. All but two studies\textsuperscript{40-41} reported the exact time interval between the finish of exercise and the first post-exercise biopsy. It ranged from immediately after exercise to 48 hrs post-exercise. Five studies reported additional delayed biopsy timepoints after the first post-exercise biopsy and these ranged from 2 hours up to 5 days after the completion of exercise\textsuperscript{34-37,41}.

**Cohort characteristics**

A summary of each of the study cohorts is shown in Table 2. 4. 1 and Table 2.4. 2. In total, the 9 studies consisted of only 99 subjects, with small sample sizes ranging from 6 to 17, with a median 10 and interquartile range 7. Overall, 53. 5% of all subjects were males, with 3 studies including only males, 3 studies consisting of females and the other
one with mixed genders. Most subjects were young adults, with the exception of 1 study which compared young and older adults, and 1 study which had a mixture of young and middle-aged subjects. Across all the cohorts, mean±SD age ranged from 21±1 to 79±3 years. Seven studies recruited healthy subjects and the other 2 recruited overweight/obese subjects. Body mass index (BMI) was reported in all but 2 studies. It ranged from 22±1 to 34±2. No other health characteristics, demographic variables, or lifestyle factors were reported.

**Outcome measures**

All studies performed the examination of JNK by semi-quantified techniques: Three studies used an immuno-kinase assay while the other 6 studies used the Western blot technique. The outcomes are summarized in Table 2.5.1 and Table 2.5.2.

Overall, in the 7 studies with healthy subjects, 6 reported the effects of acute exercise exposure and 1 reported adaptations to chronic exercise. Three studies with acute exercise and one with chronic exercise reported that JNK activity in whole muscle extracts increased immediately after exercise; One acute exercise study reported that nuclear JNK1/2 activity increased immediately after exercise; By contrast, two acute exercise studies reported that JNK activity in whole muscle extracts decreased at 14 hours and 1 day, respectively, after exercise; Finally, one acute exercise study reported that healthy young subjects had no change of JNK activity in skeletal muscle while healthy older subjects had a significant decrease in JNK activity.
Virtually all of the data were collected in healthy individuals, so the response of JNK in clinical cohorts is speculative, and limited to the 2 studies which targeted overweight/obese women. Harber investigated the response to an acute bout of aerobic exercise\textsuperscript{36}, while Schenk added aerobic chronic exercise training to a weight loss diet for 3-4 weeks\textsuperscript{40}. Harber reported that JNK activity increased immediately after acute exercise but decreased to baseline at 2 hours\textsuperscript{36}; Schenk found that chronic training added to a weight loss diet didn’t alter skeletal muscle JNK activity in these women compared to diet alone\textsuperscript{40}. No study reported relationships between alterations in JNK and other metabolic or clinical characteristics in these subjects.
DISCUSSION

We have identified a small literature pertaining to JNK and exercise adaptation, precluding definitive conclusions about the consistency, robustness, timecourse, and clinical relevance of this response. The quality of this literature is limited by the very few randomized trials, lack of standardization of JNK assay methodology utilized or reporting of precision of the assay, few chronic training studies, limited inclusion of older or clinical cohorts, and heterogeneity of exercise protocols utilized. The results are summarized in the sections which follow.

Adaptations of JNK activity to exercise

Within the 7 studies of acute exercise, skeletal muscle JNK activity increased significantly after a single bout of acute exercise exposure (both resistive and aerobic) in 4 studies of healthy subjects. By contrast, Schenk’s 2007 RCT reported that JNK activity was lower in the exercise exposure group than in non-exercising control group. Williamson reported that JNK activity decreased after exercise in older healthy adults but was unchanged in young adults. Finally, Thompson reported that JNK activity was elevated after resistive training but was reduced after aerobic training.

Study design issues may provide some insight into the heterogeneous findings with regards to acute exercise adaptation. For example, in Schenk’s 2007 acute exercise study, no pre-exercise biopsy was performed, hence it was not possible to compare both pre and post exercise JNK activity between the control and exercise groups to verify that the difference was not present before the exercise bout. Secondly, the biopsies were
performed 14 hrs after exercise and exercise-dependent JNK elevation might have already disappeared\textsuperscript{41}. Two studies in this review which also reported a decrease in JNK activity at a delayed timepoint after exercise exposure support this explanation\textsuperscript{35,37}. Notably, Thompson’s study reported heterogeneous delayed responses (at 48 hours) in 2 different muscles and 2 different modalities of exercise: an increase of JNK activity in biceps brachii after acute resistance exercise, and a decrease of JNK activity in vastus lateralis after acute aerobic exercise\textsuperscript{37}. It is possible that the alteration of JNK activity after resistive exercise is more prolonged than that after aerobic exercise but this is speculative, as no other comparative studies have been published.

Therefore, additional studies with different exercise types and various post-exercise time points of muscle biopsy are necessary to determine the post-exercise time course of JNK activity change in humans after exposure to a single bout of exercise. Additionally, as all but one of these studies were done in healthy young subjects\textsuperscript{38}, it is not known if older adults or clinical cohorts such as those with diabetes or cardiovascular disease would respond similarly. It is also not known what relevance the acute response of JNK to a bout of exercise means in relation to chronic expression or activation of JNK in skeletal muscle and its impact on insulin signalling and other metabolic pathways in these individuals.

There were only 2 studies reporting the adaptations of JNK after chronic training and they also provided heterogeneous results. Boppart’s 1999 study reported JNK activity was dramatically elevated after resistive chronic exercise. By contrast, Schenk’s 2009
RCT reported JNK activity was unaltered after a weight loss diet was not altered by concomitant aerobic exercise training.

However, in Schenk’s chronic exercise study, no baseline biopsy was performed, hence it was not possible to compare both pre and post intervention JNK activity between the control and exercise group. In addition, the timepoint of the post-exercise biopsy was not reported hence it is impossible to judge whether JNK activity was really unaltered by chronic exercise or whether the changed of JNK activity was not captured due to delayed biopsy time relative to the last bout of training.

**Relevance to metabolic health**

In subjects who may have systemic inflammation, such as obese or older subjects with chronic disease, adaptations of JNK activities remain largely unknown. The three human studies with older or overweight/obese subjects have had different findings. Harber’s study drew the conclusion that acute exercise was responsible for the decrease in JNK activity in skeletal muscle of overweight women. Williamson’s study with older healthy adults reported similar results, with a decrease in JNK after acute resistive exercise. By contrast, Schenk’s RCT of aerobic exercise added to a weight loss diet for 3-4 weeks reported no difference in JNK activity attributable to the addition of exercise.

There might be several reasons leading to this disparity. First, chronic exercise may influence skeletal muscle JNK activity differently than acute exercise. Secondly, this study involved weight loss diet intervention together with chronic exercise training.
Thirdly, only female subjects were included, and the study may have been underpowered to look at the additive effect of exercise to diet (as only 17 subjects were included in total). Therefore, further studies using both acute and chronic exercise as interventions in clinical cohorts, with and without concomitant interventions are needed to better understand adaptations of JNK in this setting.

**Qualities and Deficits of the Reviewed Literature**

A very limited number of human studies were available, and overall the quality was modest. Primary deficits of study design included few randomized controlled trials, and small sample sizes, which made type II errors more likely to occur. Additionally, precision of JNK assays was not reported at all, timing of biopsies was not standardized, not all studies included biopsies before exercise, supervision of exercise and verification that the subjects performed their exercise at the intended duration and intensity were provided in none of these studies. Finally, no study included both activated and total JNK protein levels, mRNA for JNK, and other critical components of JNK signalling pathways.

Regarding the length of the exercise intervention, most studies used acute exercise as intervention. Only two studies investigated chronic exercise. Hence the different impacts of various exercise exposures on JNK activity are largely unknown. The acute bout effect could be attenuated after chronic training, as is the case with other stressor responses, but whether this happens with JNK is unknown at this time.
As to exercise modality, resistive and aerobic trainings were not able to be directly compared with each other, as they were not performed in the same trial except for Aronson’s study\textsuperscript{33}. In this trial, progressive resistance training (PRT) was performed with the upper limbs and aerobic exercise with the lower limbs. Thus, the variable response observed could have been due to either exercise modality to differences between muscle groups studied.

Although most studies reported BMI as a basic demographic characteristic, there was only one study that reported the changes of BMI over the course of intervention. No information about habitual physical activity levels, dietary intake, body composition, systemic inflammation, insulin sensitivity, other metabolic characteristics or changes in these factors were included in any of these studies. Therefore, the relevance of the JNK adaptations, where observed, remains largely speculative at this time.

**Recommendations**

Based on the current literature, several suggestions can be made which would fill identified gaps and advance knowledge in this field. Specifically:

- More robustly-designed and larger randomized controlled trials should be performed in order to decrease the possibility of type II errors and other threats to study validity.
- Adaptations to chronic exercise or preferably to both acute and chronic training adaptations in the same cohort would be very informative.
• Intensities, volumes, frequencies and duration should be accurately recorded in order to compare the differential adaptation to exercise interventions.

• Clinical cohorts, older adults, and in particular those with metabolic and cardiovascular disease, such as those with type 2 diabetes should be studied.

• Biopsies should be performed at multiple time-points including pre-exercise, immediately after the completion of exercise, and delayed, in order to identify the timecourse of JNK activity adaptation to exercise.

• The target tissue should not be restricted to skeletal muscle as the benefits of exercise and the metabolic roles of JNK are not limited in skeletal muscle, although animal studies would be required for tissues other than muscle or fat.

• Comparisons of total JNK, phosphorylated JNK, JNK mRNA should be included in the same cohort to determine whether the mechanism of the rise in JNK activity is transcription, translation, activation, or combinations of these mechanisms.

• Studies should include potential clinical sequelae of JNK modification, including ameliorated insulin resistance and metabolic syndrome, impaired cardiovascular functions, memory and immunological functions, and demonstrate what proportion of the variation in these clinical outcomes is attributable to alterations in JNK activity. This would clarify the clinical relevance of the JNK pathway to exercise-related benefits.

In summary, the current literature does not allow definitive conclusions to be drawn about the role of JNK in exercise-mediated metabolic improvements. However, there is preliminary evidence from both human and animal studies that both acute and chronic
exercise can alter JNK expression and activation. Given the central signalling role of JNK in many tissues, it is conceivable that it underlies a significant proportion of the broad spectrum of benefits seen with physical activity for neurological, metabolic, cardiovascular, and other chronic diseases. Future investigations are clearly warranted, in order to optimize exercise prescriptions to modify JNK beneficially, as well as to define clinical cohorts most responsive to such prescriptive elements.
REFERENCES


# TABLES AND FIGURES

## Table 2.1.1 Summarization of Animal Studies-Acute Exercise Studies

<table>
<thead>
<tr>
<th>Citation</th>
<th>Animal Model</th>
<th>Tissue</th>
<th>Exercise Type</th>
<th>Training Method</th>
<th>Training time/session</th>
<th>Pre-exercise activated JNK (Mean±SD) (Arbitrary units)</th>
<th>Immediate postexercise activated JNK (Mean±SD) (Arbitrary units)</th>
<th>Delayed response of JNK (Mean±SD) (Arbitrary units)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goodyear, 1996[^10]</td>
<td>Male Sprague-Dawley Rat</td>
<td>Skeletal Muscle</td>
<td>Acute</td>
<td>Treadmill</td>
<td>10-60 min (20 m/min, 10% grade)</td>
<td>1.0±0.1</td>
<td>2.4±0.2</td>
<td>N/A</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Melling, 2006[^15]</td>
<td>12 week old male Sprague-Dawley rats</td>
<td>Heart</td>
<td>Acute</td>
<td>Treadmill</td>
<td>60min continuous running (30 m/min; 2% grade)</td>
<td>1.0±0.1</td>
<td>3.8±0.1</td>
<td>N/A</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Ropelle, 2006[^17]</td>
<td>Diet-induced obese (DIO) Wistar rats</td>
<td>Skeletal Muscle</td>
<td>Acute</td>
<td>Swimming</td>
<td>3hrs swimming (2×) 45 min break</td>
<td>5.4±0.3</td>
<td>2.1±0.1</td>
<td>N/A</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Lagranha, 2007[^14]</td>
<td>Sprague-Dawley rats</td>
<td>Neutrophils</td>
<td>Acute</td>
<td>Treadmill</td>
<td>1 hour</td>
<td>2.3±0.6</td>
<td>8.0±1.2</td>
<td>N/A</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Hunter, 2008[^12]</td>
<td>Sprague-Dawley rats</td>
<td>Left Ventricle</td>
<td>Acute</td>
<td>Treadmill</td>
<td>10 min, high intensity</td>
<td>4.0±1.1</td>
<td>6.4±0.9</td>
<td>N/A</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Hoene, 2010[^21]</td>
<td>Male C57BL/6 Mice</td>
<td>liver</td>
<td>Acute</td>
<td>Treadmill</td>
<td>60min run on the treadmill</td>
<td>3.6±0.7</td>
<td>12.4±2.3</td>
<td>4.0±1.2</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

SD: Standard deviation  
N/A: Not applicable
### Table 2.1.2 Summarization of Animal Studies-Chronic Exercise Studies

<table>
<thead>
<tr>
<th>Citation</th>
<th>Animal Model</th>
<th>Tissue</th>
<th>Exercise Type</th>
<th>Training Method</th>
<th>Training time/session</th>
<th>Pre-exercise activated JNK (Mean±SD) (Arbitrary units)</th>
<th>Immediate post exercise activated JNK (Mean±SD) (Arbitrary units)</th>
<th>Delayed response of JNK (Mean±SD) (Arbitrary units)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vichaiwong, 2009</td>
<td>Male Sprague-Dawley Rat</td>
<td>Skeletal Muscle</td>
<td>Chronic</td>
<td>Treadmill</td>
<td>Start: 15-20m/min, 0% grade, 5-10min/day Week 1-3: 25min/min, 10% grade, 40-70min/day Week 4-6: 20-25min/min, 5-15% grade, 90min/day</td>
<td>3.2±0.6</td>
<td>3.4±0.7</td>
<td>N/A</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Nakamura, 2005</td>
<td>Duchenne mice &amp; C57BL/10 mice</td>
<td>Skeletal Muscle</td>
<td>Chronic</td>
<td>Treadmill</td>
<td>N/A</td>
<td>1.0</td>
<td>N/A</td>
<td>Duchenne: C57BL/10 mice: 2.1±0.9</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Kiraly, 2010</td>
<td>Zucker diabetic fatty (ZDF) rat</td>
<td>Plasma &amp; Liver</td>
<td>Chronic</td>
<td>Wheel running</td>
<td>10 weeks</td>
<td>1.0±0.1</td>
<td>0.4±0.05</td>
<td>N/A</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Iemitsu, 2006</td>
<td>Male Sprague-Dawley Rat</td>
<td>Heart</td>
<td>Chronic &amp; Acute</td>
<td>Treadmill</td>
<td>Group 1: 4 weeks, 30min acute exercise Group 2: 8 weeks, 30min acute exercise Group 3: 12 weeks, 30min acute exercise</td>
<td>1.0±0.1</td>
<td>N/A</td>
<td>Group 1: 9.4±0.8 Group 2: 4.7±1.1 Group 3: 1.2±0.2</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

**SD**: Standard deviation  
**N/A**: Not applicable
Table 2.2  Study Quality Assessment

<table>
<thead>
<tr>
<th>Citation</th>
<th>Randomization Performed?</th>
<th>Treatment allocation concealed?</th>
<th>Groups/subjects similar at baseline?</th>
<th>Eligibility criteria specified?</th>
<th>Supervision of exercise?*</th>
<th>Drop-outs ≤ 15%</th>
<th>Intention to treat analysis reported?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aronson, D. et al, 1998(^{33})</td>
<td>N/A</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Boppart, M. D. et al, 2000(^{35})</td>
<td>N/A</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>H. S. Thompson, et al 2003(^{37})</td>
<td>N/A</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Williamson, D. et al, 2003(^{38})</td>
<td>N/A</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Chan, M. H. et al, 2004(^{39})</td>
<td>N/A</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Schenk, S. et al, 2007(^{41})</td>
<td>Yes</td>
<td>NR</td>
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<td>Yes</td>
<td>Yes</td>
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<td>M. P. Harber, et al, 2008(^{56})</td>
<td>N/A</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Boppart, M. D. et al, 1999(^{34})</td>
<td>N/A</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>NR</td>
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<td>Yes</td>
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<tr>
<td>Schenk, S. et al, 2009(^{46})</td>
<td>Yes</td>
<td>NR</td>
<td>Yes</td>
<td>Yes</td>
<td>NR</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* acute studies are assumed to have been supervised, although this was not explicitly stated by the authors in any case

N/A: not applicable  NR: Not reported

This table is based on Delphi List and a modified version of the Physiotherapy Evidence Database (PEDro) scale\(^{30-31}\)
### Table 2.3.1 Exercise Interventions-Acute Exercise Studies

<table>
<thead>
<tr>
<th>Citation</th>
<th>Study Design</th>
<th>Exercise modality</th>
<th>Specific exercise</th>
<th>Intensity</th>
<th>Volume</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aronson, D. et al, 1998&lt;sup&gt;33&lt;/sup&gt;</td>
<td>UCT</td>
<td>Aerobic-Resistance Acute exercise</td>
<td>Protocol A &amp; B: cycle ergometer Protocol C: one-legged exercise</td>
<td>70% VO2 Max</td>
<td>60min, 1 session</td>
<td>60min</td>
</tr>
<tr>
<td>Boppart, M. D. et al, 2000&lt;sup&gt;35&lt;/sup&gt;</td>
<td>UCT</td>
<td>Aerobic Acute exercise</td>
<td>Marathon</td>
<td>High intensity</td>
<td>42.2km marathon</td>
<td>3:35±0:1 hrs</td>
</tr>
<tr>
<td>H. S. Thompson, et al 2003&lt;sup&gt;37&lt;/sup&gt;</td>
<td>UCT</td>
<td>Eccentric damage &amp; Resistance Acute exercise</td>
<td>BB: Voluntary eccentric contractions of elbow flexors (preacher curl exercise apparatus) VL: Run downhill on a treadmill</td>
<td>BB: maximal voluntary eccentric contractions (MVC) VL: 77% age-predicted maximum heart rate</td>
<td>BB: two sets of 25 MVCs, a constant rate of one contraction every 15s, 2 min rest between sets* VL: Run downhill for 30min (-10°C)</td>
<td>N/A</td>
</tr>
<tr>
<td>Williamson, D. et al, 2003&lt;sup&gt;38&lt;/sup&gt;</td>
<td>UCT</td>
<td>Resistance Acute exercise</td>
<td>Knee extension</td>
<td>70% 1-RM</td>
<td>3 sets of 10 repetitions</td>
<td>N/A</td>
</tr>
<tr>
<td>Chan, M. H. et al, 2004&lt;sup&gt;39&lt;/sup&gt;</td>
<td>NRCT</td>
<td>Aerobic Acute exercise</td>
<td>Cycle ergometer</td>
<td>70%&amp; VO2 max</td>
<td>60min, 1 session</td>
<td>60min</td>
</tr>
<tr>
<td>Schenk, S. et al, 2007&lt;sup&gt;41&lt;/sup&gt;</td>
<td>RCT</td>
<td>Aerobic Acute exercise</td>
<td>Treadmill + cycle ergometer</td>
<td>65% VO2max</td>
<td>45min treadmill =45min cycle ergometer</td>
<td>90min</td>
</tr>
<tr>
<td>M. P. Harber, et al, 2008&lt;sup&gt;26&lt;/sup&gt;</td>
<td>UCT</td>
<td>Resistance Acute exercise</td>
<td>Bilateral knee extensions</td>
<td>68±1% 1-RM</td>
<td>6 sets, 10 repetitions</td>
<td>N/A</td>
</tr>
</tbody>
</table>

UCT: Uncontrolled trial  RCT: Randomized-controlled trial
BB: Biceps Brachii exercise group; VL: Vastus Lateralis exercise group

N/A: Not applicable 1-RM: 1 repetition maximum
Table 2.3.2 Exercise Interventions-Chronic Exercise Studies

<table>
<thead>
<tr>
<th>Citation</th>
<th>Study Design</th>
<th>Exercise modality</th>
<th>Specific exercise</th>
<th>Intensity</th>
<th>Volume</th>
<th>Frequency</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boppart, M. D. et al, 1999&lt;sup&gt;34&lt;/sup&gt;</td>
<td>UCT</td>
<td>Resistance Chronic exercise</td>
<td>Knee extension</td>
<td>Maximum eccentric or concentric contraction</td>
<td>20 sets/session, 10 sessions</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Schenk, S. et al, 2009&lt;sup&gt;40&lt;/sup&gt;</td>
<td>RCT</td>
<td>Aerobic Chronic exercise</td>
<td>Stationary bicycle ergometer</td>
<td>85% maximum heart rate</td>
<td>45min/time</td>
<td>3 times/week</td>
<td>3-4 weeks</td>
</tr>
</tbody>
</table>

UCT: Uncontrolled trial  
RCT: Randomized-controlled trial

N/A: Not applicable
Table 2.4.1 Cohort Characteristics-Acute Exercise Studies

<table>
<thead>
<tr>
<th>Citation</th>
<th>Subject Numbers</th>
<th>Ages (years)</th>
<th>Gender</th>
<th>Healthy Status</th>
<th>Body Mass Index (BMI) kg/m²</th>
<th>VO2 max (ml kg⁻¹ min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aronson, D. et al, 1998³⁵</td>
<td>10</td>
<td>30±12</td>
<td>M: n=8 F: n=2</td>
<td>Healthy</td>
<td>NR</td>
<td>N/A</td>
</tr>
<tr>
<td>Boppart, M. D. et al, 2000²⁵</td>
<td>14</td>
<td>32±2</td>
<td>M: n=14</td>
<td>Healthy</td>
<td>24.1±0.01</td>
<td>60±2</td>
</tr>
<tr>
<td>H. S. Thompson, et al 2003³⁷</td>
<td>8</td>
<td>21.5±1.5</td>
<td>M: n=1 F: n=7</td>
<td>Healthy</td>
<td>NR</td>
<td>N/A</td>
</tr>
<tr>
<td>Williamson, D. et al, 2003³⁸</td>
<td>16</td>
<td>Young Group: 22±1 Old Group: 79±3</td>
<td>M: n=16</td>
<td>Healthy</td>
<td>&lt;26</td>
<td>N/A</td>
</tr>
<tr>
<td>Chan, M. H. et al, 2004³⁹</td>
<td>8</td>
<td>24±2</td>
<td>M: n=8</td>
<td>Healthy</td>
<td>NR</td>
<td>49.0±3.2</td>
</tr>
<tr>
<td>Schenk, S. et al, 2007⁴¹</td>
<td>8</td>
<td>26±2</td>
<td>F: n=8</td>
<td>Healthy</td>
<td>22±1</td>
<td>65.9±0.9</td>
</tr>
<tr>
<td>M. P. Harber, et al, 2008³⁵</td>
<td>6</td>
<td>28±3</td>
<td>F: n=6</td>
<td>Overweight</td>
<td>28±3</td>
<td>N/A</td>
</tr>
</tbody>
</table>

NR: Not reported
M: Male   F: Female
WL: Weight loss group; WE: Weight loss & exercise group
Table 2.4.2 Cohort Characteristics-Chronic Exercise Studies

<table>
<thead>
<tr>
<th>Citation</th>
<th>Subject Numbers</th>
<th>Ages (years)</th>
<th>Gender</th>
<th>Healthy Status</th>
<th>Body Mass Index (BMI) kg/m²</th>
<th>VO₂ max (ml kg⁻¹ min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boppart, M. D. et al, 1999[^34]</td>
<td>12</td>
<td>Concentric group: 24±2</td>
<td>Concentric group:</td>
<td>Healthy</td>
<td>Concentric group: 23.8±1.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eccentric group: 21±1</td>
<td>M: n=4, F: n=3</td>
<td></td>
<td>Eccentric group: 23.6±1.4</td>
<td></td>
</tr>
<tr>
<td>Schenk, S. et al, 2009[^40]</td>
<td>17</td>
<td>WL: 30±3</td>
<td>F: n=17</td>
<td>Abdominally obese</td>
<td>WL: 34±2</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WE: 33±2</td>
<td></td>
<td></td>
<td>WE: 33±1</td>
<td></td>
</tr>
</tbody>
</table>

N/A: Not applicable
Table 2.5.1 Effects of Exercise on JNK outcomes-Acute Exercise Studies

<table>
<thead>
<tr>
<th>Citation</th>
<th>Time point of baseline sample collection</th>
<th>Time point of post-exercise sample collection</th>
<th>Pre-exercise activated JNK (Mean±SD) (Arbitrary units)</th>
<th>Immediate post-exercise activated JNK (Mean±SD) (Arbitrary units)</th>
<th>Delayed response (Mean±SD) (Arbitrary units)</th>
<th>Effect size (ES)</th>
<th>95% Confidence interval (CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aronson, 1998</td>
<td>Protocol A: 24hr before exercise</td>
<td>3-4min after exercise</td>
<td>1.0±0.06</td>
<td>5.9±0.63 (Combined from protocol A and B)</td>
<td>N/A</td>
<td>10.95</td>
<td>(4.48, 5.32)</td>
<td>P=0.05</td>
</tr>
<tr>
<td>Boppart, 2006</td>
<td>10 days prior marathon</td>
<td>Immediately after marathon, 1, 3, 5 days after marathon</td>
<td>8.0±0.03</td>
<td>55±1.6</td>
<td>Day1: 12±0.03 Day2: 9±0.03 Day3: 9±0.03</td>
<td>41.54</td>
<td>(29.74, 50.91)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Thompson, 2003</td>
<td>2-3 weeks before exercise</td>
<td>48hrs after exercise</td>
<td>BB: 1.00</td>
<td>N/A</td>
<td>BB: 1.95±0.22 VL: 0.45±0.04</td>
<td>N/A</td>
<td>N/A</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Williamson, 2003</td>
<td>Immediately before exercise</td>
<td>Immediately after exercise</td>
<td>Y: 0.8±0.03 O: 1.2±0.08</td>
<td>Y: 0.9±0.03 O: 0.8±0.03</td>
<td>N/A</td>
<td>Y: 3.33 O: 6.62</td>
<td>Y: (0.07, 0.13) O: (3.88, 8.64)</td>
<td>Pre &amp; post exercise: P&lt;0.05</td>
</tr>
<tr>
<td>Chan, 2004</td>
<td>Immediately before exercise</td>
<td>Immediately after exercise</td>
<td>ND: 0.15±0.02 LCHO: 0.3±0.06</td>
<td>ND: 0.48±0.05 LCHO: 0.65±0.09</td>
<td>ND: 8.67 LCHO: 4.58</td>
<td>ND: (0.29, 0.37) LCHO: (0.27, 0.43)</td>
<td>Pre &amp; post exercise: P&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Schenk, 2007</td>
<td>N/A</td>
<td>14hrs after exercise</td>
<td>N/A</td>
<td>Sedentary group: 3.4±0.35 Exercise group: 3.4±0.1</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>P=0.05</td>
</tr>
<tr>
<td>Harper, 2008</td>
<td>Immediately before exercise</td>
<td>Immediately 1min &amp; 2hrs after exercise</td>
<td>1.0</td>
<td>3.2±0.08</td>
<td>1.5±0.2</td>
<td>N/A</td>
<td>N/A</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

All the samples were skeletal muscle and obtained by skeletal muscle biopsy.

BB: Biceps Brachii exercise group; VL: Vastus Lateralis exercise group
Y: Young group; O: Old group
ND: Normal diet group  LCHO: Low carbohydrate group
WL: Weight loss group  WE: Weight loss + exercise group

Only Thompson’s study reported the exact arbitrary units of JNK activity measured. All the other results were calculated from the graphs.

Samples for JNK analysis were obtained from whole skeletal muscle homogenization except Chan’s study\textsuperscript{39} was using nuclear extraction; all the studies reported that total JNK protein levels remained unaltered.
Table 2.5.2 Effects of Exercise on JNK outcomes-Chronic Exercise Studies

<table>
<thead>
<tr>
<th>Citation</th>
<th>Time point of baseline sample collection</th>
<th>Time point of post-exercise sample collection</th>
<th>Pre-exercise activated JNK (Mean=SD) (Arbitrary units)</th>
<th>Immediate post-exercise activated JNK (Mean=SD) (Arbitrary units)</th>
<th>Delayed response (Mean=SD) (Arbitrary units)</th>
<th>Effect size (ES)</th>
<th>95% Confidence interval (CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boppert, 1999</td>
<td>(Day 1) in the morning after 1 night fasting</td>
<td>(Day 2) within 10 min after exercise, 3 hrs, 6 hrs and 24 hrs after exercise</td>
<td>CONC: 1.0 ECCE: 1.0</td>
<td>CONC: 3.8±0.23 ECCE: 15±0.87</td>
<td>CONC: 1.3±0.09 ECCE: 3.0±0.17</td>
<td>N/A</td>
<td>N/A</td>
<td>Pre &amp; post-exercise: P&lt;0.05</td>
</tr>
<tr>
<td>Schenk, S. et al. 2009</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>WL: 0.6±0.02 WE: 0.6±0.02</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

CONC: Concentric group  ECCE group: Eccentric group

All the results were calculated from the graphs.

Samples for JNK analysis were obtained from whole skeletal muscle homogenization; all the studies reported that total JNK protein levels remained unaltered.
Figure 2.1. Flow chart of study selection results

 Intervention category records identified (n = 4910833)

 Outcome category records identified (n = 36798)

 Records after duplicates removed (n = 11253)

 Records excluded (n = 11815)
  - Full-text articles excluded (n = 61)
    - Reasons:
      - No exercise exposure (n = 6)
      - No JNK analysis (n = 7)
      - Animal studies (n = 13)
      - Review (n = 22)

 Manual search (n = 2)

 Included papers (n = 9)
  - Includes studies (n = 9)

 Randomized controlled trial (n = 2)

 Uncontrolled trials (n = 7)
Appendix 1

Search Strategy: Medline (OVID)

1. exercise
2. physical activity
3. training
4. aerobic
5. resistance
6. weight lifting
7. endurance
8. fitness
9. active
10. sedentary
11. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10
12. JNK
13. c-Jun N-terminal kinase
14. 12 OR 13
15. 11 AND 14
CHAPTER 3

Skeletal Muscle c-Jun N-terminal Kinase (JNK) Activity after Acute Resistive Exercise in Older Adults with Type 2 Diabetes:

Methods of Investigating Metabolic and Clinical Correlates
ABSTRACT

Objectives
This chapter describes the methodology of the baseline sub-study of a randomized double-blind, sham-exercise controlled trial designed to assess the efficacy of power training on older adults with type 2 diabetes. This includes an overview of the study design, and focuses in particular on the method of analysis of baseline muscle tissue biopsy performed in this study, and the methods of investigating the factors in muscle and adipose tissues which would be related to exercise adaptation and insulin sensitivity according to our hypotheses.

Methods
Our cohort consisted of adults over 60 years of age with type 2 diabetes. The primary outcomes were insulin resistance and glucose homeostasis, as well as skeletal muscle metabolism, adipokines and inflammatory factors. Secondary outcomes included: adipose tissue area and distribution, regional and whole body composition, all of the components of metabolic syndrome, measures of energy expenditure, physical activity, functional performance, and quality of life. Blinded outcome measures were performed in all the outcomes.

Thigh muscle and subcutaneous adipose tissue biopsy was performed during the outcome assessments. Heat Shock Protein 72 (HSP72) and phosphor-Jun N-terminal Kinase (pJNK), Tumour Necrosis Factor-α (TNF-α), Insulin-like Growth Factor-1 (IGF-1), Interleukin-6 (IL-6) were measured in vastus lateralis muscle. High Molecular Weight
(HMW) Adiponectin, total Adiponectin, Adiponectin ratio, TNF-α, and IL-6 were measured in subcutaneous adipose tissue. Reliability testing was performed to determine the precision for Western Blots.

**Results**

The results reported here were from the first 50 subjects who finished the study in Apr 2009. In total 110 biopsies (73.3%) were performed. Muscle samples were obtained in 102 biopsies (92.7%); adipose samples were obtained in 72 biopsies (65.5%); both tissue types were obtained in 65 biopsies (59.1%). The average weight of muscle samples was 130.6 mg and the average weight of adipose samples was 78.8 mg. No adverse effects of biopsies were reported other than mild transient pain or light-headedness.

The Western blot reliability testing was performed with HSP72. As the Western blot were performed by two investigators (QYG & YW), both intra-rater and inter-rater reliability tests were performed. In QYG’s intra-rater reliability test, the average intra-trial coefficient of variation (CV) was 7.97%, the average inter-trial CV performed on different days was 9.14%. The average inter-rater CV was 1.89% between the two investigators. The shape of the Bland-Altman plots did not suggest systematic bias, and all data were within 2 SD of mean values. The mean±SD of intra-assay coefficient of repeatability (CR) was 24.84±9.77 and the mean±SD of inter-assay CR was 35.91±11.04. The mean±SD of inter-rater CR was 4.87±3.09 between the two investigators.
Conclusions

This study conforms to all design and reporting requirements for randomised controlled trials recommended by the CONSORT group. This is the first report of the detailed method, yield, and safety of both subcutaneous adipose and muscle tissue obtained in the same biopsy. As for Western Blot reliability testing, the precision of our assays was considered adequate.
INTRODUCTION

Type 2 diabetes (T2D) is the most common metabolic disease in the world\(^1\). It is rapidly becoming a global pandemic and is projected to afflict more than 300 million individuals worldwide by the year 2025\(^2\). It is the end of a series of metabolic disturbances, starting with insulin resistance (IR), then proceeding to hyperglycemia, and finally T2D\(^3\). Autoimmune destruction of pancreatic β-cells does not occur and ketoacidosis is rare, therefore insulin-dependent therapy is rarely necessary\(^4\).

Skeletal muscle and liver are critical insulin-responsive organs in maintaining glucose homeostasis. Insulin resistance of these two organs accounts for numerous abnormalities of glucose metabolism in T2D patients\(^1\). Additionally, skeletal muscle is the largest reservoir for glucose disposal\(^5\). Muscle weakness, decreased muscle mass and reduction of skeletal muscle fiber numbers and size are related to, and may precede insulin resistance, glucose intolerance, and T2D\(^6\).

Visceral obesity is a critical risk factor of insulin resistance and T2D\(^7\). Visceral adipose tissue floods the portal circulation with free fatty acids (FFA), thus exposing nonadipose tissues to fat excess\(^8\). This leads to ectopic triglycerides (TG) accumulation in muscles, liver and pancreatic beta-cells, resulting in insulin resistance and beta-cell dysfunction\(^8\). It becomes apparent that central obesity is linked to chronic inflammation\(^9\). Visceral adipose tissue and its adipose-tissue resident macrophages produce more proinflammatory cytokines such as TNF-α and less anti-inflammatory adiponectin than other adipocyte depots\(^10-11\). This results in the activation of serine threonine kinases (e.g. JNK}
activity) in insulin responsive tissues such as adipose tissue, skeletal muscle, and liver\textsuperscript{11}. JNK phosphorylates Insulin Receptor Substrate-1 (IRS-1) on Ser312 in humans (Ser307 in rodents), rendering it a poor substrate for the activated insulin receptor (IR)\textsuperscript{12}. This would thus hamper IRS-dependent glucose transporter 4 (GLUT4) translocation to the cell membrane and decrease the capacity of glucose uptake\textsuperscript{13}. However, the role of JNK in insulin resistance and the progression of type 2 diabetes hasn’t been fully described yet. Additionally, previous studies merely paid attention to pJNK, which was regarded as the activated form of JNK and there was little study concerning the role of tJNK level (including pJNK and non-phosphorylated JNK) and potential correlation between tJNK and insulin resistance-related factors. Moreover, little exploration has been made to compare the advantages and disadvantages of using pJNK and p/t JNK ratio as indicators of JNK activation in clinical trials.

This article describes the methods of baseline characteristics analyses of older adults with type 2 diabetes and correlation analyses between JNK and other crucial insulin resistance-related factors.
METHODS

I. Study Design

This study was a double-blind randomised, sham-exercise controlled clinical trial. Subjects (n=100) were randomised to the experimental (high intensity, high velocity power training) or the control group (sham low intensity, non-progressive resistance training, the subjects were blinded to the investigators’ hypothesis as to which was the experimental group) for 12 months. Blinded outcome assessments were conducted at 0, 6, and 12 months in all subjects regardless of compliance level.

Ethical approval was obtained from Ethics Review Committee (Royal Prince Alfred Hospital (RPAH) Zone), Sydney South West Area Health Service (Ethics Committee Protocol No: X04-0096) and written informed consent was obtained from all participants. The trial was registered with the Australian Clinical Trials Registry (ACTR) (ACTR No: ACTRN12606000436572). The assessments were conducted at Cumberland Campus of University of Sydney in Lidcombe New South Wales (NSW) Australia. Computerized Tomography (CT) Scans were performed at the Radiology Department of RPAH in Camperdown NSW Australia. The exercise training was conducted at either Freshwater Rehabilitation in Manly NSW Australia or the Centre for STRONG Medicine, Balmain Hospital in Balmain NSW Australia.

A. Objectives and Hypothesis

Our objective of the whole study was to determine the efficacy of power training as a disease-modifying intervention in older adults with type 2 diabetes. The objective of this
substudy is to describe baseline characteristics in our cohort and to determine the potential correlations between skeletal muscle JNK and the degree of insulin resistance/metabolic syndrome in baseline characteristics. Skeletal muscle is the largest insulin responsive organ in human body, the degree of insulin resistance of skeletal muscle has a large impact on the severity of metabolic syndrome in T2D subjects. JNK has been identified as a central mediator of insulin resistance. Therefore, we hypothesized that the expression level and kinase activity of JNK would have potential correlations with numerous insulin resistance-related characteristics.

**B. Sample Size**

Sample size estimates were driven by hypothesised differences between the experimental and control subjects in the primary outcomes of the trial: insulin sensitivity and HbA1c, based on an average of published studies of progressive resistance training in diabetes/obesity\textsuperscript{14-17}. Sample size was sufficient for testing secondary hypotheses regarding all components of metabolic syndrome as well, with > 90% power, alpha of 0.5, assuming 10% loss to follow-up (See Table 3. 1). Largest available SDs were used for conservative estimates of ES. The study recruited 100 subjects from Aug 2006 to Dec 2009, and the entire study will be finished by Jan 2011. Since the 50\textsuperscript{th} subject completed her 12-month assessments in Apr 2009 and this thesis was to be submitted in Aug 2010, it only included analysis of baseline data from the first 50 subjects.
C. Study Population

Inclusionary Criteria

Inclusionary criteria were based on diagnosed type 2 diabetes, age and physical activity level. Subjects had to be over 60 years old and sedentary (no PRT; structured exercise ≤ 1/week; less than 150min/week low or moderate-intensity walking). Subjects could be treated with diet alone, oral medications or insulin or combination at the time of enrolment.

Exclusionary Criteria

Exclusionary criteria included significant cognitive impairment, non-ambulatory status or lower extremity amputation other than toes, current alcohol or substance abuse, inability to comply with study requirements over the course of one year due to travel plans or other commitments, and specific contraindications to resistance training exercise, such as unstable cardiovascular disease, unrepaired aortic aneurysm, symptomatic hernias, proliferative diabetic retinopathy, or rapidly progressive or terminal illness. Temporary exclusions (any change in dosage or type of diabetic medications within the past 3 months, retinal laser surgery within 6 weeks, uncontrolled hypertension) were resolved prior to study enrollment and screening procedure.

D. Recruitment

Subjects were recruited from August 2006 to Oct 2009 into the study via articles and advertisements in local newspapers, community advertisements, referrals from General Practitioners (GPs) local to the training sites, and word-of-mouth. A telephone screening
questionnaire was followed by a physician history and physical examination, and cardiac stress test in potential subjects. Physician screening was completed initially. If subjects were eligible, the remainder of the baseline testing was completed. Randomization was performed at the completion of all baseline assessments and subjects would receive either high intensity power training or sham exercise control training in the following 12 months. Details of randomization and intervention were not included in this chapter because we only analyzed baseline characteristics in this substudy.

**E. Adverse Events**

A weekly questionnaire, administered by a trainer, in person or by phone was used to monitor adverse events plus changes in health status/medication use/health care utilization in all participants. Any musculoskeletal or cardiovascular event attributable to testing or training was defined \textit{a priori} as an adverse event\textsuperscript{18}. Reasons for any missed sessions that week were elicited.

**F. Outcome measures**

**Primary Outcomes**

The primary outcomes were glucose level, insulin level, insulin resistance as assessed by the HOMA2 computer model, as well as results obtained from skeletal muscle and subcutaneous adipose tissue biopsies (IGF-1, TNFα, IL-6, HSP72, pJNK and tJNK in muscle and Adiponectin, TNFα, IL-6 in adipose tissue)\textsuperscript{19-20}. Details of methods are described in the following sections.
**Secondary Outcomes**

The secondary outcomes were health status including body composition, quality of life and physical activity.

**G. Domains of Assessments**

**Insulin Sensitivity, Glucose Homeostasis**

HOMA2 computer model for insulin sensitivity (IR, %S) and beta cell function (%Beta) (C-peptide was used for HOMA calculations due to the use of long acting insulin in many subjects)\(^9\), HbA1c, C-peptide levels, insulin, fasting, 1 hour post pandrial (1hr PP), 2 hours post pandrial (2hr PP) glucose. Blood samples were taken at Cumberland Campus of University of Sydney in Lidcombe NSW Australia, and sent to Douglass Hanly Moir Pathology (DHM) (Macquarie Park, NSW, Australia, www. dhm. com. au) for analysis.

**Body Composition**

Body mass index (BMI) was calculated from fasting naked weight and stretched stature measurements\(^2\). Waist and thigh circumferences were measured according to the International Diabetes Federation (IDF) protocol\(^2\). Percent body fat and fat-free mass were estimated using bioelectrical impedance (BIA-101: RJL Systems. Detroit, MI); all subjects were measured 3 times early in the morning after a 12-hour fast. Fat mass and fat-free mass were calculated from the formula developed by Lukaski and colleagues for older adults\(^2\).
CT Scans

All CT scans were obtained with a GE Lightspeed CT Scanner (USA) at the Radiology Department of Royal Prince Alfred Hospital, Sydney, Australia.

CT scans of the abdomen: A 1 mm slice was performed at the mid-point of the iliac crest and lowest rib. This was located by palpation with the patient supine and arms rose above the head. A marker placed at the site was visible on the scout image to set up scanning coordinates. A line was drawn from the femoral notch to the marker and the linear distance was recorded. This was designed to enable replication of follow-up scans. Settings were kV: 100 and mA: 170 (depending on subject’s abdominal mass) with displayed field of view (DFOV) 45-48 (depending on subject size).

CT scans of the mid-thigh: A 1 mm slice was performed at the mid-point of the inguinal crease to the proximal pole of the patella measured with the subject supine and knee flexed. A marker placed at the site was visible on the scout image and a linear distance from the femoral notch to this marker was recorded to replicate follow-up scans. Settings were kV: 100 and mA: 170 with displayed field of view (DFOV) 25 (depending on subject size).

Image analysis: Scan images were analysed according to optical density on a Macintosh iBook G4 (Apple; Sunnyvale, CA.), by a trained investigator in a blinded manner. NIH Image software (Version 1. 63, National Institutes of Health) was programmed via specific macros to quantify cross-sectional areas of muscle, bone and adipose tissue.
Manual editing was performed according to a defined protocol. Some images required editing to remove scan bed pixels or artifact causing some parts of the muscle perimeter to extend into the subcutaneous fat and other parts to run too deeply. The addition of pixels to make the abdominal muscle continuous was necessary most of the time in order to calculate visceral adipose area. Scan images did not permit consistent reconstruction of the thigh fascial plane as described by Goodpaster\textsuperscript{24-25}, so editing involved smoothing out the muscle perimeter with a minimal number of pixels and therefore subcutaneous and intermuscular adipose tissue area differ quantitatively from reports based on manual tracing of this border. Thigh muscle attenuation was calculated using a template set up in Excel (Microsoft) based on the ‘average’ density for thigh pixels in a specific optical density range (10-113) chosen to best discriminate muscle from fat and bone.

**Questionnaires**

All questionnaires were interviewer-administered by a trained interviewer, in a private room using visual prompts. All questionnaires were widely-used, previously valuated questionnaires in cohort similar to ours. Habitual physical activity levels were assessed using the Physical Activity Scale for the Elderly (PASE) questionnaire\textsuperscript{26}. Higher values indicate higher levels of walking, leisure time, household, and occupational activity as well as structured exercise of all types (exclusive of study exercise). Health-related quality of life was assessed using Version 2 of the Medical Outcome Survey 36-item Short-Form (SF-36) questionnaire\textsuperscript{27}. Normative values are 50 on this version of the scales.
**Muscle Metabolism**

HSP72, pJNK, tJNK, TNF-α, IGF-1, IL-6 in vastus lateralis muscle were measured by Western blot. Western blotting procedures were described in the following sections.

**Adipokines, Inflammatory Markers**

High Molecular Weight (HMW) Adiponectin, total Adiponectin, Adiponectin ratio, TNF-α, and IL-6 in adipose and C-reactive protein (CRP), HMW Adiponectin, total Adiponectin, and Adiponectin ratio in serum were measured by cytometric bead array. Procedures were described in the following sections.

**H. Statistical Analysis**

Data were inspected for normality visually and statistically (-1 ≤ skewness ≤ 1), and expressed as mean and standard deviation or median and range, as appropriate. Non-normally distributed data were log-transformed prior to use with parametric statistics if possible or used with non-parametric test (spearman test) if assumptions of normality were not met despite transformation. All P values of less than 0.05 were considered statistically significant.

Calculations of effect size were adjusted via Hedges bias-corrected effect size for small sample sizes. Effect sizes were interpreted according to Cohen’s interpretation of ‘trivial’ (<0.20), ‘small’ (≥0.20 <0.50), ‘moderate’ (≥0.50 <0.80), and ‘large’ (≥0.80) effect size. Ninety-five percent confidence intervals (CIs) for the relative ES were calculated.
Relationships between variables of interest were analysed with linear regression models if the data or log-transformed data were normally distributed or with Spearman test if assumptions of normality were not met despite transformation. Statview (Version 5.0 for Windows, Cary NC: SAS Institute Inc) was used for all data analysis. All p values of less than 0.05 were considered statistically significant. Potential clinical significance of adaptations observed was judged in the light of other published literature where available.

II. Muscle and Adipose Tissue Biopsy Procedures

Muscle biopsies were performed at 3 time points: baseline, 6 months after training, and 12 months after training. In total 110 biopsies (73.3%) were performed. Muscle samples were obtained in 102 biopsies (92.7%); adipose samples were obtained in 72 biopsies (65.5%); both tissue types were obtained in 65 biopsies (59.1%). The average weight of muscle samples was 130.6mg and the average weight of adipose samples was 78.8mg. No adverse effects of biopsies were reported other than mild transient pain or light-headedness. The main reasons for biopsies not being performed were medical reasons (subjects who couldn’t or forgot to stop anticoagulants), subjects’ refusals, technical problems, and dropouts. The details are shown in Fig 3.1. In this substudy, we only analyzed the baseline skeletal muscle and adipose samples.
Subjects were required to stop any medications that might increase the risk of bleeding subsequent to the biopsy from 7 days before biopsies until 2 days after biopsy. Biopsies were not performed on subjects who could not safely stop anticoagulants due to atrial fibrillation, mechanical heart valve, recent stent procedures or recent thrombosis/embolism. Subjects took all other medications as usual on the biopsy day. Subjects were allowed to refuse the biopsy and still participate in the trial.

Muscle biopsies were performed on an assessment day, and the testing schedule is shown in Table 3. 2. The subject had a standardized breakfast and a normal lunch before the biopsy. For the breakfast, RESOURCE PLUS (Novartis, Minneapolis, Minnesota, USA), a high calorie, high protein balanced liquid nutrition, was provided. The nutrition information of RESOURCE PLUS is provided in Table 3. 3. The meal size was calculated by the following equation:

\[
\text{Meal Size (ml)} = \frac{(\text{REE (kcal/day)} \times 0.3)}{1.5}
\]

For the lunch, bread, meats, vegetables, coffee, tea and fruit juices were provided, and the subject made his/her own choices.

After lunch, the subject did tests of muscle power and endurance which took approximately 1 hour, and then underwent the muscle biopsy approximately 5 minutes after the muscle testing. Power and endurance testing protocols were described briefly below.


A. Power Testing

Equipment

Keiser pneumatic resistance training equipment (Keiser Sports Health Equipment, Inc., Fresno, CA, USA) with K400 electronics was used for muscle power and endurance testing.

Purpose

- This test measured the ability of muscles to generate force (movement) quickly.
- Force, velocity, and power were assessed using a single explosive contraction at 20, 40, 50, 60, 70, 80, 90, and 100% of the subject’s most recently measured 1RM or, at baseline, the better of the two 1RM measurements for each exercise.

Five exercises were used: bilateral horizontal leg press, seated chest press, bilateral knee extension, seated row, and seated bilateral knee flexion. Keiser A400 software calculated work and power during the concentric phase of the repetition by sampling the system pressure (force) and position (via ultrasonic position transducers) at a rate of 400 times per second. Accuracy of system pressure and position were reported by the manufacturer to be less than 1%. Power (Watts) and velocity (cm/sec) were calculated as the average respective value between 5% and 95% of the concentric phase of the repetition to eliminate noisy data at the beginning and end points of motion.

The highest average power produced throughout the loads tested was recorded as the peak power.
B. Endurance Testing

Equipment
Keiser pneumatic resistance training equipment (Keiser Sports Health Equipment, Inc., Fresno, CA) with K400 electronics was used for muscle endurance testing.

Purpose
Muscle endurance was a test of sub-maximal muscle performance, and the performance relative to this fixed workload was used to determine adaptation to the intervention.

Types of Endurance testing
90% of the subject’s current 1RM was used as the workload. If they could not complete 1 repetition, load was decreased to 80% 1RM.

C. Skeletal Muscle Biopsy
All equipment for both sampling and specimen preparation was arranged before commencing the procedure as shown in Table 3. 4, and the equipment was placed as shown in Fig 3. 2. The subject was asked to rest on a bed in a reclined position with a pillow under his/her head and one pillow supporting the lateral side of the foot on the biopsy leg with the non-dominant thigh exposed. The biopsy site was swabbed with betadine solution (MCP Operations, Virginia, QLD, Australia), after ascertaining that subject was not allergic to iodine/fish/shellfish (in which case alcohol was substituted). The subject’s non-dominant thigh was injected with local anaesthetic (1% Xylocaine HCl) into the dermis and superficial subcutaneous adipose tissue using tuberculin
(dermis) and 5ml syringes and 21G needle attached by the physician. The biopsy site was left to become anaesthetized for several minutes, during which time the subject was instructed to try to completely relax the muscles of the lower limb. A longitudinal incision of about 1 cm was made in the skin of the thigh by the physician using a scalpel. The incision was deepened so that the muscle fascia was cut if possible. The first biopsy needle was then inserted deeply into the vastus lateralis muscle by the physician. Attached to the needle were silicon tubing, a 3-way stopcock and a 50 ml syringe. The pipette tip connecting the silicon tubing to the needle was cut at an angle (approximately 30 degrees at the top) that maximized the cross-sectional area of the opening to the needle (Fig 3. 3). The 50 ml syringe and stopcock were held by Assistant 1. Sampling of muscle occurred when the physician opened the window of the biopsy needle and signalled Assistant 1 to quickly and vigorously apply suction via the 50 ml syringe. The needle window was then closed and suction was ceased. The 3-way stopcock was repositioned by the Assistant 1 to allow air to be expelled from the 50 ml syringe, and then the needle was repositioned slightly by quarter turns for second, third and fourth passes to be made on the thigh muscle, and then removed. After that, the second biopsy needle was inserted superficially under the skin through the same incision, and the similar procedures were used to obtain subcutaneous adipose tissue. Once sampling was completed, the muscle and adipose specimens were examined to determine whether an appropriate quantity of muscle and adipose tissue had been obtained. If the quantity of muscle or adipose tissue was insufficient, the sampling procedure was repeated with a third needle through the same incision. Pressure was applied to the incision by Assistant 2 as soon as the needle was removed for several minutes with sterile gauzes.
incision was then closed with Steri-strips (3M, St. Paul, MN, USA), covered with a protective pad (Cutfilm, Smith&Nephew, Mount Waverley, VIC, Australia) and an elastic pressure (PEG) bandage (Beckton Dickinson, North Ryde, NSW, Australia) applied over a folded gauze pad positioned directly over the wound. The subject remained supine until they felt comfortable and the dressings were secure and the physician cleared them to stand. A note was made if any symptoms or complications of the procedure. The subject was advised regarding post-biopsy care and given a written copy of the care instructions. The sampling procedures were demonstrated in Fig 3. 4. The subject was called the day following the biopsy to assess any related symptoms, and generally seen within 1-3 days for the initiation or resumption of exercise training.

Prior to commencement of the sampling procedure, isopentane was poured into a small container and placed in the liquid nitrogen canister until white balls began to form in isopentane (around 2-5 minutes). The isopentane was not allowed to freeze.

Muscle and adipose samples were placed on the Petri dish, and cleaned with saline to remove visible blood. The muscle sample was divided into 5 samples if possible, and weighed. The samples were placed on foil, and muscle fibres aligned longitudinally, and then placed in isopentane and left for 1 minute. Then the samples were placed in the cryovial tubes, and were frozen immediately in liquid nitrogen. The adipose sample was divided into 4 samples if possible, and weighed. Then the samples were placed in the cryovial tubes, and were frozen immediately in liquid nitrogen. After the biopsy was
finished, the samples were immediately transferred from liquid nitrogen to a -80°C freezer until the time of analyses.

III. Analysis of Muscle and Adipose Tissues

A. Cytokine Analysis

TNF-α, IGF-1, and IL-6 in muscle tissues, HMW Adiponectin, total Adiponectin, Adiponectin ratio, TNF-α, and IL-6 in adipose tissue, CRP, HMW Adiponectin, total Adiponectin, and Adiponectin ratio in serum were sent to School of Medicine and Dentistry, James Cook University (Townsville, QLD, Australia), and analyzed in the laboratory of Prof. Bernhard Baune.

Cytokine concentrations were measured using cytometric bead array (CBA, BD Biosciences, San Diego, USA; BD FACSCalibur) and results were generated using the BD CBA Analysis Software (BD Biosciences, San Diego, CA, USA). These protocols followed published protocols\textsuperscript{30-32}.

- Cytokine concentrations were measured using cytometric bead array (CBA, BD Biosciences, San Diego, USA) for the cytokines IL-6 and TNF-α.
- For the cytometric bead array six bead populations with distinct fluorescence intensities were coated with capture antibodies specific for IL-6 and TNF-α proteins.
- The six bead populations were mixed together to form the BD CBA which resolved in the FL3 channel of a flow cytometer (BD FACSCalibur).
• The capture beads, PE-conjugated detection antibodies, and recombinant standards or test samples were incubated together to form sandwich complexes.

• Following acquisition of sample data using the flow cytometer, the sample results were generated in graphical and tabular format using the BD CBA Analysis Software.

• The intra-assay coefficients of variation were 5-8% for IL-6 and 6-10% for TNF-α.

• The inter-assay coefficients of variation were 8-10% for IL-6 and 8-15% for TNF-α.

• CRP, IGF-1, HMW Adiponectin, total Adiponectin were also measured.

B. Western Blot

HSP72, phospho-JNK (pJNK) and total-JNK (tJNK) in the insulin signalling pathway were tested with Western blot. The manufacturer and catalog number of all antibodies and other main reagents are shown in Table 3.

Muscle sample homogenization

Materials:

1. Extraction buffer (2×):

   350mg Sodium Chloride (NaCl) 0.3M
   2ml Tris (1M, pH=8.0) 100mM
   2ml Ethylenediaminetetraacetic Acid (EDTA) (100mM, pH=7.0) 10mM
   10mg N-ethylmaleimide 4mM
320µl Phenylmethanesulfonyl Fluoride (PMSF) (43.5mg/ml) 4mM
400µl Igepal undiluted 2%
8µl leupeptin (5mg/ml) 2µg/ml
8µl pepstatin A (5mg/ml) 2µg/ml

The above materials were made to 20ml with MilliQ water, separated into 10 tubes, stored at -20°C.

2. Tris-buffered saline (TBS): 2. 42g Tris, 29.24g NaCl were dissolved in MilliQ water and adjusted pH to 7.5, made to 1L.

Methods:
1. The weight of the muscle was measured.
2. Muscle sample was placed in a 4 ml plastic tube, and then was added TBS at a ratio of 1mg muscle: 3µl TBS.
3. The generator (PRO200, Pro Scientific Inc, Monroe, USA) was turned on and switched to “level C”, and then homogenized for 15sec, stopped to rest for 15sec, homogenized for another 15sec.
4. The same amount of 2×extraction buffer to TBS was added and mixed.
5. The liquid was transferred to an effendorf tube, span at 4°C, and 13,000rpm for 20min.
6. Separated: 100µl in a small tube for protein assay, all other in a 1.5ml effendorf tube.

**Muscle protein concentration measurement**

Materials:
1. Reagent A: 4g Sodium Carbonate (Na$_2$CO$_3$), 0.8g Sodium Hydroxide (NaOH), 0.32g sodium tartrate, 2g Sodium Dodecyl Sulphate (SDS) were made to 200ml with MilliQ water

2. Reagent B: 2.56g CuSO$_4$ anhydrous was made to 100ml with MilliQ water

3. Folin-Ciocalteau: diluted 1:1 with MilliQ water on day of use

4. Reagent C: 100 parts of reagent A were mixed with 1 part of reagent B

5. 5% Bovine Serum Albumin (BSA): 10ml MilliQ water was added to 0.5g BSA, separated into 20 tubes, stored at -20°C.

6. Standard: 5% BSA was prepared and made dilutions: 1, 0.5, 0.25, 0.125, 0.0625 g/dl with MilliQ water.

Methods:

1. The reaction was performed in 4ml plastic tubes

2. 300µl water was added to 6µl of homogenate or standard

3. 0.9ml of reagent C was added and vortexed

4. The liquid was left for 10 min at room temperature

5. 90µl of dilute Folin-Ciocalteau was added and vortexed

6. The liquid was left for 45 min at 25 ºC in water bath

7. The value was read at 660 nm in the spectrophotometer

Reactions were done in duplicate (muscle homogenate in triplicate)

The program was set as:

1). Configure: PC configuration

2). Configure: Utilities → photometer: on

3). Acquire mode: quantitative
4). Configure: parameters

Wavelength: 660nm

Repetitions: 2

Concentration: g/dl

0-1

5). Auto zero (use water)

Protein measurement results were reported in concentrations (g/dl). Standard curve and muscle homogenate were both tested in duplicate. The average of duplicates was used as the final concentration of muscle homogenate.

**Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

Materials:

1. Acrylamide bis: 5. 84g acrylamide, 0. 16g bis were dissolved in MilliQ water and made to 20ml. Stored at 4°C, discarded after 1 month. This was used in making gels.

2. Laemli’s buffer: 3g tris, 14. 4g glycine, 1g SDS were dissolved in MilliQ water and made to 1l. Stored at 4°C. This was used in electrophoresis.

3. Tris: 1. 5M pH 8. 8 9. 08g/50ml

   0. 5M pH6. 8 3. 025g/50ml

   Stored at 4°C. These were used in making gels.

4. TBS: 2. 42g Tris, 29. 24g NaCl were dissolved in MilliQ water, adjusted pH to 7. 5, and make to 1l. Stored at 4°C. This was used in washing membranes and diluting antibodies.

5. TBST: 500µl Tween 20 was added to 1l TBS. This was used in washing membranes.
6. Sample buffer: 1. 0ml 0.5M pH6.8 Tris, 0.8ml glycerol, 1. 6ml 10% SDS, 0.4ml 2-mercaptoethanol, 0.4ml 1% bromphenol blue were added to 3. 5ml MilliQ water. Stored at room temperature. This was used to adjust protein concentrations in electrophoresis.

7. Transfer buffer: 3. 03g Tris, 14. 4g glycine and 200ml methanol were added to MilliQ water to 1l. This was used in transferring proteins to nitrocellulose membrane.

Antibodies:

1. HSP72:

Primary antibody: Anti-HSP72 (Cell Signaling), dilution: 1:2000 final (6µl antibody → 12ml 1% milk in TBS)

Secondary antibody: Anti-mouse Immunoglobulin G (IgG) (whole molecule)-Peroxidase antibody produced in sheep, dilution: 1:1000 final (12µl antibody → 12ml 1% milk in TBS)

2. pJNK:

Primary antibody: pJNK (Cell Signaling), dilution: 1:200 final (60µl antibody → 12ml 1% milk in TBS)

Secondary antibody: Anti-mouse IgG (whole molecule)-Peroxidase antibody produced in sheep, dilution: 1:300 final (40µl antibody → 12ml 1% milk in TBS)

3. tJNK:

Primary antibody: tJNK antibody (Cell Signaling), dilution: 1:500 final (12µl antibody → 6ml 1% milk in TBS)
Secondary antibody: Anti-rabbit IgG (whole molecule)-Peroxidase antibody produced in mouse, dilution: 1:6000 final (1µl antibody → 6ml 1% milk in TBS)

Methods:

1. Separating gel (10%) preparation:

   MilliQ water
   1. 5M pH8. 8 Tris
   10% SDS
   Acrylamide bis
   Ammonium Persulfate (APS) (10%) 100µl (need to be fresh)
   Tetramethylethylenediamine (TEMED) 6. 7µl (need to be fresh)

The above materials were mixed and made into separating gel. Water was added above separating gel at last.

2. Stacking gel (4%) preparation:

   MilliQ water
   0. 5M pH6.8 Tris
   10% SDS
   Acrylamide bis
   APS (10%) 50µl (need to be fresh)
   TEMED 5µl (need to be fresh)

The above materials were mixed and made into stacking gel. Comb was added at last to make wells.
3. Protein extract was thawed on ice and adjusted protein concentration with sample buffer to 1mg/ml. Samples were heated for 4 min at 95°C.

4. 5µl color marker was added in the first well, and then 20µl sample was added to each well.

5. Electrophoresis was run for 45min at 200V, 4°C in Laemli’s buffer.

6. Prepare blocking buffer and dissolve overnight:

0. 5% milk blocking buffer

- Skim milk powder 0. 25g
- TBS 50ml
- Tween 20 50µl

**Protein Transfer and Western Blot**

Proteins were transferred to nitrocellulose overnight at 30V, 4°C in transfer buffer. The protocol was described in the Bio-Rad handbook.

Probe the membrane with SNAP Protein Detection System (Millipore):

1. Next morning, the nitrocellulose was air dried for 20 min.

2. Open the blot holder lid. Thoroughly wet the white surface of the blot holder with MilliQ water.

3. Place the membrane in the centre of the blot holder with protein side down. Roll the blot membran gently to remove air bubbles.

4. Place the spacer (wetting not necessary) on top of the membrane and roll again to ensure contact of spacer with blot membrane.

5. Close the blot holder lid. Squeeze firmly at base of tab area to secure lid.
6. Open lid of the SNAP system and place blot holder in chamber, aligning blot holder tabs with notches of chamber. Close and latch lid.

7. Add 10ml 0.5% milk blocking buffer. Use knobs on the system, apply vacuum until well is completely empty. Turn vacuum off.

8. Add 3ml of primary antibody per membrane. Antibody solution must evenly cover entire blot holder surface. Incubate for 15min at room temperature. Solution will be absorbed into the holder and the surface appears dry. Apply vacuum.

9. With vacuum running continuously, wash 3 times with TBST (15ml/time). Turn vacuum off.

10. Add 3ml of secondary antibody. Antibody solution must evenly cover entire blot holder surface. Incubate for 10min at room temperature. Solution will be absorbed into the holder and the surface appears dry. Apply vacuum.

11. With vacuum running continuously, wash 3 times with TBST (15ml/time). Turn vacuum off.

**Enhanced Chemiluminescence (ECL) Visualization**

1. Reagent A (2ml) was mixed with Reagent B (2ml). The reagent was layered over blot, incubated 5 min, tipped off, placed blot on gladwrap, the blot was wrapped so any fluid was sealed.

2. Place the membrane into Bio Rad GelDoc imaging system.

3. Adjust IRS to 50, choose “while luminous” and switch on the white light to make sure the membrane is placed at an appropriate position.

4. Switch of the white light, adjust IRS to 100 and choose “custom-supersensitivity”.

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5. Expose the membrane for 800 sec, 8 times.

6. Quantify the bands according to the manufacture’s guide.

**Data Processing and Analysis**

For statistics, Subject 034 (male) who donated a large amount of muscle samples at baseline was selected as the “control” for all other subjects. In each gel, Subject 034’s baseline muscle homogenate was run in duplicate. Western blot result was reported as the average of the results on 2 gels (in each gel, the result was a ratio of one subject’s optical density compared to the average of Subject 034’s baseline optical densities).

**IV. Method of determining precision for Western Blots**

The reliability test was only done with HSP72 antibody, in order to test the accuracy of Western blot done by a single observer and the repeatability of Western blot in our lab. Baseline samples of 7 subjects who donated larger amounts of muscle samples than other subjects were selected for this test. Western blots of these 7 subjects were done on 3 different days (each run in duplicate, marked as Gel 1 and Gel 2). Table 3. 6 shows the original data of these 3 western blots.

Coefficient of variation (CV) was calculated as a measure of the reliability of the technique. Intra-trial CV was calculated as mean±SD of duplicates in each trial, and expressed as a percentage (Table 3. 7). In the inter-assay CV test, it is difficult to control the film process in Western blot, and the Western blot itself is semi-quantitative (it can just show the relative differences but not the absolute values). Therefore, the
optical densities were converted into percentages using Subject 6 as the control (Table 3. 8). The mean percentage of the 2 values in each trial was calculated as follows (Table 3 . 9). Inter-trial CV was calculated as SD/Mean of the 3 trials of each subject, and expressed as a percentage (Table 3. 9).

All Western blots were done by two observers (YW & QYG), so inter-rater reliability was reported as well (Table 3. 11). Both observers used the same skeletal muscle extracts and tested the same target protein, HSP72. The average percentages of each subjects from 3 trials performed by two observers were listed and the average CV was 1. 89%.

To assess if there was any systematic bias in the Western blot, Bland & Altman plots and statistics were calculated between Gel 1 and Gel 2 in each trial (Fig 3. 5-3. 7), between all pairs of trials (i.e., 1 vs. 2, 2 vs. 3 and 1 vs. 3) (Fig 3. 8-3. 9), and between the results of two observers (Figure 3. 10), using the software MedCalc (version 9). Coefficient of reliability (CR) was calculated as 1. 96 times the standard deviations of the differences between the two measurements (Table 3. 11). A higher CR implies lower precision. The shape of plots (distribution of dot points around mean) was inspected visually to identify any potential for systematic bias, as per Bland and Altman. Inter-trial variability had much higher mean difference than intra-trial variability as expected; examples as shown in Fig 3. 8. and Fig 3. 10. The Bland-
Altman plots showed that all data were within 2 SD of mean. The intra-trial, inter-trial and inter-observer CRs showed enough accuracy and repeatability of the Western blot in our lab.
RESULTS

The results reported here were from the first 50 subjects who finished the study in Apr 2009. In total 110 biopsies (73.3%) were performed. Muscle samples were obtained in 102 biopsies (92.7%); adipose samples were obtained in 72 biopsies (65.5%); both tissue types were obtained in 65 biopsies (59.1%). The average weight of muscle samples was 130.6 mg and the average weight of adipose samples was 78.8 mg. No adverse effects of biopsies were reported other than mild transient pain or light-headedness.

The Western blot reliability testing was performed with HSP72, the average intra-assay coefficient of variation (CV) was 7.97% and the average inter-assay CV within assays performed on different days was 9.14%. The shape of the Bland-Altman plots did not suggest systematic bias, and all data were within 2 SD of mean values. The mean±SD of intra-assay coefficient of repeatability (CR) was 24.84±9.77, and the mean±SD of inter-assay CR was 35.91±11.04.
DISCUSSION

Quality of Study Design

This study conforms to all design and reporting requirements for randomised controlled trials as recommended by CONSORT Statement\textsuperscript{36-37}. In this study, randomization to power training group and sham exercise group was performed, and treatment allocation was concealed. Eligibility criteria for subjects were specified, and the outcome assessors and subjects were double-blinded. Both power training and sham exercise were supervised. The primary analysis was of all available data and the secondary analysis included an intention-to-treat analysis with imputation for missing data.

There have been to our knowledge only 3 previous randomised controlled trials (RCTs) represented in 9 publications\textsuperscript{15,38-45} of exercise training in people with type 2 diabetes or impaired glucose tolerance with muscle biopsy. The average age of subjects in these 3 RCTs was 65 years old. Two of these 3 RCTs represented in 8 publications\textsuperscript{15,38-44} used PRT as an isolated intervention, and the other one\textsuperscript{45} included aerobic training as well as PRT. Our study was the first to use power training as a novel modality of PRT that we hypothesized would better address the multiple co-morbidities and skeletal muscle abnormalities in this particular cohort.

Muscle Biopsy Technique

Strengths

It was reported by Evans and his colleagues\textsuperscript{46} that percutaneous needle biopsy method, the one which was used in this study, increased the size of skeletal muscle specimen
compared with the previous percutaneous muscle biopsy technique described by Bergstrom\textsuperscript{47-48}. The average weight per biopsy of the muscle samples obtained in our study (first 50 subjects) was 130.6 mg, which was much higher than the average weight reported by Evans (78.5 mg). The median weight per biopsy of the adipose samples obtained in our study (first 50 subjects) was 51 mg (range 3 to 412 mg). No previous study has reported the yield from subcutaneous adipose tissue biopsy, and we were able to find only one previous publication mentioning this technique of simultaneous adipose and skeletal muscle biopsies in the English literature\textsuperscript{49}. No adverse effects of biopsies were reported in our study, despite the use of up to 3 different biopsy needles through the same incision, with up to 4 passes (cutting and aspiration with syringe) with each needle. The entire biopsy procedure from time of incision to last sample collection was approximately 2 minutes, with minimal discomfort to the subject imposed by the additional needles. The lack of bruising, bleeding, wound dehiscence or infection in our cohort may be attributable to the insistence on stopping all drugs affecting bleeding time for 7 days before and 2 days after the biopsy, firm pressure by a second assistant for several minutes after the last sampling, inspection of the site by the physician to ensure hemostasis before placing of the steri-strips, use of a compressive bandage for 24 hours after the biopsy, and prohibition of bathing the site for 2 days.

The most common reasons for biopsies not performed were anticoagulants which could not be stopped (16%, 24 times out of total 150 biopsies (50 subjects × 3 timepoints)), and dropouts (7.3%, 11 times out of total 150 biopsies). Only 2.7% (4 times out of total 150 biopsies) of biopsies were not done due to subject refusal. This rate was lower than
another similar study (10 out of 62 patients (16.1%) refused to have biopsies) performed by Castaneda and associates\textsuperscript{15}.

**Limitations**

Technical problems occurred in 4.7% of biopsies (7 times out of total 150 biopsies). Biopsies were performed, but no muscle samples were obtained in those biopsies (adipose samples were however obtained in those biopsies). At the beginning of the study, we only used one biopsy needle to perform each biopsy, so in some cases no samples were obtained or the adipose tissue obtained first blocked adequate sampling of muscle tissue during the subsequent opening of the needle. In that biopsy. To solve this problem, in the later phase of the study, three biopsy needles were prepared to perform passes 2 or 3 three times (if necessary) if the samples obtained in the first pass were not sufficient for both muscle and adipose tissue analyses.

**Western Blot Reliability Test**

**Strengths**

In Western blot reliability testing with HSP72, the average intra-assay coefficient of variation (CV) was 7.97%, the average inter-assay CV was 9.14% and the average CV between two observers’ results were 1.89%. The shape of the Bland-Altman plots did not suggest systematic bias, and all data were within 2 SD of mean values. The mean±SD of intra-assay coefficient of repeatability (CR) was 24.84±9.77, and the mean±SD of inter-assay CR was 35.91±11.04. Ochoa reported that the intra-assay CV
of Western blots was 15% or lower\textsuperscript{50}. Thus, our results indicate precision at least as good as that reported previously in the literature.

The shape of the Bland-Altman plots didn’t show any suggestion of systematic bias, and all data were within 2 SD of the mean. The mean±SD of intra-assay CR was 1.14±0.52, and the mean±SD of inter-assay CR was 35.91±11.04. The values were relatively similar across the 3 trials. Thus, the precision of our assays was considered adequate.

In this reliability study, the same control (Subject 034) was used for all samples to minimize error. To minimize the variability, all solutions used in each trial were freshly made, and all buffers were discarded immediately after using (Laemli’s buffer, transfer buffer, developer and fixer can be re-used in Western blot). Additionally, the protocol was followed exactly for precise time control in every procedure and the same time intervals were maintained between the procedures.

**Limitations**

There were only 7 subjects involved in the intra-assay reliability test, and also, in the inter-assay test, the outcome of one subject was used as control, so there were outcomes from only 6 subjects involved. These sample sizes were relatively small, and could have elevated the variability.

In other Western blot tests in this study, “control sample” was run twice in each gel, and the average of the 2 values was used as the value of control in order to optimize
reliability. The antibodies which need to be frozen and all muscle extractions were separated in small amount into different tubes before being frozen to prevent thawing more than once. The antibodies were stored as required, and the muscle extractions were stored in -80°C freezer.
REFERENCES


### TABLES AND FIGURES

Table 3.1. Sample Size Calculation.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Experimental Mean change (SD)</th>
<th>Control Mean Change</th>
<th>Effect Size (ES)</th>
<th>Beta</th>
<th>Alpha</th>
<th>Sample size estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sensitivity (HOMA2-IR)</td>
<td>-0.6 (0.8)</td>
<td>0 (0.8)</td>
<td>0.75</td>
<td>.10</td>
<td>0.05</td>
<td>78</td>
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<tr>
<td>HbA1c (%)</td>
<td>-1.2 (1.7)</td>
<td>0 (0.2)</td>
<td>0.71</td>
<td>.10</td>
<td>0.05</td>
<td>86</td>
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<tr>
<td>Waist circumference (cm)</td>
<td>-5 (7)</td>
<td>0 (7)</td>
<td>0.71</td>
<td>.10</td>
<td>0.05</td>
<td>86</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>+1.2 (.2)</td>
<td>-0.1 (0.1)</td>
<td>6.50</td>
<td>.10</td>
<td>0.05</td>
<td>4</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>+4.32 (1.6)</td>
<td>+0.62 (3.5)</td>
<td>1.06</td>
<td>.10</td>
<td>0.05</td>
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<td>HDL cholesterol</td>
<td>+8 (3)</td>
<td>0 (2)</td>
<td>1.67</td>
<td>.10</td>
<td>0.05</td>
<td>18</td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>-9.9 (1.6)</td>
<td>+7.7 (1.9)</td>
<td>9.51</td>
<td>.10</td>
<td>0.05</td>
<td>4</td>
</tr>
</tbody>
</table>

SD = Standard deviation

ES = Effect size

HOMA=Homeostatic model assessment

IR= Insulin resistance

HbA1c=Hemoglobin A1c

HDL=High-density lipoprotein
Table 3.2. Testing schedule on the biopsy day.

<table>
<thead>
<tr>
<th>TIME (h:min)</th>
<th>ASSESSMENT</th>
<th>DURATION (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:00</td>
<td>24-hour Food Recall</td>
<td>15</td>
</tr>
<tr>
<td>08:15</td>
<td>Naked Weight Measurement</td>
<td>15</td>
</tr>
<tr>
<td>08:30</td>
<td>Bioelectrical Impedance Analysis (BIA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Resting Metabolic Rate (RMR)</td>
<td>60</td>
</tr>
<tr>
<td>09:30</td>
<td>Sensory Testing</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Monofilament Test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ankle/Brachial Index (ABI)</td>
<td></td>
</tr>
<tr>
<td>10:15</td>
<td>Blood Draw #1 (Fasting)</td>
<td>15</td>
</tr>
<tr>
<td>10:30</td>
<td>Liquid Breakfast</td>
<td>5</td>
</tr>
<tr>
<td>10:45</td>
<td>Questionnaires</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>- Demographics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Geriatric Depression Scale (GDS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Trail Making A &amp; B</td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td>Blood Draw #2 (1h PP)</td>
<td>15</td>
</tr>
<tr>
<td>11:45</td>
<td>Strength Testing</td>
<td>45</td>
</tr>
<tr>
<td>12:30</td>
<td>Blood Draw #3 (2h PP)</td>
<td>15</td>
</tr>
<tr>
<td>12:45</td>
<td>Lunch</td>
<td>30</td>
</tr>
<tr>
<td>13:15</td>
<td>Power &amp; Endurance Testing</td>
<td>30</td>
</tr>
<tr>
<td>13:45</td>
<td>6-min walk test</td>
<td>15</td>
</tr>
<tr>
<td>14:00</td>
<td>Biopsy</td>
<td>30</td>
</tr>
<tr>
<td>14:30</td>
<td>Post Biopsy Care Information</td>
<td>15</td>
</tr>
</tbody>
</table>
Table 3.3. Nutrition information for RESOUCe PLUS.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Serving Size</td>
<td>237 ml (8 fl. oz.)</td>
</tr>
<tr>
<td>Calories</td>
<td>360 kcal</td>
</tr>
<tr>
<td>Protein</td>
<td>13.0g</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>52.0g</td>
</tr>
<tr>
<td>Fat</td>
<td>11.0g</td>
</tr>
<tr>
<td>Sodium</td>
<td>310mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>530mg</td>
</tr>
</tbody>
</table>
### Table 3.4. Equipment required for a single muscle biopsy.

<table>
<thead>
<tr>
<th>Equipment for Physician</th>
<th>Equipment</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle biopsy needle kits (containing</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Gloves (sterile)</td>
<td>1 pair</td>
<td></td>
</tr>
<tr>
<td>Dressing pack</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Betadine</td>
<td>10ml</td>
<td></td>
</tr>
<tr>
<td>PEG bandage</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>21 G needles</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>5 ml syringes</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>50 unit insulin syringes</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Steri-strip</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cutifilm</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Incontinence pad</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Equipment for Assistant 1</th>
<th>Equipment</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gloves (non-sterile)</td>
<td>1 pair</td>
<td></td>
</tr>
<tr>
<td>Xylocaine 1% for injection</td>
<td>10ml</td>
<td></td>
</tr>
<tr>
<td>Scalpel blade (size 11) and handle</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>50 ml syringes</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3 way stop-cocks</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Cryovial containers</td>
<td>As needed</td>
<td></td>
</tr>
<tr>
<td>Liquid nitrogen container with liquid</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Isopentane</td>
<td>30ml</td>
<td></td>
</tr>
<tr>
<td>Container for isopentane (stainless steel)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Saline (0.9% NaCl)</td>
<td>10ml</td>
<td></td>
</tr>
<tr>
<td>Petri dishes</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Forceps</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Aluminium foils (1.5cm × 1.5cm)</td>
<td>As needed</td>
<td></td>
</tr>
<tr>
<td>Xylene free pen</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Container with water for immersion of</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>muscle biopsy needle after procedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incontinence pad</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Equipment for Assistant 2</th>
<th>Equipment</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sterile gauzes</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Sterile gauzes</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Gloves (sterile)</td>
<td>1 pair</td>
<td></td>
</tr>
<tr>
<td>Incontinence pad</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Equipment for Subject</th>
<th>Equipment</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incontinence pad</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other</th>
<th>Equipment</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contaminated waste bin</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.5. Antibodies and major reagents used in Western Blot analysis.

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>Catalog No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HSP72</td>
<td>StressMarq, Victoria, BC, Canada</td>
<td>SMC-100B</td>
</tr>
<tr>
<td>p-JNK (G-7)</td>
<td>Santa Cruz, Santa Cruz, CA, USA</td>
<td>sc-6254</td>
</tr>
<tr>
<td>Anti-JNK</td>
<td>Cell Signaling, Danvers, MA, USA</td>
<td>#9252</td>
</tr>
<tr>
<td>Anti-mouse IgG (whole molecule)</td>
<td>Sigma-Aldrich, St Louis, MO, USA</td>
<td>A5906</td>
</tr>
<tr>
<td>Acrylamide</td>
<td>Bio-Rad, Hercules, CA, USA</td>
<td>161-0100</td>
</tr>
<tr>
<td>Bis</td>
<td>Bio-Rad, Hercules, CA, USA</td>
<td>161-0200</td>
</tr>
<tr>
<td>Ammonium Persulfate</td>
<td>Bio-Rad, Hercules, CA, USA</td>
<td>161-0700</td>
</tr>
<tr>
<td>Temed</td>
<td>Bio-Rad, Hercules, CA, USA</td>
<td>161-0800</td>
</tr>
<tr>
<td>Nitrocellulose Membrane</td>
<td>Amersham, Pittsburgh, PA, USA</td>
<td>RPN2020D</td>
</tr>
<tr>
<td>ECL Reagents</td>
<td>Amersham, Pittsburgh, PA, USA</td>
<td>RPN2109</td>
</tr>
<tr>
<td>Color Marker</td>
<td>Sigma-Aldrich, St Louis, MO, USA</td>
<td>027k6015</td>
</tr>
<tr>
<td>GBX developer</td>
<td>Kodak, Tokyo, Japan</td>
<td>1900943</td>
</tr>
<tr>
<td>GBX fixer</td>
<td>Kodak, Tokyo, Japan</td>
<td>1901857</td>
</tr>
</tbody>
</table>

HSP72=Heat shock protein 72

JNK= c-Jun N-terminal kinase

IgG=Immunoglobulin G

ECL=Enhanced chemiluminescence
Table 3.6. Original western blot optical densities (OD) of reliability test.

<table>
<thead>
<tr>
<th></th>
<th>Trial 1 (OD)</th>
<th>Trial 2 (OD)</th>
<th>Trial 3 (OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gel 1</td>
<td>Gel 2</td>
<td>Gel 1</td>
</tr>
<tr>
<td>Subject 1</td>
<td>8.01</td>
<td>6.53</td>
<td>6.12</td>
</tr>
<tr>
<td>Subject 2</td>
<td>6.46</td>
<td>7.12</td>
<td>6.11</td>
</tr>
<tr>
<td>Subject 3</td>
<td>5.23</td>
<td>5.70</td>
<td>6.55</td>
</tr>
<tr>
<td>Subject 4</td>
<td>5.22</td>
<td>6.25</td>
<td>6.89</td>
</tr>
<tr>
<td>Subject 5</td>
<td>6.11</td>
<td>6.81</td>
<td>6.31</td>
</tr>
<tr>
<td>Subject 6</td>
<td>4.88</td>
<td>4.31</td>
<td>4.88</td>
</tr>
<tr>
<td>Subject 7</td>
<td>5.31</td>
<td>5.78</td>
<td>6.01</td>
</tr>
</tbody>
</table>
Table 3.7. Intra-trial coefficient variations (CV).

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th></th>
<th></th>
<th>Trial 2</th>
<th></th>
<th></th>
<th>Trial 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>CV (%)</td>
<td>Mean</td>
<td>SD</td>
<td>CV (%)</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Subject 1</td>
<td>7.27</td>
<td>1.05</td>
<td>14.40</td>
<td>6.62</td>
<td>0.70</td>
<td>10.58</td>
<td>5.35</td>
<td>0.68</td>
</tr>
<tr>
<td>Subject 2</td>
<td>6.79</td>
<td>0.47</td>
<td>6.87</td>
<td>6.67</td>
<td>0.79</td>
<td>11.87</td>
<td>4.73</td>
<td>0.25</td>
</tr>
<tr>
<td>Subject 3</td>
<td>5.47</td>
<td>0.33</td>
<td>6.08</td>
<td>7.08</td>
<td>0.74</td>
<td>10.49</td>
<td>5.40</td>
<td>0.58</td>
</tr>
<tr>
<td>Subject 4</td>
<td>5.74</td>
<td>0.73</td>
<td>12.7</td>
<td>6.93</td>
<td>0.06</td>
<td>0.82</td>
<td>6.45</td>
<td>0.47</td>
</tr>
<tr>
<td>Subject 5</td>
<td>6.46</td>
<td>0.25</td>
<td>7.66</td>
<td>6.78</td>
<td>0.66</td>
<td>9.80</td>
<td>5.78</td>
<td>0.15</td>
</tr>
<tr>
<td>Subject 6</td>
<td>4.60</td>
<td>0.40</td>
<td>8.77</td>
<td>5.25</td>
<td>0.52</td>
<td>9.84</td>
<td>4.16</td>
<td>0.23</td>
</tr>
<tr>
<td>Subject 7</td>
<td>5.55</td>
<td>0.33</td>
<td>5.99</td>
<td>6.00</td>
<td>0.01</td>
<td>0.24</td>
<td>4.43</td>
<td>0.31</td>
</tr>
<tr>
<td>Average</td>
<td>-</td>
<td>-</td>
<td>8.92</td>
<td>-</td>
<td>-</td>
<td>7.66</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

OD=Optical density

CV=Coefficient variation
Table 3.8. Relative percentages of western blot density.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Trial 1 (%)</th>
<th>Trial 2 (%)</th>
<th>Trial 3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>Subject 1</td>
<td>164.14</td>
<td>151.51</td>
<td>125.41</td>
</tr>
<tr>
<td>Subject 2</td>
<td>132.38</td>
<td>165.20</td>
<td>125.20</td>
</tr>
<tr>
<td>Subject 3</td>
<td>107.17</td>
<td>132.25</td>
<td>134.22</td>
</tr>
<tr>
<td>Subject 4</td>
<td>106.97</td>
<td>145.01</td>
<td>141.19</td>
</tr>
<tr>
<td>Subject 5</td>
<td>125.20</td>
<td>158.00</td>
<td>129.30</td>
</tr>
<tr>
<td>Subject 6 *</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Subject 7</td>
<td>108.81</td>
<td>134.11</td>
<td>123.16</td>
</tr>
</tbody>
</table>

*= Subject 6 was the control in each trial. All the other optical densities were compared to subject 6 to get relative percentages of densities.
Table 3.9. Inter-trial coefficient variations (CV).

<table>
<thead>
<tr>
<th></th>
<th>Mean of</th>
<th>Mean of</th>
<th>Mean of</th>
<th>Mean (%)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1</td>
<td>157.83</td>
<td>126.08</td>
<td>128.51</td>
<td>137.47</td>
<td>17.67</td>
<td>12.85</td>
</tr>
<tr>
<td>Subject 2</td>
<td>148.79</td>
<td>127.04</td>
<td>113.74</td>
<td>129.86</td>
<td>17.69</td>
<td>13.63</td>
</tr>
<tr>
<td>Subject 3</td>
<td>119.71</td>
<td>134.85</td>
<td>129.78</td>
<td>128.11</td>
<td>7.71</td>
<td>6.02</td>
</tr>
<tr>
<td>Subject 4</td>
<td>125.99</td>
<td>132.72</td>
<td>155.04</td>
<td>137.92</td>
<td>15.21</td>
<td>11.03</td>
</tr>
<tr>
<td>Subject 5</td>
<td>141.60</td>
<td>129.27</td>
<td>139.31</td>
<td>136.73</td>
<td>6.56</td>
<td>4.8</td>
</tr>
<tr>
<td>Subject 7</td>
<td>121.46</td>
<td>114.97</td>
<td>106.58</td>
<td>114.34</td>
<td>7.46</td>
<td>6.52</td>
</tr>
<tr>
<td>Average</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.14</td>
</tr>
</tbody>
</table>

SD= Standard deviation
CV=Coefficient variation
Table 3.10. Inter-reliability testing between two assessors (YW & QYG) of Western blot

<table>
<thead>
<tr>
<th>Subject</th>
<th>YW (%)</th>
<th>QYG (%)</th>
<th>Mean (%)</th>
<th>SD (CV%)</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1</td>
<td>131.22</td>
<td>137.47</td>
<td>134.35</td>
<td>4.42</td>
<td>3.29</td>
</tr>
<tr>
<td>Subject 2</td>
<td>128.12</td>
<td>129.86</td>
<td>128.99</td>
<td>1.23</td>
<td>0.95</td>
</tr>
<tr>
<td>Subject 3</td>
<td>127.03</td>
<td>128.11</td>
<td>127.57</td>
<td>0.76</td>
<td>0.60</td>
</tr>
<tr>
<td>Subject 4</td>
<td>141.91</td>
<td>137.91</td>
<td>139.91</td>
<td>2.83</td>
<td>2.02</td>
</tr>
<tr>
<td>Subject 5</td>
<td>138.44</td>
<td>136.73</td>
<td>137.59</td>
<td>1.46</td>
<td>0.88</td>
</tr>
<tr>
<td>Subject 7</td>
<td>120.27</td>
<td>114.33</td>
<td>117.30</td>
<td>4.20</td>
<td>3.58</td>
</tr>
<tr>
<td>Average CV</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.89</td>
</tr>
</tbody>
</table>

SD=Standard deviation

CV=Coefficient variation
Table 3.11. Coefficient of repeatability (CR) for intra-trial, inter-trial and inter-observer reliability testing.

<table>
<thead>
<tr>
<th></th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-trial</td>
<td></td>
</tr>
<tr>
<td>Trial 1</td>
<td>1.74</td>
</tr>
<tr>
<td>Trial 2</td>
<td>0.93</td>
</tr>
<tr>
<td>Trial 3</td>
<td>0.76</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.14±0.52</td>
</tr>
<tr>
<td>Inter-trial</td>
<td></td>
</tr>
<tr>
<td>Trial 1 &amp; 2</td>
<td>34.22</td>
</tr>
<tr>
<td>Trial 1 &amp; 3</td>
<td>47.69</td>
</tr>
<tr>
<td>Trial 2 &amp; 3</td>
<td>26.87</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>36.26±10.58</td>
</tr>
<tr>
<td>Inter-observer</td>
<td></td>
</tr>
<tr>
<td>Subject 1 (YW&amp;QYG)</td>
<td>8.66</td>
</tr>
<tr>
<td>Subject 2 (YW&amp;QYG)</td>
<td>2.41</td>
</tr>
<tr>
<td>Subject 3 (YW&amp;QYG)</td>
<td>1.49</td>
</tr>
<tr>
<td>Subject 4 (YW&amp;QYG)</td>
<td>5.55</td>
</tr>
<tr>
<td>Subject 5 (YW&amp;QYG)</td>
<td>2.86</td>
</tr>
<tr>
<td>Subject 7 (YW&amp;QYG)</td>
<td>8.23</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.87±3.09</td>
</tr>
</tbody>
</table>

CR=Coefficient of repeatability
Fig 3.1.1 Biopsy equipment 1.
Fig 3.1.2 Biopsy Equipment 2.
Fig 3.2.1 Picture of biopsy needle, pipette tip, silicon tubing, 3-way stopcock and syringe.
Fig 3.2.2. Picture of the cut pipette tip, angled so as to maximize suction surface area and biopsy yield.
Fig 3.3.1 Biopsy Procedure 1.
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CHAPTER 4

Skeletal Muscle c-Jun N-terminal Kinase (JNK) Activity after Acute Resistive Exercise in Older Adults with Type 2 Diabetes:

Results of Metabolic and Clinical Correlates
AUTHOR DECLARATIONS

I hereby acknowledge that my contribution to the manuscript titled:

Skeletal Muscle c-Jun N-terminal Kinase (JNK) Activity after Acute Resistive Exercise in Elder Adults with Type 2 Diabetes:

Methods of Metabolic and Clinical Correlates

is accurately described, and I give permission for Qianyu Guo to submit this manuscript as part of her thesis for the fulfilment of the award of M.App.Sc

Author Contributions

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Yi Wang - subject testing, primary biopsy assistant, biopsy sample analysis, data acquisition, entry, analysis and interpretation, manuscript preparation, review of manuscript

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ABSTRACT

Objective

This chapter describes the results of the baseline sub-study of a randomized double-blind, sham-exercise controlled trial designed to assess the efficacy of power training in older adults with type 2 diabetes. The primary purpose of this sub-study was to define the state of total and activated JNK in skeletal muscle which had been exposed to an acute resistive bout of exercise. In addition, metabolic and clinical characteristics were investigated in an attempt to explain the variance and the metabolic relevance of the JNK expression (total JNK, tJNK) and activation (ratio of phosphorylated/total JNK, p/tJNK) observed. We hypothesized that greater JNK expression and activation would be related to impaired health status, chronic inflammation, metabolic disturbance and insulin resistance in our cohort.

Methods

Our cohort consisted of sedentary adults over 60 years of age with type 2 diabetes. The study recruited 103 participants from Aug 2006 to Dec 2009. Since the 50th participant completed their assessments in Apr 2009 and this thesis was to be submitted in Aug 2010, it only included analysis of baseline data from the first 50 participants which were available at the time of submission.

Insulin resistance was measured by homeostasis model assessment 2 (HOMA 2) computerized model; body composition was measured by computed tomography (CT) scan, bioelectric impedance analysis (BIA) and anthropometrics; glucose, insulin and
serum cytokines (adiponectin, c-reactive protein (CRP) were measured by serum assays, factors related to the insulin signalling pathway obtained from skeletal muscle and subcutaneous adipose tissue biopsies (Insulin-like Growth Factor-1 (IGF-1), Tumour Necrosis Factor-α (TNFα), Interleukin 6 (IL-6), Heat Shock Protein 72 (HSP72), phospho-Jun N-terminal Kinase (pJNK) and total JNK (tJNK) in muscle, and Adiponetin, TNFα, IL-6 in adipose tissue) were measured by cytokine assays and Western Blot. The secondary outcomes, including health status, medications, physical performance and quality of life, were assessed by validated questionnaires.

**Results**

35 of the 50 subjects had baseline measures of skeletal muscle JNK. As hypothesized, higher JNK was related to potentially poorer health status: number of medications/day (r=0.304, 0.081) and lower habitual physical activity (r=-0.333, p=0.55). Similarly, the relationships we found between total and activated JNK and anti-inflammatory/metabolic factors supported our hypotheses: higher tJNK was related to lower serum (r=-0.364, p=0.057) and adipose tissue (r=-0.465, p=0.060) HMW/adiponectin ratios, and higher p/tJNK was related to higher skeletal muscle IL-6 (rho=0.644, p<0.001), lower serum total (r=-0.336, p=0.081) and HMW adiponectin (r=-0.504, p=0.006), and lower HOMA β cell function (r=-0.332, p=0.055).

By contrast, the relationships we found between JNK and systemic pro-inflammatory and local anabolic factors were inconsistent with our hypothesis. Specifically, higher tJNK was related to lower serum CRP (r=-0.457, p=0.015) and higher skeletal muscle IGF-
1(r=0.641, p<0.001). Also contrary to our expectations, higher p/tJNK was related to lower serum total cholesterol (r=-0.059, p<0.001) and LDL (r=-0.401, p=0.016).

**Conclusion**

This was the first study to describe activated and total skeletal muscle JNK expression after acute resistive exercise in older adults with type 2 diabetes, and the first study to analyze potential correlations between skeletal muscle JNK and other aspects of health status, metabolism, and inflammation in this cohort. As hypothesized, higher JNK was related to more impaired health status (age, inactivity) and inversely related to anti-inflammatory adiponectin and HOMA2 β cell function. Unexpectedly, however, higher JNK was related to higher skeletal muscle IGF-1 and inversely related to CRP, serum total cholesterol and LDL.

Additional studies are warranted to investigate whether chronic exercise training in type 2 diabetes alters the expression and activation of JNK, and to determine whether beneficial adaptations in JNK may explain a portion of the variance in exercise-related metabolic benefits in this cohort.
INTRODUCTION

Type 2 diabetes (T2D) is the most common metabolic disease in the world\(^1\). It is rapidly becoming a global pandemic and is projected to afflict more than 300 million individuals worldwide by the year 2025\(^2\). It is the end of a series of metabolic disturbances, starting with insulin resistance (IR), then proceeding to hyperglycemia, and finally T2D\(^3\). Autoimmune destruction of pancreatic β-cells does not occur and ketoacidosis is rare, therefore insulin-dependent therapy is rarely necessary\(^4\).

Physical inactivity is a well-established risk factor for T2D\(^5\). Previous studies indicate that reduction of physical activity is associated with decreased skeletal muscle mass and function, impaired glucose uptake by muscle, disturbed glucose and lipid metabolism, elevated body mass index (BMI) and visceral obesity, which are all highly related to the progression of T2D\(^5\).

Two body composition changes common with aging, sarcopenia (loss of muscle mass) and excess central adiposity, are both associated with metabolic impairments. Skeletal muscle is the largest reservoir of glucose in the body\(^6\). Muscle weakness, decreased muscle mass and reduction of skeletal muscle fiber numbers and size are related to, and may precede insulin resistance, glucose intolerance, and type 2 diabetes\(^7\). Visceral obesity, also critical risk factor for insulin resistance and type 2 diabetes\(^8\), is linked to chronic inflammation\(^9\). Visceral adipose tissue and its adipose-tissue resident macrophages produce more proinflammatory cytokines such as TNF-α and IL-6 and less of the anti-inflammatory adiponectin than other adipocyte depots\(^10-11\). This results in the
activation of serine threonine kinases including c-Jun N-terminal kinase (JNK) in insulin responsive tissues such as adipose tissue, skeletal muscle, and liver\textsuperscript{11}. JNK phosphorylates Insulin Receptor Substrate-1 (IRS-1) on Ser312 in humans, rendering it a poor substrate for the activated insulin receptor (IR)\textsuperscript{12}. This would theoretically impair IRS-dependent glucose transporter 4 (GLUT4) translocation to the cell membrane and reduce glucose uptake\textsuperscript{13}. However, the role of JNK in insulin resistance and the progression of type 2 diabetes hasn’t yet been fully described.

Additionally, previous studies typically only investigated phosphorylated JNK (pJNK), which was regarded as the activated form of JNK. By comparison, there has been relatively little study of total JNK (tJNK) and the relationships between tJNK and other factors related to insulin resistance. The advantages and disadvantages of using pJNK vs. the ratio of pJNK to tJNK (p/t JNK ratio) as indicators of JNK activation are unknown. The activity of an enzyme is defined as the activity of an enzyme per milligram of total protein. It is a measure of enzyme processivity at a specific substrate concentration\textsuperscript{14}. We measured tJNK and p/tJNK in our study because of the following reasons: Most previous studies paid most attention to the role of activated JNK, hence tJNK level has received little attention. However, it is known that protein expression and degradation are relatively chronic biological processes comparing to the reversible protein phosphorylation\textsuperscript{15-17}. The balance between JNK expression and degradation might re-adjust according to pathological condition of insulin resistance therefore influence tJNK level, indicating that tJNK might represent certain insulin resistance-related abnormalities.
as well. As for p/t JNK ratio, we regarded it as a better gauge of JNK activity as it eliminates individual variance of tJNK protein levels among different subjects.

Therefore, we designed this cross-sectional sub-study using the baseline data from the Great 2 Do randomized controlled trial of power training in older adults with type 2 diabetes. We hypothesized that greater JNK expression and activation would be related to impaired health status, elevated chronic inflammation, metabolic disturbance and insulin resistance in our older adult T2D cohort.
METHODS

Study Design and Recruitment of Participants

The overall study was a double-blind randomized, sham-exercise controlled clinical trial. Participants (n=103) were randomized to the experimental (high intensity, high velocity power training) or the control group (sham low intensity, non-progressive resistance training). The participants were blinded to the investigators’ hypothesis as to which was the experimental group for 12 months. There were two aims for this baseline sub-study: one aim was to describe the baseline characteristics of health status, body composition, glucose homeostasis, insulin resistance and levels of blood lipids and cytokines, myokines and adipokines; the other aim was to explore potential correlations between skeletal muscle JNK and the above characteristics with a particular focus on metabolic factors.

Ethical approval was obtained from Ethics Review Committee (Royal Prince Alfred Hospital (RPAH) Zone), Sydney South West Area Health Service (Ethics Committee Protocol No: X04-0096) and written informed consent was obtained from all participants. The trial was registered with the Australian Clinical Trials Registry (ACTR) (ACTR No: ACTRN1260600436572).

The study recruited 103 participants from Aug 2006 to Dec 2009, and the entire study will be finished by Jan 2011. Since the 50th participant completed her 12-month assessments in Apr 2009 and this thesis was to be submitted in Aug 2010, it only
included analysis of baseline data from the first 50 participants which were available at the time of submission.

Inclusionary criteria were based on diagnosed type 2 diabetes, age and physical activity level. Participants had to be over 60 years old and sedentary (no PRT; structured exercise ≤ 1/week; less than 150 min/week low or moderate-intensity walking). Participants could be treated with diet alone, oral medications or insulin or combination at the time of enrolment. Exclusionary criteria included significant cognitive impairment, non-ambulatory status or lower extremity amputation other than toes, current alcohol or substance abuse, inability to comply with study requirements over the course of one year due to travel plans or other commitments, and specific contraindications to resistance training exercise, such as unstable cardiovascular disease, unrepaired aortic aneurysm, symptomatic hernias, proliferative diabetic retinopathy, or rapidly progressive or terminal illness. Temporary exclusions (any change in dosage or type of diabetic medications within the past 3 months, retinal laser surgery within 6 weeks, uncontrolled hypertension) were resolved prior to study enrollment and screening procedure.

**Sample Collection and Measurements of Primary Characteristics**

The primary characteristics of interest in this sub-study were blood glucose and insulin level, insulin resistance as assessed by the HOMA2 computer model, serum cytokines and lipids, as well as results obtained from skeletal muscle and subcutaneous adipose tissue biopsies (IGF-1, TNFα, IL-6, HSP72, pJNK and tJNK in muscle and Adiponectin, TNFα, IL-6 in adipose tissue). The secondary characteristics of interest were health
status, including burden of disease, body composition, quality of life and habitual physical activity level. Details are presented below.

**Insulin Sensitivity, Glucose Homeostasis**

Blood samples were taken at Cumberland Campus of University of Sydney in Lidcombe NSW Australia, and sent to Douglass Hanly Moir Pathology (DHM) (Macquarie Park, NSW, Australia, www.dhm.com.au) for analysis. Fasting blood glucose, insulin, serum lipids and high sensitivity CRP were measured using standard laboratory procedures.

**Muscle Biopsy**

Muscle biopsies were performed at 3 time points in the overall study: baseline, 6 months after training, and 12 months after training. In this sub-study, we only analyzed the baseline skeletal muscle and adipose samples. Participants were required to stop any medications that might increase the risk of bleeding subsequent to the biopsy from 7 days before biopsies until 2 days after biopsy. Each participant had a standardized breakfast and a normal lunch before the biopsy. For the breakfast, RESOURCE PLUS (Novartis, Minneapolis, Minnesota, USA), a high calorie, high protein balanced liquid nutrition, was provided. For the lunch, bread, meats, vegetables, coffee, tea and fruit juices were provided, and the participant made his/her own choices. After lunch, the participant did tests of muscle power and endurance which took approximately 1 hour, and then underwent the muscle biopsy approximately 5 minutes after the muscle testing.
The participant was asked to rest on a bed in a reclined position with a pillow under his/her head and one pillow supporting the lateral side of the foot on the biopsy leg with the non-dominant thigh exposed. The biopsy site was swabbed with betadine solution (MCP Operations, Virginia, QLD, Australia), after ascertaining that participant was not allergic to iodine/fish/shellfish (in which case alcohol was substituted). The participant’s non-dominant thigh was injected with local anesthetic (1% Xylocaine HCl) into the dermis and superficial subcutaneous adipose tissue using tuberculin (dermis) and 5ml syringes and 21G needle attached by the physician. The biopsy site was left to become anaesthetized for several minutes, during which time the participant was instructed to try to completely relax the muscles of the lower limb. A longitudinal incision of about 1 cm was made in the skin of the thigh by the physician using a scalpel. The incision was deepened so that the muscle fascia was cut if possible. The first biopsy needle was then inserted deeply into the vastus lateralis muscle by the physician. The 50 ml syringe and stopcock were held by Assistant 1. Sampling of muscle occurred when the physician opened the window of the biopsy needle and signaled Assistant 1 to quickly and vigorously apply suction via the 50 ml syringe. The needle window was then closed and suction was ceased. The 3-way stopcock was re-positioned by the Assistant 1 to allow air to be expelled from the 50 ml syringe, and then the needle was repositioned slightly by quarter turns for second, third and fourth passes to be made on the thigh muscle, and then removed. After that, the second biopsy needle was inserted superficially under the skin through the same incision, and the similar procedures were used to obtain subcutaneous adipose tissue. Once sampling was completed, the muscle and adipose specimens were examined to determine whether an appropriate quantity of muscle and
adipose tissue had been obtained. If the quantity of muscle or adipose tissue was insufficient, the sampling procedure was repeated with a third needle through the same incision. Pressure was applied to the incision by Assistant 2 as soon as the needle was removed for several minutes with sterile gauzes. The incision was then closed with Steri-strips (3M, St. Paul, MN, USA), covered with a protective pad (Cutfilm, Smith&Nephew, Mount Waverley, VIC, Australia) and an elastic pressure (PEG) bandage (Beckton Dickinson, North Ryde, NSW, Australia) applied over a folded gauze pad positioned directly over the wound. Muscle and adipose samples were placed on the Petri dish, and cleaned with saline to remove visible blood. The weights of all samples were measured and samples were soaked in pre-chilled isopentane for 1 minute. Then the samples were placed in the cryovial tubes, and were frozen immediately in liquid nitrogen. After the biopsy was finished, the samples were immediately transferred from liquid nitrogen to a -80°C freezer until the time of analyses.

**Analysis of Baseline Adipose and Skeletal Muscle Samples**

TNF-α, IGF-1, and IL-6 in muscle tissue, HMW Adiponectin, total Adiponectin, Adiponectin ratio, TNF-α, and IL-6 in adipose tissue, CRP, HMW Adiponectin, total Adiponectin, and Adiponectin ratio in serum were sent to School of Medicine and Dentistry, James Cook University (Townsville, QLD, Australia), and analyzed by Prof. Bernhard Baune.
Cytokine concentrations were measured using cytometric bead array (CBA, BD Biosciences, San Diego, USA; BD FACSCalibur) and results were generated using the BD CBA Analysis Software. These analyses followed reliable published protocols\textsuperscript{20-22}.

Skeletal muscle p-JNK, tJNK, IGF-1, and HSP72 in the insulin signalling pathway were tested with Western blot according to published protocols\textsuperscript{23} as detailed in Chapter 3: Skeletal muscle c-Jun N-terminal Kinase (JNK) activity after acute resistive exercise in older adults with type 2 diabetes: methods of metabolic and clinical correlates.

**Measurements of secondary characteristics**

Body mass index (BMI) was calculated from fasting naked weight and stretched stature measurements\textsuperscript{24}. Waist and thigh circumferences were measured according to the International Diabetes Federation (IDF) protocol\textsuperscript{25}. Percent body fat and fat-free mass were estimated using bioelectrical impedance (BIA-101: RJL Systems. Detroit, MI); all participants were measured 3 times early in the morning after a 12-hour fast. Fat mass and fat-free mass were calculated from the formula developed by Lukaski and colleagues for older adults\textsuperscript{26}.

All CT scans were obtained with a GE Lightspeed CT Scanner (USA) at the Radiology Department of Royal Prince Alfred Hospital, Sydney, Australia. Scan images were analysed according to optical density on a Macintosh iBook G4 (Apple; Sunnyvale, CA.), by a trained investigator in a blinded manner. NIH Image software (Version 1.63, National Institutes of Health) was programmed via specific macros to quantify cross-
sectional areas of muscle, bone and adipose tissue.

All questionnaires were interviewer-administered by a trained interviewer, in a private room using visual prompts. All questionnaires were widely-used, previously validated questionnaires in cohort similar to ours. Habitual physical activity levels were assessed using the Physical Activity Scale for the Elderly (PASE) questionnaire\textsuperscript{27}. Health-related quality of life was assessed using Version 2 of the Medical Outcome Survey 36-item Short-Form (SF-36) questionnaire\textsuperscript{28}.

**Statistical Analysis**

The primary analysis was of all available data with no imputation for missing values. For statistical analysis, data were inspected for normality visually and statistically (-1 ≤ skewness ≤ 1), and expressed as mean and standard deviation or median and range, as appropriate. Non-normally distributed data were log-transformed prior to use with parametric statistics if possible or used with non-parametric test (spearman test) if assumptions of normality were not met despite transformation. Values of zero were changed to 0.0001 to allow for log transformation where necessary. Relationships between variables of interest were analysed with linear regression models if the data or log-transformed data were normally distributed or with Spearman correlation coefficient if assumptions of normality were not met despite transformation. Calculations of effect size were adjusted via Hedges bias-corrected effect size for small sample sizes\textsuperscript{29}. Effect sizes were interpreted according to Cohen’s interpretation of
‘trivial’ (<0.20), ‘small’ (≥0.20 <0.50), ‘moderate’ (≥0.50 <0.80), and ‘large’ (≥0.80) effect size\textsuperscript{29}. Ninety-five percent confidence intervals (CIs) for the relative ES were calculated. All \textit{p} values of less than 0.05 were considered statistically significant. Clinical meaningfulness of differences observed was assessed by evaluation of the magnitude of the differences relative to clinical outcomes in the literature. Statview (Version 5.0 for Windows, Cary NC: SAS Institute Inc) was used for all data analysis.
RESULTS

Recruitment results

A total of 427 people were assessed for eligibility, and 103 (24.1%) of those were randomized, see Figure 4.1. Reasons for exclusion were: not meeting study criteria (5.6%), medical reasons (2.1%), too young (1.2%), too physically active (16.4%), too far to travel (8.2%), inability to commit to the study protocol (33.3%), no longer interested in participating (3.5%), work commitments (2.6%) and other reasons (3.0%). This cross-sectional baseline analysis was based on data from the first 50 participants randomized.

Baseline Characteristics

Demographics

The baseline demographic characteristics are presented in Table 4.1. Age ranged from 59 to 83 years (58% were over 65 years of age). The majority of participants were Caucasian, married, and had completed high school or tertiary level education. Approximately 80% of participants drank alcohol, and nearly 1/3 reported daily consumption. Although nearly 60% had a smoking history, less than 5% were current smokers.

Health Status

Baseline health status is presented in Table 4.2. The cohort was primarily overweight or obese (92%). Eighty percent of the participants took oral diabetic medications, 22% were on insulin, and 14% used both. Overall, there was a high burden of chronic
disease, as all participants had one or more chronic disorders (92% overweight or obese; 50% high total cholesterol; 32% raised triglycerides; 34% reduced HDL cholesterol; 70% hypertension).

**Body Composition**

Characteristics related to body composition are summarized in Table 4.3. Total abdominal fat area was almost identical between male and female participants in our cohort. However, visceral fat area was significantly higher in men than in women, and subcutaneous abdominal fat area was significantly lower in men than in women. On the other hand, subcutaneous thigh fat area was significantly higher in women than in men. Thus, the ratio of visceral fat area to subcutaneous thigh fat area (a ratio associated with excess metabolic risk) was markedly higher in men than in women.

**Factors Associated with skeletal muscle JNK**

Relationships between skeletal muscle JNK and other baseline characteristics are presented in Tables 4.5-4.6 and Figures 4.2-4.3. The correlations were divided into several relevant domains for clarity in the sections listed below.

**Demographics and Health Status**

We hypothesized that JNK would be related to impaired general health status or risk of disease. As expected, we found that higher tJNK was related to higher total number of medications/day (p<0.05), which represents a higher burden of chronic disease, and was related to lower habitual physical activity level (p<0.05). Higher p/tJNK was related to
higher age (p<0.05), again as hypothesized. We also found that p/tJNK was higher in men than in women (p<0.05), possibly consistent with the markedly higher visceral fat/subcutaneous fat ratio observed in men. Thus, both total and activated JNK were generally associated with worse health status/metabolic risk, although not to the exact same factors.

**Inflammatory Milieu and Anabolic factor**

We hypothesized that higher JNK would be related to higher pro-inflammatory factors and inversely related to anti-inflammatory and anabolic factors. Our findings were consistent with this hypotheses: higher tJNK was related to lower serum and adipose HMW/adiponectin ratios (p<0.05). Similarly, higher p/tJNK was related to lower serum total and HMW adiponectin (p<0.05). However, higher tJNK was unexpectedly related to lower serum inflammatory marker CRP (p<0.05). Finally, higher p/tJNK was related to higher skeletal muscle IL-6 (p<0.05), a myokine with both pro- and anti-inflammatory properties. Thus, apart from circulating inflammatory marker CRP, both total and activated JNK expression was generally associated with a pro-inflammatory milieu in serum, adipose tissue, and skeletal muscle. Finally, regarding JNK’s relationship to the anabolic factor IGF-1, both tJNK and p/tJNK were unexpectedly both directly related to skeletal muscle IGF-1 (p<0.05).

**Metabolic Characteristics**

We hypothesized that JNK would be related to impaired insulin sensitivity, glucose homeostasis, and disturbance of lipid metabolism, as JNK has been reported to be
elevated in type 2 diabetes compared to healthy individuals\textsuperscript{12,16}. We found a trend for higher p/tJNK to be related to lower HOMA β cell (p=0.055), consistent with our hypotheses, although not to other aspects of glucose control or insulin sensitivity we measured. Unexpectedly, higher p/t JNK was related to lower serum total cholesterol and LDL (p<0.05), although the prevalent use of statin medications in this cohort, with their marked effect on lipids via activation of the PPAR\textgamma pathway would likely overwhelm any JNK-cholesterol relationships.
DISCUSSION

Based on the proposed significance of JNK in the progression of insulin resistance, metabolic syndrome and type 2 diabetes,12,16 the overall hypothesis of this baseline sub-study was that JNK may be related to impaired health status, elevated chronic inflammation, metabolic disturbance and insulin resistance in our older adult T2D cohort. Consistent with our hypotheses, higher JNK was related to older age, more impaired health status and inversely related to anti-inflammatory adiponectin and HOMA2 β cell function. Unexpectedly, however, higher JNK was related to IGF-1 and inversely related to CRP, serum total cholesterol and LDL. The direct relationship to skeletal muscle IL-6 is complex, as IL-6 has been shown to have both pro- and anti-inflammatory roles in metabolism.

In the sections that follow, these findings are discussed in greater detail with respect to the existing literature, and possible explanations for the outcomes we did observe are offered.

Factors Related to Skeletal Muscle JNK

Health Status

Age and Numbers of Medications/day: This is the first report to our knowledge that higher tJNK is related to higher burden of disease as reflected by greater numbers of medications/day30 and that higher p/tJNK is related to older age. All participants in our cohort had T2D and many had other chronic diseases such as high cholesterol, hypertension, cardiovascular disease, depression and osteoarthritis. The above diseases share a common characteristic, that is chronic low grade inflammation31-34, which triggers
the expression and activation of JNK. This may explain why JNK is related to age and numbers of medications/day.

**Habitual physical activity level:** We now report for the first time that JNK is inversely related to habitual physical activity level, and interpret this again as consistent with our hypotheses. Generally speaking, acute stimuli tend to influence JNK activity (p/tJNK ratio) without influencing tJNK, while chronic stimuli might change tJNK level because regulation of protein expression is a relatively chronic process\(^\text{16}\). Our systematic review of the literature found that acute exercise failed to significantly change skeletal muscle tJNK protein level in any cohort\(^\text{35-41}\), while activated JNK was modified by exercise in 7 studies\(^\text{35-41}\) (Please refer to Chapter 2 for further details). Hence, the influence of acute exercise before our muscle biopsy on tJNK level in the skeletal muscle would have been negligible, even if there was any. Therefore, the tJNK level should be representative of the baseline chronic JNK protein level. The inverse relationship between skeletal muscle tJNK and PASE score could be explained by the chronic protective effects of daily physical activity against the progression of insulin resistance/T2D. For example, higher levels of physical activity have been reported by others to be associated with lower systemic inflammation\(^\text{42-43}\). This would be consistent with our finding of lower tJNK in the most active participants in our study. This lower inflammatory milieu would theoretically inhibit the synthesis and promote the degradation of JNK protein. This novel and interesting finding supports the relationships between physical activity, inflammation, JNK, and insulin resistance, and future studies should include direct measures of physical activity as well as whole body insulin sensitivity to further elucidate
the mechanism underlying the relationships observed, as well as the clinical relevance of these findings.

**Inflammatory milieu & Anabolic factor**

Low-grade chronic inflammation has been implicated as a link between JNK activation and the progression of insulin resistance/T2D\(^4^4\). According to our hypotheses, JNK should have been directly related to pro-inflammatory factors and inversely related to anti-inflammatory factors. However, our findings were not all consistent with these hypotheses.

**C-Reactive Protein (CRP):** We found that higher tJNK was related to lower serum CRP level in our cohort. This finding is inconsistent with our hypotheses. As a circulating marker of inflammation produced predominantly by liver, modest elevation of CRP can represent low-grade chronic inflammation\(^4^5\). Theoretically, CRP should be directly related to JNK therefore\(^4^6\). However, we observed the opposite relationship, and the reason for this paradoxical result is unknown at this time and warrants further investigation.

**Interleukin-6 (IL-6):** We observed that p/t JNK ratio was positively related to IL-6 protein level in the thigh skeletal muscle of older adults with T2D in our baseline sub-study, a novel finding, which must be interpreted in light of the source of the IL-6 and its known complex role in metabolic processes following exercise.
In our baseline assessment, all participants performed 1RM (approximately 5 repetitions, ≥ 70% 1RM), power (4 repetitions, ≥ 70% 1RM) and endurance (median: 8 repetitions, 90% 1RM) tests on knee extension, knee flexion and leg press, and the biopsies were performed immediately (within 5 minutes) after those tests. Thus, the participants in our study were considered to have performed an acute bout of resistance exercise (51 repetitions, ≥ 70% 1RM) before the biopsy.

Consideration of previous literature pertaining to both JNK and IL-6 expression after muscle contraction is necessary to interpret our findings. In this regard, the known adaptations of JNK to exercise in animals and humans are summarized in the systematic review (Chapter 2). In general, available results indicate that JNK activity most often increases immediately after an acute bout of exercise in healthy participants. However, the study designs and results of animal studies concerning the response of JNK to exercise were too heterogeneous to draw any solid conclusions. There have been no previous published human studies concerning the response of JNK to acute exercise in diabetics.

Furthermore, IL-6 is a cytokine which is hypothesized to have anti-inflammatory as well as pro-inflammatory effects. The myokine IL-6 is synthesized locally within skeletal muscle itself and secreted into blood. Pedersen and colleagues reported that plasma IL-6 level in T2D participants was similar to that in healthy participants, and that exercise dramatically increases plasma IL-6 level in both T2D and healthy cohorts. Exercise induced elevation of plasma IL-6 might be a result of muscle contraction...
dependent IL-6 expression and secretion into circulation\textsuperscript{58}. Finally, several studies have provided potential evidence that JNK and IL-6 may be directly related. Boppart and colleagues reported that participants with the highest JNK activity also had the highest plasma IL-6 activity after exercise training\textsuperscript{47}.

Additionally, JNK activation is accompanied by IL-6 induction in C2C12 myoblasts and the elevation of IL-6 mRNA is abolished by JNK inhibitor\textsuperscript{52}. Certain elements of AP-1 transcriptional factor complex are crucial JNK substrates and AP-1 can bind to IL-6 promoter\textsuperscript{59}. Therefore, JNK might induce IL-6 expression via AP-1 transcriptional factor complex.

Thus, the direct correlation between JNK activity and IL-6 observed in our baseline sub-study in older T2D participants may be explained by the stimulus of acute exercise. According to the evidence mentioned above, IL-6 was elevated after a single bout of exercise in T2D participants\textsuperscript{50-51,56-57}. We observed that activated JNK was directly correlated with IL-6. Notably, total JNK was not related to IL-6 in our cohort, further supporting the interpretation that acute muscle contractions led to both activation of JNK and expression of IL-6 in skeletal muscle, whereas chronic habitual physical activity level, by contrast, was inversely related to total JNK level (and unrelated to activated JNK).

**Adiponectin:** As an anti-inflammatory cytokine, adiponectin, plays a pivotal role in regulating lipid and glucose metabolism\textsuperscript{60}. Our results indicate that the serum total
adiponectin in our cohort was much lower than in healthy cohorts reported previously\textsuperscript{61-62}. In addition, we found that both JNK and p/JNK were inversely related to serum and adipose adiponectin, as hypothesized. Our results are consistent with previous findings, as JNK has been reported to negatively regulate adiponectin expression\textsuperscript{60}. Recent studies have provided detailed mechanisms underlying these associations. Specifically, peroxisome proliferator-activated-receptor (PPAR) might serve as a potential link between JNK and adiponectin, as PPAR is able to interact with the adiponectin promoter to enhance expression of adiponectin\textsuperscript{63}. In addition, PPAR can promote the secretion of adiponectin by inhibiting the expression of Erp44, an endoplasmic reticulum retaining chaperone\textsuperscript{64}. JNK was found to inhibit the transcriptional activity of PPAR by phosphorylation\textsuperscript{65}. All of these findings lend mechanistic support to the inverse relationship we observed between JNK and serum and adipose adiponectin in our cohort.

**Insulin-like growth factor-1 (IGF-1):** We now report for the first time that JNK was related to skeletal muscle IGF-1 in an older adult T2D cohort. This finding was inconsistent with our original hypotheses, as IGF-1 is regarded as a critical anabolic factor involved in enhancing the activity of IRS signaling and maintaining skeletal muscle mass, while JNK is related to reduced skeletal muscle mass and insulin signalling in T2D\textsuperscript{66-67}. This paradoxical and unexpected relationship between JNK and IGF-1 is not fully understood. One study reported that blocking JNK activity with specific inhibitors abolished the IGF-1 signaling transduction\textsuperscript{68}, while others reported that JNK activity was unaffected by IGF-1\textsuperscript{69-70}. These limited results are too heterogeneous to conclude
whether IGF-1 is involved in inducing JNK phosphorylation. Only two studies were found reporting that both IGF-1 mRNA and protein levels can be induced by acute exercise\textsuperscript{71-72}. Chronic resistance training has been reported to markedly increase skeletal muscle IGF-1 levels in frail elders however\textsuperscript{73}.

Thus, the direct correlation between skeletal muscle JNK and IGF-1 we found in older T2D participants is likely explained by the stimulus of the acute resistive exercise bout before the biopsy. However, more studies are required to understand the mechanism and relevance of the relationship between JNK and IGF-1 expression after muscle contraction in this and other cohorts.

Metabolic Characteristics

Serum Lipids: Our study reported that p/tJNK was inversely related to serum HDL, LDL and total cholesterol. The relationship between JNK and serum total cholesterol and LDL were inconsistent with our hypotheses and previous reports. JNK has been reported to regulate serum lipids level by inhibiting transcriptional activity of PPAR. It is known that PPARs can promote HDL formation and prohibit cholesterol synthesis. Activation of JNK, therefore, abolishes PPARs’ function, and would be expected to be directly related to LDL/total cholesterol and indirectly related to HDL cholesterol. However, most participants in our cohort took statins regularly. This inconsistency might because that most participants had highly-controlled serum lipids within normal ranges while taking statins regularly. The effects of statins could be strong enough to mask the regulatory impacts of JNK on serum lipids\textsuperscript{43}. 

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**Homeostatic Model Assessment 2 (HOMA2) β Cell Function:** We report for the first time that HOMA2 β cell function was inversely related skeletal muscle JNK activity in our cohort, although this inverse correlation was consistent with our hypotheses. It is known that visceral obesity is a critical risk factor for impaired β cell function because excessive lipid accumulation within the pancreas and chronic inflammation are toxic to β cells. Therefore, visceral obesity is indirectly related to HOMA2 β cell function. In addition, visceral adipose tissue and its adipose-tissue resident macrophages produce more proinflammatory cytokines, which therefore results in JNK activation within skeletal muscle. Hence central obesity provides a link for the indirect correlation between skeletal muscle JNK and HOMA2 β cell function we observed.

In addition, JNK activation is responsible for the sequestration of glucose transporter 4 (GLUT4) within the cells and the subsequent inhibition of glucose uptake by skeletal muscle. Therefore, JNK activation is partially responsible for elevated blood glucose, which is also toxic to β cells. Therefore, skeletal muscle JNK activation may remotely disturb β cell function via glucotoxicity secondary to impaired insulin resistance/glucose homeostasis.

*External validity of our findings*

Our findings are potentially generalizable to the wider diabetes population, as this was not a selective cohort of very healthy diabetics enrolled in our study. Detailed
characteristics of our cohort and comparisons with other T2D cohorts are described below to substantiate this interpretation.

**Health Status**

Generally speaking, the health status of our cohort (91% overweight or obese; 51% high total cholesterol; 31% raised triglycerides; 31% reduced HDL cholesterol; 66% hypertension) was worse than the health status of people with diabetes in Australia (59.6% overweight or obese; 51.2% high total cholesterol; 20.5% raised triglycerides; 23.1% reduced HDL cholesterol; 28.8% hypertension)\(^4\)\(^-\)\(^7\)\(^7\)\(^-\)\(^7\)\(^9\).

**Body Composition**

Notably, 92% of our subjects were overweight or obese. This percentage is higher than that of a similar age group (approximately 74% in people aged 55 or over) reported by Australian Bureau of Statistics\(^7\)\(^8\). Eighty percent of our participants took regular oral diabetic medications, and 22% of them were on insulin. Seventy-four percent of diabetic patients (age ≥55) in Australia took oral diabetic medications, and 17% were on insulin\(^7\)\(^9\). Hence, the usage of medications in our cohort was similar to medications used by people with diabetes in Australia.

Our cohort was similar to Castaneda’s randomized controlled trial of resistance training in 62 subjects with type 2 diabetes\(^8\)\(^0\)-\(^8\)\(^2\) in age, BMI, and general health status. However, Castaneda’s study was performed in Hispanic people with a poorer glycemic control (HbA1c was 8.6±0.3 in Castaneda’s study and 7.2±1.3 in our study). There was another
randomized controlled trial included resistance training with type 2 diabetes\textsuperscript{83-86}. This cohort had similar age and BMI to ours, but with a lower percentage (60\%) of oral diabetic medications usage. Thus, our findings appear to have relevance to both general community-dwelling older adults with obesity and type 2 diabetes, as well as previous RCTs of exercise in this cohort.

\textit{Limitations of the study}

This investigation only included data from the first 50 subjects recruited in this trial, as their results were available at the time of writing, and 7 of them didn't have biopsies due to medical reasons (see Chapter 3). Thus, the sample size of this article was limited, and some type II errors could have occurred.

There were no truly sedentary baseline muscle and adipose samples available. Muscle and adipose tissue biopsies were all performed after 1RM, power and endurance tests, as the purpose of the overall study was to look at the effects of long-term power training on muscle adaptations in older people with type 2 diabetes. The biggest obstacle was that our subjects were older people with impaired health status and burdened with an average of 5 chronic illnesses. Thus, at baseline, there were no resting muscle and adipose tissue biopsy samples as control. We considered the positive relationships among skeletal muscle JNK, IL-6 and IGF-1 as acute responses to a bout of resistance exercise, suggesting that muscle contraction could produce these cytokines and growth factors. However, we don't have any muscle samples before this bout of exercise to definitively substantiate this conclusion.
Although total and activated JNK protein levels were measured in the skeletal muscle, we lack data on JNK mRNA level. Therefore, it is not possible to report potential relationships between JNK transcription/translation and insulin resistance/metabolic syndrome. In addition, there are many other critical components in the insulin signaling pathways we didn’t measure, such as phosphoinositide-3 kinase (PI-3K)/Akt, Akt substrate 160 (AS160) and peroxisome proliferator-activated receptors (PPARs), which future studies should include.

Finally, we had measures of fasting insulin, glucose, and calculated insulin resistance level, which primarily reflected hepatic insulin sensitivity on the morning of the biopsy. Although the HOMA2 model of insulin resistance is related to measures of peripheral glucose uptake, it is likely that whole body insulin sensitivity measures such as a glucose clamp would have provided additional information on the metabolic relevance of the skeletal muscle and adipose tissue proteins we measured.
CONCLUSION

This study is the first clinical trial which systematically described many characteristics related to metabolic and general health status, explored the adaptations of skeletal muscle JNK to acute exercise and analyzed potential correlations between skeletal muscle JNK and other insulin resistance-related factors in serum, adipose tissue, and skeletal muscle in older people with T2D. We found that JNK had expected relationships to metabolic and general health status in most cases, and novel associations were found with habitual physical activity level.

Future studies are required for better understanding of JNK adaptations to acute and chronic exercise and its relationship to health status, inflammation, metabolic syndrome and insulin resistance in diabetes and other clinical cohorts.
ACKNOWLEDGMENTS

This study was funded by the Australian National Health and Medical Research Council, project grant 512381, Diabetes Australia, Australian Diabetes Society, and supported by the University of Sydney. Thank Dennis Keiser, of Keiser Sports Health Equipment, Inc, for development and donation of the K-400 electronics for the Keiser machines.
REFERENCES


37. Chan, M.H., *et al.* Altering dietary nutrient intake that reduces glycogen content leads to phosphorylation of nuclear p38 MAP kinase in human skeletal muscle:


78. ABS, 2004-05. *Australian Bureau of Statistics*

79. ABS, 2007-08. *Australian Bureau of Statistics*


## TABLES AND FIGURES

### Table 4.1 Baseline Demographic Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SD/Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td>Whole Cohort (n=50)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>67.8±6.2</td>
</tr>
<tr>
<td><strong>Gender (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>46%</td>
</tr>
<tr>
<td>Male</td>
<td>54%</td>
</tr>
<tr>
<td><strong>Marital Status (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>60%</td>
</tr>
<tr>
<td>Widowed</td>
<td>12%</td>
</tr>
<tr>
<td>Divorced</td>
<td>18%</td>
</tr>
<tr>
<td>Never Married</td>
<td>6%</td>
</tr>
<tr>
<td>Separated</td>
<td>4%</td>
</tr>
<tr>
<td><strong>Ethnic Origin (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>96%</td>
</tr>
<tr>
<td>South-East Asian</td>
<td>2%</td>
</tr>
<tr>
<td>Other</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Residential Status (%)</strong></td>
<td></td>
</tr>
<tr>
<td>House</td>
<td>74%</td>
</tr>
<tr>
<td>Unit</td>
<td>26%</td>
</tr>
<tr>
<td><strong>Living Situation (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Alone</td>
<td>31%</td>
</tr>
<tr>
<td>Category</td>
<td>Percentage</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Spouse/Partner</td>
<td>43%</td>
</tr>
<tr>
<td>Family</td>
<td>26%</td>
</tr>
<tr>
<td><strong>Level of Education Completed (%)</strong></td>
<td></td>
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<tr>
<td>High School</td>
<td>49%</td>
</tr>
<tr>
<td>Tertiary</td>
<td>41%</td>
</tr>
<tr>
<td>Post-Graduate Study</td>
<td>10%</td>
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<tr>
<td><strong>Work (%)</strong></td>
<td>25%</td>
</tr>
<tr>
<td><strong>Volunteer Work (%)</strong></td>
<td>41%</td>
</tr>
<tr>
<td><strong>Annual Income Distribution (%)</strong></td>
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<tr>
<td>&lt;$15,000</td>
<td>21%</td>
</tr>
<tr>
<td>$15,000-$30,000</td>
<td>28%</td>
</tr>
<tr>
<td>&gt;$30,000</td>
<td>51%</td>
</tr>
<tr>
<td><strong>Pension (%)</strong></td>
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<tr>
<td>Nil</td>
<td>63%</td>
</tr>
<tr>
<td>DVA</td>
<td>2%</td>
</tr>
<tr>
<td>Age</td>
<td>27%</td>
</tr>
<tr>
<td>Widows</td>
<td>4%</td>
</tr>
<tr>
<td>Disability</td>
<td>4%</td>
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<tr>
<td><strong>Private Health Insurance (%)</strong></td>
<td>88%</td>
</tr>
<tr>
<td><strong>Smoking-History (%)</strong></td>
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</tr>
<tr>
<td>Smoking-Current (%)</td>
<td>59%</td>
</tr>
<tr>
<td><strong>Smoking-Current (%)</strong></td>
<td>4%</td>
</tr>
<tr>
<td><strong>Drinking Habit (%)</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>20%</td>
</tr>
<tr>
<td>Frequency</td>
<td>Percentage</td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Once a month</td>
<td>6%</td>
</tr>
<tr>
<td>Two or three times a month</td>
<td>2%</td>
</tr>
<tr>
<td>Once or twice times a week</td>
<td>27%</td>
</tr>
<tr>
<td>Three to four times a week</td>
<td>12%</td>
</tr>
<tr>
<td>Almost everyday</td>
<td>33%</td>
</tr>
</tbody>
</table>

SD = Standard Deviation

Data were inspected for normality visually and statistically (-1 ≤ skewness ≤ 1) and are presented as mean and SD or median and range as appropriate.
### Table 4.2 Baseline Health Status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Health Status</strong></td>
<td>Whole Cohort (n=50)</td>
</tr>
<tr>
<td><strong>No. of Chronic Diseases</strong></td>
<td>5.1±1.9</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>70%</td>
</tr>
<tr>
<td>Osteoarthritis (%)</td>
<td>60%</td>
</tr>
<tr>
<td>High Cholesterol (%)</td>
<td>50%</td>
</tr>
<tr>
<td>IHD, MI and Angina (%)</td>
<td>20%</td>
</tr>
<tr>
<td>Sleep Apnoea (%)</td>
<td>16%</td>
</tr>
<tr>
<td>Depression (%)</td>
<td>16%</td>
</tr>
<tr>
<td>Peripheral neuropathy (%)</td>
<td>12%</td>
</tr>
<tr>
<td><strong>Metabolic Syndrome (%) IDF Criteria</strong></td>
<td></td>
</tr>
<tr>
<td>Diabetes/Raised Fasting Plasma Glucose</td>
<td>100%</td>
</tr>
<tr>
<td>Central Obesity</td>
<td>98%</td>
</tr>
<tr>
<td>IDF Diagnosis of metabolic syndrome</td>
<td>76%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>64%</td>
</tr>
<tr>
<td>Reduced HDL Cholesterol</td>
<td>34%</td>
</tr>
<tr>
<td>Raised Triglycerides</td>
<td>32%</td>
</tr>
<tr>
<td><strong>Medication (current)</strong></td>
<td></td>
</tr>
<tr>
<td>Total No. of medications/day (n)</td>
<td>5.1±2.9</td>
</tr>
<tr>
<td>Insulin (%)</td>
<td>22%</td>
</tr>
<tr>
<td>Metformin (%)</td>
<td>62%</td>
</tr>
<tr>
<td>Glibenclamide (%)</td>
<td>2%</td>
</tr>
<tr>
<td>Drug</td>
<td>Percentage</td>
</tr>
<tr>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>Glicazide</td>
<td>18%</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>2%</td>
</tr>
<tr>
<td>Glipzide</td>
<td>2%</td>
</tr>
<tr>
<td>Acarbose</td>
<td>2%</td>
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</table>

<table>
<thead>
<tr>
<th>Obesity Categories (%)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (18.5&lt;BMI&lt;24.9)</td>
<td>8%</td>
</tr>
<tr>
<td>Overweight (25&lt;BMI&lt;29.9)</td>
<td>36%</td>
</tr>
<tr>
<td>Obese I (30&lt;BMI&lt;34.9)</td>
<td>36%</td>
</tr>
<tr>
<td>Obese II (35&lt;BMI&lt;39.9)</td>
<td>12%</td>
</tr>
<tr>
<td>Obese III (BMI≥40)</td>
<td>8%</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Quality of Life</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF-36 PCS</td>
<td>45.6±7.6</td>
</tr>
<tr>
<td>SF-36 MCS</td>
<td>52.1(19.1-62.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Habitual Physical Activity Level</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>PASE score</td>
<td>125.3±60.5</td>
</tr>
</tbody>
</table>

SD = Standard Deviation
IHD = Ischemic Heart Disease
MI = Myocardial Infarction
HDL = High-density Lipoprotein
IDF = International Diabetes Federation
SF-36 = Version 2 of 36-item Short-Form health survey
PCS = Physical Component Summary
MCS = Mental Component Summary

PASE = Physical Activity Scale for the Olderly

Data were inspected for normality visually and statistically (-1 ≤ skewness ≤ 1) and are presented as mean and SD or median and range as appropriate.
### Table 4.3 Body Composition

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SD</th>
<th>Whole Cohort (n=50)</th>
<th>Male (n=27)</th>
<th>Female (n=23)</th>
<th>P Value (m vs. f)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Health Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Body Composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stretched Stature (m)</td>
<td>1.69±0.08</td>
<td>1.74±0.05</td>
<td>1.62±0.05</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Naked body weight (kg)</td>
<td>86.9(63.1-158.7)</td>
<td>91.9(64.2-140.8)</td>
<td>82.7(63.1-158.7)</td>
<td>0.090*</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.6 (22.8-54.6)</td>
<td>29.8 (23.7-42.9)</td>
<td>31.5 (22.8-54.6)</td>
<td>0.225</td>
<td></td>
</tr>
<tr>
<td>Skeletal Muscle Mass (kg)</td>
<td>30.4±4.1</td>
<td>31.8±4.0</td>
<td>28.7±3.6</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>587.4±10.0</td>
<td>63.2±7.9</td>
<td>50.6±7.7</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>32.3(10.6-82.2)</td>
<td>29.7(10.6-58.0)</td>
<td>35.5(17.2-82.2)</td>
<td>0.096*</td>
<td></td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>35.5±7.7</td>
<td>31.5±5.7</td>
<td>40.2±7.1</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td><strong>Regional Body Composition Fat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist Circumference (ISAK) (cm)</td>
<td>107.1±12.6</td>
<td>110.7±12.1</td>
<td>102.9±12.3</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>Abdominal Sagittal Length (cm)</td>
<td>27.1±3.7</td>
<td>28.4±3.2</td>
<td>25.7±3.9</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Total Abdominal Fat Area (without skin) (cm²)</td>
<td>430.4±109.6</td>
<td>434.4±95.4</td>
<td>425.9±126.2</td>
<td>0.798</td>
<td></td>
</tr>
<tr>
<td>Visceral Fat Area (cm²)</td>
<td>223.7±88.8</td>
<td>270.9±77.5</td>
<td>169.8±68.8</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous Abdominal Fat Area (cm²)</td>
<td>206.7±83.8</td>
<td>163.5±55.8</td>
<td>256.1±84.1</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Thigh Muscle Density (intramuscular lipid)</td>
<td>85.4±2.0</td>
<td>85.0±1.8</td>
<td>85.9±2.1</td>
<td>0.135</td>
<td></td>
</tr>
<tr>
<td>Total Thigh Fat Area (cm²)</td>
<td>84.9(33.6-249.7)</td>
<td>72.6(36.8-145.7)</td>
<td>107.9(33.6-249.7)</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous Thigh Fat Area (cm²)</td>
<td>69.9(24.1-230.0)</td>
<td>57.5(25.3-110.4)</td>
<td>101.7(24.1-230.0)</td>
<td>&lt;0.0001*</td>
<td></td>
</tr>
<tr>
<td>Intermuscular Thigh Fat Area (sub-fascial) (cm²)</td>
<td>13.5(2.6-36.5)</td>
<td>14.6(2.6-36.5)</td>
<td>12.9(3.9-19.5)</td>
<td>0.190*</td>
<td></td>
</tr>
<tr>
<td>Visceral Fat Area/ Subcutaneous Thigh Fat Area Ratio</td>
<td>3.3±2.2</td>
<td>4.6±1.8</td>
<td>1.9±1.5</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

**Muscle Composition**

| Thigh Muscle Area (cm²) | 109.0±23.3 | 123.5±19.5 | 91.1±13.0 | <0.0001 |
| Relative Muscle Area of Thigh (%) | 0.5±0.1 | 0.6±0.1 | 0.4±0.1 | <0.0001 |

Reginal measures were from CT scan or authors; whole body measures were from authors or bioelectrical impedance (BIA).

SD = Standard Deviation
BMI = Body Mass Index

ISAK = International Society for the Advancement of Kinanthropometry

Data were inspected for normality visually and statistically (-1 ≤ skewness ≤ 1) and are presented as mean and SD or median and range as appropriate.
### Table 4.4 Blood, Muscle and Adipose Tissue Results

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood, Muscle and Adipose Tissue Results</strong></td>
<td>Whole Cohort</td>
</tr>
<tr>
<td><strong>Glucose Homeostasis &amp; Insulin Resistance</strong> (n=50)</td>
<td></td>
</tr>
<tr>
<td>Serum Glucose fasting (mmol/L)</td>
<td>6.5 (4.6-16.4)</td>
</tr>
<tr>
<td>Serum Insulin fasting (mU/L)</td>
<td>9.2 (3.0-36.3)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.2±1.3</td>
</tr>
<tr>
<td>Serum C-peptide (nmol/L)</td>
<td>1.10±0.51</td>
</tr>
<tr>
<td>HOMA2 Beta Cell Function (%B)</td>
<td>101.7±50.3</td>
</tr>
<tr>
<td>HOMA2 Insulin Sensitivity (%S)</td>
<td>41.9 (16.1-226.9)</td>
</tr>
<tr>
<td>HOMA2 Insulin Resistance</td>
<td>2.68±1.25</td>
</tr>
<tr>
<td><strong>Lipids (n=50)</strong></td>
<td></td>
</tr>
<tr>
<td>Serum Total Cholesterol (mmol/L)</td>
<td>4.44±1.00</td>
</tr>
<tr>
<td>Serum Triglycerides (mmol/L)</td>
<td>1.4 (0.5-4.5)</td>
</tr>
<tr>
<td>Serum HDL Cholesterol (mmol/L)</td>
<td>1.2 (0.7-2.4)</td>
</tr>
<tr>
<td>Serum LDL Cholesterol (mmol/L)</td>
<td>2.4±0.8</td>
</tr>
<tr>
<td><strong>Serum (n=29)</strong></td>
<td></td>
</tr>
<tr>
<td>Serum HMW Adiponectin (ng/ml)</td>
<td>3781.1(573.9-23695.0)</td>
</tr>
<tr>
<td>Serum Total Adiponectin (ng/ml)</td>
<td>6091.0(2312.4-18620.1)</td>
</tr>
<tr>
<td>Serum HMW/Total Adiponectin Ratio</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>Serum C Reactive Protein (ng/ml)</td>
<td>1.5(0-2.7)</td>
</tr>
<tr>
<td><strong>Thigh Skeletal Muscle</strong></td>
<td></td>
</tr>
<tr>
<td>IGF-1 (pg/mg) (n=27)</td>
<td>11.7±9.8</td>
</tr>
<tr>
<td>TNF-α (pg/mg) (n=27)</td>
<td>0.0(0.0-1.4)</td>
</tr>
</tbody>
</table>
IL-6 (pg/mg) (n=27) 0.1(0.0-1.1)
HSP72 (ratio) (n=35) 1.0±0.4
pJNK (ratio) (n=35) 0.6±0.6
tJNK (ratio) (n=34) 0.5±0.4
p/tJNK(ratio) (n=34) 1.0±0.8

Thigh Subcutaneous Adipose Tissue (n=18)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMW adiponectin (ng/mg)</td>
<td>25.9(0.0-117.0)</td>
</tr>
<tr>
<td>Total adiponectin (ng/mg)</td>
<td>56.0(3.7-251.2)</td>
</tr>
<tr>
<td>HMW/Total adiponectin ratio</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>IL-6 (pg/mg)</td>
<td>6.2 (0.0-132.7)</td>
</tr>
</tbody>
</table>

SD = Standard Deviation
HbA1C = Hemoglobin A1c
HOMA2 = Homeostasis Model Assessment 2
HMW Adiponectin = High Molecular Weight Adiponectin
IGF-1 = Insulin-like Growth Factor-1
TNF-α = Tumour Necrosis Factor-α
IL-6 = Interleukin-6
HSP72 = Heat Shock Protein 72
pJNK = phosho-Jun N-terminal Kinase
HDL = High-density Lipoprotein
LDL = Low-density Lipoprotein

Data were inspected for normality visually and statistically (-1≤skewness≤1 ) and are presented as mean and SD or median and range as appropriate
Table 4.5  Associations between baseline Thigh Muscle total c-Jun N-terminal kinase (tJNK) and other characteristics

<table>
<thead>
<tr>
<th>Domain</th>
<th>r value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Health status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitual physical activities level (PASE score)</td>
<td>-0.333</td>
<td>0.055</td>
</tr>
<tr>
<td>Numbers of medications/day</td>
<td>0.304</td>
<td>0.080</td>
</tr>
<tr>
<td><strong>Inflammatory milieu</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum HMW/total adiponectin ratio</td>
<td>-0.364</td>
<td>0.057</td>
</tr>
<tr>
<td>Serum CRP</td>
<td>-0.457*</td>
<td>0.015*</td>
</tr>
<tr>
<td>Adipose HMW/total adiponectin ratio</td>
<td>-0.465</td>
<td>0.060</td>
</tr>
<tr>
<td><strong>Anabolic factor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle IGF-1</td>
<td>0.641</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

PASE Score = Physical Activity Scale for the Olderly, higher score indicates more physical activity.

IGF-1 = Insulin-like Growth Factor-1

IL-6 = Interleukin-6

HMW Adiponectin = High Molecular Weight Adiponectin

CRP = c-reactive protein

* = linear regression was used after log transformation

Data were inspected for normality visually and statistically \((-1 \leq \text{skewness} \leq 1\) ) and are presented as mean and SD or median and range as appropriate.
Table 4.6 Associations between baseline Thigh Muscle phospho/total JNK (p/t JNK) and other characteristics

<table>
<thead>
<tr>
<th>Domain</th>
<th>r value or rho</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.433</td>
<td>0.011</td>
</tr>
<tr>
<td>Gender</td>
<td>--</td>
<td>0.022***</td>
</tr>
<tr>
<td><strong>Metabolic characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.559</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.323*</td>
<td>0.063*</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.410</td>
<td>0.016</td>
</tr>
<tr>
<td>HOMA β cell function</td>
<td>-0.332</td>
<td>0.055</td>
</tr>
<tr>
<td><strong>Inflammatory milieu</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum total adiponectin</td>
<td>-0.336*</td>
<td>0.081*</td>
</tr>
<tr>
<td>Serum HMW adiponectin</td>
<td>-0.504*</td>
<td>0.006*</td>
</tr>
<tr>
<td>Skeletal muscle IL-6</td>
<td>0.644**</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td><strong>Anabolic factor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle IGF-1</td>
<td>0.548</td>
<td>0.004</td>
</tr>
</tbody>
</table>

HOMA = Homeostatic model assessment
HDL = High density lipoprotein
LDL = Low density lipoprotein
IGF-1 = Insulin-like Growth Factor-1
IL-6 = Interleukin-6
HMW Adiponectin = High Molecular Weight Adiponectin

* = linear regression was used after log transformation
**=Spearman test was used for non-distributed characteristics after logged-transformed

***=t-test was used.

Data were inspected for normality visually and statistically (-1≤skewness≤1 ) and are presented as mean and SD or median and range as appropriate.
Figure 4.1. Participant Flow-Chart

- Assessed for eligibility (n=427)
  - Excluded (n=324)
    - Not meeting inclusion criteria (n=285)
    - Not interested (n=15)
    - Other reasons (n=24)
  - Randomised (n=103)
    - Withdrawn before intervention (n=2)
    - Data unavailable (n=53)

Allocation:
- Allocated to intervention (n=25)
  - Received intervention (n=25)
  - Did not receive intervention (n=1)
- Allocated to intervention (n=23)
  - Received intervention (n=23)
  - Did not receive intervention (n=1)

Follow-up:
- Lost to follow-up (n=4)
  - Discontinued intervention (n=2)
- Lost to follow-up (n=1)
  - Discontinued intervention (n=1)

Analysis:
- Analysed (n=21)
  - Excluded from analysis (n=4)
- Analysed (n=22)
  - Excluded from analysis (n=1)
Figure 4.2.1 Relationships between skeletal muscle total c-Jun N-terminal kinase (tJNK) and health status

A: Muscle sample tJNK versus PASE score: $R=-0.333$, $P=0.055$

B: Muscle sample tJNK versus total numbers of medications/day: $R=0.304$, $P=0.080$

tJNK = total JNK
Figure 4.2.2 Relationships between skeletal muscle total c-Jun N-terminal kinase (tJNK) and inflammatory milieu

B

C
A: Muscle sample tJNK versus serum HMW/total Adiponectin ratio: $R=-0.364$, $P=0.057$

B: Muscle sample tJNK versus serum C-Reactive Protein (CRP): $R=-0.457$, $P=0.015^*$

C: Muscle sample tJNK versus adipose sample HMW/total Adiponectin ratio:

$R=-0.465$, $P=0.060$

tJNK = total JNK

* = linear regression was used after log transformation
Figure 4.2.3 Relationships between skeletal muscle total c-Jun N-terminal kinase (tJNK) and anabolic factor IGF-1

Muscle sample total-JNK versus muscle sample IGF-1 (pg/mg): R=0.641, P<0.001
Figure 4.3.1 Relationship between skeletal muscle phospho/total c-Jun N-terminal kinase and age

Muscle sample p/t JNK versus age (year): $R=0.433 \quad P=0.011$
Figure 4.3.2 Relationship between skeletal muscle phospho/total c-Jun N-terminal kinase (p/t JNK) ratio and inflammatory milieu

A: Muscle sample phospho-JNK/total-JNK versus serum total adiponectin: R=-0.336, P=0.081*

B: Muscle sample phospho-JNK/total-JNK versus serum HMW adiponectin:

C: Muscle sample phospho-JNK/total-JNK versus muscle sample IL-6 (pg/mL) baseline

A: Muscle sample phospho-JNK/total-JNK versus serum total adiponectin: R=-0.336, P=0.081*

B: Muscle sample phospho-JNK/total-JNK versus serum HMW adiponectin:
R = -0.504, P = 0.006*

C: Muscle sample phospho-JNK/total-JNK versus muscle sample IL-6: Rho = 0.712, P = 0.0004**

* = linear regression was used after log transformation

** = Spearman test was used for non-distributed characteristics after logged-transformed
Figure 4.3.3 Relationship between skeletal muscle phospho/total c-Jun N-terminal kinase (p/t JNK) ratio and metabolic characteristics

A: Muscle sample phospho-JNK/total-JNK versus HOMA2 β cell function (%β):

R=0.332, P=0.055
B: Muscle sample phospho-JNK/total-JNK versus serum total cholesterol: R=-0.559, P=0.0006

C: Muscle sample phospho-JNK/total-JNK versus serum HDL: R=-0.323, P=0.063*

D: Muscle sample phospho-JNK/total-JNK versus serum LDL: R=-0.410, P=0.016

* = linear regression was used after log transformation
Figure 4.3.4 Relationships between skeletal muscle phospho/total c-Jun N-terminal kinase (p/t JNK) ratio and anabolic factor

Muscle sample phospho-JNK/total-JNK versus muscle sample IGF-1 (pg/mg): R=0.548, P=0.004
CHAPTER FIVE

DISCUSSION
Overview of the study

The aims of this thesis were to: 1) summarize current knowledge as to how JNK adapts to exercise in humans within the systematic review, 2) to describe the characteristics of JNK in older subjects with type 2 diabetes, and 3) to investigate metabolic and other factors related to skeletal muscle JNK at baseline in participants enrolled in a randomized double-blind, sham exercise controlled trial designed to assess the efficacy of power training in type 2 diabetes (T2D).

Chapter 2 provided a systematic review of published evidence as to how JNK adapts to exercise exposure in various tissues of healthy or clinical cohorts. The data were limited as published studies primarily included healthy cohorts and the results were too heterogeneous to come to conclusions regarding JNK adaptations to exercise. In addition, only two trials included overweight/obese individuals, only one trial included healthy older adults and no study of older clinical cohorts have been published. More trials, especially robustly designed exercise studies with clinical and older cohorts, and inclusion of other physiological outcomes are needed to understand how JNK adapts to acute and chronic exercise and how it is related to metabolic outcomes.

Chapter 3 described the methodology of the baseline sub-study of the randomized double-blind, sham-exercise controlled trial designed to assess the efficacy of power training on older adults with type 2 diabetes. This chapter included an overview of the study design, methods of analysis of health status, body composition, glucose homeostasis and insulin resistance, blood lipids and cytokines; procedures of muscle and
adipose tissue biopsies, methods and precision of measurement of the factors in muscle and adipose tissues we hypothesized would be correlated to insulin sensitivity and exercise. All the protocols were based on well-validated, published methodss\textsuperscript{10-23}. Western Blot reliability was tested using Bland-Altman methodology, and the precision of our assays was considered adequate compared to published literature\textsuperscript{24}.

Chapter 4 described the results of this baseline sub-study of total and activated JNK. The baseline characteristics presented included health status, body composition, physical activity level, glucose homeostasis, insulin resistance, serum cytokines and lipids, myokines, adipokines and selected components of the insulin signaling pathways in skeletal muscle and adipose tissue. We hypothesized that greater JNK expression and activation would be related to impaired health status, chronic inflammation, metabolic disturbance and insulin resistance in our cohort. As hypothesized, higher JNK was related to more impaired health status (age, inactivity, lower HDL) and inversely related to anti-inflammatory adiponectin and HOMA2 \( \beta \) cell function. Unexpectedly, however, higher JNK was related to higher skeletal muscle IGF-1, IL-6 and inversely related to CRP, serum total cholesterol and LDL.

**Novelty of the study**

**Relationship between skeletal muscle JNK and health status**

This was the first study to report that skeletal muscle JNK was related to age, number of medications and lower habitual physical activity level. Older age and number of medications are regarded as a mark of higher burden of diseases in our cohort. Physical
inactivity is a well known risk factor for worse metabolic control in T2D, while numbers of medication represents the progression and severity of chronic diseases. Our study showed that skeletal muscle JNK may serve as a molecular indicator of increased disease burden and physical inactivity. Hence the adaptations of this specific molecule to exercise may have clinical relevance in this cohort.

**Associations between insulin signaling in skeletal muscle and impairment of pancreatic β cell function**

Most studies concerning the adaptations of JNK to exercise or the effects of exercise in subjects with T2D investigated only a single target tissue. Our study investigated not only particular components of signaling pathways in specific restricted tissues, but also the overall metabolic status, health status and body composition of the individual, allowing greater understanding of the variations in JNK expression observed.

For example, we found that skeletal muscle JNK was negatively correlated to HOMA β cell function, and offer the following explanation. Considerable evidence has suggested that chronic hyperglycemia impairs insulin production/secretion and leads to pancreatic β cell exhaustion/apoptosis. As the largest reservoir of glucose, skeletal muscle plays a dominant role in responding to insulin and controlling blood glucose level. In insulin-resistant skeletal muscle, activated JNK sequesters glucose transporter 4 (GLUT4) within the cells, subsequently reduces the amount of GLUT4 localized on the cell surface and thus inhibits glucose uptake by skeletal muscle cells. Hence, activation of JNK in skeletal muscle is at least partially responsible for hyperglycemia and glucotoxicity to
pancreatic β cells. Impairment of β cell function results in further reduction of insulin production, thereby promoting the progression of insulin resistance.

In addition, visceral obesity might link impaired β cell function and elevated skeletal muscle JNK activity together, as it might be a common underlying mechanism explaining part of the variance both factors. Overweight/obese subjects have excessive lipid accumulation and higher levels of systematic inflammation. Similar to the deleterious effects of chronic hyperglycemia, fatty acids (FA), which are essential β cell fuels in the normal state, become toxic when chronically present in excessive levels. Prolonged exposure of pancreatic β cells to FA increases basal insulin release but inhibits glucose-induced insulin secretion. Therefore, visceral obesity is indirectly related to HOMA2 β cell function. In addition, visceral adipose tissue and its adipose-tissue resident macrophages produce more pro-inflammatory cytokines, which induce JNK activation in skeletal muscle. Hence central obesity serves as an important link which may explain the novel indirect correlation between skeletal muscle JNK and HOMA2 β cell function we observed.

**Correlations of skeletal muscle JNK and myokines**

Skeletal muscle JNK was directly related to interleukin-6 (IL-6) and Insulin-like Growth Factor-1 (IGF-1). These two correlations were seen after an acute bout of resistive exercise just prior to the biopsy. These data expand evidence from animal models and healthy individuals, showing for the first time in older adults with type 2 diabetes that
skeletal muscle JNK and myokines may respond to exercise exposure and these responses appear to be related to one another.

Although the mechanisms of IL-6 regulation in skeletal muscle are largely unknown, previous studies provide some evidence that JNK may play a regulatory role in this process. Specifically, IL-6 expression has been positively correlated with JNK activity\textsuperscript{33}; IL-6 expression can be abolished by SP600125, a specific JNK inhibitor, in a dose-dependent manner\textsuperscript{34}; and c-Jun, a classic JNK substrate and transcriptional factor, can bind to IL-6 promoter and activate IL-6 gene transcription\textsuperscript{34}. Thus, our novel observation of direct relationships between the expression of all three factors appears consistent with known interactions in the literature.

**Limitations**

This investigation only included data from the first 50 subjects recruited in this trial, as their results were available at the time of writing, and 7 of them didn't have biopsies due to medical reasons (see Chapter Three). Thus, the sample size of this article was limited, and some type II errors could have occurred.

The assessments were performed by several assessors, so the inter-rater coefficient of variation could be a factor. Although not as reliable as if the same assessor was used in all the assessments, our Bland-Altman analyses suggested that reliability was acceptable.
There were no truly resting baseline muscle and adipose samples available. Muscle and adipose tissue biopsies were all performed after 1RM, power and endurance tests, as the purpose of the whole study was to look at the effects of long-term power training on muscle adaptations in older people with type 2 diabetes. The biggest obstacle was that our subjects were older people with impaired health status and burdened with an average of 5 chronic illnesses, and we did not believe they could tolerate 2 biopsies on a single day, or perform maximal strength and power testing after a biopsy. We considered the positive relationships among skeletal muscle JNK, IL-6 and IGF-1 as acute responses to a bout of resistance exercise, suggesting that muscle contractions could produce these cytokines and growth factors. However, we don't have any muscle samples before this bout of exercise to substantiate this conclusion.

Although total and activated JNK protein levels were measured in the skeletal muscle, we lack data on JNK mRNA level. Therefore, it is not possible to report potential relationships between JNK transcription/translation and insulin resistance/metabolic syndrome. In addition, there are many other critical components in the insulin signaling pathways we didn’t measure, such as phosphoinositide-3 kinase (PI-3K)/Akt, Akt substrate 160 (AS160) and peroxisome proliferator-activated receptors (PPARs), which future studies should include.

Finally, we had measures of fasting insulin, glucose, and calculated insulin resistance level, which primarily reflected hepatic insulin sensitivity on the morning of the biopsy. Although the HOMA2 model of insulin resistance is related to measures of peripheral
glucose uptake, it is likely that whole body insulin sensitivity measures such as a glucose clamp would have provided additional information on the metabolic relevance of the skeletal muscle and adipose tissue proteins we measured.

**Areas for future investigation**

**Performing biopsies both at rest and after a bout of resistance exercise**

Our results showed that skeletal muscle JNK measured after an acute bout of resistive exercise were significantly related to IL-6, IGF-1, adiponectin, c-reactive protein (CRP), HOMA β cell, cholesterol levels, age, physical activity level and numbers of medications. However, there were no resting muscle and adipose tissue biopsy samples before this bout of exercise to compare as control samples to investigate whether JNK expression we measured and/or its associations with these factors were altered by the acute exercise exposure. Thus, future studies should include 2 biopsies at each assessment timepoint if possible to assess the effects of both acute and chronic resistive exercise on insulin signaling pathways and inflammatory factors in thigh skeletal muscle and subcutaneous adipose tissues. It is likely that the resting biopsy would have to be taken at least 2 weeks prior to the exercise bout, however, as healing of the incision site generally resulted in hesitancy to exert maximal effort for at least a week in this cohort.

**Exploring molecular mechanisms behind adaptations of JNK to exercise and JNK related signaling pathways in insulin resistance**

It is still not fully understood how JNK responds to various exercise exposures and how it contributes to the progression of insulin resistance in individuals with type 2 diabetes.
Based on the fact that JNK is significantly elevated and activated in T2D compared to healthy controls\textsuperscript{35-36}, it may seem contradictory that JNK activation is increased after acute exercise exposure \textit{but at the same time} is linked to impaired insulin signaling/glucose transport. Previous studies and our novel findings provide some insight into these processes. For example, IL-6 and IGF-1 have both been shown to improve insulin sensitivity\textsuperscript{33, 37-38}, IL-6 is increased after a single bout of exercise\textsuperscript{37, 39-40}; and a single bout of exercise improves insulin sensitivity for several days\textsuperscript{41}. Because our results showed that skeletal muscle JNK was positively related to IL-6 and IGF-1, we suggest that JNK activity also increased in response to the acute exercise, and that this activation of JNK after acute exercise may play a different role than the chronic elevation of total JNK expression which is associated with inactivity in our study, and with insulin resistance in other studies. Future studies should measure both activated and total JNK, before and after exercise, along with other myokines and whole body insulin resistance to improve our understanding of these processes.

We also found that skeletal muscle JNK was negatively correlated to serum LDL, and total cholesterol. The indirect correlation with total and LDL coholesterol was unexpected, but may be related to the prevalent use of statins in this cohort, with their overwhelming effect on cholesterol metabolism. It is possible that the older and most inactive individuals (with the highest JNK levels) were using higher doses of statins, and therefore had the lowest cholesterol levels. However, the effects of statins and acute exercise on JNK and serum lipids weren’t able to be separated in our study. Future
studies in individuals not treated with statins are needed to investigate the paradoxical JNK- cholesterol relationships we observed.

**Summary**

Our systematic review of the literature and cross-sectional study reported herein suggests that JNK is an important component of metabolic processes in older adults with type 2 diabetes, and is related to inflammation, myokine expression after muscle contractions, beta cell function, and overall health status and physical activity level in this cohort. Much more information is needed about the adaptation and activation of JNK in response to acute and chronic exercise in various clinical cohorts. This will allow optimization of the specifics of the physical activity prescription to promote beneficial adaptation and improvement in insulin signalling and overall metabolic health.
REFERENCES


CHAPTER 7

Bibliography
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