THE EFFECT OF WHOLE BODY VIBRATION TRAINING ON INSULIN SENSITIVITY IN OVERWEIGHT ADOLESCENTS: A RANDOMISED CONTROLLED TRIAL

KIM ANN RAMJAN

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Discipline of Child and Adolescent Health

Sydney Medical School

The University of Sydney

2017

For Rocky, my lifelong friend

Declaration

I, **Kim Ann Ramjan**, hereby declare that to the best of my knowledge, the content of this thesis is my own work except where otherwise acknowledged, and that this thesis has not been submitted in whole or in part for an award, including a higher degree, to any other university or institution.

Kim Ramjan

June 2017

Acknowledgement

I was a recipient of an NHMRC postgraduate scholarship and Australasian Paediatric Endocrine Research Grant that provided personal financial support during the course of this research study. The VIBRATE study was also funded by a Regional Diabetes Support Scheme grant and an Australasian Paediatric Endocrine Group grant. I wish to express my gratitude to these organisations for their generous assistance.

There are many individuals who assisted me during the course of this research project to whom I wish to extend my personal thanks.

First and foremost, I would like to thank my supervisor Professor Chris Cowell and associate supervisor, A/Professor Craig Munns for their wonderful support and encouragement of my candidature and to Professor Louise Baur who was involved in the initial stages of the study design.

Special thanks to the other co-investigators of the VIBRATE study, A/Prof Sarah Garnett who assisted me with the ethics submission, study and database design and Dr Carolyn Broderick who reviewed the study participants and provided exercise prescription at The Children's Hospital Institute of Sports Medicine. I wish to thank my physician colleagues for their kind referral of participants to the VIBRATE study.

I would like to extend my appreciation of thanks to the Postgraduate Student Team, Discipline of Child and Adolescent Health at The Children's Hospital at Westmead Clinical School, particularly Ms Denise Yuille for her immense support of my PhD and

A/Prof Nicholas Wood and Prof Russell Dale for their kind assistance and guidance through my candidature.

Special mention to the wonderful people who comprised the VIBRATE team and provided me great friendship, including Srs Liz Lawrie and Kelly Winning, the clinical nurse consultants in the Endocrine Testing Unit, who assisted with the oral glucose tolerance tests; Ms Julie Briody and Ms Madeleine Thompson, nuclear medicine scientists, who performed the DXA and pQCT scans; Ms Nancy Van Doorn, exercise physiologist at CHISM who performed the fitness testing, Mrs Miriam Dawes who arranged the CHISM bookings; Mrs Kerryn Chisholm and Ms Susie Burrell, weight management dietitians who provided nutritional guidance to the participants; the Endocrine Laboratory scientists who performed the hormone assays: Ms Jenny Lee, Ms Catherine Loi, Ms Denise Cunliffe, Ms Mirijana Pupovac, Ms Darna Bradford, Ms Rose Tesoriero, Ms Oksana Markovych and Dr Jim Minchenko. Thank you also to Miss Ariana Villarosa for her expert administrative assistance with my thesis at such short notice.

The VIBRATE study would not be possible without the participation of the adolescents and their families, who took time out of their busy schedules to facilitate the research tests, to altruistically provide further knowledge to stem the tide of Type 2 diabetes, to them I extend my heartfelt thanks.

Finally, I would like to thank my family for their continual support and encouragement throughout my candidature.

Abstract

Clinical insulin resistance (IR) is commonly seen in the obese adolescent and precedes a diagnosis of Type 2 diabetes (T2DM). Early detection and management of young people at risk of T2DM is essential to prevent disease progression and reduce later cardiovascular complications. Yet, despite greater recognition of clinical IR in youth and the potential implications for later metabolic health, treatment options are limited to weight management with a minor role for adjunctive pharmacotherapy. Moreover, the best activity prescription to optimise metabolic health in these young people remains unclear. Paediatric weight management programs including aerobic based exercise have shown improvements in insulin sensitivity (Si) and metabolic profile, with or without body composition change. Resistance-based training is gaining favour as a safe therapeutic option as research demonstrates it to be equally effective and possibly easier to perform in the obese adolescent whose fitness may be decreased. Strength training may produce benefits to peripheral glucose uptake by regionally increasing lean tissue mass. However, this remains to be fully explored in adolescents with IR.

This thesis, centred on a randomised controlled trial designed to investigate whether whole body vibration training (WBVT), a novel resistance exercise could enhance insulin sensitivity in a cohort of obese adolescents, in addition to a contemporary lifestyle intervention. To investigate change in insulin sensitivity we used validated proxy measures including whole body insulin sensitivity (WBISI) and homeostasis model of insulin resistance (HOMA-IR), measures which can be readily replicated in the clinical setting. Whilst the primary outcome was change in insulin sensitivity (Si), we also examined interval change in secondary outcome measures including: anthropometry, cardiorespiratory fitness and musculoskeletal parameters.

Participants in our study were recruited from tertiary hospital clinics and derived from Greater Western Sydney (GWS), a multi-ethnic community, with high genetic metabolic risk. Detailed clinical anthropometry, medical history and examination taken at baseline confirmed that the obese participants had high-risk ethnicities for the development of T2DM and the metabolic syndrome. Extended family members who reported a history of T2DM were more likely to report additional metabolic risk factors as shown in 30.2% of first and 60.2% of second-degree relatives. Participants had central adiposity as demonstrated by increased waist circumference and waist to height ratio (WtHR), in the majority of cases exceeding national adult guidelines [1]. Adverse anthropometry was associated with poorer insulin sensitivity, increased weight and BMI. Insulin resistance was correlated with greater inflammation as measured by hs-CRP. Acanthosis nigricans (AN) could, in dark skinned individuals, detect biochemical hyperinsulinaemia but was not a reliable clinical feature in Caucasian participants.

Obesity is not gender specific, yet over two thirds of the VIBRATE participants were female, with males having the highest body mass index (BMI). This disparity in accessing healthcare during adolescence mirrors adult trends and may represent cultural, social and peer influences with regards to the recognition of obesity in young people. The average fasting insulin within the group was 33.8mU/L and significantly higher than adult cut-off levels <15mU/L, likely due to the increased weight but also exacerbated by the physiological changes occurring in puberty. Calculated proxy measures for insulin resistance showed the group to be highly insulin resistant at baseline with HOMA-IR 7.62 and WBISI 1.74, elevated, when compared to contemporary adult and adolescent published normative data. Metabolic profile indicators taken at baseline, showed high prevalence of the metabolic syndrome particularly in pubertal participants, ranging from 23-44% dependent on the definition applied. Early evidence of sexual dimorphism was

seen with males displaying higher systolic blood pressure (SBP) z score at baseline. The group demonstrated mild elevation in aspart aminotransferase (AST), which may suggest early possible hepatic derangements associated with Non Alcoholic Fatty Liver Disease (NAFLD). Triglycerides (TG), also implicated in the pathogenesis of NAFLD, positively correlated with alanine aminotransaminase (ALT), more strongly in males. Participants were inactive with increased sedentary times. Further highlighting the de-conditioning in these adolescents and not unexpectedly, cardiorespiratory fitness was reduced as determined by $VO2_{peak}$. Leonardo^{TM} Mechanography, a new technique for analysing functional muscle performance showed lower muscle force and power generation, indicating that the ability to perform day-to-day activities may be compromised.

Two techniques were employed to assess bone and mineral parameters including areal bone mineral density analysis with dual energy x-ray absorptiometry (DXA) and volumetric analysis with peripheral quantitative computed tomography (pQCT). The impact of early skeletal maturation in the obese adolescent was evident with obese individuals having higher areal bone mineral density (BMD) and bone mineral content (BMC) for age but normal values when adjusted for height. The effects of an earlier puberty, body composition and hormonal changes were demonstrated with females having a higher BMD and BMC compared to males. The integrity of the muscle bone unit was confirmed with lean tissue mass being an important predictor of BMD. Interestingly, increased waist circumference, suggestive of visceral adiposity, negatively affected both bone mineral content and bone mineral density, suggesting a possible impact of inflammation on bone accrual.

The VIBRATE randomised controlled trial, confirmed the benefits of short-term lifestyle intervention in the management of insulin resistance and obesity. With lifestyle

intervention leading to significant decrease in BMI z score (-0.8 vs. -0.08, $p<0.039$) in the lifestyle and WBVT group respectively, which was associated with significant improvements in fasting glucose (-0.3 vs. -0.0, $p<0.047$) and clinically beneficial but non-significant changes in proxy markers of insulin resistance. WBVT, despite increasing lean tissue mass by almost 0.5kg, with demonstrated positive effects to muscle bone interaction (significant increase in BMD for height) did not lead to enhancements in glucose homeostasis, demonstrating the complex nature of insulin dynamics particularly during puberty which is accompanied by physiological changes to Si. There was no association between bone and mineral turnover markers and energy homeostasis in our study, though we did see a significant association between low vitamin D levels, dark skin and greater insulin resistance (higher fasting insulin, lower WBISI and higher HOMA-IR). Adolescent obesity studies are associated with poor adherence and ours was no exception, whilst retention to the study was high with a dropout rate of just 2.3%, the compliance to the WBVT exercise intervention was poor but is likely to reflect real life challenges in the management of the adolescent with obesity.

This thesis contributes further knowledge about the metabolic characteristics, bone health, exercise capabilities and musculoskeletal limitations of the obese adolescent. This information will help to identify and target those individuals at risk of progressing to T2DM and may aid in the development of an individualised lifestyle intervention to assist in the transition of the obese adolescent to a more active lifestyle. We confirmed the role of lifestyle measures in the management of obesity and insulin resistance and provide further evidence of the safety of WBVT as a possible efficient method of improving lean tissue mass in adolescents with increased weight.

Publications arising from this work

Abstracts

Ramjan KA, Broderick C, Van Doorn N, Briody JN, Garnett SP, Winning K, Lawrie E, Burrell S, Chisholm K, Baur L, Munns CF, Cowell CT "*The Effect of Whole Body Vibration Training on Insulin Sensitivity in Overweight Adolescents: A Randomised Control Trial*" Postgraduate Student Conference 2012 Oral Presentation

Ramjan KA, Broderick C, Van Doorn N, Briody JN, Garnett SP, Winning K, Lawrie E, Burrell S, Chisholm K, Baur L, Munns CF, Cowell CT "*The Effect of Whole Body Vibration Training on Insulin Sensitivity in Overweight Adolescents: A Randomised Control Trial*" *28th* RNSH/UTS/USYD Scientific Research Meeting 2011 Oral presentation

Ramjan KA, Broderick C, Van Doorn N, Briody JN, Garnett SP, Winning K, Lawrie E, Burrell S, Chisholm K, Baur L, Munns CF, Cowell CT "*The Effect of Whole Body Vibration Training on Insulin Sensitivity in Overweight Adolescents: a Randomised Control Trial*" *APEG*-ESA ASM 2012 Poster 179

Ramjan KA, Broderick C, Van Doorn N, Briody JN, Garnett SP, Winning K, Lawrie E, Burrell S, Chisholm K, Baur L, Munns CF, Cowell CT "*The Effect of Whole Body Vibration Training on Insulin Sensitivity in Overweight Adolescents: a Randomised Control Trial*" Diabetes Outreach Meeting 2011 Oral Presentation

Ramjan KA, Broderick C, Van Doorn N, Briody JN, Garnett SP, Winning K, Lawrie E, Burrell S, Chisholm K, Baur L, Munns CF, Cowell CT *"The Effect of Whole Body Vibration Training on Insulin Sensitivity in Overweight Adolescents: a Randomised Control Trial*" Postgraduate Student Conference 2011 Oral presentation

Ramjan KA, Broderick C, Van Doorn N, Briody JN, Garnett SP, Winning K, Lawrie E, Burrell S, Chisholm K, Baur L, Munns CF, Cowell CT 2011 *"The Effect of Whole Body Vibration Training on Insulin Sensitivity in Overweight Adolescents: a Randomised Control Trial"* ENDO ASM Boston, MA, USA 2011 Poster P3-487

Ramjan KA, Broderick C, Van Doorn N, Briody JN, Garnett SP, Winning K, Lawrie E, Burrell S, Chisholm K, Baur L, Munns CF, Cowell CT *" Cardio-respiratory fitness is related to fatness, insulin sensitivity and body composition in overweight insulin resistant adolescents" APEG* ASM 2010 Oral Presentation

Ramjan KA, S Burrell, M Dunkley, S Alexander
Invited Speaker FILEX 2010 Conference Sydney Convention Centre Darling Harbour *"Paediatric Obesity, Insulin Resistance, Diet and Exercise Interventions: A Scientific Update and Implications for Exercise Professionals"* Oral presentation

Ramjan KA, Broderick C, Van Doorn N, Briody JN, Garnett SP, Winning K, Lawrie E, Burrell S, Chisholm K, Baur L, Munns CF, Cowell CT "*The Effect of Whole Body Vibration Training on Insulin Sensitivity in Overweight Adolescents: A Randomised Control Trial*" Postgraduate Student Conference 2010 Oral presentation

Ramjan KA, Broderick C, Garnett SP, Baur L, Munns CF, Cowell CT *"Metabolic, Cardiorespiratory and Musculoskeletal Parameters in Overweight Insulin Resistant Adolescents"* Young Investigator's Presentation, Australasian Paediatric Endocrine Group ASM 2009 Oral Presentation OPO6

Publications arising during candidature but not included in the thesis

Publications

Biggin, A., Briody, J. N., **Ramjan, K. A**., Middleton, A., Waugh, M. C., & Munns, C. F. (2013). Evaluation of bone mineral density and morphology using pQCT in children after spinal cord injury. *Dev Neurorehabil, 16*(6), 391-397.

Ramjan, K. A., Roscioli, T., Rutsch, F., Sillence, D., & Munns, C. F. (2009). Generalized arterial calcification of infancy: treatment with bisphosphonates. *Nat Clin Pract Endocrinol Metab, 5*(3), 167-172.

Abstracts

Kim A Ramjan, Julie N Briody, Carolyn M West, Craig FJ Munns

"*Evaluation of regional bone mineral density after zoledronic acid treatment in a child with spinal cord injury*" LWPES/ESPE 8th Joint Meeting Global Care in Pediatric Endocrinology 2009 Poster 03-044

Ramjan KA, Briody JN, Gallego PH, Waugh MC, Munns CF

"*Regional changes in bone mineral density in paediatric patients after spinal cord injury"*

APEG ASM 2008, Poster presentation

Ramjan KA, Briody JN, Gallego PH, Johannesen J, Waugh MC, Munns CF

"*Regional changes in bone mineral density in paediatric patients after spinal cord injury"*

6th International Workshop for Society of Musculoskeletal and Neuronal Interactions 2008 Cologne, Germany. Poster 027

List of abbreviations

25 OHD	25 Hydroxy Vitamin D
a	Acceleration
A	Amplitude
AA	African American
aBMD	Areal bone mineral density
ABPM	Ambulatory Blood Pressure Monitoring
ABS	Australian Bureau of Statistics
ADA	American Diabetes Association
AHA	American Heart Association
ALT	Alanine aminotransferase
AN	Acanthosis nigricans
ANZCTR	Australian New Zealand Clinical Trials Registry
AST	Aspart aminotransferase
AT	Anaerobic threshold
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content
BMC/LTM	Bone mineral content for lean tissue mass
BMD	Bone mineral density
BMI	Body Mass Index (kg/m^2)
BP	Blood pressure
BPA	Bisphenol A
CDC	Centers for Disease Control and Prevention
CDR	Cardiovascular Disease Risk
CHISM	Children's Hospital Institute of Sports Medicine
CHW	Children's Hospital at Westmead
CONSORT	Consolidated Standards of Reporting Trials
CRF	Cardiorespiratory fitness
CRP	C-reactive protein
CSA	Cross sectional area
CT	Computerised Tomography
CTX	C-terminal collagen cross-linked peptide
CV	Coefficient of variation
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DI	Disposition Index
DPD	Deoxypyridinoline crosslinks/creatinine
DPP	Diabetes Prevention Program
DXA	Dual energy x-ray absorptiometry
EDC	Endocrine disrupting chemical
EFI	Efficiency
EMCL	Extramyocellular lipid
f	Frequency
FFA	Free fatty acids
FFM	Fat free mass
Fmaxrel	Force maximal relative to weight (one leg jump)
FSIVGTT	Frequently sampled intravenous glucose tolerance test
GLUT4	Glucose transporter 4
GRFP	Ground reaction force plate

GWS	Greater Western Sydney
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostasis model assessment of insulin resistance
hs-CRP	High sensitivity C-reactive protein
HT	Hypertension
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGF-1	Insulin like growth factor 1
IGT	Impaired glucose tolerance
IMCL	Intramyocellular lipid
IOTF	International Obesity Task Force
ISI	Insulin sensitivity index
IR	Insulin resistance
LDL-C	Low-density lipoprotein cholesterol
LTM	Lean tissue mass
m1LJ	Multiple one leg jump
MHO	Metabolically healthy obese
mU/L	Milliunits per litre
MUO	Metabolically unhealthy obese
MVF	Maximal voluntary force (during one leg jump)
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NCEP III-ATP	National Cholesterol Education Program Adult Treatment Panel III
NHANES	National Health and Nutrition Examination Study
OCN	Osteocalcin
OGTT	Oral glucose tolerance test
OECD	The Organization for Economic Cooperation and Development
OSA	Obstructive sleep apnoea
P1NP	Amino terminal propeptide of type 1 collagen
PCOS	Polycystic ovary syndrome
Pmaxrel	Maximal power relative to body weight (single 2 leg jump)
pmol/L	Picomol per litre
pQCT	Peripheral quantitative computed tomography
QUICKI	Quantitative insulin sensitivity check index
RCT	Randomised controlled trial
RIA	Radioimmunoassay
s2LJ	Single two leg jump
SAAT	Subcutaneous abdominal adipose tissue
SBP	Systolic blood pressure
SD	Standard deviation
Si	Insulin sensitivity
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TG	Triglycerides
USA	United States of America
VAAT	Visceral abdominal adipose tissue
VAT	Ventilatory anaerobic threshold
vBMD	Volumetric bone mineral density
VIBRATE	RCT Effect of whole body vibration training on insulin sensitivity in overweight adolescents

VO_{2peak}	Peak rate of oxygen uptake
VLDL	Very low density lipoprotein
WBISI	Whole body insulin sensitivity index
WBVT	Whole body vibration training
WC	Waist circumference
WHO	World Health Organisation
WOCC	World Obesity Clinical Care
WtHR	Waist to height ratio

List of figures

List of tables

Table of contents

CHAPTER 1 REVIEW OF THE LITERATURE AND AIMS

1.1 Obesity

1.1.1 Prevalence and trends in obesity

Australian data suggests that 20-25% of young people under the age of 18 can be characterised as overweight or obese[2] [3], with slightly higher rates seen in the United States[4] [5]. Whilst prevalence continues to increase with age in Australian indigenous children [6], data suggests a plateau in child and adolescent obesity rates in Australia [7-9]. Similarly, other OECD countries have also shown decline in rates of abdominal [10-12] and generalised obesity[5]. In Australian children, Indigenous heritage [6], ethnicity and socio-economic status are related to obesity risk [13], [14] which is expected to increase to almost one in every three children by 2025 [15].

According to the International Obesity Taskforce (IOTF) now World Obesity Clinical Care (WOCC) definition, 10% of the world's youth, aged 5-17 years are overweight or obese, with higher prevalence rates being seen in the developed countries[16]. Disconcertingly, two national surveys showed a doubling of the prevalence of obesity and overweight in Australian children between 1985 and 1995 [17]. In the United States (USA), the prevalence of adolescent obesity in the most recent survey 2013-14 was 17% and extreme obesity 5.8%, since 2003-04 this obesity in adolescents has continued to rise despite plateauing in younger children[18]. The public health issue of overweight is also increasing in developing countries of the world[16]. Worldwide, 110 million children are affected by overweight [19] and 2013 estimates from the World Health Organisation (WHO), classified 42 million children under the age of 5 years as overweight, three quarters of these children lived in developing nations, with higher incidences being seen in urban areas [20]. Demographic trends in overweight have been seen according to socio-

economic status and ethnicity [21-23] [13] [24] and parental education [25] [26]. In developed countries, lower socioeconomic status appears to confer an increased risk of overweight (and obesity); this is in contrast to poorer nations where the prevalence is higher in children of more affluent families [16] [27-29] [24]. In the United States, children of Hispanic, African American (AA) and Native American background display higher prevalence of obesity compared to white children, confirming this socioeconomic divide [30] [4] [5]. These data demonstrate the extent of the obesity epidemic both nationally and globally, a greater awareness of which may halt this upward progression.

Apart from the physical and metabolic morbidity of the condition, weight issues, impact greatly upon the psychological health of the young person and may compromise social functioning [31] [32] [33]. Adolescents with increased BMI are at greater risk of depression and those who are morbidly obese are most vulnerable [34] [35]. Therefore, early-targeted intervention in young people may reduce immediate and long-term physical and psychosocial sequelae.

1.1.2 Aetiology of obesity

The aetiology of obesity is multi-factorial and remains poorly understood. In simple terms, overweight and obesity are due to an imbalance in energy intake and expenditure with the excess energy stored as body fat. This new body weight set point is then actively defended by a complex neuroendocrine response that leads to changes in metabolism, decreases in physical activity or increases in appetite. This phenomenon has been demonstrated in adults where resting metabolic rate and total energy expenditure decrease to counteract dietary weight loss [36] [37].

Appetite, satiety and energy balance is mediated by a complex array of neuroendocrine mediators within the hypothalamus and higher brain centres. The obese state leads to changes in regulatory hormone release from the pancreas, adipose tissue, gastrointestinal system and the brain [38].

In addition to other hormones, adipose tissue secretes leptin, an anorexigenic hormone, with multi-fold effects leading to increased satiety and energy expenditure and decreased appetite and food intake. The amount of leptin secreted is in direct proportion to the amount of fat tissue, however with well-established obesity, a state of leptin resistance develops. The gastrointestinal system is the source of additional anorexigenic hormones, including: obestatin which influences gastrointestinal motility, food intake and metabolic processes; peptide YY (PYY) which influences the secretion of neuropeptide Y from the hypothalamus and glucagon like peptide (GLP-1) which acts in the pancreas to increase insulin and decrease glucagon secretion. Ghrelin is the main orexigenic hormone secreted by the gut, it has an antagonistic effect to ghrelin leading to increased hunger [39] .

Environmental and individual factors also play a role in the development of obesity. Individual risk factors associated with obesity include: nutritional intake with increased consumption of energy dense foods, genetic predisposition, ethnicity, medical conditions and medications. Secular trends leading to decreased physical activity and an increased in sedentary activities such as television viewing also contribute to reduced energy expenditure [14]. Increasing the duration and quality of sleep [40 41], decreasing energy intake and increasing physical activity are modifiable risk factors to prevent overweight in later childhood [40]. Although single gene mutations may lead to significant obesity in isolation or in the context of an inherited genetic syndrome, they account for less than 1% of obesity referrals [42].

There is rising interest in the role of endocrine disrupting chemicals (EDCs) and their putative role in the development of obesity as shown in Figure 1.1. These products coined as "obesogens" are commonly found in the environment with exposures occurring through ingestion, dermal absorption or inhalation. Obesogens such as Bisphenol A (BPA) and phthalates, present in plastics can leach out to produce endocrine disrupting effects in humans affecting metabolic pathways for both glucose and fat homeostasis leading to T2DM and obesity. Epigenetic changes that occur through the ingestion or absorption of chemical may be combining to increase obesity prevalence rates [43-45].

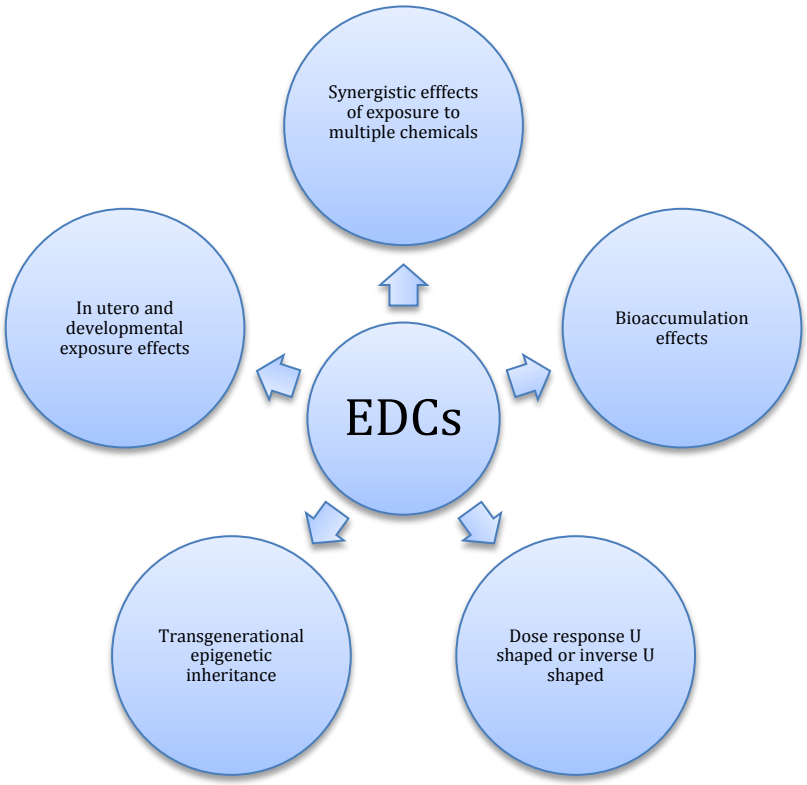


Figure 1.1 The actions of endocrine disrupting chemicals (EDCs)

The possible actions of endocrine disrupting chemicals (EDCs) in the pathogenesis of human obesity Adapted from Stojanoska *et al* [46]

An "obesogenic" environment that is, an environment conducive to overweight and obesity is thought to provide a catalyst for the phenotypic expression of overweight and obesity in individuals with and without a genetic predisposition. The global trend toward a more sedentary lifestyle along with a change in dietary patterns is thought to be the overriding influence in the worldwide development of overweight and obesity [16].

Other prenatal and early life factors that may influence the development of obesity include the intrauterine environment, birth size, rapid weight gain during infancy and early rebound of BMI [22 47 48]. Excessive weight gain during pregnancy is positively associated with heavier birth weight and increased BMI at 2 and 8 years of age. Maternal diabetes in pregnancy is also associated with heavier birth weight and the development of obesity in childhood [49]. Further studies in Australian children have confirmed the role of pregnancy related factors including maternal smoking, large birth weight as well as single child status[50]. Placental weight may mediate some of these maternal factors [51]. Socioeconomic factors such as low parental education and SES level have been shown to influence early childhood obesity rates and may contribute to the above demographic trends [52 53 26]. Parental obesity, particularly that of the mother may also increase the obesity risk of Australian children [54 55]. Furthermore, lack of parental recognition of obesity and risks to the child may also contribute to high prevalence rates [56 57]. These data reveal that whilst the problem of childhood obesity is multifactorial and complex, there are many potential areas for intervention including lifestyle modification.

1.1.3 Measurement of overweight and obesity in adolescents

An ideal clinical tool for the assessment of body fatness in young people has not yet been developed [58 59]. Furthermore, a general consensus for the measurement of adiposity has

not been reached and there are wide variations across the world, resulting in difficulties in the definition of obesity. This lack of standardisation makes it difficult to quantify the global impact of the condition and to establish worldwide trends [16 60]. The assessment of body fatness in the paediatric cohort relies on indirect measures of adiposity such as anthropometry. In research settings, a more direct analysis of body composition with tools such as dual energy x-ray absorptiometry (DXA) or underwater weighing may be possible however, these gold standard measures require specialised equipment and personnel rendering them costly and impractical for day to day clinical use. Indirect measures that include anthropometry have greater clinical utility and can be easily applied by health professionals [1].

1.1.3.1 Indirect Measurement of Adiposity

Several indirect measures of adiposity are available including Body Mass Index (BMI), waist circumference (WC), waist-hip ratio, waist-height ratio (WtHR) and skinfold thickness. Only BMI, WC and WtHR will be discussed as these techniques are employed in the VIBRATE research study.

a. BMI

The BMI is a calculated ratio and is used as a proxy measure of body fatness. The BMI is calculated by dividing the weight of the child in kilograms (kg) by the height of the child in metres^2; the result is a numeric value with units of kg/m^2. It has greater applicability in the adult population, where well-defined cut-off thresholds exist for normal, overweight, obese and morbid obesity. In adults, a higher BMI is associated with increased risk of cardiovascular health problems [61 48 62] similar trends for childhood obesity have not been clearly proven [63-66]. In Caucasian adults, a BMI greater than 25kg/m^2 is characterised as overweight and a BMI over 30kg/m^2 is classified as obese. However, these definitions may not be appropriate for all ethnic populations, for example in Asian populations, a

BMI of 23kg/m^2 and 25kg/m^2, for overweight and obesity respectively, may better characterise individuals at metabolic risk given marked variations in body composition and increased genetic susceptibility to the metabolic syndrome [16 62 67]. The percentage of body fat varies with gender, race (different distribution of body fat) and pubertal status and therefore the assessment of body fatness using BMI may not provide a true representation of total body fat in all populations [68 69 70].

To determine if an adolescent is overweight, the calculated BMI should be plotted on an age and gender specific population reference standard. There is no local reference standard in Australia and therefore the BMI for age percentile charts from the US Centers for Disease Control and Prevention (CDC) have been adopted for routine clinical use [71]. A BMI that lies above the 85th centile classifies the child as overweight; if the ratio is greater than 95th centile an adolescent can be considered obese [1 22]. BMI assessment can be performed for children aged 2-18 years using these charts.

b. Waist circumference

The WC is an easily obtained, non-invasive, surrogate measure for central adiposity and correlates well (R>0.8) with similar measures of truncal fat obtained using DXA. It provides good reliability, with low inter-observer error [16]. Waist circumference is also a strong proxy measure for visceral fat in children [72-74] and adults. The regional distribution of adiposity confers additional risk and increased WC is often associated with adverse metabolic profile [75 76 77].

The WC may also provide clues to adverse lipid partitioning and associated intramyocellular lipid (IMCL) and intrahepatic lipid (IHCL) lipid deposition [78 79]. It has a recognised correlation with visceral adipose tissue deposition (R^2 =0.64)[80]. In adults,

visceral adiposity has been associated with increased metabolic risk[73 81-83]. In contrast, in children the association between visceral fat and metabolic risk is still to be determined but it is likely to parallel those seen in adults [73 76 84 72]. The WC can independently predict decreased insulin sensitivity (Si) and it may be a strong predictor of the more metabolically adverse visceral fat component in children [85] and adults [86]. Waist circumference may also prove better than BMI z score in predicting cardiovascular risk as demonstrated in studies of Australian [75] and Greek-Cypriot children [75 87]. Watts *et al* showed that children with a WC ≥ 90th centile [88] had increased cardiovascular risk. An increased waist circumference may identify children with high metabolic risk to a greater extent that BMI measurement as it better represents visceral adiposity [72 89].

There is a lack of reference standards for waist circumference that can be partially overcome by the use of the waist to height ratio (WHtR). This measure is easy to calculate and provides an individual with clinical feedback about metabolic risk. It is calculated by dividing the waist circumference in centimetres (around a well-defined reference point e.g. umbilicus or midpoint between the costal margin or ileal crests) by the height of the adolescent in centimetres. A waist to height ratio ≥ 0.5 has been shown to correlate with cardiovascular disease risk (CDR) clustering in young people [90 91]. More recently however, some have suggested age specific cut off values for WHtR are preferable in the growing child [92 93]. An elevated WHtR was superior to BMI measurement in assessing cardiovascular risk factors [94]. Similarly, cross-sectional data from the Bogalusa Heart Study demonstrated CDR clustering with increased waist circumference. This study showed children with an android distribution of adipose tissue had higher fasting insulin levels and adverse lipid profiles, independent of race or gender[95]. In the clinical setting, the simple message "keep your waist circumference to less than half your height"[96 90] is readily understood, provides immediate feedback to the

family and young person and provides a tool to monitor progress. Whilst BMI is useful to measure overall body fatness, it is clear that centripetal adiposity is related to CDR in both adults [97] and children [76 91] and therefore the measures of WC and WtHR may allow better characterisation of childhood metabolic risk.

1.1.3.2 Direct measures of adiposity

Bioelectrical impedance analysis (BIA), Dual energy x-ray Absorptiometry (DXA), Magnetic resonance imaging (MRI), Computerised axial tomography (CT) and underwater weighing are research tools for evaluation of adiposity. They are costly and require specialised personnel [1]. DXA was the only direct measure of adiposity employed in the VIBRATE study and is discussed further.

a. Dual energy x-ray absorptiometry (DXA)

DXA is a useful tool to measure total and regional body composition and provides a direct measure of adiposity in the research setting. A DXA scan can provide information about total and regional fat, fat free mass and bone mineral compartments. The method relies on the variable attenuation of low dose x-ray radiation passing through each of these body compartments, which is recorded by specialised software. In addition to low radiation exposure, the scan is relatively easy to perform and has good accuracy and reproducibility. The DXA scanning bed has an upper weight thus limiting the ability of severely obese adolescents to be assessed via this method. Further limitations include an underestimation of percent body fat in the obese adolescent when compared to the 4-compartment method (divides the body into fat, water, protein and mineral.) [98] In the growing adolescent, changes in the hydration and density of lean tissue may affect analysis of DXA [99] . Small changes to body composition seen during short-term intervention trials may not be accurately detected by this method [100 101].

1.1.4 Complications of obesity

Obesity and overweight have significant impact on many systems of the body [33],[102]. This literature review will focus on the development of the endocrine complications of insulin resistance, its involvement in the metabolic syndrome and the development of T2DM. The musculoskeletal effects of obesity will be briefly examined along with the possible role of bone as an endocrine organ with putative influences on insulin sensitivity.

1.1.4.1 Obesity and the metabolic syndrome

In the seminal Banting Lecture, Reaven first described the "metabolic syndrome" as a constellation of metabolic risk factors that heighten an individual's risk for diabetes and cardiovascular disease (CVD) [103]. This syndrome has also become known by a variety of other names including "insulin resistance syndrome", "syndrome X" and the "multiple metabolic syndrome".

Cardiovascular risk factors such as dyslipidaemia, hypertension (HT), abdominal obesity and insulin resistance/ glucose intolerance are increased in obese individuals. These adverse cardiovascular indicators are also key defining elements of the metabolic syndrome. Obesity often precedes the development of the metabolic syndrome and insulin resistance. Presence of metabolic risk factors may predict glucose response in pre and post pubertal children [104]. The atherosclerotic process, a cardinal feature of cardiovascular disease has been shown to be present in youth with the metabolic syndrome [105]. Carotid femoral pulse wave analysis, a measure of arterial wall stiffness, is reduced in children who have increased BMI and central adiposity [106] and insulin resistance [107]. Atherosclerosis, HT and adverse lipid profile predisposes the young person to increased CVD. [60 105 108]. Furthermore, in obese adolescents, the pre diabetic state is already associated with reduced cardiovascular function[109]. Adolescent males with

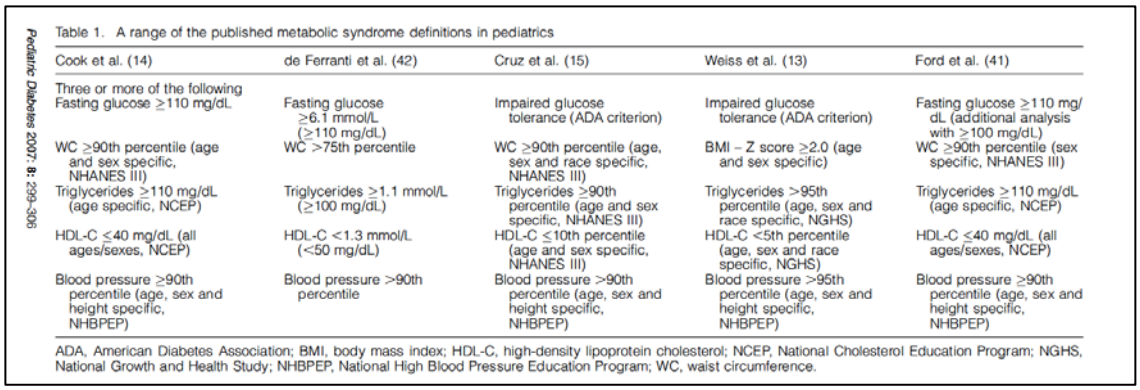
elevated BMI within the top 10th centile are 7 times more likely to develop coronary heart disease at age 25-45 years, than counterparts who are in the lowest 10th centile of the BMI charts. The excess cardiovascular risk is present even within the normal range, rising 12% for every unit increment in the BMI [66]. As the weight of an individual increases, there is a concomitant increase in risk of developing a risk factor for cardiovascular disease, with half of overweight adolescents having a least one cardiovascular risk factor [110]. This suggests that an elevated BMI in childhood contributes independently to the risk of later CVD.

Although influenced by many modifiable 'obesogenic' environmental factors, metabolic risk, may also be pre-determined by intrauterine factors including; maternal gestational diabetes [111 112], intrauterine growth retardation [49 113], genetic and ethnic specific factors. Nutritional influences such as infant feeding and the development of early adiposity rebound may also contribute to metabolic risk in later life [114 115].

There exists much confusion regarding the definition of the metabolic syndrome in the adult population, making it difficult to gauge global estimates of metabolic syndrome prevalence. In 2001, the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) [116] guidelines were released as a tool to allow clinicians to classify adults with the metabolic syndrome. It is difficult to adapt an adult definition to the paediatric population as metabolic indicators such as blood pressure; lipid profile and growth parameters are highly variable with age. In addition, the advent of puberty causes body composition change that confers a physiological decrease in insulin sensitivity. This necessitates the use of age, gender and pubertal specific values rather than single cut-off thresholds [117 118].

As no consensus guidelines for the metabolic syndrome in children exist, adult guidelines had been modified for use in the paediatric population with age specific limits. Cook *et al* reported that 6.8% of overweight adolescents and 28.7% of obese youngsters were defined as having the metabolic syndrome [119]; suggesting that the degree of overweight is an important factor in the development of the metabolic risk. Data from the National Health and Nutrition Examination Study (NHANES) 1999-2000 showed a slight increased overall prevalence in the metabolic syndrome in adolescents from 4.2% to 6.4% from the previous NHANES study conducted in 1988-1992 [120]. Similar to Cook *et al*[119], they found 32.1% of individuals with BMI>95th centile could be classified with the metabolic syndrome. Shaibi *et al* examined the prevalence of the metabolic syndrome using three different published definitions of the metabolic syndrome and discovered aggregations of risk factors consistent with the metabolic syndrome in 25.7%-39% depending on which definition was applied [121]. Various definitions of the metabolic syndrome in children and adolescents may be seen in Table 1.1.

Table 1.1 Definitions of the metabolic syndrome in children



Pediatric Diabetes 2007: 8: 299–306

Table 1. A range of the published metabolic syndrome definitions in pediatrics

Cook et al. (14)	de Ferranti et al. (42)	Cruz et al. (15)	Weiss et al. (13)	Ford et al. (41)
Three or more of the following				
Fasting glucose ≥110 mg/dL	Fasting glucose ≥6.1 mmol/L (≥110 mg/dL)	Impaired glucose tolerance (ADA criterion)	Impaired glucose tolerance (ADA criterion)	Fasting glucose ≥110 mg/dL (additional analysis with ≥100 mg/dL)
WC ≥90th percentile (age and sex specific, NHANES III)	WC >75th percentile	WC ≥90th percentile (age, sex and race specific, NHANES III)	BMI – Z score ≥2.0 (age and sex specific)	WC ≥90th percentile (sex specific, NHANES III)
Triglycerides ≥110 mg/dL (age specific, NCEP)	Triglycerides ≥1.1 mmol/L (≥100 mg/dL)	Triglycerides ≥90th percentile (age and sex specific, NHANES III)	Triglycerides >95th percentile (age, sex and race specific, NGHS)	Triglycerides ≥110 mg/dL (age specific, NCEP)
HDL-C ≤40 mg/dL (all ages/sexes, NCEP)	HDL-C <1.3 mmol/L (<50 mg/dL)	HDL-C ≤10th percentile (age and sex specific, NHANES III)	HDL-C <5th percentile (age, sex and race specific, NGHS)	HDL-C ≤40 mg/dL (all ages/sexes, NCEP)
Blood pressure ≥90th percentile (age, sex and height specific, NHBPEP)	Blood pressure >90th percentile	Blood pressure >90th percentile (age, sex and height specific, NHBPEP)	Blood pressure >95th percentile (age, sex and height specific, NHBPEP)	Blood pressure ≥90th percentile (age, sex and height specific, NHBPEP)

ADA, American Diabetes Association; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; NCEP, National Cholesterol Education Program; NGHS, National Growth and Health Study; NHBPEP, National High Blood Pressure Education Program; WC, waist circumference.

This table is taken from [122]

In an attempt to clarify the situation, the International Diabetes Federation (IDF) proposed a consensus definition of the metabolic syndrome in children in 2007, incorporating clinically relevant metabolic profile indicators in children [122] (Table 1.2). The definition clarified aspects of the metabolic syndrome in children aged 10 years and older; adult guidelines may be applied to adolescents aged ≥ 16 years [123]. Abdominal adiposity is central to the IDF definition of the metabolic syndrome in both adults and children. A further two or more metabolic profile indicators including: elevated triglycerides (TG), low levels of high-density lipoprotein cholesterol (HDL-C), increased blood pressure (BP), and elevated plasma glucose are also required to diagnose the metabolic syndrome in childhood [122].

Adverse metabolic profile indicators persist from childhood into young adulthood [124]. The Fels longitudinal study confirmed this tracking phenomenon by showing that elevated WC in boys was associated with increased WC as an adult and heightened metabolic risk. They also demonstrated a sexual dimorphism with regard to metabolic risk. The divergence of risk was different for boy and girls, occurring earlier in childhood for boys and later during puberty for girls [125].

Table 1.2 IDF definition of the metabolic syndrome in children

Table 2. The IDF definition of the at risk group and metabolic syndrome in children and adolescents

Age group (years)	Obesity (WC)	Triglycerides	HDL-C	Blood pressure	Glucose
6–<10†	≥90th percentile				
10–<16	≥90th percentile or adult cut-off if lower	≥1.7 mmol/L (≥150 mg/dL)	<1.03 mmol/L (<40 mg/dL)	Systolic BP ≥130 or diastolic BP ≥85 mm Hg	FPG ≥5.6 mmol/L (100 mg/dL)** or known T2DM
16+(Adult criteria)	WC ≥ 94cm for Europid males and ≥ 80cm for Europid females, with ethnic-specific values for other groups*)	≥1.7 mmol/L (≥150 mg/dL) or specific treatment for high triglycerides	<1.03mmol/L (<40 mg/dL) in males and <1.29mmol/L (<50 mg/dL) in females, or specific treatment for low HDL	Systolic BP ≥130 or diastolic BP ≥85 mm Hg or treatment of previously diagnosed hypertension	FPG ≥5.6 mmol/L (100 mg/dL)** or known T2DM

BP: blood pressure; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; IDF, International Diabetes Federation; T2DM, type 2 diabetes mellitus; WC, waist circumference.
*For those of South and South-East Asian, Japanese, and ethnic South and Central American origin, the cutoffs should be ≥90 cm for men, and ≥80 cm for women. The IDF Consensus group recognise that there are ethnic, gender and age differences but research is still needed on outcomes to establish risk.
†Metabolic syndrome cannot be diagnosed, but further measurements should be made if there is a family history of metabolic syndrome, T2DM, dyslipidemia, cardiovascular disease, hypertension and/or obesity.
**For clinical purposes, but not for diagnosing the MetS, if FPG 5.6-6.9 mmol/L (100-125 mg/dl) and not known to have diabetes, an oral glucose tolerance test should be performed.
Diagnosing the metabolic syndrome requires the presence of central obesity plus any two of the other four factors.

This table is taken from [122]

1.1.4.2 Obesity and insulin resistance

A direct relationship between obesity and insulin resistance has been shown in adults and children[126] [127]. Overweight individuals are more likely to have elevated fasting insulin levels due to resistance of insulin hormone action in the body which results in higher circulating levels of insulin and the development of hyperinsulinaemia[128]. Insulin resistance is a precursor to the development of pre-diabetes, including impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) that may progress to T2DM [129] [130]. An obese state, independent of the presence of insulin resistance can lead to ß cell dysfunction, with elevated 2 hour glucose levels within the normal range seen during oral glucose tolerance test in obese children and adolescents [131].

In the USA, the prevalence of pre diabetes and T2DM in US adolescents increased from 9% to 23% from 1999-2008 but other factors associated with the metabolic syndrome such as hypertension and adverse lipid profile remained stable [110]. These rates are comparable to adults where 8.3% of the population has been diagnosed with T2DM and many more are still undiagnosed [132]. A recent study has shown that during mid-

adolescence, progression from normoglycaemia to pre diabetes occurs at a rate of 1% in a random selection of the population [133].

1.1.4.3 Obesity and the musculoskeletal system

Children with obesity are at risk of orthopaedic side effects such as slipped capital femoral epiphyses, tibia vara, pes planus and scoliosis of the spine [33 102]. In addition, overweight may also influence the mechanical properties of bone with alterations in bone mineral density (BMD) and bone mineral content (BMC). In children, there are opposing viewpoints with regard to the effect of increased body mass index on bone parameters. A cross sectional study by Goulding *et al* showed that overweight and obese children had reduced BMC and areal BMD for weight when compared to normal weight controls. This unexplained difference resulted in up to 10% less BMC and bone area in obese adolescents for body weight. One possible explanation for this mismatch between BMI and bone may relate to a delay in bone development and growth during a period of rapid weight gain [134]. Overweight children are more likely to present with fractures with an increased odds ratio of 4.5 compared to healthy weight counterparts [135 136]. The increased fracture risk may be explained by altered bone structure and increased force experienced during a fall. Poor balance in the obese child may predispose them to increased falls [137]. However, other studies have demonstrated the positive effects of obesity [138] secondary to increased fat derived hormones [139]. The above findings have important implications for integrating overweight children into physical activity given possible disruption to bone properties and an increase fracture risk.

In contrast to children [140], studies in adults have mostly shown a protective effect of increased BMI on fracture risk with a positive correlation between weight and osteoporosis, BMD and fracture risk [141-143]. Furthermore, the positive influence on BMD

may be related to independent changes in fat and lean body mass. It remains unclear whether lean tissue or fat mass or both influence the apparent differences in BMD.

1.1.5 Strategies for the prevention and management of overweight

The prevention of overweight needs to commence in early life with public health strategies facilitating change in the "obesogenic" environment and modifying behaviours at the individual and family level. The environment, family and peer influences play key roles in the development of obesity and overweight [144] [145]. Therefore, intervention programmes aimed at reducing the problem of childhood overweight and obesity must mutually consider these factors. It is often difficult to extrapolate results from research studies to real life because children often achieve weight loss under the ideal conditions of a clinical trial. In real life however, many of these weight loss goals are not met often due to poor adherence. Recruitment challenges and high dropout rates are characteristic of clinical trials involving overweight adolescents [146]. This is likely due to intrinsic characteristics of the patient population, including poor mood and lack of self-esteem[32] [33]. There are often psychosocial difficulties and economic constraints inherent that make management more difficult [13] [50].

At the family level, supporting parenting skills, educating parents and promoting breastfeeding may be useful to prevent excessive weight gain in infancy and the preschool years [22]. In an RCT of 12 months duration, Golley *et al* showed young children aged 6-9 years whose parents were involved in intensive lifestyle and parenting skills courses, had a 10% reduction in BMI z-score compared to 5% decrease in the parenting skills alone and control groups [147]. This and other studies suggest a family focused intervention may be more successful for weight management. Adolescents whose parents, particularly mothers, have poor levels of education appear vulnerable to obesity [26] [47] [148].

Therefore targeting mothers for further education may prove useful to successfully manage weight.

Adolescents must grapple with peer group pressure and also struggle to gain independence. Physiologic changes including puberty may also influence body weight during adolescence. In view of these age specific tasks, counselling sessions for adolescents on their own may be more successful [149]. Increasing physical activity and altering dietary habits in the school-aged child may assist with lifestyle change and decrease prevalence of overweight and obesity. Childhood obesity management requires the dedicated input of a multidisciplinary team that will often include a physician, weight management dietitian, exercise physiologist and psychologist or social worker. The therapeutic process requires engagement with not only the child but also the family and caregivers. If family motivation is lacking then successful outcomes are less likely, particularly with the pre-pubertal child.

Weight management often presents a challenge for the children, their families and health care workers. Young children and those with mild overweight may achieve success with weight stabilisation, as ongoing growth will allow them to "grow into their weight" [150]. However, to prevent metabolic complications in severely obese adolescents lifestyle change should target weight loss with referral to specialist clinics if these measures fail [14]. Population based strategies should evaluate food advertising directed at children on the mass media. In Australia, children from ethnic minority groups, particularly Middle Eastern and European backgrounds have been found to have a higher prevalence of weight issues and therefore culturally appropriate strategies with consideration of socioeconomic variances may be required[151].

1.1.5.1 Dietary modification

Dietary modification in paediatric obesity remains controversial and there is no consensus regarding the most appropriate diet for weight management, improved insulin sensitivity [152] [153] and metabolic health [154]. Given that childhood and adolescence are associated with growth and physiological changes such as puberty, adequate nutritional intake should be not be compromised. Age specific caloric intake is generally maintained through the inclusion of foods low in total and saturated fat and cholesterol [22]. Australian guidelines suggest inclusion of fruit and vegetables, cereals, lean meats and dairy products for adequate calcium intake, water should be considered the standard beverage [155] . Breastfeeding infants born to mothers with gestational diabetes appears to have a protective effect against later obesity as shown by the results of a retrospective cohort study [156]. Children were followed up at ages 6 and 13 years and were found to have significantly lower BMI, WC, subcutaneous abdominal (SAAT) and visceral abdominal adipose tissue (VAAT) than those who breastfed less. The results suggest that any programming effects due to the exposure of intrauterine diabetes may be attenuated by breastfeeding. Health promotion encouraging breastfeeding in mothers may be an effective strategy to reduce the increased risk of obesity in offspring of mothers with diabetes [55].

Nutritional counselling using the elements of the "Stop Light/ Traffic Light" diet can effect BMI change in paediatric patients. This diet, colour codes food into red, orange and green choices based on caloric density with an emphasis on an overall less prescriptive and lower energy diet. Reduction of sugar sweetened beverages, recommendation of water as the main beverage, appropriate portion sizes and a regular breakfast are also encouraged as first line nutritional measures [102]. A family based approach with nutritional education to caregivers may be more appropriate in the

younger child [157] [158]. In the older adolescent a different approach may be required. A recent RCT showed that meal replacements to adolescents increased weight loss compared with a conventional diet after 4 months suggesting that adolescents may need more prescriptive nutritional guidance [159]. Modifying the macronutrient composition of the nutritional component of lifestyle programs has not found to be superior to standard nutritional coaching as shown by Gow *et al* [160] and reduction in overall energy intake may assist in BMI reduction [161].

Dairy products are an invaluable source of dietary calcium and are required for attainment of peak bone mass during childhood and adolescence. The intake of dairy appears to decrease during adolescence due to concerns about weight gain due to the increased caloric density of these foods [162]. A recent meta-analysis has however shown that obesity rates may decrease by 16% for each 200g/day increment in dairy intake [163]. The effects on weight loss may be mediated through increased satiety and reduced appetite [164].

1.1.5.2 Lifestyle management

The most appropriate physical activity prescription or dose of activity for health benefits in young people with overweight or obesity is unknown, however, behavioural lifestyle interventions were found to be associated with positive weight loss outcomes in a recent Cochrane Review [29]. Adolescents are encouraged to incorporate 60 minutes of moderate intensity physical activity on most days [14] [165] but a lack of time is increasingly cited as a barrier to achieving this target. Therefore, time efficient and shorter duration activities have been explored to determine their utility in paediatric based lifestyle programs. These activities may be more appealing to children who have shorter attention spans and different levels of development.

High intensity interval training (HIIT) [166] is one such method to improve muscle power. HIIT involves burst of high intensity activity with interspersed low intensity or rest periods and has been shown to increase muscle power [167] [168], decrease body fat [169] and improve cardiorespiratory fitness [170] in obese adults. In adolescents, a HIIT program had similar effects to a standard school based physical education class and maintained cardiorespiratory fitness [171]. HIIT may produce similar improvements to cardiorespiratory fitness in the obese adolescent but may lead to greater BMI reductions when compared with endurance training [172].

Decreasing sedentary activities with a concomitant increase in incidental activity may prove to have similar outcomes to increasing physical activity. Although television viewing, internet use and electronic games are engrained in the social culture, reduction in total small screen time to less than 2 hours per day may change may increase activity [165] and reduce consumption of excessive calories [173].

1.1.6 Conclusions

Childhood obesity has risen to critical rates both in Australia and internationally, with almost one in four children being classified as overweight or obese. As a consequence there is an increasing prevalence of complications such as insulin resistance, T2DM and CVD. Childhood overweight and obesity is more often than not, a precursor to body mass issues in adult life [174]. The "tracking" phenomenon has been well established in childhood overweight and obesity, meaning that adolescents will carry weight issues into adulthood. Thus, the impact to the health system will be most apparent in the next generation of young adults as overweight and obese children become adults with obesity related health problems such as T2DM, CVD and other manifestations of the metabolic

syndrome [16]. In addition to the increased risk of health problems as an adult, childhood overweight and obesity is associated with immediate serious health consequences and has a directly impacts on the psychological welfare of young people [175] [176]. Hence, early targeted intervention in the paediatric and adolescent populations may be reduce the future economic burden associated with the complications of obesity and overweight but will also lead to immediate direct health improvements for the paediatric population.

1.2 Insulin resistance

1.2.1 Definition

Insulin, secreted by beta cells in the pancreatic islets, is the primary glucose-lowering hormone. It maintains glucose homeostasis by: suppressing endogenous glucose production, inhibiting hepatic glycogenolysis and gluconeogenesis and reducing renal glucose production. It stimulates and enhances peripheral glucose utilisation by insulin sensitive tissues including muscle and fat [177]. An insulin resistant state exists when physiologically normal levels of insulin produce a reduced biological response at the tissue level, resulting in reduced insulin dependent glucose uptake. An individual with insulin resistance requires higher levels of circulating insulin to maintain euglycaemia. The liver, muscle and adipose tissue are the primary organs and tissues that determine the degree of insulin resistance.

A variety of factors contribute to the development of an insulin resistant state including: genetic predisposition, obesity, puberty, adverse fat partitioning and inflammatory cytokines[178] [179]. Insulin resistance is a term often used descriptively to describe the clinical status of the individual. Insulin resistance and Si are often used interchangeably, however, Si is a derived value obtained during dynamic biochemical testing. An individual who has

been investigated and has low Si is often highly insulin resistant and vice versa, an individual with high insulin sensitivity is less insulin resistant.

1.2.2 Aetiology and prevalence

Insulin resistance and subsequent ß cell failure are antecedents to the development of T2DM. Insulin resistance is the most common metabolic feature of the obese adolescent but may also be seen in normal weight children, particularly those with a family history of metabolic disease [61 103 180]. The prevalence of insulin resistance varies according to the measurement adopted and population studied. As BMI progresses into the obese range the prevalence of insulin resistance in the adolescent increases [181-183]. High prevalence rates of insulin resistance as measured by HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) have been described in the literature, ranging from 10-60% in overweight and 45-80% in obese children and adolescents [183-186].

Similarly, in a large cross sectional study of eighth grade students in the US, almost half had a BMI in the overweight range ≥85th centile, with mean fasting insulin of 30.1μU/mL, a level highly suggestive of biochemical insulin resistance. Of these participants, a high proportion, 40.5% had evidence of glucose dysregulation with evidence of impaired fasting glucose ≥5.6mmol/L [187]. In the USA, the NHANES study showed 51.1% of obese children (BMI>95th centile) were found to have biochemical evidence of insulin resistance [185]. A hyperbolic relationship between insulin sensitivity and insulin response preserves normal glucose tolerance; the product of the two is known as the Disposition Index (DI) [188] as shown in Figure 1.2 and will be further discussed in 1.2.3.

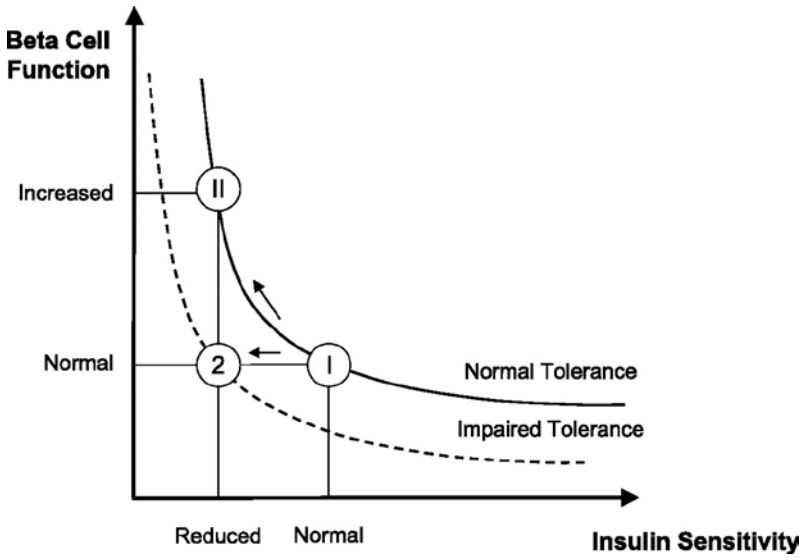


Figure 1.2 The hyperbolic relationship between insulin sensitivity and ß cell function. Different hyperbolas reflect differing degrees of tolerance. The product of ß cell function and insulin sensitivity is known as the Disposition Index = constant. (I) Normal state (II) Decreased insulin sensitivity is compensated for by increasing insulin secretion. The DI remains the same and there is normal glucose tolerance. Failure of the ß-cell to compensate with increased secretion leads to decreased insulin sensitivity and glucose intolerance as shown in (2) taken from [189].

Ethnicity, particularly African American (AA) and Hispanic background and family history of T2DM are associated with decreased Si in adolescents [190 191] and may predict progression from prediabetes to T2DM in obese adolescents[192]. Furthermore, offspring of individuals with T2DM have a higher prevalence of insulin resistance highlighting the heritability of this disease process [193 194]. After 25 year follow up period, offspring of couples with T2DM who developed T2DM had evidence of reduced Si and glucose effectiveness (S_G) at least 10 years prior to presentation with disease confirming defects in insulin dependent and independent uptake [195].

Individuals with prediabetes are more likely to progress to T2DM and have adverse cardio-metabolic profile. Adolescents with a fasting glucose level even within the upper normal range demonstrate risk factors for the metabolic syndrome including insulin

resistance; elevated BP and increased WC, independent of BMI z score [196]. Similarly, an elevated 2-hour glucose level after an oral glucose tolerance test was associated with a decline in beta cell function [197].

In adults from high-risk populations such as the Pima Indian, the progression to T2DM evolves over a protracted period of time 5-10 years [198]. Prospective analysis of studies in adults showed high variability of progression from IGT to T2DM ranging from 35.8 to 87.3 per 1000 person-years [199]. A pre-diabetic state may also co-exist in the obese, insulin resistant adolescent. Sinha *et al* found that almost one quarter of severely obese adolescents had evidence of pre-diabetes with IGT [129]. Further studies have confirmed that pre-diabetic adolescents have marked insulin resistance [200]. Disconcertingly, the progression from prediabetes to T2DM is accelerated in youth with 24% progressing from IGT to T2DM after only 2 years [192], however longitudinal data in this cohort is limited.

In utero and early life factors may also contribute to increased risk of insulin resistance in later life. Barker proposed the developmental origins of health and disease hypothesis whereby the intra-uterine environment particularly maternal nutrition and obesity can influence an individual's metabolic health in later life by altering physiology and epigenetic change [201] [202]. Intra-uterine factors such as prematurity (regardless of birth weight) [203-205]small[206] and large [207]for gestational age, maternal obesity[208] gestational diabetes [111] and twin pregnancy [209] [210]have been shown to increase the risk of developing insulin resistance and other elements of the metabolic syndrome supporting the notion of foetal programming [49] [211] [212] and epigenetic change[213] [214] [215] Individuals born small for gestational age have reduced Si that is persistent at age 20 years independent of their BMI [203] and first born children may have higher metabolic risk compared to siblings [216].

Genetic factors such as ethnicity and the in utero environment contributing to insulin resistance may be more difficult to modify. However, as a sedentary lifestyle and obesity, work in concert to exacerbate the inherited tendency towards insulin resistance, careful weight management in these patients may lessen risk. In addition, attention to maternal health and feeding practices may attenuate the presence of adverse metabolic phenotype in later life. Although limited, studies in adolescents suggest that there is more rapid progression from IGT to T2DM making this population high risk compared to adults [217]. Furthermore, longer duration of dysglycaemia may also indicate likelihood of progressive disease [218]. Therefore, screening of adolescents for evidence of clinical insulin resistance and prediabetes is essential.

1.2.3 Physiological and pathological insulin resistance

Puberty is a time of rapid somatic growth and sexual maturation. This transitional stage is accompanied by changes in body composition and an increase in truncal adiposity [219]. Physiologic changes during puberty result in a transient 30% decrease in insulin sensitivity, which peaks around Tanner stage III with recovery at Tanner stage V [220]. The exact process that underlies these changes has not been elucidated however, the β cells in the pancreas compensate by increased endogenous insulin secretion. Adolescents from AA and Hispanic backgrounds may have a blunted physiological compensatory response thus increasing their propensity to develop T2DM [221] [222]. During puberty, an increase in sex steroid secretion and physical maturity, results in increased BMI and adiposity, which worsen insulin resistance. The increased central adiposity in puberty expands the visceral adipose tissue compartment, which is pro-inflammatory and has a negative influence on Si [223].

Goran *et al* [222] showed that progression from Tanner stage I to III was accompanied by a 32% reduction in Si that could not be attributed to body composition changes or the altered hormonal milieu, apart from rising androstenedione levels. Jeffery *et al* in their longitudinal study showed that the insulin resistance often attributed to pubertal change actually precedes any pubertal biochemical signals and physical change by 3-4 years [224]. The above theories do not explain the nadir of Si and recovery at Tanner Stage V. The sequential changes in Si during puberty appear to best correspond to the pubertal growth spurt that is accompanied by increases in growth hormone (GH) and insulin like growth factor-1 (IGF-1) levels [225] [226].

Puberty is a time of physiological stress and it is not surprising that most cases of childhood T2DM are diagnosed in late adolescence particularly in those children with exogenous obesity. During puberty, metabolism shifts in favour of fat oxidation rather than glucose oxidation, resulting in lowered glucose utilisation, with decompensation more likely [226].

Pathological insulin resistance may result from inherited gene defects or acquired as part of the metabolic syndrome. Insulin resistance is a key pathology of the metabolic syndrome or syndrome X as coined by Reaven [103]. The metabolic syndrome is an aggregation of multiple risk factors that cumulatively increase the risk of CVD. Insulin resistance may also be found in certain rare genetic syndromes including Type A leprechaunism and Rabsen-Mendenhall syndromes due to mutations in the insulin receptor gene. The insulin resistance is often severe in nature and may present from birth leading to early death.

1.2.4 Pathophysiology of Insulin Resistance

Insulin, secreted by the pancreatic ß cell is involved in the maintenance of glucose homeostasis. In healthy individuals, normal glucose tolerance is maintained by dynamic interplay between endogenous glucose production and insulin dependent and independent glucose uptake.

1.2.3 Assessment of insulin sensitivity

Given the variety of tissues involved in insulin action and glucose homeostasis, the assessment of ß cell function remains a challenge. Single measures of glucose and insulin provide limited information and fail to account for the dynamic processes occurring as the ß cell responds to the rise and fall in postprandial glucose. The glucose tolerance of an individual is related to both the ß cell function and insulin sensitivity, which in turn are related by a hyperbolic relationship. The product is known as a disposition index (DI) (Figure 1.1). A low disposition index is a marker of poor ß cell compensation.

Insulin resistance often develops insidiously in an attempt to maintain blood glucose levels within the normal range and is influenced by several factors including: increasing overweight, in-utero programming, lifestyle factors and genetic predisposition. In the early stages, the ß cell compensates with increased insulin secretion. The compensatory hyperinsulinaemia and reduced hepatic clearance of insulin may manifest in higher fasting insulin levels[227] [228]. The raised insulin levels may lead to the development of clinical features such as acanthosis nigricans (AN) or polycystic ovary syndrome (PCOS) in females. With worsening insulin resistance, the ß cell decompensates which may unmask abnormalities in glucose tolerance with eventual progression to a pre-diabetic state and T2DM. As an adolescent becomes more insulin resistant the compensatory

insulin release from the pancreas may not be sufficient to preserve the status quo, resulting in fasting or post-prandial hyperglycaemia.

In the post-absorptive state and during fasting, glucose uptake occurs in the brain, red blood cells, renal medulla and gastrointestinal tract via insulin-independent processes. This uptake is matched to endogenous hepatic glucose production with the fasting plasma glucose primarily representing the liver production of glucose that is modified by circulating levels of glucose and insulin [229]. In the fed state, rising levels of glucose and amino acids trigger insulin release leading to active glucose disposal by skeletal muscle, gut and liver and to a much lesser extent adipose tissue with concomitant decreased hepatic gluconeogenesis and glycogenolyis. Approximately, 70-75% of insulin mediated glucose disposal in the post-prandial state occurs in the peripheral tissues, predominately skeletal muscle with minimal amounts taken up by adipose tissue. The release of insulin results in active translocation of glucose transporters to the cell membrane to facilitate glucose disposal and this is the rate-limiting step in the glucose metabolic pathway. The cellular mechanism of insulin resistance is likely to be multifactorial but may include insulin receptor and pathway signalling defects of varying aetiology; obesity; sedentary lifestyle and genetic factors Aberrations in the insulin-signalling pathway may include: reduced number of insulin receptors at the cell membrane as seen in obese individuals; pathological conformation changes in the receptor after insulin binding due to the effect of plasma membrane glycoproteins; post translation impairments including phosphorylation defects from free fatty acid accumulation and modulating effects of adipocytokines including tumour necrosis factor-α (TNF- α) and interleukin 6 (IL-6) [230]. There are varying degrees of insulin resistance and insulin secretory dysfunction in individuals with disorders of glucose homeostasis; knowledge of the differential contribution may be useful in treatment [231].

1.2.5 Adipose tissue as an endocrine organ

Obesity is associated with increased total fat. The adipose tissue contributes minimally to glucose disposal however provides a major substrate for the generation of potentially harmful free fatty acids which contribute to insulin resistance and impaired ß cell function through lipotoxicity.

Adipose tissue is now considered to play an active role in the development of obesity and eventual insulin resistance and secretes over a hundred products involved in energy homeostasis. These hormones and adipocytokines are involved in energy regulation and have both paracrine or local effects and endocrine actions in distant target organs. Leptin and adiponectin are two products secreted by adipose tissue. Adiponectin, derived from adipose tissue cells, improves Si and is cardio-protective [232]; a deficiency of leptin leads to pathological obesity and increased food intake [22]. Adolescents with T2DM have much lower adiponectin levels compared with leaner individuals with type 1 diabetes [233]. Adiponectin reduces insulin resistance by increasing glucose uptake, enhancing FFA oxidation and reduces endogenous hepatic glucose production [234].

In healthy individuals, insulin has varied physiologic effects including: inhibition of lipolysis, reduced hepatic gluconeogenesis and increased peripheral glucose disposal in skeletal muscle. With increasing obesity and worsening insulin resistance, insulin fails to completely suppress lipolysis and gluconeogenesis and there is a reduced uptake of glucose in the periphery.

FFAs play an integral role in the development of insulin resistance (Figure 1.3) and T2DM (Figure 1.4). In the insulin resistant state, FFAs are more readily available as the anti-lipolytic effect of insulin is reduced, particularly in the visceral compartment. This

results in FFA release into the hepatic vein and exposure of the hepatic cells in the liver to high FFA concentrations[235]. In the pancreas, there is a hyperbolic relationship between insulin secretion and sensitivity. High levels of FFAs may impair ß cell activity and may blunt any compensatory responsiveness as shown in Figure 1.3.

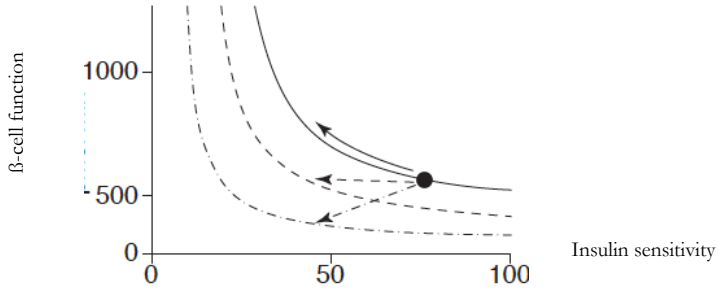


Figure 1.3 The role of free fatty acids in the development of insulin resistance

Taken and adapted from [235]and [236] Top arrow - ß-cell sensitivity to glucose is preserved by up-regulation of secretion. Middle arrow - (upper dashed line) lipid infusion results in insulin resistance and blockage of ß-cell compensation that results in a horizontal and left shift. Bottom arrow - (lower dashed curve) lipid infusion results in insulin resistance and lipotoxicity of ß-cell.

The greater availability of FFAs act in skeletal muscle to further worsen the degree of insulin resistance and have deleterious effects on glucose homeostasis by providing energy and substrate for gluconeogenesis [227]. In addition, FFAs have independent lipotoxic effects on pancreatic ß cell. Adipose tissue that accumulates in skeletal muscle and liver is a source of FFAs and adipocytokines. Adipocytokines released by adipose tissue may trigger an inflammatory cascade resulting in macrophage infiltration of adipose tissue. C-reactive protein is a marker of inflammation, however lower levels can be associated with obesity related inflammation and can be measured via a high sensitive assay hs-CRP, which in adults correlates with cardiovascular risk [237 238]. Adolescents with

increased central adiposity have elevated levels of hs-CRP further demonstrating that visceral fat is pro-inflammatory [239].

1.2.6 Lipid partitioning and insulin resistance

The location of fat within the body determines metabolic risk and this may explain why some obese adults have less metabolic co-morbidities [240]. Fat is partitioned into 2 main compartments. Visceral abdominal adipose tissue (VAAT) surrounds the organs, omentum and mesenteric layers. VAAT is associated with greater insulin resistance compared with subcutaneous adipose tissue (SAAT) as it is less sensitive to insulin and therefore undergoes lipolysis contributing to serum FFAs to a greater extent than SAAT. These FFAs are released into the portal vein contributing to hepatic insulin resistance [230] [235]. Intrahepatocellular lipid (IHCL) and intramyocellular lipid (IMCL), the accumulation of lipid within the liver and muscle respectively, disrupt glucose access to muscle cells, and interfere with glucose metabolism heightening the insulin resistance state. This is discussed further in section 1.2.6.1.

Similar to adults, obese adolescents of equal BMI can differ in terms of metabolic risk [241], suggesting that regional fat distribution, particularly central adiposity important in risk determination in the paediatric cohort [242]. Weiss *et al*, found when they compared two groups of adolescents with similar BMI, those with higher levels of intramyocellular and visceral fat were more insulin resistant and had lower levels of adiponectin [241]. In another study, obese adolescent girls displayed reduced fatty oxidation, impaired glucose disposal and reduced glucose oxidation in response to an insulin infusion compared to young women of normal weight [243]. In this cohort, those with greater visceral adiposity had higher fasting insulin, higher stimulated insulin secretion and greater overall insulin resistance. The deleterious effect of visceral fat accumulation was seen in a multi-ethnic

cohort of obese adolescents who were stratified into groups based on amount of fat in the visceral compartment. Adolescents in the highest tertile demonstrated high metabolic risk but were not necessary the most overweight, with the authors suggesting the phenotype was akin to partial lipodystrophy [244]. Weight loss accompanied by reduced VAAT may be associated with improvements in Si [245]. In contrast to the above findings, Maffeis *et al* found that this relationship may not be consistent in pre-pubertal children as lower Si showed correlation with subcutaneous fat [246].

It is important to note that BMI whilst clinically useful and easily measured provides a good surrogate measure of overall adiposity and an estimate of subcutaneous fat, which is largely metabolically inert. On the other hand, it is the VAAT and fatty deposits within the liver and muscle, which heightens metabolic risk and is more difficult to measure with anthropometric techniques [247]. Therefore, waist circumference, rather than BMI, may correlate better with metabolic risk allowing a more accurate representation of adverse fat distribution [91 248]. Racial factors and ethnicity are also important contributors to the development of insulin resistance, due to differing proportions of VAAT as shown in children [249] and adults [250 251]. In AA children, IMCL varies independently of insulin resistance unlike Americans of European extraction. AA have less visceral fat, which is associated with inflammation and insulin resistance, but have higher incidence of T2DM most likely due to a lower threshold of visceral fat required for an insulin resistant state [252]

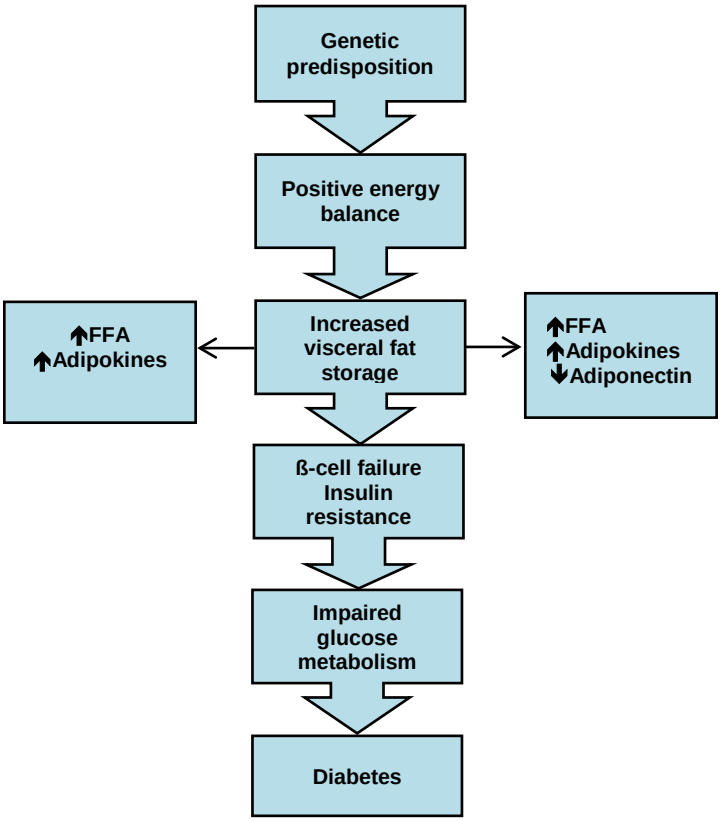


Figure 1.4 The role of free fatty acids (FFA) in the development of T2DM
adapted from [230]

1.2.6.1 Intramyocellular lipid (IMCL) and Extramyocellular lipid (EMCL)

The accrual of IMCL in the skeletal muscle affects insulin-mediated glucose uptake by interfering with glucose transporter 4 (GLUT 4) receptor transport in the cell membrane; a similar process is also responsible for hepatic insulin resistance [253]. FFAs accumulate in skeletal muscle and through a series of intracellular events, preventing GLUT4 translocation to the cell membrane. The increased circulating levels of FFAs generated from lipolysis lead to mitochondrial dysfunction and impaired lipid oxidation, which in turn perpetuates the vicious cycle by leading to accumulation of further IMCL. The net result is decreased glucose transport, glycogen synthesis and a blunting of the insulin-signalling cascade [230].

IMCL can be differentiated from EMCL using proton nuclear magnetic resonance spectroscopy. Studies have shown ICML has a higher correlation with insulin sensitivity when compared to the visceral compartment, independent of obesity. Paradoxically, elite athletes who are highly insulin sensitive have increased IMCL to meet their higher oxidative requirements during intense activity suggesting actual location and size of the lipid particles may also modulate the clinical presentation of insulin resistance.

Adolescents who are insulin resistant have higher visceral and IMCL deposits [241]. There is a direct relationship between the accumulation of IMCL and the degree of insulin resistance when assessed with surrogate markers of insulin sensitivity [254]. Recent studies investigating children prior to puberty have demonstrated a correlation between IMCL deposition, metabolically adverse visceral fat and markers of the metabolic syndrome [78]. Indirect assessment of visceral adiposity might be possible through WC measures.

Exercise training of skeletal muscle can improve Si without significant weight loss or change in body composition likely the result of increased expression of GLUT 4 proteins in the cell membrane [255 256]. Enhanced Si is evident in insulin resistant individuals after a single episode of training as insulin and exercise activate glucose uptake by different mechanisms. The hallmark of hepatic insulin resistance is the inability of insulin to inhibit gluconeogenesis with concomitant changes in lipid metabolism. Hepatocellular dysfunction occurs when lipid accumulates within the hepatocytes. This contributes to insulin resistance and in severe cases may lead to non-alcoholic fatty liver disease (NAFLD). Intra-abdominal, visceral fat rather than generalised obesity seems to contribute more to hepatic lipid accumulation. Lipid accumulation within the hepatocyte impairs suppression of hepatic glucose production by insulin, clinically this may manifest

as an elevated fasting glucose level. There is also an associated dyslipidaemia with increased very low-density lipoprotein cholesterol and reduced HDL-C from increased clearance. Interestingly, patients with insulin receptor mutations do not manifest the metabolic sequelae seen with IHCL despite the known correlation of insulin resistance and IHCL.

1.2.7 Insulin resistance and prediabetes

Prediabetes is an intermediate state of glucose intolerance whereby the glucose level is above the normal range but does not satisfy the criteria for T2DM. A single abnormal reading during formal testing is sufficient to make a diagnosis of prediabetes. The pre-diabetic state includes several entities including: impaired fasting glucose (IFG) and or/ impaired glucose tolerance (IGT) (Table 1.3). Individuals with prediabetes are at high risk of progressing to T2DM but may also revert to normal glucose tolerance [257].

IFG and IFG represent carbohydrate dysregulation and are due to different aetiology. Individuals with IFG have a high degree of liver insulin resistance and relatively normal muscle insulin sensitivity; conversely patients with IGT have significant contribution of muscle insulin resistance but demonstrate only mild hepatic insulin resistance. The differing pathophysiology may require therapeutic interventions that specifically target the site of greatest insulin resistance.

Table 1.3 Definition of glucose tolerance states

Categories of glucose tolerance	Normal	IFG	T2DM
Fasting plasma glucose mmol/L		5.6-6.9	≥7.0
2 hour plasma glucose mmol/L	<7.8	7.8-11.1	≥11.1
Haemoglobin A1c %	<5.7	5.7-6.4	≥6.5

Adapted from [258 259]
IFG (Impaired fasting glucose) T2DM (Type 2 Diabetes Mellitus)

1.2.8 Measurement of insulin resistance

Early targeted intervention of adolescents at high risk of insulin resistance is necessary to delay progression to T2DM. Therefore, reliable methods to quantify insulin resistance and reference standards are necessary in the paediatric population. To date, due to insufficient paediatric studies in this area and the variety and variability of tests, no such practical standard exists. Furthermore, there are no clear guidelines with regards to normal and abnormal results.

The euglycaemic-hyperinsulinaemic clamp is considered the gold standard to which other measures of insulin are compared [260]. Several validated derived proxy measures have been developed to estimate Si with variable correlation with clamp studies. The frequently sampled intravenous glucose tolerance test (FSIVGTT) with minimal modelling [261 262] MINIMOD analysis [263] is also considered a gold standard and shows high correlation with the gold standard [264-266].

Some relatively less invasive measures of insulin resistance include: fasting plasma insulin level, HOMA-IR, Quantitative Insulin Sensitivity Check Index (QUICKI), Whole Body Insulin Sensitivity Index (WBISI or Matsuda Index), fasting plasma Glucose-to-Insulin Ratio (G/I) [266]. These derived proxy measures have the advantage of being economical as

well as simple and easy to perform; this is in contrast with clamp and FSIVGTT studies, which are intensive, expensive and require specialised personnel. However, very few paediatric studies have been conducted to validate these derived measures [267-270] and consequently there is no consensus with regard to the optimal surrogate measures in this population. In this thesis, insulin sensitivity (Si) will be used when describing measures derived from these calculations.

1.2.8.1 Invasive measures of insulin sensitivity

Invasive measures of Si are expensive and time consuming and most often performed in research settings. Three such methods, the euglycaemic-hyperinsulinaemic clamp, frequent-sample IV glucose tolerance test and oral glucose tolerance test are discussed below.

a. Euglycaemic-hyperinsulinaemic clamp study

The euglycaemic-hyperinsulinaemic clamp is considered the gold standard technique for quantifying the degree of insulin resistance and determining Si. There are two variations of the glucose clamp: the hyperinsulinaemic-euglycaemic and hyperglycaemic clamp. The hyperinsulinaemic-euglycaemic clamp is used to measure peripheral (skeletal muscle) Si whilst the hyperglycaemic clamp assesses β cell function and insulin secretion [260]. During the hyperinsulinaemic-euglycaemic clamp, glucose disposal predominately occurs in skeletal muscle (~80-90%). A simultaneous intravenous infusion of insulin ensures that endogenous hepatic glucose production is suppressed, allowing calculation of peripheral Si that is predominately determined by skeletal muscle [271].

b. FSIVGTT (with Minimal model analysis MINIMOD)

The FSIVGTT is another invasive method [262] and via a minimal model analysis [263] produces a result which correlates well with the gold standard clamp study; the result reflects a mixture of hepatic and peripheral Si [261 262].

c. Oral glucose tolerance test

The oral glucose tolerance test (OGTT) can be used to diagnose diabetes when there is clinical suspicion of glucose impairment, particularly in the overweight adolescent with clinical signs of insulin resistance such as AN or in a female with features of PCOS. An adolescent may be referred for an OGTT when they show an elevated fasting glucose 5.5-6.9mmol/L or a random level suspicious of glucose intolerance 5.5-11.0mmol/L. The OGTT is affected by carbohydrate intake, fasting period and activity and is generally undertaken first thing in the morning after an overnight fast. Venesection occurs at the commencement of the test prior to the glucose load of 1.75g/kg (max 75g), bloods are taken 2 hours later; some protocols involve 30 minute sampling during the two-hour period. First phase insulin secretion and hepatic Si determine the rise in plasma glucose over the first hour of the test, whereas later decline in glucose is due to the peripheral Si of the muscle and second phase insulin secretion. Values of insulin and glucose obtained during the test can be used to calculate derived indices of Si, which have reasonable correlation with the clamp [254 268]. The fasting and 2-hour blood glucose levels are used for diagnosis of normal, pre-diabetes and frank diabetes.

During oral ingestion, 30-40% of glucose uptake occurs in the splanchnic circulation due to incomplete suppression of hepatic glucose production via this route. Therefore during the OGTT, 60-70% of the glucose is taken up by skeletal muscle tissue in the periphery. The fasting glucose measure gives an indication of hepatic glucose output. Values

obtained during the test and particularly the 2-hour levels are the combined result of peripheral glucose uptake and endogenous hepatic glucose production. Although there are no established reference ranges for the 1-hour level, there is increasing evidence that the value may have some clinical utility. A 1-hour level of 8.6mmol/L has been proposed as a cut-off to identify those who may be at risk of the development of T2DM and additional cardiovascular risk [272 273]. The results of an OGTT may vary within the same individual. There is a coefficient of variation of 8% and 20% respectively for the fasting and 2 hour levels respectively [274]. This high variation makes the test less than perfect. The OGTT allows for the measurement of two derived surrogate indices of Si: 1) the whole body insulin sensitivity index (WBISI) and the 2) insulin sensitivity index (ISI) [275]. The WBISI has been validated in both adults [276] and children [254] to show good correlation with the gold standard clamp technique with R=0.78.

d. Whole body insulin sensitivity index (WBISI)/ Matsuda Index

The whole body insulin sensitivity (Matsuda Index) is calculated with measures obtained during the oral glucose tolerance test and the correlation with the clamp in adult studies is approximately R=0.70, as it reflects a combination of hepatic and peripheral insulin resistance [276]. The WBISI has also been validated in the paediatric population and it shows similar correlation with clamp studies, R=0.78 [254]. Similar correlations have been shown in adult studies [276 277]. The oral glucose tolerance test is more acceptable to patients as there is less risk of complications and it is less labour intensive than the clamp technique. It is therefore a good option for clinical use and for screening individuals at high risk of dysglycaemia.

Due to the relative ease of performing an oral glucose tolerance test in our study venue we chose to use the Matsuda or WBISI index to determine the primary study outcome.

The results of this test would also identify as part of routine clinical care any individuals with T2DM who may require further definitive treatment. The WBISI is a commonly used validated proxy measure of the hyperinsulinaemic-euglycaemic clamp with high correlation [276] and excellent reproducibility on repeated measurement [278].

1.2.8.2 Less invasive measures derived from fasting blood samples

Less invasive measures of insulin resistance include the fasting plasma insulin level, HOMA-IR, Quantitative Insulin Sensitivity Check Index (QUICKI) and Fasting Plasma Glucose-to-Insulin Ratio (G/I) also known as the insulinogenic index [266]. These derived proxy measures have the advantage of being economical as well as simple and easy to perform. These surrogate measures have been found to have excellent correlation with the gold standard clamp studies in non-diabetic individuals [268]. Their ease of applicability in large populations allow for widespread use in epidemiological studies of insulin resistance. HOMA and QUICKI have been shown to better reflect hepatic insulin resistance [279].

Studies performed in adolescents at two different ages, 13 and 15 years, showed only modest correlation of these measures with the gold standard clamp derived M sensitivity, with values ranging from 0.48 (fasting insulin and FGIR)~0.54 (QUICKI), with similar correlations at age 15 years, with the exception of the FGIR which showed much lower correlation of 0.25 [270]. Experts have agreed that the fasting surrogate measures such as the HOMA-IR do not offer any additional benefit over the fasting insulin, which is highly correlated ~0.99 with HOMA-IR [280].

a. Fasting plasma insulin concentration

Fasting plasma insulin levels can be obtained after an overnight fast and high levels indicate biochemical insulin resistance. Although, this method is easy to perform and inexpensive, there are some limitations to its applicability in the clinical setting. A single fasting insulin level may vary considerably in an individual as it represents not only the degree of insulin resistance but also reflects pulsatile secretion from the pancreas and is participant to the pharmacokinetic processes of distribution and clearance; variation in any of these elements will alter the result [281]. The fasting insulin result is only reliable in individuals with normal glucose tolerance as those with glucose dysregulation have diminished ß cell production of insulin and will exhibit lower fasting levels due to the underlying pathology.

The fasting insulin level is subject to inter-assay variation and there is no standard reference range for children [282]. In addition, there is wide variation seen in the correlation between the fasting insulin level and clamp studies in children. Paediatric studies comparing the fasting insulin level to clamp derived Si results have shown a wide variation with correlations ranging from 0.42-0.91 [268 270]. Studies in non-diabetic adults have wide ranges with fasting insulin cut off levels ranging between 7.2mU/L [283]-18mU/L [284]. Further limiting the diagnostic use of a fasting insulin level is the lack of consensus regarding the cut off value for insulin resistance in the paediatric cohort. The American Heart Association (AHA) provides the following clinical guidelines for fasting plasma insulin levels which may prove useful for screening high risk individuals if there is a strong clinical suspicion: Normal <15 mU/L, borderline high 15-20 mU/L, high>20 mU/L [285]. Most studies to date in both children and adults utilise the fasting insulin or

HOMA-IR index as outcome measures. Ten and Maclaren suggested a fasting level >15 μU/mL or >75μU/mL at 2 hours was indicative of insulin resistance [286]. In contrast, a recent consensus statement examining childhood insulin resistance has discouraged routine clinical use of the fasting insulin level [280]. However, research suggests that the use of the fasting insulin measure may be still provide a reliable estimate in adults [128] and in early adolescent pubertal participants [287], independent of ethnicity. Despite the many limitations, the fasting insulin level may be useful in large population based research studies to evaluate biochemical insulin resistance in patients with normal glucose tolerance.

b. HOMA-IR

Given the limitation of a single fasting glucose measure particularly in patients with impairments in glucose tolerance, this measure adds a further dimension incorporating the fasting glucose level. The HOMA-IR (homeostasis model assessment of insulin resistance) is easily calculated from fasting measures of insulin and glucose [fasting insulin (μU/mL)] x [fasting glucose (mmol/L)]/22.5 [288]. It is a validated proxy for assessing insulin sensitivity from a single fasting blood glucose and insulin level and can be used in clinical settings, provided ß cell function is not severely impaired.

The HOMA-IR index correlates well with clamp derived glucose disposal [276 288] and Si derived from the FSIVGTT [289]. In adolescents this correlation was moderate with r=0.49~0.53, being higher in older and heavier children [270]. Highlighting the variability of research results, Gungor *et al* [268], showed higher correlation for measures derived from fasting insulin and glucose in their paediatric study, including 156 children from AA and Caucasian backgrounds, with r=0.91~0.92 for HOMA-IR, QUICKI and fasting insulin respectively. Higher Si is associated with a lower value, normal values between 1.21-1.45,

with levels between 2.61-2.89 in insulin resistant individuals [288]. However further studies have shown values up to 3.8 may in fact be normal in adults [290]. Several other surrogate measures of Si exist, however the HOMA-IR shows greater consistency in the evaluation of insulin resistance in adolescents [291].

Although is it widely accepted that obese and overweight adolescents are more likely to be insulin resistant, young people whose BMI lies in the upper normal range may also be at risk. A cross-sectional analysis in American children of varying BMI, showed a trend of increasing HOMA-IR as BMI progressed from the 75th centile (OR 4.277) to above the 95th centile (OR 17.907) [182]. Screening for children with BMI scores within the upper normal range may detect insulin resistance and provide new opportunities for early intervention in T2DM. In adolescents, a normal HOMA-IR does not exclude insulin resistance [292] and therefore an individual should proceed to further investigation in the setting of additional risk factors. Although a practical method, the calculation of the HOMA-IR is based on solitary basal measures of glucose and insulin and may not provide the best assessment of insulin resistance of obesity that may become more apparent under dynamic conditions of insulin stimulated secretion and peripheral glucose uptake and may not confer any advantage over the fasting insulin alone. HOMA-IR correlates highly with the fasting insulin level R=0.95 and the addition of the fasting glucose levels may add little discriminative power as they vary in a relatively tight range in comparison to the fasting insulin level.

c. QUICKI

The QUICKI method of determining insulin resistance also incorporates the fasting blood glucose and insulin measurements. It is considered a more accurate representation of insulin sensitivity when compared to HOMA-IR and fasting insulin. It is calculated

using the following formula: (1/[log[fasting insulin (μU/mL)]+log[fasting glucose (mg/dL)] [293 294]. It can be used in both non-diabetic and diabetic patients however it is less accurate in those who are insulin sensitive. Similar to adult studies, Uwaifo *et al* showed that in children the QUICKI index had better correlation with Si derived from the clamp studies with R=0.69[267]

1.2.9 Clinical insulin resistance

Routine clinical examination findings may allude to the presence of underlying insulin resistance. Irregular menstrual cycles in the female adolescent or general dermatological findings of AN are important discriminating features in the clinical assessment.

1.2.9.1 Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) can be defined by various criteria. The Rotterdam criteria, includes oligo-anovulation, clinical and/or biochemical hyper-androgenism and polycystic ovaries [295]. Obese adolescents are at risk of developing PCOS that may lead to infertility and increased cardiovascular risk. Most adolescent females with PCOS have features of the metabolic syndrome or insulin resistance. This syndrome is characterised by clinical and biochemical androgen excess, polycystic ovaries and menstrual irregularity. Insulin resistance is present in approximately 50% of young women with this disorder and the presence of PCOS is an indicator of heightened cardiovascular risk. Weight reduction and the use of insulin sensitizing agents such as metformin may improve ovulation by reducing androgen levels [296]. Metformin administered to premenarchal girls with early adrenarche and low birth weight delayed the onset of PCOS features with greater effectiveness when given at a younger age (8-12 years rather than 12-14 years of age) [297].

1.2.9.2 Acanthosis nigricans

Acanthosis nigricans (AN) is a clinical feature often present in overweight individuals with insulin resistance. AN develops in the skin flexures of the neck, axillae and groin and transforms the appearance of the skin with hyperpigmentation, hyperkeratosis and papillomatosis by causing epidermal growth. It has been proposed as a clinical marker of insulin resistance in obese children as it is associated with higher fasting insulin levels and measures of insulin resistance [298]. Acanthosis nigricans in adolescents appears to be independently associated with maternal gestational diabetes [299], non-Caucasian background, female gender, severe obesity [299 300 301]. The presence of AN in adolescents, may also indicate underlying features of the metabolic syndrome [302] and glucose dysregulation with a quarter of participants with evidence of dysglycaemia [303]. Whilst most studies suggest AN may discern the individual at high metabolic risk some suggest that the sign may be unreliable and state the degree of body fat and anthropometry measures (BMI SD score $\geq$ 3.0) be used instead to stratify risk and to target children for further testing [301].

1.2.10 Treatment of insulin resistance and prediabetes

Despite the increased rates of insulin resistance and prediabetes in the paediatric population, there are limited therapeutic options due to a paucity of clinical trials in this area. Most therapeutic strategies in high-risk adults have involved lifestyle change to effect weight loss [304 305].

Most children and adults who develop T2DM are obese. Furthermore, increased adiposity is responsible for over 50% of the variance of insulin sensitivity in children. Dietary modification can lead to weight reduction and improved Si. In adults, modest weight loss of 5-10% can have proven health benefits including beneficial effects on

cardiovascular risk and other components of the metabolic syndrome [306]. Pharmacological treatments are limited to the off label use of metformin although some research has been conducted in the use of weight loss medications. Exercise can improve Si without significant weight change by altering muscle sensitivity. The Look AHEAD study showed that an intensive lifestyle in adults had long term, sustained effects on weight loss, glycaemic control and cardiovascular risk factors after four years duration [307]. In the Diabetes Prevention Program (DPP), lifestyle intervention was superior to pharmacological treatment in preventing the progression to T2DM in adult patients with pre-existing evidence of dysglycaemia [304]. Medications such as metformin predominantly target hepatic insulin resistance, whereas weight loss may provide dual benefits at both the hepatic and skeletal level.

1.2.10.1 Dietary management of insulin resistance

Dietary guidelines in the paediatric population are limited to the traffic light guide, hypocaloric diet or healthy eating advice. Evaluation of individual dietary prescription in this cohort remains difficult as it is often undertaken as part of a lifestyle intervention incorporating an exercise component [308]. Compounding these difficulties is the fact that dietary modification in adolescents often leads to weight loss [309] [310] that may independently influence Si. The appropriate dietary prescription for prevention of T2DM is still being investigated in both adults and adolescents. An increased protein, low carbohydrate diet in conjunction with a structured exercise program proved more successful in producing weight and fat loss in obese women with insulin resistance after 10 weeks. These changes were accompanied by reductions in fasting glucose [311]. In contrast, a recent study by Garnett *et al* showed that energy restriction rather than macronutrient (protein and carbohydrate) variation led to improved Si in adolescents after 12 months [152].

Free fatty acids are implicated in the pathogenesis of insulin resistance and therefore altering the dietary fat content may influence insulin sensitivity. In a review of 41 studies conducted in adults, no clear relationship could be found between Si and quality of fat ingested, the negative findings and difficulty in interpretation being attributed to various methodology differences [312]. A recent systematic review showed that lipid profile could be improved in young people with dietary only intervention but that measures of glucose homeostasis including Si showed greater improvements with exercise [154].

Individually, high fibre and low glycaemic load diets result in weight loss and may improve Si in overweight adolescents [313] [314] [315]. A 6-week intervention of psyllium supplementation altered body fat composition and reduced LDL cholesterol but did not enhance insulin sensitivity or reduce BMI in obese males [316]. Given the interrelationship between BMI and insulin sensitivity, it is difficult to discern whether the change in insulin status is due to the diet intervention or the associated weight loss and certainly adult studies have shown variable results [314]. These studies demonstrate the inherent difficulties when assessing the optimal dietary prescription for obese adolescents with insulin resistance and pre-diabetes as many interventions are confounding by weight loss and difficulties with poor compliance, making the true effects of the diet difficult to elucidate.

1.2.10.2 Exercise and insulin resistance

Exercise is a non-pharmacological treatment to augment insulin sensitivity and can improve whole body insulin sensitivity in adults with T2DM [317]. A healthy lifestyle with attention to diet and incorporating regular physical activity is essential for weight management and good cardiovascular health. In adolescents, exercise has been shown to

improve insulin sensitivity [318-320], assist with weight reduction and has positive effects on cardio-respiratory parameters [321]. Unfortunately, adolescents have become more sedentary and this has contributed to the development of overweight issues. According to a United States Survey of school students, approximately 62% of young children did not engage in any after school structured physical activity [322].

The DPP found that in adults with IGT, exercise was superior when compared to metformin in reducing the progression to T2DM, with 58% reduction in lifestyle arm compared with 31% in the metformin group [323]. Obese insulin resistant adults with normal glucose tolerance demonstrated more efficient glucose disposal after 6 weeks of physical training with lower fasting insulin, improved hepatic glucose suppression and increased peripheral glucose uptake [324]. Physical activity is associated with greater glucose transport across the cell membrane resulting in enhanced skeletal and hepatic insulin sensitivity. Skeletal muscle contributes greatly to peripheral and whole body insulin sensitivity; therefore efficient glucose uptake at the cellular level will reduce progression to T2DM. Aerobic exercise studies have demonstrated physiological adaptations leading to improved muscle quality with activation of the insulin signalling cascade and glucose transport. Longer duration exercise interventions may result in altered body composition and fitness, both of which affect Si. Short-term studies, which don't influence the parameters above, therefore provide information with regard to the independent effect of exercise on gluco-regulation. Studies in adults with diabetes have shown that short duration of aerobic activity (ranging from a single bout to 10 days) led to improved whole body insulin sensitivity [317], with Winnick *et al* [325] demonstrating that these changes were due to improved peripheral skeletal muscle glucose uptake. These changes are thought to be due to increased insulin independent uptake in the muscle as well as increased GLUT 4 protein concentration which facilitates insulin mediated glucose

uptake [326]. Further studies have shown that exercise without significant change in weight or body composition can result in lower insulin levels in both adults [327] and children [328 329].

Most studies have focused on aerobic training of muscle characterised by low force, multiple repetitive contractions [321 330 331]. Improvements in Si with short term moderate intensity aerobic activity resulted in significantly enhanced direct measures of Si ranging from 32% [332]-37% [333], higher improvements of 59% may be seen in the absence of weight loss by increasing the intensity of training [331]. However, relatively little is known about the effects of resistance training involving a smaller number of muscle contractions with higher force development. There are few studies examining the effect of resistance training in the paediatric population. However, the limited literature suggests positive effects on body composition with reduced fat, increased lean tissue [334] and possibly greater enhancement in Si compared with aerobic exercise [335]. In healthy adults, a short duration strength-training program led to an attenuated insulin response during oral glucose tolerance, suggestive of improved Si, which correlated with a gain in lean tissue during the study [336]. Similarly, a cross-sectional study of over one thousand adolescents demonstrated that greater muscular strength is associated with Si [337].

Lee *et al* attempted to distinguish the effect of aerobic versus anaerobic exercise in the adolescent cohort and discovered that any exercise type (aerobic and resistance) led to decreased hepatic and visceral fat, however only resistance training was associated with a 27% improvement in Si after the 3 month intervention [338]. Yaspelkis *et al* showed in small animals that resistance and aerobic training led to similar qualitative changes with increased GLUT 4 proteins in the muscle, independent of the amount of lean tissue [256].

Obese adolescents with hyperinsulinaemia have decreased levels of physical fitness when compared to obese counterparts when matched for BMI [339]. Children with T2DM who have reduced Si demonstrated reduced $VO2_{peak}$ and early evidence of cardiovascular impairment [340]. Elevated insulin levels, may independently affect the cardiovascular system, causing fluid retention and raised blood pressure with increased heart rate due to increased sympathetic nervous activity. Insulin may suppress fatty acid oxidation and thereby might reduce the amount of energy substrates required for muscle activity [341]. Insulin causes vasodilation in skeletal muscle [342] [343] this physiologic action is compromised in patients with T2DM [344]and insulin resistance [235] and may explain reduced exercise ability in these individuals. Exercise leads to improved Si in children and adolescents, often without significant change in body composition [328] [330] [345]. Furthermore, van der Heijden *et al* showed that the enhanced Si could be attributed to increased peripheral uptake at the muscle and improved hepatic insulin sensitivity [331].

Cardiovascular fitness (CRF), rather than total body composition may better predict Si in children, providing evidence of qualitative changes at the muscle level and enhanced peripheral glucose uptake [346]. High level of CRF and muscular strength are associated with greater Si in children [347] and adults [348]. In post-pubertal females, the degree of physical fitness as measured by $V02_{max}$ (maximal oxygen consumption) predicted insulin sensitivity to a greater extent than the amount of body fat and BMI [349].

The role of unorthodox forms of resistance training, such as whole body vibration training (WBVT) on weight loss and Si is unclear and difficult to quantify due to a variety of training protocols [350]. Initial studies in small animals showed favourable effects with fat loss [351] that have been recently confirmed by others[352]. Untrained females who participated in WBVT had small increases in fat free mass (FFM) but no change in total

body fat or weight [353]. Studies in obese adults show that fat loss may occur in combination with resistance or aerobic training effects but may be minimal [354] with possible effects on BMI [355] and visceral adiposity [356]. A study in T2DM patients found significantly reduced anthropometry measures after 12 weeks WBVT compared with controls [357]. Weight loss may occur through increased lipolysis and reduced adipogenesis with sympathetic nervous system activation; gains in FFM or by increasing energy expenditure [358]. However, the effects may also be mediated through multiple interactions between the musculoskeletal, endocrine, nervous and circulatory systems.

Resistance training, involves higher forces but a small number of repetitive contractions. This training leads to changes in the physiological properties of the skeletal muscle by upregulating GLUT 4 receptors, activating and enhancing the insulin-signalling cascade, whereas in aerobic training, the oxidative capacity of the muscle is increased. This may improve the quality of the skeletal muscle with favourable effects on glucose metabolism, independent of changes in muscle bulk and mass [256]. This is pertinent given that skeletal muscle accounts for majority of insulin mediated peripheral glucose disposal.

Resistance training programs can lead to significant body composition changes in overweight children within short periods of time. Sgro *et al* showed a reduction in fat mass within an 8-week period that was sustained after cessation of the training program. Four months of training led to improved muscle performance with demonstrated significant change in strength and power, but no changes in body mass index or bone mineral content was detected [359]. After 10 weeks of resistance training, young male adolescents showed improved Si during an oral glucose tolerance with concomitant changes in lean tissue mass [336].

1.2.11 Pharmacological management of insulin resistance

a. Metformin

Metformin, a biguanide is the main pharmacological treatment of paediatric T2DM [360]. It is an anti-hyperglycaemic, insulin sensitising agent, whose effects may be mediated through increased peripheral glucose disposal and reduced hepatic gluconeogenesis and gastrointestinal absorption, whilst maintaining insulin levels low [361 362]. Metformin does not have any effect on insulin secretion or ß cell function. In obese adults with T2DM, metformin treatment is associated with favourable effects on glycaemic control, weight, appetite and lipid profile [363 364 365]. In addition, it has positive effects on gluco-regulation in at risk obese adults decreasing fasting glucose and insulin levels [363]. In adults, the use of metformin reduced progression from IGT to T2DM [323]. Adolescents and adult women with PCOS and insulin resistance, show enhanced Si and androgen profile after treatment with metformin [366].

The clinical indication of metformin has also been extended to "off label" use in obese paediatric patients with insulin resistance, often in conjunction with lifestyle prescription. A meta-analysis of three studies comparing an intervention of metformin with or without lifestyle change found favourable metabolic effects with decreased fasting insulin, lowered HOMA-IR and reduced BMI [367]. These findings were confirmed in another study in younger, severely obese children aged 6-12 years [368]. Others have failed to show change in weight but have demonstrated improved Si [369]. New data from the TODAY study [370] confirmed the therapeutic effect of metformin monotherapy and showed it to be successful in maintaining glycaemic control in 50% of adolescent patients with T2DM aged 10-17 years. Interestingly, the adjunctive use of rosiglitazone, a pioglitazone enhanced this effect on glycaemic control with no additional benefit of pharmacotherapy

and lifestyle measures over medication-based treatment alone. However, in adults, thiazolidinedione class of drugs are associated with a high risk of cardiovascular morbidity.

Metformin has been trialled in adolescents in a randomised placebo control trial with no significant difference in weight loss of the group compared to placebo, there was a greater BMI reduction in females suggesting gender differences in response [371]. In adults with IGT, metformin in conjunction with lifestyle measures can halt the progression to T2DM [323]. Studies in children have been conflicting. The addition of metformin to a very low calorie diet in obese adolescents can lead to greater weight and fat loss and enhanced measures of insulin sensitivity, compared to controls [372]. Freemark *et al* [373] showed a small decrease in BMI SD and reduced fasting glucose and insulin levels with 6 months of metformin treatment in a small group of obese adolescents; however the baseline BMI in the treatment group. There were no changes in Si as assessed by minimal model but fasting glucose to insulin, QUICKI and HOMA-IR did reduce slightly. Another study of 28 obese adolescents showed improved body composition and BMI but no change in insulin sensitivity [374]. In combination with a lifestyle program, some studies have shown some benefit with reduced BMI and enhanced insulin sensitivity [375] and others have shown no change in either parameter [371]. A systematic review showed a moderate effect of metformin treatment on BMI and insulin resistance measures. Only five trials met the inclusion criteria, however the meta-analysis showed that metformin reduced BMI by 1.42kg/m^2 and reduced HOMA-IR by 2.01, however the analysis involved short-term studies with small numbers from high-risk populations [376]. The most common side effects related to treatment with metformin appear to be mild and mainly due to gastrointestinal upset [368].

There are conflicting data with the regard to the use of metformin in adolescents with insulin resistance. Most studies have simultaneously incorporated a lifestyle program making it difficult to ascertain the beneficial effect of the pharmacological treatment. When used alone with placebo in obese adolescents, metformin assisted with weight loss and reduced fasting insulin levels [372] [373]. In addition to these effects, Srinivasan *et al* showed a positive effect on body composition but no change in insulin sensitivity [374]. The small body of literature would suggest that metformin might reduce progression to T2DM when used an adjunct to a lifestyle intervention due to its proven effects on fasting glucose and insulin. Clarson *et al* showed improved BMI in insulin resistant adolescents but no change in measures of insulin resistance, but improvements in lipid profile [377]

b. Other medications

Thiazolidinedione enhance insulin sensitivity by altering the distribution of adipose tissue from the visceral compartment to the subcutaneous compartment. They also reduced IHCL and IMCL and reduce ectopic storage of fat in theses organs. Sibutramine and orlistat are used in adults to assist with weight management. Studies in adolescents have shown significant change in weight loss in the short term particularly in combination with lifestyle change however, gastrointestinal side effects with orlistat and tachycardia with sibutramine may preclude the use of these agents in adolescents [378].

1.3 Whole Body Vibration Training

Whole body vibration training (WBVT), a novel form of resistance training has been mostly researched in adult populations. It can improve muscle strength [379-381], power [382] and flexibility [383-385]producing similar [380] or superior results compared to resistance exercise but in shorter period of time[386] [387]. The effects of WBVT on bone morphology is

unclear with positive results in small animals [388 389] and postmenopausal women at risk of osteoporosis [390] which might be mediated through a decrease in bone resorption, [391] enhanced muscle bone interaction through increase muscle amount and strength [381 392] or direct effects [358 388]. Recent reviews however, suggest a weak effect of WBVT on bone or muscle parameters [393] with some demonstrating no effects [394 395] this may be attributed to the variability in study protocols [396].

Trained athletes may benefit from the strength effects of WBVT [397 398] and there is growing evidence that untrained adults may also be able to boost muscle performance [380] particularly when combined with other resistance activities [379 399]; longer duration trials may produce greater results [400].

The low impact nature of this training may provide a non-pharmacological way of improving both muscle and bone parameters in vulnerable populations. The amplitude (A), frequency (f) and acceleration (a) are three characteristics of the vibration that can be modified to suit different patient groups. Although, assessing the effectiveness of WBVT exercise in research trials can be difficult due to the variability in study protocols [350 393 401].

The WBVT platform delivers sinusoidal high frequency low amplitude vertical vibrations. The platforms are capable of delivering vibration via two main methods (Figure 1.5), including a side to side alternation as used in this study or vertical vibration, which is thought to be less physiological and results in greater transmission of vibration to the upper body and head [402]. If the stimulus is applied to the lower limb (i.e. person stands on the platform for training) then there is regional activation of muscles of the lower limb and trunk, particularly leg extensors to dampen the stimulus [403 404]. The plantar flexor muscles may be strengthened more than the proximally located muscles due to the

proximity to the vibration stimulus. Muscle activation occurs unconsciously through the tonic vibration reflex in combination with the stretch reflex with some modulation from the higher centres. The frequency generally ranges from 15-60Hz and amplitude (1-15mm), there are a variety of training protocols. The optimal duration of intervention is still participant to speculation. However, most studies describe times ranging from 1-30 minutes [393] [405].

The neuromuscular effects of WBVT remain unclear. In animal studies, the vibration stimulus activates the tonic vibration reflex mediated by the golgi tendon organs and Ib afferents in the muscle spindle. In humans the same stimulus may inhibit the H reflex [406] [407] or stimulate the tonic vibration and muscle reflexes [408] [409]. Using animal studies, Matthews proposed that WBVT elicited the well-known stretch reflex (Figure 1.6) and that the vibration led to stretch of the 1a afferents of the muscle spindle [410].

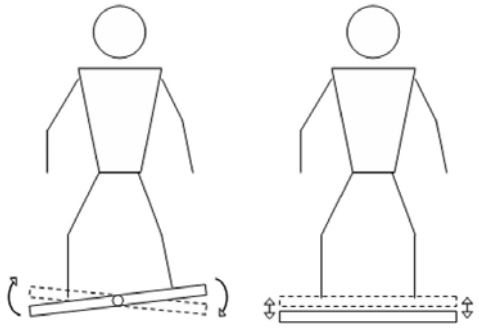
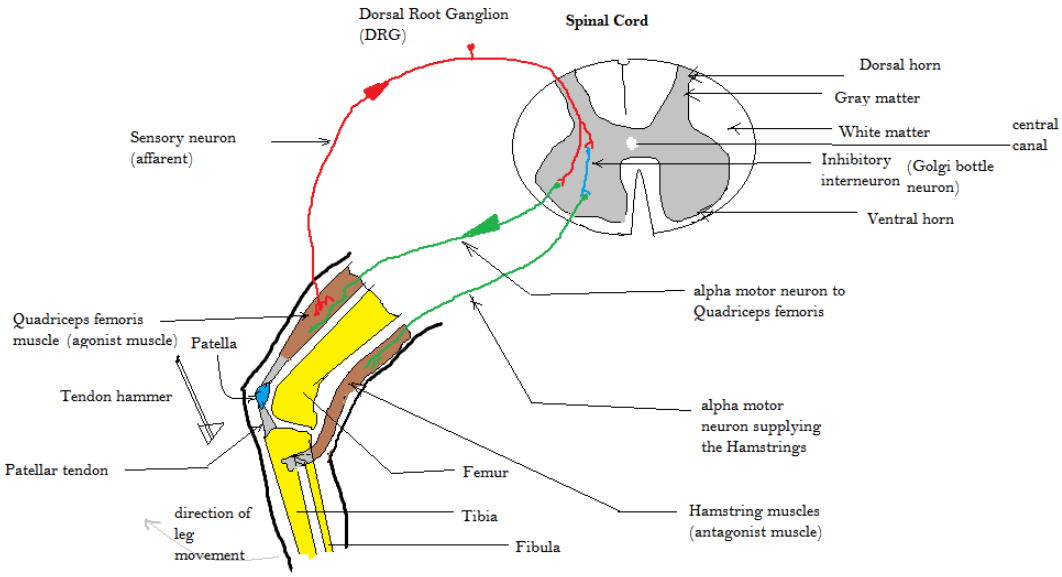


Figure 1.5 Methods of whole body vibration training (WBVT)

The two different methods of delivering whole body vibration training are shown above.

Side to side alternating as used in Galileo™ 2000 (left) and vertical vibration transmission (right) adapted from [402]



taken from [411]

Figure 1.6 Stretch reflex arc

The stimulus delivered by the tendon hammer causes stretch in the muscle spindle which leads to increased activity in Ia afferents and an increase in the activity of α motor neurons that innervate the same muscle. Ia afferents also excite the motor neurons that innervate synergistic muscles (Quadriceps femoris) and inhibit the motor neurons that innervate antagonists (Hamstrings).

Several studies have shown that this is also applicable to humans and have used electromyography to investigate the effect of an applied vibration to muscle and have found that as well as producing a motion artefact [412], vibration appears to produce an EMG signal similar to that evoked by passive stretching [413]. It is postulated that the early changes in muscle force are due to heightened excitability of muscle spindle Ia afferents, which activates motor neurons and results in muscle contraction through the tonic vibration reflex as well an increased sensitivity of the stretch reflex (Figure 1.7) [353 414]. WBVT may modulate spinal cord neuro-excitability, through inhibition of the H reflex as shown in patients with spinal cord injury and without voluntary muscle function [406]. Longer periods of training may stimulate hormones such as growth hormone and

testosterone that facilitate muscle development [415]. Similar to resistance training effects, muscle hypertrophy could also occur as a local adaptation.

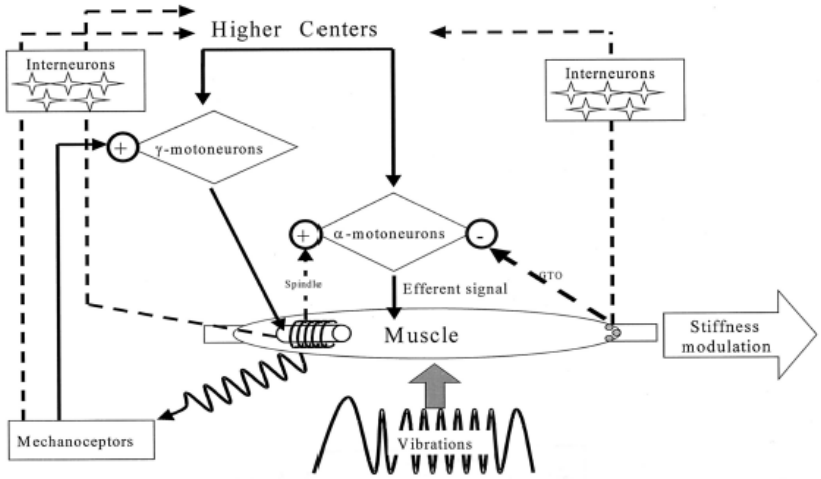


Figure 1.7 Effect of whole body vibration on spinal reflex and higher centres

The quick change in muscle length and the joint rotation caused by vibration trigger both alpha and gamma motor neurons to fire to module muscle stiffness. Higher centres are also involved via a long loop. Taken from [387]

WBVT has a good safety profile and has been utilised in studies in children with physical disability. However, most studies have investigated the musculoskeletal effects of the vibration exercise and have not carefully examined the possible effects on other organ systems. Isolated case reports of side effects have included two reports of intraocular lens dislocation in elderly adults [416] and one possible case of vitreous haemorrhage [417]. A young athlete with asymptomatic nephrolithiasis developed significant morbidity following a bout of vibration [418].

1.3.1 Bone effects

Exercise can improve bone mineral density and bone health in children [419], however compliance with exercise regimes may be difficult in this time poor society. Exercise provides a known benefit on peak bone mass in young people and may assist in reducing pathological fractures from osteoporosis in later life [420]. Bone remodelling occurs in

response to strain which leads to slight bone deformation [421]. WBVT can deliver a low mechanical stimulus to alter bone morphometry and bone strength with significantly less strain on the bone than that delivered by physical exercise and possibly within shorter periods of time. The effects were non-dose dependent and not uniform through the skeletal with greatest gains seen in the proximal tibial metaphyses and no effect in the femur or vertebra of the animals [388]. Aeronautical space programs have used WBVT to limit bone loss associated with weightlessness based on the results of studies in volunteers simulating similar conditions. One study conducted in healthy male volunteers undertaking 56 days of bed rest resulted in preserved muscle size and function and reduced bone loss compared to a control group [422].

Initial studies examining the use of WBVT on the skeleton were performed in small animals. The most striking results were seen in the hind limb bones of sheep demonstrated a 34.2% increase in proximal femur trabecular density compared to a control group. Similar results with >30% improvement in trabecular bone volume was seen in the tibia of adult mice subjected to a series of increasing accelerations [388]. Further animal studies by Xie *et al* [423], showed that brief periods of WBVT could improve bone formation and reduce bone resorption by approximately 30%, whilst still maintaining bone quality in the growing skeleton, suggesting this may have a useful role in reducing incidence of osteoporosis by assisting peak bone formation in adolescents and young adults. Mice subjected to conditions of unloading that simulated the weightlessness experienced in space or paralysis maintained remodelling parameters in the skeleton similar to control rats [424].

Several RCTs have examined the effect of WBVT in populations at risk of bone disease. In post-menopausal females randomised to WBVT, resistance or control groups, WBVT

was the only intervention to significantly improve BMD at the hip by 0.93%, $p<0.05$, after the 6 months intervention. Interestingly, in the same study, isometric and dynamic muscle strength were improved by 15% and 16%, $p<0.01$ respectively [425]. A double blind, placebo controlled study in a similar population showed a similar trend with mild improvements in femoral neck BMD 2.17%, $p=0.06$, in highly compliant participants followed prospectively for a year [390]. A year of WBVT for just 10 minutes per day led to mild improvements in femoral cortical and vertebral trabecular bone after a year of WBVT in young women with low BMD [426].

Studies in children have been limited to small pilots, however Ward *et al* [427], confirmed improvements in bone quality in children with physical disability with an increase of 6.3% in proximal tibia volumetric trabecular bone mineral density after 6 months compared to a reduction in those children using a placebo device, despite compliance of 44% to the exercise protocol [427]. A recent RCT study examining 26 adolescents with and without Down syndrome, showed 20 weeks of WBVT increased BMC and BMD in both groups but to larger extent in those without Down Syndrome [428]. A 10-week study in overweight Latino males[429], showed that increases in BMC and BMD might be due to decreased bone resorption. Regional changes in vBMD and areal BMD were demonstrated in healthy children with forearm loading after 12 weeks WBVT [430]. A 6-month study in children with cerebral palsy showed cortical bone parameters could be improved[431]. In contrast, another 6 month pilot study of children with cerebral palsy did not show any positive change in bone mineral density [432].

1.3.2 Muscle effects

WBVT incorporated into paediatric physiotherapy programs may result in functional improvements in children with cerebral palsy [432] [433] and other children with reduced

mobility [434]. In children with this disability, Stark *et al* [433], showed improvements in bone mineral density, bone mineral content and muscle mass after undertaking 6 months of therapy including 3 months of home based WBVT with the Galileo® platform. Similarly brief periods of WBVT led to improved muscle force and tilt table tolerance in recently immobilised children with fractures due to osteogenesis imperfecta [435]. A recent study in children with Down syndrome demonstrated a trend to increased lean tissue mass (LTM) after 20 weeks compared with a control arm[436].

In postmenopausal women a program of 24 weeks of WBVT led to greater increases in the height of countermovement jump compared to resistance training and controls with concomitant enhancements in isometric and dynamic knee extensor muscle strength [437]. Young untrained female participants involved in a 6 month WBVT intervention demonstrated similar strength gains to women who undertook a mixed aerobic and resistance training program [353]. Blottner *et al* demonstrated preservation of isometric plantar flexion force, likely due to increased soleus muscle fibre size in young males subjected to 56 days of bed rest. In postmenopausal women postural balance, isometric strength (15%) and dynamic strength (16.5%) were seen after 6 months of WBV training compared with a control group [425].

Muscle efficiency with improvements in muscle force during jump testing has been shown in adults with cystic fibrosis [438]. Peak average power during squat jump can be increased by 6.8% and 7.3% in trained and untrained participants after a single bout of very high, 50Hz vibration suggesting that this may be an alternative to traditional methods of power training for elite athletes [439]. Chronic WBVT can improve muscle strength when compared to a passive control group however, in a systematic review

Nordlund showed that in 4 out of 5 studies, WBVT proved no superior benefit when compared to controls performing similar exercise [401].

In addition to muscle effects, it has been suggested that neuromuscular efficiency is improved with this form of training particularly through enhanced and improved coordination of motor units [440] [415]. However detrimental effects on nerve conduction times have been observed in monkeys with variation of frequency of vibration [441]. These positive results in the children with physical disability suggest that WBVT may have a clinical role in physiotherapy regimes and is safe for use in the vulnerable group. Taken together, these studies suggest that functional improvements in trained and untrained individuals and attenuation of muscle atrophy in conditions of disuse such as prolonged immobilisation.

1.3.3 Circulation effects

Reduced tissue perfusion at high frequency of vibration particularly with hand held devices is a well described occupational hazard known as the hand arm vibration syndrome [442]. Long term occupational exposure to high frequency vibration may lead to vertebral morbidity and neurological side effects [442]. Whole body vibration training protocols adopted in human research trials have utilised frequencies much lower than those delivered in the occupational setting. The nature of the vibration is also different being delivered as sinusoidal rather than vertically. It appears that the lower frequency and shorter duration vibration may be beneficial and appears to enhance local skeletal muscle blood flow as demonstrated in Doppler flow studies of the popliteal artery at 26Hz [443]. In addition to the effects on arterial flow, peripheral blood circulation is enhanced by improved venous drainage and lymphatic flow [444].

1.3.4 Metabolic and body composition effects of WBVT

WBVT is a form of efficient resistance exercise, which has been shown to increase muscle blood flow and mass. The vibration platforms have often been marketed for their weight loss properties. Some recent studies conducted in adults demonstrate small amounts of fat loss[355] and anthropometry change[355 445]. There are some studies, which show increased energy expenditure, carbohydrate and fat oxidation and well as increased oxygen consumption [446]. These physiological changes may alter body composition and may influence metabolism, particularly glucose homeostasis. Studies conducted in patients with T2DM have shown improvement in HbA1c[447] and adiposity [357]. It is important to note that the study by Lee et al [447] was conducted over a 6-week period and therefore the stated difference in HbA1c may not be accurate given that HbA1c has a recommended measurement interval of 3 months due to the life span to the red blood cell.

Murine studies have demonstrated positive effects on body composition with moderate decreases of 34.2% in torso fat. Studies in mice have indicated low magnitude mechanical stimulus in the form of WBVT may reduce differentiation of mesenchymal stem cells into the adipocyte lineage by 22%; with a trend to improved glucose tolerance and improvement in metabolic profile indicators such as free fatty acids and triglycerides [351]. Untrained females showed slight gains in lean tissue mass after twenty-four weeks of WBV (35-40hZ, 2.5-5.0mm) however body fat and weight was not altered [353]. A randomised control trial in adults showed that WBVT in conjunction with a diet intervention produced sustained weight loss of 5% after 12 months, with a greater loss of VAT compared to an aerobic fitness intervention [448]. A review of the potential weight loss properties of this exercise showed minimal change to body composition but difficulties with comparison due to the variety of protocols [350].

Further studies are required to confirm this result, however, if WBVT does lead to greater VAT weight loss it may translate into positive metabolic outcomes. Therefore, it appears that energy expenditure may be positively affected by WBVT on its own or in conjunction with an exercise regime. There are limited studies in both animals and humans that examine the effects of WBVT on body composition. To date are no studies in overweight insulin resistant children which examine body composition and insulin sensitivity change.

1.3.5 Hormonal effects

Hormones may mediate some of the positive effects of WBVT on muscle and bone. WBVT may alter the secretion of hormones. In addition to the direct mechanical effects of WBVT on the skeletal muscle, several authors have discovered that several hormones may fluctuate about this form of exercise; some of these hormones influence osteoblastic and osteoclastic activity. Growth hormone, insulin like growth factor 1 (IGF-1), cortisol and testosterone all influence bone cell activity. Bosco *et al* [415], found that 10 repetitions of WBVT of 1 min duration in young males resulted in increased plasma testosterone and growth hormone levels with decreased levels of cortisol. Di Loreto showed that a similar regime in a similar aged cohort resulted in additional findings of plasma glucose level at 30 minutes and sympathetic stimulation with increased secretion of noradrenaline but unlike the previous study did not find any change in GH, IGF-1 or cortisol or adrenaline levels [449]. They concluded that due to the lack of effect on the endocrine system, that this type of exercise would not ameliorate fat mass in obese participants.

There is little paediatric evidence as to the optimum duration and frequency of WBVT. Effects on metabolic parameters have been shown after 6 weeks in adults however most studies indicate that a longer duration is required for change to muscle and bone

parameters. We chose a 3-month intermediate period due to the inclusion of participants obese adolescents) who generally display high attrition rates during research interventions of extended duration.

1.4 Bone as an endocrine organ

The role of bone in the regulation of glucose homeostasis remains unclear. A close endocrine interaction between bone and glucose metabolism can already be seen through insulin's anabolic effects on bone, mediated by insulin like growth factor (IGF-1)[450]. Emerging evidence investigating bone-derived proteins and 25-hydroxyvitamin D (25OHD) have shown a possible reciprocal relationship between bone and energy metabolism, suggesting that bone may function as an endocrine organ [451].

1.4.1 Insulin sensitivity and bone

Studies in small animals have shown a link between bone and energy metabolism. Leptin derived from adipose tissue and insulin via IGF-1 can independently regulate bone. Osteocalcin (OCN) released by the osteoblast can increase insulin secretion via a direct effect on the pancreatic beta cell [452]. Osteocalcin is derived from the osteoblast and is involved in bone formation. Women with gestational diabetes, a state associated with insulin resistance and glycaemic stress have elevated levels of OCN during pregnancy, which return to nondiabetic levels 3 months after the pregnancy. Observational cohort studies in humans have confirmed a similar relationship. Total OCN has been related to the metabolic syndrome in adults. Osteocalcin undergoes post-translational modification and results in a carboxylated and undercarboxylated form [453]. The ratio between undercarboxylated and carboxylated OCN shows a strong association to insulin sensitivity measures in children. It is thought that the undercarboxylated form plays a

direct role in mediating insulin sensitivity by affecting adiponectin, which has insulin sensitising properties [454].

1.4.2 Vitamin D

Vitamin D is measured as 25-hydroxyvitamin D (25OHD) in the plasma. Vitamin D plays an important role in bone health and as a regulator of calcium homeostasis. Plasma levels are influenced by many factors including: ethnicity, dietary intake and sun exposure. Markers of increased adiposity including elevated BMI are also associated with hypovitaminosis D in both adolescents and adults [455]. Vitamin D is fat soluble, and is sequestered in adipose tissue that may explain the inverse association between fat and low circulating levels.

Recent studies have also focussed on the possible extra-skeletal effects of 25OHD including a putative role in glucose homeostasis. The optimal 25OHD levels for extra-skeletal benefit are unknown. Low vitamin D levels in adults has been associated with insulin resistance [456], lower ß cell function and presence of T2DM [456 457]. Independent of adiposity, adolescents with low 25OHD levels were found to have higher HBA1c and lower insulin sensitivity as measured by QUICKI [458]. An inverse association has been found between Vitamin D and insulin sensitivity as assessed by clamp studies [459]. However, Rajakumar *et al* [460] showed no relationship of serum 25OHD to insulin sensitivity as measured by clamp in a cross sectional of youth with normal glucose tolerance. No link between 25OHD deficiency and cardiovascular and metabolic disease was shown in a recent umbrella review of 137 clinical outcomes [461]. It is possible that low vitamin D levels may be a marker of poor underlying health or obesity acting as

confounders in observational research studies. Interventional RCTs in children failed to show that Vitamin D supplementation improved cardio-metabolic parameters [462].

Insulin resistance and obesity are pro-inflammatory states as indicated by the elevated hs-CRP levels, a putative reason for the observed relationship between hypovitaminosis D and glucose and cardio metabolic outcomes may be related to this inflammatory process given that macrophages, a key component of this cascade have receptors for 1,25 Vitamin D [463-465].

Recent consensus suggest a 25OHD level >50nmol/L to prevent childhood nutritional rickets [466]. The therapeutic target to optimise skeletal and extra skeletal manifestations remains unclear; however in North America a level above 50nmol/L is considered a therapeutic target [467 468].

1.4.3 Osteocalcin

Osteocalcin (OCN) is protein hormone formed by osteoblasts in bone. OCN is carboxylated with glutamic acid during post-translational modification to Gla-OCN. Decarboxylation by activated osteoclasts results in under-carboxylated OCN Glu-OCN. It is thought that the metabolic effects of osteocalcin are mediated by the undercarboxylated fraction. OCN may modulate energy balance in several ways including proliferation of ß cells and stimulation of insulin secretion possibly through increased adiponectin expression in fat cells. Animal studies showed that OCN knockout mice had decreased ß cell proliferation and glucose intolerance with insulin resistance [451]. Further studies in humans have not been equally as convincing. In older adults, indirect measures of insulin sensitivity such as HOMA-IR have been associated with higher total OCN. Adults with elevated BMI have lower under carboxylated OCN. There is limited data in

children. A study of young adults using clamp derived measures of Si showed a significant association with total and carboxylated OCN with measures of adiposity including BMI and SBP but borderline relationship to insulin sensitivity, after correcting for BMI [469].

1.5 Aims and objectives

Childhood obesity and insulin resistance is increasing and despite further knowledge in this field, there are many questions to be answered regarding the most appropriate management for the obese insulin adolescent. The study was conducted to determine the efficacy and acceptability of WBVT, a novel resistance based exercise intervention in association with a standard lifestyle prescription. By addressing the following aims, we will provide further knowledge to healthcare providers in the area of paediatric obesity and insulin resistance about the efficacy of lifestyle programs and whether novel types of resistance exercise are suitable for adolescents.

Primary aim

To explore whether WBVT could improve insulin sensitivity in obese adolescents with clinical insulin resistance.

Hypothesis

We employed a randomised controlled trial designed to test the hypothesis that three months of whole body vibration training in addition to a prescribed diet and exercise intervention would enhance the effect of the lifestyle interventions by improving insulin sensitivity and metabolic profile in overweight adolescents with hyperinsulinaemia. We speculated that WBVT would lead to an increase in fat free mass (FFM) that would increase non-insulin mediated peripheral glucose uptake. The intervention effect of

WBVT is discussed in Chapter 4 – "Does Whole Body Vibration Training improve insulin sensitivity in overweight adolescents?"

Secondary aims

1. Provide further information about recruitment patterns to an obesity intervention trial and describe any noticeable differences.
2. Highlight clinical examination and ethnographic features common to adolescents with insulin resistance in Greater Western Sydney (GWS) and determine the presence of metabolic co-morbidities in the participants and their relatives.
3. Determine any anthropometric similarities seen in adolescents with insulin resistance and to examine any gender differences.
4. Review any modifiable dietary and lifestyle features present in the obese adolescent.
5. To examine correlations and interrelations between baseline biochemical metabolic profile indicators and clinical examination features.
6. To provide a cross-sectional snapshot of the bone health of obese, insulin resistant adolescents in a multi-ethnic demographic using clinical densitometry and pQCT.
7. Evaluate relationships between bone parameters, bone turnover markers, Vitamin D, anthropometry and measures of insulin sensitivity.
8. Determine the cardiorespiratory profile of the obese insulin resistant adolescent and discover any intervention effect.
9. Examine possible limitations to exercise by using Mechanography, a functional assessment tool for muscle strength and power.

Chapter 3 – "What are the metabolic characteristics of obese adolescents living in Greater Western Sydney?" study addresses aims 1-5. Chapter 5 "Does obesity or insulin

resistance affect musculoskeletal health?" further explores aims 6-7. Aims 8-9 are addressed in Chapter 6 "Are there limitations to exercise in the obese insulin resistant adolescent?"

CHAPTER 2 METHODS

2.1 Overview

This thesis is centred around the VIBRATE study, a randomised controlled trial (RCT), designed primarily to investigate the effect of three months of whole body vibration training (WBVT) in addition to a prescribed diet and exercise intervention on insulin sensitivity in overweight adolescents with hyperinsulinaemia. We hypothesised that WBVT, a unique form of resistance exercise would enhance insulin sensitivity by improving muscle mass, thereby assisting peripheral glucose uptake to a greater degree than lifestyle intervention alone. In addition, secondary outcome measures explored included: body composition change (fat, muscle and bone); muscle performance (power and force); aerobic fitness and metabolic profile indicators. The participants were prescribed the same lifestyle intervention (including a diet and exercise component) with half of the participants being randomised to the WBVT intervention at the commencement of the study. The trial was registered with the Australian New Zealand Clinical Trials Registry (ANZCTR) ACTRN12608000509369.

Ethics approval

The study was conducted at The Children's Hospital at Westmead (CHW) and received local Ethics Committee approval (approval number 07/CHW/20) prior to being conducted. The parents and/or legal guardians gave written informed consent and the young people gave their assent to participate in this research study.

2.2 Outcome measures

i. Primary

The primary outcome of the study was change in insulin sensitivity (Si) during the 3-month trial. This was estimated using the validated proxy measure of whole body insulin

sensitivity index (WBISI) according to the formula: 10000/√[(fasting insulin x fasting glucose) x (mean 2hr glucose x mean 2hr insulin)] [254 276] from measures derived from an oral glucose tolerance test conducted at baseline and at 3 months. Although conducted over a short time frame of 3 months, it was appreciated that potential confounding variables may have included: changes in adiposity, pubertal status, physical fitness and activity levels and adherence to WBVT.

ii. Secondary

Secondary outcome measures examined the following changes over 3 months: body composition (anthropometry, BMI, WtHR); musculoskeletal parameters derived from DXA and pQCT; born turnover markers (urine deoxypyridinoline cross links (DPD), Procollagen type 1 N propeptide (P1NP), Osteocalcin (OCN), C-terminal telopeptide of type 1 collagen (CTX)); metabolic profile indicators (blood pressure, hs-CRP, total cholesterol, HDL-cholesterol and triglycerides); physical fitness measures (VO_{2peak} and exercise time) and single and two leg jump force and power derived from Leonardo® Mechanography.

2.3 Role of the candidate

The candidate designed the study with guidance from the co-investigators, submitted four successful grant applications and obtained ethics clearance prior to implementing the trial at CHW. This involved participant recruitment, co-ordination of clinical testing, performing the clinical evaluation and Mechanography, being trained in various techniques including PQCT (by Ms Julie Briody, Department of Nuclear Medicine, The Children's Hospital at Westmead) and use of the Galileo® Home vibration platform and Leonardo Mechanograph® GRFP, by a representative from Novotec Medical GmbH, interpretation and analysis of data with feedback to the participants, their families and referring doctors at the conclusion of the trial. Professor Christopher Cowell,

A/Professor Craig Munns and Professor Louise Baur, provided academic supervision to the candidate, during the conduct of the trial.

2.4 Participants

i. Inclusion criteria

Participants recruited to the VIBRATE study were adolescents aged 10 to 18 years who were referred to the Endocrinology and Weight Management clinics at CHW. Potential candidates were overweight or obese as defined by the International Obesity Task Force (IOTF)[60] with evidence of clinical insulin resistance with acanthosis nigricans (AN) [470], and/ or raised fasting insulin levels >120pmol/L (20mU/L) but <300pmol/L (50mU/L) [the lower cut-off was based on the 90th centile derived from local CHW endocrinology clinic data of insulin resistant adolescents] [471]. In November 2008, the lower cut off was revised to 90pmol/L (15mU/L) in line with quoted paediatric reference ranges for hyperinsulinaemia suggested by the American Heart Association (AHA)[285]. Participants with evidence of pre diabetes as defined by the American Diabetes Association (ADA)[472] with fasting blood glucose: 5.6-6.9mmol/L and/ or 2 hour post load glucose level 7.8-11.1mmol/L were also considered for participation to achieve recruitment targets.

ii. Exclusion criteria

The following excluded the participant from the study:

- Type 1 or Type 2 diabetes
- Secondary causes of obesity
- Weight greater than 120kg (due to technical issues with DXA machine and weight threshold of the Galileo® Home vibration platform)
- Psychiatric disturbance or significant medical illness
- Inability to take part in physical activity

- Taking weight loss medications, insulin sensitisers or medications known to cause and/or affect weight gain
- Pregnancy

iii. Sample size

The sample size calculation was based on previous analysis of whole body insulin sensitivity obtained from local clinic data at CHW [471]. We assumed a clinical significant difference in WBISI of 0.6 SD between each treatment arm during the trial. In order to have an 80% chance of detecting a significant difference at the two-sided 5% level, a total of 17 participants would be required in each arm. Initial sample size of 42, accounted for a dropout rate of 20%, based on previous obesity studies [personal communication Prof L Baur]. This figure is consistent with local data that had demonstrated dropout rates of 12-18% in children participating in paediatric obesity trials [147]. After review by the local Ethics Committee, a 40% drop out rate was suggested which increased the total number of participants to 48 (24 adolescents in each arm of the study). The actual dropout rate of 2.3% was less than anticipated and therefore we were given permission to close the study early when 43 participants had been enrolled.

iv. Randomisation

Treatment allocation to the intervention and standard arm of the trial was performed centrally by minimisation [473] using computer-based software, MINIM [474]. Minimisation ensured that prognostic factors were continually balanced between the study groups. The tally of participants was updated during the study to ensure balanced allocation. To account for potential confounding influences, participants were randomised into treatment groups stratified by gender (male or female) and pubertal status (pre pubertal Tanner stage I-II and post pubertal Tanner stage III-V) [475]. A Consolidated Standards of Reporting Trials (CONSORT) flowchart for the VIBRATE study is shown in Figure 2.1.

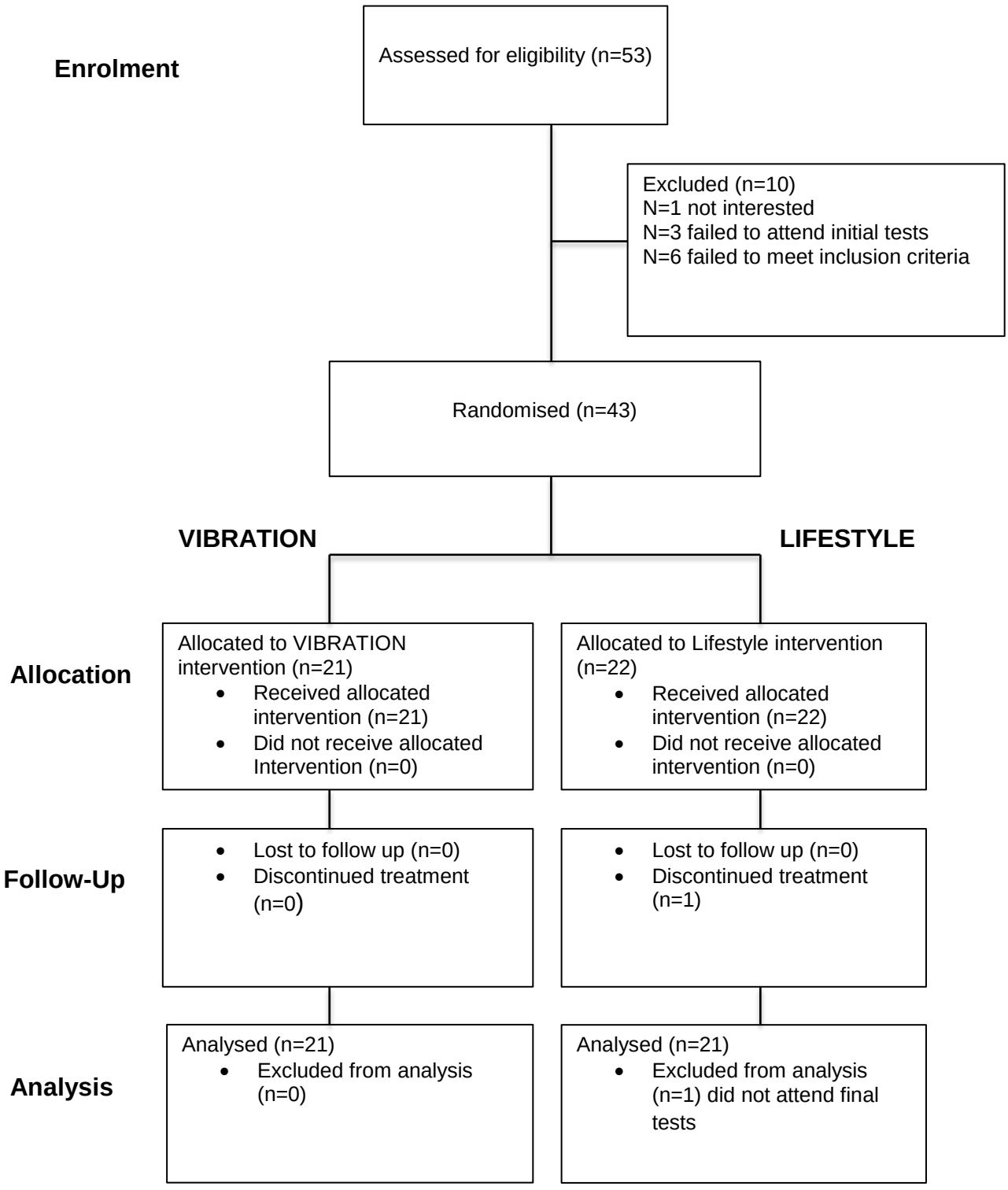


Figure 2.1 CONSORT diagram for the VIBRATE study

vi. Blinding

The candidate was not blinded to the allocated intervention to allow for adverse event reporting. Assessors of all primary and secondary outcome measures were blinded to the treatment allocated including: the two clinical nurse consultants who assisted with the oral glucose tolerance tests, anthropometry and blood pressure measurements; the nuclear medicine scientists who performed the dual energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT); the exercise physiologist and sports medicine physician at CHISM (Children's Hospital Institute of Sports Medicine) and person assisting with the Leonardo Mechanography™ tests.

vii. Statistical analysis

Intent to treat analysis was performed. All data shown are expressed as mean ± SD or have been converted to percentages. Statistical analysis was performed with SPSS software version 18.0 (SPSS, Chicago, IL). If the data showed normal distribution, independent samples Student t tests were used to determine differences between the groups. Non-normally distributed data was log transformed to normality; if this was not possible then a Mann Whitney test was conducted. Individual changes over the 3-month period were analysed with paired t tests. Bivariate correlations were performed to examine strength and direction of associations between two variables with Pearson's correlation coefficient reported for normally distributed variables and Spearman's rho reported for nonparametric analysis. Scatter plots were created and provided with the regression line. Partial correlations were performed as above controlling to control for the effect of other contributory variables. To study the linear relationship between a dependent variable and several independent variables a multivariate linear regression analysis was performed with potentially relevant independent variables.

2.5 Recruitment

The study was advertised through multiple methods including: word of mouth at weekly CHW endocrinology departmental meetings; via a generic paragraph developed for inclusion in electronic bulletins such as The Bandaged Bear Bulletin (CHW staff weekly email hospital communiqué) and Paediatric Pot-Pourri (Royal Australasian College of Paediatricians weekly e-bulletin); through small flyers placed in the waiting area of the Bandaged Bear Outpatients clinic at CHW and by letters to local General Practitioners (GPs); postal recruitment however, was not required (Appendix A). Potential participants were referred to the study by the treating clinician after fulfilling the inclusion criteria. Upon referral, the candidate met/ phoned the family to gauge interest. A written patient information sheet (Appendix B) was provided at first contact or sent in the mail after telephone contact. A follow-up phone call a week later was made to discuss concerns and to confirm interest and availability for the recruitment interview.

i. Recruitment interview

At the first visit, the candidate obtained written informed consent/ assent and performed a full physical examination and pubertal staging [475]. Further personal and family medical history plus demographic information was recorded on two specially developed clinical evaluation forms (Appendix C). The candidate assisted the participant and their family to complete dietary checklists and surveys (Appendix D). Randomisation to the treatment arm occurred at this visit. A second visit was scheduled for baseline anthropometry, investigations (OGTT, pQCT, DXA, Leonardo® mechanography, $V0_{2peak}$ fitness test) sports medicine and nutrition consultations.

2.6 Interventions

All participants were prescribed standardised lifestyle measures. Those randomised to the WBVT intervention were loaned a Galileo® Home platform and instructed on safe use by the candidate prior to commencement. The duration of the trial was 3 months.

a. Lifestyle Intervention

All participants received a lifestyle intervention, which comprised individualised nutritional coaching by two experienced paediatric weight management dietitians specialised in the care of children and adolescents with obesity and insulin resistance. The Team at CHISM provided the sports medicine consultation and fitness assessment. All participants had clearly defined weight loss and physical activity goals. This was a contemporary clinical intervention offered to all overweight patients of the endocrine clinic at CHW. The schedule for the lifestyle intervention can be seen in Figure 2.2.

Nutrition coaching

At baseline, each participant and their parent/s met with one of two specialised weight management dietitians. Nutrition intervention employed a structured meal plan tailored on an individual basis and incorporating an energy restriction of 500-1000kJ/ day (6000-7000kJ for adolescents aged 10-14 years; 7000-8000kJ for those 10-18 years). The coaching model employed is routinely used by the Weight Management Services at CHW to assist with motivation and adherence to lifestyle recommendations. At the initial meeting, the dietitian introduced their role, gauged interest for nutritional change and provided an individualised eating plan based on a moderate carbohydrate (CHO) diet (Appendix E). Nutrient targets included: 40-45% total CHO, 30% protein, 25% fat (<10% saturated) aiming for 30-45g total carbohydrate, 20-30g total protein, 10-20g total fat for main meals with 20-30g total carbohydrate, 10-20g total protein, 5-10g total fat for snacks. Standard checklists were adhered to during this consultation with an

emphasis on eating a regular breakfast, discussion of hungry periods during the day, increased protein at meals, snacks choices and reduction of carbohydrate intake at night. Participants were encouraged to drink water as the main beverage. The dietitian called the participant and their family 24 hours later to discuss any concerns. A follow-up face-to-face appointment with the dietitian was scheduled during week 2 of the study. The dietitian contacted the family by phone during weeks 6 and 10 to monitor progress. The timeline for nutritional counselling can be seen in Figure 2.2.

Exercise prescription and fitness testing

Participants were referred to CHISM for aerobic fitness testing and an exercise prescription. Participants were encouraged to keep an exercise diary or calendar, limit small screen time to less than 2 hours per day, engage in 30-40 minutes of moderate intensity physical activity four times per week and participate in a team sport. A sample prescription can be seen in APPENDIX F. A follow up sports medicine consultation was scheduled at the conclusion of the study during week 12. The exercise program incorporated an age-appropriate mix of both aerobic and resistance training. In addition, an increase in incidental physical activity and active transport with concomitant decrease in sedentary behaviours was encouraged.

	Wk 0	Wk 2	Wk 4	Wk 6	Wk 8	Wk 10	Wk 12
Clinical assessment							
Tanner Stage	★						★
Medical review	★						★
Anthropometry							
Height	★						★
Weight	★						★
Waist	★						★
Metabolic tests							
OGTT	★						★
hsCRP, lipid profile, LFTs	★						★
Bone turnover markers							
Urine deoxypyridinoline	★						★
P1NP	★						★
CTX	★						★
Body composition							
DEXA	★						★
PQCT	★						★
Leonardo™ Mechanography							
Single leg jump	★						★
Two leg jump	★						★
Lifestyle intervention							
Nutrition coaching	★	★		★		★	
Fitness test $VO2_{peak}$	★						★
Exercise prescription	★						
Therapeutic phone contact		★	★	★	★	★	
Checklists & surveys							
CLASS	★						
Diet checklist	★						

Figure 2.2 VIBRATE study measurement schedule

b. Whole body vibration training

Participants assigned to the intervention arm were supplied with and instructed by the candidate on the safe use of a side alternating whole body vibration platform (Figure 2.3), Galileo Sport® (Novotec GmbH, Pforzheim, Germany). Written instructions were also attached to each platform as a quick reference guide. The training protocol was home based with parental supervision. The 12-week WBVT program was outlined in a training diary provided at randomisation and included a one-week period of increasing training intensity to prevent injury (APPENDIX G). Each daily training session incorporated 3x3 minute blocks of WBVT at variable frequencies (starting at 12Hz for first 3 minutes and increasing to 18-20Hz for the 2nd and 3rd blocks, interspersed with 2x3 minute rest periods; amplitude on the platform varied from 0-6mm, depending on the position of the legs).

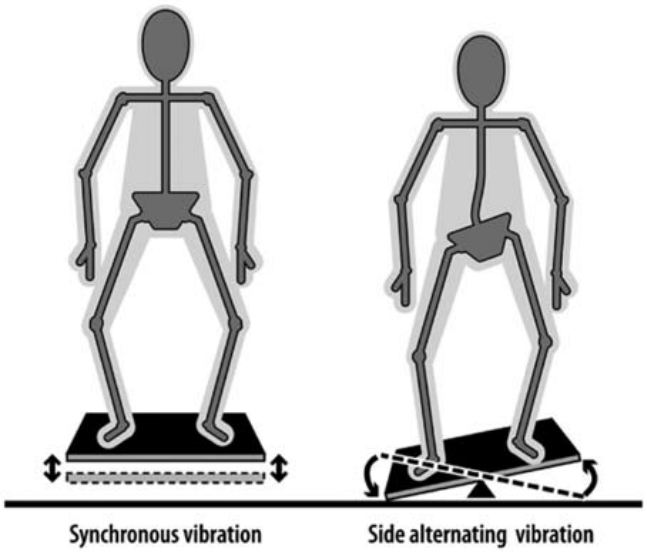


Figure 2.3 Difference between side-alternating and vertical vibration training taken from [409] The Galileo Sport® (Novotec GmbH, Pforzheim, Germany) was used in this study and provides side alternating vibration training (right side); this movement is more physiological).

Figure 2.4 demonstrates the commonly used terminology to describe vibration training. Legs were bent at 45 degrees at the knee with foot placement on standard marker 2 imprinted on the platform surface by the manufacturer (Figure 2.5). The position was chosen to maximally exercise the large muscles of the legs and buttock and to prevent excessive vibration transmission to the spine. The training was performed in socks or thin-soled shoes to minimise dampening of the vibration. This vibration protocol was adapted from previous research studies [427] [434] in the paediatric cohort due to efficacy and tolerability. It was also piloted in a cross section of overweight insulin resistant children derived from our endocrine clinic for tolerability and feasibility prior to commencement of the study. The device had a factory inbuilt electronic timer that recorded the number of minutes in use and which was used in conjunction with the vibration training diary and fortnightly therapeutic contact to assess compliance to the WBVT intervention and determine any specific difficulties or adverse events. A home visit was organised during week one to assess correct use of the vibration plate.

The vibration platform was located in the patient's family home and use by other family members was discouraged, however, if this occurred the participant or parent was to record this time in the vibration diary. Figure 2.6 shows a participant performing WBVT in their home. Full adherence with the prescribed training protocol would constitute a usage of 739 minutes. At the completion of the training period, the candidate checked the platform compliance meter and subtracted time used/ if any, by other family members (as recorded in the vibration training diary). The actual compliance time for each participant was calculated by subtracting this amount from 739 minutes. Percentage compliance time was calculated by multiplying by 100, to obtain a percentage value (%).

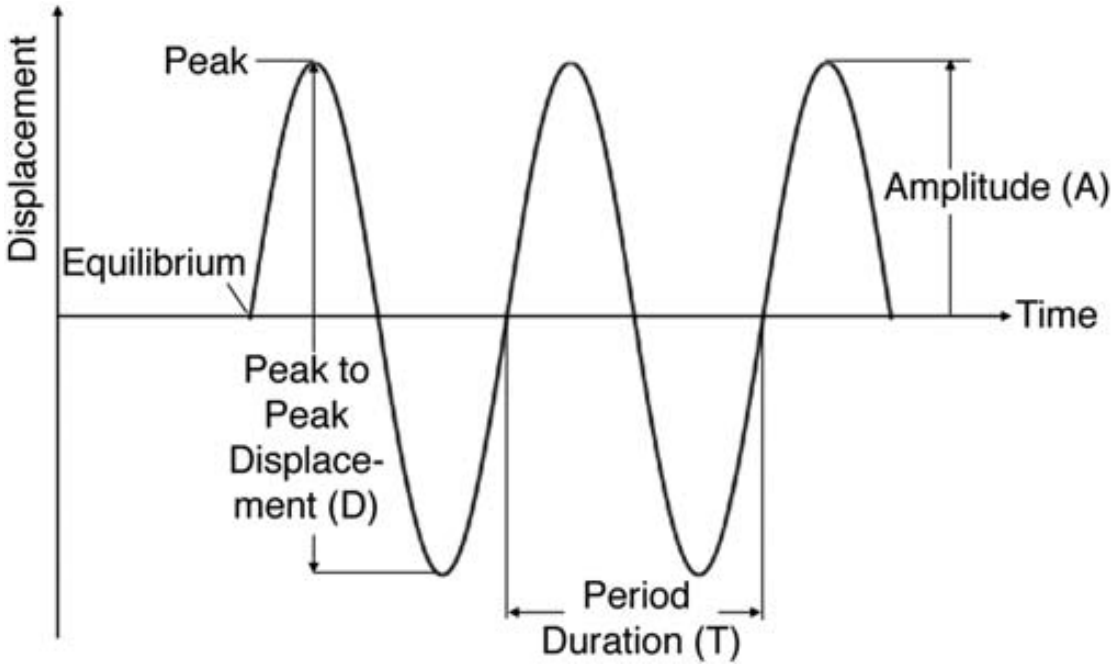


Figure 2.4 Variables in whole body vibration training

Amplitude (A), frequency 1/T, displacement (P) taken from [476]

Figure 2.5 Whole body vibration platform used in VIBRATE study

Galileo® Sport with foot placement markers (0,1,2,3) and remote control taken from [477] with permission.

Figure 2.6 Participant in the VIBRATE study

Participant in the VIBRATE trial using the Galileo Home ™ platform in their bedroom (photograph taken by Adam Hollingworth, Sun Herald 19/10/2008, page 27).

c. Therapeutic contact

The candidate provided therapeutic phone contact to each participant on a fortnightly basis according to the schedule (Figure 2.2) to monitor adherence to the program, troubleshoot any issues raised by the young person and to record any adverse events. A standardised coaching protocol was employed for therapeutic contact.

2.7 Details of measurements

Measurements taken during the trial are listed below and included in Figure 2.2.

a. Clinical Evaluation

Medical and family history

In addition to the medical and family history, the candidate collected demographic information at the recruitment interview using the clinical evaluation form (Appendix C).

Pubertal staging

To classify pubertal status, Tanner and Whitehouse staging [475] was performed by the candidate at baseline and at the end of the study.

Acanthosis nigricans

The presence of acanthosis nigricans at the neck and axillae was noted and quantitatively graded at baseline and 3 months using the Burke scale [470]. Skin colour was noted according to the Fitzpatrick scale [478].

Blood pressure

An automated blood pressure device (Dinamap 1846SX; Critikon Inc, Tampa, FL) was used to measure blood pressure (mmHg) according to standard procedures, on the right arm with an appropriately sized cuff, whilst the adolescent was seated [479]. Three measures were taken and the average of the last two measures was used for analysis.

Anthropometry

Height, weight and waist circumference were measured by one of two endocrine clinical nurse consultants according to standard procedure [480]; both were blinded to the study intervention. Weight was measured once to the nearest 0.1kg, in light clothing using digital scales (Wedderburn, Summer Hill, NSW, Australia). Three height measurements were recorded to the nearest 0.1cm using a wall mounted Harpenden Stadiometer (Holtain Ltd, Crymmych, Dyfed, Wales, UK). The average of the three height measures was used in calculation of body mass index and data analysis. Horizontal waist circumference at the umbilicus was measured using a flexible steel tape to the nearest 0.1cm, using the left hand under technique.

The waist to height ratio (WHtR), was calculated as: waist circumference (cm)/ height (cm), with a ratio of <0.5 considered ideal to reduce cardiovascular risk [90]. The BMI was calculated according to the formula: weight in kg/height $(m)^2$. Height, weight and BMI were converted to z-scores with the LMS excel add in [481] using the reference data from the Center for Disease Control (CDC)[132]. Waist to height ratio, rather than waist circumference z-scores was used for analysis as there are no Australian national standards for waist circumference in children and it has been shown that WHtR exhibits similar CVD risk clustering to the measurement of central adiposity [91].

b. Body composition

Dual energy x-ray absorptiometry (DXA)

Body composition was assessed using DXA as it has high reproducibility, with low coefficients of variation in paediatric patients [482]. Previous studies at CHW have established extensive normative DXA data on children and adolescents [483 484].

DXA (Prodigy, GE-Lunar Corporation, Madison, WI, USA) with proprietary enCORE® software version 8.6 was used to measure body composition including: total and regional (trunk, legs, arms) bone mineral density; bone mineral content (BMC), bone area, lean tissue mass (LTM) and fat at baseline and 3 months. The DXA scans were performed by two experienced scientists (Ms Julie Briody and Mrs Madeleine Thompson) within the Department of Nuclear Medicine at The Children's Hospital at Westmead. The manufacturer recommended scan mode was used for scan acquisition. For this enCORE® version, the "Standard" mode was used for participants with an average tissue thickness less than < 13 cm, and the "Thick" mode was used for participants with tissue thicknesses over 13cm. Participant tissue thickness was estimated by the enCORE® software from the participant's height and weight.

Prior to the scan, any x-ray attenuating materials on the participant were removed. Participant positioning on the scanner table was standardised. Participants were instructed to lie supine on the centre of the scanner table, with both arms close to the body and hands curled into loose fists. A participant whose width was close to, or exceeded, 60 cm (the maximum scan width) was tightly swaddled with a sheet to reduce their width and permit inclusion of their whole body in the scan. A vertical reference line on the centre of the table and a horizontal line at the top of the table provided landmarks for central positioning. Velcro straps at the ankles were fastened to prevent

movement artefacts. This technique has been used in previous obesity studies at CHW [485]. A sample DXA scan may be seen in Figure 2.7. A "Standard" scan required the participants to lie still for less than 4 minutes (< 10 minutes if "Thick" scan mode was used). The effective radiation dose for each scan was less than 1 uSv which is less than that normally received daily from natural sources of radiation (effective dose of 6 μ Sv/day).

Variables analysed included changes in bone mineral content (BMC) relative to body height and projected bone area, lean body mass and fat mass. Analysis of skeletal subregions (head, upper limbs, trunk, pelvis, lower limbs) was performed to assess the site-specificity of the changes. Z-scores were calculated using in house data based on previously published studies. The in vivo coefficients of variation for total body BMC, total BMD, LTM and %fat mass were 0.74, 1.1, 0.82, and 1.59%, respectively [483 486].

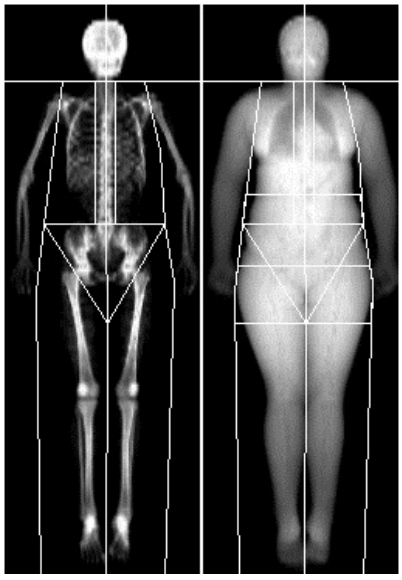


Figure 2.7 DXA scan of participant in the VIBRATE study

Reference lines in white delimit the regions for calculation of regional fat and bone mineral density

c. Metabolic profile measures

Oral glucose tolerance test

Patients were admitted to the Turner Day Stay ward at CHW, after an overnight fast and underwent a standard oral glucose tolerance test (OGTT). Anaesthetic cream was placed over a large vein in the antecubital fossa or hand. A 22g cannula was inserted for blood glucose and insulin sampling. At baseline bloods were withdrawn from the cannula for electrolytes, LFTs, fasting insulin, glucose, C-reactive protein (CRP) and lipid profiles, and analysed in the Biochemistry Laboratory at The Children's Hospital at Westmead. Children were given a glucose containing drink Lucozade^{TM} 1.75g/kg to a maximum dose of 75g and consumed this within 5 minutes. Bloods were taken for insulin and glucose at 30, 60, 90 and 120 min. Plasma glucose concentration was measured immediately on the Dade Dimension ARX (Dade Behring Inc, IL, USA) using the hexokinase-glucose-6-phosphate dehydrogenase method with plasma insulin levels determined by commercial radioimmunoassay kits, inter and intra-assay coefficient of variation 6.0 and 4.4%, respectively (Millipore, Missouri, USA). An extra 10mL of blood was centrifuged and divided in 4 microtubes and stored at -80^{o}C for batch analysis of bone serum turnover markers.

Whole body insulin sensitivity index (ISI) was calculated using the formula:

ISI = 10,000/ √[(fasting insulin (IU/ml) x fasting glucose (mg/dl)] x (mean 2hr insulin (IU/ml) x mean 2 hr glucose(mg/dl))] [276]

Surrogate measures of insulin resistance

The insulin and glucose value at baseline (time 0) was used to calculate a surrogate measure of insulin resistance.

The homeostasis model of insulin resistance (HOMA-IR) was calculated using the formula: HOMA-IR = fasting insulin (mU/L) x fasting glucose (mmol/L)/ 22.5

Metabolic blood profile

Blood was collected at baseline and 3 months for analysis in the Biochemistry Laboratory at CHW according to standard technique. The following bloods were analysed:

- Glucose measurements were analysed on a Vitros DT60 analyser (Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA)
- Metabolic bloods including hs-CRP and lipid profile were analysed on the Vitros 5, 1 FS analyser (Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA).
- Plasma insulin levels determined by commercial radioimmunoassay kits (Millipore, Missouri, USA).

Serum creatinine, urea and LFTs were analysed in the Biochemistry laboratory using standard automatic enzymatic methods.

d. Bone measures

Peripheral Quantitative Computed Tomography

Peripheral quantitative computed tomography (pQCT) of the non-dominant tibia was performed using a Stratec XCT-2000L (Stratec Medizintechik GmbH, Pforzheim, Germany) with proprietary software version 6.00B 0.61 for measurements and analyses. Scans were performed at baseline and 3 months. The participant was required to stay still for about 5 minutes. The foot was placed in a rest and strapped into place for the examination. The effective radiation dose of the pQCT was < 0.3 microSv per scan.

Total effective radiation dose for this study was < 3 microSv. The length of the tibia was measured with a standard measuring tape from the medial malleolus to the medial femoral condyle. [487] A scout view of the distal epiphyses allowed for consistent placement of a reference line in the middle of the distal cortical end of the tibia. The scan site was in the diaphyseal region, 66% of the limb length proximal to the reference position. The following parameters were measured at the tibial diaphysis: muscle cross sectional area (MCSA, mm^2) and polar stress strain index (pSSI, mm^3); in addition cortical volumetric BMD (vBMD) and cortical bone mineral content (BMC) were calculated and converted to z scores at this site. Conversion from pQCT raw data to sex- and age-matched z scores was based on published paediatric reference data [488].

The tibia was acquired with a voxel size of 0.4mm and a scan speed of 20 mm/s was used. The slice thickness of the machine was 2.4 mm. For analysis of the images, a single operator (Ms Julie Briody) manually placed regions of interest around the tibia, fibula and whole leg cross section and then used the LOOP analysis function. The settings for CALCBD and CORTBD were based on those used by Moyer-Mileur [488]. For the vBMD and BMC, CORTBD settings were Cort Mode 1 and Threshold 711 mg/cm^3. CORTBD settings for Strength–strain index (SSI) were Cort Mode 1 and a Threshold of280 mg/cm^3. The parameters used for the muscle analysis (for removal of subcutaneous fat) were: CALCBD Contour Mode 3, Peel Mode 1, Threshold 50 mg/cm^3 and a F03F05 Filter (this noise suppression filter helps to differentiate muscle, fat and bone and combines a 3x3 median filter with a threshold range of -500 to 500 mg/cm3 with a 5x5 median filter with a threshold range of -500 to 300 mg/cm^3). In some patients this analysis failed so an additional F05 filter was required.

Bone turnover markers

Urine

The second urine sample of the day was collected and covered with aluminium foil to protect it from light. The sample was analysed for deoxypyridinoline cross-links by the Endocrinology laboratory at CHW using chemiluminescent competitive immunoassay kits (Immulite®1000, Siemens Healthcare, UK) that has a coefficient of variation (CV) of 9.5% at 98.8 nmol/l to 17.5% at 23.0 nmol/l.

Serum

The following bone turnover markers were analysed in batches by the staff in the Endocrinology Laboratory at CHW:

- Osteocalcin was analysed by chemiluminescent immunoassay using the IMMULITE® 1000 (Siemens Healthcare, UK).
- 25 Hydroxyvitamin Vitamin D levels were determined by using an extraction radioimmunoassay commercial kit (DiaSorin Inc, MN, USA) with serum CV 12.6–10.8% (19.8–280.0 nmol/L) and 9.7–9.5% (45.0–154.5 nmol/L) for kit controls.
- CTX and PINP were measured via chemiluminescent immunoassay (CTX-I CrossLaps® assay kit, IDS Ltd, UK) with manufacturer within run coefficient of variation 2.1-4.9% and (Intact PINP assay kit, IDS Ltd, UK), respectively on the IDS-iSYS Multi-Discipline Automated Analyser (Immunodiagnostic Systems Ltd, England, UK) with manufacturer within run coefficient of variation of 2.6-3.0%.

The manufacturer's reference range for adults was used for reporting the normative data ranges.

e. Fitness and muscle performance

$VO2_{peak}$ fitness test

At baseline, participants performed a supervised $VO2_{peak}$ fitness test on a Trackmaster TMX425 treadmill according to a standard Bruce Protocol [489-491]. In the standard Bruce protocol, the starting point is 1.7 mph at a 10% grade. Stage 2 is 2.5 mph at a 12% grade. Stage 3 is 3.4 mph at a 14% grade, with continual increments in grade and speed at 3-minute increments. This protocol includes 3-minute periods to allow achievement of a steady state before workload is increased. This test was repeated at the conclusion of the trial to evaluate change in aerobic fitness. VO_{2peak} was obtained by continuously analysing gas exchange variables using the Medgraphics CPX/D breath-by-breath gas exchange system (Medgraphics Corporation, St Paul, Minnesota). Participants breathed through a mouthpiece attached to a lightweight pneumotach and in line analysers measured O2 and CO2. Anaerobic threshold was determined using the V-slope method. Peak oxygen uptake was defined by a heart rate greater than 185 beats per minute and peak Respiratory Exchange Ratio (RER) exceeded 1.10; lower values for heart rate and RER were unlikely to reflect exhaustive exercise.

A paediatric exercise physiologist blinded to the treatment intervention conducted the testing and encouraged each child to continue until the point of severe fatigue. The end point was determined to have occurred when the child refused to continue despite verbal encouragement. The participant was able to stop the test at any time by pressing the emergency stop button or by placing their legs on the stationary part of the treadmill. At the end of the test, each participant was questioned about the reason for cessation i.e. short of breath, muscular fatigue or other reason.

Leonardo® mechanography

Although muscle CSA derived from pQCT has been used as a surrogate measure of muscle strength, the correlation between muscle CSA and muscle strength is unknown. Muscle strength in the shin was measured using ground reaction force testing. Peak jump force (N), Power (kW) and efficiency (s) of jump was assessed in all participants at baseline and 3 months during the single leg and two-leg jump on the Leonardo Mechanograph® Ground Reaction Force Plate (GRFP) (Novotec Medical GmbH, Pforzheim, Germany). The child was instructed to jump as high as possible. Three jumps were performed and the jump that resulted in the maximum peak jump force was used for analysis. The raw score and values adjusted for weight and height were evaluated. The countermovement jump or single two leg jump (s2LJ) provides an assessment of anaerobic peak power output and can be matched for mass, age and gender. The child was also instructed to repeatedly hop on the fore foot without making heel contact. This multiple 1 leg jump (m1LJ) estimates the maximal muscular force of the lower limb and allows evaluation of the muscle tendon complex and energy storage capacity [492] [493]. A screen capture of the data provided during Mechanography can be seen in Figure 2.8.

Figure 2.8 Leonardo® Mechanography taken with permission from Novotec Medical

Screen shot of data obtained during the multiple one leg jump showing power (kW) and force (kN)

f. Surveys, checklists and diary

The participants completed these tasks with parental or guardian assistance.

Diet checklist

In the absence of appropriate biomarkers for dietary intake, diet checklists similar to those used in previous studies assessed adherence to the prescribed diet [485]. Each participant completed a 7 day diet checklist (APPENDIX C) at baseline and 3 months, the checklist had been adapted from previous studies [494] and used in a recent study of insulin resistant adolescents [153].

Fitness and physical activity

Physical activity and sedentary behaviours were measured by a validated questionnaire (APPENDIX C), Children's Leisure Activities Study Survey (CLASS). CLASS assesses a

range of physical activity and sedentary behaviours, has been validated for use among children aged 5-6 and 10-12 years, and has been used by adolescents 13-16 years in the Nepean Study [495].

Whole body vibration training diary

The adherence to WBVT was assessed by documentation in the WBVT training diary (APPENDIX G). Participants were asked to note any specific issues during the training session and record use of the platform by other family members, to assist with determining compliance.

2.8 Summary

This chapter provides the methods for the VIBRATE study including the primary and secondary study aims, patient recruitment, schedule of the interventions and the methods employed for data collection and statistical analysis. Chapter 3 will examine the demographic and metabolic features of the cohort, Chapter 4 will provide results from the RCT examining the effect of WBVT on the primary outcome of insulin sensitivity and will also include analysis of the secondary outcomes of metabolic profile indicators; Chapter 5 will explore the results of body composition and musculoskeletal variables and Chapter 6 will provide further data pertaining to the cardiorespiratory fitness of the cohort.

CHAPTER 3

What are the metabolic characteristics of obese insulin resistant adolescents living in Greater Western Sydney?

3.1 Introduction

In the obese adolescent, insulin resistance clusters with other co-morbid metabolic conditions such as PCOS, NAFLD and hypertension (HT). Many children are diagnosed with biochemical or clinical insulin resistance after referral to a specialist for evaluation. Further knowledge about the adolescent at risk of metabolic syndrome will allow for early, targeted intervention of metabolic risk factors and allow rational investigation/ screening of those who may have accompanying insulin resistance.

At baseline, all participants in the VIBRATE study underwent comprehensive clinical assessment and anthropometrical assessment. Fasting serum metabolic profile indicators were taken during the OGTT. To evaluate sedentary behaviours and activity validated activity surveys were completed and nutrition checklists done to review daily intake of common food groups.

The purpose of this chapter is to provide a snapshot of the obese adolescent in Greater Western Sydney (GWS) by fulfilling the following aims:

- Provide further information about the recruitment pattern and describe any noticeable differences.
- Highlight clinical examination and ethnographic features common to adolescents with insulin resistance in Greater Western Sydney (GWS).
- Demonstrate the prevalence of co-existent features of the metabolic syndrome in obese adolescents and their close relatives.
- Determine any anthropometric similarities seen in adolescents with insulin resistance and to examine any gender differences.

- Review any modifiable dietary and lifestyle features present in the obese adolescent?
- To examine correlations and interrelations between baseline biochemical metabolic profile indicators and clinical examination features.

3.2 Demographics

3.2.1 Study participants

Of the 43 overweight and/ or obese adolescents sequentially enrolled in VIBRATE study, 14 (32.6%) were male and 29 (67.4%) were female. Composition of the groups can be seen in Figure 3.1. Participants ranged in age from 10.1 to 17.4 years at randomisation; mean age of the participants was 13.3 years (SD±2.1). All had the presence of acanthosis nigricans and/ or elevated fasting insulin levels >15mU/L [285] in addition to an increased BMI which was at least in the overweight range [60], as per study inclusion criteria.

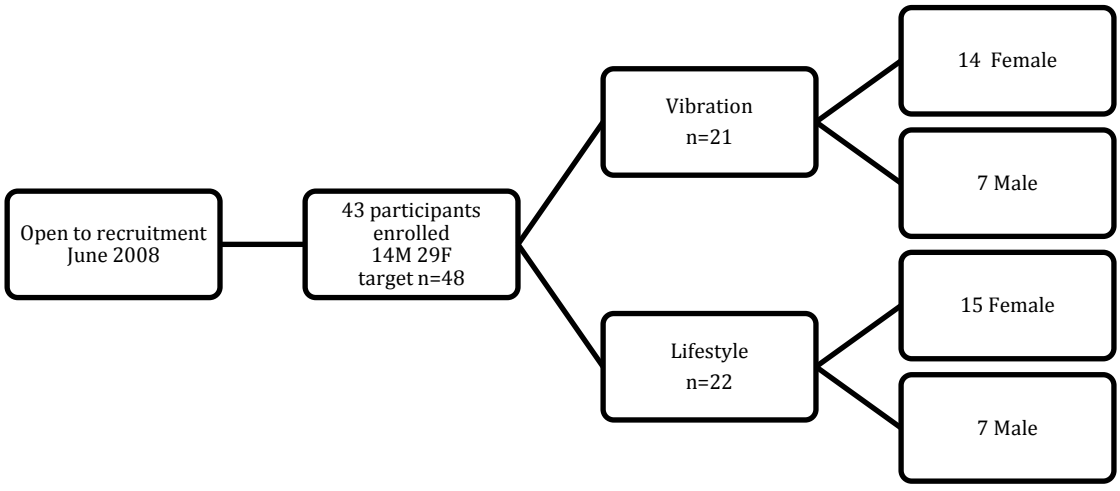


Figure 3.1 Recruitment and randomisation for the VIBRATE study

This diagram shows the composition of the intervention groups.

3.2.2 Demographic and ethnicity data

All participants and their families lived in the GWS region, comprised of 14 local government areas as seen in Figure 3.2.

Figure 3.2 Local government areas of Greater Western Sydney

Map showing the 14 local government areas of Greater Western Sydney taken from [496]

Ethnicity data was self-reported and categorised according to the child and parental birthplace and divided into categories consistent with the Australian Bureau of Statistics (ABS) Census Classifications Index [497]. The majority of participants in the study identified as being from populations at high risk of developing the metabolic syndrome including: Middle Eastern, 13 participants (30%); South East Asian, 6 participants (19%); Indian, 6 participants (19%); Southern European, 4 participants (9%); Pacific Islander, 2 participants (5%) and African 1 participant (2%); 8 adolescents (19%) identified as Caucasian Australian (Figure 3.3).

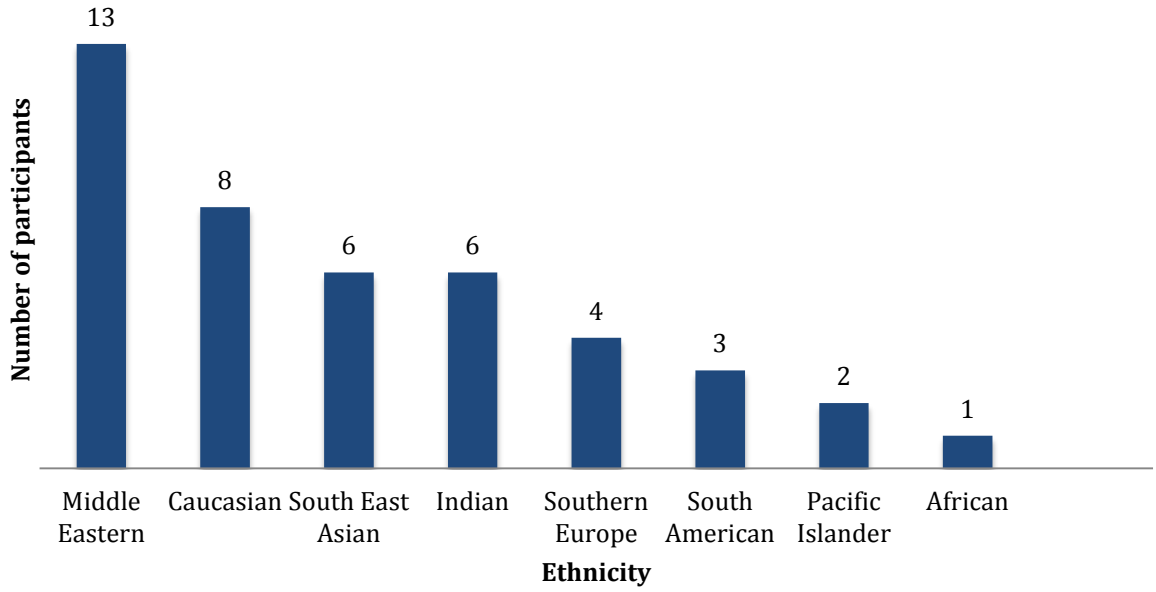


Figure 3.3 Ethnicity of participants in VIBRATE study

Self/ parental reported ethnicity as per ABS Census Classifications Index [497]

3.3 Metabolic profile

3.3.1 Metabolic syndrome in participants

At baseline, 43 participants were assessed for features of the metabolic syndrome using data obtained from anthropometry (WC, BMI z-score) and metabolic bloods (HDL-C, TG, fasting glucose). Selected paediatric definitions of the metabolic syndrome were applied to the data as shown in Figure 3.4. The prevalence of the metabolic syndrome in the cohort ranged from 23-44% depending on the criteria used for the analysis. The modified NCEP ATP III definition identified the highest proportion of individuals and the most females with the metabolic syndrome compared to the other classifications. The prevalence of the metabolic syndrome was similar between the genders, 21-43% of males and 21-45% of females had sufficient clustering of risk factors to identify them as having the metabolic syndrome. Post-pubertal females were more likely to have enough

risk factors to be diagnosed with the metabolic syndrome. Adolescents of Middle Eastern ethnicity were more likely to have more than one risk factor for metabolic disease (Figure 3.5).

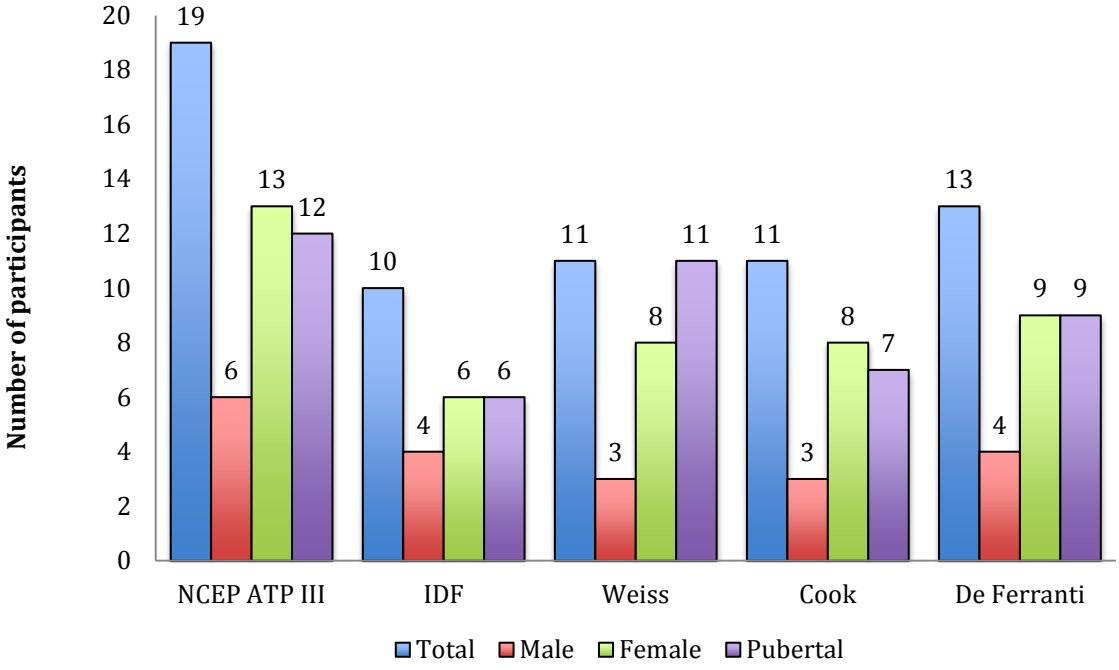


Figure 3.4 Metabolic syndrome in the participants

This histogram shows the number of adolescents in the VIBRATE study with features of the metabolic syndrome according to several paediatric definitions.

NCEP ATP III (National Cholesterol Education Program Adult Treatment Panel III) [116]; IDF (International Diabetes Federation) [122]; Weiss [241]; Cook [498] and De Ferranti [499]. The modified NCEP ATP III criteria includes three of the following measures: waist circumference >90th centile for age and sex; systolic or diastolic blood pressure >90th centile for age, sex and height; TG >1.24mmol/L; HDL-C <1.03mmol/L; fasting glucose >5.55mmol/L.[116]. The IDF definition for metabolic syndrome in children and adolescents include: elevated waist circumference >90th centile for age (adult age and ethnicity specific cut-off used for adolescents>16 years; triglycerides >1.7mmol/L; HDL-cholesterol<1.03mmol/L; SBP >130mmHg or DBP >85mmHg; fasting glucose >5.6mmol/L.

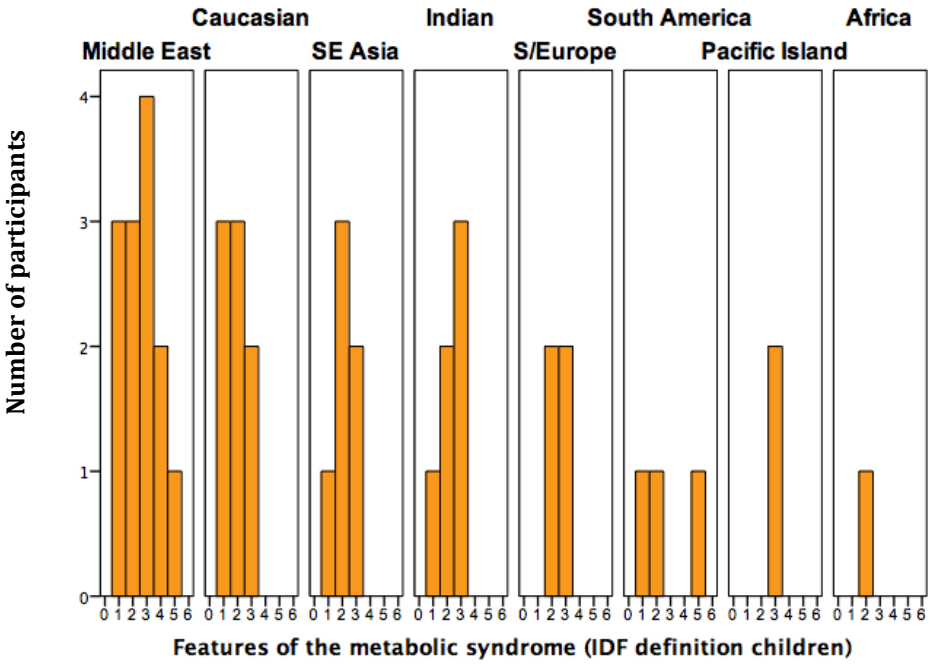


Figure 3.5 IDF[122] features of the metabolic syndrome according to ethnicity

The IDF definition for metabolic syndrome in children and adolescents include: elevated waist circumference >90th centile for age (adult age and ethnicity specific cut-off used for adolescents>16 years; triglycerides >1.7mmol/L; HDL-cholesterol<1.03mmol/L; SBP >130mmHg or DBP >85mmHg; fasting glucose >5.6mmol/L.

3.3.2 Features of the metabolic syndrome in close relatives

To obtain this data, the parent/ caregiver was asked about family history of metabolic disease. There was a significant clustering of features of the metabolic syndrome in both first and second degree relatives as illustrated in Figure 3.6. Obesity was reported most commonly amongst the first-degree relatives followed by T2DM. Amongst second-degree relatives, which included grandparents, uncles and aunts, the most common metabolic disease was T2DM (26 participants), followed by HT (22 participants) and CVD (19 participants).

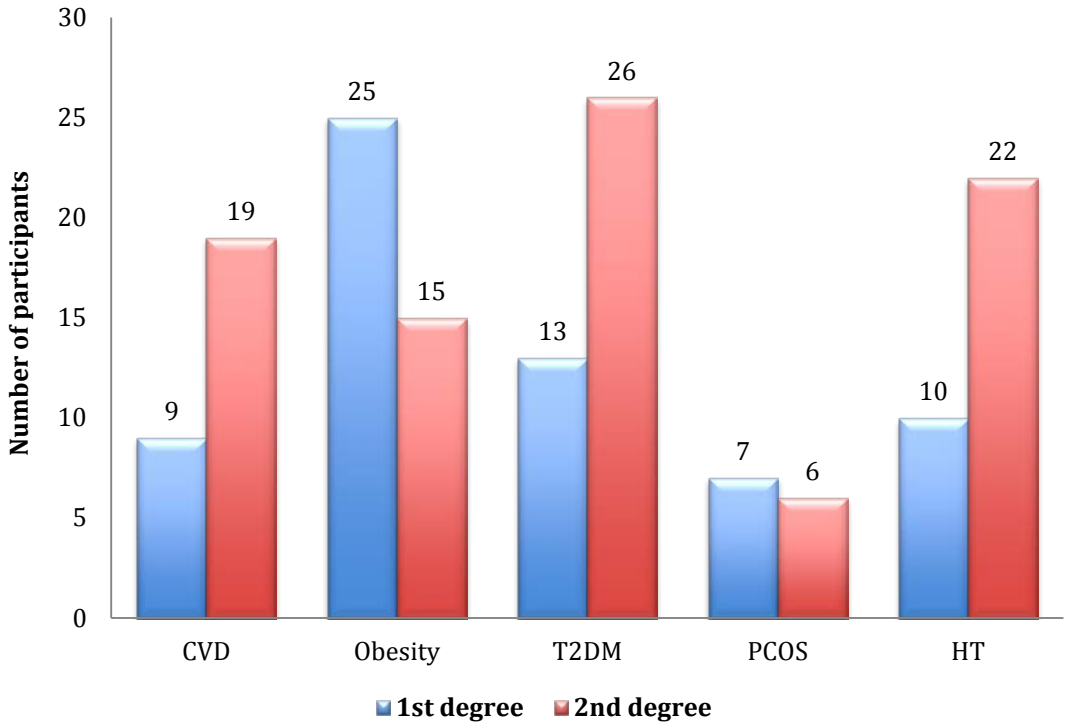


Figure 3.6 Metabolic diseases in first and second-degree family members

Histogram showing metabolic disease in first and second-degree family members (Obesity, CVD Cardiovascular disease, Type 2 diabetes T2DM, Polycystic ovary syndrome PCOS, Hypertension HT)

3.4 Clinical measurement and assessment

3.4.1 Baseline anthropometry and pubertal status

Baseline anthropometry and metabolic indicators according to gender and pubertal status can be seen in Table 3.2. At baseline there were no significant differences in average age or anthropometry between the sexes or intervention groups, apart from baseline height in males which was higher and approached significance p=0.06. Adolescents had a mean BMI in the obese range [500] with central adiposity indicated by elevated waist circumference and waist to height ratio. Almost two thirds (63%) of the study population was female, with most being in late puberty (Tanner stage III-V). Overall there were significantly more late pubertal individuals recruited to the study as indicated in Table 3.1.

Table 3.1 Pubertal status of the participants

	Pubertal status of the participants N=43			
Pubertal status	**Early**	**Late**	**Total**	**T test**
Males	6	8	14	0.01*
Females	10	19	29	<0.001*

*indicates significant result chi squared test Early (Tanner I-II) Late (Tanner III-V)

Table 3.2 Baseline anthropometry and blood pressure

Measure	Group (N=43)	Male	Female	Early puberty (n=16)	Late puberty (n=27)
Age (years)	13.3 (0.3)	13.8 (2.0)	13.1 (2.1)	11.3 (1.0)	14.5 (1.6)[b#]
Weight (kg)	82.5 (2.4)	82.7 (15.0)	82.4 (16.1)	72.4 (10.4)[b#]	88.4 (15.1)
Height (cm)	161.6 (1.4)	165.3 (8.8)	159.8 (8.8)	155.6 (8.6)	165.1 (7.5)[b#]
BMI (kg/m^2)	31.5 (0.8)	30.2 (4.2)	32.2 (5.4)	29.6 (3.0)	32.5 (5.8)
BMI z-score	2.1 (0.4)	2.1 (0.5)	2.2 (0.4)	2.2 (0.3)	2.1 (0.5)
WC (cm)	102.4 (1.8)	102.1 (12.1)	102.6 (12.2)	99.4 (9.3)	104.2 (13.3)
WtHR	0.63 (0.01)	0.62 (0.08)	0.64 (0.08)	0.64 (0.1)	0.63 (0.1)
Pubertal stage Early: Late	16: 27	7: 7	9: 20	16	27
Systolic BP (mmHg)	119.5 (2.0)	121.1 (13.7)	118.7 (13.2)	116.4 (12.2)	121.3 (13.7)
Diastolic BP (mmHg)	65.9 (1.2)	69.8 (8.4)	64.0 (7.4)[a*]	63.3 (7.0)	67.4 (8.5)
SBP z-score	1.0 (0.9)	1.5 (0.9)[a*]	0.7 (0.9)	0.8 (0.8)	1.1 (1.0)
DBP z-score	0.4 (1.2)	0.4 (1.4)	0.4 (1.2)	0.5 (1.2)	0.4 (1.3)

Data are presented as mean ± SD score early Tanner stages I-II late Tanner stage III-V *p<0.05 #p<0.01
[a]Indicates significant result males versus females
[b]indicates significant results early versus late puberty *p<0.05 #p<0.01

3.4.2 Blood pressure

Females had significantly lower diastolic blood pressure (p=0.03) compared to males at the start of the study as seen in Table 3.2. Males had higher SBP z score compared with females. The gender disparity was more evident, particularly for SBP z score when pubertal participants were selected as shown in Table 3.3.

Table 3.3 Blood pressure comparison in pubertal participants

	N = 27	DBP (mmHg)	SBP (mmHg)	DBP z-score	SBP z-score
Male	8	74 (8)	124 (14)	0.4 (1.6)	1.8 (0.9)
Female	19	65 (7)	120 (14)	0.4 (1.2)	0.8 (0.9)
P value		0.014*	0.504	0.976	0.009**

Mean (SD) Student t test *p<0.05 **p<0.01

Participants had evidence of diastolic hypertension (Figure 3.7): 15/43 participants had an elevated diastolic blood pressure (DBP) at baseline; 5 participants had pre hypertension (DBP 90-95th centile for age) and 10/43 (23.3%) participants measured a DBP>95th centile for age, in the hypertensive range.

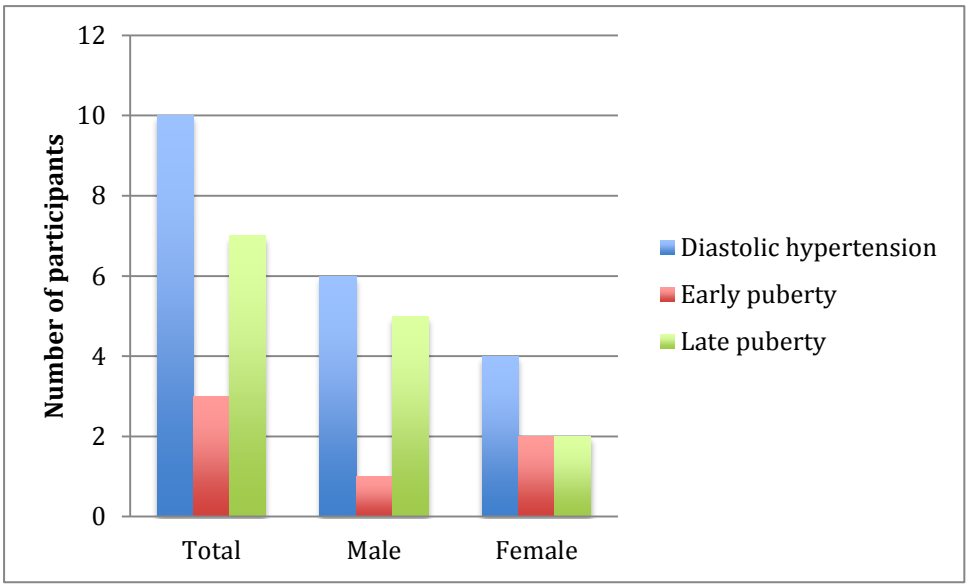


Figure 3.7 Diastolic hypertension (mmHg) in the participants n=43

Blood pressure measured in mmHg Early Puberty (Tanner I-II) and Late Puberty (Tanner III-V)

Systolic blood pressure (SBP) measurement showed similar trends (Figure 3.8) with 3/43 participants classified with pre hypertension and 10/43 (23.3%) participants with systolic hypertension. The majority of those presenting with elevated blood pressure were late pubertal males.

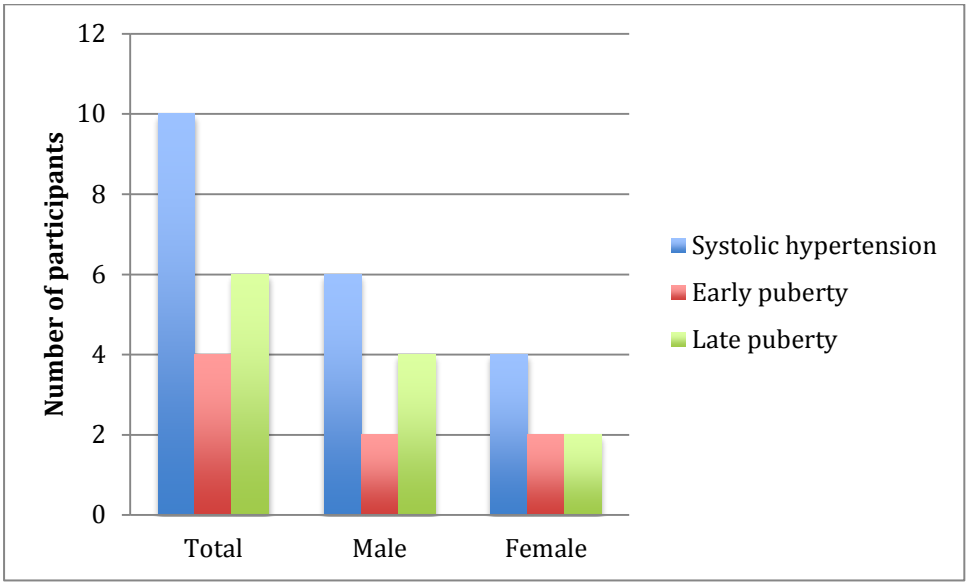


Figure 3.8 Grade 1 Systolic hypertension (mmHg) in the participants n=43

Blood pressure measured in mmHg Early Puberty Tanner I-II and Late Puberty Tanner III-V

3.4.3 Acanthosis nigricans

Almost two thirds (28/43) of the cohort presented with AN and all had dark skin (Fitzpatrick grade IV-VI [478]). None of the Caucasian or Southern European participants had this dermatological feature.

3.5 Biochemical measures

3.5.1 Metabolic profile indicators

At the start of the study all participants had fasting blood drawn for measurement of fasting lipid profile, fasting insulin and glucose and hs-CRP. The fasting insulin and glucose level was used to calculate the surrogate measure of insulin resistance, HOMA-IR. Table 3.4 provides the biochemical measures obtained at baseline in the group, stratified according to gender and pubertal status. There were no gender differences for fasting measures of glucose, insulin or lipid profile at the commencement to the study. The calculated proxy index of insulin resistance (HOMA-IR) was high across the groups.

a. Liver function tests

Liver function tests analysis revealed elevated transaminases with mean AST increased above the normal range. ALT levels whilst still in the normal range were significantly higher in males when compared to females (p=0.007). Liver function tests show mean for total, intervention groups and gender AST and ALT was elevated outside the normal range.

b. Lipid profile

There was a high prevalence of dyslipidaemia at baseline, 25/43 (58%) participants had an abnormal lipid profile with elevation of total cholesterol (TC), low-density lipoprotein C (LDL-C), triglycerides (TG) and/or evidence of low high-density lipoprotein-C (HDL-C) fraction at baseline.

c. hs- CRP

There was no difference between hs-CRP at baseline for gender or pubertal status.

Table 3.4 Biochemical measures of metabolic profile indicators

	Whole group (n=43)	Males (n=14)	Females (n=29)	Early Puberty	Late Puberty
Fasting glucose (mmol/L)	5.0 (0.5)	5.0 (0.4)	5.0 (0.5)	5.2 (0.5)	4.9 (0.4)
2 hr glucose (mmol/L)	6.2 (1.2)	6.2 (0.7)	6.2 (1.3)	6.1 (0.8)	6.3 (1.4)
Fasting insulin (pmol/L)	203 (58)	201 (48)	204 (63)	203 (59)	203 (58)
2 hr insulin (pmol/L)	1047 (594)	1133 (548)	1004 (620)	969 (571)	1092 (613)
HOMA-IR	7.6 (2.5)	6.5 (1.6)	6.6 (2.4)	6.8 (2.3)	6.5 (2.1)
WBISI	1.74 (0.8)	1.6 (0.5)	1.8 (1.0)	1.9 (0.9)	1.6 (0.8)
hs-CRP (mg/L)	6.1 (6.2)	5.4 (4.3)	6.4 (7.0)	6.5 (3.1)	5.1 (3.0)
Lipid Profile					
Total cholesterol (mmol/L)	4.2 (0.9)	4.3 (0.9)	4.1 (0.9)	4.3 (0.7)	4.2 (0.9)
LDL cholesterol (mmol/L)	2.6 (0.7)	2.8 (0.8)	2.6 (0.7)	2.6 (0.6)	2.7 (0.8)
HDL-C (mmol/L)	1.1 (0.3)	1.1 (0.6)	1.1 (0.5)	1.1 (0.3)	1.1 (0.2)
TG (mmol/L)	1.1 (0.5)	1.3 (0.8)	1.1 (0.5)	1.2 (0.6)	1.1 (0.5)
Liver Function tests					
Aspart Transaminase (AST)	55 (30)	59 (18)	53 (34)	62 (34)	51 (26)
Alanine Transaminase (ALT)	31 (20)	46 (27)**[a]	24 (9)	28 (8)	33 (24)**[b]
Alkaline Phosphatase	188 (103)	216 (93)	175 (106)	265 (76)	143 (89)

Data are presented as mean±SD *indicates Student t-test p<0.05 **indicates p<0.01
[a] indicates baseline difference males versus females [b]indicates baseline difference early versus late puberty

Normal ranges
Total cholesterol 3.0-5.2mmol/L
HDL cholesterol >1.0 mmol/L
LDL cholesterol 1.7-3.4 mmol/L
Triglycerides <1.5mmol/L
AST 10-50 U/L
ALT 0-45 U/L
ALP 50-350 U/L
Fasting glucose <5.6mmol/L
CRP mg/L normal (0-10)
HOMA-IR <1.5 [288]

d. Glucose

At baseline, fasting glucose displayed a normal Gaussian distribution (Figure 3.9) within the range 3.9-5.9mmol/L and there were no gender or group differences. Six participants had evidence of pre-diabetes with IFG with levels ≥ 5.6mmol/L. The mean 2 hr glucose was similar for the group with no differences for gender or pubertal status.

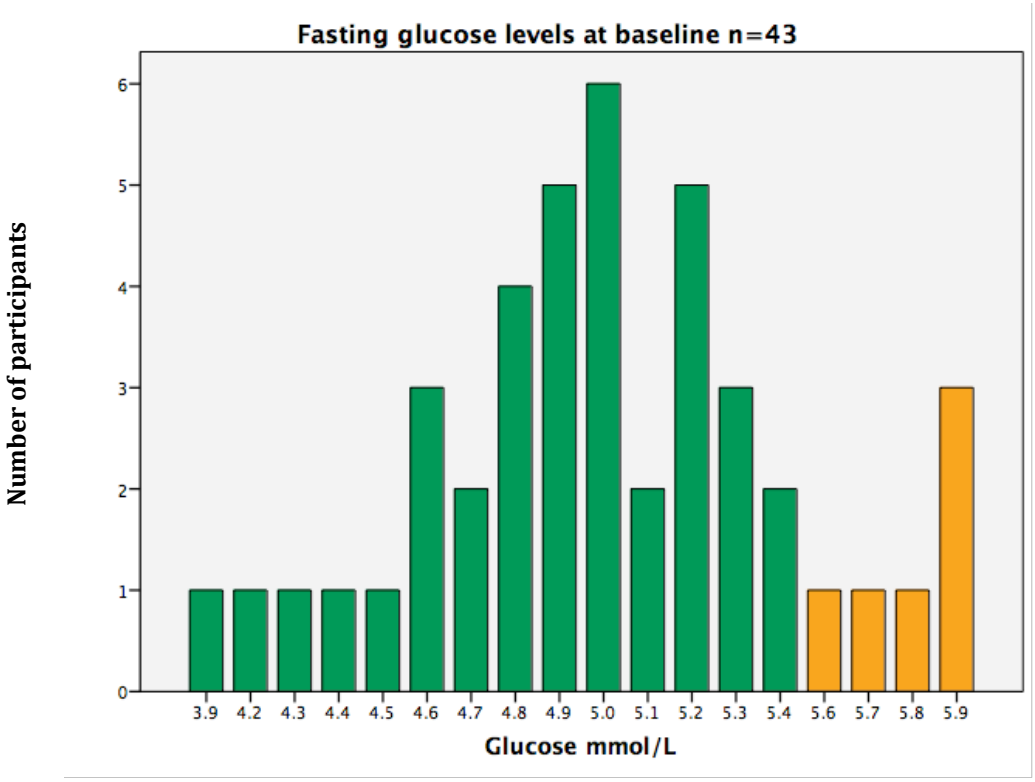


Figure 3.9 Distribution of fasting glucose levels for the VIBRATE participants

e. Fasting insulin and HOMA-IR

As a group the mean (SD) fasting insulin was elevated at 203pmol/L (58) with no gender or pubertal differences. The group was highly insulin resistant as per inclusion criteria and as indicated by the mean HOMA-IR level of 7.6 (2.5).

3.6 Research investigations

3.6.1 Whole body insulin sensitivity index (WBISI)

Consistent with what is known about physiological insulin resistance which peaks in Tanner stage 3, later pubertal participants (Tanner 3-5) had lower WBISI compared with early pubertal participants (Tanner 1-2) as seen in Figure 3.10 and higher WC was associated with reduced insulin sensitivity (Figure 3.11).

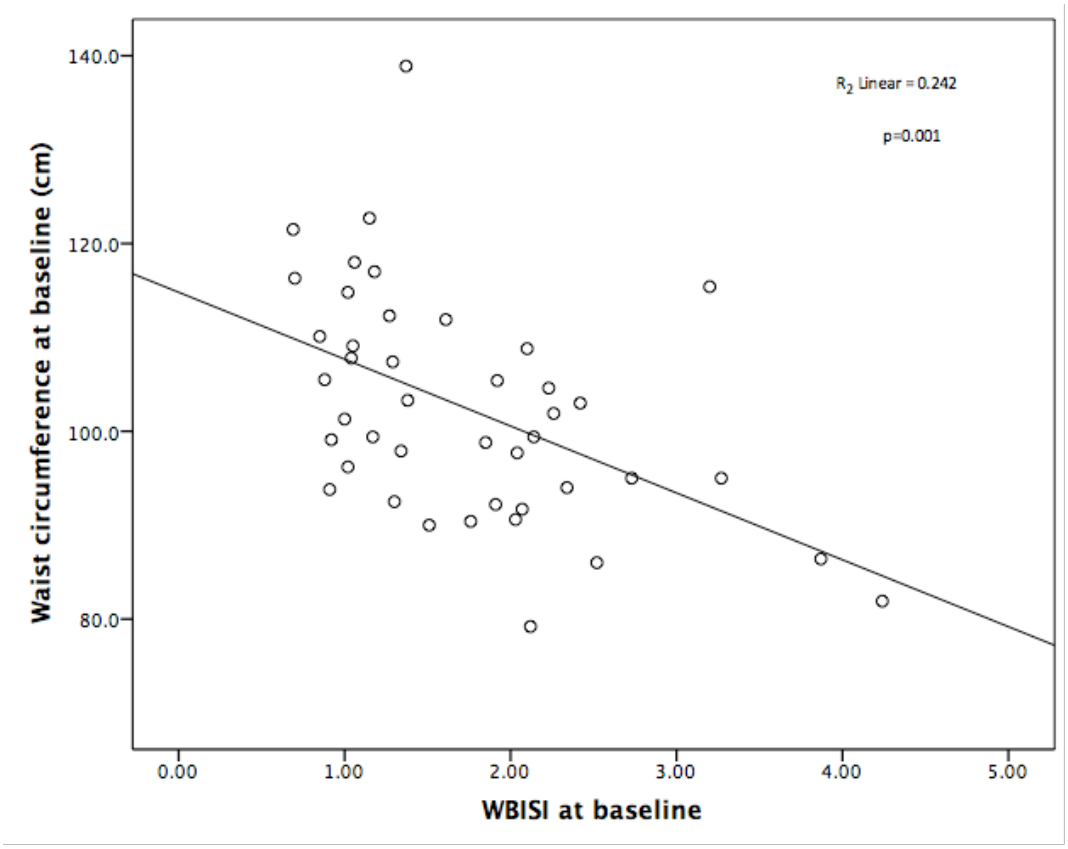


Figure 3.10 Relationship between waist circumference and insulin sensitivity

This plot shows a negative correlation between increasing waist circumference and insulin sensitivity.

3.6.2 CLASS activity questionnaire

Males and females showed no difference in levels of activity or sedentary behaviour times at baseline. Pubertal status did influence these lifestyle behaviours (Table 3.5).

Table 3.5 CLASS leisure and activity data as baseline

	Total N=41	Males N=14	Females N=27	Early Puberty N=16	Late Puberty N=25
%Sedentary time week	21.9 ± 12.0	24.8 ± 13.3	20.4 ± 11.1	20.0 ± 6.4	23.4 ± 14.4
%Active time week	4.6 ± 3.1	5.1 ± 3.5	4.3 ± 3.0	4.2 ± 3.0	4.8 ± 3.3
Active time (min)	460 ± 317	513 ± 356	433 ± 299	418 ± 297	487 ± 333
Sedentary time week (min)	2208 ±1206	2500 ± 1342	2057 ± 1126	1975 ± 641	2358 ± 1452

Completion of the CLASS questionnaire in 41 participants at baseline showed no difference in activity or sedentary times for boys and girls. However, % time engaged in sedentary activities far exceeded %active time per week, which amounted to <5% in all participants.

3.7 Correlations between metabolic indicators and anthropometry

Increased anthropometry measures (BMI, WC, WtHR) were positively correlated with greater insulin resistance (HOMA-IR) and adverse lipid profile with increased TG. Increased TG showed positive association with hepatocyte injury with increase in transaminases (AST and ALT). An inflammatory state was associated with increased BMI, elevated SBP and insulin resistance (HOMA-IR) at baseline as shown in Table 3.6.

Table 3.6 Correlation between metabolic profile indicators at baseline

	WH	Waist	HOMA-IR	TG	HDL	LDL	TG	AST	ALT	SBP	DBP	CRP
BMI z-score	0.755**	0.646**	0.346*	0.378*	NS	0.335*	NS	NS	NS	NS	NS	0.318*
W/H		0.889**	0.342*	0.431**	0.453**	0.362*	NS	NS	NS	NS	NS	NS
Waist			0.383*	0.401**	0.372*	0.384*	NS	NS	NS	NS	NS	NS
HOMA-IR				NS	NS	NS	NS	NS	NS	NS	NS	0.431**
TC					NS	0.891**	0.503**	NS	NS	0.325*	NS	NS
HDL						NS	NS	NS	NS	NS	NS	NS
LDL							NS	NS	NS	0.316*	NS	NS
TG								0.346*	0.331*	NS	NS	NS
AST									NS	NS	NS	NS
ALT										NS	0.433**	NS
SBP											0.523**	0.435**
DBP												NS

Pearson's correlation coefficient significance ≤0.01** (2 tailed); * ≤0.05 (2 tailed) NS not significant

BMI Body mass index WtHR waist to height ratio HOMA-IR Homeostasis model assessment of insulin resistance TC Total cholesterol HDL High-density lipoprotein-C LDL Low density lipoprotein TG Triglycerides AST Aspart Transaminase ALT Alanine transaminase SBP Systolic blood pressure DBP Diastolic blood pressure

3.8 Discussion

The results in this chapter present a cross sectional snapshot of the unique metabolic and ethnographic risk factors of the overweight adolescent living in Greater Western Sydney.

The study population was derived from a multi-ethnic demographic of GWS. More than 80% of the study participants had origins from populations considered to be at high risk of the metabolic syndrome [501] with only 19% identifying as Caucasian. Almost one third of adolescents in this study were from a Middle Eastern or North African background (Figure 3.3). The prevalence of high-risk ethnicities within our cohort demonstrates the genetic susceptibility to insulin resistance and components of the metabolic syndrome [502].

A family history of cardiovascular risk factors and T2DM is known to contribute to individual disease risk [503-505]. In the VIBRATE cohort, there was clustering of the metabolic syndrome in both first and second-degree relatives; obesity was more prevalent in first-degree relatives and the metabolic sequelae of obesity (T2DM, HT and CVD) in second degree relatives (Figure 3.6). For overseas born Australians, those born in South Eastern Europe, North Africa and the Middle East are considered to be at higher risk of developing T2DM [506]. Holdenson *et al* also included the South Pacific Islands, Central and Southern Asia in their evaluation of high-risk ethnicities. Of concern, the prevalence of T2DM in Middle Eastern and North African adults was highest amongst all groups in Australia with odds ratios of 3.60 and 2.43 for males and females respectively [501]. The ethnicities of the insulin resistant adolescents in our study almost mirror the local Australian data in adults.

Within our cohort, eight participants had an overweight mother and ten reported an overweight father; six participants reported that both parents were obese. Whilst multifactorial, the inheritance of obesity is likely to be high with parental obesity more than doubling risk of developing overweight in later life [174]. Some studies suggest that childhood obesity is closely aligned to parental weight status [47 507-509], however we did not see this in our study and this may be due to the body composition differences in our multicultural cohort with a predominance of central adiposity.

The prevalence of T2DM in the extended family of our participants was increased with 30.2% of first-degree and 60.5% of second-degree relatives reportedly with the disease (Figure 3.6). Our results are consistent with other studies, showing a strong genetic inheritability of T2DM, whereby children are more likely to be overweight or obese, if the parental BMI particularly maternal weight is increased [54 174]. Our data may also

provide some insight into the timeline of pathogenesis of T2DM and cardiovascular disease progression with the first appearance of obesity and insulin resistance in adolescence and early adulthood and loss of compensatory changes and the development of T2DM and cardiovascular disease in the second generation. A parental history of T2DM has been found to increase prevalence of BMI and other adverse anthropometric measures such as increased WC)[510], skinfold thickness and BMI [511], a similar association has been seen in children whose parents have the metabolic syndrome [512].

Female adolescents accounted for 67.4% of our study population and this may be related to several factors including: the differences in health seeking behaviours amongst males and females and the differential perception of weight as an issue between the genders. Females are more likely to seek medical attention throughout life and these behaviours are often developed during adolescence. Adolescent females have a more accurate perception of their weight status [513] [514] [515] [516] [517] compared to adolescent males and therefore more likely to seek assistance through weight loss programs or try to lose weight [517].

Weight perception is multifactorial and includes cultural and social influences. The perception of the weight as a marker of attractiveness [518] may encourage recruitment of female adolescents into weight related research programs, however we did not examined this in our study. Parental concern is a major determinant in weight management of the young person [519]. Studies have shown that parents are more likely to raise concerns about their overweight in their daughters [520], if they themselves are obese or suffer from a chronic disease [56]. Overweight adolescents in general have more difficulty discriminating their weight status compared to obese individuals but females were more likely to accurately perceive their weight as being above the healthy weight range [521]. Recognition

of overweight is a fundamental step towards weight loss through lifestyle and behavioural change.

In addition to external concerns about body weight, many overweight adolescents are referred for tertiary investigation due to the presence of acanthosis nigricans (AN), a significant and sometimes unsightly discolouration of the skin in visible area.

Gender attitudes to healthcare could explain differences we observed during the recruitment phase. Australian and international studies show that adolescent females were more likely to seek help from their close relatives or a health practitioner compared to their male counterparts. Booth *et al* found that late adolescent males aged 13-16 years were least likely to access health care [522]. During the adolescent period, males access health care less frequently than females whose health seeking behaviours show an increased uptake [523].

There are many socio-cultural determinants of weight. These ethnic differences in weight perceptions may positively and negatively influence the health related behaviours of adolescents [513]**.** We had a disproportionate number of individuals from ethnic minority groups and most of the participants 81.4% presented with a BMI in the obese range (Table 3.2). It raises the question about the late presentation of these children rather than at the earliest signs of overweight. The delay in presentation may be due to self and family perceptions of overweight [57] [56]. Weight may be gauged comparatively to relatives and hence not considered to be an issue. In a large study by Brener *et al*, a quarter of overweight individuals self-reported in the underweight range [514]. Within cultural groups there may be disparate views of increased weight. In the USA, African American (AA) women were more likely to accept a higher BMI as being the norm compared to white

counterparts [524], whilst obese Hispanic adolescent males were misperceived by family as being of normal weight [57]. Increased body weight has been considered a sign of health and prosperity particularly in the Pacific nations [525] and this may delay presentation to health professionals until co-morbidities develop. A majority of the participants derived from a Middle Eastern background, where traditionally and for religious reasons many are unable to participate in physical activity [526], whether these reasons continue to be relevant for second generation Australian children remains to be determined.

The average BMI in our participants was in the obese range by adult standards at 31.5kg/m^2. As BMI does not account for the percentage or distribution of body fat, it has been suggested that Indigenous Australians and those from an Asian background have lower BMI thresholds for overweight and obesity to reduce cardiovascular risk [527]. This is because they have a higher percentage of body fat than white people for the same age, sex and BMI [67]. Ethnicity is also a determinant of body shape and composition and may account for the increased waist circumference in both males and females in our study. The average waist circumference of 102cm is in excess of the national adult recommendations [14]. In addition to lower BMI thresholds for high-risk populations, the waist circumference measure is likely to provide a better assessment of metabolic risk and adverse adiposity.

The average fasting insulin level in the group was 203pmol/L (33.8mU/L), much higher than the inclusion criteria to the study of 90pmol/L (15mU/L) (Table 3.4). Whilst there are no paediatric reference ranges, levels greater than 20mU/L are likely to signify high insulin resistance in a child [285]. The HOMA –IR at baseline was high averaging 7.62, higher than the cut-offs (5.22 and 3.82 pubertal boys and girls) proposed by Kurtolglu [528] and considerably higher than the suggested adult limit of 2.5 [266 288]. WBISI was low at

1.74 with levels <2.5 suggestive of insulin resistance [266]; some researchers suggest that the degree of insulin resistance is more closely linked to metabolic risk [529]. Six participants (14%) (Figure 3.9) in our study already had evidence of impaired glucose tolerance (IGT), a pre-diabetic state suggesting that progression to T2DM may occur more quickly in this age group and require more aggressive intervention [61 192]. Since the start of our study, an international working party [280] has indicated that the fasting insulin and proxy measures poorly define individual risk of insulin resistance likely due to pulsatile variation and physiological changes during puberty.

There was a high prevalence of the metabolic syndrome in our study population ranging from 23-44% (Figure 3.5) depending on the various definitions applied, this is not an unexpected finding given the interrelationship between the pathophysiology of insulin resistance and the metabolic syndrome. We also identified more pubertal than pre-pubertal individuals with metabolic syndrome indicating that even earlier recognition and action during childhood may be required to prevent metabolic decline during adolescence.

There is no standard definition of the metabolic syndrome in children, therefore use of adult guidelines may lead to under or overestimation of the actual prevalence depending on the cut off levels. In addition, the effect of puberty should be considered in any definition of the metabolic syndrome in an adolescent due to presence of physiological insulin resistance and variability in metabolic profile indicators [118]. Our data is consistent with overseas studies of high-risk paediatric populations where prevalence rates of the metabolic syndrome ranged from 33-39% [530 499 498]. In the USA, AA children, who are at high metabolic risk of T2DM may not be correctly identified because of less dysglycaemia [531]. Therefore in some populations with known high risk treating known

CVD risk factors may prove more beneficial to future health than waiting for the a clustering of components that fall under the umbrella of the metabolic syndrome [531].

A strong association between puberty and metabolic health occurs in overweight and obese children. The hormonal milieu associated with puberty has been shown to convert previously metabolic healthy obese (MHO) children into metabolic unhealthy obese (MUO) children [117]. A considerable proportion of participants in the study were female and a greater majority of patients were pubertal which has possibly conferred an additional metabolic stress. Interestingly, the modified NCEP III-ATP definition of the metabolic syndrome identified a greater proportion of female participants with the metabolic syndrome. This definition had the lowest cut off for triglycerides compared to the other definitions presented in this thesis. Therefore consideration of the triglyceride fraction may be another discriminating factor and assist to correctly identify those at highest risk. We did not see any difference between serum lipids in early or late puberty but it is known that gender differences in the lipid profile develop during this time with reductions in the HDL-C in males [532]. The changes in the various lipid fractions may be influenced by sex steroid hormones particularly testosterone [533].

All adolescents in our study classified with dark skin (Fitzpatrick Type IV to VI) [478] presented with the dermatological finding of AN, whereas this was absent in children with light skin. This indicates that whilst AN may be a reliable clinical indicator of underlying insulin resistance in dark skinned individuals [300], children from a Caucasian background or who have lighter skin may be missed and not referred for further evaluation by primary health care providers.

Adolescent females in our cohort had a lower absolute diastolic blood pressure (DBP) compared with males who had a higher SBP z score (Table 3.3). The difference in SBP between the genders is expected, as sexual dimorphism in this trait is known to have origins in adolescence. This result may also suggest later hypertension in males could be prevented by lifestyle modification during the adolescent years. At baseline, we discovered a high prevalence of prehypertension (19% of cohort) and Grade 1 systolic (24%) (Figure 3.8) and diastolic (24%) hypertension (Figure 3.7), particularly in pubertal males [534]. The recognition of hypertension is important in young people as it is co-morbidly seen in one quarter of youth with T2DM [535] and may accelerate atherosclerotic processes [536]. The Framingham Heart Study showed that DBP was the best predictor of coronary events later in life in younger people compared to the SBP (more predictive of events in older persons) [537], indicating that closer consideration to both blood pressure components may be required in younger persons. One of the limitations to our BP data is that we used an automated blood pressure method rather than the manual technique that would have allowed better discrimination of the fourth and fifth Korotkoff sounds and DBP [534]. Also, we only measured BP on one occasion rather than at different time points. The use of ambulatory blood pressure monitoring would have allowed us to better evaluate this cardiovascular risk factor and remove the element of "white coat hypertension" which is often present in a clinical setting.

The mean AST level was increased in both genders and across pubertal stages in our participants. Elevated transaminases are a surrogate indicator of non-alcoholic fatty liver disease (NAFLD), strongly clustering with obesity and T2DM. We found that ALT levels positively correlated with total cholesterol and triglycerides in both sexes but seemed to do so more strongly in boys. The male participants had significantly higher mean ALT levels when compared with females. Our reference range for ALT is not gender

dependent and some have proposed that lower thresholds: ALT>30IU/L and 19IU/L for boys and girls, respectively, as males have higher liver enzymes than females [538 539]. Using these gender specific cut off values a disconcerting number of participants: 20/29 females and 11/14 males raised transaminases however, our in house reference range demonstrated only 5 participants with an elevated ALT level above the normal range.

The clinical significance of these results cannot be determined given we did not perform contemporary liver ultrasounds. NAFLD is a recognised complication of rising obesity rates in young people and is associated with elevated liver transaminases. Nadeau et al [540] found a prevalence of 14.3% elevated ALT and 14.3% elevated AST with a higher occurrence in Hispanic and in males. The aetiology of NAFLD remains unclear however accumulation of triglycerides within the hepatocyte is a key pathogenic step in this disease [541]. We showed a positive correlation between triglycerides and to a lesser extent total cholesterol and liver transaminases (ALT and AST) which is similar to other studies that have shown hypertriglyceridemia as significant risk factor for NAFLD [540]. Dietary checklists were completed at baseline but were found to be incomplete and based on recall and therefore not reliable for analysis.

Participants had an inactive lifestyle as evidenced by low activity times and high percentage of sedentary behaviours with small screen times exceeding 2 hours per day. Inactivity is associated with increased weight, adverse lipid profile, elevated systolic blood pressure and T2DM [542-545]. The cardiorespiratory fitness of the participants is further evaluated in Chapter 6.

The implementation of lifestyle change in children may alleviate the later health burden expected due to cardiovascular disease and the metabolic syndrome. The American Heart

Association (AHA) Scientific Statement on Cardiovascular Health in Childhood recommended that key lifestyle factors be addressed to improve the cardiovascular health of children and adolescents. The areas targeted for improvement included: regular physical activity with a reduction in sedentary activity; weight management and dietary counselling; clinical assessment of insulin resistance and T2DM risk; early detection and management of hypertension and dyslipidaemia [285]. Ho and colleagues confirmed that metabolic risk factors could be ameliorated through lifestyle interventions which included dietary modification with a structured supervised exercise program potentiating these effects [154]. Clinicians should encourage young adolescents to be physically active as a decline in fitness into adulthood is linked to T2DM [546] and health practitioners may need to be mindful of peers, family and neighbourhood when encouraging behavioural change [145].

CHAPTER 4

Does Whole Body Vibration Training improve insulin sensitivity in overweight adolescents?

4.1 Introduction and Aims

Overweight remains a major health concern in Australian adolescents [547] [33] despite national [7] and international evidence [548] to suggest rates are stabilising. With the rise in obesity there has been a significant increase in the prevalence of metabolic co-morbidities including insulin resistance, a known precursor to the development of T2DM, metabolic syndrome and early CVD [549] [550]. Early recognition and management of those at risk of T2DM can prevent disease progression. Physical activity, lifestyle change and selected pharmacotherapy may enhance Si through weight loss [280] and through weight independent body composition change [328] [551].

Whilst there are many metabolic and other health benefits of exercise [552], the most appropriate type of physical activity for young people at risk of T2DM remains to be elucidated [553]. Aerobic or anaerobic exercise may enhance metabolic health due to effects on different physiological pathways which may occur independent of weight loss [328] [551]. Increased muscle mass may increase the peripheral glucose disposal of glucose but an exercise regime which involves an aerobic component may cause changes in the ability of the muscle to response to insulin through post receptor binding effects. Enhanced Si, with weight loss and exercise are likely to have different physiological mechanisms [554].

Whole body vibration training is a form of resistance training that has received a lot of recent interest regarding its possible weight loss benefits, purported to occur with minimal effort and maximal efficiency. However, interventional research studies in adults have only demonstrated at best, very modest [355] [555] fat loss. Similar to other forms of resistance training, WBVT leads to muscle activation [556] inducing changes in muscular strength, performance [404] and metabolism [557]. Muscle plays a key role in post-prandial glucose disposal [558] and certainly studies in adolescents have demonstrated that changes

at the muscle level may be associated with enhanced insulin sensitivity [337 559]. There are no studies examining the possible effect of WBVT on glucose regulation. Therefore we explored whether WBVT would improve insulin sensitivity in obese adolescents with clinical insulin resistance either with associated body composition change or independent of weight loss when compared to a lifestyle intervention.

The management of obesity in young people is challenging and therefore, an examination of the adherence and retention of participants in our study will be discussed to advise future obesity intervention programs. The results for the primary outcome of insulin sensitivity and secondary effects on the anthropometry and metabolic profile indicators will be provided in this chapter. The effects of WBVT on musculoskeletal architecture and turnover and body composition will be discussed in Chapter 5. The effects of the intervention on functional performance indices of cardiorespiratory fitness and muscle performance will be examined and discussed in Chapter 6.

The primary aim of the study was to test within an RCT the hypothesis that three months of whole body vibration training in addition to a prescribed diet and exercise intervention would enhance the effects of the lifestyle interventions by improving insulin sensitivity in overweight adolescents with hyperinsulinaemia. The details of the randomised groups can be seen in Chapter 3. We speculated that the WBVT intervention would increase muscle mass in the lower limbs and would enhance peripheral non-insulin mediated glucose uptake compared to those adolescents who received lifestyle intervention (diet and exercise) alone. Secondary effects on anthropometry, body composition and metabolic profile indicators will also be examined in this chapter.

4.2 Participant flow during the trial

The recruitment inclusion and exclusion criteria for the study have been previously discussed in Chapter 2.1.3. Fifty three adolescents were assessed for study eligibility, one adolescent declined to participate, three failed to attend baseline investigations on two separate occasions and six did not meet inclusion criteria as follows: one adolescent was diagnosed with T2DM during baseline investigation; four had fasting insulin levels outside the specified range and one adolescent had a weight greater than 120kg.

The study opened in June 2008 with the aim of enrolling forty-eight participants and closed to recruitment in October 2010 after permission from the local ethics committee due to a lower than anticipated dropout rate. Forty-three participants were randomised into the study by minimisation [473], 22 participants were allocated to the standard lifestyle intervention with all receiving this intervention over 3 months as per protocol. One participant in the standard arm declined to attend for final testing due to higher school examinations. All 21 participants allocated to the WBVT intervention completed the trial. Gender and pubertal status were equally represented in the two intervention arms. A CONSORT (Consolidated Standards of Reporting Trials) flow diagram [560 561] can be seen in Chapter 2 (Figure 2.1).

The study had high 3-month retention with a dropout rate of just 2.3%, considerably less than the anticipated attrition rate of 40%.

4.3 Intervention effects

4.3.1 Effect on anthropometry and body composition

a. Anthropometry

Baseline anthropometry can be seen in Table 4.1. At the start of the study, the two intervention groups were homogeneous to anthropometry measures.

Table 4.1 Anthropometry at baseline and 3 months

	Baseline			3 months		
	Lifestyle	**Vibration**	**P value**	**Lifestyle**	**Vibration**	**P value**
N	22	21		21	21	
Age (years)	13.25 ± 2.13	13.41 ± 2.11	0.80	13.36 ± 2.0	13.74 ± 2.12	0.60
Gender. No. (%)						
Female	15 (68.2)	14 (66.7)	0.92	14 (66.7)	14 (66.7)	
Male	7 (31.8)	7 (33.3)		7 (33.3)	7 (33.3)	
Height (cm)	160.7 ± 9.9	162.5 ± 8.3	0.52	161.5 ± 9.9	163.5 ± 8.3	0.45
Weight (kg)	81.6± 19.1	83.4 ± 11.1	0.70	79.4 ± 18.9	84.3 ± 12.3	0.32
BMI (kg/m^2)	31.4 ± 5.9	31.6 ± 4.2	0.90	30.4 ± 6.3	31.5 ± 4.54	0.29
BMI z-score	2.10 ± 0.5	2.14 ± 0.4	0.79	2.00 ± 0.5	2.14 ± 0.38	0.38
Waist (cm)	102.4 ± 14.8	102.5 ± 8.8	0.97	98.6 ± 16.1	100.9 ± 10.2	0.57
Waist-height-ratio	0.64 ± 0.1	0.63 ± 0.1	0.80	0.62 ± 0.1	0.62 ± 0.1	0.80

Data presented as mean ± SD * indicates a significant result $p<0.05$ ** indicates a significant result $p<0.01$ Student t test

Between group differences

The effect of the interventions on anthropometry can be seen in Table 4.2.

There was a tendency to greater raw and z-score weight loss in the lifestyle group when compared to the vibration arm although these results did not reach significance ($p=0.08$ and $p=0.07$ respectively). Lifestyle change resulted in a significantly reduced BMI z-score -0.1 ($p=0.039$), with decreased absolute BMI -0.8kg/m^2 ($p=0.05$); there were no significant changes in height between the two groups at the end of the study $p=0.5$, suggesting that the weight loss in the lifestyle group contributed to the observed BMI changes.

Table 4.2 Absolute 3-month treatment effects on anthropometry

Δ change over 3 months			
	Lifestyle	**WBVT**	**P value**
Weight (kg)	-0.9 ± 3.7	0.9 ± 2.7	0.08
Weight z-score	-0.04 ± 0.1	0.02 ± 0.1	0.07
Waist (cm)	-2.8 ± 4.2	-1.5 ± 3.4	0.6
Waist/height ratio (<0.5)	-0.02 ± 0.03	-0.01 ± 0.02	0.25
Height (cm)	1.1 ± 0.9	1.2 ± 1.0	0.5
BMI (kg/m^2)	-0.1 ± 0.1	-0.02 ± 0.1	0.05
BMI z-score	-0.8 ± 1.2	-0.08 ± 0.8	0.039*

Data presented as mean ± SD * indicates a significant result p<0.05 - independent samples student t-test p<0.05

Measures of central adiposity including waist circumference and waist to height ratio were slightly reduced at the end of the study in both groups (Lifestyle -2.8cm vs. WBVT -1.5cm; p=0.6) but this did not reach significance. Mean waist circumference remained elevated in both groups at the end of the trial (98.6 cm vs. 100.9 cm; p=0.570, Lifestyle vs. WBVT) in excess of the national Australian guidelines for adult males and females [1].

Lifestyle effects

When the groups were examined separately and longitudinally, there were significant changes pre and post intervention in anthropometry including reductions (mean ± % change) in waist circumference (-2.82cm, -2.8%; p=0.005); WHR (-0.02, -3.1%; p=0.001) and absolute BMI (-0.8kg/m^2, -2.6%; p=0.009). In contrast, the only significant change in the WBVT group was a lower waist to height ratio (-0.01, -1.6%; p=0.008). As expected during adolescence, both groups demonstrated linear growth after 3 months with significant changes in height (1.2 cm, 0.8%, p<0.01) vs. (1.1cm, 0.7%; p<0.01) in the WBVT and lifestyle groups respectively.

DXA

Table 4.3 Adiposity at baseline for the total cohort and intervention groups

	Total N=42		Lifestyle N=21		Vibration N=21	
Gender	M	F	M	F	M	F
	N=13	N=29	N=6	N=15	N=7	N=14
%Total fat	38.1 (7.4)	47.5 (6.3)*	34.3 (6.3)*	49.4 (4.6)	43.4 (4.6)	46.1 (6.2)
Fat z-score	3.2 (1.1)	3.1 (0.8)	2.7 (0.9)	3.4 (0.7)	4.0 (0.7)**	2.9 (0.8)
%Fat trunk	40.9 (6.4)	49.1 (5.1)*	37.5 (7.1)**	50.5 (4.5)	43.8 (4.1)	47.7 (5.4)

DATA PRESENTED AS MEAN (SD) * SIGNIFICANT RESULT STUDENT T TEST P<0.01 OR **P<0.001 WITH COMPARISON MADE BETWEEN GENDER IN EACH GROUP I.E. TOTAL, LIFESTYLE AND VIBRATION. N=42 GROUP TOTAL AS ONE PARTICIPANT IN LIFESTYLE ARM WAS UNABLE TO HAVE DXA SCAN.

b. Body composition change between the groups

At baseline, females had more total and trunk fat compared with males as shown in Table 4.3. Males in the lifestyle group has significantly less total and trunk fat compared with females in this group. In the vibration group, males had greater fat z-score compared with females at baseline.

The significant decrease in BMI z score in the lifestyle group, did not result in altered body composition with no change in total body fat or lean tissue as seen in Table 4.4. Participants who performed the WBVT training showed considerable but non-significant gains in total lean tissue mass 184.6 g (lifestyle) vs. 588.4g (WBVT); p=0.500) as seen in Table 4.5.

pQCT

pQCT analysis revealed significantly decreased muscle fat cross-sectional area at the 66% site in the lower limb in the LIFESTYLE arm, -175.7mm^2 vs. -16.9mm^2 ; p=0.029. Conversely, greater increases in muscle cross-sectional area were seen in the VIBRATION group 153.4mm^2 vs. 73.4mm^2, but this was not significant p=0.559.

Table 4.4 Change in DXA body composition measures after 3 months

Data mean ± SD	Lifestyle	Vibration	P value
Total body fat (%)	-0.94 ± 2.37	-0.14 ± 1.82	0.229
Trunk fat (%)	-0.83 ± 3.50	0.33 ± 2.65	0.234
Trunk lean tissue mass (g)	19.76 ± 1464.49	114.1 ± 1186.84	0.820
Total lean tissue mass (g)	184.57 ± 2142.39	588.44 ± 1678.04	0.500
Lean tissue mass/ height z-score	-0.25 ± 0.81	-0.09 ± 0.43	0.431
Leg fat (%)	-1.03 ± 2.10	-0.75 ± 1.67	0.637
Leg lean tissue (g)	38.18 ± 726.19	411.49 ± 562.92	0.070

Data presented as mean ±SD Independent samples Student t test

Table 4.5 Within group soft tissue change

	Lifestyle			Vibration		
Parameter	**Pre**	**Post**	**Δ change**	**Pre**	**Post**	**Δ change**
Total fat %	45.1 (8.6)	44.1 (9.0)	-1 (1.2)	45.2 (5.7)	45.0 (6.7)	-0.1 (1.0)
Total fat z-score	3.2 (0.8)	3.0 (0.8)	-0.2 (0.3)*	3.2 (0.9)	3.2 (1.0)	0.0 (0.2)
Trunk fat %	46.8 (8.0)	46.0 (8.1)	-0.8 (3.5)	46.4 (5.2)	46.7 (6.5)	-0.3 (2.6)
LTM/ Ht z-score	1.6 (2.4)	1.4 (2.4)	-0.3 (0.8)	1.8 (1.9)	1.7 (1.9)	-0.1 (0.4)
Leg fat%	47.4 (10.1)	46.4 (11.0)	-1.0 (2.1)*	48.0 (7.1)	47.2 (7.7)	-0.8 (1.8)
Legs LTM (g)	14460 (3466)	14498 (3320)	-38 (727)	14869 (1938)	15280 (2150)	412** (562)

Mean (SD) * significant result independent Student t test p<0.05 ** p<0.01 comparing pre/post values in each group

LTM/Ht Lean tissue mass for height

4.3.2 Effect on insulin sensitivity and related parameters

At baseline there were no significant differences between the intervention groups for insulin sensitivity, glucose or insulin (Table 4.6). Both groups were highly insulin resistant with (mean ± SD) HOMA-IR score 7.6 (2.9) vs. 7.6 (2.1) and demonstrated reduced insulin sensitivity with calculated WBISI 1.9 (0.9) vs. 1.6 (0.8); p=0.4, in the lifestyle and WBVT groups respectively.

Within group longitudinal analysis revealed clinically favourable albeit non-significant changes in insulin sensitivity within the lifestyle group with improved final HOMA-IR and WBISI (Table 4.7)

Table 4.6 Baseline and final insulin sensitivity and glycaemic markers

	Baseline			Final		
	Lifestyle	**WBVT**	**P value**	**Lifestyle**	**WBVT**	**P value**
Fasting glucose (mmol/L)	5.1 ± 0.5	4.9 ± 0.3	0.2	4.8 ± 0.6	4.9 ± 0.4	0.6
Fasting Insulin pmol/L	200 ± 62	207 ± 56	0.7	189 ± 105	236 ± 95	0.1
HOMA IR	7.6 ± 2.9	7.6 ± 2.1	0.8	6.9 ± 4.6	8.6 ± 3.7	0.2
WBISI	1.9 ± 0.9	1.6 ± 0.8	0.4	2.1 ± 0.9	1.7 ± 0.9	0.2
Acanthosis nigricans score	2.36 ± 1.4	2.67 ± 1.4	0.5	2.23 ± 1.3	2.62 ± 1.3	0.3

Mean ± SD independent samples student t-test p

After 3-months, fasting glucose had decreased significantly by -0.3mmol/L (p=0.047) in the lifestyle cohort but there were no other significant absolute improvements in insulin sensitivity measures as seen in Table 4.7.

Table 4.7 Absolute changes in insulin sensitivity and glycaemic markers

Absolute change over 3 months			
	Lifestyle	**WBVT**	**p value**
Fasting glucose (mmol/L)	-0.3 ± 0.5	0.00 ± 0.3	0.047*
Fasting insulin (pmol/L)	-10 ± 105	-29 ± 99	0.222
HOMA-IR	-0.98 ± 4.17	0.93 ± 3.17	0.100
WBISI	0.25 ± 0.46	0.10 ± 0.66	0.537

*significant result - independent samples student t-test $p<0.05$

Given that compliance was sub-optimal in the WBVT intervention group. The WBVT group was divided into high and low compliance groups based on the median of platform time usage. A comparison between the high compliance and standard group revealed no significant difference in the primary outcome measure, WBISI and the lifestyle intervention. However, it must be noted compliance to the lifestyle intervention is unknown and based on verbal telephone reports from patient and family.

Table 4.8 Metabolic profile indicators at baseline and after 3 months

	Pre intervention			Post intervention			Absolute change		
	Lifestyle	**Vibration**	**P value**	**Lifestyle**	**Vibration**	**P value**	**∆Lifestyle**	**∆Vibration**	**P value**
N	22	21		21	21		21	21	
Age (years)	13.25 ± 2.13	13.41 ± 2.11	0.800	14.79 ± 2.13	15.03 ± 2.27	0.729			
Gender. No. (%)									
Female	15 (68.2)	14 (66.7)	0.918	14 (66.7)	14 (66.7)				
Male	7 (31.8)	7 (33.3)		7 (33.3)	7 (33.3)				
BP systolic (mmHg)	121 ± 14	118 ± 13	0.493	119 ± 11	119 ± 14	0.961	-3 ± 14	1 ± 14	0.916
BP diastolic (mmHg)	67 ± 8	64 ± 8	0.264	66 ± 9	64 ± 7	0.355	-1 ± 11	-1 ± 10	0.418
Cholesterol mmol/L									
Total	4.24 ± 0.99	4.14 ± 0.72	0.700	4.11 ± 0.72	4.23 ± 0.54	0.564	-0.2 ± 0.6	0.1 ± 0.4	0.204
HDL	1.09 ± 0.29	1.10 ± 0.21	0.946	1.05 ± 0.21	1.10 ± 0.16	0.478	-0.04 ± 0.2	0.0 ± 0.2	0.582
LDL	2.66 ± 0.91	2.60 ± 0.56	0.798	2.57 ± 0.67	2.53 ± 0.45	0.827	-0.06 ± 0.5	-0.01 ± 0.4	0.766
Triglycerides	1.20 ± 0.62	1.09 ± 0.45	0.515	1.06 ± 0.48	1.25 ± 0.47	0.227	-0.14 ± 0.5	0.14 ± 0.4	0.036*
Liver function tests u/l									
AST	52 ± 28	58 ± 32	0.523	55 ± 29	65 ± 36	0.344	3 ± 43	7 ± 51	0.768
ALT	33 ± 19	30 ± 21	0.617	26 ± 16	30 ± 23	0.507	-7 ± 17	0 ± 8	0.139
C-reactive protein (mg/L)	7.04 ± 8.22	5.10 ± 3.02	0.316	4.70 ± 4.06	5.64 ± 4.85	0.502	-2.6 ± 7.2	0.29 ± 4.3	0.129

Student t test * indicates a significant result p<0.05 ** indicates a significant result p<0.01 groups compared pre/post
Normal ranges
Total cholesterol 3.0-5.2mmol/L
HDL cholesterol 0.8-1.5mmol/L
LDL cholesterol 1.7-3.4
Triglycerides 0.6-1.7mmol/L
AST 10-50 U/L
ALT 0-45 U/L
ALP 50-350 U/L
Glucose range <5.6mmol/L
C reactive protein <10mg/L
AST aspart aminotransferase
ALT alanine aminotransferase

4.3.3 Effect on metabolic profile indicators

Adolescents had a normal lipid profile at baseline with mean levels within normal ranges for total cholesterol, triglycerides, HDL and LDL cholesterol as shown in Table 4.8 At the start of the study 21 (11M) participants had abnormal liver function tests with mean AST in both groups outside the normal range 52u/l (28) vs. 58u/l (32), normal range 10-50, possibly reflecting early liver function enzyme derangements.

Lipid profile improved in the lifestyle group with significant change in triglycerides -0.14mmol/L (0.5) vs. 0.14mmol/L (0.4), p=0.036. C reactive protein levels were elevated at baseline, consistent with the degree of obesity and insulin resistance, after 3 months there was an improvement in the lifestyle arm -2.6mg/L (7.2) vs. 0.29mg/L (4.34) but this was not significant p=0.1

Table 4.9 Partial correlations between anthropometry, fasting measures of insulin resistance and selected metabolic profile indicators for group at baseline (n=43)

Controlled variables Gender Pubertal status	HOMA IR	WBISI	Glucose	Insulin	hs-CRP	SBP
Waist	0.404**	-0.479**	0.345*	0.339*	NS	NS
Weight	0.495**	-0.484**	NS	0.375*	0.401*	0.418**
BMI	0.441**	-0.416**	0.468**	0.335*	0.353**	NS
W/H ratio	0.342*	-0.408**	0.424**	NS	NS	NS
HOMA IR		-0.866***	0.594***	0.968***	0.428**	NS
WBISI	-0.866***		-0.498**	-0.863***	-0.323*	NS
hs-CRP	0.428**	-0.323*	NS	0.388*		0.463**

p< 0.05 level (2 tailed) ** p< 0.01 level (2 tailed) ***p<0.001(2 tailed)
hs-CRP high sensitivity C-reactive protein W/H waist to height ratio HOMA-IR homeostasis model assessment of insulin resistance WBISI whole body insulin sensitivity index SBP systolic blood pressure

4.4 Correlations between anthropometry, insulin sensitivity and selected serum metabolic profile indicators

Significant associations between anthropometry measures and insulin sensitivity were seen at baseline including: positive associations between HOMA-IR and waist, weight, BMI and WtHR ratio; conversely WBISI showed more significant negative correlations with the same parameters. Fasting glucose and insulin were also moderately correlated with BMI and central adiposity measures (Table 4.9). The hs-CRP, a marker of low-grade inflammation was positively associated with increased weight, BMI, systolic blood pressure, insulin resistance HOMA-IR and negatively correlated with WBISI.

Partial correlations controlling for gender and pubertal status revealed significant associations between absolute changes for the entire group (N=42) at the end of the trial including: moderate correlation between raw ΔBMI with Δwaist circumference and Δhs-CRP. Changes in HOMA-IR were significantly correlated with Δhs-CRP. The change in serum triglycerides correlated with ΔALT levels. As expected, absolute changes in fasting insulin and glucose correlated with changes in the proxy measures HOMA-IR and WBISI (which are directly derived from these levels). These results can be seen in Table 4.10.

Table 4.10 Partial correlations between absolute changes in metabolic variables and anthropometry (adjusted for BMI and puberty)

Δ value	Insulin	Glucose	BMI	Waist	WBISI	HOMAIR	ALT	hs-CRP
Insulin	-	NS	NS	NS	0.697** *	0.975***	NS	0.478**
Glucose	NS	-	NS	NS	NS	0.371*	NS	NS
BMI kg/m^2	NS	NS	-	0.409**	NS	NS	NS	0.419**
WBISI	-0.697***	NS	NS	NS	-	-0.645***	NS	NS
Waist cm	NS	NS	0.409**	-	NS	NS	NS	0.466**
hs-CRP	0.478**		0.419**	0.466**	NS	0.493**	NS	-
TG	NS	NS	NS	NS	NS	NS	0.346 *	0.346*

p< 0.05 level (2 tailed) ** p< 0.01 level (2 tailed) ***p<0.001(2 tailed)
All bloods reported are fasting measures
BMI body mass index WBISI whole body insulin sensitivity index
HOMA IR Homeostasis model assessment of insulin resistance
ALT Alanine transaminase hs CRP C-reactive protein

4.5 Side effects, adherence to intervention and safety

No adverse events were recorded in either intervention arm; this was monitored during the fortnightly phone contact. Participants were encouraged to be compliant to the allocated intervention via a standard phone coaching method. Adherence to the lifestyle component of the study was obtained verbally during the phone contact.

Compliance to the vibration intervention ranged from 50 minutes (7%) to 1353 minutes (183%). The mean compliance time as determined from the inbuilt timer and vibration diary entries averaged 460 ± 325 minutes (mean ± SD). Full compliance to the training program was indicated by a training time of 743 minutes. Three (14%) participants were fully compliant to the therapy and a further 3 adolescents (14%) were poorly adherent to the prescribed regime with <25% of predicted platform use. Males had a higher average use of the platform, 72% versus 58%, but this was not significant.

4.6 Discussion

In this randomised control trial, we investigated whether a novel resistance based exercise, whole body vibration training (WBVT), could enhance insulin sensitivity Si in our cohort of obese adolescents with clinical insulin resistance. To our knowledge, this is the first randomised control trial designed to investigate the metabolic and anthropometric effects of WBVT in the obese adolescent population.

Our results confirm the benefit of lifestyle intervention in the management of paediatric obesity with significant BMI reductions in those who received this treatment, exclusively. The study also demonstrated that significant changes to anthropometry measures are possible in a relatively short time period and in real life settings, with only occasional therapeutic contact. Our results are applicable to the real life setting. For example, an

exercise prescription incorporating the NHMRC guidelines [14] along with nutritionist involvement may assist in BMI reduction in the obese adolescent in the primary care setting.

The addition of WBVT to standard lifestyle prescription did not enhance insulin sensitivity and did not lead to additional weight loss but did lead to an increase in peripheral lean tissue mass within this group at the end of the study.

Our results are not surprising, given that lifestyle change with adjunctive use of pharmacotherapy is regarded as first line in the management of paediatric obesity and insulin resistance [280] [154]. In established cases of paediatric T2DM, a combination of metformin plus lifestyle intervention did not result in weight loss and was not more effective than metformin monotherapy. Both the aforementioned treatments were less effective than dual pharmacotherapy with rosiglitazone and metformin. This highlights the need for a multipronged approach to T2DM but may indicate that the disease process is more aggressive in younger patients and needs to be managed accordingly [562].

After 3 months, those participants in the lifestyle arm demonstrated a BMI change of -0.8kg/m^2 (-2.6%) equating to a significant BMI z score change of -0.1 (-4.8%), this result is comparable to a recent study in obese adolescents which showed BMI SDS change of -0.1 to -0.2 [563] with an unsupervised home based intervention over a longer period of 8 months. The observed BMI loss in our lifestyle arm was accompanied by minimal change in body composition with only a slight loss of total body fat ~1% (p=0.229). Paediatric studies have determined that a BMI z-score change of 0.25 equates to a fat loss of 2.9% [564] and certainly our results show equivalence with these data. A study from our own centre with a supervised physical activity component demonstrated -

6.8% and -2.4%, BMI and %fat loss, respectively and improved WBISI score of 0.2 [152] after 1 year.

The decreased BMI within the lifestyle group was associated with significant pre and post group changes in central adiposity (WC and WtHR) and body composition (lower total fat z score). Within the WBVT group, the only significant difference in anthropometry pre and post intervention was the WtHR, which in the absence of significant BMI may be attributed to increased linear growth, which occurred similarly in both adolescent groups. It is interesting that whilst both groups were provided the same lifestyle program the WBVT group did not show appreciable change in BMI. The WBVT intervention may have been viewed as a "magic cure" and easy method of weight reduction and may have influenced compliance to the lifestyle intervention in this group.

In agreement with the literature, we showed that positive BMI changes were associated with clinically favourable albeit non-significant enhancements in insulin sensitivity in the lifestyle participants [332 563 154 152] with improved WBISI (0.25 versus 0.10; p=0.537) and HOMA IR (-0.98 versus 0.93; p=0.2) in the lifestyle and WBVT groups, respectively. Our results are similar to other paediatric studies which show that metabolic health parameters can be improved without significant differences in anthropometry or body composition [328 329 563 551].

Lifestyle intervention with an exercise and dietary component resulted in lower fasting blood glucose levels (p=0.047) at the end of the study, this is similar to observations in obese adults which have shown reduced hepatic insulin resistance and lower glucose production with exercise training and caloric restriction [565]. The significant changes in the fasting glucose may represent an improved metabolic health status and reduced diabetes

risk as research has shown that glucose levels within the normal range correlate with cardiovascular health in children and adults [196] and may indicate progression to T2DM in adulthood [566] [197].

We hypothesised that WBVT would lead to regional changes in body composition with greater accrual of lean tissue mass in the lower limbs with possible increased peripheral glucose disposal through non-insulin dependent mechanisms. We also believed the exercise could serve a dual purpose by increasing muscular strength [400] and allow for more active participation in physical activity. As we did not directly observe the physical activity of the participants during the trial, it remains unclear whether WBVT had any conditioning effect in these adolescents. However, speculated changes in lean tissue were observed after three months in this group. We found that total lean body tissue increased by 588g (WBVT) vs. 185g (Lifestyle); p=0.50. When pre and post intervention levels were compared within the WBVT group, lean tissue increased in the legs by 412g (2.8%) above baseline (P=0.003). Further results regarding muscle and bone change will be discussed in Chapter 5. This resistance exercise did not show any effect on adipose tissues measures on DXA analysis.

Our results are consistent with data from Roelants *et al* [353] that showed a 2.2% increase in lean tissue with 24 weeks of WBVT in a similarly untrained cohort, with no change to fat parameters. Whilst our results are consistent with the above, they are discordant with small animal [351] and recent adult studies showing modest loss in VAT [448] and improved anthropometry with WBVT [355]. The significant gains in peripheral lean tissue may well have been due to a direct effect of this form of training on muscle spindles and enhanced neuromuscular excitation [387] [567].

Lean tissue changes in the lower limbs were accompanied by changes measurable on pQCT analysis including significantly decreased fat cross sectional area (tibial 66% site) in lifestyle participants and non-significant increases in muscle cross sectional area in those undertaking WBVT. Intramyocellular lipids are implicated in the development of skeletal muscle resistance and decrease with weight loss, therefore the significantly decreased levels we observed with the lifestyle program may have contributed to the enhancements in Si we saw in this group after 3 months [568]. Muscle hypertrophy and an increase in muscle cross sectional area would be anticipated structural changes in the muscle of the lower limbs being targeted by this intervention [381 393 569]. It is possible that some of the increased weight seen in the WBVT group at the end of the study could be attributed to the gains in lean tissue.

Compliance to the WBVT exercise was inconsistent; despite the novelty of the intervention, the short exercise program (15 minutes per day) and the ability to perform this in the home environment. The variable compliance to WBVT may have reflected this group's compliance to the standard lifestyle prescription and reflects general motivational issues in this age group to activities conducted in real life and unsupervised settings. An intervention such as WBVT done with relatively little effort and prescribed as an adjunct to standard lifestyle change may be viewed as a "magic cure" by those participants and may be seen as preferable to the relatively more difficult lifestyle program. Non-adherence to the lifestyle component could account for the static or increased weight and BMI measures within this group. The compliance of both groups to the standard exercise and nutritional coaching is unclear, as there was no formal method to assess daily activity and implementation of nutritional advice apart from self-report during fortnightly therapeutic contact.

In addition, to the insulin resistance state accompanying obesity, some adolescents in our study were pubertal which confers an additional state of physiological insulin resistance. This underlying process is likely to influence any examination of Si particularly long-term studies where Si may spontaneously improve as the adolescent transitions through puberty. Similarly, cardiovascular risk factors show similar variation during puberty [570]. Despite attempting to control for the effects of physiological variation by randomisation according to gender and pubertal status, it is possible that individual variation in both Si and other metabolic factors as participants progressed towards a higher Tanner stage may have influenced our primary and secondary outcome results independent of any weight change.

Furthermore, our study demographic was multicultural and we did not stratify according to ethnicity and thereby genetic variations in insulin sensitivity may not have been fully appreciated when we evaluated our results[287 571]. Consideration of ethnicity of the study population is important in weight loss trials as the literature suggest that those from Caucasian backgrounds may have more successful outcomes [572]. In an attempt to correctly identify adolescents at risk of disease progression we included adolescents with elevated fasting insulin and clinical features of acanthosis nigricans. Since our trial was conducted, the use of a single fasting insulin measure to define an insulin resistant state has been discouraged due to difficulties with interpretation during puberty and high inter-assay variability [280].

Our study confirms that cardiovascular risk factors cluster in the obese adolescent. We demonstrated a correlation (after correcting for the effects of puberty and gender) of BMI and central adiposity with Si measures, which in turn was associated with metabolic profile indicators. The interrelation between obesity, insulin resistance and adverse

cardiovascular health profile measures observed again at the end of the study with correlations showing absolute change in BMI, waist and HOMA-IR was associated with change in the serum hs-CRP. This result highlights the anti-inflammatory effects of an exercise intervention and weight loss on underlying chronic inflammatory mediators. As the lifestyle group showed positive changes in BMI, anthropometry and measures of insulin resistance, we would expect that metabolic profile indicators would also show positive changes. Indeed, we demonstrated clinically measurable but non-significant reductions in hs-CRP -2.6mg/L and ALT -4mmol/L and significant improvements in triglyceride levels -0.14mmol/L, ($p=0.036$). Insulin resistance is a pro-inflammatory state associated with high circulating level of C-reactive protein [573] and any improvements in Si and body composition should lead to an attenuation of this underlying inflammatory state as we observed.

The decreased BMI or the adoption of a healthier diet with a reduced intake of saturated fats and/or the addition of Omega-3 polyunsaturated fatty acids may in part be responsible for the change in triglyceride levels [574]. Additionally, triglycerides are involved in pathogenesis of hepatic insulin resistance, which directly influences fasting glucose levels. Reduced triglycerides in our cohort are likely due to altered nutrition habits in this cohort and weight loss in this group. Both groups received nutritional coaching and it is interesting to note that changes to the lipid profile was only observed in one group, suggesting either poor compliance to aspects of the lifestyle program or additional factors such as transition through puberty determining this metabolic profile indicator.

Participation of obese adolescents in research studies is associated with many barriers at various stages in the process. The use of the whole body vibration platform was seen as a novel intervention and the possible randomisation into this arm of the study sparked

curiosity amongst the children and their families. We approached only 48 families to obtain our recruitment target of 43, indicating that most were keen to participate. Possible reasons for the recruitment success include the fact that adolescents were already attending specialist clinics for management of obesity related co-morbidity and had significant concerns about personal health and risks of developing T2DM. In addition, many had a strong family history of obesity and metabolic disease and may have been encouraged to participate by their caregivers.

Motivating young obese adolescents to engage in physical activity programs can often be difficult due to body image issues and poor fitness levels. Many studies have shown that once involved in a research program, the dropout rates within this cohort are considerable. The VIBRATE study had a good 3 month study retention, with only 1 participant withdrawing for final testing. The low dropout rate of 2.3% is considerably less than similar Australian RCTs involving obese adolescents where attrition can amount to as much as 20-30% [575]. The male participant who withdrew from the study was older and had the highest BMI amongst the 14 males at 36.8kg/m2, it is known that in adolescent studies, adolescents with higher BMI contribute more to attrition rates [576] [577]. The low dropout rate may be related to frequent and personalised therapeutic monitoring during the progress of the study and the use of small non-monetary incentives at the completion of major testing encounters. The provision of small incentives has been found to be the most effective strategy to minimise dropout rates in this age target group [578].

The participants had contact with one study co-ordinator (KR) throughout the trial and this allowed a good rapport to be developed between the researcher and the participant/ family that may have contributed to the high retention rates during the study. Smith *et al*

2014, determined a "good relationship between facilitator and participant" as crucial to the smooth running of any research program involving overweight adolescents [579]. Research staff, play an integral role in minimising drop out during intervention studies. Allowing the adolescent to have ownership in the trial and outlining the health benefits of weight loss and the possible reduction in the risk of T2DM may improve compliance.

There were no adverse events during the trial and the novel intervention was well tolerated by all participants. To lessen unnecessary transmission of the vibration through the spine we ensured participants were correctly trained about the correct use of the platform including the adoption of a flexed training stance [580]. The positioning of the participant on the platform [393] [403], either standing upright or with flexed stance, the inclusion of rest periods interspersed with training [581] and training protocol [405], may have differential effects on bone and/or muscle parameters and therefore may have affected some of our outcome measures.

The adolescents' compliance to the vibration intervention was variable and ranged from 7-183% of predicted, indicating that some individuals were highly committed to this resistance training whereas others only used it for a couple of days during the three months. The adherence likely reflects the fact that unsupervised interventions require a highly motivated participant and or parent. It is unclear whether some of the use was by other family members as actual compliance times were extrapolated from the inbuilt compliance monitor and vibration diary entries. Similar RCT studies of a vibration intervention in adults have recommended that unsupervised interventions are not likely to produce positive results [582]. Similarly, it is difficult to determine whether the participants were compliant with the lifestyle component as this was gauged by weekly telephone contact and not direct participation in a structured observed exercise program.

Of concern, the waist circumference of these young people remained elevated well above Australian recommendations for adult measurements. Central adiposity reflects the accumulation of visceral fat deposits known to contribute to increased metabolic risk and is likely to be carried in young adulthood. In our study, change in BMI was associated with change in waist circumference, suggesting that manipulation of either weight status or height could ameliorate waist measures. Therefore weight interventions during the pubertal transition are crucial because both of these factors can be altered and lead to significant BMI decreases. Weight loss is key to ameliorating both insulin resistance and the degree of overweight and indeed is considered a cornerstone to the management as highlighted in consensus statements [280] [29]. Most research involves participation in various research programs similar to the VIBRATE study which incorporates a lifestyle program including elements of nutritional education and increased activity.

There may be several reasons as to why we did not see any change in insulin sensitivity between the two groups, including poor motivation and adherence to treatment, small sample size and short study duration. In addition, the development of insulin resistance involves a complex interplay of many organ systems as well as environmental and genetic factors. The program length may have been too short to elicit change as most WBVT studies now involve 6-9 month interventions. Both interventions in this study were given via prescription rather than directly monitored and whilst reflect "real life settings" are likely to suffer from poor adherence in this adolescent age group. Our WBVT training program and protocol may not have effectively targeted muscle training. Furthermore, since our study protocol was performed, the use of fasting insulin and glucose derived proxy measures has since been discouraged from routine use. Essentially the use of a fasting insulin level is now not recommended in clinical practice settings as there are

known difficulties with both interpretation of result and variability of assay measures. We acknowledge that this is an issue particularly when interpreting the fasting insulin and HOMA-IR results. Despite this the use of the WBISI continues in research settings and is a validated proxy measure and shows good correlation and good reproducibility with the gold standard clamp study.

This is the first randomised controlled trial to investigate effects of whole body vibration training on insulin sensitivity in an obese adolescent cohort. Although in this study we did not demonstrate any significant enhancement in insulin sensitivity on group comparison at the conclusion of the 3-month trial, there were considerable positive metabolic gains in anthropometry, insulin resistance and glucose homeostasis after a short time period in the lifestyle arm. Our study, confirms the benefit of lifestyle intervention in the management of both insulin resistance and obesity. Cumulatively the small changes that resulted within a short period of time are likely to have improved underlying metabolic health. Adherence is a major consideration when an intervention is conducted in a home based and everyday setting and therefore programs need to provide age appropriate and interesting activities in addition to establishing rapport with participants and their families.

CHAPTER 5

Does obesity or insulin resistance affect adolescent musculoskeletal health?

5.1 Introduction

The adolescent years are a crucial period for the establishment of peak bone mass [420 583 584]. Similar to obesity [585], bone health parameters established during childhood track into adulthood and may affect lifetime risk of osteoporosis [586]. Skeletal integrity and bone strength continually adapt to mechanical strain through a process of bone modelling and remodelling, this is most apparent during puberty where longitudinal growth of bone and increased muscle force render bone vulnerable [587 588]. Efforts to optimise peak bone mass during the formative years may reduce the later incidence of skeletal fractures [589 590].

Obesity in adults is associated with increased bone mineral density [591], in part due to the biomechanical loading conferred by greater weight and stronger muscle forces exerted on the skeleton [592 593]. In addition to the biomechanical factors, hormones such as leptin and oestradiol released from fat tissue may positively affect bone development [139]. Conversely, adipocytokines released from visceral adipose deposits may negatively affect bone mineralisation [594]. Independent of the above, further detrimental effects on bone accrual may occur in insulin resistance which often co-exists in the obese patient [595 596 597 598].

The effect of obesity, visceral adiposity and hyperinsulinaemia on the bone health of young people remains unclear. Some studies show that overweight in children is associated with increased bone mass [138 599] which may be related to advanced skeletal development [600 601], greater lean tissue mass [602] and the effect of hormones from adipose deposits [139]. However, other studies propose that bone mass [134] and BMD [603] are actually reduced in obese adolescents making them vulnerable to increased fractures during this period of maximal growth [135 140]. The difficulty in determining any conclusive relationship is in part due to limitations with clinical densitometry that underestimates areal BMD (aBMD) in the rapidly growing child whose bones are continually changing shape and

size [604]. Therefore any evaluation of BMD in the adolescent must consider the physiological processes of growth and puberty in addition to other external modulators.

The osteoblast, integral to bone formation, expresses insulin receptors and may play an integral role in the link between skeletal system and energy regulation [605]. The effect may be mediated by osteocalcin, a protein produced by the osteoblast [606]. Similarly, 25OHD, another integral hormone in bone and mineral metabolism has proposed roles in the development of T2DM, metabolic disease and the pathogenesis of CVD [607] [608] [609]. The metabolic and glucose regulating effects of Vitamin D may be mediated by interaction on its receptors in adipocytes [610], muscle cells [611] and by indirect effects on pancreatic insulin secretion through the activated form of Vitamin D 1,25 (OH) D2.

Lifestyle measures, incorporating dietary restriction remains central to the management of increased weight and related co-morbidities. However, weight loss achieved through these health measures may have detrimental effects on BMD [612] [613] [614]. Physical activity interventions often prescribed in conjunction with dietary modification increase BMD in both adults and children [590] [419] and may attenuate the decreases in BMD seen with weight loss [615]. The most appropriate physical activity prescription to produce weight loss, improve metabolic status and optimise peak bone mass during adolescence remains unclear.

Resistance training creates greater mechanical strain leading to higher bone deformation, repair and remodelling and greater bone accrual [616] [617]. WBVT is a resistance exercise, with an anabolic effect on bone comparable to more intense resistance training as demonstrated in obese adults [355]. The effect of WBVT on BMD related parameters in the paediatric population is inconsistent, as most studies have involved children with

underlying bone health issues and training protocols have varied. Despite this lack of clarity, some studies have demonstrated improved BMD [434] whilst others have shown functional improvements in mobility without positive bone effects [432]. The musculoskeletal effects of WBVT in overweight, insulin resistant youth is unclear.

This chapter aims to provide a cross-sectional snapshot of the bone health of obese, insulin resistant adolescents in a multi-ethnic demographic. The secondary effects of the study interventions, WBVT and lifestyle intervention were examined using clinical densitometry and pQCT. We evaluated relationships between bone parameters, bone turnover markers, Vitamin D, anthropometry and measures of insulin sensitivity to further evaluate the role of the skeleton in energy metabolism and the possible effects of body composition on the bone health.

5.2 Bone architecture

5.2.1 Dual energy x-ray absorptiometry

a. Baseline

There were no differences in DXA parameters between the intervention groups at baseline as seen in Table 5.1. There was no evidence of any underlying muscle or bone pathology with normal BMC for LTM. All DXA parameters (Total BMC and BMD, BMD legs and BMD trunk) were normal relative to weight apart from males who had low weight adjusted total BMC and BMD. DXA values for height were normal but were high compared to age matched controls. There were significant gender differences at baseline and females displayed higher z scores for several parameters including: BMC for weight and age; total BMD and regionally had evidence of higher BMD in the lower limbs and trunk for certain z-score parameters as shown in Table 5.1.

Table 5.1 Baseline DXA parameters by group and gender

	Entire Group n=42	Standard N=21	Vibration N=21	P value	Male N=13	Female N=29	P value
BMC/ LTM	-0.3 ± 2.0	-0.2 ± 2.4	-0.4 ± 1.6	0.783	-0.3 ± 2.2	-0.4 ± 1.6	0.889
Total BMC							
Age	2.4 ± 1.9	2.2 ± 2.1	2.6 ± 1.6	0.454	1.3 ± 1.1	2.9 ± 1.9	0.002**
Ht	1.0 ± 1.4	1.0 ± 1.3	1.0 ± 1.5	0.968	0.5 ± 1.6	1.2 ± -.2	0.139
Wt	-1.2 ± 1.7	-1.1 ± 1.7	-1.2 ± 1.8	0.790	-2.4 ± 1.3	-0.6 ± 1.6	0.001**
BMD total							
Age	1.8 ± 1.3	1.7 ± 1.4	1.9 ± 1.2	0.750	1.1 ± 1.0	2.1 ± 1.3	0.02*
Ht	0.5 ± 1.2	0.4 ± 1.1	0.6 ± 1.3	0.711	0.4 ± 1.4	0.5 ± 1.1	0.669
Wt	-1.6 ± 1.2	-1.6 ± 1.2	-1.5 ± 1.2	0.917	-2.0 ± 1.3	-1.4 ± 1.1	0.113
BMD Legs							
Age	2.0 ± 1.3	2.0 ± 1.4	2.3 ± 1.2	0.487	2.0 ± 1.2	2.2 ± 1.4	0.600
Ht	1.1 ± 1.3	1.1 ± 1.1	1.2 ± 1.6	0.858	1.1 ± 1.5	1.1 ± 1.3	0.987
Wt	-0.6 ± 1.2	-0.8 ± 1.1	-0.8 ± 1.3	0.997	-1.4 ± 1.2	-0.5 ± 1.1	0.02*
BMD Trunk							
Age	1.6 ± 1.4	1.5 ± 1.6	1.7 ± 1.2	0.605	1.0 ± 0.8	1.8 ± 1.5	0.028*
Ht	0.2 ± 0.4	0.1 ± 0.4	0.2 ± 0.5	0.622	0.1 ± 0.5	0.2 ± 0.4	0.552
Wt	-0.9 ± 1.4	-1.0 ± 1.6	-0.9 ± 1.1	0.947	-1.6 ± 0.8	-0.7 ± 1.4	0.01*

Data presented as age, height and weight z-scores; BMC/LTM total z score
*significant result with *p<0.05 **p<0.01 on independent samples Student t test

b. Intervention effects

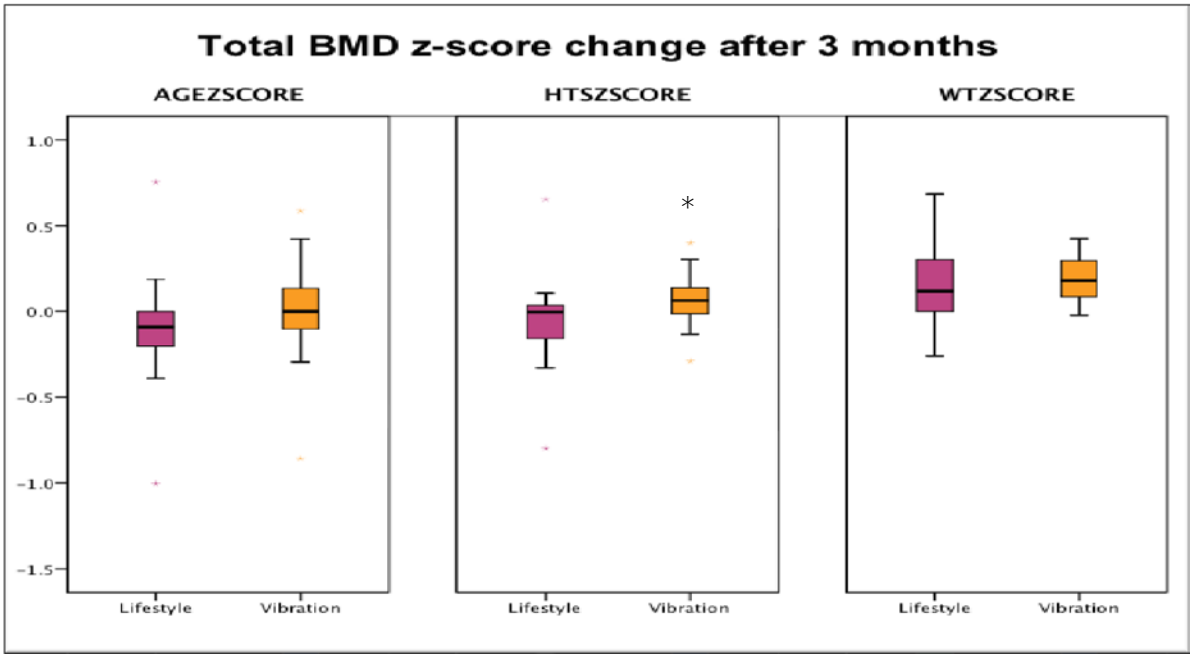
Comparing the two groups, WBVT had significant increase in total BMD height z score (p=0.021) as seen in Figure 5.1 and Table 5.2. Total BMD raw score was also increased in this group (p=0.040) after 3 months. The changes in total BMD in the WBVT group were associated with regional changes with higher BMD legs for age z score (p=0.036) compared with the lifestyle arm (Table 5.2). Notably, LTM showed clinically significant increases (588g vs. 184g) in this group after 3 months but this did not achieve statistical significance (p=0.5).

Table 5.2 DXA intervention effects after 3 months (absolute change)

	Lifestyle	Vibration	P value
N	21	21	
DXA			
Bone mineral content/ Lean tissue mass (BMC/LTM) Z-score	0.365 ± 1.15	0.302 ± 1.02	0.852
Bone mineral density (legs) – age z-score	-0.020 ± 0.34	-0.002 ± 0.33	0.036[a]
Bone mineral density (legs) – height z-score	-0.147 ± 0.32	0.033 ± 0.31	0.052
Bone mineral density (legs) – weight z-score	0.09 ± 0.305	0.22 ± 0.21	0.104
Bone mineral density (total) – raw score g/cm^2	0.006 ± 0.02	0.014 ± 0.01	0.040[a]
Bone mineral density (total) – age z-score	-0.10 ± 0.32	0.004 ± 0.29	0.300
Bone mineral density (total) – height z-score	-0.05 ± 0.25	0.07 ± 0.15	0.021[a]
Bone mineral content – raw value (g)	94.89 ± 151.54	104.61 ± 150.06	0.836
Bone mineral content – height z-score	0.090 ± 0.66	0.16 ± 0.57	0.667
Lean tissue mass (g)	184.57 ± 2142.39	588.44 ± 1678.04	0.500
Lean tissue mass/ height z-score	- 0.25 ± 0.81	-0.09 ± 0.43	0.431
Leg fat (%)	-1.03 ± 2.10	-0.75 ± 1.67	0.637
Leg lean tissue (g)	38.18 ± 726.19	411.49 ± 562.92	0.070
Total body fat (%)	-0.94 ± 2.37	-0.14 ± 1.82	0.229
Total body fat age z-score	-0.15 ± 0.32	-0.04 ± 0.25	0.217
Trunk fat (%)	-0.83 ± 3.50	0.33 ± 2.65	0.234
Trunk lean tissue mass (g)	19.76 ± 1464.49	114.1 ± 1186.84	0.820

[a] indicates a significant result

Figure 5.1 Change in BMD at the end of the study



Boxplot showing absolute change in BMD Ht z is significantly increased in the WBVT arm

*P<0.05 Student t test

c. Longitudinal within group changes

Within group changes can be seen in Table 5.3. There were contrasting regional changes in BMD. Within the lifestyle group, BMD (legs) showed decreased age p=0.016 and ht z scores p=0.047. In contrast, the WBVT group demonstrated increased BMD (legs) p<0.001 and BMD (trunk), p=0.005 for weight z-scores. Significant positive increases in total BMC (weight) in both groups (p=0.013 and 0.002, in lifestyle and vibration group respectively) were accompanied by increased total BMD (weight) after 3 months as might be expected during the pubertal growth phase.

Table 5.3 Within group changes during 3-month intervention

	Lifestyle Pre	Lifestyle Post	P value	Vibration Pre	Vibration Post	P value
BMC/ LTM	-0.2 ± 2.4	0.1 ± 2.2	0.161	-0.4 ± 1.6	-0.1 ± 1.9	0.189
Total BMC						
Age	2.2 ± 2.1	2.3 ± 1.9	0.496	2.6 ± 1.6	2.7 ± 1.9	0.390
Ht	1.0 ± 1.3	1.0 ± 1.1	0.394	1.0 ± 1.5	1.1 ± 1.6	0.213
Wt	-1.1 ± 1.7	-0.7 ± 1.9	0.013*	-1.2 ± 1.8	-0.8 ± 1.7	0.002*
BMD total						
Age	1.7 ± 1.4	1.6 ± 1.3	0.187	1.9 ± 1.2	1.9 ± 1.3	0.953
Ht	0.4 ± 1.1	0.4 ± 1.2	0.380	0.6 ± 1.3	0.6 ± 1.3	0.064
Wt	-1.6 ± 1.2	-1.4 ± 1.3	0.014*	-1.6 ± 1.2	-1.4 ± 1.2	<0.001**
BMD Legs						
Age	2.0 ± 1.4	1.8 ± 1.5	0.016*	2.3 ± 1.2	2.3 ± 1.2	0.984
Ht	1.1 ± 1.1	1.0 ± 1.0	0.047*	1.2 ± 1.6	1.2 ± 1.6	0.636
Wt	-0.8 ± 1.1	-0.7 ± 1.1	0.198	-0.8 ± 1.3	-0.6 ± 1.3	<0.001**
BMD Trunk						
Age	1.5 ± 1.6	1.4 ± 0.3	0.086	1.7 ± 1.2	1.8 ± 1.3	0.448
Ht	0.1 ± 0.4	0.1 ± 0.4	0.174	0.2 ± 0.5	0.3 ± 0.5	0.223
Wt	-1.0 ± 1.6	-0.8 ± 1.7	0.289	-0.9 ± 1.1	-0.7 ± 1.1	0.005*

Significant result *p<0.05 **p<0.001 using paired samples t test

5.2.2 Peripheral quantitative computed tomography

a. Baseline

The adolescents had normal results on pQCT. There were group differences despite equal randomisation according to gender and pubertal status. The WBVT group had significantly greater total tibial cross sectional area (raw and z-score) and higher strength strain indices (pSSI). Gender differences were seen with males having significantly stronger bones and increased cross sectional area compared with females (Table 5.4).

Table 5.4 Baseline PQCT parameters by group and gender

66% tibia site	Whole group N=41	Lifestyle N=20	Vibration N=21	Males N=14	Females N=27
Cortical BMC	307 ± 58	295 ± 64	319 ± 51	332 ± 66	294 ± 50
Cortical BMC z-score	1.1 ± 1.7	0.8 ± 1.9	1.4 ± 1.5	1.3 ± 2.8	0.9 ± 0.8
Cortical CSA (cm^2)	286 ± 49	274 ± 54	298 ± 42	313 ± 52	272 ± 42[b]
Relative CSA (cm^2)	51 ± 7	52 ± 7	50 ± 7	50 ± 7	51 ± 7
Total CSA (cm^2)	570 ± 95	536 ± 98	603 ± 80[a]	635 ± 80	537 ± 84[b]
Total CSA z-score	1.2 ± 1.6	0.7 ± 1.2	1.8 ± 1.8[a]	1.8 ± 2.4	1.0 ± 0.9
pSSI	2334 ± 530	2147 ± 563	2512 ± 439[a]	2630 ± 528	2181 ± 471[b]
pSSi z-score	2.2 ± 2.3	1.5 ± 2.2	2.8 ± 2.4	3.5 ± 3.5	1.5 ± 1.0a
Cortical vBMD (mg/cm^3)	1072 ± 46	1075 ± 49	1069 ± 45	1080 ± 47	1055 ± 42
Total vBMD (mg/cm^3)	620 ± 82	629 ± 80	611 ± 84	629 ± 79	603 ± 88
Cortical vBMD z-score	0.9 ± 1.3	1.1 ± 1.5	0.7 ± 0.9	1.4 ± 1.8	0.6 ± 0.7
CSA fat mm^2	4508 ± 1304	4471 ± 1473	4543 ± 1158	3785 ± 936	4869 ± 1325
CSA muscle mm^2	6396 ± 1101	6127 ± 1159	6651 ± 1005	6592 ± 805	6298 ± 1225

[a] significant result $p<0.05$ [b] significant result $p<0.001$ on Student t test comparing standard vs. WBVT and males vs. females
BMC bone mineral content (mg), CSA cross sectional area PSSI strength strain index vBMD volumetric bone mineral density mg/cm^3

b. Intervention effects

Cortical BMC was preserved in the WBVT arm group. The cross sectional area of fat was significantly decreased in the lifestyle group after 3 months (Table 5.5).

Table 5.5 Change in pQCT variables after 3-month interventions

Tibia 66% site	Lifestyle N=21	Vibration N=21	P value
pQCT			
Cortical bone mineral content (mg)	1.11 ± 7.46	5.21 ± 9.40	0.138
Cortical bone mineral content z-score	-0.18 ± 0.24	-0.02 ± 0.23	0.042[a]
Cortical cross sectional area (cm^2)	1.88 ± 5.33	4.12 ± 8.12	0.314
Relative cross sectional area (cm^2)	-1.31 ± 3.11	0.31 ± 2.13	0.060
Total cross sectional area (cm^2)	19.08 ± 37.58	2.51 ± 24.52	0.104
Total cross sectional area z-score	0.25 ± 0.90	-0.13 ± 0.58	0.222
PSSI strength strain index	102.84 ± 213.29	57.87 ± 88.75	0.109
PSSI strength strain index z-score	0.28 ± 1.21	0.00 ± 0.47	0.381
Cortical volumetric bone mineral density vBMD mg/cm^3	-2.72 ± 14.63	2.81 ± 9.96	0.588
Cortical volumetric bone mineral density z-score	-0.02 ± 0.53	-0.07 ± 0.27	0.344
Total volumetric bone mineral density (mg/cm^3)	-12.15 ± 37.63	3.94 ± 20.22	0.167
Cross sectional area fat mm^2	-175.69± 226.25	-16.92 ± 211.58	0.029[a]
Cross sectional area muscle mm^2	73.36 ± 489.27	153.37 ± 349.53	0.559

5.3 Bone and mineral metabolism

5.3.1 Serum markers

a. Vitamin D

Vitamin D status was similar across the intervention groups at baseline as shown in Table 5.6. Thirty-eight samples were sufficient for analysis with 5 samples being unsuitable. As a group the participants n=38 (14M) appeared 25OHD sufficient with a mean level >50nmol/L. However, on subgroup analysis, 9 participants had levels <50nmol/L: seven had mild Vitamin D insufficiency, 1 male participant had moderate deficiency and one veiled female had severe deficiency with a serum level of 12nmol/L. All participants with Vitamin D<50nmol/L had dark skin. Individuals with dark skin had significantly lower 25OHD levels, 46nmol/L versus 72nmol/L ($p<0.005$). Almost one quarter of sampled participants (n=9) was considered Vitamin D deficient according to commonly used Australian criteria [618]. The US definition identified 63% of participants as being insufficient or deficient [619].

Table 5.6 Vitamin D and bone turnover markers at baseline

Measure	Entire group	Lifestyle	Vibration	Dark N=18 6m	Fair N=25 8m	P Value Dark Vs. Fair	P value Lifestyle Vs. Wbvt
25 OHD (nmol/L) n=38	61 ± 22	56 ± 22	66 ± 21	46 ± 21	72 ± 14	P<0.005*	0.16
OCN nmol/L n=43	4.2 ± 3.1	4.1 ± 3.4	4.3 ± 3.6	3.3 ± 2.1	4.8 ± 3.6	0.12	0.78
PINP (ng/mL) n=43	447 ± 355	430 ± 377	463 ± 338	372 ± 297	500 ± 388	0.25	0.76
CTx (ng/mL) n=43	1.8 ± 1.1	1.7 ± 1.2	1.8 ± 1.0	1.4 ± 0.7	2.1 ± 1.3	0.05	0.91
Urine DPD N=37	12.6 ± 6.6	12.2 ± 6.0	12.9 ± 7.3	13.0 ± 7.7	12.3 ± 5.9	0.78	0.76

CTx (C-terminal collagen cross-linked peptide) 0.033-6.000ng/mL
DPD (free deoxypyridinoline crosslinks/creatinine): 3.0-7.4nmol/L creatinine range 4.5-26nM/mM later 6.8-40 females 3.4-38.8 young female older male 1.1-26
PINP (amino-terminal propeptide of type 1 collagen) Manufacturer assay normal range adults (2-230ng/mL)
25OHD (25-hydroxyvitaminD): sufficiency >50nmol/L
OCN (Osteocalcin) 1.7-7.7nmol/L

b. Bone formation markers

Bone formation markers OCN and P1NP showed no differences at baseline for skin colour or group. Subgroup analysis showed that males n=14 had significantly (p=0.021) higher mean serum OCN levels at baseline compared to females.

c. Bone resorption markers

Bone resorption markers serum CTX and urine DPD were not different at baseline and showed no difference between those participants with dark or light skin despite the presence of 25OHD insufficiency in 50% of participants with dark skin.

5.3.2. Urine markers

Free urine deoxypyridinoline crosslinks were assessed in relation to creatinine, giving the urine free deoxypyridinoline crosslinks/ creatinine ratio. The entire group (mean n=37) was similar to the intervention subgroups and skin pigmentation groups. Samples were

missing due to the inability to urinate for the test and some were not analysed due to contamination by red blood cells in females.

5.3.3 Intervention effects on 25OHD and bone turnover markers

There was much variability of 25OHD levels during the three-month trial period. Of those participants with complete pre/ post 25OHD data five adolescents had spontaneous improvement in 25OHD levels with four becoming sufficient after 3 months. The 25OHD levels of two participants dropped into the insufficient range at final testing. After 3 months there was no difference in 25OHD levels as shown in Table 5.7. The only bone turnover marker to show significant changes was OCN and this was decreased in the WBVT arm (p=0.024). Within the groups, bone formation was increased in the lifestyle group when osteocalcin was analysed however, P1NP did not show a similar increase.

Table 5.7 Intervention effects on Vitamin D and bone turnover markers

Marker	Lifestyle	Vibration	P value
25 OHD (nmol/L) N=38	6 (17)	-2 (12)	0.109
CTX N=42	0.1 (0.5)	-0.15 (0.7)	0.201
P1NP (ug/L) N=42	-9 (115)	-39 (155)	0.486
Osteocalcin (nmol/L) N=41	0.3 (1.1)	-0.7 (1.6)	0.024*
Urine DPD (nmol/L) N=36	4.2 (9.9)	0.4 (6.0)	0.178

*indicates significant result using independent samples Student t test p<0.05 Data presented as mean (SD)
CTx (C-terminal collagen cross-linked peptide) 0.033-6.000ng/mL
DPD (free deoxypyridinoline crosslinks/creatinine): 3.0-7.4nmol/L creatinine range 4.5-26nM/mM later 6.8-40 females 3.4-38.8 young female older male 1.1-26
PINP (amino-terminal propeptide of type 1 collagen) Manufacturer assay normal range adults (2-230ng/mL)
25OHD (25-hydroxyvitaminD): sufficiency >50nmol/L
OCN (Osteocalcin) 1.7-7.7nmol/L

5.4 Relationships between bone and insulin sensitivity

Participants with dark skin and those with 25OHD levels <50nmol/L were significantly more insulin resistant and had decreased peripheral insulin sensitivity as shown in Table 5.8. CTX was significantly increased in the light skin group suggesting increased bone turnover however urinary measures of bone resorption did not show this same trend.

Table 5.8 Relationship of baseline Vitamin D and skin colour to bone turnover and insulin sensitivity markers

Vitamin D status/ skin colour	Deficient	Sufficient	P value	Dark	Light	P value
	9 (1M)	29 (11M)		18 (6M)	25 (8M)	
25OHD (nmol/L)N=38	37 (12)	68 (18)	P<0.01**	46 (21)	72 (14)	<0.01**
HOMA-IR N=43	9.8 (2.0)	7.1 (2.2)	0.002**	8.6 (2.2)	6.9 (2.4)	0.021*
Fasting insulin (pmol/L) N=43	250 (48)	188 (50)	0.003**	227 (53)	186 (56)	0.022*
WBISI N=43	1.24 (0.6)	1.8 (0.8)	0.042*	1.37 (0.5)	2.00 (0.9)	0.012*
Fasting glucose (mmol/L) N=43	5.3 (0.5)	5.0 (0.4)	0.102	5.1 (0.4)	5.0 (0.5)	0.242
Osteocalcin (nmol/L) N=43	3.4 (2.2)	3.9 (2.4)	0.544	3.3 (2.1)	4.8 (3.6)	0.116
P1NP (ug/L) N=43	382 (336)	416 (268)	0.760	372 (297)	500 (388)	0.248
CTX (ng/L) N=43	1.3 (0.8)	1.8 (1.0)	0.138	1.4 (0.7)	2.1 (1.3)	0.032*
Urine DPD N=37	16.0 (8.7)	11.5 (5.5)	0.099	13.0 (7.7)	12.3 (6.0)	0.776

* p<0.05 **p<0.01 Independent samples student t test
Fasting glucose normal <5.6mmol/L, fasting insulin <90pmol/L
CTx (C-terminal collagen cross-linked peptide) 0.033-6.000ng/mL
DPD (free deoxypyridinoline crosslinks/creatinine): 3.0-7.4nmol/L creatinine range 4.5-26nM/mM later 6.8-40 females 3.4-38.8 young female older male 1.1-26
PINP (amino-terminal propeptide of type 1 collagen) Manufacturer assay normal range adults (2-230ng/mL)
25OHD (25-hydroxyvitaminD): sufficiency >50nmol/L
OCN (Osteocalcin) 1.7-7.7nmol/L

5.4.1 Correlations and regression models

Table 5.9 DXA correlations between body composition and anthropometry

Variables	BMC (raw)		BMD (raw)		BMD/Ht	
	R	p	R	p	R	p
Age	0.528	<0.001**	0.723	<0.001**	0.411	0.007*
Height	0.726	<0.001**	0.486	0.001*	-0.198	0.209
Weight	0.328	0.012*	0.594	<0.001**	0.289	0.063
Waist	-0.092	0.560	0.220	0.162	0.152	0.335
W/H ratio	0.425	0.005*	-0.02	0.901	0.232	0.140
BMI	-0.041	0.796	0.372	0.012*	0.479	0.001*
Puberty	0.566	<0.001**	0.670	<0.001**	0.359	0.020*
% LTM	0.309	0.046*	0.252	0.107	0.051	0.751
Gender	0.145	0.359	0.061	0.699	-0.068	0.669

Spearman's correlation coefficients against anthropometry and body composition
* significant <0.05 **p<0.001

Correlations were performed between anthropometry, body composition and selected DXA variables (Table 5.9). Bone mass as determined by raw BMC score showed strong positive correlation with height and moderate positive correlations with age, pubertal status and weak positive correlations with %LTM and weight. BMD raw score demonstrated strong positive correlations with age and pubertal status; moderate positive correlations with weight and height and weak correlation with BMI. BMD when adjusted for height z score only showed moderate positive correlations with age and BMI with weaker positive association with pubertal status.

5.4.2 Partial Correlations

Table 5.10 Partial correlations between raw values of BMD and BMC and selected measures of insulin sensitivity and anthropometry

Variable Controlling for age, height, weight, puberty and gender code	BMD (raw)	BMC (raw)
Waist	-0.314	-0.413*
WtHR ratio	-0.308	-0.414*
CRP	-0.137	0.040
Insulin	-0.111	-0.286
WBISI	0.159	0.194
HOMA-IR	0.023	-0.045
%Sedentary time	0.247	0.117

*p<0.05 significant result

Regression analysis

Regression models to examine the effect of the independent variables of age, Ht, weight, WtHR, pubertal status, BMI, %LTM, waist, WBISI and BMI z-score on bone measures of BMD and BMC were calculated (Table 5.11). Significant predictors for BMC (height) included waist, BMI at baseline and absolute weight adjusted R^2=0.495, P<0.001.

Table 5.11 Regression models for explaining variance in BMD and BMC for height

		BMD height z-score						BMC for height z-score			
Model	Variable	Coefficient ß±s.e.	95% CI	P	R^2	Model	Variable	Coefficient ß±s.e.	95% CI	P	R^2
1	BMI	0.10 (0.04)	0.024 to 0.174	0.01**	0.16	1	Waist	-0.04 (0.02)	-0.08 to -0.001	0.047*	0.10
2	BMI	0.22 (0.05)	0.12 to 0.32	<0.01**	0.36	2	Waist	-0.12 (0.03)	-0.018 to -0.07	<0.01	0.41
	Waist	-0.07 (0.02)	-0.12 to -0.03				BMI	0.25 (0.06)	0.13 to 0.36		
3	BMI	0.25 (0.05)	0.15 to 0.35	<0.01**	0.44	3	Waist	-0.10 (0.03)	-0.15 to -0.05	<0.01	0.50
	Waist	-0.07 (0.021)	-0.11 to -0.02				BMI	0.32 (0.06)	0.20 to 0.45		
	%LTM	0.05 (0.02)	0.01 to 0.10				Weight	-0.05 (0.02)			

To examine whether an interaction occurred between waist, BMI, weight and bone variables, an interaction term was calculated waist*BMI, to reduce collinearity, centred variables were calculated for the product and the individual variables. The individual

variables remained significant but the product of the variables was not significant, suggesting no interaction. Similarly, waist and BMI were also associated with BMD (height) z-score, however %LTM was an additional predictor in this model adjusted R^2=0.435, p<0.0001. Proxy measures of insulin sensitivity were not predictors in any models.

5.5 Discussion

We examined the longitudinal effects of clinical insulin resistance and obesity on the bone health of adolescents at baseline and in response to two separate interventions over three months. Cross-sectional analysis revealed that participants as a group had higher total aBMD and BMC compared to same age peers but normal aBMD for height and weight with the exclusion of males who had low BMC/aBMD adjusted for weight. The increased aBMD and BMC for age could be attributed to early puberty and skeletal advancement in obese adolescents [620] due to higher circulating IGF-1, oestradiol and leptin levels [600 601]; with further maturational influences from hyperinsulinaemia [621]. In this study we did not perform contemporary bone age x-rays or measure serum IGF-1, oestradiol or leptin but we did show strong correlations between BMC and height; aBMD (raw score) with age/ puberty and BMD (Ht) with age/BMI and can infer that these hormones were actively involved.

At baseline, there were gender differences between total BMC and BMD (age and weight) with pubertal females demonstrating higher BMC and BMD compared with their male counterparts. This difference is likely due to the fact that boys accrue more bone and lean tissue in the post-pubertal period [622] under the influence of testosterone [623]. Furthermore, bone mineral accrual is closely aligned with the attainment of peak height velocity and this is known to occur earlier in females [620 624]. Faulkner *et al* [625] estimated

maximal growth around 12.67± 0.99 years in females and 14.14 ± 1.05 years in males. The mean age of males and females in our study was 13.8 ± 2.0 and 13.1 ± 2.1 years respectively suggesting that most females in our study had already attained peak growth velocity whilst this was yet to occur for the males. Further evidence of sexual dimorphism was evident with males displaying decreased BMC and BMD for weight with z-scores -2.4 and -2.0 respectively, demonstrating variable effect of increasing adiposity between the sexes during puberty [626], with bone mineral accrual in females being greatly influenced by the secretion of fat derived hormones such as oestradiol and leptin [623 627 628]; with excess adiposity being less important to bone mineralisation in males [623 629]. In males particularly, the relatively low bone mass for weight may contribute to an increased fracture risk with relative skeletal weakness as bones increase in area but are incompletely mineralised [135 625]. During normal pubertal development, there is often a lag between the BMC accrual with increases in muscle accompanied by subsequent increases in bone development [625] and in obese children, concomitant rapid increases in weight may result in increased porosity and decreased strength with failure to compensate for increased loading [134]. This lag in bone BMC accrual may contribute to the observed increased risk of fractures during adolescence [630].

As bone is primarily affected by changes in lean tissue mass, it fails to adequately adapt to an increase in biomechanical loading conferred by excess adiposity, which doesn't participate in muscle bone interaction. Bone mineral content for lean tissue mass (BMC/ LTM) was normal for the group and both genders at baseline indicating that there was no underlying bone or muscle pathology.

Central to our hypothesis was that WBVT might lead to an increase in LTM and thereby enhance peripheral insulin sensitivity through non-insulin mediated glucose uptake in the

muscle. The resistance activity was efficacious, with substantial increases in LTM, amounting to almost 600g and significantly higher BMD parameters including: total aBMD (raw and height) and legs aBMD (age) albeit without associated change to insulin sensitivity. This result reinforces the importance of the muscle bone interaction [421] [587] and is consistent with studies demonstrating the influence of LTM on BMD in both obese [134], insulin resistant [631] and normal weight children [632]. Our regression model also confirmed %LTM as an important predictor of BMD together with waist circumference and BMI. Waist, BMI and weight were important predictors of BMC.

WBVT has been shown to alter body composition [445], muscle mass and function [395] [633-635] and BMD. However in older adults where this intervention has been mostly studied, there exists only weak evidence for an effect on bone and muscle parameters [386] [393] [394]. Determining the actual nature of the effect of WBVT is difficult due to the variability in study protocols and diversity of populations studied [393], particularly in children where most WBVT trials have been undertaken in those with disabling medical conditions [636] [637] with few results in healthy children [430]. The mixed results we observed on bone and muscle parameters could be due to the WBVT protocol which included: a flexed knee stance; 3 rest periods and higher vibration frequency ranging from 18-24 Hz. The adequate intervention period for WBVT has been suggested to be at least 8 weeks' duration with frequency settings between 20-30Hz [393]. The addition of rest periods into the training program has been shown to enhance the osteogenic effect on the bone [581] whilst the flexed knee positioning is thought to maximises resistance training effects to the muscle by dampening transmission to the spine [638].

We included physical activity and dietary coaching in our study with the aim of improving insulin sensitivity through weight loss and body composition change. The

lifestyle arm performed an aerobic based intervention and demonstrated significantly decreased BMI and fasting glucose, clinical improvements in Si and significant increases in OCN, a marker of bone formation. This result is consistent with paediatric [604 639] and adult studies [640 641] which have shown that weight loss improved Si and increased total OCN levels, suggesting a possible role of OCN in energy regulation [642-644]. Simple correlations between OCN, WBISI and fasting glucose were weak correlations and may be due to measurement of total rather than undercarboxylated osteocalcin (uOCN), which is thought to be more active in glucose regulation [645 646]. Despite this, selected studies have indicated an association between total OCN with Si [641 647] and metabolic markers [648 649 650]. Unlike other studies [391] the increased BMD in the WBVT group could not be accounted for by a decrease in bone turnover with no significant differences in bone resorptive measures. There was no association between BMC or aBMD and proxy markers of insulin sensitivity controlling for height, weight, age, gender or pubertal status.

Analysis of the bone turnover markers may have been affected by: sample degradation; the high within person variation of up to 12% for the serum and urine markers of bone turnover [651]; variation of OCN with age [652], gender and growth [653] and fasting status [654]. OCN is highly variable in puberty and mirrors peak growth velocity, which is gender specific [653 655].

Recent evidence suggests that an increased waist circumference in adolescents may adversely affect BMC and BMD [656 657]. We found that increased waist circumference and WtHR ratio in our participants was negatively associated with raw BMD and BMC after controlling for the effects of (age, height, puberty and gender) and any interaction effects, possibly due to pro-inflammatory adipocytokines produced by visceral adipose deposits [594 658 659] and decreased osteoblast proliferation in those with insulin resistance [660].

Furthermore, we showed that increased total and central adiposity was associated with lower circulating 25OHD, a hormone integral to bone and mineral metabolism.

Areal BMD may be overestimated in young obese children due to larger bones. To overcome this limitation, we investigated bone parameters with pQCT at the 66% site of the tibia. We found that males had evidence of larger and stronger bones. Cortical BMC was preserved in adolescents who undertook the vibration intervention but was reduced in the lifestyle group who also showed a significant decrease in BMI z-score [661] which may more accurately represent subtle changes in fat loss rather than DXA soft tissue analysis [564]. The preservation of BMC could be attributed to the resistance training effects of the WBVT with enhanced muscle bone interaction or the relative loss of BMC in the lifestyle group, who also demonstrated significant changes with a reduction in both BMI and leg fat cross-sectional area on pQCT. In adults, weight loss induced by dietary restriction is associated with decreased BMD [662] [663] [664] but may be attenuated with the addition of an exercise intervention [665] and the possible effect of exercise on the muscle bone unit with increased bone turnover. The decreased fat cross sectional area in the lifestyle group may be secondary to aerobic enhanced fat oxidation in this group who received an aerobic based activity prescription [666]. These regional changes within the muscle may in part account for the enhanced peripheral Si within the lifestyle arm.

In line with Australian findings, adolescents in our study who had dark skin were more likely to be 25OHD insufficient [667]. Individuals who were 25OHD deficient and/or had dark skin were significantly more insulin resistant at baseline. This result could be secondary to sequestration of 25OHD in adipose tissue [668], an increased risk of insulin resistance and T2DM in ethnic minority groups with darker skin, higher sedentary behaviours with less sun exposure or a direct interaction of 25OHD with glucose and

metabolic health independent of body composition [458 607 669-671 672] and seasonal variation. Of the obese and overweight participants sampled at baseline, 24% presented with 25OHD deficiency. It is difficult to ascertain prevalence rates amongst adolescents but apparent deficiency may be due to the higher adiposity in the obese [673] with biochemical Vitamin D deficiency in up to 50% of severely obese children [674].

Acanthosis nigricans (AN) was an inclusion criterion for our study; this cutaneous marker of clinical insulin resistance has been associated with lower 25OHD levels in those with increased BMI [675]. However, despite our findings, the role of Vitamin D in the metabolic syndrome and evolution of T2DM remains unclear [461]. The optimal value of 25OHD for extra-skeletal benefit is unknown and is confounded by variables such as increased adiposity, sunlight exposure and dietary variables that affect both metabolic risk and circulating 25OHD levels [462]. However, some studies have indicated that higher than currently recommended levels may be required to produce changes to insulin sensitivity [676] and that supplementation of 25OHD might produce similar HOMA-IR reductions to metformin treatment [677].

PQCT analysis did not reveal any gender differences with regard to cortical BMD; cortical or total vBMD but had stronger bones with larger CSA compared with females. This may be related to lean tissue gains during puberty [678] and known gender morphological differences with higher pQCT parameters of strength and bone cross section in males [488 679].

Obesity presents a significant mechanical challenge to the musculoskeletal system of adolescents. Fracture rates in this population are higher and the presence of visceral adiposity and complications of the metabolic syndrome such as hyperinsulinism may

further compromise bone geometry at a time when accrual of peak bone mass is vital. Knowledge about the effects of Vitamin D, exercise, weight loss and bone mediators of glucose homeostasis is integral to optimise both skeletal and metabolic outcome and to reduce immediate and later fracture risk.

Paediatric recommendations suggest that BMI should be targeted to manage co-morbidities such as insulin resistance however weight loss is known to be detrimental to BMC and BMD in adults. Therefore, incorporating activities such as resistance training, known to be more osteogenic to bone would be an important non-pharmacological strategy to attenuate any bone loss that might occur with these measures.

The aBMD and BMC values when adjusted for height were normal in this study. Therefore, aBMD and BMC comparisons made for height and evaluation of total BMC may provide a better indicator of bone health in obese children who are likely to attain peak height velocity at an earlier age due to accelerated skeletal development [680].

There are differing opinions regarding optimal Vitamin D intake at various ages and no universal agreement regarding the optimal serum concentration of 25-hydroxyvitamin D for maintenance of bone health and possible extra-skeletal health benefits. Recent consensus guidelines have been published which recommend at 25OHD level >50nmol/L to prevent nutritional rickets in young people [466].

Our results show an association between Vitamin D levels and measures of insulin resistance. However, 25OHD is likely to be sequestered in excess adipose tissue of the obese adolescent and may not be completely bioavailable for bone and mineral metabolism. Optimising Vitamin D levels in obese adolescents may prove an adjunctive

treatment to lifestyle intervention but further prospective studies in obese children need to be conducted. We have demonstrated that lean tissue and bone mineral density can be improved within a short period of time suggesting that WBVT may be an efficient way of producing resistance-training effects with minimal cardiovascular impact in untrained, obese adolescent who often lacks motivation for change.

CHAPTER 6

Are there limitations to exercise in the obese insulin resistant adolescent?

6.1 Introduction and Aims

Regular physical activity in adults can prevent T2DM [323], improve metabolic profile indicators [307] and can benefit skeletal health [552]. There remains however, a paucity of research into the cardio-metabolic benefits of exercise in overweight children [308], but it is clear that aerobic exercise [321] in particular, plays a central role in weight management in children [14 681]. Current data in obese adolescents suggest that insulin sensitivity can be enhanced with [682 683] or without significant body composition change [328 345 551], with greater effect in the older adolescent with more severe insulin resistance [320].

The exact physical activity prescription to halt the progression to T2DM in at risk obese children remains unclear. In the paediatric population, aerobic based activities have been traditionally incorporated into lifestyle programs with positive effects on weight loss, body composition and cardiorespiratory fitness [321]. In addition to weight loss [154], aerobic activities can increase cardiorespiratory reserve [543], which is independently associated with greater insulin sensitivity [684]. Resistance-training activities, once thought to be detrimental to the growing skeleton are gaining popularity as an exercise modality in the obese adolescent with recognised effects on muscle strength [685-687]. Strength activities increase lean tissue and may enhance metabolism within muscle resulting in greater glucose uptake [256 338] that may prove a useful adjunct in the management of paediatric insulin resistance.

There are many barriers to the active participation of obese children in physical activity including but not limited to: poor levels of physical fitness and decreased cardiorespiratory reserve, motivational factors, poor muscle fitness and strength [33]. Exercise capacity in children, may be further compromised, by the pathophysiological effects of underlying hyperinsulinaemia [341 688]. In obese adolescents with greater lean

tissue, strength exercises may increase self-esteem and confidence for further physical activities, as they are likely to outperform normal weight peers.

In this chapter, we present the baseline cardiorespiratory profile of the obese insulin resistant adolescent and examine any effect on exercise ability after a 3 month aerobic and strength program which incorporated the novel resistance technique of WBVT. To further explore the possible limitations to exercise in these youth, we examined muscle strength and power using Mechanography, a dynamic research tool developed to test parameters of motor function during activities of daily living.

6.2 Cardio-respiratory fitness

Baseline

All participants (n=43) underwent treadmill fitness testing at baseline. There were no significant differences between the intervention groups for all measures of cardiorespiratory fitness, anthropometry, metabolic profile indicators or proxy markers of insulin sensitivity at the start of the trial (Table 6.1). The average $VO2_{peak}$ (mL/kg/min) was low representing around 85% predicted for age and gender. Of note, $VO2_{peak}$ adjusted for LTM produced results comparable to healthy peers on average 63.7mL/kg_{LTM}/min. Duration of the exercise testing was short and most participants were not able to exercise beyond ten minutes. Females had significantly higher anaerobic threshold and achieved better VO2% predicted, averaging almost 88% of expected values, suggesting better CRF. There was no difference between VO2 adjusted for LTM between the genders (Table 6.2).

Table 6.1 Baseline and final measures of cardiorespiratory fitness, anthropometry & metabolic profile

	Whole group		Lifestyle		Vibration	
Cardiorespiratory fitness	**Pre N=43**	**Post N=39**	**Pre N=22**	**Post N=20**	**Pre N=21**	**Post N=19**
Exercise time (min)	8.6 (1.8)	9.1 (2.2)[a]	8.6 (2.0)	9.0 (1.5)[a]	8.7 (1.5)	9.3 (2.2)
$VO2_{peak}$	26.8 (7.0)	27.6 (8.0)	26.8 (8.2)	27.6 (8.9)	27.0 (6.3)	27.6 (7.3)
$VO2_{peak}$ ($mL/kg_{LTM}/min$)	63.7 (22.5)	65.3 (22.5)	66.3 (22.7)	67.7 (23.1)	61.1 (22.5)	63.0 (22.3)
$\%VO2_{peak}$ predicted	84.4 (20.8)	86.8 (25.0)	87.3 (20.0)	86.7 (20.2)	81.7 (21.7)	87.0 (29.4)
Anaerobic Threshold	55.5 (13.1)	47.2 (13.1)[b]	57.7 (12.0)	46.8 (12.0)[a]	53.5 (14.0)	47.7 (13.8)
Anthropometry	**Pre N=43**	**Post N=42**	**Pre N=22**	**Post N=21**	**Pre N=21**	**Post N=21**
BMI z-score	2.1 (0.4)	2.1 (0.44)[b]	2.1 (0.5)	2.0 (0.5)[a]	2.1 (0.4)	2.0 (0.4)
BMi kg/m^2	31.5 (5.1)	31.0 (5.4)[b]	31.1 (5.9)	30.3 (6.2)[a]	31.6 (4.2)	31.5 (4.5)
Weight (kg)	81.9 (15.2)	81.9 (16.0)	81.6 (19.1)	79.4 (18.9)	83.4 (11.1)	84.3 (12.3)
Waist height ratio	0.63 (0.1)	0.61 (0.1)[b]	0.64 (0.1)	0.61 (0.1)[a]	0.63 (0.1)	0.62 (0.1)[a]
Metabolic Indicators	**Pre N=43**	**Post N=42**	**Pre N=22**	**Post N=21**	**Pre N=21**	**Post N=21**
DBP z-score	1.1 (0.9)	0.9 (0.9)	1.2 (0.9)	1.0 (1.0)	0.9 (0.9)	0.7 (0.8)
SBP z-score	0.4 (1.3)	0.4 (1.2)	0.6 (1.3)	0.4 (1.0)	0.2 (1.3)	0.4 (1.4)
HOMA-IR	7.6 (2.5)	7.8 (4.3)	7.7 (2.8)	6.9 (4.6)	7.6 (2.1)	8.6 (3.7)
Fasting glucose (mmol/L)	5.0 (0.4)	4.9 (0.5)[a]	5.1 (0.5)	4.8 (0.6)[a]	4.9 (0.3)	4.9 (0.4)
Fasting insulin (pmol/L)	202 (58)	212 (102)	199 (62)	189 (105)	206 (56)	236 (95)
WBISI	1.8 (0.8)	1.9 (0.9)	1.8 (0.9)	2.1 (0.9)	1.6 (0.8)	1.7 (0.9)

[a] indicates significant difference $p<0.05$ between pre and post training values within each group
[b] indicates significant difference at <0.01 level

Table 6.2 Cardiorespiratory and mechanography measures at baseline

	Male (n=14)	Female (n=29)
Age (years)	13.8 (2.0)	13.1 (2.1)
Height (cm)	165.3 (9.0)	160 (8.8)
Weight (kg)	82.7 (15)	82.4 (16.1)
BMI (kg/m2)	30.2 (4.2)	32.2 (5.4)
BMI z-score	2.1 (0.5)	2.2 (0.4)
Body fat%	42.8 (8.6)	46.3 (6.2)
$VO2_{peak}$	29.0 (8.1)	25.7 (6.7)
VO2 % predicted	72.0 (20.2)	87.8 (20.5)[a]
$VO2_{peak}$ ($mL.FFMkg^{-1}.min^{-1}$)	64.7 (21.1)	63.3 (23.4)
Anaerobic threshold %	44.4 (11.2)	59.4 (11.7)[a]
Exercise time (min)	9.1 (2.0)	8.4 (1.7)
Muscle force (N) m1LJ	2.0 (0.3)	1.9 (0.4)
Muscle power (W) s2LJ	3.0 (0.9)	2.5 (0.6)
Power efficiency % s2LJ	84.2 (17.8)[a]	74.5 (12.2)
Maximal power relative to weight (w) s2LJ	36.5 (11.9)[a]	30.8 (5.7)
$Fmvrel_{m1LJ}$ (g) m1LJ	2.5 (0.4)	2.3 (0.3)

[a]indicates significant difference males and females ($p<0.05$)
$VO2_{peak}$ peak oxygen uptake during fitness test, FFM fat free mass
$Fmvrel_{m1LJ}$ Maximal voluntary force relative to body weight N Newton W watt

Final

At the completion of the trial, results were available for n=39 participants (1M withdrew from trial, 2 M and 1 F declined testing). Results are presented in Table 6.2 for the participants who completed the study. The lifestyle intervention was associated with positive change in anthropometry (decreased BMI, BMI z-score and WtHR ratio) with decreased fasting glucose. This group was also able to exercise longer and showed significant decrease in anaerobic threshold. The WBVT was only associated with a change to central adiposity with decrease in the WtHR ratio. On group comparison, there were no differences for any of the cardiorespiratory parameters (Table 6.3).

Table 6.3 Absolute change in cardiorespiratory parameters in both intervention groups after 12 weeks

Parameter	Lifestyle	Vibration
N = 39	**19**	**20**
$VO2_{peak}$ (mL/min)	0.7 (5.5)	0.6 (5.9)
$VO2_{peak}$ (% predicted)	-0.6 (16.5)	5.3 (23.9)
$VO2_{peak/LTM}$ (mL/kg_{LTM}/min)	0.3 (9.5)	1.1 (12)
Exercise time (sec)	26.2 (55.8)	33.5 (118.2)
Anaerobic threshold %	-10.9 (17.1)	-5.8 (15.8)
N=42	**21**	**21**
BMI z-score	-0.1 (0.1)*	-0.02 (0.1)
Waist height ratio	-0.02 (0.02)	-0.01 (0.01)
BMI kg/m^2	-0.8 (1.2)	-0.1 (1.0)

Data presented as mean (SD) *indicates a significant result p<0.05

6.3 Mechanography

Baseline

Pre/ post mechanography data can be seen in Table 6.4. All participants (n=43) performed mechanography at baseline with no significant differences between intervention groups. At the end of the trial, complete data was available for 41 participants (1 M withdrew from trial, 1 M declined testing).

Although absolute muscle force and power was similar between the genders, males had higher muscle efficiency and were able to generate more power per unit of weight (Table 6.2 above). Both arms of the study had poor muscle function highlighted by the low force and power EFI SDS scores during the s2LJ. Whilst, higher forces were generated with eccentric muscle contraction during the m1LJ, the peak force SDS score during the m1LJ was -2.7 SDS in both groups indicating very low muscle force capability. Absolute muscle force was strongly association to weight R=0.746, with direct increases in hopping force as the weight of the adolescent increased (Figure 6.1).

Final

At the end of the study, the lifestyle group, showed a decrease in body weight and BMI z-score and this was accompanied by increased power EFI_{SDS} (4% Lifestyle vs. 1.5% WBVT) and significant increase in Pmaxrel compared to WBVT at the end of the study; both of these measures are sensitive to weight changes. Other changes to muscle force and power parameters were negligible between and within the groups.

Table 6.4 Mechanography results for intervention groups at baseline and at completion

	Lifestyle		**Vibration**	
	Pre N=22	**Post N=21**	**Pre N=21**	**Post N=20**
Single Two-Leg Jump				
Muscle force (kN)	1.86 (0.5)	1.88 (0.4)	2.0 (0.3)	2.0 (0.7)
Force EFI $_{SDS}$	-1.4	No change	-1.8	No change
Muscle power (w)	2.60 (0.8)	2.64 (0.8)	2.7 (0.7)	2.61 (0.5)
Power EFI %	77.5 (18)	80.3 (13)	77.5 (11)	84.3 (12)
Power EFI $_{SDS}$	-1.8	Gain 4%	-2.0	Gain 1.5%
Pmaxrel (w)[a]	32.5 (10)	33.6 (10)	32.8 (7)	32.4 (7)
Multiple One-Leg Jump				
MVF (g)	2.4 (0.4)	2.4 (0.4)	2.4 (0.2)	2.4 (0.4)
Peak force SDS	-2.7	No change	-2.7	Loss 1.5%

All SDS data comparisons kindly provided by Dr Rainer Rawer (Novotec Medical GmbH)
MVF maximal voluntary force during one legged jump, peak ground reaction force in relation to body weight
EFI_{SDS} Efficiency SDS normalised to age and gender matched controls
[a] indicates significant between group difference $p<0.05$

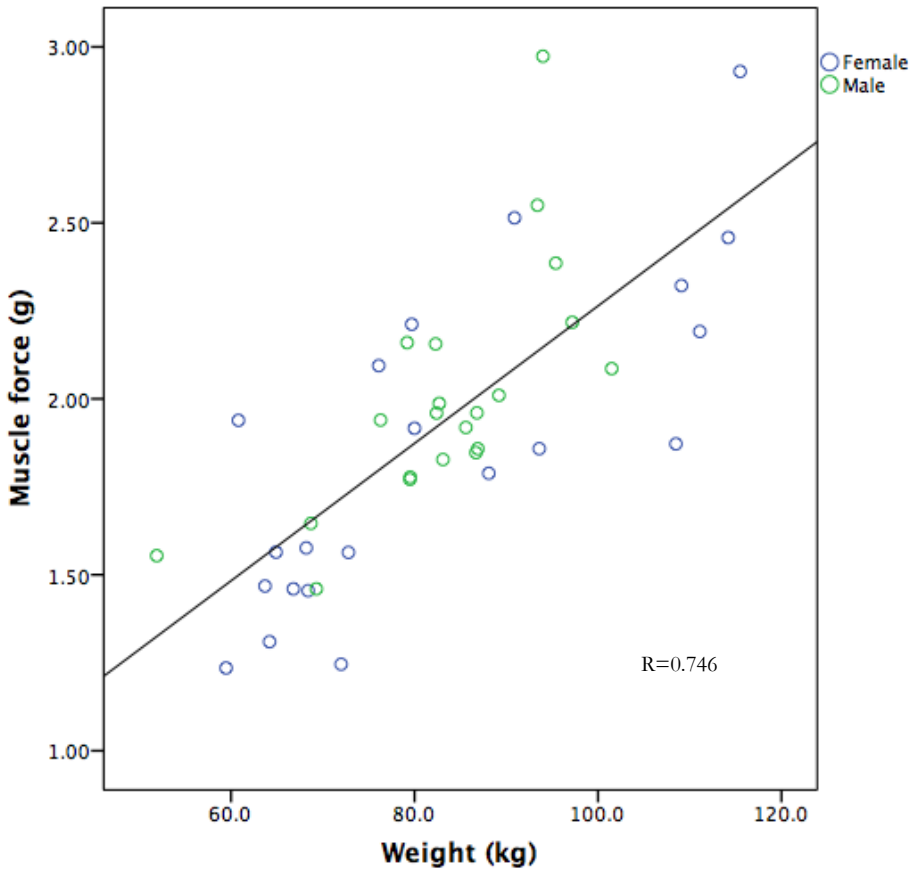


Figure 6.1 Maximum ground reaction force s2LJ in relation to body mass (kg)
Green circles (males) and blue circles (females) and mean linear fit.

6.4 Correlations between fitness, mechanography and metabolism

Duration of exercise showed strong positive correlation to overall fitness capacity, VO_{2peak} (Table 6.5) and functional muscle measures of relative power and efficiency. Relative muscle force per kg body weight, demonstrated moderate positive correlation to cardiorespiratory parameters. Similarly, VO_{2peak} displayed strong to moderate correlations with mechanography measures. Increased weight negatively affected endurance and cardiopulmonary fitness.

Table 6.5 Correlations between muscle parameters, vitamin D and cardiorespiratory fitness at baseline (N=43)

Pearson's Correlation *r*	$VO2_{peak}$	$VO2_{peak}$ %	Anaerobic Threshold	Muscle Force (kN)	Muscle Power (w)	Power EFI %	Weight (kg)	Pmaxrel (w)	FMVrel (g)
Ex. Time	0.699**	0.459**	-0.045	-0.095	0.253	0.654**	-0.483**	0.628**	0.502**
$VO2_{peak}$		0.754**	-0.132	-0.272	0.052	0.530**	-0.624**	0.567**	0.556**
$VO2_{peak}$ %	0.754**		0.266	-0.252	-0.052	0.306*	-.0490**	0.351*	0.359*
Anaerobic Threshold	-0.132	0.266		-0.058	0.027	0.127	0.063	-0.019	-0.166
25OHD (n=38)				0.081	0.147	0.207		0.368*#	0.423**

**Correlation is significant at 0.01 level (2-tailed) *Correlation is significant at 0.05 level (2-tailed)
Power EFI Power efficiency, VO_2 maximal uptake 0_2 at exercise peak Pmaxrel maximal power for kg body weight FMVrel maximal force for kg body weight 25OHD Vitamin D nmol/L # Spearman's rho

On simple bivariate correlations (Table 6.6), exercise capacity as measured by VO_{2peak} appeared to negatively correlate with several markers of glucose homeostasis including fasting glucose and HOMA-IR; a positive association was seen between WBISI and VO_{2peak}. To further explore this relationship and to determine possible confounding by BMI, puberty, age and central adiposity, further partial correlations (Table 6.7) were performed controlling for these variables. The previously seen association between indicators of glucose sensitivity was not seen, however an association between fasting

glucose and %anaerobic threshold was discovered. Relative muscle power and force (adjusted per kg body weight) were both positively correlated with baseline 25OHD levels.

Table 6.6 Correlations between insulin sensitivity and cardiorespiratory fitness

	Insulin	Glucose	HOMA-IR	WBISI	Ex time	$VO2_{Peak}$	Anaerobic Threshold	$VO2_{peak}$ LTM
Insulin		0.373*	0.965**	-0.840**	-0.252	-0.277	0.080	0.264
Glucose	0.373*		0.592**	-0.434**	-0.094	-0.317*	0.197	0.285
HOMA-IR	0.965**	0.592**		-0.827**	-0.244	-0.302*	0.118	-0.281
WBISI	-0.840**	-0.434**	-0.827**		0.297	0.360*	-0.003	0.317*
Ex time	-0.252	-0.094	-0.244	0.297		0.699**	-0.045	0.641**
$VO2_{peak}$	-0.277	-0.317*	-0.302*	0.360*	0.699**		-0.132	0.927**
Anaerobic Threshold	0.080	0.197	0.118	-0.003	-0.045	-0.132		-0.150
%Fat	-0.206	-0.302	-0.019	0.128	-0.135	-0.172	-0.076	-0.320**

**Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed)

Table 6.7 Partial correlations between insulin sensitivity and cardiorespiratory fitness

Partial correlations		Exercise time	$VO2_{peak}$	Anaerobic threshold	WBISI	HOMA-IR
Controlled variables **BMI, gender, waist, puberty**	**Ex time**		0.460**	-0.037	0.013	0.040
	$VO2_{peak}$	0.460**		-0.066	0.058	0.003
	Anaerobic Threshold	-0.037	-0.066		-0.122	0.174
	WBISI	0.013	0.058	-0.122		-0.848**
	HOMA-IR	0.040	0.003	0.174	-0.848**	
	Glucose	0.259	-0.017	0.318*	-0.381*	0.485**
	Insulin	-0.030	-0.031	0.119	-0.851**	0.967**

**Correlation is significant 0.01 level (2-tailed) *Correlation is significant 0.05 level (2-tailed)

6.5 Discussion

We investigated the effects of a lifestyle and strength intervention on the cardiorespiratory fitness, muscle performance, body composition and insulin sensitivity of obese, insulin resistant adolescents. The 3-month lifestyle intervention led to improved anthropometry: BMI -0.8kg/m^2 (-2.6%), BMI z-score -0.1 (-4.8%) and waist/ height ratio -0.03 (-4.7%) which was accompanied by clinical improvement in measures of insulin sensitivity: WBISI 1.8 to 2.1 (+0.3, 16.7%); HOMA-IR 7.7 to 6.9 (-0.8, +10.4%) and fasting glucose 5.1mmol/L to 4.8mmol/L (-0.3,-5.9%; $p<0.05$). Consistent with contemporary guidelines during the VIBRATE study, both groups were prescribed 3-4 sessions (45-60minutes) of moderate intensity aerobic based physical activity per week. The magnitude of the BMI z-score reduction is in keeping with recent systematic reviews and meta-analyses indicating similar exercise prescriptions (mean 3-4 x week, 43-46 minutes per session) may anticipate a 3-3.6% BMI z-score reduction within a timeframe of 13-16 weeks [689]. The absolute BMI loss of 0.8kg/m^2 was similar to adolescent males who undertook an eight-week lifestyle observed intervention [682]. As demonstrated in our lifestyle group, improvements in insulin sensitivity occurred in conjunction with anthropometry changes [321 332] but are also possible with changes to body composition without significant weight loss [328 551 690].

At study end, the lifestyle group had a significantly lower BMI z score: mean (SD) -0.1 (0.1). Hunt *et al* proposed that a BMI z-score loss of -0.25 might equate to -2.9% reduction in total body fat loss [564]. In comparison, we estimated that the BMI z-score reduction of -0.1 was associated with a -0.6% (3.5) total body fat loss as measured by DXA. However, there are limitations to the measurement of soft tissues in obese participants, particularly with smaller changes [100 101]. Despite the subtle body composition change, our results confirm that minimal changes in BMI and body composition in the

course of a lifestyle program, can lead to meaningful gains in metabolic health, which can ameliorate T2DM risk [75 328 551 563 690-692]. Presently, lifestyle interventions remain the mainstay of paediatric obesity management and are central to the treatment of metabolic co-morbidities such as insulin resistance [280 321 693]. A single exercise session in sedentary adolescents can have insulin sensitising effects for up to 17 hours [694], reiterating the importance for daily physical activity. Since our study, the Australian guidelines for physical activity have changed and suggest that adolescents include 60 minutes per day of moderate to vigorous physical activity with a mix of aerobic and strength activities during the week [695]. Greater activity per week has been associated with favourable cardio-metabolic outcome and this may influence the results of future obesity studies [544].

At baseline, all participants had reduced cardiorespiratory fitness (CRF) with an average VO_{2peak} of 26.8 mL.kg-1.min-1, representing approximately 85% of predicted values for age and gender. Our results demonstrating a lower VO_{2peak}, is in concordance with research in similarly obese cohorts where VO_{2peak} ranged from 23.9 [696] to 25.7mL.kg-1.min-1 [697]. Females had greater VO_{2peak} with higher anaerobic threshold, an indication of better fitness, and greater $\%VO_{2peak}$ predicted compared with males. This was despite both groups having similar anthropometry and males demonstrating greater muscle power and efficiency at baseline. The sexual dimorphism observed with regards to the CRF may be due to the usual increase in CRF between 12-14 years in females, with males expected to increase CRF later in their adolescence [684 698]. Muscle power and force are strongly linked to the ability to exercise on the treadmill as we showed strong correlations between relative Mechanography variables and exercise duration and intensity.

The assessment of CRF in obese adolescents is difficult due to standardisation of VO_{2peak} for weight that naturally defaults to obese adolescents who have relatively lower measures of VO_{2peak} compared with normal weight peers. Fat mass is thought to contribute minimally to CRF with lean tissue being directly related to fitness ability. Therefore, some have suggested that VO_{2peak} be standardised per kg of LTM to provide more relevant results for comparison [699] [700] [347]. However as we found in our cohort, this method of standardisation results in an overestimation of VO_{2peak} for adolescents [701] and the lack of appropriate reference ranges makes comparison difficult. Pinto and colleagues demonstrated a 20% reduction in cardiac output in adolescents with T2DM compared to normal weight controls VO2/ FFM indices were compared [702]. This suggests that cardiac function may limit ability to participate in currently recommending exercise prescriptions involving moderate intensity activity.

In adults, better cardio-pulmonary fitness correlates with better insulin sensitivity and can improve glycaemic control [703]. The effect of a higher CRF on insulin sensitivity in youth remains unclear due to physiological changes in adolescence and the variability of study methods. Young children with high CRF demonstrate better insulin sensitivity [704] and reduced central adiposity [705]. The superiority of aerobic or resistance based programs in obese adolescents also remains to be determined. Aerobic based programs in obese adolescents can improve CRF independent of body composition with enhancement of insulin sensitivity [551]. Conversely, some have shown that highly insulin resistant children [339] and adolescents [706] may demonstrate lower exercise capacity, independent of BMI, with improvements in insulin sensitivity accompanied by greater exercise capacity [707].

We did not show any significant improvements in VO_{2peak} with either lifestyle or WBVT intervention however the participants had better exercise endurance with an increase in

fitness time, more evident in the lifestyle arm. The use of the fitness time may provide an alternative indicator of the successfulness of the intervention, particularly as we showed strong correlations (r=0.699) between exercise time and overall fitness capacity (VO_{2peak}).

The WBVT, strength training, did not lead to any additional enhancement to mechanography or insulin sensitivity parameters above that of the lifestyle intervention. However, body composition improved with WBVT producing substantial increases in FFM ~0.5kg in our obese adolescents. It is unclear whether this could be attributed to the intervention or maturational changes due to advancing pubertal stage. Nevertheless, WBVT can increase LTM as shown in older women [555] and younger participants [379] but others demonstrate minimal [633] or negative results [708]. Fat loss may be minimally reduced by WBVT with body fat impedance analysis (BIA) showing reductions ranging from -0.2% [354] to -7% [448] with possible changes to central measures of adiposity [355]. Fjeldstad *et al* [555] showed in older participants that WBVT in conjunction with resistance training led to measurable fat loss on DXA amounting to -3.1% compared with a resistance and control group. The effect of WBVT on glucose metabolism is unclear, Lee *et al* [447] showed a small significant decrease in HbA1c -0.06% in their group of patients with diabetes with WBVT against an exercising and non-exercising control this is in contrast to a study in obese Latino boys showing no change in HOMA-IR measures after 8 weeks of WBVT [429]. Despite the increase in LTM we did not show any enhancement to glucose physiology or peripheral insulin sensitivity in our cohort. Overall the effect on WBVT on weight and body composition remains to be elucidated and present literature is difficult due to the limited studies confined mainly to adults and lack of standardisation [350 358 381 393].

Resistance interventions are being included along with aerobic interventions into paediatric exercise programs due to comparable, if not superior, effects on body

composition change [709]. Monteiro *et al* [553] showed that an aerobic plus resistance intervention produced slightly greater body fat reductions than aerobic activity alone (-2.9% versus -3.6%, respectively). Lee *et al* showed in obese adolescent males [338] equivalent body fat losses in their intervention groups (-2.5 vs. -2.6%, aerobic versus resistance). They concluded that the increased insulin sensitivity in their resistance group could be attributed to the observed increase in LTM. Whilst we showed an increase in LTM in the WBVT arm this was not significant and was not accompanied by enhancement of insulin sensitivity parameters.

Both of the aforementioned studies and the systematic review and meta-analysis by Garcia-Hermoso [709] included studies in which the intervention was directly observed. Our results better reflect real life conditions where variable adherence to the dietary, aerobic lifestyle and WBVT components is likely to have influenced our observed outcomes. Furthermore, we are mindful that both groups undertook a dietary intervention and any weight loss resulting from the nutritional component may have had independent and variable effects on insulin sensitivity and other metabolic profile indicators [154 710].

The metabolic effects of exercise in adolescents [711 712] are not as clear-cut and physical activity may not be as effective in adolescents with established T2DM [370]. In younger children, Eisenmann showed that cardiorespiratory fitness was correlated to measures of Si [704]. We observed weak correlations between fasting glucose, HOMA-IR and WBISI but these correlations were no longer seen when corrected for body composition (BMI, waist circumference), gender and pubertal status. The initial association may be confounded by the dual interaction of increased BMI on ability to exercise and the adverse metabolic risk profile conferred by increased weight. Molnar *et al* [339] showed that hyperinsulinaemia was correlated to lower exercise ability, however in this cohort,

distribution of body fat was not accounted for, particularly central adiposity, which strongly correlates with metabolic risk [706] [713].

As part of the fitness test we measured the ventilatory anaerobic threshold (VAT). The VAT indicates the point where an individual switches from aerobic to anaerobic metabolism and provides an indication of fitness. At baseline, the VAT was higher in females and decreased in the lifestyle arm, which undertook a predominately aerobic based training. With increased physical training we would expect a rise in anaerobic threshold. However, the utility of this measure remains unclear in children and adolescents because of maturational changes occurring during growth and puberty with increased accrual of LTM. In addition, the presence of hyperinsulinaemia may disrupt the normal metabolic response to exercise [341]; preventing fatty oxidation within muscle with reliance on carbohydrate metabolism, and this may have influenced the physiological parameters seen during the fitness testing. The VAT was higher at baseline in females and showed significant decreases in the group as a whole at the end of the study. Others have shown a physiological decrease of this parameter in mid-adolescence occurring slightly earlier in females (12-13 years) than males. As our study population was two-thirds females, this decrease may in part explain this result [698]. In addition weight was negatively correlated to all CRF parameters apart from the anaerobic threshold. Increased BMI may cause physical limitations to a more active lifestyle transition. Anecdotally, the major reason for terminating the fitness test was reported muscle fatigue suggesting strength exercises may be necessary prior to undertaking a fitness program.

To further explore the dynamic function of the muscle, we used mechanography and confirmed that muscle force and power were very low at baseline, with minimal change

with either training intervention. We confirmed the findings of Lang *et al* [714] that showed that muscle force strongly correlated with weight of the adolescent.

The only parameter to show significant increase after 12 weeks was maximal power relative to weight (which increased by virtue of weight loss in the lifestyle group). Participants were unable to generate much force (Fmaxrel) during the m1LJ, which requires an eccentric contraction through one leg during a hopping jump. The Fmaxrel which represents the force during the m1LJ, has been shown to be relatively constant between the ages of 6-19 years with mean 3.33g (SD 0.3) [154], this is compared to a mean of 2.4g (SD0.4) in our cohort. This result may indicate that adolescents may not have had adequate time to compensate for the increased load conferred by obesity or that they lacked co-ordination for the activity. The Mechanography results may indicate that improvements in muscle function (either through weight loss or strength exercises) need to occur before obese adolescents embark on more physically active sports. As over two thirds of our study population were adolescent females and we previously showed a high prevalence of Vitamin D insufficiency, optimising levels of this fat soluble hormone may assist in muscle function. Similar to Ward *et al* [715], we showed an association between this 25OHD and muscle force and power relative to weight.

Obese adolescents are often reluctant to participate in physical activity due to poor self-esteem and decreased levels of cardiorespiratory fitness. Understanding the exercise performance and fitness levels of the obese adolescent can allow for tailored individualised exercise programs. The inclusion of resistance activities can also improve self-confidence and adherence in young people [716 717]. Fitness testing integrates variables from several physiological systems including respiratory, cardiovascular function, autonomic and musculoskeletal. Therefore, performing exercise testing in obese

adolescents with obesity and insulin resistance may provide a sensitive indicator to subtle dysregulation due to underlying disease and may provide earlier opportunities to intervene.

CHAPTER 7 SUMMARY AND CONCLUSIONS

In this randomised controlled trial, our primary aim was to investigate whether a novel resistance intervention, WBVT, could enhance the effect of standard lifestyle measures in high-risk obese, insulin resistant young people. However, obesity has multi-systemic effects, an understanding of which is important for clinical management. Therefore, in addition to the results of the RCT we also examined the metabolic, anthropometric, musculoskeletal and cardiorespiratory health of the VIBRATE cohort.

In Chapter 3, we reviewed the baseline demographic, metabolic and anthropometric characteristics of 43 overweight adolescents with features of clinical insulin resistance from Greater Western Sydney, who comprised the VIBRATE cohort. Our inclusion criteria identified a group of insulin resistant youth as shown by the proxy measures of HOMA-IR and WBISI; some already had evidence of pre-diabetes. The majority of participants recruited to the study were female raising concerns about differential uptake of healthcare between genders and suggestive of the increased awareness of body image issues in teenage girls. Disconcertingly, the male participants in this study had the greatest BMI but were least represented in the study. The participants came from high-risk ethnicities for T2DM and the metabolic syndrome and had strong family histories of the same in first and second-degree relatives. We confirmed that acanthosis nigricans (AN), a cutaneous feature of insulin resistance, could correctly identify underlying biochemical insulin resistance in adolescents with dark skin but could not be used as a discriminating factor in Caucasian individuals who were equally insulin resistant but lacked this feature. We demonstrated the uncertainty surrounding the definition of metabolic syndrome in children and adolescents but were able to show high prevalence rates consistent with current paediatric research, however this varied dependent on the definition we applied. Insulin resistance, clustered strongly with features of the metabolic syndrome, particularly in those of a Middle Eastern background. Our data highlights the

need for future definitions of the metabolic syndrome in young persons to account for pubertal status and gender, as lipid profile, insulin resistance and blood pressure show variability during this period. This was demonstrated in our cohort whereby adolescents already showed gender and puberty related divergence of blood pressure, with male adolescents having higher SBP z-score and females lower diastolic blood pressure, a pattern known to continue until the menopausal years in females. Many participants had evidence of pre-hypertension.

Risk factors for the metabolic syndrome occur well before a diagnosis of T2DM and may combine to increase later CVD risk therefore they should be managed during puberty and as they manifest. Subtle liver dysfunction with mild elevation of liver transaminases was demonstrated which might suggest early changes of NAFLD. Therefore, primary health care providers may need to opportunistically provide preventative health advice, particularly to males who may not seek medical care as often as females. Finally in this chapter, we confirmed the adolescents' sedentary lifestyle with relatively little time engaged in active movement.

In Chapter 4, we examined the effect of WBVT on insulin sensitivity of obese adolescents. The results of the RCT did not show any enhancement in insulin sensitivity measures, over and above the lifestyle intervention. We determined that a short-term lifestyle intervention that provided nutritional coaching and an aerobic exercise prescription could effect BMI change and was accompanied by clinical improvement in markers of insulin sensitivity. The improvements in peripheral insulin sensitivity without increases in LTM, suggest the complex aetiology of insulin resistance and the requirements for a multi-faceted approach. The approach should include dietary change, weight loss and exercise. WBVT, a novel resistance exercise was safe, well tolerated and

an efficient form of neuromuscular training leading to regional increases in LTM, which potentiated the effect of the muscle bone interaction with noticeable change in BMD/LTM. We retained all but one participant to the end of the trial, our high adherence compared with other similar obesity intervention trials indicate that the program was interesting and that a good rapport was developed between the research team, the participants and families. Despite the frequent therapeutic contact and motivational strategies, we found that adherence to the WBVT in an everyday setting, to be poor considering the activity prescription of 15 minutes per day. Current recommendations now discount the use of the fasting insulin measurement in the evaluation of insulin resistance in obese adolescents. Whilst the WBISI is a validated proxy measure of the gold standard euglycaemic clamp, future studies would be best to measure Si with methods that allow careful evaluation of insulin dynamics such as the FSIVGTT from which the disposition index can be obtained.

In Chapter 5, we examined the baseline musculoskeletal and soft tissue characteristics of the obese adolescent using DXA and pQCT. In our study, obese adolescents had increased BMC and BMD for age (normal for weight and height) apart from males where excess adiposity did not confer additional mechanical loading benefits as shown by decreased BMC and BMD z-score. This suggests that assessment of bone health parameters against height would be more appropriate in overweight adolescents who are more physically mature. The integrity of the muscle bone unit was intact with normal BMD/LTM values and no disruption from the pathological changes related to peripheral insulin resistance. Elevated waist circumference was a notable feature of our cohort, but we showed that central adiposity might independently associate with less favourable bone and mineral parameters. Moreover, many of the obese participants were Vitamin D deficient, a hormone vital to bone and mineral metabolism. Increased BMI and waist

circumference were associated with Vitamin D insufficiency with contributory effects from fat tissue sequestration, a lack of sun exposure, a sedentary lifestyle and dark skin colour. Exploring the skeletal pancreas axis, we showed individuals with low 25OHD or dark skin had more unfavourable proxy measures of insulin resistance suggesting 25OHD might play a role in glucose homeostasis. However, no such relationship was demonstrated with regard to bone turnover markers, particularly OCN, recently implicated in energy regulation.

Lastly in Chapter 6, we examined the cardio-respiratory fitness of our cohort and showed decreased fitness capacity and poor tolerance to exercise related to decreased muscle force and power generation on Mechanography. These individuals showed muscle deconditioning for the increased weight. The vitamin D deficient state was also independently related to the poorer muscle function. Neither intervention led to improvements in cardio-respiratory parameters but relative muscle force and power were improved secondary to weight loss. Transition to an active lifestyle in the obese adolescent is limited by increased weight, poor cardiorespiratory function, low musculoskeletal performance and hormonal influences such as 25OHD deficiency.

To the clinician at the frontline, our data has confirmed the use of the simple clinical examination of BMI, waist circumference, waist to height ratio and acanthosis nigricans combined with a history of familial metabolic disease, to identify individuals at high risk of insulin resistance and the metabolic syndrome. In order to prevent T2DM progression, lifestyle measures including dietary and exercise prescription can lead to clinically relevant improvement in weight and metabolic profile indicators, such as insulin sensitivity. The adolescent transition may provide a window of opportunity for future metabolic health with linear growth passively decreasing BMI. The message of weight stability rather than

loss could be encouraged in the pubertal child with mild overweight as they grow into their weight. Obese adolescents, with co-morbidities may require input from a multidisciplinary team to achieve BMI change through weight reduction, to produce metabolic enhancements with 5% weight loss shown to be beneficial in adults[718].

Diabetes and osteoporosis are both likely to lead to chronic and disabling health problems. Given the emerging data between bone and energy metabolism, improving bone and muscle parameters through activity and supplementing deficient micronutrients such as Vitamin D, may have dual effects on both skeletal health and glucose homeostasis in the obese adolescent.

Whilst the optimum physical activity intervention for the obese adolescent with insulin resistance remains to be determined, we have shown that resistance training in the form of WBVT is safe. WBVT may be efficacious in increasing muscle quantity and strength and may assist in the transition of the obese adolescent to a more active lifestyle by providing greater self-confidence. The results from this study can inform future exercise based programs in obese adolescents who are often reluctant to participate in physical activity due to poor self-esteem and decreased levels of cardiorespiratory fitness. Consideration of cultural factors, an understanding of fitness parameters and functional assessment of muscle will permit tailored exercise prescriptions. This in combination with adolescent friendly environments and interesting activities may help to improve adherence in everyday settings.

We recommend future research replicate the study with a larger sample size. We observed clinically significant increases in lean tissue that did not reach significance possibly due to the small sample size in the intervention arm. Larger numbers and the

addition of a control group of normal weight adolescents would allow comparison of the effect of puberty on muscle and bone parameters. As the intervention was conducted in the home environment, the recruitment of other family members such as siblings and parents may expand the available knowledge of the effect of WBVT at various ages. This would better inform the public of its use as a home based exercise tool given its retail popularity and affordability.

The addition of a group of adolescents with T2DM would allow us to further explore the limitations to exercise in the obese insulin resistant adolescent compared with the adolescent with T2DM. Further investigations could add functional MRI studies to better clarify the cardiac and muscle limitations to exercise.

Future studies utilising WBVT should be conducted over longer periods of time as recent research suggests positive results with longer duration trials of at least 6 months. The WBVT program should be directly observed, particularly in the adolescent in whom adherence is likely to confound results. To better investigate the effectiveness of WBVT against strength training or HIIT in addition to a control group may be informative. The study protocol and training stance could be varied to examine differential changes to muscle and bone parameters.

Our study included a multi-ethnic demographic of adolescents at risk of T2DM who were also undergoing pubertal change. Therefore, conducting the study in late pubertal or post-pubertal adolescents may provide further information about the enhancement of insulin sensitivity, as a result of peripheral lean tissue gains and attenuate the effect of physiological insulin resistance during adolescence. Stratifying groups according to ethnicity may allow further discrimination of interventional changes. The addition of

bone age skeletal investigation would provide a benchmark for comparison of cardiorespiratory and muscle and bone parameters and would allow for more accurate analysis.

Prospective studies should also explore factors to optimise healthcare access amongst obese male adolescents, due to the noticeable patterns of gender disparity and emergence of metabolic risk factors seen in our study.

CHAPTER 8 REFERENCES

1. NHMRC. National Health and Medical Research Council (2013) Clinical practice guidelines for the management of overweight and obesity in adults, adolescents and children in Australia. Melbourne: National Health and Medical Research Council.
2. Booth ML, Dobbins T, Okely AD, et al. Trends in the prevalence of overweight and obesity among young Australians, 1985, 1997, and 2004. Obesity (Silver Spring) 2007;**15**(5):1089-95.
3. Australian Bureau of Statistics. National Health Survey: First Results, 2014-15, accessed June 2016. [Available from:http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/bySubject/4364.0.55.001~2014-15~Main Features~Children's risk factors~31.]
4. Ogden CL, Carroll MD, Flegal KM. High body mass index for age among US children and adolescents, 2003-2006. Jama 2008;**299**(20):2401-5.
5. Ogden CL, Carroll MD, Kit BK, et al. Prevalence of childhood and adult obesity in the United States, 2011-2012. Jama 2014;**311**(8):806-14.
6. Dyer SM, Gomersall JS, Smithers LG, et al. Prevalence and Characteristics of Overweight and Obesity in Indigenous Australian Children: A Systematic Review. Crit Rev Food Sci Nutr 2015:0.
7. Olds TS, Tomkinson GR, Ferrar KE, et al. Trends in the prevalence of childhood overweight and obesity in Australia between 1985 and 2008. Int J Obes (Lond) 2010;**34**(1):57-66.
8. Nichols MS, Silva-Sanigorski A, Cleary JE, et al. Decreasing trends in overweight and obesity among an Australian population of preschool children. Int J Obes (Lond) 2011;**35**(7):916-24.
9. Martin K, Rosenberg M, Pratt IS, et al. Prevalence of overweight, obesity and underweight in Western Australian school-aged children; 2008 compared with 2003. Public Health Nutr 2014;**17**(12):2687-91.
10. Xi B, Mi J, Zhao M, et al. Trends in abdominal obesity among U.S. children and adolescents. Pediatrics 2014;**134**(2):e334-9.
11. de Wilde JA, Verkerk PH, Middelkoop BJ. Declining and stabilising trends in prevalence of overweight and obesity in Dutch, Turkish, Moroccan and South Asian children 3-16 years of age between 1999 and 2011 in the Netherlands. Arch Dis Child 2014;**99**(1):46-51.
12. Schmidt Morgen C, Rokholm B, Sjoberg Brixval C, et al. Trends in prevalence of overweight and obesity in danish infants, children and adolescents--are we still on a plateau? PLoS One 2013;**8**(7):e69860.
13. O'Dea JA, Dibley MJ. Prevalence of obesity, overweight and thinness in Australian children and adolescents by socioeconomic status and ethnic/cultural group in 2006 and 2012. Int J Public Health 2014;**59**(5):819-28.
14. NHMRC. Clinical Practice Guidelines for the Management of Overweight and Obesity in Adults, Adolescents and Children in Australia (2013). 2013.
15. Haby MM, Markwick A, Peeters A, et al. Future predictions of body mass index and overweight prevalence in Australia, 2005-2025. Health Promot Int 2012;**27**(2):250-60.
16. Lobstein T, Baur L, Uauy R. Obesity in children and young people: a crisis in public health. Obes Rev 2004;**5 Suppl 1**:4-104.
17. Magarey AM, Daniels LA, Boulton TJ. Prevalence of overweight and obesity in Australian children and adolescents: reassessment of 1985 and 1995 data against new standard international definitions. Med J Aust 2001;**174**(11):561-4.

18. Ogden CL, Carroll MD, Lawman HG, et al. Trends in Obesity Prevalence Among Children and Adolescents in the United States, 1988-1994 Through 2013-2014. Jama 2016;**315**(21):2292-9.
19. Haslam DW, James WP. Obesity. Lancet 2005;**366**(9492):1197-209.
20. World Health Organisation. Childhood overweight and obesity. Last accessed June 2016. [Available from: http://www.who.int/dietphysicalactivity/childhood/en/.
21. Saxena S, Ambler G, Cole TJ, et al. Ethnic group differences in overweight and obese children and young people in England: cross sectional survey. Arch Dis Child 2004;**89**(1):30-6.
22. Daniels SR, Arnett DK, Eckel RH, et al. Overweight in children and adolescents: pathophysiology, consequences, prevention, and treatment. Circulation 2005;**111**(15):1999-2012.
23. Gordon-Larsen P, Adair LS, Popkin BM. The relationship of ethnicity, socioeconomic factors, and overweight in US adolescents. Obes Res 2003;**11**(1):121-9.
24. Gonzalez-Casanova I, Sarmiento OL, Pratt M, et al. Individual, family, and community predictors of overweight and obesity among colombian children and adolescents. Prev Chronic Dis 2014;**11**:E134.
25. Eidsdottir S, Kristjansson A, Sigfusdottir ID, et al. Secular trends in overweight and obesity among Icelandic adolescents: do parental education levels and family structure play a part? Scand J Public Health 2013;**41**(4):384-91.
26. Ruiz M, Goldblatt P, Morrison J, et al. Impact of Low Maternal Education on Early Childhood Overweight and Obesity in Europe. Paediatr Perinat Epidemiol 2016;**30**(3):274-84.
27. Shrewsbury V, Wardle J. Socioeconomic status and adiposity in childhood: a systematic review of cross-sectional studies 1990-2005. Obesity (Silver Spring) 2008;**16**(2):275-84.
28. Wang Z, Patterson CM, Hills AP. Association between overweight or obesity and household income and parental body mass index in Australian youth: analysis of the Australian National Nutrition Survey, 1995. Asia Pac J Clin Nutr 2002;**11**(3):200-5.
29. Oude Luttikhuis H, Baur L, Jansen H, et al. Interventions for treating obesity in children. Cochrane Database Syst Rev 2009(1):CD001872.
30. Strauss RS, Pollack HA. Epidemic increase in childhood overweight, 1986-1998. Jama 2001;**286**(22):2845-8.
31. Lumeng JC, Gannon K, Cabral HJ, et al. Association between clinically meaningful behavior problems and overweight in children. Pediatrics 2003;**112**(5):1138-45.
32. Pulgaron ER. Childhood obesity: a review of increased risk for physical and psychological comorbidities. Clin Ther 2013;**35**(1):A18-32.
33. Sanders RH, Han A, Baker JS, et al. Childhood obesity and its physical and psychological co-morbidities: a systematic review of Australian children and adolescents. Eur J Pediatr 2015;**174**(6):715-46.
34. Topcu S, Orhon FS, Tayfun M, et al. Anxiety, depression and self-esteem levels in obese children: a case-control study. J Pediatr Endocrinol Metab 2016;**29**(3):357-61.
35. Goodman E, Whitaker RC. A prospective study of the role of depression in the development and persistence of adolescent obesity. Pediatrics 2002;**110**(3):497-504.
36. Fothergill E, Guo J, Howard L, et al. Persistent metabolic adaptation 6 years after "The Biggest Loser" competition. Obesity (Silver Spring) 2016;**24**(8):1612-9.

37. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. N Engl J Med 1995;**332**(10):621-8.
38. Boguszewski CL, Paz-Filho G, Velloso LA. Neuroendocrine body weight regulation: integration between fat tissue, gastrointestinal tract, and the brain. Endokrynol Pol 2010;**61**(2):194-206.
39. Eyster KM. Dysfunctional hormonal regulation of metabolism in obesity. S D Med 2011;**Spec No**:18-21.
40. Wilkie HJ, Standage M, Gillison FB, et al. Multiple lifestyle behaviours and overweight and obesity among children aged 9-11 years: results from the UK site of the International Study of Childhood Obesity, Lifestyle and the Environment. BMJ Open 2016;**6**(2):e010677.
41. Watanabe E, Lee JS, Mori K, et al. Clustering patterns of obesity-related multiple lifestyle behaviours and their associations with overweight and family environments: a cross-sectional study in Japanese preschool children. BMJ Open 2016;**6**(11):e012773.
42. Reinehr T, Hinney A, de Sousa G, et al. Definable somatic disorders in overweight children and adolescents. J Pediatr 2007;**150**(6):618-22, 22 e1-5.
43. Foraita R, Gunther F, Gwozdz W, et al. Does the FTO gene interact with the socioeconomic status on the obesity development among young European children? Results from the IDEFICS study. Int J Obes (Lond) 2015;**39**(1):1-6.
44. Reuter CP, Rosane De Moura Valim A, Gaya AR, et al. FTO polymorphism, cardiorespiratory fitness, and obesity in Brazilian youth. Am J Hum Biol 2016;**28**(3):381-6.
45. Rzehak P, Saffery R, Reischl E, et al. Maternal Smoking during Pregnancy and DNA-Methylation in Children at Age 5.5 Years: Epigenome-Wide-Analysis in the European Childhood Obesity Project (CHOP)-Study. PLoS ONE 2016;**11**(5):e0155554.
46. Stojanoska MM, Milosevic N, Milic N, et al. The influence of phthalates and bisphenol A on the obesity development and glucose metabolism disorders. Endocrine 2017;**55**(3):666-81.
47. Parrino C, Vinciguerra F, La Spina N, et al. Influence of early-life and parental factors on childhood overweight and obesity. J Endocrinol Invest 2016.
48. Goosby BJ, Cheadle JE, McDade T. Birth weight, early life course BMI, and body size change: Chains of risk to adult inflammation? Soc Sci Med 2016;**148**:102-9.
49. Whitaker RC, Dietz WH. Role of the prenatal environment in the development of obesity. J Pediatr 1998;**132**(5):768-76.
50. Gopinath B, Baur LA, Burlutsky G, et al. Socio-economic, familial and perinatal factors associated with obesity in Sydney schoolchildren. J Paediatr Child Health 2012;**48**(1):44-51.
51. Ouyang F, Parker MG, Luo ZC, et al. Maternal BMI, gestational diabetes, and weight gain in relation to childhood obesity: The mediation effect of placental weight. Obesity (Silver Spring) 2016;**24**(4):938-46.
52. Wake M, Hardy P, Canterford L, et al. Overweight, obesity and girth of Australian preschoolers: prevalence and socio-economic correlates. Int J Obes (Lond) 2007;**31**(7):1044-51.
53. Booth ML, Wake M, Armstrong T, et al. The epidemiology of overweight and obesity among Australian children and adolescents, 1995-97. Aust N Z J Public Health 2001;**25**(2):162-9.
54. Wang Z, Patterson CM, Hills AP. Association between overweight or obesity and household income and parental body mass index in Australian youth: analysis of

the Australian National Nutrition Survey, 1995. Asia Pac J Clin Nutr 2002;**11**(3):200-5.
55. Bider-Canfield Z, Martinez MP, Wang X, et al. Maternal obesity, gestational diabetes, breastfeeding and childhood overweight at age 2 years. Pediatr Obes 2016.
56. Wright DR, Lozano P, Dawson-Hahn E, et al. Parental Predictions and Perceptions Regarding Long-Term Childhood Obesity-Related Health Risks. Acad Pediatr 2016.
57. Twarog JP, Politis MD, Woods EL, et al. Is obesity becoming the new normal? Age, gender and racial/ethnic differences in parental misperception of obesity as being 'About the Right Weight'. Int J Obes (Lond) 2016.
58. Himes JH. Challenges of accurately measuring and using BMI and other indicators of obesity in children. Pediatrics 2009;**124 Suppl 1**:S3-22.
59. Power C, Lake JK, Cole TJ. Measurement and long-term health risks of child and adolescent fatness. Int J Obes Relat Metab Disord 1997;**21**(7):507-26.
60. Cole TJ. Establishing a standard definition for child overweight and obesity worldwide: international survey. Bmj 2000;**320**(7244):1240-40.
61. Weiss R, Kaufman FR. Metabolic complications of childhood obesity: identifying and mitigating the risk. Diabetes Care 2008;**31 Suppl 2**:S310-6.
62. Lam BC, Koh GC, Chen C, et al. Comparison of Body Mass Index (BMI), Body Adiposity Index (BAI), Waist Circumference (WC), Waist-To-Hip Ratio (WHR) and Waist-To-Height Ratio (WHtR) as predictors of cardiovascular disease risk factors in an adult population in Singapore. PLoS One 2015;**10**(4):e0122985.
63. Dietz WH. Health consequences of obesity in youth: childhood predictors of adult disease. Pediatrics 1998;**101**(3 Pt 2):518-25.
64. Lammi N, Moltchanova E, Blomstedt PA, et al. Childhood BMI trajectories and the risk of developing young adult-onset diabetes. Diabetologia 2009;**52**(3):408-14.
65. Freedman DS, Katzmarzyk PT, Dietz WH, et al. The relation of BMI and skinfold thicknesses to risk factors among young and middle-aged adults: the Bogalusa Heart Study. Ann Hum Biol 2010;**37**(6):726-37.
66. Tirosh A, Shai I, Afek A, et al. Adolescent BMI trajectory and risk of diabetes versus coronary disease. N Engl J Med 2011;**364**(14):1315-25.
67. Misra A, Vikram NK, Gupta R, et al. Waist circumference cutoff points and action levels for Asian Indians for identification of abdominal obesity. Int J Obes (Lond) 2006;**30**(1):106-11.
68. Daniels SR, Khoury PR, Morrison JA. The utility of body mass index as a measure of body fatness in children and adolescents: differences by race and gender. Pediatrics 1997;**99**(6):804-7.
69. Lear SA, Humphries KH, Kohli S, et al. The use of BMI and waist circumference as surrogates of body fat differs by ethnicity. Obesity (Silver Spring) 2007;**15**(11):2817-24.
70. Carroll JF, Chiapa AL, Rodriquez M, et al. Visceral fat, waist circumference, and BMI: impact of race/ethnicity. Obesity (Silver Spring) 2008;**16**(3):600-7.
71. Kuczmarski RJ, Ogden CL, Guo SS, et al. 2000 CDC Growth Charts for the United States: methods and development. Vital Health Stat 11 2002(246):1-190.
72. Zhang YX, Zhao JS, Chu ZH. Children and adolescents with low body mass index but large waist circumference remain high risk of elevated blood pressure. Int J Cardiol 2016;**215**:23-5.
73. Karatzi K, Moschonis G, Polychronopoulou MC, et al. Cutoff points of waist circumference and trunk and visceral fat for identifying children with elevated inflammation markers and adipokines: The Healthy Growth Study. Nutrition 2016.

74. Lee S, Bacha F, Gungor N, et al. Waist circumference is an independent predictor of insulin resistance in black and white youths. J Pediatr 2006;**148**(2):188-94.
75. Watts K, Bell LM, Byrne SM, et al. Waist circumference predicts cardiovascular risk in young Australian children. J Paediatr Child Health 2008;**44**(12):709-15.
76. Sardinha LB, Santos DA, Silva AM, et al. A Comparison between BMI, Waist Circumference, and Waist-To-Height Ratio for Identifying Cardio-Metabolic Risk in Children and Adolescents. PLoS One 2016;**11**(2):e0149351.
77. Maffeis C, Banzato C, Brambilla P, et al. Insulin resistance is a risk factor for high blood pressure regardless of body size and fat distribution in obese children. Nutr Metab Cardiovasc Dis 2010;**20**(4):266-73.
78. Brumbaugh DE, Crume TL, Nadeau K, et al. Intramyocellular lipid is associated with visceral adiposity, markers of insulin resistance, and cardiovascular risk in prepubertal children: the EPOCH study. J Clin Endocrinol Metab 2012;**97**(7):E1099-105.
79. Larson-Meyer DE, Newcomer BR, Ravussin E, et al. Intrahepatic and intramyocellular lipids are determinants of insulin resistance in prepubertal children. Diabetologia 2011;**54**(4):869-75.
80. Brambilla P, Bedogni G, Moreno LA, et al. Crossvalidation of anthropometry against magnetic resonance imaging for the assessment of visceral and subcutaneous adipose tissue in children. Int J Obes (Lond) 2006;**30**(1):23-30.
81. Despres JP, Nadeau A, Tremblay A, et al. Role of deep abdominal fat in the association between regional adipose tissue distribution and glucose tolerance in obese women. Diabetes 1989;**38**(3):304-9.
82. Despres JP, Moorjani S, Ferland M, et al. Adipose tissue distribution and plasma lipoprotein levels in obese women. Importance of intra-abdominal fat. Arteriosclerosis 1989;**9**(2):203-10.
83. Despres JP, Moorjani S, Tremblay A, et al. Relation of high plasma triglyceride levels associated with obesity and regional adipose tissue distribution to plasma lipoprotein-lipid composition in premenopausal women. Clin Invest Med 1989;**12**(6):374-80.
84. Bauer KW, Marcus MD, El ghormli L, et al. Cardio-metabolic risk screening among adolescents: understanding the utility of body mass index, waist circumference and waist to height ratio. Pediatr Obes 2015;**10**(5):329-37.
85. Lee S, Bacha F, Arslanian SA. Waist circumference, blood pressure, and lipid components of the metabolic syndrome. J Pediatr 2006;**149**(6):809-16.
86. Kawada T, Andou T, Fukumitsu M. Waist circumference, visceral abdominal fat thickness and three components of metabolic syndrome. Diabetes Metab Syndr 2016;**10**(1):4-6.
87. Savva SC, Tornaritis M, Savva ME, et al. Waist circumference and waist-to-height ratio are better predictors of cardiovascular disease risk factors in children than body mass index. Int J Obes Relat Metab Disord 2000;**24**(11):1453-8.
88. Eisenmann JC. Waist circumference percentiles for 7- to 15-year-old Australian children. Acta Paediatr 2005;**94**(9):1182-5.
89. Clemente AP, Netto BD, de Carvalho-Ferreira JP, et al. [Waist circumference as a marker for screening nonalcoholic fatty liver disease in obese adolescents]. Rev Paul Pediatr 2016;**34**(1):47-55.
90. Garnett SP, Baur LA, Cowell CT. Waist-to-height ratio: a simple option for determining excess central adiposity in young people. Int J Obes (Lond) 2008;**32**(6):1028-30.

91. Graves L, Garnett SP, Cowell CT, et al. Waist-to-height ratio and cardiometabolic risk factors in adolescence: findings from a prospective birth cohort. Pediatr Obes 2013.
92. Schwandt P, Haas GM. Is the ratio waist circumference to height (WHtR) of 0.5 a universal measure for abdominal adiposity in children and adolescents? Int J Obes (Lond) 2016.
93. Inokuchi M, Matsuo N, Takayama JI, et al. Waist-to-height ratio centiles by age and sex for Japanese children based on the 1978–1981 cross-sectional national survey data. Int J Obes (Lond) 2015;**40**(1):65-70.
94. Kahn HS, Imperatore G, Cheng YJ. A population-based comparison of BMI percentiles and waist-to-height ratio for identifying cardiovascular risk in youth. J Pediatr 2005;**146**(4):482-8.
95. Freedman DS, Serdula MK, Srinivasan SR, et al. Relation of circumferences and skinfold thicknesses to lipid and insulin concentrations in children and adolescents: the Bogalusa Heart Study. Am J Clin Nutr 1999;**69**(2):308-17.
96. McCarthy HD, Ashwell M. A study of central fatness using waist-to-height ratios in UK children and adolescents over two decades supports the simple message--'keep your waist circumference to less than half your height'. Int J Obes (Lond) 2006;**30**(6):988-92.
97. Adegbija O, Hoy WE, Wang Z. Waist circumference values equivalent to body mass index points for predicting absolute cardiovascular disease risks among adults in an Aboriginal community: a prospective cohort study. BMJ Open 2015;**5**(11):e009185.
98. Fuller NJ, Jebb SA, Laskey MA, et al. Four-component model for the assessment of body composition in humans: comparison with alternative methods, and evaluation of the density and hydration of fat-free mass. Clin Sci (Lond) 1992;**82**(6):687-93.
99. Lohman TG. Applicability of body composition techniques and constants for children and youths. Exerc Sport Sci Rev 1986;**14**:325-57.
100. Knapp KM, Welsman JR, Hopkins SJ, et al. Obesity increases precision errors in total body dual-energy x-ray absorptiometry measurements. Journal of Clinical Densitometry 2015;**18**(2):209-16.
101. Rajamanohara R, Robinson J, Rymer J, et al. The effect of weight and weight change on the long-term precision of spine and hip DXA measurements. Osteoporos Int 2011;**22**(5):1503-12.
102. Batch JA, Baur LA. 3. Management and prevention of obesity and its complications in children and adolescents. The Medical journal of Australia 2005;**182**(3):130-5.
103. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;**37**(12):1595-607.
104. Tobisch B, Blatniczky L, Barkai L. Cardiometabolic risk factors and insulin resistance in obese children and adolescents: relation to puberty. Pediatr Obes 2015;**10**(1):37-44.
105. Berenson GS, Srinivasan SR, Bao W, et al. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 1998;**338**(23):1650-6.
106. Sakuragi S, Abhayaratna K, Gravenmaker KJ, et al. Influence of adiposity and physical activity on arterial stiffness in healthy children: the lifestyle of our kids study. Hypertension 2009;**53**(4):611-6.
107. Ho M, Benitez-Aguirre PZ, Donaghue KC, et al. Arterial elasticity in obese adolescents with clinical features of insulin resistance. Diab Vasc Dis Res 2015;**12**(1):62-9.

108. Berenson GS, Dalferes E, Jr., Savage D, et al. Ambulatory blood pressure measurements in children and young adults selected by high and low casual blood pressure levels and parental history of hypertension: the Bogalusa Heart Study. Am J Med Sci 1993;**305**(6):374-82.
109. Eklioglu BS, Atabek ME, Akyurek N, et al. Prediabetes and Cardiovascular Parameters in Obese Children and Adolescents. J Clin Res Pediatr Endocrinol 2016;**8**(1):80-5.
110. May AL, Kuklina EV, Yoon PW. Prevalence of cardiovascular disease risk factors among US adolescents, 1999-2008. Pediatrics 2012;**129**(6):1035-41.
111. Pettitt DJ, Nelson RG, Saad MF, et al. Diabetes and obesity in the offspring of Pima Indian women with diabetes during pregnancy. Diabetes Care 1993;**16**(1):310-4.
112. Dabelea D, Pettitt DJ. Intrauterine diabetic environment confers risks for type 2 diabetes mellitus and obesity in the offspring, in addition to genetic susceptibility. J Pediatr Endocrinol Metab 2001;**14**(8):1085-91.
113. Phillips DI, Jones A, Goulden PA. Birth weight, stress, and the metabolic syndrome in adult life. Ann N Y Acad Sci 2006;**1083**:28-36.
114. Pettitt DJ, Forman MR, Hanson RL, et al. Breastfeeding and incidence of non-insulin-dependent diabetes mellitus in Pima Indians. Lancet 1997;**350**(9072):166-8.
115. Eriksson JG, Forsen T, Tuomilehto J, et al. Early adiposity rebound in childhood and risk of Type 2 diabetes in adult life. Diabetologia 2003;**46**(2):190-4.
116. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama 2001;**285**(19):2486-97.
117. Reinehr T, Wolters B, Knop C, et al. Strong effect of pubertal status on metabolic health in obese children: a longitudinal study. J Clin Endocrinol Metab 2015;**100**(1):301-8.
118. Reinehr T. Metabolic Syndrome in Children and Adolescents: a Critical Approach Considering the Interaction between Pubertal Stage and Insulin Resistance. Curr Diab Rep 2016;**16**(1):8.
119. Cook S, Weitzman M, Auinger P, et al. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. Arch Pediatr Adolesc Med 2003;**157**(8):821-7.
120. Duncan GE, Li SM, Zhou XH. Prevalence and trends of a metabolic syndrome phenotype among u.s. Adolescents, 1999-2000. Diabetes Care 2004;**27**(10):2438-43.
121. Shaibi GQ, Goran MI. Examining metabolic syndrome definitions in overweight Hispanic youth: a focus on insulin resistance. J Pediatr 2008;**152**(2):171-6.
122. Zimmet P, Alberti KG, Kaufman F, et al. The metabolic syndrome in children and adolescents - an IDF consensus report. Pediatr Diabetes 2007;**8**(5):299-306.
123. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med 2006;**23**(5):469-80.
124. Katzmarzyk PT, Perusse L, Malina RM, et al. Stability of indicators of the metabolic syndrome from childhood and adolescence to young adulthood: the Quebec Family Study. J Clin Epidemiol 2001;**54**(2):190-5.
125. Sun SS, Liang R, Huang TT, et al. Childhood obesity predicts adult metabolic syndrome: the Fels Longitudinal Study. J Pediatr 2008;**152**(2):191-200.

126. Kursawe R, Eszlinger M, Narayan D, et al. Cellularity and adipogenic profile of the abdominal subcutaneous adipose tissue from obese adolescents: association with insulin resistance and hepatic steatosis. Diabetes 2010;**59**(9):2288-96.
127. Sinaiko AR, Donahue RP, Jacobs DR, Jr., et al. Relation of weight and rate of increase in weight during childhood and adolescence to body size, blood pressure, fasting insulin, and lipids in young adults. The Minneapolis Children's Blood Pressure Study. Circulation 1999;**99**(11):1471-6.
128. Ter Horst KW, Gilijamse PW, Koopman KE, et al. Insulin resistance in obesity can be reliably identified from fasting plasma insulin. Int J Obes (Lond) 2015.
129. Sinha R, Fisch G, Teague B, et al. Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. N Engl J Med 2002;**346**(11):802-10.
130. Giannini C, Caprio S. Progression of -cell dysfunction in obese youth. Curr Diab Rep 2013;**13**(1):89-95.
131. Yeckel CW, Taksali SE, Dziura J, et al. The normal glucose tolerance continuum in obese youth: evidence for impairment in beta-cell function independent of insulin resistance. J Clin Endocrinol Metab 2005;**90**(2):747-54.
132. Centers for Disease Control and Prevention. National diabetes fact sheet: national estimates and general information on dabetes and prediabetes in the United States, 2011. Atlanta, GA: U.S. Dpeartment of Health and Human Services, Centers for Disease Control and Prevention 2011. [Last accessed June 2016 http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf.]
133. Mirbolouk M, Derakhshan A, Charkhchi P, et al. Incidence and predictors of early adulthood pre-diabetes/type 2 diabetes, among Iranian adolescents: the Tehran Lipid and Glucose Study. Pediatr Diabetes 2016.
134. Goulding A, Taylor RW, Jones IE, et al. Overweight and obese children have low bone mass and area for their weight. Int J Obes Relat Metab Disord 2000;**24**(5):627-32.
135. Goulding A, Jones IE, Taylor RW, et al. More broken bones: a 4-year double cohort study of young girls with and without distal forearm fractures. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2000;**15**(10):2011-8.
136. Taylor ED, Theim KR, Mirch MC, et al. Orthopedic complications of overweight in children and adolescents. Pediatrics 2006;**117**(6):2167-74.
137. Goulding A, Jones IE, Taylor RW, et al. Dynamic and static tests of balance and postural sway in boys: effects of previous wrist bone fractures and high adiposity. Gait Posture 2003;**17**(2):136-41.
138. Leonard MB, Shults J, Wilson BA, et al. Obesity during childhood and adolescence augments bone mass and bone dimensions. Am J Clin Nutr 2004;**80**(2):514-23.
139. Maggio AB, Belli DC, Puigdefabregas JW, et al. High bone density in adolescents with obesity is related to fat mass and serum leptin concentrations. J Pediatr Gastroenterol Nutr 2014;**58**(6):723-8.
140. Whiting SJ. Obesity is not protective for bones in childhood and adolescence. Nutr Rev 2002;**60**(1):27-30.
141. Nguyen TV, Center JR, Eisman JA. Osteoporosis in elderly men and women: effects of dietary calcium, physical activity, and body mass index. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2000;**15**(2):322-31.
142. Dimitri P, Bishop N, Walsh JS, et al. Obesity is a risk factor for fracture in children but is protective against fracture in adults: a paradox. Bone 2012;**50**(2):457-66.

143. Asomaning K, Bertone-Johnson ER, Nasca PC, et al. The association between body mass index and osteoporosis in patients referred for a bone mineral density examination. J Womens Health (Larchmt) 2006;**15**(9):1028-34.
144. Larson NI, Wall MM, Story MT, et al. Home/family, peer, school, and neighborhood correlates of obesity in adolescents. Obesity (Silver Spring) 2013;**21**(9):1858-69.
145. Salvy SJ, Miles JN, Shih RA, et al. Neighborhood, Family and Peer-Level Predictors of Obesity-Related Health Behaviors Among Young Adolescents. J Pediatr Psychol 2016.
146. Nguyen B, McGregor KA, O'Connor J, et al. Recruitment challenges and recommendations for adolescent obesity trials. J Paediatr Child Health 2012;**48**(1):38-43.
147. Golley RK, Magarey AM, Baur LA, et al. Twelve-month effectiveness of a parent-led, family-focused weight-management program for prepubertal children: a randomized, controlled trial. Pediatrics 2007;**119**(3):517-25.
148. Shrewsbury VA, Steinbeck KS, Torvaldsen S, et al. The role of parents in pre-adolescent and adolescent overweight and obesity treatment: a systematic review of clinical recommendations. Obes Rev 2011;**12**(10):759-69.
149. Mellin LM, Slinkard LA, Irwin CE, Jr. Adolescent obesity intervention: validation of the SHAPEDOWN program. J Am Diet Assoc 1987;**87**(3):333-8.
150. Barlow SE, Expert C. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics 2007;**120 Suppl 4**:S164-92.
151. Booth ML, Macaskill P, Lazarus R, et al. Sociodemographic distribution of measures of body fatness among children and adolescents in New South Wales, Australia. Int J Obes Relat Metab Disord 1999;**23**(5):456-62.
152. Garnett SP, Gow M, Ho M, et al. Improved insulin sensitivity and body composition, irrespective of macronutrient intake, after a 12 month intervention in adolescents with pre-diabetes; RESIST a randomised control trial. BMC Pediatr 2014;**14**:289.
153. Garnett SP, Gow M, Ho M, et al. Optimal macronutrient content of the diet for adolescents with prediabetes; RESIST a randomised control trial. J Clin Endocrinol Metab 2013;**98**(5):2116-25.
154. Ho M, Garnett SP, Baur LA, et al. Impact of dietary and exercise interventions on weight change and metabolic outcomes in obese children and adolescents: a systematic review and meta-analysis of randomized trials. JAMA Pediatr 2013;**167**(8):759-68.
155. Council NHaMR. Australian Dietary Guidelines. Canberra: National Health and Medical Research Council, 2013.
156. Crume TLPHD, Ogden LPHD, Maligie MBS, et al. Long-Term Impact of Neonatal Breastfeeding on Childhood Adiposity and Fat Distribution Among Children Exposed to Diabetes In Utero. Diabetes Care 2011;**34**(3):641-45.
157. Kalarchian MA, Levine MD, Arslanian SA, et al. Family-based treatment of severe pediatric obesity: randomized, controlled trial. Pediatrics 2009;**124**(4):1060-8.
158. Kalarchian MA, Levine MD, Marcus MD. Structured Dietary Interventions in the Treatment of Severe Pediatric Obesity: A Pilot Study. Bariatr Surg Pract Patient Care 2013;**8**(2):58-60.
159. Ho M, Gow M, Halim J, et al. Effect of a prescriptive dietary intervention on psychological dimensions of eating behavior in obese adolescents. Int J Behav Nutr Phys Act 2013;**10**:119.

160. Gow ML, Ho M, Burrows TL, et al. Impact of dietary macronutrient distribution on BMI and cardiometabolic outcomes in overweight and obese children and adolescents: a systematic review. Nutr Rev 2014;**72**(7):453-70.
161. Joslowski G, Halim J, Goletzke J, et al. Dietary glycemic load, insulin load, and weight loss in obese, insulin resistant adolescents: RESIST study. Clin Nutr 2015;**34**(1):89-94.
162. O'Sullivan TA, Bremner AP, Bremer HK, et al. Dairy product consumption, dietary nutrient and energy density and associations with obesity in Australian adolescents. J Hum Nutr Diet 2015;**28**(5):452-64.
163. Wang W, Wu Y, Zhang D. Association of dairy products consumption with risk of obesity in children and adults: a meta-analysis of mainly cross-sectional studies. Ann Epidemiol 2016;**26**(12):870-82 e2.
164. Vien S, Luhovyy BL, Patel BP, et al. Pre- and within-meal effects of fluid dairy products on appetite, food intake, glycemia, and regulatory hormones in children. Appl Physiol Nutr Metab 2017;**42**(3):302-10.
165. Australian Government. Australia's Physical Activity & Sedentary Behaviour Guidelines for Young People (13 -17 years) Canberra: Department of Health; 2014 Last accessed June 2016. [Available from: http://www.health.gov.au/internet/main/publishing.nsf/Content/F01F92328EDADA5BCA257BF0001E720D/$File/brochure PA Guidelines_A5_13-17yrs.PDF.
166. Astrand I, Astrand PO, Christensen EH, et al. Intermittent muscular work. Acta Physiol Scand 1960;**48**:448-53.
167. Gibala MJ, McGee SL. Metabolic adaptations to short-term high-intensity interval training: a little pain for a lot of gain? Exerc Sport Sci Rev 2008;**36**(2):58-63.
168. Sculthorpe NF, Herbert P, Grace F. One session of high-intensity interval training (HIIT) every 5 days, improves muscle power but not static balance in lifelong sedentary ageing men: A randomized controlled trial. Medicine (Baltimore) 2017;**96**(6):e6040.
169. Fisher G, Brown AW, Bohan Brown MM, et al. High Intensity Interval- vs Moderate Intensity- Training for Improving Cardiometabolic Health in Overweight or Obese Males: A Randomized Controlled Trial. PLoS One 2015;**10**(10):e0138853.
170. Lanzi S, Codecasa F, Cornacchia M, et al. Short-term HIIT and Fat max training increase aerobic and metabolic fitness in men with class II and III obesity. Obesity (Silver Spring) 2015;**23**(10):1987-94.
171. Martin R, Buchan DS, Baker JS, et al. Sprint interval training (SIT) is an effective method to maintain cardiorespiratory fitness (CRF) and glucose homeostasis in Scottish adolescents. Biol Sport 2015;**32**(4):307-13.
172. Corte de Araujo AC, Roschel H, Picanco AR, et al. Similar health benefits of endurance and high-intensity interval training in obese children. PLoS One 2012;**7**(8):e42747.
173. Leech RM, McNaughton SA, Timperio A. Clustering of diet, physical activity and sedentary behaviour among Australian children: cross-sectional and longitudinal associations with overweight and obesity. Int J Obes (Lond) 2015;**39**(7):1079-85.
174. Whitaker RC, Wright JA, Pepe MS, et al. Predicting obesity in young adulthood from childhood and parental obesity. N Engl J Med 1997;**337**(13):869-73.
175. Wang Y, Lobstein T. Worldwide trends in childhood overweight and obesity. International Journal of Pediatric Obesity 2006;**1**(1):11-25.

176. Zeller MH, Modi AC. Development and initial validation of an obesity-specific quality-of-life measure for children: sizing me up. Obesity (Silver Spring) 2009;**17**(6):1171-7.
177. Williams RH, & Larsen, P. R. *Williams textbook of endocrinology*. 11th ed. ed. Philadelphia, Pa: Saunders, 2008.
178. Gonzalez-Jimenez E, Schmidt-RioValle J, Montero-Alonso MA, et al. Influence of Biochemical and Anthropometric Factors on the Presence of Insulin Resistance in Adolescents. Biol Res Nurs 2016.
179. Cree-Green M, Triolo TM, Nadeau KJ. Etiology of insulin resistance in youth with type 2 diabetes. Curr Diab Rep 2013;**13**(1):81-8.
180. Romualdo MC, Nobrega FJ, Escrivao MA. Insulin resistance in obese children and adolescents. J Pediatr (Rio J) 2014;**90**(6):600-7.
181. Cruz ML, Shaibi GQ, Weigensberg MJ, et al. Pediatric obesity and insulin resistance: chronic disease risk and implications for treatment and prevention beyond body weight modification. Annu Rev Nutr 2005;**25**:435-68.
182. Rodden A, Diaz V, Mainousiii A, et al. Insulin Resistance in Adolescents. J Pediatr 2007;**151**(3):275-79.
183. Yi KH, Hwang JS, Kim EY, et al. Prevalence of insulin resistance and cardiometabolic risk in Korean children and adolescents: a population-based study. Diabetes Res Clin Pract 2014;**103**(1):106-13.
184. Manios Y, Moschonis G, Kourlaba G, et al. Prevalence and independent predictors of insulin resistance in children from Crete, Greece: the Children Study. Diabet Med 2008;**25**(1):65-72.
185. Lee JM, Okumura MJ, Davis MM, et al. Prevalence and determinants of insulin resistance among U.S. adolescents: a population-based study. Diabetes care 2006;**29**(11):2427-32.
186. Bahillo-Curieses MP, Hermoso-Lopez F, Martinez-Sopena MJ, et al. Prevalence of insulin resistance and impaired glucose tolerance in a sample of obese Spanish children and adolescents. Endocrine 2012;**41**(2):289-95.
187. Baranowski T, Cooper DM, Harrell J, et al. Presence of diabetes risk factors in a large U.S. eighth-grade cohort. Diabetes Care 2006;**29**(2):212-7.
188. Bergman RN, Phillips LS, Cobelli C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest 1981;**68**(6):1456-67.
189. Cobelli C, Toffolo GM, Dalla Man C, et al. Assessment of beta-cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose tests. Am J Physiol Endocrinol Metab 2007;**293**(1):E1-E15.
190. Arslanian SA, Bacha F, Saad R, et al. Family history of type 2 diabetes is associated with decreased insulin sensitivity and an impaired balance between insulin sensitivity and insulin secretion in white youth. Diabetes Care 2005;**28**(1):115-9.
191. Danadian K, Balasekaran G, Lewy V, et al. Insulin sensitivity in African-American children with and without family history of type 2 diabetes. Diabetes Care 1999;**22**(8):1325-9.
192. Weiss R, Taksali SE, Tamborlane WV, et al. Predictors of changes in glucose tolerance status in obese youth. Diabetes Care 2005;**28**(4):902-9.
193. Ferrannini E, Gastaldelli A, Miyazaki Y, et al. beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. J Clin Endocrinol Metab 2005;**90**(1):493-500.

194. Pratipanawatr W, Pratipanawatr T, Cusi K, et al. Skeletal muscle insulin resistance in normoglycemic subjects with a strong family history of type 2 diabetes is associated with decreased insulin-stimulated insulin receptor substrate-1 tyrosine phosphorylation. Diabetes 2001;**50**(11):2572-8.
195. Martin BC, Warram JH, Krolewski AS, et al. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet 1992;**340**(8825):925-9.
196. Di Bonito P, Sanguigno E, Forziato C, et al. Fasting plasma glucose and clustering of cardiometabolic risk factors in normoglycemic outpatient children and adolescents. Diabetes care 2011;**34**(6):1412-4.
197. Gutknecht DR. Higher values of fasting plasma glucose within the normal range were associated with increased risk of type 2 diabetes. Evid Based Med 2008;**13**(6):186.
198. Saad MF, Knowler WC, Pettitt DJ, et al. The natural history of impaired glucose tolerance in the Pima Indians. N Engl J Med 1988;**319**(23):1500-6.
199. Edelstein SL, Knowler WC, Bain RP, et al. Predictors of progression from impaired glucose tolerance to NIDDM: an analysis of six prospective studies. Diabetes 1997;**46**(4):701-10.
200. Weiss R, Dufour S, Taksali SE, et al. Prediabetes in obese youth: a syndrome of impaired glucose tolerance, severe insulin resistance, and altered myocellular and abdominal fat partitioning. Lancet 2003;**362**(9388):951-7.
201. Barker DJ, Osmond C, Simmonds SJ, et al. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. Bmj 1993;**306**(6875):422-6.
202. Nicholas LM, Morrison JL, Rattanatray L, et al. The early origins of obesity and insulin resistance: timing, programming and mechanisms. Int J Obes (Lond) 2016;**40**(2):229-38.
203. Hofman PL, Regan F, Jackson WE, et al. Premature birth and later insulin resistance. N Engl J Med 2004;**351**(21):2179-86.
204. Derraik JG, Mathai S, Chiavaroli V, et al. Preterm birth is associated with an intergenerational effect on cardio-metabolic risk. Clin Endocrinol (Oxf) 2015;**83**(3):439-40.
205. Gluckman PD, Cutfield W, Hofman P, et al. The fetal, neonatal, and infant environments-the long-term consequences for disease risk. Early human development 2005;**81**(1):51-9.
206. Hofman PL, Cutfield WS. Insulin sensitivity in people born pre-term, with low or very low birth weight and small for gestational age. J Endocrinol Invest 2006;**29**(1 Suppl):2-8.
207. Chiavaroli V, Derraik JG, Hofman PL, et al. Born Large for Gestational Age: Bigger Is Not Always Better. J Pediatr 2016;**170**:307-11.
208. Derraik JG, Ayyavoo A, Hofman PL, et al. Increasing maternal prepregnancy body mass index is associated with reduced insulin sensitivity and increased blood pressure in their children. Clin Endocrinol (Oxf) 2015;**83**(3):352-6.
209. Jefferies CA, Hofman PL, Knoblauch H, et al. Insulin resistance in healthy prepubertal twins. J Pediatr 2004;**144**(5):608-13.
210. Jefferies CA, Hofman PL, Wong W, et al. Increased nocturnal blood pressure in healthy prepubertal twins. J Hypertens 2003;**21**(7):1319-24.
211. Levy-Marchal C, Jaquet D. Long-term metabolic consequences of being born small for gestational age. Pediatr Diabetes 2004;**5**(3):147-53.
212. Grella PV. Low birth weight and early life origins of adult disease: insulin resistance and type 2 diabetes. Clin Exp Obstet Gynecol 2007;**34**(1):9-13.

213. Hofman PL, Regan F, Jefferies CA, et al. Prematurity and programming: are there later metabolic sequelae? Metab Syndr Relat Disord 2006;**4**(2):101-12.
214. Duque-Guimaraes DE, Ozanne SE. Nutritional programming of insulin resistance: causes and consequences. Trends in endocrinology and metabolism: TEM 2013;**24**(10):525-35.
215. Mathai S, Cutfield WS, Derraik JG, et al. Insulin sensitivity and beta-cell function in adults born preterm and their children. Diabetes 2012;**61**(10):2479-83.
216. Ayyavoo A, Savage T, Derraik JGB, et al. First-born children have reduced insulin sensitivity and higher daytime blood pressure compared to later-born children. Journal of Clinical Endocrinology & Metabolism 2013;**98**(3):1248-53.
217. Goran MI, Ball GD, Cruz ML. Obesity and risk of type 2 diabetes and cardiovascular disease in children and adolescents. J Clin Endocrinol Metab 2003;**88**(4):1417-27.
218. Goran MI, Lane C, Toledo-Corral C, et al. Persistence of pre-diabetes in overweight and obese Hispanic children: association with progressive insulin resistance, poor beta-cell function, and increasing visceral fat. Diabetes 2008;**57**(11):3007-12.
219. Taylor RW, Grant AM, Williams SM, et al. Sex differences in regional body fat distribution from pre- to postpuberty. Obesity (Silver Spring) 2010;**18**(7):1410-6.
220. Moran A, Jacobs DR, Jr., Steinberger J, et al. Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes 1999;**48**(10):2039-44.
221. Saad RJ, Danadian K, Lewy V, et al. Insulin resistance of puberty in African-American children: lack of a compensatory increase in insulin secretion. Pediatr Diabetes 2002;**3**(1):4-9.
222. Goran MI, Gower BA. Longitudinal study on pubertal insulin resistance. Diabetes 2001;**50**(11):2444-50.
223. Staiano AE, Katzmarzyk PT. Ethnic and sex differences in body fat and visceral and subcutaneous adiposity in children and adolescents. Int J Obes (Lond) 2012;**36**(10):1261-9.
224. Jeffery AN, Metcalf BS, Hosking J, et al. Age before stage: insulin resistance rises before the onset of puberty: a 9-year longitudinal study (EarlyBird 26). Diabetes Care 2012;**35**(3):536-41.
225. Arslanian SA, Kalhan SC. Correlations between fatty acid and glucose metabolism. Potential explanation of insulin resistance of puberty. Diabetes 1994;**43**(7):908-14.
226. Hannon TS, Janosky J, Arslanian SA. Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. Pediatr Res 2006;**60**(6):759-63.
227. Matthaei S, Stumvoll M, Kellerer M, et al. Pathophysiology and pharmacological treatment of insulin resistance. Endocr Rev 2000;**21**(6):585-618.
228. Sesti G. Pathophysiology of insulin resistance. Best Pract Res Clin Endocrinol Metab 2006;**20**(4):665-79.
229. DeFronzo RA, Ferrannini E, Simonson DC. Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. Metabolism 1989;**38**(4):387-95.
230. Wilding JP. The importance of free fatty acids in the development of Type 2 diabetes. Diabet Med 2007;**24**(9):934-45.
231. Armato J, DeFronzo RA, Abdul-Ghani M, et al. Successful treatment of prediabetes in clinical practice: targeting insulin resistance and beta-cell dysfunction. Endocrine Practice;**18**(3):342-50.
232. Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001;**86**(5):1930-5.

233. Maahs DM, Hamman RF, D'Agostino R, Jr., et al. The association between adiponectin/leptin ratio and diabetes type: the SEARCH for Diabetes in Youth Study. J Pediatr 2009;**155**(1):133-5, 35 e1.
234. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;**8**(11):1288-95.
235. Bergman RN, Ader M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. Trends in endocrinology and metabolism: TEM 2000;**11**(9):351-6.
236. Boden G, Chen X, Rosner J, et al. Effects of a 48-h fat infusion on insulin secretion and glucose utilization. Diabetes 1995;**44**(10):1239-42.
237. Danesh J, Wheeler JG, Hirschfield GM, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004;**350**(14):1387-97.
238. Danesh J, Whincup P, Walker M, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. Bmj 2000;**321**(7255):199-204.
239. Ford ES, Ajani UA, Mokdad AH. The metabolic syndrome and concentrations of C-reactive protein among U.S. youth. Diabetes Care 2005;**28**(4):878-81.
240. Bluher M. The distinction of metabolically 'healthy' from 'unhealthy' obese individuals. Current opinion in lipidology 2010;**21**(1):38-43.
241. Weiss R, Taksali SE, Dufour S, et al. The "obese insulin-sensitive" adolescent: importance of adiponectin and lipid partitioning. J Clin Endocrinol Metab 2005;**90**(6):3731-7.
242. Prince RL, Kuk JL, Ambler KA, et al. Predictors of metabolically healthy obesity in children. Diabetes Care 2014;**37**(5):1462-8.
243. Caprio S, Hyman LD, Limb C, et al. Central adiposity and its metabolic correlates in obese adolescent girls. Am J Physiol 1995;**269**(1 Pt 1):E118-26.
244. Taksali SE, Caprio S, Dziura J, et al. High visceral and low abdominal subcutaneous fat stores in the obese adolescent: a determinant of an adverse metabolic phenotype. Diabetes 2008;**57**(2):367-71.
245. Goran MI, Gower BA. Abdominal obesity and cardiovascular risk in children. Coronary artery disease 1998;**9**(8):483-7.
246. Maffeis C, Manfredi R, Trombetta M, et al. Insulin sensitivity is correlated with subcutaneous but not visceral body fat in overweight and obese prepubertal children. J Clin Endocrinol Metab 2008;**93**(6):2122-8.
247. Lara-Castro C, Garvey WT. Intracellular lipid accumulation in liver and muscle and the insulin resistance syndrome. Endocrinol Metab Clin North Am 2008;**37**(4):841-56.
248. Lee JA, Laurson KR. Obesity and Insulin Resistance Screening Tools in American Adolescents: National Health and Nutrition Examination Survey (NHANES) 1999 to 2010. Can J Diabetes 2016.
249. Goran MI, Nagy TR, Treuth MS, et al. Visceral fat in white and African American prepubertal children. Am J Clin Nutr 1997;**65**(6):1703-8.
250. Araneta MR, Barrett-Connor E. Ethnic differences in visceral adipose tissue and type 2 diabetes: Filipino, African-American, and white women. Obes Res 2005;**13**(8):1458-65.
251. Wang L, Sacks FM, Furtado JD, et al. Racial differences between African-American and white women in insulin resistance and visceral adiposity are associated with differences in apoCIII containing apoAI and apoB lipoproteins. Nutr Metab (Lond) 2014;**11**(1):56.

252. Goran MI, Bergman RN, Gower BA. Influence of total vs. visceral fat on insulin action and secretion in African American and white children. Obes Res 2001;**9**(8):423-31.
253. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. Lancet 2010;**375**(9733):2267-77.
254. Yeckel CW. Validation of Insulin Sensitivity Indices from Oral Glucose Tolerance Test Parameters in Obese Children and Adolescents. Journal of Clinical Endocrinology & Metabolism 2004;**89**(3):1096-101.
255. Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity. Annu Rev Med 1998;**49**:235-61.
256. Yaspelkis BB, 3rd. Resistance training improves insulin signaling and action in skeletal muscle. Exerc Sport Sci Rev 2006;**34**(1):42-6.
257. Twigg SM, Kamp MC, Davis TM, et al. Prediabetes: a position statement from the Australian Diabetes Society and Australian Diabetes Educators Association. Med J Aust 2007;**186**(9):461-5.
258. Craig ME, Jefferies C, Dabelea D, et al. ISPAD Clinical Practice Consensus Guidelines 2014. Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes 2014;**15 Suppl 20**:4-17.
259. American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;**37 Suppl 1**:S81-90.
260. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;**237**(3):E214-23.
261. Bergman RN, Hope ID, Yang YJ, et al. Assessment of insulin sensitivity in vivo: a critical review. Diabetes Metab Rev 1989;**5**(5):411-29.
262. Bergman RN, Prager R, Volund A, et al. Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. J Clin Invest 1987;**79**(3):790-800.
263. Pacini G, Bergman RN. MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput Methods Programs Biomed 1986;**23**(2):113-22.
264. Coates PA, Luzio SD, Brunel P, et al. Comparison of estimates of insulin sensitivity from minimal model analysis of the insulin-modified frequently sampled intravenous glucose tolerance test and the isoglycemic hyperinsulinemic clamp in subjects with NIDDM. Diabetes 1995;**44**(6):631-5.
265. Pratt-Phillips SE, Geor RJ, McCutcheon LJ. Comparison among the euglycemic-hyperinsulinemic clamp, insulin-modified frequently sampled intravenous glucose tolerance test, and oral glucose tolerance test for assessment of insulin sensitivity in healthy Standardbreds. American journal of veterinary research 2015;**76**(1):84-91.
266. Monzillo LU, Hamdy O. Evaluation of insulin sensitivity in clinical practice and in research settings. Nutr Rev 2003;**61**(12):397-412.
267. Uwaifo GI, Fallon EM, Chin J, et al. Indices of insulin action, disposal, and secretion derived from fasting samples and clamps in normal glucose-tolerant black and white children. Diabetes Care 2002;**25**(11):2081-7.
268. Gungor N, Saad R, Janosky J, et al. Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. J Pediatr 2004;**144**(1):47-55.
269. Conwell LS, Trost SG, Brown WJ, et al. Indexes of insulin resistance and secretion in obese children and adolescents: a validation study. Diabetes Care 2004;**27**(2):314-9.

270. Schwartz B, Jacobs DR, Jr., Moran A, et al. Measurement of insulin sensitivity in children: comparison between the euglycemic-hyperinsulinemic clamp and surrogate measures. Diabetes Care 2008;**31**(4):783-8.
271. DeFronzo RA, Gunnarsson R, Bjorkman O, et al. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest 1985;**76**(1):149-55.
272. Abdul-Ghani MA, Lyssenko V, Tuomi T, et al. Fasting versus postload plasma glucose concentration and the risk for future type 2 diabetes: results from the Botnia Study. Diabetes Care 2009;**32**(2):281-6.
273. Abdul-Ghani MA, Abdul-Ghani T, Ali N, et al. One-hour plasma glucose concentration and the metabolic syndrome identify subjects at high risk for future type 2 diabetes. Diabetes Care 2008;**31**(8):1650-5.
274. Mooy JM, Grootenhuis PA, de Vries H, et al. Intra-individual variation of glucose, specific insulin and proinsulin concentrations measured by two oral glucose tolerance tests in a general Caucasian population: the Hoorn Study. Diabetologia 1996;**39**(3):298-305.
275. Soonthornpun S, Setasuban W, Thamprasit A, et al. Novel insulin sensitivity index derived from oral glucose tolerance test. J Clin Endocrinol Metab 2003;**88**(3):1019-23.
276. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes care 1999;**22**(9):1462-70.
277. Stumvoll M, Mitrakou A, Pimenta W, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care 2000;**23**(3):295-301.
278. Maki KC, Rains TM, Dicklin MR, et al. Repeatability of indices of insulin sensitivity and secretion from standard liquid meal tests in subjects with type 2 diabetes mellitus or normal or impaired fasting glucose. Diabetes Technol Ther 2010;**12**(11):895-900.
279. Abdul-Ghani MA, Matsuda M, Balas B, et al. Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. Diabetes Care 2007;**30**(1):89-94.
280. Levy-Marchal C, Arslanian S, Cutfield W, et al. Insulin resistance in children: consensus, perspective, and future directions. J Clin Endocrinol Metab 2010;**95**(12):5189-98.
281. Ferrannini E, Mari A. How to measure insulin sensitivity. J Hypertens 1998;**16**(7):895-906.
282. Chevenne D, Trivin F, Porquet D. Insulin assays and reference values. Diabetes Metab 1999;**25**(6):459-76.
283. Lindahl B, Asplund K, Hallmans G. High serum insulin, insulin resistance and their associations with cardiovascular risk factors. The northern Sweden MONICA population study. Journal of internal medicine 1993;**234**(3):263-70.
284. Laakso M. How good a marker is insulin level for insulin resistance? Am J Epidemiol 1993;**137**(9):959-65.
285. Williams CL. Cardiovascular Health in Childhood: A Statement for Health Professionals From the Committee on Atherosclerosis, Hypertension, and Obesity in the Young (AHOY) of the Council on Cardiovascular Disease in the Young, American Heart Association. Circulation 2002;**106**(1):143-60.
286. Ten S. Insulin Resistance Syndrome in Children. Journal of Clinical Endocrinology & Metabolism 2004;**89**(6):2526-39.
287. Adam TC, Hasson RE, Lane CJ, et al. Fasting indicators of insulin sensitivity: effects of ethnicity and pubertal status. Diabetes Care 2011;**34**(4):994-9.

288. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;**28**(7):412-9.
289. Fukushima M, Taniguchi A, Sakai M, et al. Assessment of insulin sensitivity: comparison between simplified evaluations and minimal model analysis. Diabetes Care 2000;**23**(7):1038-9.
290. Ascaso JF, Romero P, Real JT, et al. [Insulin resistance quantification by fasting insulin plasma values and HOMA index in a non-diabetic population]. Medicina clinica 2001;**117**(14):530-3.
291. Keskin M. Homeostasis Model Assessment Is More Reliable Than the Fasting Glucose/Insulin Ratio and Quantitative Insulin Sensitivity Check Index for Assessing Insulin Resistance Among Obese Children and Adolescents. Pediatrics 2005;**115**(4):e500-e03.
292. Sahin NM, Kinik ST, Tekindal MA. OGTT results in obese adolescents with normal HOMA-IR values. J Pediatr Endocrinol 2013;**26**(3-4):285-91.
293. Muniyappa R, Lee S, Chen H, et al. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab 2008;**294**(1):E15-26.
294. Chen H, Sullivan G, Quon MJ. Assessing the predictive accuracy of QUICKI as a surrogate index for insulin sensitivity using a calibration model. Diabetes 2005;**54**(7):1914-25.
295. Rotterdam EA-SPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;**19**(1):41-7.
296. Buggs C, Rosenfield RL. Polycystic ovary syndrome in adolescence. Endocrinology & Metabolism Clinics of North America 2005;**34**(3):677-705, x.
297. Ibanez L, Lopez-Bermejo A, Diaz M, et al. Early metformin therapy (age 8-12 years) in girls with precocious pubarche to reduce hirsutism, androgen excess, and oligomenorrhea in adolescence. Journal of Clinical Endocrinology & Metabolism 2011;**96**(8):E1262-7.
298. Guran T, Turan S, Akcay T, et al. Significance of acanthosis nigricans in childhood obesity. J Paediatr Child Health 2008;**44**(6):338-41.
299. Brickman WJ, Binns HJ, Jovanovic BD, et al. Acanthosis nigricans: a common finding in overweight youth. Pediatric dermatology 2007;**24**(6):601-6.
300. Kluczynik CEN, Mariz LS, Souza LCF, et al. Acanthosis nigricans and insulin resistance in overweight children and adolescents. An Bras Dermatol 2012;**87**(4):531-7.
301. Nguyen TT, Keil MF, Russell DL, et al. Relation of acanthosis nigricans to hyperinsulinemia and insulin sensitivity in overweight African American and white children. J Pediatr 2001;**138**(4):474-80.
302. Kong AS, Vanderbloemen L, Skipper B, et al. Acanthosis nigricans predicts the clustering of metabolic syndrome components in Hispanic elementary school-aged children. J Pediatr Endocrinol 2012;**25**(11-12):1095-102.
303. Brickman WJ, Huang J, Silverman BL, et al. Acanthosis nigricans identifies youth at high risk for metabolic abnormalities. J Pediatr 2010;**156**(1):87-92.
304. Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;**344**(18):1343-50.
305. Alberti KG, Zimmet P, Shaw J. International Diabetes Federation: a consensus on Type 2 diabetes prevention. Diabet Med 2007;**24**(5):451-63.

306. Tsigos C, Hainer V, Basdevant A, et al. Management of obesity in adults: European clinical practice guidelines. Obes Facts 2008;**1**(2):106-16.
307. Wing RR. Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes mellitus: four-year results of the Look AHEAD trial. Arch Intern Med 2010;**170**(17):1566-75.
308. Ho M, Garnett SP, Baur L, et al. Effectiveness of lifestyle interventions in child obesity: systematic review with meta-analysis. Pediatrics 2012;**130**(6):e1647-71.
309. Duckworth LC, Gately PJ, Radley D, et al. RCT of a high-protein diet on hunger motivation and weight-loss in obese children: an extension and replication. Obesity (Silver Spring) 2009;**17**(9):1808-10.
310. Gately PJ, King NA, Greatwood HC, et al. Does a high-protein diet improve weight loss in overweight and obese children? Obesity (Silver Spring) 2007;**15**(6):1527-34.
311. Kreider RB, Rasmussen C, Kerksick CM, et al. A carbohydrate-restricted diet during resistance training promotes more favorable changes in body composition and markers of health in obese women with and without insulin resistance. Phys Sportsmed 2011;**39**(2):27-40.
312. Galgani JE, Uauy RD, Aguirre CA, et al. Effect of the dietary fat quality on insulin sensitivity. Br J Nutr 2008;**100**(3):471-9.
313. Ebbeling CB, Leidig MM, Sinclair KB, et al. A reduced-glycemic load diet in the treatment of adolescent obesity. Arch Pediatr Adolesc Med 2003;**157**(8):773-9.
314. Thomas DE, Elliott EJ, Baur L. Low glycaemic index or low glycaemic load diets for overweight and obesity. The Cochrane database of systematic reviews 2007(3):CD005105.
315. Steffen LM, Jacobs DR, Jr., Murtaugh MA, et al. Whole grain intake is associated with lower body mass and greater insulin sensitivity among adolescents. Am J Epidemiol 2003;**158**(3):243-50.
316. de Bock M, Derraik JG, Brennan CM, et al. Psyllium supplementation in adolescents improves fat distribution & lipid profile: a randomized, participant-blinded, placebo-controlled, crossover trial. PLoS One 2012;**7**(7):e41735.
317. Arciero PJ, Vukovich MD, Holloszy JO, et al. Comparison of short-term diet and exercise on insulin action in individuals with abnormal glucose tolerance. J Appl Physiol 1999;**86**(6):1930-5.
318. Shields N. Exercise training decreases fasting insulin levels and improves insulin resistance in children and adolescents. J Physiother 2014;**60**(3):165.
319. Mendelson M, Michallet AS, Monneret D, et al. Impact of exercise training without caloric restriction on inflammation, insulin resistance and visceral fat mass in obese adolescents. Pediatr Obes 2015;**10**(4):311-9.
320. Fedewa MV, Gist NH, Evans EM, et al. Exercise and insulin resistance in youth: a meta-analysis. Pediatrics 2014;**133**(1):e163-74.
321. Alberga AS, Frappier A, Sigal RJ, et al. A review of randomized controlled trials of aerobic exercise training on fitness and cardiometabolic risk factors in obese adolescents. Phys Sportsmed 2013;**41**(2):44-57.
322. Centers for Disease C, Prevention. Physical activity levels among children aged 9-13 years--United States, 2002. MMWR - Morbidity & Mortality Weekly Report 2003;**52**(33):785-8.
323. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;**346**(6):393-403.
324. DeFronzo RA, Sherwin RS, Kraemer N. Effect of physical training on insulin action in obesity. Diabetes 1987;**36**(12):1379-85.

325. Winnick JJ, Sherman WM, Habash DL, et al. Short-Term Aerobic Exercise Training in Obese Humans with Type 2 Diabetes Mellitus Improves Whole-Body Insulin Sensitivity through Gains in Peripheral, not Hepatic Insulin Sensitivity. Journal of Clinical Endocrinology & Metabolism 2007;**93**(3):771-78.
326. Houmard JA, Hickey MS, Tyndall GL, et al. Seven days of exercise increase GLUT-4 protein content in human skeletal muscle. J Appl Physiol (1985) 1995;**79**(6):1936-8.
327. Kriska AM, Pereira MA, Hanson RL, et al. Association of physical activity and serum insulin concentrations in two populations at high risk for type 2 diabetes but differing by BMI. Diabetes Care 2001;**24**(7):1175-80.
328. Bell LM, Watts K, Siafarikas A, et al. Exercise alone reduces insulin resistance in obese children independently of changes in body composition. J Clin Endocrinol Metab 2007;**92**(11):4230-5.
329. Kim Y, Park H. Does Regular Exercise without Weight Loss Reduce Insulin Resistance in Children and Adolescents? Int J Endocrinol 2013;**2013**:402592.
330. Nassis GP, Papantakou K, Skenderi K, et al. Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. Metabolism 2005;**54**(11):1472-9.
331. van der Heijden GJ, Toffolo G, Manesso E, et al. Aerobic exercise increases peripheral and hepatic insulin sensitivity in sedentary adolescents. J Clin Endocrinol Metab 2009;**94**(11):4292-9.
332. Many G, Hurtado M-E, Tanner C, et al. Moderate-intensity aerobic training program improves insulin sensitivity and inflammatory markers in a pilot study of morbidly obese minority teens. Pediatr Exerc Sci 2013;**25**(1):12-26.
333. Conwell LS, Trost SG, Spence L, et al. The feasibility of a home-based moderate-intensity physical activity intervention in obese children and adolescents. British journal of sports medicine 2010;**44**(4):250-5.
334. McGuigan MR, Tatasciore M, Newton RU, et al. Eight weeks of resistance training can significantly alter body composition in children who are overweight or obese. J Strength Cond Res 2009;**23**(1):80-5.
335. Damaso AR, da Silveira Campos RM, Caranti DA, et al. Aerobic plus resistance training was more effective in improving the visceral adiposity, metabolic profile and inflammatory markers than aerobic training in obese adolescents. J Sports Sci 2014;**32**(15):1435-45.
336. Miller WJ, Sherman WM, Ivy JL. Effect of strength training on glucose tolerance and post-glucose insulin response. Med Sci Sports Exerc 1984;**16**(6):539-43.
337. Jimenez-Pavon D, Ortega FB, Valtuena J, et al. Muscular strength and markers of insulin resistance in European adolescents: the HELENA Study. Eur J Appl Physiol 2012;**112**(7):2455-65.
338. Lee S, Bacha F, Hannon T, et al. Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys: a randomized, controlled trial. Diabetes 2012;**61**(11):2787-95.
339. Molnar D, Porszasz J. The effect of fasting hyperinsulinaemia on physical fitness in obese children. Eur J Pediatr 1990;**149**(8):570-3.
340. Nadeau KJ, Zeitler PS, Bauer TA, et al. Insulin resistance in adolescents with type 2 diabetes is associated with impaired exercise capacity. J Clin Endocrinol Metab 2009;**94**(10):3687-95.
341. Martin MJ, Horwitz DL, Nattrass M, et al. Effects of mild hyperinsulinemia on the metabolic response to exercise. Metabolism 1981;**30**(7):688-94.

342. Montero D. Hemodynamic actions of insulin: beyond the endothelium. Front Physiol 2013;**4**:389.
343. Baron AD. Hemodynamic actions of insulin. Am J Physiol 1994;**267**(2 Pt 1):E187-202.
344. Lalande S, Gusso S, Hofman PL, et al. Reduced leg blood flow during submaximal exercise in type 2 diabetes. Med Sci Sports Exerc 2008;**40**(4):612-7.
345. Shaibi GQ, Cruz ML, Ball GD, et al. Effects of resistance training on insulin sensitivity in overweight Latino adolescent males. Med Sci Sports Exerc 2006;**38**(7):1208-15.
346. Allen DB, Nemeth BA, Clark RR, et al. Fitness is a stronger predictor of fasting insulin levels than fatness in overweight male middle-school children. J Pediatr 2007;**150**(4):383-7.
347. Ahn B, McMurray R, Harrell J. Scaling of VO2max and its relationship with insulin resistance in children. Pediatr Exerc Sci 2013;**25**(1):43-51.
348. Haufe S, Engeli S, Budziarek P, et al. Cardiorespiratory fitness and insulin sensitivity in overweight or obese subjects may be linked through intrahepatic lipid content. Diabetes 2010;**59**(7):1640-7.
349. Kasa-Vubu JZ, Lee CC, Rosenthal A, et al. Cardiovascular fitness and exercise as determinants of insulin resistance in postpubertal adolescent females. J Clin Endocrinol Metab 2005;**90**(2):849-54.
350. Cochrane DJ. Is vibration exercise a useful addition to a weight management program? Scand J Med Sci Sports 2012;**22**(6):705-13.
351. Rubin CT, Capilla E, Luu YK, et al. Adipogenesis is inhibited by brief, daily exposure to high-frequency, extremely low-magnitude mechanical signals. Proc Natl Acad Sci U S A 2007;**104**(45):17879-84.
352. Huang CC, Tseng TL, Huang WC, et al. Whole-body vibration training effect on physical performance and obesity in mice. International journal of medical sciences 2014;**11**(12):1218-27.
353. Roelants M, Delecluse C, Goris M, et al. Effects of 24 weeks of whole body vibration training on body composition and muscle strength in untrained females. Int J Sports Med 2004;**25**(1):1-5.
354. Wilms B, Frick J, Ernst B, et al. Whole body vibration added to endurance training in obese women - a pilot study. Int J Sports Med 2012;**33**(9):740-3.
355. Zaki ME. Effects of whole body vibration and resistance training on bone mineral density and anthropometry in obese postmenopausal women. J Osteoporos 2014;**2014**:702589.
356. Vissers D, Hens W, Taeymans J, et al. The effect of exercise on visceral adipose tissue in overweight adults: a systematic review and meta-analysis. PLoS One 2013;**8**(2):e56415.
357. Sanudo B, Alfonso-Rosa R, Del Pozo-Cruz B, et al. Whole body vibration training improves leg blood flow and adiposity in patients with type 2 diabetes mellitus. Eur J Appl Physiol 2013;**113**(9):2245-52.
358. Cristi-Montero C, Cuevas MJ, Collado PS. Whole-body vibration training as complement to programs aimed at weight loss. Nutr Hosp 2013;**28**(5):1365-71.
359. Sgro M, McGuigan MR, Pettigrew S, et al. The effect of duration of resistance training interventions in children who are overweight or obese. J Strength Cond Res 2009;**23**(4):1263-70.
360. Jones KL, Arslanian S, Peterokova VA, et al. Effect of metformin in pediatric patients with type 2 diabetes: a randomized controlled trial. Diabetes Care 2002;**25**(1):89-94.

361. Prager R, Schernthaner G, Graf H. Effect of metformin on peripheral insulin sensitivity in non insulin dependent diabetes mellitus. Diabete Metab 1986;**12**(6):346-50.
362. Gerich JE. Oral hypoglycemic agents. N Engl J Med 1989;**321**(18):1231-45.
363. Fontbonne A, Charles MA, Juhan-Vague I, et al. The effect of metformin on the metabolic abnormalities associated with upper-body fat distribution. BIGPRO Study Group. Diabetes Care 1996;**19**(9):920-6.
364. Lee A, Morley JE. Metformin decreases food consumption and induces weight loss in subjects with obesity with type II non-insulin-dependent diabetes. Obes Res 1998;**6**(1):47-53.
365. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. N Engl J Med 1995;**333**(9):541-9.
366. Arslanian SA, Lewy V, Danadian K, et al. Metformin therapy in obese adolescents with polycystic ovary syndrome and impaired glucose tolerance: amelioration of exaggerated adrenal response to adrenocorticotropin with reduction of insulinemia/insulin resistance. J Clin Endocrinol Metab 2002;**87**(4):1555-9.
367. Quinn SM, Baur LA, Garnett SP, et al. Treatment of clinical insulin resistance in children: a systematic review. Obes Rev 2010;**11**(10):722-30.
368. Yanovski JA, Krakoff J, Salaita CG, et al. Effects of metformin on body weight and body composition in obese insulin-resistant children: a randomized clinical trial. Diabetes 2011;**60**(2):477-85.
369. Wiegand S, l'Allemand D, Hubel H, et al. Metformin and placebo therapy both improve weight management and fasting insulin in obese insulin-resistant adolescents: a prospective, placebo-controlled, randomized study. Eur J Endocrinol 2010;**163**(4):585-92.
370. Zeitler P, Hirst K, Pyle L, et al. A clinical trial to maintain glycemic control in youth with type 2 diabetes. N Engl J Med 2012;**366**(24):2247-56.
371. Love-Osborne K, Sheeder J, Zeitler P. Addition of metformin to a lifestyle modification program in adolescents with insulin resistance. J Pediatr 2008;**152**(6):817-22.
372. Kay JP, Alemzadeh R, Langley G, et al. Beneficial effects of metformin in normoglycemic morbidly obese adolescents. Metabolism 2001;**50**(12):1457-61.
373. Freemark M, Bursey D. The effects of metformin on body mass index and glucose tolerance in obese adolescents with fasting hyperinsulinemia and a family history of type 2 diabetes. Pediatrics 2001;**107**(4):E55.
374. Srinivasan S. Randomized, Controlled Trial of Metformin for Obesity and Insulin Resistance in Children and Adolescents: Improvement in Body Composition and Fasting Insulin. Journal of Clinical Endocrinology & Metabolism 2006;**91**(6):2074-80.
375. Atabek ME, Pirgon O. Use of metformin in obese adolescents with hyperinsulinemia: a 6-month, randomized, double-blind, placebo-controlled clinical trial. J Pediatr Endocrinol Metab 2008;**21**(4):339-48.
376. Park MH, Kinra S, Ward KJ, et al. Metformin for obesity in children and adolescents: a systematic review. Diabetes Care 2009;**32**(9):1743-5.
377. Clarson CL, Mahmud FH, Baker JE, et al. Metformin in combination with structured lifestyle intervention improved body mass index in obese adolescents, but did not improve insulin resistance. Endocrine 2009;**36**(1):141-6.
378. Garcia Diaz E, Martin Folgueras T. Systematic review of the clinical efficacy of sibutramine and orlistat in weigth loss, quality of life and its adverse effects in obese adolescents. Nutr Hosp 2011;**26**(3):451-7.

379. Osawa Y, Oguma Y. Effects of resistance training with whole-body vibration on muscle fitness in untrained adults. Scand J Med Sci Sports 2013;**23**(1):84-95.
380. Delecluse C, Roelants M, Verschueren S. Strength increase after whole-body vibration compared with resistance training. Medicine & Science in Sports & Exercise 2003;**35**(6):1033-41.
381. Machado A, Garcia-Lopez D, Gonzalez-Gallego J, et al. Whole-body vibration training increases muscle strength and mass in older women: a randomized-controlled trial. Scand J Med Sci Sports 2010;**20**(2):200-7.
382. Wilcock IM, Whatman C, Harris N, et al. Vibration training: could it enhance the strength, power, or speed of athletes? Journal of Strength & Conditioning Research 2009;**23**(2):593-603.
383. Sands WA, McNeal JR, Stone MH, et al. Flexibility enhancement with vibration: Acute and long-term. Med Sci Sports Exerc 2006;**38**(4):720-5.
384. Gerodimos V, Zafeiridis A, Karatrantou K, et al. The acute effects of different whole-body vibration amplitudes and frequencies on flexibility and vertical jumping performance. J Sci Med Sport 2010;**13**(4):438-43.
385. Di Giminiani R, Manno R, Scrimaglio R, et al. Effects of individualized whole-body vibration on muscle flexibility and mechanical power. Journal of Sports Medicine & Physical Fitness 2010;**50**(2):139-51.
386. Bemben DA, Palmer IJ, Bemben MG, et al. Effects of combined whole-body vibration and resistance training on muscular strength and bone metabolism in postmenopausal women. Bone 2010;**47**(3):650-6.
387. Cardinale M, Bosco C. The use of vibration as an exercise intervention. Exercise and sport sciences reviews 2003;**31**(1):3-7.
388. Rubin C, Turner AS, Bain S, et al. Anabolism. Low mechanical signals strengthen long bones. Nature 2001;**412**(6847):603-4.
389. Christiansen BA, Silva MJ. The effect of varying magnitudes of whole-body vibration on several skeletal sites in mice. Annals of biomedical engineering 2006;**34**(7):1149-56.
390. Rubin C, Recker R, Cullen D, et al. Prevention of postmenopausal bone loss by a low-magnitude, high-frequency mechanical stimuli: a clinical trial assessing compliance, efficacy, and safety. J Bone Miner Res 2004;**19**(3):343-51.
391. Turner S, Torode M, Climstein M, et al. A randomized controlled trial of whole body vibration exposure on markers of bone turnover in postmenopausal women. J Osteoporos 2011;**2011**:710387.
392. Von Stengel S, Kemmler W, Bebenek M, et al. Effects of whole-body vibration training on different devices on bone mineral density. Med Sci Sports Exerc 2011;**43**(6):1071-9.
393. Mikhael M, Orr R, Fiatarone Singh MA. The effect of whole body vibration exposure on muscle or bone morphology and function in older adults: a systematic review of the literature. Maturitas 2010;**66**(2):150-7.
394. Liphardt AM, Schipilow J, Hanley DA, et al. Bone quality in osteopenic postmenopausal women is not improved after 12 months of whole-body vibration training. Osteoporos Int 2015;**26**(3):911-20.
395. Verschueren SM, Bogaerts A, Delecluse C, et al. The effects of whole-body vibration training and vitamin D supplementation on muscle strength, muscle mass, and bone density in institutionalized elderly women: a 6-month randomized, controlled trial. J Bone Miner Res 2011;**26**(1):42-9.
396. Prisby RD, Lafage-Proust M-H, Malaval L, et al. Effects of whole body vibration on the skeleton and other organ systems in man and animal models: what we know and what we need to know. Ageing Res Rev 2008;**7**(4):319-29.

397. Fagnani F, Giombini A, Di Cesare A, et al. The effects of a whole-body vibration program on muscle performance and flexibility in female athletes. Am J Phys Med Rehabil 2006;**85**(12):956-62.
398. Oosthuyse T, Viedge A, McVeigh J, et al. Anaerobic power in road cyclists is improved after 10 weeks of whole-body vibration training. J Strength Cond Res 2013;**27**(2):485-94.
399. Osawa Y, Oguma Y. Effects of whole-body vibration on resistance training for untrained adults. J Sports Sci Med 2011;**10**(2):328-37.
400. Rehn B, Lidstrom J, Skoglund J, et al. Effects on leg muscular performance from whole-body vibration exercise: a systematic review. Scand J Med Sci Sports 2007;**17**(1):2-11.
401. Nordlund MM, Thorstensson A. Strength training effects of whole-body vibration? Scand J Med Sci Sports 2007;**17**(1):12-7.
402. Abercromby AFJ, Amonette WE, Layne CS, et al. Vibration exposure and biodynamic responses during whole-body vibration training. Medicine & Science in Sports & Exercise 2007;**39**(10):1794-800.
403. Rees SS, Murphy AJ, Watsford ML. Effects of whole-body vibration exercise on lower-extremity muscle strength and power in an older population: a randomized clinical trial. Phys Ther 2008;**88**(4):462-70.
404. Roelants M, Verschueren SM, Delecluse C, et al. Whole-body-vibration-induced increase in leg muscle activity during different squat exercises. J Strength Cond Res 2006;**20**(1):124-9.
405. Jordan MJ, Norris SR, Smith DJ, et al. Vibration training: an overview of the area, training consequences, and future considerations. J Strength Cond Res 2005;**19**(2):459-66.
406. Sayenko DG, Masani K, Alizadeh-Meghrazi M, et al. Acute effects of whole body vibration during passive standing on soleus H-reflex in subjects with and without spinal cord injury. Neurosci Lett 2010;**482**(1):66-70.
407. Ritzmann R, Kramer A, Gollhofer A, et al. The effect of whole body vibration on the H-reflex, the stretch reflex, and the short-latency response during hopping. Scand J Med Sci Sports 2013;**23**(3):331-9.
408. Rittweger J, Mutschelknauss M, Felsenberg D. Acute changes in neuromuscular excitability after exhaustive whole body vibration exercise as compared to exhaustion by squatting exercise. Clin Physiol Funct Imaging 2003;**23**(2):81-6.
409. Rittweger J. Vibration as an exercise modality: how it may work, and what its potential might be. Eur J Appl Physiol 2010;**108**(5):877-904.
410. Matthews PB. Reflex activation of the soleus muscle of the decerebrate cat by vibration. Nature 1966;**209**(5019):204-5.
411. Wikipedia. [Available from: https://en.wikipedia.org/wiki/Patellar_reflex -/media/File:Patellar_tendon_reflex_arc.png] Last accessed June 2016.
412. Abercromby AFJ, Amonette WE, Layne CS, et al. Variation in neuromuscular responses during acute whole-body vibration exercise. Medicine & Science in Sports & Exercise 2007;**39**(9):1642-50.
413. Ritzmann R, Kramer A, Gruber M, et al. EMG activity during whole body vibration: motion artifacts or stretch reflexes? Eur J Appl Physiol 2010;**110**(1):143-51.
414. Cardinale M, Pope MH. The effects of whole body vibration on humans: dangerous or advantageous? Acta Physiol Hung 2003;**90**(3):195-206.
415. Bosco C, Iacovelli M, Tsarpela O, et al. Hormonal responses to whole-body vibration in men. Eur J Appl Physiol 2000;**81**(6):449-54.

416. Vela JI, Andreu D, Diaz-Cascajosa J, et al. Intraocular lens dislocation after whole-body vibration. J Cataract Refract Surg 2010;**36**(10):1790-1.
417. Bertschinger DR, Dosso A. [Vitreous hemorrhage and whole-body vibration training--is there an association?]. Journal Francais d Opthalmologie 2008;**31**(8):e17.
418. Monteleone G, De Lorenzo A, Sgroi M, et al. Contraindications for whole body vibration training: a case of nephrolitiasis. Journal of Sports Medicine & Physical Fitness 2007;**47**(4):443-5.
419. Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials. Bone 2007;**40**(1):14-27.
420. Matkovic V, Jelic T, Wardlaw GM, et al. Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. J Clin Invest 1994;**93**(2):799-808.
421. Frost HM. Bone "mass" and the "mechanostat": a proposal. Anat Rec 1987;**219**(1):1-9.
422. Rittweger J, Beller G, Armbrecht G, et al. Prevention of bone loss during 56 days of strict bed rest by side-alternating resistive vibration exercise. Bone 2010;**46**(1):137-47.
423. Xie L, Jacobson JM, Choi ES, et al. Low-level mechanical vibrations can influence bone resorption and bone formation in the growing skeleton. Bone 2006;**39**(5):1059-66.
424. Rubin C, Xu G, Judex S. The anabolic activity of bone tissue, suppressed by disuse, is normalized by brief exposure to extremely low-magnitude mechanical stimuli. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2001;**15**(12):2225-9.
425. Verschueren SM, Roelants M, Delecluse C, et al. Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: a randomized controlled pilot study. J Bone Miner Res 2004;**19**(3):352-9.
426. Gilsanz V, Wren TA, Sanchez M, et al. Low-level, high-frequency mechanical signals enhance musculoskeletal development of young women with low BMD. J Bone Miner Res 2006;**21**(9):1464-74.
427. Ward K, Alsop C, Caulton J, et al. Low magnitude mechanical loading is osteogenic in children with disabling conditions. J Bone Miner Res 2004;**19**(3):360-9.
428. Matute-Llorente A, Gonzalez-Aguero A, Gomez-Cabello A, et al. Effect of whole-body vibration training on bone mass in adolescents with and without Down syndrome: a randomized controlled trial. Osteoporos Int 2015.
429. Erceg DN, Anderson LJ, Nickles CM, et al. Changes in Bone Biomarkers, BMC, and Insulin Resistance Following a 10-Week Whole Body Vibration Exercise Program in Overweight Latino Boys. International journal of medical sciences 2015;**12**(6):494-501.
430. Binkley TL, Parupsky EC, Kleinsasser BA, et al. Feasibility, compliance, and efficacy of a randomized controlled trial using vibration in pre-pubertal children. J Musculoskelet Neuronal Interact 2014;**14**(3):294-302.
431. Wren TA, Lee DC, Hara R, et al. Effect of high-frequency, low-magnitude vibration on bone and muscle in children with cerebral palsy. J Pediatr Orthop 2010;**30**(7):732-8.
432. Ruck J, Chabot G, Rauch F. Vibration treatment in cerebral palsy: A randomized controlled pilot study. J Musculoskelet Neuronal Interact 2010;**10**(1):77-83.

433. Stark C, Nikopoulou-Smyrni P, Stabrey A, et al. Effect of a new physiotherapy concept on bone mineral density, muscle force and gross motor function in children with bilateral cerebral palsy. J 2010;**10**(2):151-8.
434. Semler O, Fricke O, Vezyroglou K, et al. Preliminary results on the mobility after whole body vibration in immobilized children and adolescents. J Musculoskelet Neuronal Interact 2007;**7**(1):77-81.
435. Semler O, Fricke O, Vezyroglou K, et al. Results of a prospective pilot trial on mobility after whole body vibration in children and adolescents with osteogenesis imperfecta. Clin Rehabil 2008;**22**(5):387-94.
436. Gonzalez-Aguero A, Matute-Llorente A, Gomez-Cabello A, et al. Effects of whole body vibration training on body composition in adolescents with Down syndrome. Res Dev Disabil 2013;**34**(5):1426-33.
437. Roelants M, Delecluse C, Verschueren SM. Whole-body-vibration training increases knee-extension strength and speed of movement in older women. J Am Geriatr Soc 2004;**52**(6):901-8.
438. Roth J, Wust M, Rawer R, et al. Whole body vibration in cystic fibrosis--a pilot study. J 2008;**8**(2):179-87.
439. Ronnestad BR. Acute effects of various whole-body vibration frequencies on lower-body power in trained and untrained subjects. Journal of Strength & Conditioning Research 2009;**23**(4):1309-15.
440. Blottner D, Salanova M, Puttmann B, et al. Human skeletal muscle structure and function preserved by vibration muscle exercise following 55 days of bed rest. Eur J Appl Physiol 2006;**97**(3):261-71.
441. Floyd WN, Broderson AB, Goodno JF. Effect of whole-body vibration on peripheral nerve conduction time in the rhesus monkey. Aerosp Med 1973;**44**(3):281-5.
442. Bovenzi M. Health risks from occupational exposures to mechanical vibration. La Medicina del lavoro 2006;**97**(3):535-41.
443. Kerschan-Schindl K, Grampp S, Henk C, et al. Whole-body vibration exercise leads to alterations in muscle blood volume. Clin Physiol 2001;**21**(3):377-82.
444. Stewart JM, Karman C, Montgomery LD, et al. Plantar vibration improves leg fluid flow in perimenopausal women. American journal of physiology Regulatory, integrative and comparative physiology 2005;**288**(3):R623-9.
445. Milanese C, Piscitelli F, Zenti MG, et al. Ten-week whole-body vibration training improves body composition and muscle strength in obese women. International journal of medical sciences 2013;**10**(3):307-11.
446. Garatachea N, Jimenez A, Bresciani G, et al. The effects of movement velocity during squatting on energy expenditure and substrate utilization in whole-body vibration. Journal of Strength & Conditioning Research 2007;**21**(2):594-8.
447. Lee K, Lee S, Song C. Whole-body vibration training improves balance, muscle strength and glycosylated hemoglobin in elderly patients with diabetic neuropathy. Tohoku J Exp Med 2013;**231**(4):305-14.
448. Vissers D, Verrijken A, Mertens I, et al. Effect of long-term whole body vibration training on visceral adipose tissue: a preliminary report. Obes Facts 2010;**3**(2):93-100.
449. Di Loreto C, Ranchelli A, Lucidi P, et al. Effects of whole-body vibration exercise on the endocrine system of healthy men. Journal of endocrinological investigation 2004;**27**(4):323-7.
450. Hock JM, Centrella M, Canalis E. Insulin-like growth factor I has independent effects on bone matrix formation and cell replication. Endocrinology 1988;**122**(1):254-60.

451. Lee NK, Karsenty G. Reciprocal regulation of bone and energy metabolism. Journal of musculoskeletal & neuronal interactions 2008;**8**(4):351.
452. Lee NK, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. Cell 2007;**130**(3):456-69.
453. Lee AJ, Hodges S, Eastell R. Measurement of osteocalcin. Ann Clin Biochem 2000;**37**(Pt 4):432-46.
454. Prats-Puig A, Mas-Parareda M, Riera-Perez E, et al. Carboxylation of osteocalcin affects its association with metabolic parameters in healthy children. Diabetes Care 2010;**33**(3):661-3.
455. Cheng S, Massaro JM, Fox CS, et al. Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. Diabetes 2010;**59**(1):242-8.
456. Forouhi NG, Luan J, Cooper A, et al. Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. Diabetes 2008;**57**(10):2619-25.
457. Kayaniyil S, Vieth R, Retnakaran R, et al. Association of vitamin D with insulin resistance and beta-cell dysfunction in subjects at risk for type 2 diabetes. Diabetes Care 2010;**33**(6):1379-81.
458. Alemzadeh R, Kichler J, Babar G, et al. Hypovitaminosis D in obese children and adolescents: relationship with adiposity, insulin sensitivity, ethnicity, and season. Metabolism 2008;**57**(2):183-91.
459. Chiu KC, Chu A, Go VL, et al. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr 2004;**79**(5):820-5.
460. Rajakumar K, de las Heras J, Lee S, et al. 25-hydroxyvitamin D concentrations and in vivo insulin sensitivity and beta-cell function relative to insulin sensitivity in black and white youth.[Erratum appears in Diabetes Care. 2012 Oct;35(10):2108]. Diabetes Care 2012;**35**(3):627-33.
461. Theodoratou E, Tzoulaki I, Zgaga L, et al. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. Bmj 2014;**348**:g2035.
462. Dolinsky DH, Armstrong S, Mangarelli C, et al. The association between vitamin D and cardiometabolic risk factors in children: a systematic review. Clin Pediatr (Phila) 2013;**52**(3):210-23.
463. Riek AE, Oh J, Bernal-Mizrachi C. 1,25(OH)2 vitamin D suppresses macrophage migration and reverses atherogenic cholesterol metabolism in type 2 diabetic patients. J Steroid Biochem Mol Biol 2013;**136**:309-12.
464. Riek AE, Oh J, Sprague JE, et al. Vitamin D suppression of endoplasmic reticulum stress promotes an antiatherogenic monocyte/macrophage phenotype in type 2 diabetic patients. J Biol Chem 2012;**287**(46):38482-94.
465. Oh J, Riek AE, Darwech I, et al. Deletion of macrophage Vitamin D receptor promotes insulin resistance and monocyte cholesterol transport to accelerate atherosclerosis in mice. Cell Rep 2015;**10**(11):1872-86.
466. Munns CF, Shaw N, Kiely M, et al. Global Consensus Recommendations on Prevention and Management of Nutritional Rickets. The Journal of clinical endocrinology and metabolism 2016;**101**(2):394-415.
467. Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. The Journal of clinical endocrinology and metabolism 2011;**96**(1):53-8.
468. Holick MF. Vitamin D deficiency. The New England journal of medicine 2007;**357**(3):266-81.

469. Polgreen LE, Jacobs DR, Jr., Nathan BM, et al. Association of osteocalcin with obesity, insulin resistance, and cardiovascular risk factors in young adults. Obesity (Silver Spring) 2012;**20**(11):2194-201.
470. Burke JP, Hale DE, Hazuda HP, et al. A quantitative scale of acanthosis nigricans. Diabetes Care 1999;**22**(10):1655-9.
471. Garnett SP, Srinivasan S, Birt SG, et al. Evaluation of glycaemic status in young people with clinical insulin resistance; fasting glucose, fasting insulin or an oral glucose tolerance test? Clin Endocrinol (Oxf) 2010;**72**(4):475-80.
472. American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care 2008;**31 Suppl 1**:S55-60.
473. Altman DG, Bland JM. Treatment allocation by minimisation. Bmj 2005;**330**(7495):843.
474. Evans SR, P; Day, S. Minim: allocation by minimisation in clinical trials 2014 [Available from: http://www-users.york.ac.uk/~mb55/guide/minim.htm.
475. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976;**51**(3):170-9.
476. Rauch F, Sievanen H, Boonen S, et al. Reporting whole-body vibration intervention studies: recommendations of the International Society of Musculoskeletal and Neuronal Interactions. J Musculoskelet Neuronal Interact 2010;**10**(3):193-8.
477. Novotec Medical. Galileo Sport [Available from: http://www.galileowholebodyvibration.com.au/products-basic.html
478. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol 1988;**124**(6):869-71.
479. Garnett SP, Baur LA, Srinivasan S, et al. Body mass index and waist circumference in midchildhood and adverse cardiovascular disease risk clustering in adolescence. Am J Clin Nutr 2007;**86**(3):549-55.
480. Norton K WN, Carter L, Kerr D, Gore C, Marfell-, M. J. *Measurement techniques in anthropometry*. . Sydney: University of NSW Press, 1996.
481. Pan HC, T. lmsGrowth, a Microsoft Excel add-in to access growth references based on the LMS method (Version 2.68). 2009. Available online: http://www.healthforallchildren.co.uk/ (accessed on 26 April 2011). Pan, H.; Cole, T. lmsGrowth, a Microsoft Excel add-in to access growth references based on the LMS method (Version 2.68). 2009. Available online: http://www.healthforallchildren.co.uk/ (accessed on 26 April 2011).
482. Margulies L, Horlick M, Thornton JC, et al. Reproducibility of pediatric whole body bone and body composition measures by dual-energy X-ray absorptiometry using the GE Lunar Prodigy. Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry 2005;**8**(3):298-304.
483. Ogle GD, Allen JR, Humphries IR, et al. Body-composition assessment by dual-energy x-ray absorptiometry in subjects aged 4-26 y. Am J Clin Nutr 1995;**61**(4):746-53.
484. Cowell CT, Briody J, Lloyd-Jones S, et al. Fat distribution in children and adolescents--the influence of sex and hormones. Horm Res 1997;**48 Suppl 5**:93-100.
485. Garnett SP, Baur LA, Noakes M, et al. Researching Effective Strategies to Improve Insulin Sensitivity in Children and Teenagers - RESIST. A randomised control trial investigating the effects of two different diets on insulin sensitivity in young people with insulin resistance and/or pre-diabetes. BMC Public Health 2010;**10**:575.

486. Lu PW, Briody JN, Ogle GD, et al. Bone mineral density of total body, spine, and femoral neck in children and young adults: a cross-sectional and longitudinal study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 1994;**9**(9):1451-8.
487. Spender QW, Cronk CE, Charney EB, et al. Assessment of linear growth of children with cerebral palsy: use of alternative measures to height or length. Dev Med Child Neurol 1989;**31**(2):206-14.
488. Moyer-Mileur LJ, Quick JL, Murray MA. Peripheral quantitative computed tomography of the tibia: pediatric reference values. Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry 2008;**11**(2):283-94.
489. Bruce RA, Blackmon JR, Jones JW, et al. Exercising Testing in Adult Normal Subjects and Cardiac Patients. Pediatrics 1963;**32**:SUPPL 742-56.
490. Bruce RA. Exercise testing of patients with coronary heart disease. Principles and normal standards for evaluation. Ann Clin Res 1971;**3**(6):323-32.
491. Unnithan VB, Murray LA, Timmons JA, et al. Reproducibility of cardiorespiratory measurements during submaximal and maximal running in children. Br J Sports Med 1995;**29**(1):66-71.
492. Fricke O, Weidler J, Tutlewski B, et al. Mechanography--a new device for the assessment of muscle function in pediatrics. Pediatric research 2006;**59**(1):46-9.
493. Novotec Medical. https://www.galileo-training.com/de-english/products/leonardo-mechanograph/mechanography/test-procedures-assessments.html - m1lh
494. Noakes M, Keogh JB, Foster PR, et al. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. Am J Clin Nutr 2005;**81**(6):1298-306.
495. Telford A SJ, Jolley D, Crawford D. Reliability and validity of physical activity questionnaires for children: the Children's Leisure Activities Study Survey (CLASS). Pediatr Exerc Sci 2004;**16**:64-79.
496. NSW Government Department of Industry. GWS local government areas [Available from: http://www.industry.nsw.gov.au] Last accessed June 2017.
497. Statistics Australia. Census Dictionary Australia 2006. 2006 (2901.0).
498. Cook S, Auinger P, Li C, et al. Metabolic syndrome rates in United States adolescents, from the National Health and Nutrition Examination Survey, 1999-2002. J Pediatr 2008;**152**(2):165-70.
499. de Ferranti SD, Gauvreau K, Ludwig DS, et al. Prevalence of the metabolic syndrome in American adolescents: findings from the Third National Health and Nutrition Examination Survey. Circulation 2004;**110**(16):2494-7.
500. Lobstein T, Jackson-Leach R. Child overweight and obesity in the USA: prevalence rates according to IOTF definitions. Int J Pediatr Obes 2007;**2**(1):62-4.
501. AIHW: Holdenson Z CL, Phillips G & Waters A-M 2003a. A picture of diabetes in overseas-born Australians. Bulletin No. 9. AIHW Cat. No. AUS 38. Canberra: AIHW.
502. IDF Diabetes Atlas Brussels, Belgium: International Diabetes Federation; 2015 [7th Edition:[Available from: http://www.diabetesatlas.org] Last accessed June 2017.
503. Sabo RT, Lu Z, Deng X, et al. Parental and offspring associations of the metabolic syndrome in the Fels Longitudinal Study. Am J Clin Nutr 2012;**96**(3):461-6.
504. Bener A, Darwish S, Al-Hamaq AO, et al. The potential impact of family history of metabolic syndrome and risk of type 2 diabetes mellitus: In a highly endogamous population. Indian J Endocrinol Metab 2014;**18**(2):202-9.

505. Lipinska A, Koczaj-Bremer M, Jankowski K, et al. Does family history of metabolic syndrome affect the metabolic profile phenotype in young healthy individuals? Diabetol Metab Syndr 2014;**6**:75.
506. A AIoHaW. Diabetes. Australian Facts 2008. Australian Institute of Health and Welfare, Canberra: IDiabetes Series No. 8. 2008.
507. Kosti RI, Panagiotakos DB, Tountas Y, et al. Parental Body Mass Index in association with the prevalence of overweight/obesity among adolescents in Greece; dietary and lifestyle habits in the context of the family environment: the Vyronas study. Appetite 2008;**51**(1):218-22.
508. Koomanaee S, Tabrizi M, Naderi N, et al. Parental Anthropometric Indices and Obesity in Children. Acta Med Iran 2016;**54**(4):270-5.
509. Khurana I, Kaspi A, Ziemann M, et al. DNA methylation regulates hypothalamic gene expression linking parental diet during pregnancy to the offspring's risk of obesity in Psammomys obesus. Int J Obes (Lond) 2016.
510. Berenson GS, Bao W, Srinivasan SR. Abnormal characteristics in young offspring of parents with non-insulin-dependent diabetes mellitus. The Bogalusa Heart Study. Am J Epidemiol 1996;**144**(10):962-7.
511. Morrison JA, Friedman LA, Wang P, et al. Metabolic syndrome in childhood predicts adult metabolic syndrome and type 2 diabetes mellitus 25 to 30 years later. J Pediatr 2008;**152**(2):201-6.
512. Pankow JS, Jacobs DR, Jr., Steinberger J, et al. Insulin resistance and cardiovascular disease risk factors in children of parents with the insulin resistance (metabolic) syndrome. Diabetes Care 2004;**27**(3):775-80.
513. Alwan H, Viswanathan B, Paccaud F, et al. Is Accurate Perception of Body Image Associated with Appropriate Weight-Control Behavior among Adolescents of the Seychelles. J Obes 2011;**2011**:817242.
514. Brener ND, Eaton DK, Lowry R, et al. The association between weight perception and BMI among high school students. Obes Res 2004;**12**(11):1866-74.
515. Tienboon P, Rutishauser IH, Wahlqvist ML. Adolescents' perception of body weight and parents' weight for height status. J Adolesc Health 1994;**15**(3):263-8.
516. Yan AF, Zhang G, Wang MQ, et al. Weight perception and weight control practice in a multiethnic sample of US adolescents. South Med J 2009;**102**(4):354-60.
517. Wang Y, Liang H, Chen X. Measured body mass index, body weight perception, dissatisfaction and control practices in urban, low-income African American adolescents. BMC Public Health 2009;**9**:183.
518. Colabianchi N, Ievers-Landis CE, Borawski EA. Weight preoccupation as a function of observed physical attractiveness: ethnic differences among normal-weight adolescent females. J Pediatr Psychol 2006;**31**(8):803-12.
519. McKee C, Long L, Southward LH, et al. The Role of Parental Misperception of Child's Body Weight in Childhood Obesity. J Pediatr Nurs 2016;**31**(2):196-203.
520. Moore LC, Harris CV, Bradlyn AS. Exploring the relationship between parental concern and the management of childhood obesity. Matern Child Health J 2012;**16**(4):902-8.
521. Yang K, Turk MT, Allison VL, et al. Body mass index self-perception and weight management behaviors during late adolescence. J Sch Health 2014;**84**(10):654-60.
522. Booth ML, Bernard D, Quine S, et al. Access to health care among Australian adolescents young people's perspectives and their sociodemographic distribution. J Adolesc Health 2004;**34**(1):97-103.
523. Marcell AV, Klein JD, Fischer I, et al. Male adolescent use of health care services: where are the boys? J Adolesc Health 2002;**30**(1):35-43.

524. Thomas S, Ness RB, Thurston RC, et al. Racial differences in perception of healthy body weight in midlife women: results from the Do Stage Transitions Result in Detectable Effects study. Menopause 2013;**20**(3):269-73.
525. Davis J, Busch J, Hammatt Z, et al. The relationship between ethnicity and obesity in Asian and Pacific Islander populations: a literature review. Ethn Dis 2004;**14**(1):111-8.
526. Kanter R, Caballero B. Global gender disparities in obesity: a review. Adv Nutr 2012;**3**(4):491-8.
527. Consultation WHOE. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004;**363**(9403):157-63.
528. Kurtoglu S, Hatipoglu N, Mazicioglu M, et al. Insulin resistance in obese children and adolescents: HOMA-IR cut-off levels in the prepubertal and pubertal periods. J Clin Res Pediatr Endocrinol 2010;**2**(3):100-6.
529. Reinehr T, de Sousa G, Andler W. Longitudinal analyses among overweight, insulin resistance, and cardiovascular risk factors in children. Obes Res 2005;**13**(10):1824-33.
530. Weiss R, Dziura J, Burgert TS, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med 2004;**350**(23):2362-74.
531. Deboer MD. Ethnicity, obesity and the metabolic syndrome: implications on assessing risk and targeting intervention. Expert Rev Endocrinol Metab 2011;**6**(2):279-89.
532. Morrison JA, Sprecher DL, Biro FM, et al. Serum testosterone associates with lower high-density lipoprotein cholesterol in black and white males, 10 to 15 years of age, through lowered apolipoprotein AI and AII concentrations. Metabolism 2002;**51**(4):432-7.
533. Guven A, Sanisoglu SY. Pubertal progression and serum lipid profile in obese children. J Pediatr Endocrinol Metab 2008;**21**(2):135-46.
534. National High Blood Pressure Education Program Working Group on High Blood Pressure in C, Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004;**114**(2 Suppl 4th Report):555-76.
535. Rodriguez BL, Dabelea D, Liese AD, et al. Prevalence and correlates of elevated blood pressure in youth with diabetes mellitus: the SEARCH for diabetes in youth study. J Pediatr 2010;**157**(2):245-51 e1.
536. Wadwa RP, Urbina EM, Anderson AM, et al. Measures of arterial stiffness in youth with type 1 and type 2 diabetes: the SEARCH for diabetes in youth study. Diabetes Care 2010;**33**(4):881-6.
537. Franklin SS, Larson MG, Khan SA, et al. Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. Circulation 2001;**103**(9):1245-9.
538. Kim HC, Nam CM, Jee SH, et al. Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. BMJ 2004;**328**(7446):983.
539. Prati D, Taioli E, Zanella A, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 2002;**137**(1):1-10.
540. Nadeau KJ, Ehlers LB, Zeitler PS, et al. Treatment of non-alcoholic fatty liver disease with metformin versus lifestyle intervention in insulin-resistant adolescents. Pediatr Diabetes 2009;**10**(1):5-13.
541. AlKhater SA. Paediatric non-alcoholic fatty liver disease: an overview. Obes Rev 2015;**16**(5):393-405.

542. Ascenso A, Palmeira A, Pedro LM, et al. Physical activity and cardiorespiratory fitness, but not sedentary behavior, are associated with carotid intima-media thickness in obese adolescents. Eur J Pediatr 2016;**175**(3):391-8.
543. Hay J, Wittmeier K, MacIntosh A, et al. Physical activity intensity and type 2 diabetes risk in overweight youth: a randomized trial. Int J Obes (Lond) 2016;**40**(4):607-14.
544. Janssen I, Wong SL, Colley R, et al. The fractionalization of physical activity throughout the week is associated with the cardiometabolic health of children and youth. BMC Public Health 2013;**13**:554.
545. Martinez-Gomez D, Eisenmann JC, Warnberg J, et al. Associations of physical activity, cardiorespiratory fitness and fatness with low-grade inflammation in adolescents: the AFINOS Study. Int J Obes (Lond) 2010;**34**(10):1501-7.
546. Dwyer T, Magnussen CG, Schmidt MD, et al. Decline in physical fitness from childhood to adulthood associated with increased obesity and insulin resistance in adults. Diabetes care 2009;**32**(4):683-7.
547. Sabin MA, Kao KT, Juonala M, et al. Viewpoint article: Childhood obesity--looking back over 50 years to begin to look forward. J Paediatr Child Health 2015;**51**(1):82-6.
548. Skinner AC, Skelton JA. Prevalence and trends in obesity and severe obesity among children in the United States, 1999-2012. JAMA Pediatr 2014;**168**(6):561-6.
549. DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991;**14**(3):173-94.
550. Nelson RA, Bremer AA. Insulin resistance and metabolic syndrome in the pediatric population. Metabolic Syndrome & Related Disorders 2010;**8**(1):1-14.
551. Nassis G, Papantakou K, Skenderi K, et al. Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. Metabolism: clinical and experimental 2005;**54**(11):1472-79.
552. Hunter P. Exercise in a bottle: Elucidating how exercise conveys health benefits might lead to new therapeutic options for a range of diseases from cancer to metabolic syndrome. EMBO Rep 2016.
553. Monteiro PA, Chen KY, Lira FS, et al. Concurrent and aerobic exercise training promote similar benefits in body composition and metabolic profiles in obese adolescents. Lipids Health Dis 2015;**14**:153.
554. Hulver MW, Zheng D, Tanner CJ, et al. Adiponectin is not altered with exercise training despite enhanced insulin action. Am J Physiol Endocrinol Metab 2002;**283**(4):E861-5.
555. Fjeldstad C, Palmer IJ, Bemben MG, et al. Whole-body vibration augments resistance training effects on body composition in postmenopausal women. Maturitas 2009;**63**(1):79-83.
556. Bosco C, Colli R, Introini E, et al. Adaptive responses of human skeletal muscle to vibration exposure. Clin Physiol 1999;**19**(2):183-7.
557. Rittweger J, Schiessl H, Felsenberg D. Oxygen uptake during whole-body vibration exercise: comparison with squatting as a slow voluntary movement. Eur J Appl Physiol 2001;**86**(2):169-73.
558. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care 2009;**32 Suppl 2**:S157-63.
559. Lee S, Kim Y, White DA, et al. Relationships between insulin sensitivity, skeletal muscle mass and muscle quality in obese adolescent boys. Eur J Clin Nutr 2012;**66**(12):1366-8.

560. Moher D, Schulz KF, Altman D, et al. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. Jama 2001;**285**(15):1987-91.
561. Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. Lancet 2001;**357**(9263):1191-4.
562. Narasimhan S, Weinstock RS. Youth-onset type 2 diabetes mellitus: lessons learned from the TODAY study. Mayo Clin Proc 2014;**89**(6):806-16.
563. Springer F, Ballweg V, Schweizer R, et al. Changes in whole-body fat distribution, intrahepatic lipids, and insulin resistance of obese adolescents during a low-level lifestyle intervention. Eur J Pediatr 2015;**174**(12):1603-12.
564. Hunt LP, Ford A, Sabin MA, et al. Clinical measures of adiposity and percentage fat loss: which measure most accurately reflects fat loss and what should we aim for? Arch Dis Child 2007;**92**(5):399-403.
565. Haus JM, Solomon TP, Marchetti CM, et al. Free fatty acid-induced hepatic insulin resistance is attenuated following lifestyle intervention in obese individuals with impaired glucose tolerance. J Clin Endocrinol Metab 2010;**95**(1):323-7.
566. Brambilla P, La Valle E, Falbo R, et al. Normal Fasting Plasma Glucose and Risk of Type 2 Diabetes. Diabetes Care 2011.
567. Rauch F. Vibration therapy. Dev Med Child Neurol 2009;**51 Suppl 4**:166-8.
568. Berggren JR, Hulver MW, Dohm GL, et al. Weight loss and exercise: implications for muscle lipid metabolism and insulin action. Med Sci Sports Exerc 2004;**36**(7):1191-5.
569. Schoenfeld BJ, Ogborn DI, Krieger JW. Effect of repetition duration during resistance training on muscle hypertrophy: a systematic review and meta-analysis. Sports Med 2015;**45**(4):577-85.
570. Reinehr T, Toschke AM. Onset of puberty and cardiovascular risk factors in untreated obese children and adolescents: a 1-year follow-up study. Arch Pediatr Adolesc Med 2009;**163**(8):709-15.
571. Dowling AR, Nedorezov LB, Qiu X, et al. Genetic factors modulate the impact of pubertal androgen excess on insulin sensitivity and fertility. PLoS One 2013;**8**(11):e79849.
572. Baxter KA, Ware RS, Batch JA, et al. Predicting success: factors associated with weight change in obese youth undertaking a weight management program. Obes Res Clin Pract 2013;**7**(2):e147-e54.
573. Festa A, D'Agostino R, Jr., Howard G, et al. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation 2000;**102**(1):42-7.
574. Juarez-Lopez C, Klunder-Klunder M, Madrigal-Azcarate A, et al. Omega-3 polyunsaturated fatty acids reduce insulin resistance and triglycerides in obese children and adolescents. Pediatr Diabetes 2013;**14**(5):377-83.
575. Warren JM, Golley RK, Collins CE, et al. Randomised controlled trials in overweight children: practicalities and realities. Int J Pediatr Obes 2007;**2**(2):73-85.
576. Denzer C, Reithofer E, Wabitsch M, et al. The outcome of childhood obesity management depends highly upon patient compliance. Eur J Pediatr 2004;**163**(2):99-104.
577. Pinelli L, Elerdini N, Faith MS, et al. Childhood obesity: results of a multicenter study of obesity treatment in Italy. J Pediatr Endocrinol Metab 1999;**12 Suppl 3**:795-9.

578. Seymour K. Using incentives: Encouraging and recognising participation in youth research. Youth Studies Australia 2012;**Volume 31**(3):51-59.
579. Smith KL, Straker LM, McManus A, et al. Barriers and enablers for participation in healthy lifestyle programs by adolescents who are overweight: a qualitative study of the opinions of adolescents, their parents and community stakeholders. BMC Pediatr 2014;**14**:53.
580. Abercromby AF, Amonette WE, Layne CS, et al. Vibration exposure and biodynamic responses during whole-body vibration training. Med Sci Sports Exerc 2007;**39**(10):1794-800.
581. Robling AG, Hinant FM, Burr DB, et al. Shorter, more frequent mechanical loading sessions enhance bone mass. Med Sci Sports Exerc 2002;**34**(2):196-202.
582. Emerenziani GP, Meucci M, Gallotta MC, et al. Whole body vibration: unsupervised training or combined with a supervised multi-purpose exercise for fitness? J Sports Sci 2014;**32**(11):1033-41.
583. Theintz G, Buchs B, Rizzoli R, et al. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. The Journal of clinical endocrinology and metabolism 1992;**75**(4):1060-5.
584. Weaver CM. Adolescence: the period of dramatic bone growth. Endocrine 2002;**17**(1):43-8.
585. Simmonds M, Llewellyn A, Owen CG, et al. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. Obes Rev 2016;**17**(2):95-107.
586. Foley S, Quinn S, Jones G. Tracking of bone mass from childhood to adolescence and factors that predict deviation from tracking. Bone 2009;**44**(5):752-7.
587. Rauch F, Schoenau E. The developing bone: slave or master of its cells and molecules? Pediatric research 2001;**50**(3):309-14.
588. Turner CH, Burr DB. Basic biomechanical measurements of bone: a tutorial. Bone 1993;**14**(4):595-608.
589. Turner JG, Gilchrist NL, Ayling EM, et al. Factors affecting bone mineral density in high school girls. N Z Med J 1992;**105**(930):95-6.
590. Daly RM, Rich PA, Klein R. Influence of high impact loading on ultrasound bone measurements in children: a cross-sectional report. Calcified tissue international 1997;**60**(5):401-4.
591. Yang S, Shen X. Association and relative importance of multiple obesity measures with bone mineral density: the National Health and Nutrition Examination Survey 2005-2006. Arch Osteoporos 2015;**10**:14.
592. Maimoun L, Mura T, Leprieur E, et al. Impact of obesity on bone mass throughout adult life: Influence of gender and severity of obesity. Bone 2015.
593. Frost HM, Schonau E. The "muscle-bone unit" in children and adolescents: a 2000 overview. Journal of pediatric endocrinology & metabolism : JPEM 2000;**13**(6):571-90.
594. Campos RM, Lazaretti-Castro M, Mello MT, et al. Influence of visceral and subcutaneous fat in bone mineral density of obese adolescents. Arq Bras Endocrinol Metabol 2012;**56**(1):12-8.
595. Farr JN, Khosla S. Determinants of bone strength and quality in diabetes mellitus in humans. Bone 2016;**82**:28-34.
596. Ivaska KK, Heliovaara MK, Ebeling P, et al. The effects of acute hyperinsulinemia on bone metabolism. Endocrine connections 2015;**4**(3):155-62.

597. Abrahamsen B, Rohold A, Henriksen JE, et al. Correlations between insulin sensitivity and bone mineral density in non-diabetic men. Diabet Med 2000;**17**(2):124-9.
598. Mathen PG, Thabah MM, Zachariah B, et al. Decreased Bone Mineral Density at the Femoral Neck and Lumbar Spine in South Indian Patients with Type 2 Diabetes. J Clin Diagn Res 2015;**9**(9):OC08-12.
599. El Hage R, Moussa E, Jacob C. Bone mineral content and density in obese, overweight, and normal-weighted sedentary adolescent girls. The Journal of adolescent health : official publication of the Society for Adolescent Medicine 2010;**47**(6):591-5.
600. Klein KO, Larmore KA, de Lancey E, et al. Effect of obesity on estradiol level, and its relationship to leptin, bone maturation, and bone mineral density in children. J Clin Endocrinol Metab 1998;**83**(10):3469-75.
601. Klein KO, Newfield RS, Hassink SG. Bone maturation along the spectrum from normal weight to obesity: a complex interplay of sex, growth factors and weight gain. Journal of pediatric endocrinology & metabolism : JPEM 2016;**29**(3):311-8.
602. Petit MA, Beck TJ, Shults J, et al. Proximal femur bone geometry is appropriately adapted to lean mass in overweight children and adolescents. Bone 2005;**36**(3):568-76.
603. El Hage Z, Theunynck D, Jacob C, et al. Bone mineral content and density in obese, overweight and normal weight adolescent boys. J Med Liban 2013;**61**(3):148-54.
604. Binkovitz LA, Henwood MJ. Pediatric DXA: technique and interpretation. Pediatr Radiol 2007;**37**(1):21-31.
605. Fulzele K, DiGirolamo DJ, Liu Z, et al. Disruption of the insulin-like growth factor type 1 receptor in osteoblasts enhances insulin signaling and action. J Biol Chem 2007;**282**(35):25649-58.
606. Lee NK, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. Cell 2007;**130**(3):456-69.
607. Rosen CJ, Adams JS, Bikle DD, et al. The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. Endocr Rev 2012;**33**(3):456-92.
608. Juonala M, Voipio A, Pahkala K, et al. Childhood 25-OH vitamin D levels and carotid intima-media thickness in adulthood: the cardiovascular risk in young Finns study. J Clin Endocrinol Metab 2015;**100**(4):1469-76.
609. Shaw NJ, Mughal MZ. Vitamin D and child health: part 2 (extraskeletal and other aspects). Arch Dis Child 2013;**98**(5):368-72.
610. Shi H, Norman AW, Okamura WH, et al. 1alpha,25-Dihydroxyvitamin D3 modulates human adipocyte metabolism via nongenomic action. FASEB J 2001;**15**(14):2751-3.
611. Girgis CM, Clifton-Bligh RJ, Turner N, et al. Effects of vitamin D in skeletal muscle: falls, strength, athletic performance and insulin sensitivity. Clin Endocrinol (Oxf) 2014;**80**(2):169-81.
612. Van Loan MD, Johnson HL, Barbieri TF. Effect of weight loss on bone mineral content and bone mineral density in obese women. Am J Clin Nutr 1998;**67**(4):734-8.
613. Gossain VV, Rao DS, Carella MJ, et al. Bone mineral density (BMD) in obesity effect of weight loss. J Med 1999;**30**(5-6):367-76.
614. Tirosh A, de Souza RJ, Sacks F, et al. Sex Differences in the Effects of Weight Loss Diets on Bone Mineral Density and Body Composition: POUNDS LOST Trial. The Journal of clinical endocrinology and metabolism 2015;**100**(6):2463-71.

615. Ryan AS, Nicklas BJ, Dennis KE. Aerobic exercise maintains regional bone mineral density during weight loss in postmenopausal women. J Appl Physiol (1985) 1998;**84**(4):1305-10.
616. Lohman T, Going S, Pamenter R, et al. Effects of resistance training on regional and total bone mineral density in premenopausal women: a randomized prospective study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 1995;**10**(7):1015-24.
617. Layne JE, Nelson ME. The effects of progressive resistance training on bone density: a review. Med Sci Sports Exerc 1999;**31**(1):25-30.
618. Munns C, Zacharin MR, Rodda CP, et al. Prevention and treatment of infant and childhood vitamin D deficiency in Australia and New Zealand: a consensus statement. The Medical journal of Australia 2006;**185**(5):268-72.
619. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of clinical endocrinology and metabolism 2011;**96**(7):1911-30.
620. Aksglaede L, Juul A, Olsen LW, et al. Age at puberty and the emerging obesity epidemic. PLoS One 2009;**4**(12):e8450.
621. Pinhas-Hamiel O, Benary D, Mazor-Aronovich K, et al. Advanced bone age and hyperinsulinemia in overweight and obese children. Endocr Pract 2014;**20**(1):62-7.
622. Nguyen TV, Maynard LM, Towne B, et al. Sex differences in bone mass acquisition during growth: the Fels Longitudinal Study. Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry 2001;**4**(2):147-57.
623. Schoenau E. Bone mass increase in puberty: what makes it happen? Horm Res 2006;**65 Suppl 2**:2-10.
624. Rogol AD, Roemmich JN, Clark PA. Growth at puberty. The Journal of adolescent health : official publication of the Society for Adolescent Medicine 2002;**31**(6 Suppl):192-200.
625. Faulkner RA, Davison KS, Bailey DA, et al. Size-corrected BMD decreases during peak linear growth: implications for fracture incidence during adolescence. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2006;**21**(12):1864-70.
626. Gultekin T, Akin G, Ozer BK. Gender differences in fat patterning in children living in Ankara. Anthropol Anz 2005;**63**(4):427-37.
627. Carvalho WR, Goncalves EM, Ribeiro RR, et al. Influence of body composition on bone mass in children and adolescents. Rev Assoc Med Bras 2011;**57**(6):662-7.
628. Schiessl H, Frost HM, Jee WS. Estrogen and bone-muscle strength and mass relationships. Bone 1998;**22**(1):1-6.
629. Pollock NK, Laing EM, Baile CA, et al. Is adiposity advantageous for bone strength? A peripheral quantitative computed tomography study in late adolescent females. Am J Clin Nutr 2007;**86**(5):1530-8.
630. Hedstrom EM, Svensson O, Bergstrom U, et al. Epidemiology of fractures in children and adolescents. Acta Orthop 2010;**81**(1):148-53.
631. Afghani A, Cruz ML, Goran MI. Impaired glucose tolerance and bone mineral content in overweight latino children with a family history of type 2 diabetes. Diabetes Care 2005;**28**(2):372-8.
632. Manzoni P, Brambilla P, Pietrobelli A, et al. Influence of body composition on bone mineral content in children and adolescents. Am J Clin Nutr 1996;**64**(4):603-7.

633. Osawa Y, Oguma Y, Onishi S. Effects of whole-body vibration training on bone-free lean body mass and muscle strength in young adults. J Sports Sci Med 2011;**10**(1):97-104.
634. Lee BK, Chon SC. Effect of whole body vibration training on mobility in children with cerebral palsy: a randomized controlled experimenter-blinded study. Clin Rehabil 2013;**27**(7):599-607.
635. Eid MA. Effect of Whole-Body Vibration Training on Standing Balance and Muscle Strength in Children with Down Syndrome. Am J Phys Med Rehabil 2015;**94**(8):633-43.
636. Stark C, Hoyer-Kuhn HK, Semler O, et al. Neuromuscular training based on whole body vibration in children with spina bifida: a retrospective analysis of a new physiotherapy treatment program. Childs Nerv Syst 2015;**31**(2):301-9.
637. Vry J, Schubert IJ, Semler O, et al. Whole-body vibration training in children with Duchenne muscular dystrophy and spinal muscular atrophy. Eur J Paediatr Neurol 2014;**18**(2):140-9.
638. Rubin C, Pope M, Fritton JC, et al. Transmissibility of 15-hertz to 35-hertz vibrations to the human hip and lumbar spine: determining the physiologic feasibility of delivering low-level anabolic mechanical stimuli to skeletal regions at greatest risk of fracture because of osteoporosis. Spine (Phila Pa 1976) 2003;**28**(23):2621-7.
639. Reinehr T, Roth CL. A new link between skeleton, obesity and insulin resistance: relationships between osteocalcin, leptin and insulin resistance in obese children before and after weight loss. Int J Obes (Lond) 2010;**34**(5):852-8.
640. Hinton PS, Rector RS, Thomas TR. Weight-bearing, aerobic exercise increases markers of bone formation during short-term weight loss in overweight and obese men and women. Metabolism 2006;**55**(12):1616-8.
641. Fernandez-Real JM, Izquierdo M, Ortega F, et al. The relationship of serum osteocalcin concentration to insulin secretion, sensitivity, and disposal with hypocaloric diet and resistance training. J Clin Endocrinol Metab 2009;**94**(1):237-45.
642. Ducy P. The role of osteocalcin in the endocrine cross-talk between bone remodelling and energy metabolism. Diabetologia 2011;**54**(6):1291-7.
643. Fernandez-Real JM, Ricart W. Osteocalcin: a new link between bone and energy metabolism. Some evolutionary clues. Curr Opin Clin Nutr Metab Care 2011;**14**(4):360-6.
644. Chao D, Espeland MA, Farmer D, et al. Effect of voluntary weight loss on bone mineral density in older overweight women. Journal of the American Geriatrics Society 2000;**48**(7):753-9.
645. Yeap BB, Alfonso H, Chubb SA, et al. Higher serum undercarboxylated osteocalcin and other bone turnover markers are associated with reduced diabetes risk and lower estradiol concentrations in older men. The Journal of clinical endocrinology and metabolism 2015;**100**(1):63-71.
646. Clemens TL, Karsenty G. The osteoblast: an insulin target cell controlling glucose homeostasis. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2011;**26**(4):677-80.
647. Pollock NK, Bernard PJ, Gower BA, et al. Lower Uncarboxylated Osteocalcin Concentrations in Children with Prediabetes Is Associated with {beta}-Cell Function. J Clin Endocrinol Metab 2011.
648. Zhou M, Ma X, Li H, et al. Serum osteocalcin concentrations in relation to glucose and lipid metabolism in Chinese individuals. European journal of endocrinology / European Federation of Endocrine Societies 2009;**161**(5):723-9.

649. Fukumoto S, Martin TJ. Bone as an endocrine organ. Trends in endocrinology and metabolism: TEM 2009;**20**(5):230-6.
650. Pittas AG, Lau J, Hu FB, et al. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. The Journal of clinical endocrinology and metabolism 2007;**92**(6):2017-29.
651. Coates P. Bone turnover markers. Aust Fam Physician 2013;**42**(5):285-7.
652. Nimptsch K, Hailer S, Rohrmann S, et al. Determinants and correlates of serum undercarboxylated osteocalcin. Annals of nutrition & metabolism 2007;**51**(6):563-70.
653. Kanbur NO, Derman O, Sen TA, et al. Osteocalcin. A biochemical marker of bone turnover during puberty. International journal of adolescent medicine and health 2002;**14**(3):235-44.
654. Paldanius PM, Ivaska KK, Hovi P, et al. The effect of oral glucose tolerance test on serum osteocalcin and bone turnover markers in young adults. Calcified tissue international 2012;**90**(2):90-5.
655. Sen AT, Derman O, Kinik E. The relationship between osteocalcin levels and sexual stages of puberty in male children. The Turkish journal of pediatrics 2000;**42**(4):281-5.
656. Junior IF, Cardoso JR, Christofaro DG, et al. The relationship between visceral fat thickness and bone mineral density in sedentary obese children and adolescents. BMC Pediatr 2013;**13**:37.
657. Pollock NK, Bernard PJ, Gutin B, et al. Adolescent obesity, bone mass, and cardiometabolic risk factors. The Journal of pediatrics 2011;**158**(5):727-34.
658. Wei J, Ferron M, Clarke CJ, et al. Bone-specific insulin resistance disrupts whole-body glucose homeostasis via decreased osteocalcin activation. J Clin Invest 2014;**124**(4):1-13.
659. Campos RM, de Mello MT, Tock L, et al. Interaction of bone mineral density, adipokines and hormones in obese adolescents girls submitted in an interdisciplinary therapy. Journal of pediatric endocrinology & metabolism : JPEM 2013;**26**(7-8):663-8.
660. Fulzele K, Clemens TL. Novel functions for insulin in bone. Bone 2012;**50**(2):452-6.
661. Reinehr T, Kiess W, Kapellen T, et al. Insulin sensitivity among obese children and adolescents, according to degree of weight loss. Pediatrics 2004;**114**(6):1569-73.
662. Villareal DT, Fontana L, Das SK, et al. Effect of Two-Year Caloric Restriction on Bone Metabolism and Bone Mineral Density in Non-Obese Younger Adults: A Randomized Clinical Trial. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2016;**31**(1):40-51.
663. Zibellini J, Seimon RV, Lee CM, et al. Does Diet-Induced Weight Loss Lead to Bone Loss in Overweight or Obese Adults? A Systematic Review and Meta-Analysis of Clinical Trials. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2015;**30**(12):2168-78.
664. Shapses SA, Sukumar D. Bone metabolism in obesity and weight loss. Annu Rev Nutr 2012;**32**:287-309.
665. Villareal DT, Fontana L, Weiss EP, et al. Bone mineral density response to caloric restriction-induced weight loss or exercise-induced weight loss: a randomized controlled trial. Arch Intern Med 2006;**166**(22):2502-10.
666. Melanson EL, MacLean PS, Hill JO. Exercise improves fat metabolism in muscle but does not increase 24-h fat oxidation. Exerc Sport Sci Rev 2009;**37**(2):93-101.

667. Munns CF, Simm PJ, Rodda CP, et al. Incidence of vitamin D deficiency rickets among Australian children: an Australian Paediatric Surveillance Unit study. The Medical journal of Australia 2012;**196**(7):466-8.
668. Wortsman J, Matsuoka LY, Chen TC, et al. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;**72**(3):690-3.
669. Ashraf AP, Huisingh C, Alvarez JA, et al. Insulin resistance indices are inversely associated with vitamin D binding protein concentrations. J Clin Endocrinol Metab 2014;**99**(1):178-83.
670. Ganji V, Zhang X, Shaikh N, et al. Serum 25-hydroxyvitamin D concentrations are associated with prevalence of metabolic syndrome and various cardiometabolic risk factors in US children and adolescents based on assay-adjusted serum 25-hydroxyvitamin D data from NHANES 2001-2006. Am J Clin Nutr 2011;**94**(1):225-33.
671. Nunlee-Bland G, Gambhir K, Abrams C, et al. Vitamin D deficiency and insulin resistance in obese African-American adolescents. J Pediatr Endocrinol 2011;**24**(1-2):29-33.
672. Reyman M, Verrijn Stuart AA, van Summeren M, et al. Vitamin D deficiency in childhood obesity is associated with high levels of circulating inflammatory mediators, and low insulin sensitivity. Int J Obes (Lond) 2014;**38**(1):46-52.
673. Paxton GA, Teale GR, Nowson CA, et al. Vitamin D and health in pregnancy, infants, children and adolescents in Australia and New Zealand: a position statement. The Medical journal of Australia 2013;**198**(3):142-3.
674. Smotkin-Tangorra M, Purushothaman R, Gupta A, et al. Prevalence of vitamin D insufficiency in obese children and adolescents. Journal of pediatric endocrinology & metabolism : JPEM 2007;**20**(7):817-23.
675. Slyper AH, Kashmer L, Huang WM, et al. Acanthosis nigricans, vitamin D, and insulin resistance in obese children and adolescents. J Pediatr Endocrinol 2014;**27**(11-12):1107-11.
676. von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. The British journal of nutrition 2010;**103**(4):549-55.
677. Belenchia AM, Tosh AK, Hillman LS, et al. Correcting vitamin D insufficiency improves insulin sensitivity in obese adolescents: a randomized controlled trial. Am J Clin Nutr 2013;**97**(4):774-81.
678. Wetzsteon RJ, Petit MA, Macdonald HM, et al. Bone structure and volumetric BMD in overweight children: a longitudinal study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2008;**23**(12):1946-53.
679. Burrows M, Liu D, Moore S, et al. Bone microstructure at the distal tibia provides a strength advantage to males in late puberty: an HR-pQCT study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2010;**25**(6):1423-32.
680. Heidemann M, Holst R, Schou AJ, et al. The influence of anthropometry and body composition on children's bone health: the childhood health, activity and motor performance school (the CHAMPS) study, Denmark. Calcified tissue international 2015;**96**(2):97-104.
681. Richardson L, Paulis WD, van Middelkoop M, et al. An overview of national clinical guidelines for the management of childhood obesity in primary care. Prev Med 2013;**57**(5):448-55.

682. Ben Ounis O, Elloumi M, Ben Chiekh I, et al. Effects of two-month physical-endurance and diet-restriction programmes on lipid profiles and insulin resistance in obese adolescent boys. Diabetes Metab 2008;**34**(6 Pt 1):595-600.
683. Savoye M, Shaw M, Dziura J, et al. Effects of a weight management program on body composition and metabolic parameters in overweight children: a randomized controlled trial. Jama 2007;**297**(24):2697-704.
684. Kasa-Vubu JZ. Cardiovascular Fitness and Exercise as Determinants of Insulin Resistance in Postpubertal Adolescent Females. Journal of Clinical Endocrinology & Metabolism 2004;**90**(2):849-54.
685. Alberga AS, Farnesi BC, Lafleche A, et al. The effects of resistance exercise training on body composition and strength in obese prepubertal children. Phys Sportsmed 2013;**41**(3):103-9.
686. Schranz N, Tomkinson G, Olds T. What is the effect of resistance training on the strength, body composition and psychosocial status of overweight and obese children and adolescents? A Systematic review and meta-analysis. Sports Med 2013;**43**(9):893-907.
687. Dietz P, Hoffmann S, Lachtermann E, et al. Influence of exclusive resistance training on body composition and cardiovascular risk factors in overweight or obese children: a systematic review. Obesity facts 2012;**5**(4):546-60.
688. Morinder G, Larsson UE, Norgren S, et al. Insulin sensitivity, VO2max and body composition in severely obese Swedish children and adolescents. Acta Paediatr 2009;**98**(1):132-8.
689. Kelley GA, Kelley KS, Pate RR. Exercise and BMI in Overweight and Obese Children and Adolescents: A Systematic Review and Trial Sequential Meta-Analysis. Biomed Res Int 2015;**2015**:704539.
690. van der Heijden GJ, Wang ZJ, Chu ZD, et al. A 12-week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, Hispanic adolescents. Obesity (Silver Spring) 2010;**18**(2):384-90.
691. Lee JM, Davis MM, Woolford SJ, et al. Waist circumference percentile thresholds for identifying adolescents with insulin resistance in clinical practice. Pediatr Diabetes 2009;**10**(5):336-42.
692. Bell LM, Byrne S, Thompson A, et al. Increasing body mass index z-score is continuously associated with complications of overweight in children, even in the healthy weight range. J Clin Endocrinol Metab 2007;**92**(2):517-22.
693. Sabin MA, Kiess W. Childhood obesity: Current and novel approaches. Best Pract Res Clin Endocrinol Metab 2015;**29**(3):327-38.
694. Short KR, Pratt LV, Teague AM, et al. Postprandial improvement in insulin sensitivity after a single exercise session in adolescents with low aerobic fitness and physical activity. Pediatr Diabetes 2013;**14**(2):129-37.
695. Health AGDo. Australia's Physical Activity and Sedentary Behaviour Guidelines for Young People (13-17 years). 2014.
696. Gow ML, van Doorn N, Broderick CR, et al. Sustained improvements in fitness and exercise tolerance in obese adolescents after a 12 week exercise intervention. Obes Res Clin Pract 2016;**10**(2):178-88.
697. Eisenmann JC, Guseman EH, Morrison K, et al. Graded Exercise Testing in a Pediatric Weight Management Center: The DeVos Protocol. Child Obes 2015;**11**(6):657-63.
698. Ahmad F, Kavey RE, Kveselis DA, et al. Responses of non-obese white children to treadmill exercise. J Pediatr 2001;**139**(2):284-90.
699. Toth MJ, Goran MI, Ades PA, et al. Examination of data normalization procedures for expressing peak VO2 data. J Appl Physiol (1985) 1993;**75**(5):2288-92.

700. Dencker M, Wollmer P, Karlsson MK, et al. Body fat, abdominal fat and body fat distribution related to VO(2PEAK) in young children. Int J Pediatr Obes 2011;**6**(2-2):e597-602.
701. Janz KF, Burns TL, Witt JD, et al. Longitudinal analysis of scaling VO2 for differences in body size during puberty: the Muscatine Study. Med Sci Sports Exerc 1998;**30**(9):1436-44.
702. Pinto TE, Gusso S, Hofman PL, et al. Systolic and diastolic abnormalities reduce the cardiac response to exercise in adolescents with type 2 diabetes. Diabetes Care 2014;**37**(5):1439-46.
703. Yang Z, Scott CA, Mao C, et al. Resistance exercise versus aerobic exercise for type 2 diabetes: a systematic review and meta-analysis. Sports Med 2014;**44**(4):487-99.
704. Eisenmann JC, DuBose KD, Donnelly JE. Fatness, fitness, and insulin sensitivity among 7- to 9-year-old children. Obesity (Silver Spring) 2007;**15**(8):2135-44.
705. Stigman S, Rintala P, Kukkonen-Harjula K, et al. Eight-year-old children with high cardiorespiratory fitness have lower overall and abdominal fatness. Int J Pediatr Obes 2009;**4**(2):98-105.
706. Maggio AB, Bou Puigdefabregas JW, Schwitzgebel VM, et al. Insulin secretion response during oral glucose tolerance test is related to low cardiorespiratory fitness in obese adolescents. J Pediatr Endocrinol Metab 2015;**28**(5-6):539-44.
707. McMurray RG, Bauman MJ, Harrell JS, et al. Effects of improvement in aerobic power on resting insulin and glucose concentrations in children. Eur J Appl Physiol 2000;**81**(1-2):132-9.
708. Song GE, Kim K, Lee DJ, et al. Whole body vibration effects on body composition in the postmenopausal korean obese women: pilot study. Korean J Fam Med 2011;**32**(7):399-405.
709. Garcia-Hermoso A, Sanchez-Lopez M, Martinez-Vizcaino V. Effects of Aerobic Plus Resistance Exercise on Body Composition Related Variables in Pediatric Obesity: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Pediatr Exerc Sci 2015;**27**(4):431-40.
710. Goodpaster BH, Kelley DE, Wing RR, et al. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. Diabetes 1999;**48**(4):839-47.
711. Watts K, Beye P, Siafarikas A, et al. Exercise training normalizes vascular dysfunction and improves central adiposity in obese adolescents. Journal of the American College of Cardiology 2004;**43**(10):1823-7.
712. Watts K, Beye P, Siafarikas A, et al. Effects of exercise training on vascular function in obese children. J Pediatr 2004;**144**(5):620-5.
713. McMurray RG, Hosick PA, Bugge A. Importance of proper scaling of aerobic power when relating to cardiometabolic risk factors in children. Ann Hum Biol 2011;**38**(5):647-54.
714. Lang I, Busche P, Rakhimi N, et al. Mechanography in childhood: references for grip force, multiple one-leg hopping force and whole body stiffness. J 2013;**13**(2):227-35.
715. Ward KA, Das G, Berry JL, et al. Vitamin D status and muscle function in post-menarchal adolescent girls. Journal of Clinical Endocrinology & Metabolism 2009;**94**(2):559-63.
716. Faigenbaum AD, Westcott WL, Loud RL, et al. The effects of different resistance training protocols on muscular strength and endurance development in children. Pediatrics 1999;**104**(1):e5.
717. Sothern MS, Loftin JM, Udall JN, et al. Inclusion of resistance exercise in a multidisciplinary outpatient treatment program for preadolescent obese children. South Med J 1999;**92**(6):585-92.

718. Franz MJ, Boucher JL, Rutten-Ramos S, et al. Lifestyle weight-loss intervention outcomes in overweight and obese adults with type 2 diabetes: a systematic review and meta-analysis of randomized clinical trials. J Acad Nutr Diet 2015;**115**(9):1447-63.

APPENDICES

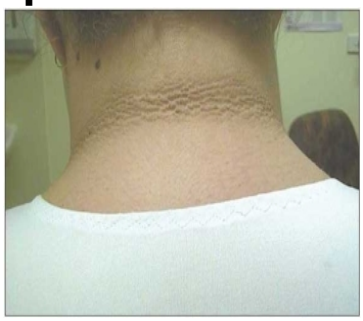
APPENDIX A

Is your child overweight or obese?

Are they between 10- 16 years?

Has your doctor told you that your child is at risk of Type 2 diabetes or has signs of insulin resistance?

If so, you may be interested in a new research trial being conducted in the Institute of Endocrinology and Diabetes at The Children's Hospital at Westmead



This child has signs of insulin resistance (there is a darkening of the neck called acanthosis nigricans)

What is insulin resistance?

Overweight, Acanthosis Nigricans (darkening of skin behind neck as in picture) and polycystic ovaries may mean your child has insulin resistance and therefore may be eligible to participate in our study.

More about our study

We are conducting a 3 month research trial to look at the effects of vibration training on insulin resistance in overweight adolescents. Your child will still receive the current standard therapies prescribed by your Endocrinologist for this condition but in addition half of the children recruited will be given a vibration plate to use at home for three months.

For more information please ask your Endocrinologist for an information sheet or call Dr Kim Ramjan on (02) 9845-3151

the children's hospital at Westmead

Sydney West Area Newsletter/ CHW Bandaged Bear Bulletin

Vibration Training and Insulin Resistance Study for Overweight Adolescents

Prof Cowell, Dr Craig Munns, Dr Sarah Garnett and Dr Kim Ramjan are conducting a new randomised control research trial into the effects of whole body vibration training and insulin resistance in overweight adolescents. This 3 month study is now recruiting young people aged 10-18 years who have clinical signs of insulin resistance for e.g. acanthosis nigricans, high fasting insulin levels, overweight and polycystic ovarian syndrome. The participants will be all receive the current standard care treatment as prescribed by Endocrinologists at The Children's Hospital at Westmead. In addition half of the participants will be randomised to receive vibration training. Participants in the vibration arm will be given a vibration platform to take home and use during the 3 month trial. If you have any patients that would like to take part in this study please contact Dr Kim Ramjan (02) 9845- 3151 or email kimr@chw.edu.au.

the children's hospital at Westmead

Corner Hawkesbury Road
and Hainsworth Street
Locked Bag 4001
Westmead NSW 2145
Sydney Australia
DX 8213 Parramatta
Tel +61 2 9845 0000
Fax +61 2 9845 3489
www.chw.edu.au
ABN 53 188 579 090

Dr Kim Ramjan
Endocrine Research Fellow
Institute of Endocrinology and Diabetes
The Children's Hospital at Westmead
Locked Bag 4001
Westmead
NSW 2145

Dr Smith
General Practitioner
Smith's Medical Practice
37 Smithville Road
Smithfield
NSW 2087

23rd November 2008

Dear Dr Smith,

The Endocrinology Departments of The Children's Hospital at Westmead, Campbelltown Hospital and RPAH are conducting two randomised control trials to determine the most effective management of adolescents with insulin resistance and/or pre-diabetes. The study coordinators are Dr Kim Ramjan and Dr Sarah Garnett and the co-investigators are listed below.

These studies are aimed at young people aged between 10 and 18 years with clinical signs of insulin resistance; overweight or obese with one or more of the following: acanthosis nigricans, PCOS, hypertension, dyslipidaemia, non-alcoholic fatty liver disease. All participants will receive diet and exercise interventions and some will be randomised to the novel vibration plate.

If you would like to refer any patients for the study please contact Dr Kim Ramjan, or Dr Sarah Garnett at The Children's Hospital at Westmead on 02 9845 3151 or 02 9845 3152 or email: kimr@chw.edu.au or sarahg@chw.edu.au. I have enclosed a flyer for the study should you wish to display this in your surgery. Thank you for your time and consideration of these research studies.

Yours sincerely,

Dr Kim Ramjan

Enclosure (1)
Investigators; A/Prof Chris Cowell, Prof Louise Baur, Dr Craig Munns, Dr Helen Woodhead, A/Prof Kate Steinbeck

the children's hospital at Westmead

Corner Hawkesbury Road
and Hainsworth Street
Locked Bag 4001
Westmead NSW 2145
Sydney Australia
DX 8213 Parramatta
Tel +61 2 9845 0000
Fax +61 2 9845 3489
www.chw.edu.au
ABN 53 188 579 090

INFORMATION SHEET
Whole Body Vibration Training for adolescents with insulin resistance

Investigators:
Dr K Ramjan, Institute of Endocrinology & Diabetes, CHW. Phone 02 9845 3151
Dr S Garnett, Division of Research, CHW. Phone: 02 9845 3152
Dr C Munns, Institute of Endocrinology & Diabetes, CHW. Phone 02 9845 3200
A/Prof C Cowell, Institute of Endocrinology & Diabetes, CHW. Phone: 02 9845 3200

We would like you to consider participating in a research study that will be conducted in The Institute of Endocrinology & Diabetes at The Children's Hospital at Westmead.

What is the study about?

This is a three month study looking at the effect whole body vibration training in young people who are overweight and are showing signs of insulin resistance. Signs include overweight and obesity, high insulin levels, acanthosis nigricans (dark pigmentation often found on the neck, under the arms or in the groin) and polycystic ovarian syndrome, high blood pressure and high blood fats.

Who can participate in the study?

10 to 18 year olds who are overweight or obese who have clinical signs of insulin resistance. Your doctor will let you know if you can take part in the study.

What will the study involve?

You will be put in either a standard care or standard care plus whole body vibration training group. All participants will be prescribed a diet and physical activity program, this is standard treatment in our clinic for all adolescents who are overweight. If you are allocated to the standard care plus whole body vibration training group, you will be asked to stand on a vibration platform at home for 15 minutes a day for three months. We will lend you the platform at no cost for three months.

Standard Care at The Children's Hospital at Westmead

Diet Intervention: You and your parent will be seen by one of our Weight Management dieticians at the beginning of the study. This is routine care for overweight children in our clinic. The dietician will review your diet and give you and your parent some guidelines about how to modify your diet if necessary. The dietician will arrange a follow up after 2 weeks and will be in contact by phone twice during the three months to see how things are going at home.

Physical Activity: You will be referred to The Children's Hospital Institute of Sports Medicine (CHISM), which is next to the hospital. You will have a fitness assessment and will be given an exercise prescription. The team at CHISM will follow you after 1 and 2 months to monitor your progress.

Vibration training group

If you are allocated to this group, we will lend you a vibration plate to use at home at no cost for the duration of the study (3 months). You will be required to stand on the vibration plate for 15 minutes a day and perform some basic stretches (we will teach them to you). We will ask you to keep a vibration training diary.

Extra Tests

To see if vibration training is useful in overweight adolescents with insulin resistance, we will need to do some further tests at the start of the study and at the end of the three months. The tests at the beginning of the study would need to be done anyway as they are part of routine care.

Details of tests

APPENDIX B – Patient Information Sheets

1) Insulin sensitivity: Will be measured by an Oral Glucose Tolerance Test (OGTT). This will involve coming to Turner Day Stay Ward at The Children's Hospital at Westmead at 8am after an overnight fast. After height and weight an IV cannula will be placed in one arm. Local anaesthetic cream will be used so that discomfort will be minimised. You will be asked to drink a glass of Lucozade™ (a sugary drink). The cannula will be used to take blood at the beginning of the study and every 30 minutes for 2 hours. During the test you can watch a video, listen to music, read a book etc. At the end of the test you will be given lunch on the ward. This test will conducted by a doctor and an experienced nurse.
2) Blood test (fasting): We will need to collect 15 ml of blood (approximately 3 teaspoons) through the cannula after it is inserted. No additional blood tests will be necessary.
3) Body composition: Will be measured by Dual energy X ray absorptiometry (DXA). This scan is non-invasive, painless and measures the amount of body fat compared to muscle in the body. Two Xray beams are passed through the body to give an image of soft tissue on the computer. This will involve you lying still on a table for approximately 10 minutes while we perform the scan. We will also measure the muscle and bone in your leg with a pQCT (Peripheral Quantitative Computer Tomography) scan. This is non invasive and painless. You will need to place your lower leg on a leg rest in the machine; it will be strapped in place if necessary. The test will take 5 minutes. These scans involve exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2000-3000 microsieverts (mSv) each year. The effective dose from this study is less than 5.6 microSv. At this dose, no harmful effects of radiation have been demonstrated and the risk is negligible.
4) Fitness test: This will involve running on a treadmill at The Children's Hospital Institute of Sports Medicine. You will be asked to exercise until you feel you are exhausted. Monitoring of heart rate will be undertaken. The test can be stopped at any time. This will take approximately 30 minutes.
5) Grading of acanthosis nigricans: The degree of acanthosis nigricans will be assessed by your doctor. In addition, photographs of the affected skin areas will be taken to assess if the treatment helps the skin condition.
6) Anthropometry: We would like to measure your height, weight and waist circumference.
7) Questionnaires: We would like you to complete questionnaires about your diet and physical activity and your vibration training (if applicable). This will take approximately 15-20minutes. You may need the help of your parents to complete these forms.
Are there any benefits for my participation in the study?
You will be informed of your results which will be explained to you in detail. The results may help you manage your insulin resistance.
Are there any side-effects and risk associated with this study?
There are no side effects or risks associated with this study. However, the effects of the small dose of radiation received in this study could affect an unborn baby. You should not become pregnant while on this study. We can provide further pregnancy counselling if requested. The vibration is very gentle, but some adolescents may find the feeling uncomfortable. At all times you will be able to stop the vibration if you become uncomfortable. When the vibration finishes some adolescents may have a tingling feeling in their legs and feet. While not painful, you may not like this feeling. The blood tests may cause some local pain and bruising, but we will apply some numbing cream to the area.
Other information:
Your anonymity and confidentiality will be maintained. In order to meet these requirements we will only report grouped data. Your information will be kept confidential and stored for a period of 5-10 years after publication, after which time information will be destroyed. Participation in this project is voluntary and if you decide not to take part or decide to withdraw at any time this will not otherwise affect your care at the Hospital.
If you have any questions about the conduct of this study, please do not hesitate to discuss them with Kim Ramjan (02 9845 3151) or any of the other investigators listed above.

APPENDIX B – Patient Information Sheets

This project has been approved by The Children's Hospital at Westmead Ethics Committee. If you have any concerns about the conduct of this study, please do not hesitate to contact Carolyn Casey, Secretary of the Ethics Committee (02 9845 1316).
This Information Sheet is for you to keep. We will also give you a copy of the signed consent form.

the children's hospital at Westmead

Corner Hawkesbury Road
and Hainsworth Street
Locked Bag 4001
Westmead NSW 2145
Sydney Australia
DX 8213 Parramatta
Tel +61 2 9845 0000
Fax +61 2 9845 3489
www.chw.edu.au
ABN 53 188 579 090

INFORMATION SHEET
Whole Body Vibration Training for adolescents with insulin resistance

Investigators:
Dr K Ramjan, Institute of Endocrinology & Diabetes, CHW Phone 02 9845 3151
Dr S Garnett, Division of Research, CHW. Phone: 02 9845 3152
Dr C Munns, Institute of Endocrinology & Diabetes, CHW. Phone 02 9845 3200
A/Prof C Cowell, Institute of Endocrinology & Diabetes, CHW. Phone: 02 9845 3200

We would like you to consider letting your child participate in a research study that will be conducted in The Institute of Endocrinology & Diabetes at The Children's Hospital at Westmead.

What is the study about?

This is a three month study looking at the effect of whole body vibration training in young people who are overweight and are showing signs of insulin resistance. Signs include overweight and obesity, high insulin levels, acanthosis nigricans (dark pigmentation often found on the neck, under the arms or in the groin) and polycystic ovarian syndrome, high blood pressure and high blood fats.

Who can participate in the study?

10 to 18 year olds who are overweight or obese who have clinical signs of insulin resistance.

What will the study involve?

Your child will be put in either a standard care or standard care plus whole body vibration training group. All participants will be prescribed a diet and physical activity program; this is standard treatment in our clinic for all adolescents who are overweight. If your child is allocated to the standard care plus whole body vibration training group, they will be asked to stand on a vibration platform at home for 15 minutes a day for three months. We will lend your child the plate at no cost for three months.

Standard Care at The Children's Hospital at Westmead

Diet Intervention: You and your child will be seen by one of our Weight Management dieticians at the beginning of the study. This is routine care for overweight children in our clinic. The dietician will review your child's diet and give you some guidelines about how to modify your child's diet if necessary. The dietician will arrange a follow up after 2 weeks and will be in contact by phone twice during the three months to see how things are going at home.

Physical Activity: Your child will be referred to The Children's Hospital Institute of Sports Medicine (CHISM), which is next to the hospital. Your child will have a fitness assessment and will be given an exercise prescription. The team at CHISM will observe you child after 1 and 2 months to monitor progress.

Vibration training group

If your child is allocated to this group, we will lend them a vibration plate to use at home at no cost for the duration of the study (3 months). Your child will be required to stand on the vibration plate for 15 minutes a day and perform some basic stretches (we will teach them to your child). We will ask your child to keep a vibration training diary.

Extra Tests

To see if vibration training is useful in overweight children with insulin resistance, we will need to do some further tests at the start of the study and at the end of the three months. The tests at the beginning of the study would need to be done anyway as they are part of routine care.

Details of tests

1) Insulin sensitivity will be measured by an Oral Glucose Tolerance Test (OGTT). This will involve bringing your child to Turner Day Stay ward at The Children's Hospital at Westmead at 8am after an overnight fast. After height and weight an IV cannula will be placed in one arm. Local anaesthetic cream will be used so that discomfort will be minimised. Your child will be asked to drink a glass Lucozade™ (a sugary drink). The cannula will be used to take blood at the beginning of the study and every 30 minutes for 2 hours. During the test your child can watch a video, listen to music, read a book etc. At the end of the test your child will be given lunch on the ward. This test will conducted by a doctor and an experienced nurse.
2) Blood test (fasting): We will need to collect 15 ml of blood (approximately 3 teaspoons) from the cannula after it has been inserted. No additional blood tests will need to be taken.
3) Body composition: will be measured by Dual energy X ray absorptiometry (DXA). This scan is non-invasive, painless and measures the amount of body fat compared to muscle in the body. Two Xray beams are passed through the body to give an image of soft tissue on the computer. This will involve your child lying still on a table for approximately 10 minutes while we perform the scan. We will also measure the muscle and bone in your child's leg with a pQCT (Peripheral Quantitative Computer Tomography) scan. This is non invasive and painless. Your child will need to place their lower leg on a leg rest in the machine; it will be strapped in place if necessary. The test will take 5 minutes. These scans involve exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2000-3000 microsieverts (mSv) each year. The effective dose from this study is less than 5.6 microSv. At this dose, no harmful effects of radiation have been demonstrated and the risk is negligible.
4) Fitness test: This will involve running on a treadmill at The Children's Hospital Institute of Sports Medicine. Your child will be asked to exercise until they feel they are exhausted. Monitoring of heart rate will be undertaken. The test can be stopped at any time. This will take approximately 30 minutes.
5) Grading of acanthosis nigricans: The degree of acanthosis nigricans will be assessed by your child's doctor. In addition, photographs of the effected skin areas will be taken to assess if the treatment help the skin condition.
6) Anthropometry: We would like to measure your child's height, weight and waist circumference.
7) Questionnaires: We would like your child to complete questionnaires about their diet and physical activity and their vibration training (if applicable). Your child may require 15-20 minutes of your time to assist them to complete these forms.

Are there any benefits for my child participating in the study?

You will be informed of your child's results which will be explained to you in detail. The results may help you manage insulin resistance in your child.

Are there any side-effects and risk associated with this study?

There are no side effects or risks associated with this study. However, the effects of the small dose of radiation received in this study could affect an unborn baby. Your child should not become pregnant while on this study. If requested we can provide counselling regarding pregnancy. The vibration is very gentle, but some children may find the feeling uncomfortable. At all times you will be able to stop the vibration if your child becomes uncomfortable. When the vibration finishes some children may have a tingling feeling in their legs and feet. While not painful, some children may not like this feeling. The blood tests may cause some local pain and bruising, but we will apply some numbing cream to the area.

Other information:

Your child's anonymity and confidentiality will be maintained. In order to meet these requirements we will only report grouped data. Your information will be kept confidential and stored for a period of 5-10 years after publication, after which time information will be destroyed. Participation in this project is voluntary and if you decide not to take part or decide to withdraw at any time this will not otherwise affect your child's care at the Hospital. If you have any questions about the conduct of this study, please do not hesitate to discuss them with Kim Ramjan (02 9845 3151) or any of the other investigators listed above.

This project has been approved by The Children's Hospital at Westmead Ethics Committee. If you have any concerns about the conduct of this study, please do not hesitate to contact Carolyn Casey, Secretary of the

Ethics Committee (02 9845 1316). This Information Sheet is for you to keep. We will also give you a copy of the signed consent form.

Clinical Evaluation Form APPENDIX C

Child's Anthropometry

Date of evaluation (dd/mm/yy)_____/_____/_____ Measured by (circle): KR/ SG/ SB/ Consultant

	Trial 1	Trial 2	Trial 3	Average
Height (cm)				
Weight (kg)				
Waist Circumference (cm)				

Acanthosis Nigricans & Hirsutism & PCOS & Fatty Liver

Acanthosis Nigricans Don't Know/No/Yes

Sites □ Neck □ Axilla □ Knuckles □ Knee

Grade at neck □ 1 □ 2 □ 3 □ 4

Hirsutism Don't Know/No/ Yes

Sites □ Face □ Abdomen □ Legs

Measured by (circle) KR/ Consultant

PCOS Don't Know/No/Yes

Fatty Liver Don't Know/No/Yes

Blood Pressure

Blood pressure measured (circle) Yes/ No

Cuff Size: □ Child □ Small Adult □ Adult □ Don't Know

Blood Pressure

BP Systolic 1 _____BP Systolic 2_____ BP Systolic 3 _____

BP Diastolic1_____BP Diastolic 2_____ BP Diastolic3_____

Pubertal Staging

Pubertal staging performed (circle) Don't know/No/Yes
Measured/ Self reported

Acne (circle) Don't know/No/Yes

Girls

Menarche attained Don't know/ No/Yes Date ____/_____/____

Breast Stage □ 1 □ 2 □ 3 □ 4 □ 5

Pubic Hair □ 1 □ 2 □ 3 □ 4 □ 5

Boys

Testes (ml) Right _____ml

Testes (ml) Left _____ml

Genital Stage □ Don't know □ 1 □ 2 □ 3 □ 4 □ 5

Pubic Hair □ Don't know □ 1 □ 2 □ 3 □ 4 □ 5

APPENDIX C – Data collection forms

Clinical Evaluation Form

Child Medical History

Does the child have any other medical problems? (circle)Yes/ No

Please list any other medical problems:

_____

Family History

Overweight/ Obesity

Is any one else in your family overweight? (circle) Don't know/No/Yes

If yes, please choose from list: Sibling/ Mother/ Father/ Grandparent/ Aunt/ Uncle

What is their current treatment: Don't know/Lifestyle/ Metformin/ Orlistat (Xenical)/ Sibutramine (Reductil)

Diabetes

Is there a family history of diabetes? (circle) Don't know/No/Yes

If yes, please choose from list: Sibling/ Mother/ Father/ Grandparent/ Aunt/ Uncle

What type of diabetes? (circle) Don't know/Type 1/ Type 2/ Other

What is their current treatment: Don't Know /Lifestyle/Oral hypoglycaemics/Insulin

Hypertension

Is there a family history of hypertension? (circle) Don't know/No/Yes

If yes, please choose from list: Sibling/ Mother/ Father/ Grandparent/ Aunt/ Uncle

What is their current treatment: Don't know/Lifestyle/ Oral Antihypertensive

Cardiovascular Disease

Is there a family history of cardiovascular disease? Don't know/No/Yes

If yes, please choose from list: Sibling/ Mother/ Father/ Grandparent/ Aunt/ Uncle

Polycystic Ovarian Syndrome

Is there a family history of PCOS? Don't know/No/Yes

If yes, please choose from the list: (circle): Don't know/ Sister/ Mother/ Aunt/ Grandmother

Interview Form

Study:

Please complete the following details and circle appropriate responses

Child's Details

Child MRN_____ Child Forename_____ Child Surname _____

Date of Birth (dd/mm/yy)_____/_____/_____

Address_____ Suburb _____ Postcode _____

Home Phone (____)_____ Mobile Number_____ Email _____

Country of Birth (circle):

Australia/ New Zealand/Pacific Islander	NW Europe (UK, Scandinavia, Germany, France)
SE Europe (Italy/ Greece/ Hungary/Poland)	N Africa & Middle East
SE Asia (Thailand, Vietnam, Malaysia, Indonesia)	NE Asia (China/Japan/ Korea)
Southern & Central Asia (India, Sri Lanka)	North America
South America	Sub Saharan Africa

Other (specify) _____

If Australia, Aboriginal or Torres Strait Yes/ No

If New Zealand, NZ Maori/ Pacific Islander Yes/ No

Language spoken at home (circle): English/ Italian/ Greek/ Cantonese/ Arabic/ Vietnamese/ Mandarin

Other language (specify) _____

Other Comments _____

Mother's Details

Forename_____ Surname_____ Date of Birth (dd/mm/yy)____/____/_____

Address_____Suburb_____

Postcode_____ Home Phone (____)_____Work Phone (____)_____

Mobile Phone _____Email_____

Country of Birth (circle):

Australia/ New Zealand/Pacific Islander	NW Europe (UK, Scandinavia, Germany, France)
SE Europe (Italy/ Greece/ Hungary/Poland)	N Africa & Middle East
SE Asia (Thailand, Vietnam, Malaysia, Indonesia)	NE Asia (China/Japan/ Korea)
Southern & Central Asia (India, Sri Lanka)	North America
South America	Sub Saharan Africa

Other (specify) _____

If Australia, Aboriginal or Torres Strait Yes/ No

If New Zealand, NZ Maori/ Pacific Islander Yes/ No

Language spoken at home (circle): English/ Italian/ Greek/ Cantonese/ Arabic/ Vietnamese/ Mandarin

Other language (specify) _____

Other Comments _____

Interview Form (Continued)

Study:

Please complete the following details and circle appropriate responses

Father's Details

Forename_____ Surname_____ Date of Birth (dd/mm/yy)____/____/_____

Address_____Suburb_____

Postcode_____ Home Phone (____)_____Work Phone (____)_____

Mobile Phone _____Email_____

Country of Birth (circle):

Australia/ New Zealand/Pacific Islander	NW Europe (UK, Scandinavia, Germany, France)
SE Europe (Italy/ Greece/ Hungary/Poland)	N Africa & Middle East
SE Asia (Thailand, Vietnam, Malaysia, Indonesia)	NE Asia (China/Japan/ Korea)
Southern & Central Asia (India, Sri Lanka)	North America
South America	Sub Saharan Africa
Other (specify)	_____
If Australia, Aboriginal or Torres Strait	Yes/ No
If New Zealand, NZ Maori/ Pacific Islander	Yes/ No
Language spoken at home (circle):	English/ Italian/ Greek/ Cantonese/ Arabic/ Vietnamese/ Mandarin
Other language (specify)	_____

Other Comments _____

Relative/ Next of Kin Details

Forename_____ Surname_____

Address_____Suburb_____

Postcode_____ Home Number (____)_____Mobile Phone _____

GP Details

Are you happy for GP to know of participation in study (circle) Yes/No

Forename_____ Surname_____

Address_____Suburb_____

Postcode_____ Work Phone (____)_____Other Number (____)_____

Mobile Phone _____

the children's hospital at Westmead

Dietary Checklist

HOW TO FILL IN THE CHECKLIST

You will need to fill the checklist in each day for 7 days.

Put a tick [✓] in the box if you have eaten the food listed in amount advised.
Example [✓lamb] or [✓apple].

If you have eaten the food, but have had more or less than advised, write in the amount eaten. *Example* [✓lamb 80g cooked] or [✓apple 200g].

Put a cross (X) if you have not eaten that food at all.

List ALL foods eaten (allowed or not!) that are not specified on the checklist under OTHER. *Example* [diet soft drink 1 can]

Name:_____

Record Number: ☐☐☐☐

Date of Appointment: ☐☐/☐☐/☐☐
If you have any questions, please contact Kim Ramjan (02) 9845 3151

APPENDIX D

WEEK:…… DATE:……	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Cereal or toast							
Eggs							
Dairy (milk or yoghurt)							
Protein @ morning tea eg cheese, milk							
Bread, wholegrain							
Lunch protein (50-100g: palm size) eg fish/ham							
Salad veg (circle number of serves)	0 1 2 3 4+	0 1 2 3 4+	0 1 2 3 4+	0 1 2 3 4+	0 1 2 3 4+	0 1 2 3 4+	0 1 2 3 4+
Dinner protein (150g raw weight: hand size) eg beef, chicken, fish							
Potato, rice, pasta (1 cup)							
Veg (circle number of serves)	0 1 2 3 4+	0 1 2 3 4+	0 1 2 3 4+	0 1 2 3 4+	0 1 2 3 4+	0 1 2 3 4+	0 1 2 3 4+
Fruit (circle number of serves)	0 1 2 3+	0 1 2 3+	0 1 2 3+	0 1 2 3+	0 1 2 3+	0 1 2 3+	0 1 2 3+
Good fats eg oil, nuts, avocado							
Treats eg chocolate, chips							
OTHER – list							
Exercise (circle number of minutes)	0 30 60	0 30 60	0 30 60	0 30 60	0 30 60	0 30 60	0 30 60
1 day off	No…Yes	No…Yes	No…Yes	No…Yes	No…Yes	No…Yes	No…Yes

Leisure and Activity Questionnaire

Please take time to answer all the questions.
It will take about 10 - 15 minutes.

Name:_____

Record Number: ☐☐☐☐

Date of Appointment: ☐☐/☐☐/☐☐

Thanks for your help in completing this survey.

If you have any questions, please contact Kim Ramjan (02) 9845 3151

Q1 In a typical WEEK in the LAST MONTH (do NOT include school holidays), which of the following PHYSICAL activities do you USUALLY do and for how long Monday-Friday and Saturday-Sunday?

PLEASE INCLUDE ACTIVITIES THAT YOU DO BEFORE SCHOOL, DURING RECESS AND LUNCH, AFTER SCHOOL AND WEEKENDS. DO NOT CIRCLE ACTIVITIES THAT YOU DO IN SCHOOL PE, ONLY INCLUDE ACTIVITIES THAT YOU DO IN SCHOOL SPORT FOR FOUR WEEKS OR MORE. FOR EXAMPLE, YOU WOULD CIRCLE 'SOCCER' IF YOU DO IT DURING SCHOOL SPORT FOR FOUR WEEKS OR MORE PER TERM.

In a typical WEEK which activities do you usually do?	Do you usually do this activity?	MONDAY - FRIDAY		SATURDAY - SUNDAY	
		Number of times Monday-Friday	Total hours/minutes Monday-Friday	Number of times Saturday & Sunday	Total hours/minutes Saturday & Sunday
EXAMPLE: Cycling	No Yes	2	40mins	1	15mins
Aerobics	No Yes				
Dance	No Yes				
Calisthenics/gymnastics	No Yes				
Martial Arts eg Karate	No Yes				
Tennis/ Bat tennis	No Yes				
Aussie Rules Football	No Yes				
Rugby League	No Yes				
Touch Football	No Yes				
Rugby Union	No Yes				
Soccer	No Yes				
Basketball	No Yes				
Cricket	No Yes				
Netball	No Yes				

GO TO NEXT PAGE

In a typical WEEK which activities do you usually do?	Do you usually do this activity?	MONDAY - FRIDAY			
		Number of times Monday-Friday	Total hours/minutes Monday-Friday	Number of times Saturday & Sunday	Total hours/minutes Saturday & Sunday
Baseball/softball	No Yes				
Swim laps	No Yes				
Swim for fun	No Yes				
Play with bats/rackets/ golf clubs	No Yes				
Skateboarding	No Yes				
Roller blading	No Yes				
Ride Scooter	No Yes				
Ride Bicycle (not for transport)	No Yes				
Ride Bicycle for transport (not to or from school)	No Yes				
Skip rope	No Yes				
Trampolining	No Yes				
Ball Games (eg, shooting hoops, kick-to-kick)	No Yes				
Hand ball	No Yes				
Play golf	No Yes				
Horse Riding	No Yes				
Walk the dog	No Yes				
Walk for exercise	No Yes				
Walk for transport (not to or from school)	No Yes				
Hockey	No Yes				
Rowing	No Yes				
Ten Pin Bowling	No Yes				
Weight training for fitness and strength	No Yes				
Jogging, running or cross country	No Yes				

GO TO NEXT PAGE

In a typical WEEK which activities do you usually do?	Do you usually do this activity?	MONDAY - FRIDAY		SATURDAY - SUNDAY	
		Number of times Monday-Friday	Total hours/minutes Monday-Friday	Number of times Saturday & Sunday	Total hours/minutes Saturday & Sunday
Household chores (eg, sweeping, scrubbing floors, chopping wood)	No Yes				
Other Clubs (eg: Scouts / Guides / Youth groups) Please state; ----------	No Yes				
Travel by walking to school (to and from school = 2 times)	No Yes				
Travel by cycling to school (to and from school = 2 times)	No Yes				
Physical Education (PE) class ACTIVITY ONLY (NOT Personal Development PD class)	No Yes				
Other (please state) ----------					

GO TO NEXT PAGE

Notice that this question is different from the last one, because it asks you how many hours/minutes you do each activity and not how many times

Q2 In a typical WEEK in the LAST MONTH (do NOT include school holidays), which of the following LEISURE activities do you USUALLY do and for how long Monday-Friday and Saturday-Sunday?

In a typical WEEK which leisure activities do you usually do?	Do you usually do this activity?	Total hours/minutes Monday-Friday	Total hours/minutes Saturday & Sunday
EXAMPLE: Watching Television/ videos/DVDs	No (Yes)	15hrs	6hrs 30mins
Watching Television/ videos/DVDs	No Yes		
Playing Playstation/ Nintendo/ XBOX / Computer games	No Yes		
Using the Computer/ internet (not games)	No Yes		
Doing homework	No Yes		
Sitting talking	No Yes		
Talking on the phone	No Yes		
Listening to music	No Yes		
Playing board games/cards	No Yes		
Playing a musical instrument	No Yes		
Reading books, magazines	No Yes		
Doing art & craft (eg. painting, models, beads, sewing, drawing)	No Yes		
Model Railways / Model Planes / cars / Warhammer	No Yes		
Other (list) _____			

Dietary Protocols – Moderate Carbohydrate
Moderate CHO diet
Nutrient Targets:
40-45% Total CHO
30% protein
25% fat (<10% saturated)

Meal Targets
30-45g total carbohydrate
20-30g total protein
10-20g total fat
Mid Meal Targets
20-30g total carbohydrate
10-20g total protein
5-10g total fat

Food Targets:
Bread – 2 slices/day
Fruit – 2 pieces
Dairy – 4 serves
Fats & Oils – 3 serves

Main Dietary Principles
Breakfast
Low GI carbohydrate + protein eaten together at each meal and mid meal
75-100g lean protein at lunch
100-150g lean protein at dinner
No fruit juice, soft drink, cordial
1 meal off week
Wholegrain low GI carbohydrates: 2 CHO exchanges @ meals/ 1-2 @ mid meals
20g total carbohydrate in the PM meal
No food after 9pm
3 cups vegetables/salad/day

Sample Meal Plan – Moderate CHO
Breakfast
2 x wholegrain bread + 2 poached eggs
1 Up & Go Energise Breakfast drink
¾ cup wholegrain cereal + 1 cup low fat milk + 1 fruit

Mid Morning
4 wholegrain crackers + 2 slices reduced fat cheese + 1 fruit
Low fat flavoured milk + 1 fruit
Protein rich snack bar +1 fruit

Lunch
75-100g lean protein +
Wrap style bread + salad

Afternoon Snack
1 dairy + serve nuts
Billabong/Paddle Pop + 15 almonds/walnuts
Nut Bar + Glass of low fat milk

Dinner
100-150g lean protein +
1 potato in jacket OR ½ corn cob OR dessert
3 cups vegetables OR salad

Dessert
<400kJ reduced fat ice cream
100g thick style yoghurt + free fruit

Dietetic Intervention Outline: Initial Consultation 30-40 minutes

1) Introduction	Dietitian to introduce self	5 mins
2) Gauge interest	Questionnaire/Weight goals	5 mins
3) Describe program	Overall summary of diet	5 mins
4) Individual plan	Satisfying checklist	5 mins
5) Resources	overview	5 mins
6) Final questions	Follow up scheduled	5 mins

Phone/email follow up – 24 hours later

Checklist –
Breakfast
Hunger
Protein at meals and mid meals
Reduced CHO at night
Extras
Fluids
Questions

Exercise advice for _____

Keep an exercise diary

Limit small screen time (TV, computer, internet, Playstation, Nintendo) to less than 2 hours per day

Exercise during your "hungry periods" such as after school

Try and do 3-4 exercise sessions per week.
Each session should be approximately 45-60 minutes of moderate intensity exercise. Walking alternating with jogging is a good activity:

Week 1 5 minutes (4 minutes of walking, 1 minute jogging)
(Repeat for whole 45-60 minute session)

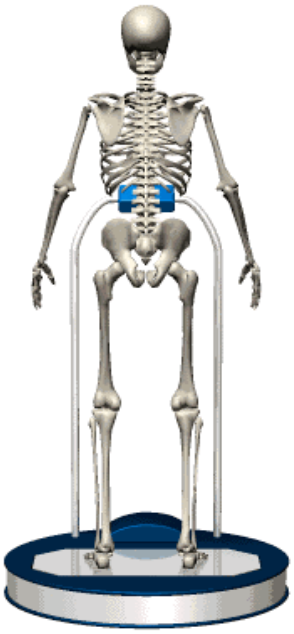
Each week try and increase your jogging time by 30 seconds

Week 2 5 minutes (3.5 minutes walking, 1.5 minutes jogging)
Week 3 5 minutes (3 minutes walking, 2 minutes jogging)

……So by week 8 you should be running most of the time

Try and do some competitive sport all year round

Summer: Boys & Girls- Basketball, touch football, rowing,
Six a side soccer, cricket, futsal
Winter: Boys - Soccer, rugby union/ League, basketball
Girls - Soccer, netball, basketball



Vibration Training Activity Diary

Instructions
Please fill in this diary each day while you are training
Record the time you spent on the vibration plate
Record the frequency (green number displayed on vibration plate)
Make a note of any problems you had during the week

Record Number □□□□□□□□
Name:_____

Date started vibration training □□ /□□ /□□

If you have any questions please call Dr Kim Ramjan (02) 9845 – 3151
Office Use Only: Data Entered □□□□ Date □□ /□□ /□□

APPENDIX G

Vibration Training Schedule Week 1

Day	Date	Vibration 1	Completed		Rest 1	Vibration 2	Completed		Rest 2	Vibration 3	Completed		Rest 3	*Official Use Only*
			Time	Frequency			Time	Frequency			Time	Frequency		
Mon		1 min; 12Hz			3 min	1 min; 12Hz			3 min	1 min; 12Hz			3 min	
Tues		1 min; 12Hz			3 min	1 min; 12Hz			3 min	1 min; 18Hz			3 min	
Wed		2 min; 12Hz			3 min	2 min; 18Hz			3 min	2 min; 18Hz			3 min	
Thurs		2 min; 12Hz			3 min	2 min; 18-20Hz			3 min	3 min; 18-20Hz			3 min	
Fri		3 min; 12Hz			3 min	3 min; 18-20Hz			3 min	3 min; 18-20Hz			3 min	
Sat		3 min; 12Hz			3 min	3 min; 18-20Hz			3 min	3 min; 18-20Hz			3 min	
Sun		3 min; 12Hz			3 min	3 min; 18-20Hz			3 min	3 min; 18-20Hz			3 min	

Please write time and frequency after you have done your training

Comments Week 1

_____

_____

_____

_____

_____

_____

Please write time and frequency after you have done your training