Metabolic Syndrome and Metabolic Syndrome Components in Young Adults

Paul Brian Nolan

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Abstract

Metabolic Syndrome represents a series of metabolic disorders most likely underpinned by insulin resistance, which increases the risk of developing clinical disease such as type II diabetes and cardiovascular disease. Metabolic syndrome is prevalent in 5-7% of young adults although estimates vary based on regional, ethnicity, and gender differences. The primary lifestyle behaviours that lead to development of metabolic syndrome, obesity and physical inactivity, increase substantially in the transition from adolescence to adulthood (18-25 years).

Young adulthood is a key time period in developing behaviours that will either increase or decrease the future risk of developing clinical disease. Less than optimal risk factors as a young adulthood are associated with an increase in the lifetime risk of developing clinical disease. Therefore identifying young adults with metabolic syndrome components may represent an important strategic approach of identifying young adults with a high lifetime risk of clinical disease. However, investigations examining metabolic syndrome in young adults and the usefulness of metabolic syndrome to identify young adults with a high lifetime risk of disease are rare.

A pooled analysis of 11 international studies (total n= 26,609) was conducted to examine the current prevalence of metabolic syndrome and metabolic syndrome components in apparently healthy young adults. Studies were included if participants were aged between 18-30 years old, and not sampled on the basis of any particular anthropometric characteristics. The key findings of this analysis were that 4.8-7.0% of young adults had metabolic syndrome, with low HDL-C being the most prevalent component (26.9-41.2%).

Apparently healthy young adults (n=268, 45.9% male) attending the University of Auckland (n=124) or Western State Colorado University (n=144) were investigated for metabolic syndrome prevalence. Metabolic syndrome was present in 14.2% of all participants with significant regional and ethnic differences in the prevalence of metabolic syndrome (NZ 4.8%, USA 22.2%; Caucasian 2.4 – 9.5%, Asian 12.0%, Hispanic 53.3%, Black American 35.0%, Maori/Pacific Island 7.1%). There were also significant differences in the prevalence of metabolic syndrome components between countries and ethnicities with low HDL-C being the most prevalent metabolic syndrome component overall (HDL-C 30.2%, BP 22.0%, TG 17.9%, WC 14.5%, FBG 9.23%). Approximately 40% of young adults had at least one component of

metabolic syndrome despite being "apparently healthy". Using questionnaire data and submaximal estimates of cardiorespiratory fitness, predictive modelling suggested that young adults with increased body mass index, a low physical activity level, increased daily sitting time, and low cardiorespiratory fitness, had increased odds of having metabolic syndrome. An isotemporal substitution analysis indicated that replacing 30 minutes of sitting time with either walking or moderate physical activity for 30 minutes would reduce the odds of having metabolic syndrome.

The results from the pooled analysis and prevalence study suggest that low HDL-C is the most prevalent component of metabolic syndrome, which requires further investigation to examine the significance of low HDL-C as a young adult. One of the potential early signs of cardiovascular disease may be microvascular endothelial-mediated dysfunction. Therefore, microvascular endothelial function was examined using laser Doppler flowmetry and iontophoresis in young adults with the low HDL-C component (male < 1.04mmol·L^{-1}, female < 1.30mmol·L^{-1}, n=15) of metabolic syndrome and normal levels of *HDL-C (control group n=28) to determine if any impairment in endothelial-mediated vasodilation occurs in the microvasculature. The results indicated that there was no reduction in endothelial–mediated vasodilation when the low HDL-C component was present, suggesting no difference in endothelial function in the microvasculature despite having one of the components of metabolic syndrome. Therefore, further work is required to determine the role of HDL-C in metabolic syndrome and subsequent clinical disease.*

In summary, this thesis provides novel information that indicates that many apparently healthy young adults already have metabolic syndrome, or components of metabolic syndrome, with low HDL-C being the most prevalent component (approximately 30% of young adults). The impact of having low HDL-C as a young adult is unclear; however it does not appear to be associated with an impaired microvascular endothelium dilatory capacity. Further research should focus on determining the role HDL-C plays in the development of metabolic syndrome.

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THE UNIVERSITY OF AUCKLAND
SCHOOL OF GRADUATE STUDIES

Co-Authorship Form

Graduate Centre
The ClockTower – East Wing
22 Princes Street, Auckland
Phone: +64 9 373 7599 ext 81321
Fax: +64 9 373 7610
Email: postgraduate@auckland.ac.nz
www.postgrad.auckland.ac.nz

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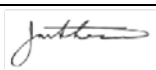
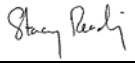
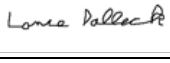
CO-AUTHORS

Name	Nature of Contribution
Dr Graeme Carrick-Ranson	Provided edits to original draft of the manuscript. Provided advice regarding results and susbequent discussion.
Assoc Prof James W Stinear	Provided edits to original draft of the manuscript. Provided guidance on grammar and editing
Dr Stacey A Reading	Provided edits to oringal draft of the manuscript. Significant contribution in helping interpret the results of the study
Dr Lance C Dalleck	Significant role in development of PhD project and inclusion of this analysis. Provided edits to original draft and thoughts to interpretation of study.

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

Name	Signature	Date
Dr Graeme Carrick-Ranson		25th Aug, 2017
Assoc Prof James W Stinear		18th Aug, 2017
Dr Stacey A Reading		17/08/2017
Assoc Prof Lance C Dalleck		24/08/2017

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Glossary

Abbreviation	Term
ACh	Acetylcholine chloride
AHA	American Heart Association
AT RISK	One or two metabolic syndrome components present
BMI	Body mass index
BP	Blood pressure
CHD	Coronary heart disease
COX	Cyclooxygenase
CRF	Cardiorespiratory fitness
CRP	C-reactive protein
CVC	Cutaneous vascular conductance
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
EDHF	Endothelium-derived hyperpolarizing factor
eNOS	Endothelial nitric oxide synthase
FBG	Fasting blood glucose
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
HDL-P	High-density lipoprotein particles
HR	Heart rate
IDF	International Diabetes Federation
IL-6	Interleukin-6
IL-18	Interleukin-18
LDF	Laser Doppler flowmetry
LDL-C	Low-density lipoprotein cholesterol
MAP	Mean arterial pressure
MetSyn	Metabolic syndrome
NCEP/ATPIII	National Cholesterol Education Program/Adult Treatment Panel III
NEFA	Non-esterified fatty acid
NHANES	National health and nutrition examination survey
NHBLI	National Health and Blood Institute
NO	Nitric oxide

OECD	Organisation for Economic Co-operation and Development
PA	Physical activity
PRESENT	Three or more metabolic syndrome components present (metabolic syndrome)
PU	Perfusion units
QCST	Queens College step test
RHR	Resting heart rate
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SkBF	Skin blood flow
SNP	Sodium nitroprusside
SR-BI	Scavenger receptor type BI
T2DM	Type two diabetes mellitus
TG	Triglyceride
TNF-α	Tumour necrosis factor alpha
TOTAL	Total cholesterol
VLDL	Very low-density lipoprotein
WC	Waist circumference
WHO	World Health Organization
ZERO	No metabolic syndrome components present

Chapter 1. **Introduction**

1.1. Brief Rationale for Thesis

Metabolic Syndrome (MetSyn) is a clustering of metabolic disorders that are associated with a high lifetime risk for cardiovascular disease (CVD)[1]. However, there is sparse research regarding MetSyn in young adults (18 – 25 years) despite alarming trends in two of the primary characteristics underpinning MetSyn; physical activity (PA) and obesity. Therefore, the primary aims of this thesis were to: 1) identify the prevalence of MetSyn in young adults; 2) determine the lifestyle factors that are associated with MetSyn components and MetSyn in young adults; 3) determine if microvascular endothelial dysfunction is present in young adults with the low high-density lipoprotein cholesterol (HDL-C) component of MetSyn.

1.1.1 **Metabolic Syndrome**

MetSyn is hypothesised to occur due to obesity and/or chronic physical inactivity leading to insulin resistance, increased circulating non-esterified fatty acids (NEFAs), and reduced adipocyte NEFA uptake. The increase in circulating NEFAs modifies local and systemic metabolic regulation and adversely affects organ function (e.g., muscle, liver, kidney, heart). The overall systemic effects are hyperinsulinemia, increased inflammation and a prothrombotic state that accelerates the development of vascular related diseases (e.g., coronary heart disease (CHD), type two diabetes mellitus (T2DM), stroke)[2].

MetSyn is determined from a clustering of three or more of the following components: large waist circumference (WC), elevated triglycerides (TGs), low HDL-C, raised blood pressure (BP), and elevated fasting blood glucose (FBG)[3]. The clustering characteristic of MetSyn implies a narrow range of possible origins in the development of MetSyn and its components.

However, multiple metabolic pathways are simultaneously affected leading to disruption in normal physiological function to maintain homeostasis. Furthermore, people with one MetSyn component are at risk of developing more MetSyn components and MetSyn. Early identification of people at risk of developing MetSyn (i.e., those with one or two components of MetSyn) may be a powerful public health intervention as a delayed onset, arrest, or reversal, of MetSyn components will likely reduce future CVD, T2DM, and stroke.

1.1.2 **There are Alarming Rates of Obesity and Physical Activity in Young Adults**

Young adulthood, encompassing the chronological ages of 18-25 years, is a distinct period during which both positive and negative health behaviours are established and maintained into adult life[4]. Alarmingly, unhealthy behaviours are prevalent in young adults with few participating in regular PA. Only 57% of adults aged between 18-44 years old in the United States of America (USA) (Centre for Disease Control 2014 Health Survey), and 50% of young New Zealand (NZ) adults (18-24 years old) report being physically active (Ministry Of Health, www.health.govt.nz). Furthermore, 40% of USA adults (20-39 years old)[5] are overweight and a further 20% of males and 16% females are obese[6]. In NZ, 49% of young adults (18-24 years) are overweight or obese (Ministry Of Health, www.health.govt.nz). Therefore, two of the main modifiable risk factors of MetSyn are common in a large proportion of young adults in the USA and NZ. Identification and intervention in young adults with MetSyn and its components is important so that current unhealthy behaviours can be replaced with healthy behaviours to help prevent the appearance of further MetSyn components and the deleterious health effects of MetSyn.

1.1.3 **Metabolic Syndrome in Young Adults**

MetSyn research in young adults is sparse despite a reported prevalence of MetSyn between 0.6% and 13.0%[7]. Prevalence of at least one component of MetSyn ranges from 25%[8] to 62%[9]

although differences in the ethnic distribution in the populations studied[10], and in the MetSyn criteria[11], may contribute to the differences in reported prevalence. No studies have been conducted specifically on young NZ adults limiting the usefulness of evaluating MetSyn in young NZ adults to identify those with an increased lifetime risk of CVD, T2DM, and stroke. Thus it is not currently known how social, cultural, ethnicity, economic, and environmental differences in NZ, may have contributed to a reduced or increased prevalence of MetSyn in young NZ adults. Furthermore, no studies have been conducted in young adults from a broad range of ethnicities thus limiting the applicability of the current studies to more heterogeneous populations. This is an important area to research as different country, regional, and ethnic specific prevalence of MetSyn exist[12].

1.1.4 **Outline of thesis**

The first study investigated 268 apparently healthy (free from diagnosed metabolic disease) young adults in NZ and the USA for MetSyn components to determine the prevalence of MetSyn. The main results reported are that 14% of apparently healthy young adults have three or more MetSyn components (i.e., MetSyn) and 41% of young adults have at least one MetSyn component. The most prevalent MetSyn component was low HDL-C. Secondary analyses reveal differences in prevalence of MetSyn between USA and NZ young adults, and people from different ethnicities. In particular, Hispanic male and female, and Black American female participants, had a significantly higher prevalence of MetSyn. This study highlighted that many apparently healthy young adults already have one or more MetSyn components. Furthermore, there were consistent small, but significant differences, in BP, anthropometric, lipid, and glucose profiles, when one or two MetSyn components were present. This finding may indicate that multiple small, but potentially significant physiological effects, are already present in young adults with one or two MetSyn components. The lifestyle behaviours associated with young adults having MetSyn components or MetSyn are examined in Chapter Five.

Lifestyle and other physiological variables such as cardiorespiratory fitness (CRF) was analysed in a series of multinomial logistic regression analyses to determine which lifestyle-related variables, if any, predicted having MetSyn components as a young adult. There were increased odds of having MetSyn in young adults with an increased body mass index (BMI), a low CRF, low reported levels of PA, and higher reported levels of daily sitting time. The full results of these analyses are reported in Chapter Five. This study provides important information regarding lifestyle behaviours that are associated with having MetSyn and MetSyn components as a young adult.

The second study investigated microvascular reactivity in the skin of young adults with the low HDL-C component of MetSyn. This study was conducted because of the high prevalence of low HDL-C in young adults as demonstrated in the first study, and a pooled analysis that was also conducted as part of this thesis (Chapter Three)[13]. HDL-C is hypothesized to be a key molecule in the process of reverse cholesterol transport (RVCT), promoting endothelial repair, and has anti-atherogenic and vascular protective effects[14]. Furthermore, microvascular endothelial dysfunction may be present before signs and symptoms of CVD are exhibited and could be the earliest detectable sign in the process of vascular disease. Given that the CVD process starts early in life[15] and due to the relationship between HDL-C and CVD[16], the significance, if any, of having low HDL-C as a young adult is an important area of research. Therefore, it was hypothesized that young adults with low HDL-C would have an attenuated increase in skin blood flow (SkBF) in response to iontophoresis of acetylcholine chloride (ACh); an endothelial-dependent vasodilator in blood vessels. However, the results from this study suggest that SkBF response was not attenuated in young adults with low HDL-C compared to young adults with normal levels of HDL-C. Therefore, people with low HDL-C do not appear to exhibit signs of microvascular endothelial dysfunction. Further research is required to investigate the significance, if any, of having low HDL-C as a young adult.

The overall findings of this thesis are that there is a high prevalence of MetSyn components in apparently healthy young adults. The most prevalent component of MetSyn in young adults was low HDL-C. However, people with low HDL-C do not have evidence of endothelial dysfunction in the skin. Maintaining a healthy BMI, reducing sitting time, increasing PA and having average or above average CRF, is likely to reduce the odds of having MetSyn as a young adult.

1.2. Summary

Two cross-sectional studies were completed from 2013 – 2017 with three primary aims:

1) To determine the prevalence of MetSyn and MetSyn components in apparently healthy young adults from two countries and different ethnicities.

2) To determine the association between PA levels, sitting time, CRF, BMI, and the odds of having MetSyn or MetSyn components as a young adult.

3) To determine if young adults with the low HDL-C component of MetSyn have reduced endothelial mediated SkBF response.

Chapter 2. **Literature Review**

This literature review is divided into three main parts. First, a brief discussion is provided on why young adults (18-25 years) are an important population to research in the context of a lifetime risk of clinical disease. Second, an overview on MetSyn is provided including the suspected causes and controversies surrounding MetSyn. Lastly, an overview is provided of lifestyle behaviours in young adults that are related to an increased risk of developing MetSyn.

2.1. Young Adulthood – A Unique Period of Life

Young adulthood is a period of life in which individuals experience increased independence but also decreased residential and social stability[17]. Young adulthood is an understudied period of life[18] but represents a critically important period to establish healthy lifestyle behaviours and reduce the lifetime risk of clinical disease. Young adulthood has been suggested as a good period of time to target for the prevention of future CVD because young adults have the capability of making independent lifestyle choices and may be easier to influence than children whose choices are largely made by their guardians[18,19]. Furthermore both positive and negative health behaviours are often established as a young adult and these lifestyle practices are maintained into adult life[4]. Therefore, understanding current lifestyle practices and any risk factors for clinical disease that are already prevalent in young adults is an important area of research. Understanding current risk factor status in young adults will enable targeted prevention strategies[19] to help reduce future rates of largely preventable diseases.

Traditional 10-year CVD risk models heavily weight chronological age in the algorithm used to calculate risk, essentially rendering them meaningless in distinguishing CVD risk in young adults[20]. Unfortunately this lack of consideration results in a large number of young adults with

a high lifetime risk of CVD not being identified until CVD risk factors (e.g., hypertension) or clinical disease are well established. For example, in one study that specifically investigated young adults and cardiovascular risk (Coronary Artery Risk Development in Young Adults (CARDIA)), young adults with low 10-year risk and low lifetime risk of CVD had lower levels of subclinical disease than the individuals with low 10-year risk but high lifetime risk as young adults[20]. In a follow up analysis of the CARDIA cohort, there was a strong association between maintaining a healthy lifestyle (defined as: BMI < 25 $kg \cdot m^{-2}$, no or moderate alcohol intake, higher healthy diet score, higher PA score, and never smoking) in young adulthood and a low CVD risk profile in middle age. Therefore evaluating CVD risk factors in young adults and evaluating their lifetime risk of CVD may be useful in identifying young adults who have a great opportunity and ample time to reverse CVD risk factor(s).

Many young adults already have CVD risk factors and unhealthy lifestyle behaviours. Fortunately, a healthy lifestyle change as a young adult is associated with a decreased risk of subclinical CVD in middle age[21]. In this study, young adults (18-30 years, n = 3538) were assessed for five healthy lifestyle factors (BMI, alcohol intake, diet, PA, smoking), and when followed up in middle age, the association with change in coronary artery calcification was assessed if they changed from having an unhealthy lifestyle factor to a healthy lifestyle factor (e.g., stopped smoking, became physically active etc.). The odds of having coronary artery calcification were reduced by 15% for each lifestyle factor that was reversed from unhealthy as a young adult to healthy in middle age. Therefore, not only is maintaining a healthy lifestyle in young adults important for maintaining a low CVD risk factor profile into middle age, adopting a healthier lifestyle (achieving a healthy BMI, increasing PA, absence of smoking, low alcohol intake, healthy diet) as a young adult appears to be a powerful strategy to reduce future CVD.

Before effective interventions to improve lifestyle behaviours can be designed and administered, young adults who have increased lifetime risk need to be identified in a manner that is easily scalable. MetSyn has been proposed as helping identify those who have a high lifetime risk of CVD and require intervention to help promote adoption of a healthy lifestyle[1]. This concept is clearly highlighted in previous work in CVD, as individuals who avoid developing CVD risk factors by the age of 50 have a 5-8% lifetime risk of developing CVD. Conversely, people who have one or more non-optimal CVD risk factors have a five-fold increase in lifetime risk of CVD (27-36%)[22]. Furthermore, young adults with a high lifetime risk but low 10-year risk of CVD are much further along the continuum of CVD development than those with lower lifetime risk factor profiles at an early age[20]. Therefore, identifying young adults with MetSyn and components of MetSyn has good clinical utility in helping to identify young adults who may have a high lifetime risk of CVD.

2.2. Metabolic Syndrome

MetSyn represents a multitude of metabolic disorders and has been described as a "multiplex cardiovascular risk factor" referring to the clustering of multiple components that confer the diagnosis of MetSyn[1]. The clustering characteristic makes MetSyn distinct from individual CVD risk factors such as smoking, hypertension etc.[1] and this clustering doubles the risk of stroke or heart attack[23,24], increases the risk of developing T2DM approximately six-fold[25,26], and increases the risk of CVD mortality substantially more than that of the individual MetSyn components[2,27]. A significant range of other clinical diseases is also associated with MetSyn (Table 2.1)[28]. MetSyn is a progressive disorder with an increasing incidence in all MetSyn components occurring with advancing age[1]. Therefore, early identification and intervention for people with components of the MetSyn represents an important step in reducing future MetSyn and the associated burden of CVD and T2DM.

Table 2.1. Diseases and Conditions Associated with Metabolic Syndrome[28]

CVD	T2DM
Stroke	Non-alcoholic fatty liver disease
Polycystic ovary syndrome	Obstructive sleep apnoea
Hypogonadism	Lipodystrophy
Microvascular disease	Chronic kidney disease

CVD – Cardiovascular Disease
T2DM – Type Two Diabetes Mellitus

Making lifestyle changes that promote favourable adaptations in all MetSyn components is the initial preventative approach for MetSyn[29]. Conversely, poor lifestyle behaviours such as physical inactivity are associated with having MetSyn. Therefore, MetSyn is related to lifestyle and is largely preventable in the majority of people. Preventing or reversing MetSyn in young adults via improvements in lifestyle (increasing PA, attaining healthy BMI, non-smoker, low alcohol intake, healthy diet)[21] is an excellent strategy to reduce the risk of future clinical disease.

2.2.1 **The History and Definition of Metabolic Syndrome**

In 1988, Dr Gerald Reaven, delivered the Banting lecture on the role of insulin in human disease[30] and proposed insulin resistance as an underlying cause of CVD[31]. Originally termed "Syndrome X", Reaven proposed that resistance to insulin-stimulated glucose uptake initiates a series of detrimental changes in the body (e.g., abnormal regulation of free-fatty acid metabolism, hypertension) that are involved in the development of CVD. The empirical data presented by Dr Reaven generated great scientific interest and multiple research groups started to research what is contemporarily known as MetSyn[32]. However, due to ambiguity

surrounding what the syndrome specifically encompassed and how to assess the presence of the syndrome, multiple research groups started researching MetSyn with different methodologies and definitions of MetSyn. These multiple lines of inquiry created significant confusion and debate surrounding the usefulness of MetSyn as an entity beyond that of its individual components. Furthermore, the determination of the cause(s) of the syndrome were confounded as the syndrome itself was not defined clinically until 1998 by the World Health Organization (WHO)[33]. Therefore, MetSyn has been a difficult concept to comprehensively understand due to multiple definitions, considerable disagreement between research groups, and ambiguity surrounding the origin(s) of MetSyn.

2.2.2 **Metabolic Syndrome: Evolution of a Definition**

One of the most important characteristics of MetSyn is the "clustering" of components that individually have been linked to CVD. Reaven's proposal that the clustering of hypertension, hyperinsulinemia, impaired glucose tolerance, increased plasma TGs, and decreased HDL-C, indicated insulin resistance was an important development in the prevention of CVD. With insulin resistance purported as an underlying cause of CVD, a single unified target to reduce future rates of CVD was apparent. Rather than treat hypertension, hyperinsulinemia, impaired glucose tolerance, and dyslipidaemia as separate CVD risk factors, focussing treatment on insulin resistance was hypothesised to reduce MetSyn and subsequent CVD.

Although the proposed treatment for insulin resistance was weight reduction and increased PA[34], central obesity was only included in the definition of MetSyn by Kaplan a year after Dr Reaven's lecture due to the strong link between central obesity and CVD[35]. Dr Reaven's "Syndrome X" had evolved into the "deadly quartet" and now included upper body obesity, glucose intolerance, hypertriglyceridemia, and hypertension[35]. Further research confirmed the

potential importance of insulin resistance to the syndrome and the "deadly quartet" was redefined as the "syndrome of insulin resistance"[36].

While this early research was important in advancing the understanding of insulin resistance as a potential underlying cause of a broad range of diseases, there was a lack of research and consensus regarding the specific cause(s) of MetSyn, how to clinically define MetSyn, and the prognostic significance of MetSyn. Significant debate ensued from various research groups and organizations and is summarised concisely in Edwin Gale's editorial in Diabetologia titled "The myth of the metabolic syndrome"[32]. In this editorial, Gale aptly describes how the physiologists tried to find the mechanisms linking insulin resistance to vascular disease, the epidemiologists tried to define causation, association, risk assessment and classification, the clinicians tried to figure out how useful MetSyn was in treating patients, the pharmaceutical companies found a problem for which they had medicine to sell, and finally, the medical organizations had a new syndrome to plant a flag on and claim as their own. Best left described in Gale's own writings, MetSyn had become:

"…a problem that defies definition, and competing groups of investigators who each want to define it differently" (pg. 1680, Gale, 2005).

"All that is needed is a suitably mystical concept for them to argue about, and such a concept exists: it is called insulin resistance" (pg. 1680, Gale, 2005).

Fortunately, consensus between formerly opposing medical organizations was achieved and in 2009 a harmonized definition for MetSyn was agreed upon[3]. However, it is useful to highlight the history of the proposed MetSyn definitions as many research groups, and presumably clinicians, still use these definitions currently.

2.2.3 **Multiple Metabolic Syndrome Definitions Emerge**

The WHO developed the first international definition of MetSyn in 1998[30] that included insulin resistance plus at least two of the following risk factors: hypertension, elevated TGs, low HDL-C, obesity, and microalbuminuria[33]. The WHO definition was intended as a working definition to create consistency between research groups as there was no international agreement on defining MetSyn at the time. Unfortunately, the justification for each criterion for the various components included in the WHO MetSyn definition was not discernible in the original WHO paper and is presumably a mixture of Reaven and Kaplan's proposals a decade earlier.

In 2001, a different definition of MetSyn was recommended by the National Cholesterol Education Program/Adult Treatment Panel III (NCEP/ATPIII). The NCEP/ATPIII proposed that any three of the following five risk factors was diagnostic of MetSyn: abdominal obesity, elevated TGs, low HDL, elevated BP, and raised FBG[29]. The NCEP/ATPIII definition represented a departure from routinely assessing insulin resistance and microalbuminuria. The change from the WHO definition had the desired effect of making the clinical diagnosis of MetSyn easier. However, the NCEP/ATPIII definition was primarily concerned with the goal of the NCEP/ATPIII that was related to low density lipoprotein cholesterol (LDL-C) management rather than MetSyn *per se*. The addition of MetSyn in the 2001 guideline was to help clinicians increase the focus and detection of people with multiple CVD risk factors that would benefit from more intensive LDL-C lowering treatment[29]. Therefore, the NCEP/ATPIII definition was never intended for the sole purpose of defining MetSyn. Nevertheless, this is a popular MetSyn definition and multiple research groups have used, and continue to use, the NCEP/ATPIII definition of MetSyn.

In 2005, the International Diabetes Federation (IDF) attempted to form a single, universal definition of MetSyn that would be easy to use in clinical practice[37]. In a significant but

contentious move, the IDF proposed the requirement of central obesity using ethnic specific cut points to identify people with MetSyn. The requirement of central obesity was suggested by the IDF as WC was strongly linked to the other components of MetSyn and is an early aetiological step in the insulin resistance cascade and thus MetSyn. The requirement of obesity in MetSyn prompted significant debate surrounding the cause of MetSyn and challenged the insulin resistance dogma. Furthermore, another definition of MetSyn was proposed by the American Heart Association (AHA)/National Health and Blood Institute (NHBLI) in the same year that aligned closely with the NCEP/ATPIII definition and did not have a requirement for central obesity[38]. Therefore a critical question arose: is MetSyn a central obesity related issue (IDF definition) or is having three or more MetSyn components enough for the diagnosis of MetSyn (AHA/NHBLI definition)?

Fortunately, after some robust debate[34], a harmonized definition of MetSyn was published in 2009[3] that integrated the recent AHA/NHBLI and IDF definitions. The harmonized paper is notable for the collaboration of people from the IDF and the AHA/NHBLI. The result of this harmonization was the removal of the obesity requirement in the IDF definition in favour of any three of five components (Table 2.2). However, the WC component was now defined using the IDF ethnic specific cut points including the lowering of the Europid cut point to a WC roughly equating to a BMI of 25 $kg \cdot m^{-2}$ rather than 30 $kg \cdot m^{-2}$. This harmonized definition of MetSyn appears to have satisfied major medical organizations regarding how to identify people with MetSyn both in clinical practice and research.

2.2.4 **Summary of Metabolic Syndrome Definitions**

The concept of MetSyn has endured robust debate, while a significant amount of time and research have resulted in a harmonized definition that currently satisfies major medical organizations. Although the exact mechanisms are still unclear, there is consensus that the

MetSyn is a useful concept in identifying people that have a raised lifetime risk of clinical disease. The use of the harmonized definition will result in greater consistency between studies and enhance the understanding of MetSyn as it relates to clinical and empirical research.

Table 2.2. Metabolic Syndrome Criteria according to Harmonized3, IDF[37], NCEP-ATPIII[29] and AHA/NHBLI criteria[38]

	Harmonized	IDF	NCEP/ATPIII	AHA/NHBLI
	Any three or more of:	**WC ≥ 94cm (male)** **WC ≥ 80cm (female)**	**Any three or more of:**	**Any three or more of:**
WC	Ethnic Specific Cut points	And two or more of:	≥102 cm (male) ≥88 cm (female)	≥102 cm (male) ≥88 cm (female)
HDL	<1.03 mmol·L^{-1} (male) <1.29 mmol·L^{-1} (female) OR taking medication for reduced HDL-C	<1.03 mmol·L^{-1} (male) <1.29 mmol·L^{-1} (female) OR taking medication for reduced HDL-C	<1.03 mmol·L^{-1} (male) <1.29 mmol·L^{-1} (female) OR taking medication for reduced HDL-C	<1.03 mmol·L^{-1} (male) <1.29 mmol·L^{-1} (female) OR taking medication for reduced HDL-C
TG	≥ 1.7 mmol·L^{-1} or medication for elevated TGs	≥ 1.7 mmol·L^{-1} or medication for elevated TGs	≥ 1.7 mmol·L^{-1} or medication for elevated TGs	≥ 1.7 mmol·L^{-1} or medication for elevated TGs
BP	≥130 mmHg SBP OR ≥ 85 mmHg DBP OR on BP lowering medication	≥130 mmHg SBP OR ≥ 85 mmHg DBP OR on BP lowering medication	≥130 mmHg SBP OR ≥ 85 mmHg DBP OR on BP lowering medication	≥130 mmHg SBP OR ≥ 85 mmHg DBP OR on BP lowering medication
FBG	≥5.6 mmol·L^{-1} OR antidiabetic medication	≥5.6 mmol·L^{-1} OR antidiabetic medication	≥6.1 mmol·L^{-1} OR antidiabetic medication	≥5.6 mmol·L^{-1} OR antidiabetic medication

MetSyn – Metabolic Syndrome; WC – Abdominal Obesity; HDL - Atherogenic Dyslipidaemia (Low HDL); TG - Atherogenic Dyslipidaemia (Raised Triglycerides); BP - Raised Blood Pressure; FBG – Raised Fasting Glucose

2.3. Pathogenesis of Metabolic Syndrome

MetSyn is proposed to develop when "metabolic susceptibility" factors are present and interact with increased adiposity[1]. Metabolic susceptibility factors include insulin signalling defects, adipose tissue disorders, mitochondrial defects, physical inactivity, aging, polygenic variations in ethnicities and individuals, and certain medications[1]. Most of the susceptibility factors are not modifiable (e.g., aging, polymorphisms) but obesity, and physical inactivity, are the two main modifiable contributors to the development of MetSyn[39,40]. High levels of physical inactivity and weight gain in people who are metabolically susceptible will likely result in MetSyn.

2.3.1 **The Insulin Resistance Model of Metabolic Syndrome**

While the exact mechanisms involved in the development of MetSyn remain elusive, insulin resistance[41], central adiposity, and/or a combination of both[1], are hypothesised to be the underlying cause. However, insulin resistance is not present in all people with MetSyn, and not all obese individuals have MetSyn[40]. Therefore, the relationship between insulin resistance, obesity, and MetSyn is complex[40] and not completely understood. However, despite a lack of understanding of the exact mechanisms in the development of MetSyn, it is clear that people with MetSyn have an increased risk of developing clinical disease.

2.3.2 **Insulin Resistance and Waist Circumference**

Insulin resistance is defined as a normal or elevated insulin level associated with an attenuated biological response to insulin[42]. A major contributor to the development of insulin resistance is increased circulating NEFAs[43]. Increased NEFA circulation leads to hypertrophy of intra-abdominal adipocytes that subsequently become resistant to insulin[44]. The effect is an increase in lipolytic activity that in turn promotes inflammatory cytokine production promoting further

increases in lipolytic activity. The net result of these interactions is an enlarged WC and further release of NEFAs into systemic circulation. In skeletal muscle, increased circulating NEFA concentration disrupts insulin signalling and insulin dependent glucose transport[43]. Collectively, the net systemic effect is insulin resistance and increased inflammation.

2.3.3 **Insulin Resistance and Triglycerides**

Increased circulating NEFAs saturate the liver, promoting TG rich very low-density lipoprotein (VLDL) formation[45]. In normal physiological conditions, insulin inhibits VLDL secretion into systemic circulation, but it is unknown if this important metabolic pathway is preserved in the insulin resistant liver[43]. Therefore, the increased TG concentration observed in MetSyn is potentially explained by hepatic stimulation and release of TGs.

2.3.4 **Insulin Resistance and High-Density Lipoprotein Cholesterol**

The increase in TG formation decreases the cholesterol content of HDL-C. This effect is primarily due to a reduction of cholesteryl ester of the lipoprotein core modifying the composition and metabolism of the HDL molecule. The change in metabolism of HDL may also attenuate or even reverse the anti-inflammatory effects of HDL. In the presence of high TG concentrations, HDL may enhance vascular inflammation, increase lipid oxidation, and increase plaque growth and thrombosis[46]. However, in the context of MetSyn, the lower HDL-C concentration is hypothesised to be primarily mediated via TG's effects of reducing the cholesteryl rich HDL[41].

2.3.5 **Insulin Resistance and Fasting Blood Glucose**

Insulin resistance eventually leads to an increased FBG via two mechanisms. First, with insulin resistance, insulin's effectiveness in suppressing gluconeogenesis is reduced leading to increased hepatic glucose output. Second, adipose and skeletal muscle tissue's ability to

"uptake" glucose from the blood is reduced for a given insulin concentration. Consequently, increased insulin secretion is required to maintain euglycemia but this insulin compensation response eventually fails. A proposed cause of this maladaptive response is that prolonged exposure of the pancreas to increased NEFA concentrations leads to a decrease in insulin secretion[47] and consequently to an increase in FBG.

2.3.6 **Insulin Resistance and Hypertension**

There are several mechanisms that may explain the relationship between insulin resistance and hypertension. First, insulin promotes blood vessel vasodilation in normal-weight individuals[48], but this response is attenuated in insulin-resistant individuals[49]. Second, endothelial production of nitric oxide (NO), a powerful vasodilatory substance, may be impaired in the setting of insulin resistance[42]. Third, insulin may stimulate sympathetic nervous system activity (i.e., release of epinephrine/norepinephrine)[50] promoting vasoconstriction in central and peripheral vascular beds. Finally, insulin promotes sodium reabsorption in the kidney[51] that also increases the retention of water which contributes to the increased BP observed in MetSyn. Therefore, in insulin-resistance, there is an attenuated vasodilator and increased vasoconstrictor response, and an increase in sodium and fluid retention resulting in increased systolic and diastolic blood pressure (DBP).

2.3.7 **Insulin Resistance and Metabolic Syndrome Summary**

In summary, the insulin resistance model of MetSyn suggests that in a "metabolically susceptible" person, increased adiposity as a result of a chronic positive energy balance leads to insulin resistance. Insulin resistance results in an increase in circulating NEFA resulting in further increases in adiposity (WC component). Hepatic exposure to increased NEFA enhances TG-rich VLDL formation (TG component) that lowers HDL-C (HDL component). The eventual failure of the compensatory hyperinsulinemia response to progressive insulin

resistance, results in inadequate clearance of glucose by skeletal muscle, and increased gluconeogenesis. A combination of a decrease in glucose clearance and an increase in glucose formation results in a rise in FBG (FBG component). Simultaneously, vasodilating properties of insulin are reduced, fluid retention is increased, sympathetic constriction of vascular beds is increased, and NO production is impaired in the endothelium, resulting in an increase in BP (BP component).

2.3.8 **Metabolic Syndrome leads to a Pro-thrombotic and Pro-inflammatory state**

MetSyn results in a pro-thrombotic and pro-inflammatory state. Confirmation of increased inflammation in people with MetSyn is observed as an increase in the concentration of inflammatory molecules such as C-reactive protein (CRP), tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and interleukin-18(IL-18)[28]. Many of these inflammatory markers have independent relationships with each of the MetSyn components and as the number of MetSyn components increase, there is an increase in the concentration of several inflammatory markers (e.g., CRP, TNF-α, IL-18)[28]. Increased coagulation markers in the blood such as fibrinogen and plasminogen activator inhibitor-1 have also been observed in people with MetSyn suggesting an increased thrombotic state[28]. All of the inflammatory and coagulation markers respond favourably to weight loss and increased PA[28] providing further evidence that lifestyle intervention should be the primary intervention in people with MetSyn.

2.4. The Prevalence of Metabolic Syndrome

The National Health and Nutrition Examination Survey (NHANES) from 2006 reported an overall age-adjusted MetSyn prevalence (≥ 20 years of age) of 34% in US adults[10]. The prevalence of MetSyn is lower in younger adults with 20% of 20-39 year old US adults having MetSyn compared to 41% in adults 40-59 years of age. MetSyn prevalence in other countries and regions varies with a reported prevalence of 29% and 32% in South Asian men and

women[52], 10% in Hong Kong Chinese men and women[53], and 16% and 14% in European men and women[54].

In New Zealanders aged between 35-74 years, the reported prevalence of MetSyn in Pacific Island (39%, n=1006) and Maori people (32%, n=996) is greater than their European counterparts (13%, n=2020)[55]. MetSyn is present in greater than 50% of Maori and Pacific Island people aged over 40 years, with approximately 80% of people assessed having at least 2 components of the MetSyn[56]. While still high, the prevalence in European men (25%) and women (13%) over the age of 40 years was much lower.

2.4.1 **Metabolic Syndrome Prevalence in Young Adults**

As part of this thesis, a pooled analysis of MetSyn prevalence in young adults (18-30 years) from multiple countries and ethnicities was performed and is presented in Chapter Three[13]. The main results from this study is that MetSyn prevalence ranges between 4.8-7.0% depending on the definition used (n=26,609 young adults age 18-30 years). MetSyn prevalence ranged from 0.5% - 22.0% between the individual studies included.

2.5. Physical Activity in the USA and NZ

PA levels typically decline from late adolescence into young adulthood and remain relatively stable until 64 years of age when a further decline occurs[57]. Approximately half of USA and NZ young adults meet the *minimum* recommended PA guidelines to improve or maintain health. In the USA, 56.7% of adults aged 18-44 engage in 150 minutes of moderate aerobic activity or 75 minutes of vigorous aerobic activity in bouts of 10 minutes or more each week (Centre for Disease Control 2014 Health Survey). In NZ, PA rates in 18-24 year old adults have decreased slightly from approximately 57% in 2011, to 50% in 2015 (Ministry of Health,

www.health.govt.nz). Therefore, there is significant proportion (approximately 50%) of young adults not engaged in PA in the USA and NZ.

2.5.1 **Physical Inactivity in the USA and NZ**

A recent consensus from the sedentary behaviour research network defines physical inactivity as *"an insufficient physical activity level to meet present physical activity recommendations"* (Table 5, pg. 9, Tremblay et al[58]). Physical inactivity is associated with adverse cardiovascular and metabolic effects independent of PA[59]. The global prevalence of physical inactivity is approximately 17.7%[60]. This is similar to 28% of 18-44 year old US adults, while approximately 13% of NZ 18-24 year olds report not performing a single ten minute bout of moderate or vigorous activity in the prior seven days. Therefore one in nine young NZ adults and one in four young USA adults do not perform any moderate or vigorous PA for at least ten continuous minutes. Moreover, trends over the past 20 years indicate physical inactivity has substantially increased. For example, a recent report highlighted a substantial increase in women (19.1 - 51.7%) and men (11.4 - 43.5%) reporting no leisure time PA[61]. Clearly, this lifestyle aspect can be improved but a significant amount of work remains on how to reverse the increasing rates of physical inactivity.

2.5.2 **Sedentary Behaviour**

Sedentary behaviour refers to *"…any waking behaviour characterized by an energy expenditure ≤1.5 metabolic equivalents, while in a sitting, reclining or lying posture."*(Table 5, pg. 9, Tremblay et al.[58]). Sedentary behaviour is independently related to an increased risk of the development of many MetSyn components. For example, a large trial in Australian adults (2031 male, 2033 female aged 25 years and over) that met the recommended PA guidelines reported a significant detrimental dose-response relationship of television viewing time with WC, systolic blood pressure (SBP), and 2hr postprandial plasma glucose concentration. This

detrimental relationship was also apparent between television-viewing time and FBG, TGs, and HDL-C in women[62]. Therefore it appears that increased sedentary time is independently associated with many of the MetSyn components in men and women even when meeting minimum PA guidelines.

2.5.3 **Physical Inactivity, Sedentary Behaviour and Metabolic Syndrome**

MetSyn is associated with physical inactivity. In a study of Danish adults, there was a two-fold increase in the odds ratio of having MetSyn in adults who were inactive during leisure time compared to the most active participants[63]. Similarly, a meta-analysis including 21,393 adults aged over 18 years reported increased time spent being sedentary with an odds ratio of 1.73 of having MetSyn[64]. These findings provide compelling evidence that physical inactivity is strongly associated with MetSyn and therefore increasing PA levels may be a particularly effective strategy to reduce the current prevalence and future incidence of MetSyn.

2.5.4 **Physical Activity and Metabolic Syndrome**

While PA may only have a small impact on individual CVD risk factors (e.g., BP 3-5 mmHg), the collective effects across multiple CVD risk factors combine to significantly reduce CVD risk in a magnitude (30-50%) that is similar to the absence of smoking[65]. A similar systemic effect is reported regarding PA and MetSyn. Lifestyle interventions for people with MetSyn have a combined reduction in MetSyn (odds ratio - 3.81), outperforming pharmacological interventions that reduce MetSyn (odds ratio - 1.59)[66]. The Finnish diabetes prevention study also reported a successful reversal and prevention of MetSyn in middle-aged adults who increased their moderate-to-vigorous leisure time PA over an average time period of 4.1 years[67]. This beneficial effect was independent of changes in BMI. Therefore, increasing PA in people at risk of developing MetSyn appears effective in reducing MetSyn.

However, PA interventions that result in lifelong increased PA are difficult to implement and may require more intensified approaches. A Cochrane analysis in 2005 reported moderate increases in self-reported PA with improved effects when a low-level of modest professional-guidance was available for support[68]. While not PA *per se*, a recent trial using Facebook™ for weight management was successful in reducing total body mass in overweight and obese individuals by 4.8% in 24 weeks[69]. Trials that are less intensive in their approach do not seem to improve PA over time. Adults with MetSyn receiving PA advice at the time of diagnosis (n=473) were not successful in meeting PA recommendations three years later[70]. Furthermore, multi-dimensional intervention studies do not appear to be successful in increasing PA community-wide although high quality studies in this area are sparse[71]. Therefore, more work is required to design effective PA interventions for people with MetSyn. Technologies such as social media may play an important role in providing long term support and access to reduce and prevent MetSyn but long duration, high quality, follow up studies are required.

2.6. Prevalence of Obese and Overweight Adults in the USA and NZ

In 2012, the Organisation for Economic Co-operation and Development (OECD) reported that the USA (35.3%), Mexico (32.4%) and NZ (31.3%) had the highest rates of obesity in OECD countries (OECD Health Statistics 2014). A further 33% of USA adults and 35% of NZ adults were considered overweight. Together, a staggering 69% of USA adults and 67% of NZ adults are overweight or obese. In adults 20-39 years of age, 40% of US adults are overweight[5] with a further 20% of US males and 16% of US females considered obese[6]. In NZ, 22% of 18-24 year old adults are obese, which represents an increase of 6% since 2006 (Ministry Of Health, www.health.govt.nz). Nearly half (49%) of NZ adults aged 18-24 years are considered either overweight or obese; a 9% increase since 2006. With obesity being a primary driver of

MetSyn[43], the high prevalence of overweight and obesity in young adults represents a significant public health problem in both countries.

Park et al. (2003) reported a five-fold increase in odds for overweight adults, 25-fold increase in odds for obese adults, and a staggering 68-fold increase in the odds of having MetSyn in adults with a BMI greater than 35 $kg \cdot m^{-2}$ compared to normal weight individuals[39]. These figures are alarming as it has been suggested that young adulthood is associated with an overall trend towards obesity[72]. For example, (n = ~7630) 13% of non-obese adolescents became obese in young adulthood whereas less than (n = ~ 2165) 2% of obese adolescents became non-obese over a five year follow-up period[73]. The scope of the problem is amplified when you consider (n=9795) 22.1% of the entire adolescent cohort were already considered obese. Furthermore, a recent cohort analysis of 92,837 men and women who had a weight recorded when 18-21 years, report a mean increase in total body mass of 12.6 kg over the following 30-35 years clearly highlighting the difficulties of maintaining a stable body mass with aging. The key message from this paper however was that this increased weight gain is associated with a significantly increased risk of developing disease[74]. Therefore, we are in the middle of an obesity crisis with young adulthood being a period of time where significant increases in BMI are often observed[75]. Reducing the age-related increase in BMI starting in young adulthood is likely to reduce future clinical disease.

2.6.1 **Obesity Studies and Metabolic Syndrome**

However difficult it may be to prevent increases in BMI as people age, it is still an interventional strategy worth pursuing. Preventing further increases in BMI in young adults appears effective in reducing the progression of MetSyn. Lloyd-Jones and colleagues reported that, in 18-30 year old adults, a BMI increase of less than 2 $kg \cdot m^{-2}$ over a period of fifteen years is associated with a minimal progression of MetSyn or MetSyn components regardless of initial

BMI[76]. Therefore, the prevention of further weight gain rather than weight loss *per se*, might be a potent primary prevention intervention and important public health message to attenuate future rates of MetSyn and subsequent clinical disease.

2.6.2 **Energy Balance and Metabolic Syndrome**

While obesity and physical inactivity may be the two primary drivers of MetSyn, they are both related entities. A report on obesity trends, PA, and caloric intake, covering the period from 1988 - 2010[61] reported that a decrease in PA was the behaviour that best explained a mean increase of 0.37% per year in BMI in the USA adult population. No significant increase in caloric intake was reported during this time period, but a substantial decrease in PA was associated with an increase in mean BMI and WC. Younger women had the largest increase in obesity prevalence during this time. This report clearly highlights that much of the increase in obesity prevalence is due to decreasing PA that likely results in a positive energy balance (i.e., energy intake exceeds energy expenditure). Consequently, obesity and MetSyn develop over time. Therefore it appears that more needs to be done to increase PA and decrease physical inactivity if obesity levels and consequently, MetSyn levels, are to be decreased.

2.7. Literature Review Summary

MetSyn is associated with an increased risk of a broad number of diseases. While exact mechanisms remain unknown, MetSyn is most likely underpinned by insulin resistance and is largely a result of low levels of PA and increased obesity in people who are metabolically susceptible. Identification of people with MetSyn is purported to identify people with a high lifetime risk for developing CVD above that of traditional CVD risk models. Therefore, identifying young adults with MetSyn components and therefore a higher lifetime risk of CVD will allow targeted interventions to prevent or reverse MetSyn and reduce lifetime risk of CVD. MetSyn is prevalent in 4.8 – 7.0% of young adults and up to 60% may have one or more

components of MetSyn. Alarmingly, approximately half of US and NZ young adults are overweight or obese and approximately half are physically inactive and these rates appear to be increasing. There is a need to address this period of young adulthood and identify those populations and attributable lifestyle behaviours that will best help prevent future clinical disease. Furthermore, due to the established prevalence of MetSyn components in young adults, there is also a need to investigate whether significant physiological dysfunction is detectable.

Chapter 3. Prevalence of Metabolic Syndrome and Metabolic Syndrome Components in Young Adults: A Pooled Analysis

This chapter is a slightly modified version of an article that was accepted for publication in the "Preventive Medicine Reports" journal in July 2017[13]. The article contains pooled data from studies that reported MetSyn and MetSyn component prevalence in 18-30 year old adults who were apparently healthy and sampling was not based on any anthropometric characteristic or presence of disease. The findings from this pooled analysis established the global prevalence of MetSyn and MetSyn components in young adults from a broad range of countries and ethnicity.

3.1. Introduction

MetSyn is an asymptomatic, pathophysiological state characterised by obesity, insulin resistance, hypertension, dysglycaemia, and dyslipidaemia[3]. While several criteria and definitions have been used to identify MetSyn[3,29,37,38]; it is generally agreed that a combination of three or more of the following components must be present: large WC, elevated TGs, low HDL-cholesterol, raised BP, and elevated FBG.

The IDF estimates that ≈25% of the world's population has MetSyn[77] although this estimate varies widely due to the age, ethnicity, and gender of the population studied[78]. Having a slightly raised value of a MetSyn component at a younger age increases the future risk for MetSyn later in life[10]. Therefore it is important to establish the prevalence of MetSyn components in young adults (18-30 years), as the presence of a MetSyn component could represent a lifetime of increased CVD risk. Moreover, the early identification of MetSyn components could lead to targeted interventions to prevent the development of the syndrome, and thus reduce CVD risk in later life.

Therefore, a pooled analysis of previous literature that examined the prevalence of MetSyn and its components in young adults was performed with the purpose of determining: 1) the global prevalence of MetSyn in young adults, and 2) the most prevalent MetSyn component in this population.

3.2. Methods

PubMed, SCOPUS and Medline were searched using the terms "Metabolic Syndrome", "Prevalence", and "Young Adults" combined with the Boolean operator "AND". The search was repeated using the term "College Students" instead of "Young Adults" and the results combined. Duplicates from the returned reference lists were discarded and the list was consolidated into one list from the three databases.

Abstracts from the returned references were downloaded and were kept if the abstract indicated that the article may contain data relating to MetSyn and apparently healthy young adults. The remaining articles were downloaded in full and analysed for specific data relating to MetSyn in young adults. Studies were included if 1) Participants were sampled on the basis of being apparently healthy, free of chronic conditions, or having specific anthropometric characteristics; 2) Data were available in the age range of 18-30 years old; 3) The prevalence for MetSyn was supplied or able to be calculated; 4) The NCEP-ATP III criteria[29], Revised NCEP-ATPIII (referred to here as AHA/NHBLI) criteria[38], IDF criteria[37] or the harmonized criteria[3] for MetSyn were used (Table 3.1). In addition, the article had to be written in English and accessible either through open access or our institution's library subscription that has access to 1200+ databases and 98,000 e-journals. All papers were cross checked to ensure that data were not used across multiple studies.

Data relating to MetSyn and MetSyn components in 18-30 year old adults were extracted from the reviewed articles and MetSyn and MetSyn component prevalence was calculated using the four different definitions. The results were tabulated (Table 3.2).

Table 3.1. Metabolic Syndrome Criteria According to Harmonized[3], IDF[37], NCEP-ATPII[29] and AHA/NHBLI Criteria[38]

	Harmonized	IDF	NCEP-ATPIII	AHA/NHBLI
	Any three or more of:	WC ≥94cm (male) WC ≥80cm (female)	Any three or more of:	Any three or more of:
WC	Ethnic Specific	And two or more of:	≥102cm (male) ≥88cm (female)	≥102cm (male) ≥88cm (female)
HDL	<1.03 mmol·L^{-1} (male) <1.29 mmol·L^{-1} (female) OR taking medication for reduced HDL	<1.03 mmol·L^{-1} (male) <1.29 mmol·L^{-1} (female) OR taking medication for reduced HDL	<1.03 mmol·L^{-1} (male) <1.29 mmol·L^{-1} (female) OR taking medication for reduced HDL	<1.03 mmol·L^{-1} (male) <1.29 mmol·L^{-1} (female) OR taking medication for reduced HDL
TG	≥ 1.7 mmol·L^{-1} or medication for elevated TG	≥ 1.7 mmol·L^{-1} or medication for elevated TG	≥ 1.7 mmol·L^{-1} or medication for elevated TG	≥ 1.7 mmol·L^{-1} or medication for elevated TG
BP	≥130mmHg systolic BP or ≥ 85mmHg diastolic BP or on BP-lowering medication	≥130mmHg Systolic BP or ≥ 85mmHg Diastolic BP or on BP-lowering medication	≥130mmHg Systolic BP or ≥ 85mmHg Diastolic BP or on BP-lowering medication	≥130mmHg Systolic BP or ≥ 85mmHg Diastolic BP or on BP-lowering medication
FBG	≥5.6 mmol·L^{-1} or antidiabetic medication	≥5.6 mmol·L^{-1} or antidiabetic medication	≥6.1 mmol·L^{-1} or antidiabetic medication	≥5.6 mmol·L^{-1} or antidiabetic medication

MetSyn – Metabolic Syndrome; WC – Abdominal Obesity; HDL - Atherogenic Dyslipidaemia (Low HDL); TG - Atherogenic Dyslipidaemia (Raised Triglycerides); BP - Raised Blood Pressure; FBG – Raised Fasting Glucose

3.3. Results

From the initial search, 1276 unique citations were returned, of which 992 studies were immediately discarded based on the title or abstract. The remaining 284 studies were evaluated against the inclusion criteria. Thirty-four papers were included in the final review with 11 studies[8,79–88] providing MetSyn data based on multiple definitions. Data from 26,609 different people aged between 18-30 years from 17 countries were available for analysis; see table for individual and grouped prevalence data. Please note that the combined number of observations for all MetSyn definitions is greater than 26,609 due to the inclusion of studies using multiple definitions.

Overall MetSyn prevalence was 4.8% (NCEP-ATPIII, n = 333/6889) 5.2% (AHA/NHBLI, n = 643/12473), 7.0% (IDF, n = 971/13953) and 6.5% (harmonized, n = 430/6578). Atherogenic dyslipidaemia defined as low HDL was the most prevalent MetSyn component regardless of the criteria used (26.9-41.2%) followed by raised BP (16.6-26.6%), abdominal obesity (6.8-23.6%), atherogenic dyslipidaemia defined as raised TGs (8.6-15.6%), and raised fasting glucose (2.8-15.4%) (Figure 3.1).

Table 3.2. Prevalence of Metabolic Syndrome and Metabolic Syndrome Components in 26,609 Young Adults

	Author	Country	Total (n)	MetSyn	WC	HDL	TG	BP	FBG
Harmonized	Al Dhaheri et al, 2016[89]	UAE	555	38 (6.8)	101 (18.2)	271 (48.8)	8 (1.4)	30 (5.4)	54 (9.7)
	Bennett et al, 2014[90]	Jamaica	746	6 (0.8)	108 (14.5)	343 (46.0)	4 (0.5)	154 (20.6)	8 (1.1)
	Ferguson et al, 2010[91]	Jamaica	839	10 (1.2)	134 (16.0)	393 (46.8)	5 (0.6)	56 (6.7)	10 (1.2)
	Gavrila et al, 2011[82] *	Spain	292	18 (6.2)	81 (27.7)	49 (16.8)	24 (8.2)	48 (16.4)	11 (3.8)
	Gupta et al, 2009[92]	India	486	12(2.5)	51 (10.5)	150 (30.9)	27 (5.6)	15 (3.1)	31 (6.4)
	Huang et al, 2015[93]	Taiwan	355	24 (6.8)	63 (17.7)	46 (20.6)	32 (9.0)	123 (34.6)	4 (1.1)
	Kaduka et al, 2012[94]	Kenya	90	9 (10.0)	17 (18.9)	47 (52.2)	3 (3.3)	51 (56.7)	1 (1.1)
	Lin et al, 2014[95]	China	323	22 (6.8)	180 (55.7)	63 (19.5)	40 (12.4)	20 (6.2)	22 (6.8)
	Martins et al, 2015[86] *	Brazil	2031	242 (9.0)	646 (31.8)	851 (41.9)	254 (12.5)	465 (22.9)	73 (3.6)
	Sy et al, 2014[88] *	Philippines	861	108 (12.5)	173 (20.1)	467 (54.2)	171 (19.9)	127 (14.8)	144 (16.7)
	Overall		6578	430 (6.5%)	1554 (23.6%)	2707 (41.2%)	568 (8.6%)	1089 (16.6%)	358 (5.4%)
NCEP –ATPIII	Erem et al, 2008[96]	Turkey	1306	93 (7.1)	182 (13.9)	318 (21.3)	183 (14.0)	424 (32.5)	25 (1.9)
	Gundogan et al, 2009[83] *	Turkey	84	10 (11.9)	25 (29.8)	19 (22.6)	24 (28.6)	20 (23.8)	11 (13.1)
	Huang et al, 2004[97]	USA	163	1 (0.6)	3 (1.8)	22 (13.5)	4 (2.5)	2 (1.2)	3 (1.8)
	Li et al, 2010[85] *	China	2532	101 (4.0)	79 (3.1)	742 (29.3)	241 (9.5)	519 (20.5)	58 (2.3)
	Manjunath et al, 2014[98]	India	473	41 (8.7)	76 (16.1)	184 (38.9)	37 (7.8)	123 (26.0)	42 (8.9)
	Mikkola et al, 2007[87] *	Finland	1099	38 (3.5)	51 (4.6)	212 (19.3)	31 (2.8)	565 (51.4)	25 (2.3)
	Sidorenkov et al, 2010[99]	Russia	862	23 (2.7)	19 (2.2)	266 (30.9)	83 (9.6)	149 (17.3)	6 (0.7)
	Sinha et al, 2013[100]	India	85	8 (9.4)	18 (21.2)	53 (62.4)	18 (21.2)	5 (5.9)	7 (8.2)
	Soysal et al, 2005[101]	Turkey	285	18 (6.3)	14 (4.9)	40 (14.0)	115 (40.4)	25 (8.8)	13 (4.6)
	Overall		6889	333 (4.8%)	467 (6.8%)	1856 (26.9%)	736 (10.7%)	1832 (26.6%)	190 (2.8%)
IDF	Bener et al, 2009[102] *	Qatar	203	16 (7.9)	16 (7.9)	43 (21.2)	30 (14.8)	34 (16.7)	8 (3.9)
	da Costa et al, 2011[103]	Brazil	711	28 (3.9)	90 (12.7)	313 (44.0)	35 (4.9)	71 (10.0)	35 (1.4)
	da Silveira et al, 2010[81] *	Brazil	3599	240 (6.7)	618 (17.2)	694 (19.3)	598 (16.6)	883 (24.5)	1322 (36.7)
	Gavrila et al, 2011[82] *	Spain	292	18 (6.2)	81 (27.7)	49 (16.8)	24 (8.2)	48 (16.4)	11 (3.8)
	Gundogan et al, 2009[83] *	Turkey	84	16 (19.0)	25 (29.8)	19 (22.6)	24 (28.6)	20 (23.8)	11 (13.1)

	Hildrum et al, 2007[84] *	Norway	1615	19 (1.2)	414 (25.6)	459 (28.4)	221 (13.7)	499 (30.9)	179 (11.1)
	Huang et al, 2007[8] *	USA	300	2 (0.7)	8 (2.7)	73 (24.3)	27 (9.0)	11 (3.7)	27 (9.0)
	Kanitkar et al, 2015[104]	India	250	55 (22.0)	139 (55.6)	93 (37.2)	71 (29.2)	21 (8.4)	44 (17.6)
	Li et al, 2010[85] *	China	2532	147 (5.8)	79 (3.1)	742 (29.3)	241 (9.5)	519 (20.5)	58 (2.3)
	Martins et al, 2015[86] *	Brazil	2031	242 (11.9)	646 (31.8)	851 (41.9)	254 (12.5)	465 (22.9)	73 (3.6)
	Mikkola et al, 2007[87] *	Finland	1099	75 (6.8)	134 (12.2)	212 (19.3)	31 (2.8)	565 (51.4)	221 (20.1)
	Sy et al, 2014[88] *	Philippines	861	108 (9.1)	173 (20.1)	467 (54.2)	171 (19.9)	127 (14.8)	144 (16.7)
	Tope et al, 2013[80] *	USA	376	35 (9.3)	43 (11.4)	73 (19.4)	21 (5.6)	38 (10.1)	42(11.2)
	Overall		13953	971 (7.0%)	2466 (17.7%)	4088 (29.3%)	1750 (12.5%)	3301 (23.7%)	2150 (15.4%)
AHA/NHBLI	Bener et al, 2009[102] *	Qatar	203	15 (7.4)	16 (7.9)	43 (21.2)	30 (14.8)	34 (16.7)	8 (3.9)
	Cheserek et al, 2014[105]	China	200	1 (0.5)	4 (2.0)	19 (9.5)	13 (6.5)	24 (12.0)	6 (3.0)
	da Silveira et al, 2010[81] *	Brazil	3599	213 (5.9)	269 (7.5)	694 (19.3)	598 (16.6)	883 (24.5)	610 (16.9)
	Dalleck et al, 2012[106]	USA	207	14 (6.8)	12 (5.8)	98 (47.3)	28 (13.5)	34 (16.4)	15 (7.2)
	De Kroon et al, 2008[107]	Netherlands	642	48 (7.5)	78 (12.1)	187 (29.1)	50 (7.8)	274 (42.7)	75 (11.7)
	Fernandes et al, 2011[7]	USA	189	7 (3.7)	14 (7.4)	38 (20.1)	33 (17.5)	4 (2.1)	14 (7.4)
	Gavrila et al, 2011[82] *	Spain	292	10 (3.4)	31 (10.6)	49 (16.8)	24 (8.2)	48 (16.4)	11 (3.8)
	Hildrum et al, 2007[84] *	Norway	1615	19 (1.2)	414 (25.6)	459 (28.4)	221 (13.7)	499 (30.9)	179 (11.1)
	Huang et al, 2007[8] *	USA	300	4 (1.3)	8 (2.7)	73 (24.3)	27 (9.0)	11 (3.7)	27 (9.0)
	Morrell et al, 2013[108]	USA	1610	81 (5.0)	209 (130)	467 (29.0)	258 (16.0)	403 (25.0)	64 (4.0)
	Morrell et al, 2012[109]	USA	2103	103 (4.9)	94 (4.5)	538 (25.6)	350 (16.6)	681 (32.4)	177 (8.4)
	Shahbazian et al, 2013[110]	Iran	203	13 (6.4)	26 (12.8)	85 (41.9)	47 (23.2)	1 (0.5)	35 (17.2)
	Sharifi et al, 2009[111]	Iran	934	70 (7.5)	31 (9.7)	714 (76.4)	246 (26.3)	56 (6.0)	74 (7.9)
	Tope et al, 2013[80] *	USA	376	45 (12.0)	43 (11.4)	73 (19.4)	21 (5.6)	38 (10.1)	42 (11.2)
	Overall		12473	643 (5.2%)	1309 (10.5%)	3537 (28.4%)	1946 (15.6%)	2990 (24.0%)	1337 (10.7%)

Data are expressed as n (%)

MetSyn – Metabolic Syndrome; WC – Abdominal Obesity; HDL - Atherogenic Dyslipidaemia (Low HDL); TG - Atherogenic Dyslipidaemia (Raised Triglycerides); BP - Raised Blood Pressure; FBG – Raised Fasting Glucose

*** Indicates study used more than one definition of MetSyn and is included multiple times.**

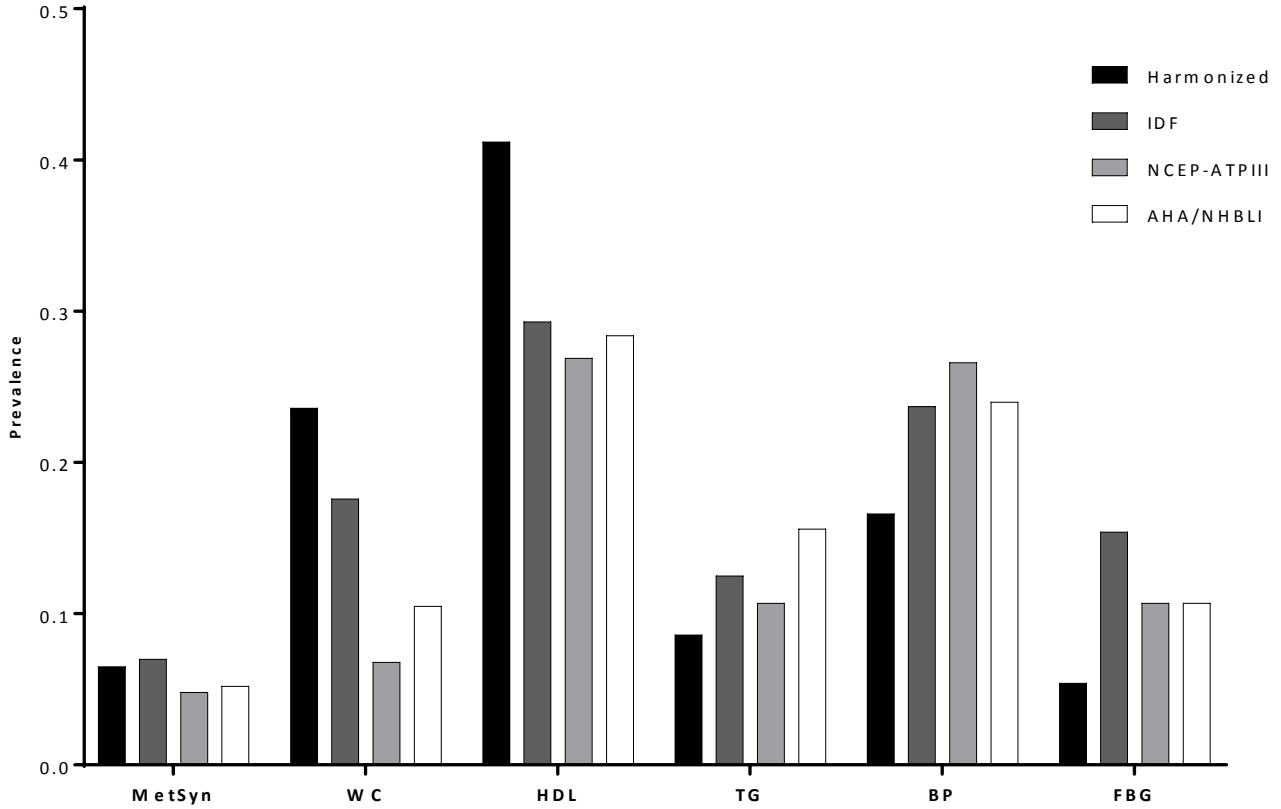


Figure 3.1. Prevalence of Metabolic Syndrome and Metabolic Syndrome Components in 26,609 Young Adults According to Four Metabolic Syndrome Criteria

MetSyn – Metabolic Syndrome; WC – Abdominal Obesity; HDL - Atherogenic Dyslipidaemia (Low HDL); TG - Atherogenic Dyslipidaemia (Raised Triglycerides); BP - Raised Blood Pressure; FBG – Raised Fasting Glucose

3.4. Discussion

This study provides new information about MetSyn in young adults in two important ways. First, pooled analysis of a large sample suggests that 5-7 percent of young adults have MetSyn. While the prevalence is less than the IDF estimated prevalence in all adults of 25% worldwide[77], the development of MetSyn early in adulthood can lead to an elevated lifetime burden of CVD risk. Second, one third of all participants had at least one component of MetSyn with low HDL being the most prevalent component. This latter finding raises the possibility that low HDL may be a key marker identifying early pathology associated with the development of MetSyn.

Prevention of the development of the first MetSyn component may have significant public health benefits as the presence of one component is predictive of the development of MetSyn[112]. Low HDL cholesterol occurs primarily due to increased TG formation reducing cholesterol content of the lipoprotein core[41]. Accordingly, it was expected that a higher prevalence of raised TG levels would be observed in the current findings; however, this did not occur. We speculate that this could reflect currently unknown mechanisms regarding HDL metabolism or a TG cut-off point not calibrated to changes in HDL levels in young adults. Regardless, while low HDL is not universally exhibited in all young adults with at least one MetSyn component, our findings demonstrate that low HDL is the most frequently exhibited MetSyn component regardless of MetSyn definition and may indicate the initiation of pathophysiological processes that underpin the development of MetSyn for many young adults. Further research should be undertaken to identify why low-HDL is the most common component of MetSyn in young adults.

MetSyn component prevalence was lower than reported for European adults from a more diverse and older aged population than the current study[113]. Approximately 45% of 19-39 year

old adults had the BP component, 25% the WC component, 25% TG component, and 20% with HDL component of MetSyn[113]. Vishram et al. also report an increased prevalence of BP and WC in males with increased age with a peak prevalence of elevated TGs and reduced HDL in the 40-49 year age bracket with a subsequent decline in older age ranges (50-59 and 60-78 years). A similar pattern was observed in females except TG prevalence increased with age and only HDL prevalence is decreased in ages above 40-49 years. Therefore, prevalence of MetSyn components in young adults are expected to increase up to the age of 50.

While overall MetSyn prevalence was similar between the four MetSyn definitions, a wide range of prevalence was present for each MetSyn component. Differences in WC prevalence can be partially explained by the use of ethnic specific thresholds in the harmonized and IDF definitions but the difference in HDL, BP, and FBG prevalence, cannot be explained by the definition. Therefore, it is possible that these observations indicate young adults from different ethnicities are more prone to develop different components of MetSyn. Therefore, a possibility worth exploring is that all MetSyn component thresholds may be ethnic specific and thus specific ethnic thresholds for each MetSyn component may need to be developed to accurately assess MetSyn. This is similar to current recommendations of using ethnic specific thresholds for WC.

3.5. Conclusion

MetSyn prevalence ranges from 5-7% in young adults. Low HDL is the most prevalent component of MetSyn in young adults and thus may also be the first detectable component of MetSyn in many young adults. Exploring the importance and significance of low HDL in young adults may have considerable public health benefit as interventions aimed at improving low HDL cholesterol levels could reduce future incidence of MetSyn and subsequent clinical disease.

Chapter 4. Prevalence of Metabolic Syndrome in Young Adults from Various Ethnicities

The following chapter discusses a study that investigated the prevalence of MetSyn in young adults in Auckland, NZ and Gunnison, Colorado, USA. Two hundred and sixty eight young adults (18-24 years) were recruited. All procedures were written and developed as part of this thesis. Being cognisant of the cost, participant time, researcher time, and validity of measures, the assessment was designed to be performed within one hour and use minimal resources. Research assistants were required to watch and read a manual of procedures produced in both video and written forms. The manual of procedures is attached as Appendix A.

4.1. Introduction

MetSyn encompasses a broad range of metabolic abnormalities[114] and is estimated to be present in 20-30% of adults of all ages worldwide[12]. MetSyn prevalence varies with age, having been reported at 6.6% for young adults (18-30 years) and 34.6% for adults (≥70 years) [115]. There is a two-fold increase in risk of developing CVD and a five-fold increase in risk of developing T2DM highlighting the clinical importance of early identification of MetSyn[3]. The early identification of the presence of MetSyn components allows for a greater opportunity of reversal and prevention of subsequent MetSyn and clinical disease.

MetSyn is diagnosed by evaluating five clinical indicators: elevated BP, atherogenic dyslipidaemia defined as either low HDL cholesterol or elevated TGs, large WC, and raised FBG, with the presence of three or more criteria necessary for establishing a diagnosis of MetSyn[3].

Young adulthood (18-24 years) is a period of life in which individuals experience increased independence but also decreased residential and social stability[17] that challenges young adults' ability to practice healthy lifestyle behaviours. This is problematic as unhealthy lifestyle practices such as physical inactivity, a poor diet, and weight gain, all play a role in the development of MetSyn components[38]. Previous research has suggested that lifestyle interventions involving diet and exercise in young adulthood and middle age could prevent development of many cardiovascular and metabolic diseases in large numbers of individuals[22]. Therefore, young adulthood is an important time for health promotion and lifelong disease prevention[116]. However, to date, studies that have comprehensively examined MetSyn component prevalence in young adult populations are rare.

The primary aim of this study was to establish the prevalence and impact on metabolic health indicators of MetSyn and MetSyn components in a young adult population. Secondary analyses were performed to determine if prevalence and metabolic health parameters differ based on country gender, and ethnicity.

4.2. Methods

A total of 268 students (54% female) individuals aged 18-24 years participated in this study. At the time of study, the participants were enrolled students at the University of Auckland, and resided in the city of Auckland, New Zealand (n=124) or were enrolled in Western State Colorado University, while residing in Gunnison, Colorado, USA (n=144). Participants that had known underlying metabolic conditions such as polycystic ovary syndrome, T2DM, or were pregnant or lactating at the time of assessment were excluded from this study. The protocol for the study was approved by the University of Auckland Human Participants Ethics Committee (Protocol number: 012554) and the Western State Colorado University Institutional Review Board (Protocol number HRC2013-0261R3). Written informed consent

was obtained from all participants after being provided a participant information sheet (Appendix B (NZ) and C (USA)) and afforded the opportunity to ask any relevant questions regarding involvement in the study, and have their questions answered to their satisfaction.

4.2.1 **Laboratory Measures**

To ensure consistency in measurement between testing sites, all research assistants were supplied a manual in written and video form that described the measurement techniques to be used. Participants attended the laboratory after an overnight fast. After at least 5 minutes' rest, resting heart rate (RHR), upright seated SBP, and DBP were measured using an automated sphygmomanometer (Life brand, Ontario, Canada; Omron HEM-705CP, Japan).

WC was measured in the standing position at the narrowest margin between the iliac crest and the 11^{th} and 12^{th} ribs using either a metal tape (Lufkin W606PM, USA) or cloth tape with spring loaded handle (Creative Health Products, Ann Arbor, MI). Height and body mass were measured via a stadiometer (SECA217, Germany; WB-3000 Digital Physician's Scale) and calibrated digital scales (SECA770, Germany) respectively.

All measures were repeated twice and the mean of the two measures was recorded as the final value. When two measures were outside the accepted levels of measurement (BP ± 5 mmHg, heart rate (HR) ± 5 bpm, WC ± 1.0 cm, mass ± 0.1 kg, height ± 1 cm) a third measure was performed and the median of the three measurements was recorded and used for data analysis.

4.2.2 **Blood Lipid and Glucose Measurement**

Blood lipid and glucose measures were analysed via a Cholestech LDX system (Alere Inc., Waltham, MA). Blood sampled by dermal puncture was immediately transferred to an LDX cassette for analysis. TG level, HDL-C, FBG, LDL-C and total cholesterol were directly measured or estimated via the Friedewald formula[117]. When blood lipids were outside the

detectable upper or lower limit of the Cholestech LDX system (n=33), the last detectable value for that variable was substituted to estimate LDL concentration. For example, TGs are not detectable <0.51 mmol·L^{-1} on the Cholestech LDX system, therefore 0.50 mmol·L^{-1} was substituted as the TG value in these cases. This procedure did not affect the ability to detect MetSyn components.

4.2.3 **Metabolic Syndrome Evaluation**

MetSyn components were determined by evaluating participant measurements against the thresholds recommended in the harmonized definition of MetSyn (Table 4.1)[3]. After evaluation of data, participants were classified as having no components (ZERO), 1-2 components (AT RISK) or three or more components (PRESENT).

Table 4.1. Harmonized Metabolic Syndrome Criteria[l3]

MetSyn is present when 3 or more of the following components are present:				
WC	**HDL-C**	**TG**	**BP**	**FBG**
Caucasian *Male: ≥94cm* *Female ≥80cm* Asian *Male ≥90cm* *Female ≥80cm* Hispanic; Black American; Maori/Pacific Island *Male ≥102cm* *Female ≥88cm*	Male *<1.03 mmol·L^{-1}* Female *<1.29 mmol·L^{-1}* OR *medication for low HDL-C*	*≥1.7 mmol·L^{-1}* OR *medication for elevated TGs*	SBP *≥130 mmHg* OR DBP *≥ 85 mmHg* OR *BP lowering medication*	*≥5.6 mmol·L^{-1}* OR *antidiabetic medication*

MetSyn – Metabolic Syndrome; WC – Abdominal Obesity; HDL - Atherogenic Dyslipidaemia (low HDL-C); TG - Atherogenic Dyslipidaemia (raised triglycerides); BP - Raised Blood Pressure; FBG – Raised Fasting Glucose

4.2.4 **Gender and Ethnicity Classification**

Participants self-identified their gender as either male or female, and identified their ethnicity by selecting one from the following list: NZ Caucasian, USA Caucasian, Asian, Hispanic, Black or African American (referred to as Black American in this thesis), Māori/Pacific Island.

4.2.5 **Statistical Procedures**

4.2.6 **Prevalence of Metabolic Syndrome Components**

To determine if there were MetSyn component prevalence differences, pairwise comparisons of MetSyn components were performed via non-parametric binomial testing. Prevalence of each MetSyn component was compared with the prevalence of the other MetSyn components

(e.g., HDL-C vs TG, HDL-C vs BP). A Bonferroni correction was applied to account for multiple comparisons of the five MetSyn components. Therefore, the alpha level of significance was set at $p<0.005$ (0.05/10) for these analyses.

4.2.7 **Differences in Metabolic Health Indicators and MetSyn presence**

Group differences in metabolic health indicators between ZERO, AT RISK, and PRESENT groups were analysed. Due to violation of the assumption of homogeneity of variance and unequal sample sizes in each group, a Welch one-way analysis of variance (ANOVA) was performed. Data were transformed using an inverse-normal transformation[118] due to violation of assumption of normality. After transformation, the Shapiro-Wilk test was performed on the transformed data. Transformed age and TG variables still violated the normality assumption (both $p<0.05$) but are included in the analysis. Due to violation of the assumption of homogeneity of variance, Games-Howell post-hoc testing was performed to isolate where group differences existed. Statistical significance for group differences was set at $p<0.05$. For ease of interpretation, non-transformed values are presented in Table 4.2.

4.2.8 **Secondary Analyses**

4.2.9 **Gender and Ethnicity Differences in Metabolic Syndrome and Metabolic Syndrome Component Prevalence**

To determine if statistically significant differences in the prevalence of MetSyn components based on ethnicity existed, multiple z-tests for proportions analyses were performed. Each ethnicity was compared with every other ethnicity for MetSyn and MetSyn component prevalence. A Bonferroni correction was applied to these analyses to account for multiple comparisons of the six ethnicities. Therefore, the alpha level of significance was adjusted to $p<0.0033$ (0.05/15) for these analyses.

A separate analysis was conducted to compare the prevalence of MetSyn and its components for each site. These analyses were performed using multiple z-tests for proportions and statistical significance was set at $p<0.05$.

4.2.10 **Ethnicity Differences in Metabolic Health Indicators**

A Welch one-way ANOVA was performed utilising the same method as described above except the group comparisons were different ethnicities rather than MetSyn components. Games-Howell post-hoc testing was performed to identify group differences. Statistical significance for group differences was set at $p<0.05$.

4.2.11 **Country differences in Metabolic Health Indicators**

An independent samples t-test was performed to determine if group differences exist in metabolic health data collected at the two different sites. Statistical significance was set at $p<0.05$.

All statistical analyses were performed using SPSS Statistics 23 software (IBM Corporation, New York, USA).

4.3. Results

4.3.1 **Participant Characteristics**

A total of 268 adults (20 ± 1 years; range 18 – 24 years) participated in this study. The NZ cohort represented 46.2% (n=124) of the cohort (total n=268, 45.9% male). Over 30% of participants identified as either NZ or USA Caucasian, 11.2% Hispanic, 9.3% Asian, 7.5% Black American, and 5.2% Māori/Pacific Islander. Group mean data (Table 4.2) revealed that overall the young adult population's metabolic health parameters were within normal ranges

Forty two percent of all participants (n=112) had either MetSyn (n=38, 14.2%), one component (n=49, 18.3%) or two components (n=25, 9.3%) of MetSyn.

Table 4.2. Resting Heart Rate, Blood Pressure, Anthropometric, Lipid, and Blood Glucose Profile

	Total (n=268)	**NZ** (n=124)	**USA** (n=144)	**p value** (NZ vs USA)
Age (yrs)	20(1)	21(2)	20(1)	0.084
RHR (bpm)	66(10)	65(11)	66(8)	0.332
SBP (mmHg)	119(11)	118(11)	121(11)*	0.019
DBP (mmHg)	74(9)	73(8)	74(8)	0.512
BMI ($kg \cdot m^{-2}$)	23.7(4.2)	22.8(3.8)	24.4(4.3)*	0.001
WC (cm)	80.0(10.7)	73.7(9.1)	85.5(8.5)*	<0.001
HDL-C ($mmol \cdot L^{-1}$)	1.40(0.40)	1.41(0.41)	1.39(0.38)	0.716
TG ($mmol \cdot L^{-1}$)	1.14(0.64)	0.85(0.54)	1.40(0.56)*	<0.001
FBG ($mmol \cdot L^{-1}$)	4.88(0.49)	4.77(0.43)	4.99(0.50)*	<0.001
LDL-C ($mmol \cdot L^{-1}$)	2.49(0.68)	2.28(0.77)	2.67(0.49)*	<0.001
TOTAL ($mmol \cdot L^{-1}$)	4.41(0.73)	4.09(0.80)	4.67(0.51)*	<0.001

Data are presented as mean (±S.D.)
RHR – Resting Heart Rate; SBP – Systolic Blood Pressure; DBP – Diastolic Blood Pressure; BMI – Body Mass Index; WC – Waist Circumference; HDL-C – High-Density Lipoprotein; TG – Triglyceride; FBG – Fasting Blood Glucose; LDL-C – Low-Density Lipoprotein Cholesterol; TOTAL – Total Cholesterol
*** p<0.05 (NZ vs USA)**

4.3.2 **Prevalence of Metabolic Syndrome**

MetSyn was present in 14.2% of the entire cohort (Table 4.3). The USA cohort had a significantly higher prevalence of MetSyn (22.2%, Table 4.3). MetSyn was identified in 53% of Hispanic, 35% of Black American, 12% of Asian, 12% of Caucasian, and 7% of Māori/Pacific Island participants (Table 4.4).

Table 4.3. Metabolic Syndrome Components and Metabolic Syndrome Status

	Entire Cohort (n=268)	NZ (n=124)	USA (n=144)
WC (%)	14.5	6.5	21.5*
HDL (%)	30.2	27.4	32.6
TG (%)	17.9	7.3	27.1*
BP (%)	22.0	20.2	23.6
FBG (%)	9.3	3.2	14.6*
ZERO (%)	58.2	58.9	57.6
AT RISK (%)	27.6	36.3	20.1
PRESENT (%)	14.2	4.8	22.2*

MetSyn – Metabolic Syndrome; WC – Abdominal Obesity; HDL - Atherogenic Dyslipidaemia (Low HDL-C); TG - Atherogenic Dyslipidaemia (Raised Triglycerides); BP - Raised Blood Pressure; FBG – Raised Fasting Glucose; ZERO – No MetSyn Component; AT RISK – One or two MetSyn components; PRESENT – MetSyn.
*** p<0.05 (NZ vs USA)**

4.3.3 **Metabolic Syndrome Component Prevalence**

In the entire cohort, low HDL-C was the most prevalent MetSyn component (n=81, 30.2%), followed by BP, TG, WC, and FBG (Table 4.3, Figure 4.1). Country differences in prevalence existed between multiple MetSyn components with the USA cohort having a significantly higher prevalence of WC, TG, and FBG (Table 4.3). Ethnicity differences also existed in multiple MetSyn components with Hispanic participants having higher prevalence of all MetSyn components compared to NZ and USA Caucasian participants. Black American participants had higher HDL-C, TG, and FBG prevalence compared to the NZ and USA Caucasian participants (Table 4.4). Maori/Pacific Island participants had a high prevalence of

low HDL-C (71.4%) that was not associated with a concomitant increase in TG prevalence (14.3%).

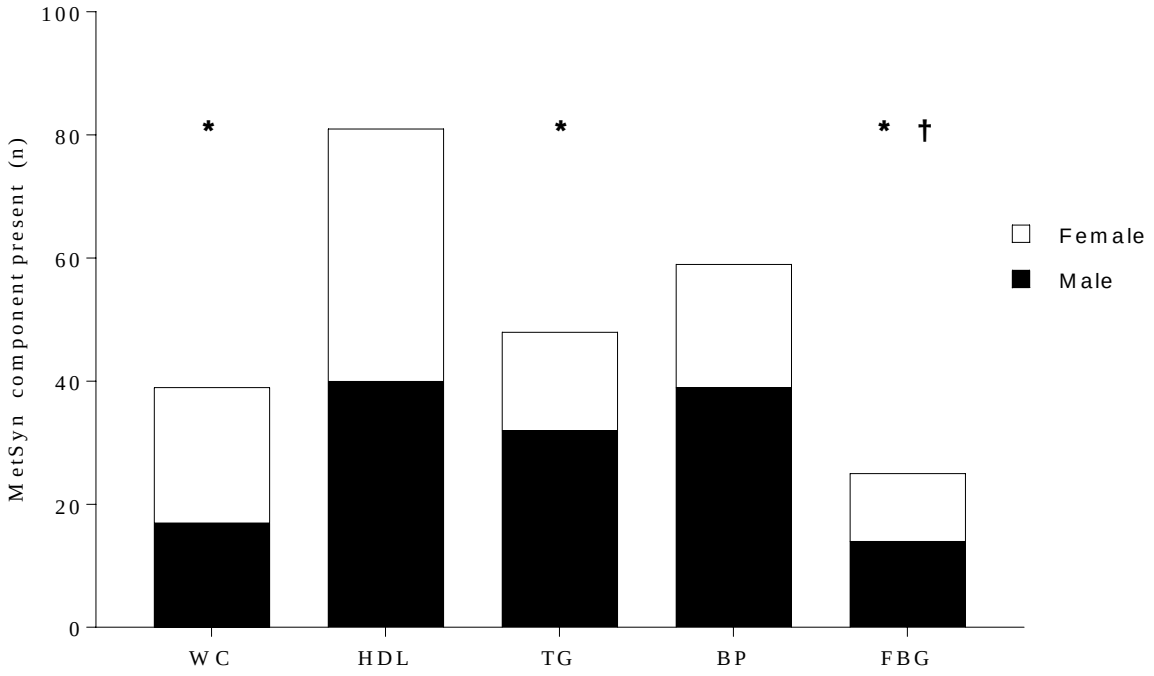


Figure 4.1. Prevalence of Metabolic Syndrome Components in Young Adults

MetSyn – Metabolic Syndrome; WC – Abdominal Obesity; HDL - Atherogenic Dyslipidaemia (Low HDL-C); TG - Atherogenic Dyslipidaemia (Raised Triglycerides); BP - Raised Blood Pressure; FBG – Raised Fasting Glucose

***Group prevalence is significantly different from HDL ($p<0.005$)**

†Group prevalence is significantly different from BP ($p<0.005$)

Table 4.4. Ethnicity Stratification of Metabolic Syndrome Components and Metabolic Syndrome Status

	NZ Caucasian (n=84)	USA Caucasian (n=95)	Asian (n=25)	Hispanic (n=30)	Black American (n=20)	Māori / Pacific Islander (n=14)
WC	4.8	15.8	12.0	43.3*†	15.0	7.1
HDL-C	19.0	16.8	32.0	66.7*†	55.0*†	71.4*†
TG	3.6	12.6	16.0	56.7*†¥	50.0*†	14.3
BP	21.	11.6	16.0	53.3*†	35.0	21.4
FBG	2.4	3.2	4.0	43.3*†¥	25.0*†	7.1
ZERO	64.3	72.6	60.0	23.3*†	40.0	21.4*†
AT RISK	33.3	17.9	28.0	23.4	25.0	71.5†£
PRESENT	2.4	9.5	12.0	53.3*†¥	35.0*†	7.1

Data are presented as % of each ethnicity
MetSyn – Metabolic Syndrome; WC – Abdominal Obesity; HDL - Atherogenic Dyslipidaemia (Low HDL-C); TG - Atherogenic Dyslipidaemia (Raised Triglycerides); BP - Raised Blood Pressure; FBG – Raised Fasting Glucose; ZERO – No MetSyn Component; AT RISK – One or two MetSyn components; PRESENT – MetSyn.
*** p<0.0033 compared to "NZ Caucasian"**
† p<0.0033 compared to "USA Caucasian"
¥ p<0.0033 compared to "Asian"
£ p<0.0033 compared to "Hispanic"

4.3.4 **Metabolic Health Indicators and Metabolic Syndrome Status**

Table 4.5 displays metabolic health parameters for participants who have no MetSyn components (ZERO), one or two MetSyn components (AT RISK), and MetSyn (PRESENT). There were small but significant differences in multiple hemodynamic, anthropometric, lipid,

and glucose, parameters when MetSyn components were present (all $p \leq 0.048$). Furthermore, the differences were increased when more MetSyn components were present (Table 4.5).

Table 4.5. Resting Heart Rate, Blood Pressure, Anthropometric, Lipid, and Blood Glucose Profile and Metabolic Syndrome Status

	ZERO (n=156)	**AT RISK** (n=74)	**PRESENT** (n=38)
Age (yrs)	20(1)	20(1)	21(1)
RHR (bpm)	63(9)	66(10)	75(7)*†
SBP (mmHg)	114(8)	123(11)*	134(6)*†
DBP (mmHg)	70(6)	75(9)*	85(5)*†
BMI ($kg \cdot m^{-2}$)	22.0(2.8)	23.9(3.6)*	30.0(4.1)*†
WC (cm)	76.3(8.4)	79.3(8.5)*	96.5(7.4)*†
HDL-C ($mmol \cdot L^{-1}$)	1.61(0.33)	1.16(0.34)*	1.00(0.15)*†
TG ($mmol \cdot L^{-1}$)	0.89(0.32)	1.11(0.51)	2.21(0.78)*†
FBG ($mmol \cdot L^{-1}$)	4.69(0.34)	4.93(0.42)*	5.59(0.44)*†
LDL-C ($mmol \cdot L^{-1}$)	2.35(0.64)	2.52(0.64)	3.03(0.64)*†
TOTAL ($mmol \cdot L^{-1}$)	4.36(0.71)	4.19(0.67)	5.02(0.57)*†

Data are presented as mean (±S.D.)
RHR – Resting Heart Rate; SBP – Systolic Blood Pressure; DBP – Diastolic Blood Pressure; BMI – Body Mass Index; WC – Waist Circumference; HDL-C – High-Density Lipoprotein; TG – Triglyceride; FBG – Fasting Blood Glucose; LDL-C – Low-Density Lipoprotein Cholesterol; TOTAL – Total Cholesterol
***p<0.05 compared to ZERO**
†p<0.05 compared to AT RISK

4.3.5 **Country, Ethnic, and Gender, Differences in Metabolic Health Indicators**

Significant differences also existed in multiple metabolic health indicators between countries (Table 4.2, all $p \leq 0.019$) and ethnic groups (Table 4.6, all $p \leq 0.011$). The Hispanic group in particular, and the Black American group to a lesser degree, had significantly different group metabolic health parameters with the most notable difference being a larger BMI than the

Caucasian and Asian groups. The Maori/Pacific Island group had a higher BMI than the Caucasian and Asian groups; however, they did not have the same elevation in mean TG levels observed in the Black American and Hispanic groups. Gender differences are presented in Table 4.7 and highlight that Hispanic male and female, and Black American female participants have a significantly higher prevalence of MetSyn components.

Table 4.6. Ethnicity Group Breakdown of Resting Heart Rate, Blood Pressure, Anthropometric, Lipid, and Blood Glucose Profile

	NZ Caucasian (n=84)	**USA Caucasian** (n=95)	**Asian** (n=25)	**Hispanic** (n=30)	**Black American** (n=20)	**Māori / Pacific Islander** (n=14)
Age (yrs)	21(2)	20(1)	21(2)	20(1)	20(1)	20(1)
RHR (bpm)	64(11)	63(8)	68(11)	72(6)*†	69(9)	64(10)
SBP (mmHg)	119(11)	117(9)	115(12)	129(10)*†¥	125(11)	122(8)
DBP (mmHg)	73(9)	71(7)	72(8)	81(8)*†¥	76(10)	77(8)
BMI ($kg \cdot m^{-2}$)	22.1(2.7)	23.1(3.8)	22.7(4.1)	28.5(5.4)*†¥	25.1(3.2)*†	26.4(4.2)*†¥
WC (cm)	72.5(6.9)	82.7(8.2)*	74.0(11.0)	93.7(9.6)*†¥	85.8(8.2)*¥	80.4(8.4)*£
HDL-C ($mmol \cdot L^{-1}$)	1.48(0.39)	1.50(0.41)	1.40(0.45)	1.14(0.21)*†	1.18(0.28)*†	1.05(0.30)*†
TG ($mmol \cdot L^{-1}$)	0.80(0.35)	1.11(0.50)*	1.00(0.52)	2.06(0.81)*†¥	1.63(0.63)*†¥	0.91(0.45)£$
FBG ($mmol \cdot L^{-1}$)	4.76(0.39)	4.79(0.42)	4.81(0.44)	5.46(0.48)*†¥	5.24(0.52)*†	4.70(0.43)£$
LDL-C ($mmol \cdot L^{-1}$)	2.21 (0.72)	2.51(0.46)*	2.48(1.04)	2.82(0.47)*†	2.87(0.44)*†¥	2.82(0.85)
TOTAL ($mmol \cdot L^{-1}$)	4.06(0.77)	4.51(0.37)*	4.34(1.21)	4.90(0.47)*†¥	4.79(0.45)*†¥	4.29(0.92)

Data are presented as mean (±S.D.)

RHR – Resting Heart Rate; SBP – Systolic Blood Pressure; DBP – Diastolic Blood Pressure; BMI – Body Mass Index; WC – Waist Circumference; HDL-C – High-Density Lipoprotein; TG – Triglyceride; FBG – Fasting Blood Glucose; LDL-C – Low-Density Lipoprotein Cholesterol; TOTAL – Total Cholesterol

*** p<0.05 compared to "NZ Caucasian"**

† p<0.05 compared to "USA Caucasian"

¥ p<0.05 compared to "Asian"

£ p<0.05 compared to "Hispanic"

$ p <0.05 compared to "Black American"

Table 4.7. Ethnic Differences in Metabolic Syndrome Components and Metabolic Syndrome Status based on Gender

Male (n=123)						
Ethnicity (n)	**NZ C** (33)	**USA C** (49)	**Asian** (10)	**Hispanic** (17)	**Black** (8)	**M/PI** (6)
WC (%)	0.0	12.2	20.0	52.9*	0.0	0.0
HDL-C (%)	21.2	16.3	50.0	64.7*$	50.0	83.3*$
TG (%)	3.0	20.4	40.0$	70.6*$	50.0$	16.7
BP (%)	36.4	18.4	20.0	70.6*	37.5	16.7
FBG (%)	0.0	4.1	10.0	52.9*	25.0	0.0
ZERO (%)	54.5	73.5	40.0	17.6*	37.5	0.0
AT RISK (%)	45.5*	14.3	40.0	17.6	25.0	100.0
PRESENT (%)	0.0	12.2	20.0	64.7*	37.5	0.0
Female (n=145)						
Ethnicity (n)	**NZ C** (51)	**USA C** (46)	**Asian** (15)	**Hispanic** (13)	**Black** (12)	**M/PI** (8)
WC (%)	7.8	19.6	6.7	30.8	25.0	12.5
HDL-C (%)	17.6	17.4	20.0	69.2**$$	58.3	62.5
TG (%)	3.9	4.3	0.0	38.5**$$	50.0**$$	12.5
BP (%)	11.8	4.3	13.3	30.8	33.3**	25.0
FBG (%)	3.9	2.2	0.0	30.8**$$	25.0	12.5
ZERO (%)	70.6	71.7	73.3	30.8	41.7	37.5
AT RISK (%)	25.5	21.7	20.0	30.8	25.0	50.0
PRESENT (%)	3.9	6.5	6.7	38.5**$$	33.3**	12.5

MetSyn – Metabolic Syndrome; WC – Abdominal Obesity; HDL - Atherogenic Dyslipidaemia (Low HDL-C); TG - Atherogenic Dyslipidaemia (Raised Triglycerides); BP - Raised Blood Pressure; FBG – Raised Fasting Glucose; ZERO – No MetSyn Component; AT RISK – One or two MetSyn components; PRESENT – MetSyn. NZ C – NZ Caucasian; USA C – USA Caucasian; Black – Black American; M/PI – Maori/Pacific/Island

*** p<0.0033 Different from USA C Male**

$ p<0.0033 Different from NZ C Male

$$p<0.0033 Different from NZ C Female

****p<0.0033 Different from USA C Female**

4.4. Discussion

The primary finding is that 40% of apparently healthy young adults in the current study had at least one component of MetSyn, and 14% met the criteria for having MetSyn. This suggests that MetSyn components are prevalent in a seemingly healthy population. It also underscores that MetSyn components are prevalent in early adulthood and efforts to reverse or mitigate progression of these components could reduce future disease burden. Furthermore, with increasing number of MetSyn components, there was a significant difference in the absolute values of HR, BP, body size, and lipid profiles. In terms of young adults with the greatest presence and risk of developing MetSyn, Hispanic males and females, and Black American females have a disproportionately high prevalence of MetSyn when compared to their Caucasian, Asian, and Māori/Pacific Island people.

4.4.1 **Metabolic Syndrome and Metabolic Syndrome Component Prevalence**

Approximately one in seven (14.2%) participants have MetSyn in the current study, which is higher than previously reported in populations of a similar age range[84,92,93,108,119]. Secondary analyses revealed that there was a higher prevalence of MetSyn in the USA (22.2%) compared to the NZ (4.8%) cohort. Further analysis also revealed that there was very high prevalence of MetSyn in Hispanic males (64.7%) and females (38.5%), and Black American females (38.5%) who were all part of the USA cohort. Surprisingly, despite reports of a higher metabolic syndrome prevalence in older Maori and Pacific Island adults[55], young Maori/Pacific Island adults had a relatively low prevalence of MetSyn (7.1%) in the current study.

The finding of a higher prevalence of MetSyn in the Hispanic and Black American cohorts in this multi-site, multi-ethnic cohort is an important finding, as all participants volunteered on the basis of being free from apparent disease. Therefore, when self-selected, apparently healthy young Hispanic male and females, and Black American females, have a higher prevalence of

MetSyn than their Caucasian, Asian, and Maori/Pacific Island counterparts. The exact reasons for the difference between ethnicities and countries are not discernible in this study and require further investigation.

A plausible explanation for these differences in prevalence could be that the pathophysiological process underpinning development of MetSyn may be ethnic specific. In the current study, the Hispanic group had an increased BMI, elevated TGs, low HDL-C, elevated FBG, elevated SBP and DBP but the Caucasian, Asian, and Māori/Pacific Islander groups did not exhibit the same characteristics. Whilst BMI was elevated in the Māori/Pacific Islander group, FBG and TGs were similar to the Caucasian and Asian groups. Conversely, Māori/Pacific Island people's low HDL-C prevalence was much higher and closer to the Hispanic and Black American groups. Furthermore, the reported prevalence of low HDL-C in the Māori/Pacific Island group supports the results reported by Gentles and colleagues[55] in 35-74 year old Māori/Pacific Island adults suggesting that low HDL might occur early in adulthood and persist into older adulthood. If future larger studies confirm these findings, a critical question could be whether different ethnicities and populations have different physiological origins of MetSyn.

An interesting finding in the Maori/Pacific Island group in relation to low HDL-C was found despite low numbers of participants in these ethnicities. A low HDL-C is hypothesised to occur due to the presence of high TGs. Indeed, in the Hispanic (HDL-C, 66.7%; TG, 56.7%) and Black American (HDL-C, 55.0%, TG, 50.0%) groups, the prevalence levels of these two factors were similar. In contrast, the Maori/Pacific Island group, the prevalence of HDL-C (71.4%) and TG (14.3%) were dissimilar and therefore were not supportive of this hypothesis. This finding may suggest that the current low HDL-C thresholds may not be appropriate for young Maori/Pacific Island adults. However, this finding needs to be confirmed in a larger and more diverse (i.e. separate Maori and Pacific Island sub groups) sample than the current study.

The ethnicity composition in the current sample is comparable to the larger USA and NZ populations. In the USA, the Hispanic ethnicity represents 16.3% of the population and Black Americans represent 12.6% of the USA population. In NZ, 69% of people identify as NZ European (Caucasian), 14.6% Māori, 9.2% Asian and 6.9% Pacific Islander. In the current study the ethnicity composition of the NZ sample is roughly similar although there is a smaller representation of Māori and Pacific Island participants. The proportion of ethnicities examined in the current study strengthens the generalizability of the study's findings and closely reflects the proportion of the population where the participants reside. Therefore, while MetSyn prevalence is higher in the current study than previous reports in young adults[84,92,93,108,119], it may be more representative of young adults in NZ and USA. Moreover, this result also suggests that Hispanic and Black American populations may have a disproportionately higher prevalence of MetSyn at a young age.

4.4.2 **Group Metabolic Health Indicators**

Metabolic health indicators were significantly different when MetSyn components were present. The PRESENT group had a significantly different hemodynamic, anthropometric, lipid, and blood glucose profile, compared to the AT RISK group. However, the AT RISK group also had a significantly different hemodynamic, anthropometric, lipid, and blood glucose profile compared to the ZERO group. This finding is not surprising given the definition of MetSyn and the clustering of components although the difference between the ZERO and AT RISK groups is interesting. This finding suggests that significant metabolic abnormalities may already be occurring as there are small differences in a broad range of metabolic health indicators when one or two MetSyn components are present. While the differences are small (e.g., BMI +1.9 $kg \cdot m^{-2}$, TGs + 0.22 $mmol \cdot L^{-1}$), over a lifetime it is possible these small differences may be important in terms of development of clinical disease. Further research into

the significance of small changes in multiple metabolic health parameters in young adults and risk of future clinical disease is required.

Previous reports indicate that the presence of just one MetSyn component increases the likelihood of developing MetSyn with advancing age with up to a 5-fold increase in incidence of MetSyn across a 40 to 50 year lifespan[113]. Therefore, it is likely that many of the AT RISK group will develop MetSyn in the future. To test this hypothesis, research focussing on interventions designed to prevent further decline or reverse MetSyn and MetSyn components in young adults who exhibit even one or two components of MetSyn will be required.

4.4.3 **Study Limitations**

The present study employed a cross-sectional design, therefore causality between the presence of MetSyn components and other metabolic health indicators cannot be determined. Moreover, this approach does not allow us to know the length of time in which the MetSyn components had been present. Second, measurements were taken at two different geographical sites with multiple researchers involved in the assessment of MetSyn components. As previously described, a video and written manual was supplied by the lead researcher to minimize differences in measurement technique. There were no significant differences in proportions of MetSyn components between females and males from the two Caucasian samples (Table 4.7) indicating similar group characteristics in the most comparable ethnicity from each site for both genders. Furthermore, the Colorado site is at an altitude of ~2,300 m which has been shown to influence insulin sensitivity[120], which may have consequently affected MetSyn component prevalence. Third, data are presented relating to the prevalence of MetSyn components on the basis of country and ethnicity. There were low numbers in some ethnic groups so some caution is advised regarding the interpretation of prevalence for each ethnicity. However, this interesting finding is appropriate to include due to the higher prevalence of MetSyn reported

in this study. Lastly, only students attending University were recruited and therefore the current findings may not be generalizable to all 18-24 year old adults. It is therefore possible that differences in the socioeconomic status of 18-24 year old adults attending University compared to age-similar young adults who do not attend could contribute to a different prevalence of MetSyn than those reported in the current study.

4.5. Conclusion

MetSyn and its components are prevalent in apparently healthy young adults. The prevalence of MetSyn differs between countries, ethnicities, while some MetSyn components appear to be more prevalent than others. Hispanic male and females, and Black American females had a higher prevalence of MetSyn and Hispanic participants had a significantly higher prevalence of all MetSyn components. Young adults with just one or two MetSyn components had a different BP, anthropometric, lipid, and blood glucose profile, in comparison to young adults with no MetSyn components. These metabolic health differences were larger when MetSyn was present. Further research is required to evaluate interventions designed to reverse and improve MetSyn components in young adults with one, two, or more components to improve and attain optimal metabolic health. Additionally, further research is required to determine if different pathophysiological processes underpin the development of MetSyn in different countries and ethnicities.

Chapter 5. Predictors of Metabolic Syndrome and Metabolic Syndrome Components in Young Adults

In Chapter Four, the prevalence of MetSyn and MetSyn components in young adults was presented. As part of the larger data collection, measures of CRF, PA levels in various domains of intensity, and weekly sitting time were assessed in all participants. In this chapter, the CRF, PA, sitting time, and BMI are examined in two multinomial logistic regression analyses to determine predictors of MetSyn components and MetSyn in young adults. A secondary "substitution" analysis was also performed to model the effect of "substituting" 30 minutes of sitting time for 30 minutes of either walking, moderate, or vigorous PA. Accordingly, the primary aim of this chapter is to provide important information regarding the major predictors of MetSyn and its components in young adults. A secondary purpose of this chapter is to model how the replacement of sitting time with PA of different intensity may result in a reduced prevalence of MetSyn in young adults.

5.1. Introduction

Obesity and physical inactivity are often cited as primary contributors to the development and progression of MetSyn[39]. Conversely, weight loss and increased PA are viewed as the primary interventions to help reverse MetSyn or prevent MetSyn incidence[31]. However, there are relatively few studies examining the predictors of MetSyn in young adults[109]. Previous research in young adults has shown a relationship between low CRF and low levels of PA with MetSyn[108]. The impact of sitting time has rarely been investigated in younger adults despite the relationship of increased sitting time and disease being established in older adults[115,121]. Of particular interest to this study is the independent relationship of PA, CRF, and sitting time with MetSyn and MetSyn components in young adults. Understanding the association of these

parameters with MetSyn in young adults will help guide the development of interventions and public health messages that may help to reduce the future incidence of MetSyn.

Therefore, the primary aim of this study was to determine the association of PA, BMI, CRF, and sitting time, with MetSyn and MetSyn components in young adults. A secondary analysis was performed to model how replacing 30 minutes of sitting with 30 minutes of PA would change the odds of having MetSyn or MetSyn components. This analysis was performed to highlight future research interventions that may be worth investigating in young adults.

5.2. Methods

5.2.1 **Participant Characteristics and Laboratory Measures**

Data were recorded from the same participants as described in Chapter Three (Section 4.2). In brief, using standardised procedures, MetSyn components[3] were assessed following an overnight fast. Participants were categorised as having no components (ZERO), one or two components (AT RISK), or three or more components that constitute MetSyn (PRESENT). In addition to the measures previously described (Chapter Four, Section 4.2.3), PA, sitting time, and CRF were also assessed in the analyses detailed below.

A one-way ANOVA was performed in the same manner as previously described (Chapter Four, Section 4.2.7) to determine if differences in group means existed in participant characteristics.

5.2.2 **Measurement of Physical Activity and Sitting time**

The short form international physical activity questionnaire (IPAQ) was administered to assess PA. The IPAQ asks four questions relating to how much time the participant has spent in three different PA domains (vigorous, moderate, walking) and sitting time over the preceding seven days. Data were processed according to standardised IPAQ protocols (www.ipaq.ki.se) and

participants were categorised into **LOW, MODERATE,** and **HIGH PA,** categories based on the following criteria:

LOW - Individuals who did not meet the criteria for **MODERATE** or **HIGH PA**

MODERATE – Any one of the following criteria:

- Completed three or more days of vigorous activity of at least 20 minutes per day
- Completed five or more days of moderate-intensity activity or walking of at least 30 minutes per day
- Completed 5 or more days of any combination of walking, moderate-intensity activity or vigorous intensity activities and achieved a minimum of at least 600 MET-min/week

HIGH – Any of the following criteria:

- Completed vigorous-intensity activity on at least three days and accumulating at least 1500 MET-minutes/week
- Completed seven or more days of any combination of walking, moderate-intensity or vigorous intensity activities and achieved a minimum of at least 3000 MET-minutes/week

As there are no established normative data for sitting time, sitting time data were partitioned into quartiles. Therefore, sitting quartiles were defined as the following:

- SIT quartile one: ≤ 4 hrs of sitting time per day
- SIT quartile two: 4 – 6 hrs of sitting time per day
- SIT quartile three: 6 – 8 hrs of sitting time per day
- SIT quartile four: ≥ 8 hrs of sitting time per day

5.2.3 **Measurement of Cardiorespiratory Fitness**

To assess CRF, participants performed the Queen's College Step Test (QCST)[122]. The QCST required participants to step up onto a 41.3 cm box for three minutes in time to a pre-determined cadence according to gender (96 steps per minutes for males, 88 steps per minute for females).

Within five seconds of completion of the three minutes of stepping, the participant's radial pulse was counted for 15 seconds while the participant stood still. The HR was used in one of the following gender-based equations to estimate maximal oxygen uptake (VO_2max):

Male = 111.33 – (0.42*HR)

Female = 65.81 – (0.1947*HR)

For the purpose of determining predictors of MetSyn and MetSyn components, CRF levels were characterized as either *below average* (male < 41.5 $mL \cdot kg^{-1} \cdot min^{-1}$, female < 34.1 $mL \cdot kg^{-1} \cdot min^{-1}$), *average* (male: 41.5 – 50.9 $mL \cdot kg^{-1} \cdot min^{-1}$, female 34.1 – 40.6 $mL \cdot kg^{-1} \cdot min^{-1}$) or *above average* (male ≥ 51.0 $mL \cdot kg^{-1} \cdot min^{-1}$, female ≥ 40.7 $mL \cdot kg^{-1} \cdot min^{-1}$) based on reported population norms for young adults[108].

To allow comparison of group mean CRF scores, each individual's calculated VO_2max value was referenced to the predicted VO_2max value based on age and gender using the following equations:

Male = (18.4-(0.16*age))*3.5 [123]

Female = (14.7-(0.13*age))*3.5 [124]

Therefore, the CRF values in Tables 5.1 and 5.2 are expressed as a percentage of predicted CRF (CRF %).

5.2.4 **Multinomial Logistic Regression**

A multinomial logistic regression analysis was performed to test the following hypotheses:

H1: gender, increased BMI, lower levels of PA category, lower CRF, and increased sitting time are associated with an increased odds ratio of having MetSyn components.

H2: gender, increased BMI, lower levels of PA category, lower CRF, and increased sitting time are associated with an increased odds ratio of having MetSyn.

For the purpose of the regression, the reference group for the dependent variable was the ZERO group. PA, sitting quartile, and gender were categorical variables. HIGH PA, SIT quartile four, above average CRF, and female gender were the reference categories for the associated independent variable. BMI was entered as a continuous variable.

5.2.5 **Secondary Analysis - Isotemporal Substitution Model**

To determine the effect of replacing 30 minutes of daily sitting time with 30 minutes of either daily vigorous, moderate, or walking PA, an isotemporal substitution model was created based on the technique described by Mekary et al.[125]. The isotemporal substitution models the odds ratio of being in the AT RISK or PRESENT group if 30 minutes of sitting time is replaced by 30 minutes of differing intensities of PA (walking, moderate, vigorous). This type of analysis could provide important information for the development of effective interventions for young adults with MetSyn or MetSyn components.

Therefore, multinomial regression was performed in a similar manner as previously described (*Section 5.2.4*). However, in this analysis, sitting time was removed as an independent variable, and the PA categories were recalculated into 30 minute units of time (e.g., 150 minutes became 5 units (150 ÷ 30)). In addition, total possible activity time (all PA categories plus sitting time) was added into the analysis. Gender, BMI, CRF were kept in the analysis to control for the effect of these variables on the dependent variable. By performing the analysis without sitting time but including total possible activity time, the PA categories represent the consequence of substituting 30 minutes of sitting time with that intensity of PA while controlling for the other independent variables. Therefore, any statistically significant result ($p<0.05$) for a particular PA category indicates that there is a significant change in the odds ratio of being AT RISK or

PRESENT if 30 minutes of sitting time is replaced with 30 minutes of that particular PA category.

All analyses were performed using SPSS Statistics 23 software (IBM Corporation, New York, USA) and figures were created using Prism software (GraphPad Software Inc., California, USA).

5.3. Results

5.3.1 **Participant Characteristics**

There were 156 participants in the ZERO group, 74 in the AT RISK group, and 38 in the PRESENT group. The cohort characteristics are presented in Table 5.1 and the group (ZERO, AT RISK, PRESENT) characteristics are presented in Table 5.2. Please note that there is replication from Chapter Four for these tables for ease of understanding and the provision of the pertinent data.

Table 5.1. Resting Heart Rate, Blood Pressure, Anthropometric, CRF, PA, Sitting Time, Lipid and Blood Glucose Profile

Age (yrs)	20 ± 1
RHR (bpm)	66 ± 10
SBP (mmHg)	119 ± 11
DBP (mmHg)	74 ± 9
BMI ($kg \cdot m^{-2}$)	23.7 ± 4.2
WC (cm)	80.0 ± 10.7
HDL-C ($mmol \cdot L^{-1}$)	1.40 ± 0.40
TG ($mmol \cdot L^{-1}$)	1.14 ± 0.64
FBG ($mmol \cdot L^{-1}$)	4.88 ± 0.49
LDL-C ($mmol \cdot L^{-1}$)	2.49 ± 0.68
Total ($mmol \cdot L^{-1}$)	4.41 ± 0.73
CRF (%)	93.7 ± 13.2
PA ($MET\text{-}min \cdot wk^{-1}$)	2324 ± 2062
Sit Time ($min \cdot wk^{-1}$)	2602 ± 1150

Data are presented as mean (± S.D).
RHR – Resting Heart Rate; SBP –Systolic Blood Pressure; DBP – Diastolic Blood Pressure; BMI – Body Mass Index; WC – Waist Circumference; HDL-C – High-Density Lipoprotein Cholesterol; TG - Triglycerides; LDL-C – Low Density Lipoprotein Cholesterol; Total – Total Cholesterol; FBG – Fasting Blood Glucose; CRF – Cardiorespiratory Fitness; PA – Physical Activity.

Table 5.2. Resting Heart Rate, Blood Pressure, Anthropometric, Cardiorespiratory Fitness, Physical Activity, Sitting Time, Lipid, and Blood Glucose Profile, based on Metabolic Syndrome Status

Variable	ZERO (n=156)	AT RISK (n=74)	PRESENT (n=38)
Age (yrs)	20 ± 1	20 ± 1	21 ± 1
RHR (bpm)	63 ± 9	66 ± 10	75 ± 7*†
SBP (mmHg)	114 ± 8	123 ± 11*	134 ± 6*†
DBP (mmHg)	70 ± 6	75 ± 9*	85 ± 5*†
BMI ($kg \cdot m^{-2}$)	22.02 ± 2.8	23.9 ± 3.6*	30.0 ± 4.1*†
WC (cm)	76.3 ± 8.4	79.3 ± 8.5*	96.5 ± 7.4*†
HDL-C ($mmol \cdot L^{-1}$)	1.61 ± 0.33	1.16 ± 0.34*	1.00 ± 0.15*†
TG ($mmol \cdot L^{-1}$)	0.89 ± 0.32	1.11 ± 0.51	2.21 ± 0.78*†
FBG ($mmol \cdot L^{-1}$)	4.69 ± 0.34	4.93 ± 0.42*	5.59 ± 0.44*†
LDL-C ($mmol \cdot L^{-1}$)	2.35 ± 0.64	2.52 ± 0.64	3.03 ± 0.64*†
TOTAL ($mmol \cdot L^{-1}$)	4.36 ± 0.71	4.19 ± 0.67	5.02 ± 0.57*†
CRF (%)	96.9 ± 11.3	93.8 ± 13.7	80.5 ± 11.2*†
PA ($MET\text{-}min \cdot wk^{-1}$)	2447 ± 1689	2961 ± 2630	582 ± 1047*†
Sit Time ($min \cdot wk^{-1}$)	2267 ± 1119	3068 ± 1166*	3075 ± 692*

Data are presented as mean ± S.D.
ZERO – No MetSyn Component; AT RISK – One or two MetSyn components; PRESENT – MetSyn. RHR – Resting Heart Rate; SBP –Systolic Blood Pressure; DBP – Diastolic Blood Pressure; BMI – Body Mass Index; WC – Waist Circumference; HDL-C – High-Density Lipoprotein Cholesterol; TG - Triglycerides; LDL-C – Low Density Lipoprotein Cholesterol; Total – Total Cholesterol; FBG – Fasting Blood Glucose; CRF – Cardiorespiratory Fitness; PA – Physical Activity.
***p<0.05 compared to ZERO**
†p<0.05 compared to ZERO

5.3.2 **Multinomial Regression Model**

For every 1 $kg \cdot m^{-2}$ increase in BMI, there was a 1.2-fold increase in the odds of being in the AT RISK (p=0.003) group and a 2.2-fold increase in the odds of being in the PRESENT group (p<0.001). Figure 5.1 displays the odds ratio of being in the AT RISK or PRESENT group for all categorical predictors. MODERATE PA (p=0.012) but not LOW PA (p=0.845) was associated with a significant reduction in the odds of being in the AT RISK group compared to HIGH PA. However, LOW PA (p=0.001) but not MODERATE PA (p=0.603) was associated with an increase in the odds of being in the PRESENT group. The lowest SIT quartile was associated with lower odds of being in the AT RISK (p<0.0005) group but not the PRESENT (p=0.969) group. Males had significantly reduced odds of being in the PRESENT (p=0.032) group compared to females. Average CRF was associated with increased odds of being in the AT RISK (p=0.031) but not the PRESENT (p=0.749) group. Below average CRF was associated with increased odds of being in the PRESENT (odds ratio 9.921, p=0.070) group and the AT RISK (odds ratio 2.903, p=0.104) group although it did not reach significance. Please note that the odds ratios are presented relative to the ZERO group (Table 5.3).

5.3.3 **Isotemporal Substitution Analysis Model**

Isotemporal substitution analysis suggests that there was no significant effect of reducing or increasing the odds of having one or two MetSyn components (AT RISK) when substituting 30 minutes of sitting time with the equivalent amount of time of any intensity of PA (walking PA p=0.672; moderate PA p=0.631; vigorous PA p=0.191). However, there was a significant reduction in the odds of being in the PRESENT group if 30 minutes of sitting time is replaced with either walking PA (p=0.048) or moderate intensity PA (p=0.019). There was no significant effect of substituting 30 minutes of sitting time with vigorous PA (p=0.319) (Table 5.4).

Table 5.3. Odds Ratio of Being in AT RISK or PRESENT Group Based on Gender, Body Mass Index, Cardiorespiratory Fitness, Physical Activity Category, and SIT quartile

	β	S.E	Wald	df	p value	Odds Ratio	95% C.I.	
							Lower	Upper
AT RISK								
Male Gender	0.145	0.368	0.155	1	0.693	1.156	0.562	2.377
BMI	0.168	0.056	9.027	1	0.003	1.183	1.060	1.319
CRF Below Average	1.066	0.656	2.640	1	0.104	2.903	0.803	10.497
CRF Average	0.820	0.380	4.660	1	0.031	2.270	1.078	4.778
Low PA	0.121	0.618	0.038	1	0.845	1.128	0.336	3.791
Mod PA	-0.868	0.346	6.281	1	0.012	0.420	0.213	0.828
SIT Quart 1	-2.094	0.531	15.545	1	<0.001	0.123	0.044	0.349
SIT Quart 2	-0.537	0.430	1.558	1	0.212	0.584	0.251	1.358
SIT Quart 3	-0.643	0.457	1.977	1	0.160	0.526	0.215	1.288
PRESENT								
Male Gender	-2.508	1.167	4.616	1	0.032	0.081	0.008	0.802
BMI	0.732	0.153	22.828	1	<0.001	2.079	1.540	2.808
CRF Below Average	2.295	1.266	3.287	1	0.070	9.921	0.830	118.555
CRF Average	0.400	1.253	0.102	1	0.749	1.492	0.128	17.401
Low PA	3.472	1.062	10.682	1	0.001	32.197	4.014	258.241
Mod PA	-0.538	1.033	0.271	1	0.603	0.584	0.077	4.426
SIT Quart 1	0.059	1.533	0.001	1	0.969	1.061	0.053	21.424
SIT Quart 2	-0.108	1.200	0.008	1	0.928	0.898	0.085	9.426
SIT Quart 3	1.765	1.123	2.471	1	0.116	5.839	0.647	52.712

AT RISK – One or two MetSyn components; PRESENT – MetSyn.
BMI – Body Mass Index; CRF – Cardiorespiratory Fitness; PA – Physical Activity; SIT – Sitting Time
β – Beta Co-efficient, S.E – Standard error

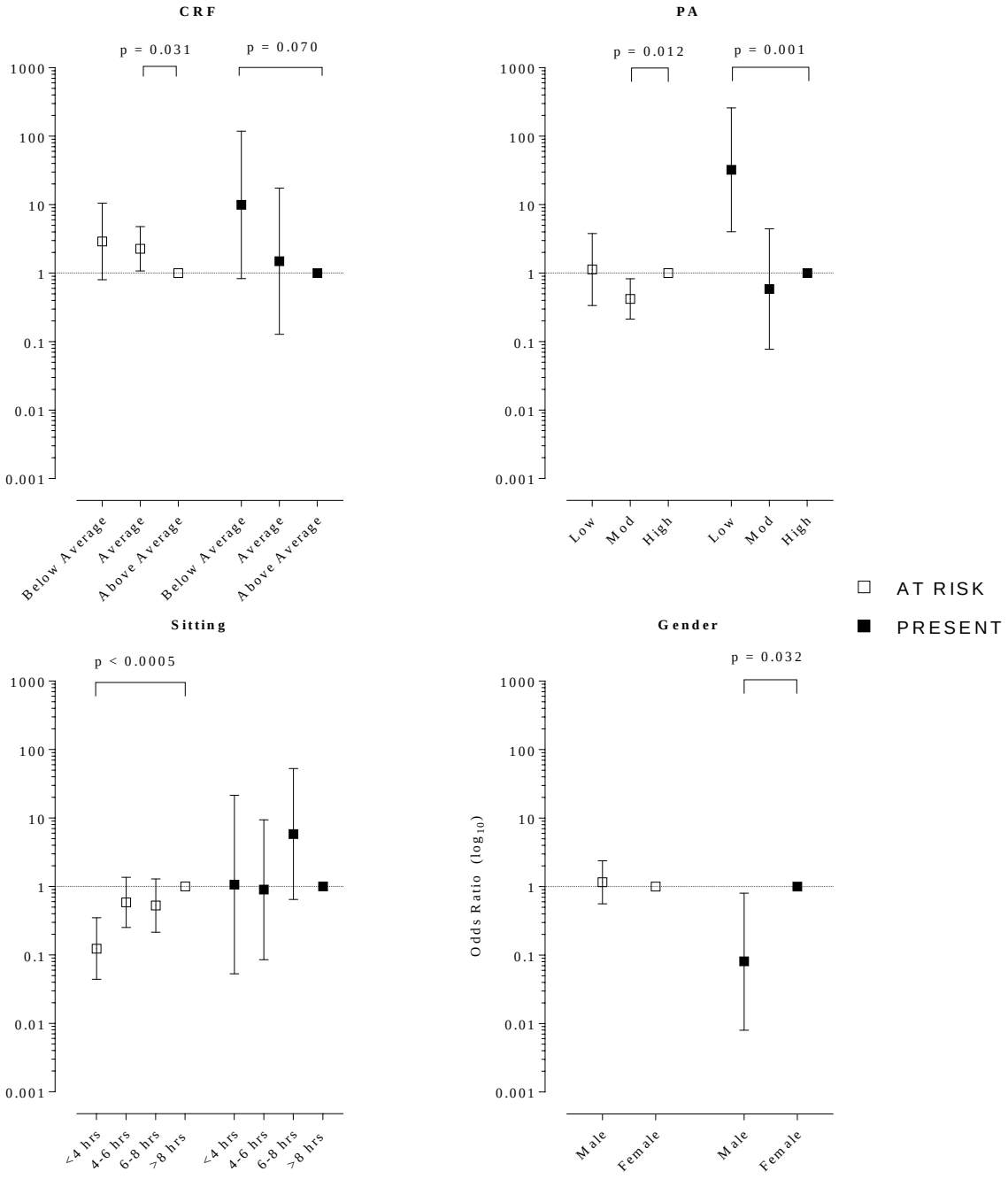


Figure 5.1. Odds Ratio (± 95% C.I.) of Metabolic Syndrome (PRESENT), and Metabolic Syndrome Components (AT RISK) in Young Adults

AT RISK – One or two MetSyn components; PRESENT – MetSyn.
CRF – Cardiorespiratory Fitness; PA – Physical Activity

Table 5.4. Isotemporal Substitution of Sitting time with 30 minutes/day increase in either Walking, Moderate, or Vigorous Physical Activity, and AT RISK or PRESENT status

	Walking PA		Moderate PA		Vigorous PA	
	Odds Ratio	95% C.I.	Odds Ratio	95% C.I.	Odds Ratio	95% C.I.
AT RISK	1.053	0.829 – 1.337	1.101	0.744 – 1.629	1.264	0.890 – 1.795
PRESENT	0.425	0.182 – 0.993*	0.043	0.003 – 0.590**	0.502	0.129 – 1.947

ZERO – No MetSyn Component; AT RISK – One or two MetSyn components; PRESENT – MetSyn.
*** p=0.048**
****p=0.019**

5.4. Discussion

The novel and key findings are that sitting less than four hours per day, being moderately active, and having above average CRF is associated with a reduction in the odds of MetSyn components. Conversely, an increased BMI is associated with increased odds of MetSyn or MetSyn components. Low levels of PA were also associated with increased odds of MetSyn. Additionally, people with below average CRF had increased odds (9.921) of having MetSyn although this did not reach statistical significance (p=0.07). Being a male was also associated with lower odds of MetSyn but not MetSyn components.

The following section details each of the variables entered into the regression analysis and discusses the results presented here with current research that reported the variable (e.g., BMI), MetSyn and MetSyn components, and young adults.

5.4.1 **Increased BMI is Associated with Increased Odds of Metabolic Syndrome and Metabolic Syndrome Components**

An increase of 1 $kg \cdot m^{-2}$ in BMI was associated with a 1.2-fold increase in the odds of having one or two components of MetSyn and a 2.2-fold increase in the odds of having MetSyn. This is unsurprising due to the influence that central obesity (and therefore increasing BMI) has on

the development of MetSyn[43]. These current findings are consistent with previous examinations in South Korean adults (43 ± 15-16 years)[126], and USA adults (≥20 years of age)[39] that showed an increased MetSyn prevalence with an increased BMI albeit in older aged populations than the current study. Based on the current findings, BMI also appears to be a significant predictor of MetSyn development in young adults.

The association between BMI and MetSyn in young adults is an important finding as approximately 40% of US adults (20-39 years) are overweight[127] and a further 20% of males and 16% of females are considered obese[6]. The prevalence of an increased BMI is very similar in NZ with 49% of young NZ adults (18-24 years) currently overweight or obese[128]. Therefore, many young adults in the USA and NZ have a significant predictor for the development of MetSyn.

Only 16.3% of young adults (18-30 years) either decrease or maintain a stable BMI over 15 years compared to 73.9% who have an increase of greater than 2 $kg \cdot m^{-2}$ [76]. Furthermore, there is a median success rate of 15% for weight loss maintenance or further weight reduction in studies that followed participants for more than three years with a tendency for improved rates of success in people who were actively followed up or had group therapy as part of the weight loss intervention[129]. Furthermore, a prospective study in nearly 5000 men (n=2406) and women (n=2569) aged 18-74 demonstrated that a 2.25 kg increase in total body mass over 16 years was associated with a 20% increase in risk factor sum (i.e., combined clustering of risk factors)in men and 37% in women[130]. Perhaps more intriguing is that the same study showed that a 2.25 kg weight loss over a 16 year period was associated with greater reductions in risk factor sum (male: - 48%; female - 40%)[130]. Therefore, efforts to attenuate or prevent the associated weight increase with increasing age represents an excellent opportunity to decrease the future incidence of MetSyn.

5.4.2 **Different Levels of PA have Different Relationships with the Odds of Having Metabolic Syndrome**

Moderate PA was significantly associated with 2.4-fold lower odds of having one or two MetSyn components compared to high PA. However, moderate PA was not associated with a decrease or increase in the odds of having MetSyn. The relationship of low PA and MetSyn components and MetSyn was different. Low PA was not significantly associated with having one or two MetSyn components but was associated with a 32-fold increase in the odds of having MetSyn compared to high PA.

Two studies investigating PA in young adults reported findings similar to those observed in the current study. Lopez-Martinez et al.[131] reported in 275 Spanish University students (20 ± 4 years) that weekly participation in 20 minutes of vigorous exercise was associated with a lower risk of MetSyn. In the current study, both moderate and high levels of PA included 20 minutes of vigorous activity on 3 or more days per week. Furthermore, only the low PA category had a significant increase in the odds of having MetSyn. Thus, the current findings support those of Lopez-Martinez's in a similarly aged population albeit in different countries.

A negative association with higher intensities and total mean volume (MET-hours) of PA and MetSyn prevalence in 24 year old adults has been previously reported by Salonen et al.[121]. In addition to the increased odds ratio in people reporting low PA, both the ZERO and AT RISK groups have significantly higher MET-min/wk values than the PRESENT group ($p<0.05$) (Table 2). Therefore, the current study also supports the findings of Salonen et al., and strengthens the evidence regarding the association of PA and MetSyn prevalence specifically in young adults. Together, increasing the intensity and overall duration of PA is associated with lower prevalence of MetSyn in young adults.

5.4.3 **Young Adults that Sit Less have Lower Odds of Metabolic Syndrome Components**

Sitting less than four hours per day was associated with an 8-fold reduction in the odds of having MetSyn components but not MetSyn. The current finding conflicts with a recent meta-analysis of over 21,000 adults (≥18 years) that showed increased sedentary behaviour time increased the odds of MetSyn by 73% (Odds Ratio = 1.73)[64]. The differences reported in the meta-analysis and the current study may be due to different sedentary behaviour outcomes analysed (sitting time, TV watching time, sedentary time etc.), different data collection methods, and different age groups studied.

The current study collected sitting time via the short form IPAQ. Previous research comparing sitting time using the IPAQ and sitting time recorded via an accelerometer (activPAL) showed a poor association between the two measures (intraclass correlations = 0.112-0.275) with the IPAQ tending to overestimate sitting time[132]. Therefore, it is possible that the use of the IPAQ to record sitting time provided only a rough estimate of sitting time and was prone to recall issues affecting the accuracy of the recorded data.

Furthermore, data in the current study were divided into quartiles of sitting due to a lack of known threshold levels for sitting time. It is plausible that the use of quartiles in the current study combined with the poor criterion validity of the IPAQ may have contributed to a reduced ability to detect true differences in sitting time between participants. It is acknowledged that the standard deviations for reported sitting time in the current study are large.

Although there are limitations in the current study, the finding that a low sitting time reduced the odds of having MetSyn components but not MetSyn warrants investigation. One intriguing possibility for this finding is that young adults may require further years of prolonged sitting time to develop three or more components of MetSyn. Accordingly, if sitting time remains high as young adults' age, the odds ratio of having MetSyn may increase. Another possibility is that

low sitting time only partially protects from MetSyn. Consequently, while a reduced sitting time may result in reduced odds in the initial development of MetSyn components in young adults, reducing sitting time is only partially effective in reducing MetSyn. Further unhealthy lifestyle behaviours such as low PA levels leading to decreased CRF and weight gain may be required for the development of MetSyn.

One more issue regarding sitting time is worth exploring. Sitting time pattern was not assessed in the current study. Previous research has reported increased interruptions of sedentary time independent of total sedentary time and moderate-vigorous activity is associated with improved WC, TGs, and FBG in older adults[133]. Unfortunately, the current study provides no data to examine this concept as participants were asked to report total sitting time only. Further research investigating differences in sitting patterns, interaction of sitting and PA, and MetSyn would be an interesting area of research to explore as it may not be primarily the total sedentary time, but the distribution of sedentary time that increases or reduces the odds of having MetSyn in young adults.

5.4.4 **Lower CRF is Associated with Increased Odds of Metabolic Syndrome**

Average CRF was associated with a 2.3-fold increase in the odds of having one or two MetSyn components compared to values that are above average CRF. Below average CRF had a 9.9-fold increase in the odds of having MetSyn compared to above average CRF, although this result did not reach statistical significance (p=0.07). The finding of lower levels of CRF being associated with increased odds of MetSyn has previously been demonstrated in University students in the USA[108]. Furthermore, in a longitudinal study over 23 years from 13 years of age, people who developed MetSyn had a marked decrease in CRF that was evident from the age of 18 onwards compared to young adults who maintained their CRF[134]. Therefore, young adults with lower levels of CRF appear to be at increased risk of developing MetSyn. Increasing

CRF may be a pertinent target for preventing MetSyn and is achievable with modest increases in PA. However, further research is still required to delineate the specific exercise dose, exercise pattern, mode, and intensity to prevent MetSyn development.

5.4.5 **Young Males Have Decreased Odds of Metabolic Syndrome**

Surprisingly, males had lower odds of having MetSyn than females in the current study. This finding conflicts with Sumner et al., who used NHANES data (n=41,474) and showed that males under the age of 50 had a higher prevalence of MetSyn than females[115]. However, in the same study, the prevalence of MetSyn in people 18-29 years specifically, was 8.0% in males and 5.3% in females representing only a small difference in prevalence of MetSyn between sexes in young adults (i.e., a 2.7% difference). Differences in sample size, MetSyn definition, ethnicities included, and sampling criteria, may explain the difference in findings between Sumner et al. and the current study.

Key questions regarding gender and MetSyn remain. Ervin show that MetSyn component prevalence is different in young adults of different genders[10]. With increasing age, females have an increased prevalence of all components of MetSyn whereas males only have an increase in the prevalence of hypertension and hyperglycaemia. Given that MetSyn could be made up of sixteen different combinations of components (e.g., HDL-C, TGs, WC; HDL-C, TGs, FBG etc.), and the prevalence of different components is different between males and females, it may be that there is a difference in aetiology of MetSyn in males and females. If this contention is correct, a key question could be: do MetSyn components respond differently to interventions in males and females?

5.4.6 **Substituting 30 minutes of Sitting time with 30 minutes of Walking or Moderate PA Reduces the Odds of having Metabolic Syndrome**

The secondary analysis modelled the "substitution" of 30 minutes of sitting behaviour for either walking PA, moderate PA, or vigorous PA, and highlighted that replacing 30 minutes of sitting time each day with 30 minutes of walking PA or moderate PA, significantly reduced the odds of having MetSyn. This finding supports the minimum recommended guidelines of performing moderate PA (including brisk walking) for 30 minutes on most if not all days of the week for improvements in general health[135]. Therefore, the key concept demonstrated in the current findings is that replacing sitting time with moderate or walking activity will reduce the odds of MetSyn in young adults.

5.4.7 **Limitations**

A few limitations exist in this study and are important to acknowledge. First, PA, sitting time, and CRF were estimated albeit using validated techniques. Whilst accelerometry and maximal graded exercise test are the gold standard techniques for the measurement of these variables, accelerometry and a maximal graded exercise test were not feasible to conduct due to a combination of resourcing, funding, and cognisance of participant and researcher time. However, the IPAQ was chosen as it has been recommended for national monitoring of PA[136], and the QCST was chosen as it has been shown to be a valid assessment of CRF in young adults[122]. In addition, participants from Colorado performed the QCST at altitude that may have had an effect on CRF in these participants. However, all participants in Colorado were habitually residing at altitude and therefore any effects of altitude on the CRF value are likely to be minimal.

Lastly, University students were exclusively recruited. Thus, the results may not be applicable to a broader range of young adults in different occupations or lifestyles. However, as detailed

in Chapter Four, Section 4.4.2, the inclusion of two countries and multiple ethnicities that are approximately representative of both the source populations helps to strengthen the generalizability of the current findings. Therefore, this study is unique in that it is one of the only studies on young adults where data have been collected in two countries.

5.5. Summary

In summary, the current findings indicate that simple, easy to implement lifestyle practices may have tangible benefits in preventing MetSyn components and MetSyn. Moderate PA, above average CRF and sitting less than four hours per day are associated with lower odds of having MetSyn components. Moreover, being female, a low level of PA, and below average CRF is associated with increased odds of having MetSyn. Every $1kg \cdot m^{-2}$ was associated with a significant increase in the odds of having either MetSyn components or MetSyn indicating a significant association of weight and MetSyn. Lastly, replacing 30 minutes of sitting time with 30 minutes of walking or moderate PA may be effective in reducing the odds of having MetSyn. This is an important and feasible public health message to promote to young adults.

Chapter 6. Low HDL-C and Skin Blood Flow Response to Iontophoresis

In the first study described in Chapter Four, approximately 30% of all participants had low HDL-C as defined by the harmonized definition of MetSyn[3]. Similarly, the pooled analysis investigating MetSyn and MetSyn component prevalence in young adults (Chapter Three) also identified low HDL-C as the most prevalent component (27 – 41%) of MetSyn in young adults (18 -30 years). Clearly, low HDL-C is a key component of MetSyn in the young adult population.

The relationship between low HDL-C and *future* CVD risk is well established in that those who have low HDL-C also have increased rates of CVD[137,138]. The relationship between low HDL-C and *current* CVD "status" is not known. However, since CVD development occurs along a continuum with the CVD process beginning early in life, it seems likely that those with low HDL-C will also have other early signs of CVD. One of the earliest detectable signs of CVD development is endothelial dysfunction[139] that may manifest as a reduction in vasodilatory capacity within the microvasculature (i.e., microvascular dysfunction). Therefore, based on the finding that many young adults have low HDL-C (i.e., the Chapter 3 pooled analysis outcome and the prevalence study result described in Chapter 4) and that low HDL-C is associated with increased CVD risk coupled with the fact that endothelial cell dysfunction often precedes overt CVD symptoms, the primary purpose of the experiments described in this chapter were to assess the impact of low HDL-C on microvascular reactivity in young adults.

6.1. Background

A large number of apparently healthy young adults have low HDL-C which is one of several components of MetSyn. Given there is a well-established inverse relationship between HDL-C levels and CVD risk[138], the high prevalence of low HDL-C in young adults is of significant concern. Furthermore, previous research suggests that evidence of early CVD is present in the large blood vessels of young adults[140]. Autopsy studies indicate raised fatty streaks are present in the aorta of 20% of adults 15-19 years old, and approximately 40% of people 30-34 years old[15]. Moreover, the amount of intimal surface involved with the raised fatty streaks was associated with low levels of HDL-C, increased age, high levels of LDL-C and TGs, hypertension, obesity, and impaired glucose tolerance[15]. Therefore, a large number of young adults develop lesions that narrow the lumen of conduit arteries even though overt CVD symptoms (e.g., ischemic pain) are not yet present. Detection of fatty streak development in the arterial lumen would identify those with low HDL-C who are at risk for developing symptomatic CVD; however, it is only possible to locate these lesions post-mortem.

Microvascular endothelial dysfunction described as an "…attenuated endothelium-dependent vasodilation, augmented vasoconstriction, and micro vessel structural remodelling that occurs simultaneously in multiple vascular beds" (pg. 370, Holowatz et al.[139]), is hypothesised to preclude development of atherosclerotic plaque formation[141]. Since HDL-C is known to affect normal endothelial cell function[142], it is possible that low HDL-C contributes to impaired endothelial cell signalling and ultimately microvascular dysfunction. Thus, identifying low-HDL-C mediated endothelial cell dysfunction in the microvasculature would provide a functional correlate to a prevalent MetSyn component and underscore its importance in the overall development of CVD[139].

Endothelial dysfunction appears to be a systemic process and occurs in multiple vascular beds simultaneously[141]. Endothelial-mediated microvascular reactivity has been shown to be reduced in people with T2DM[143], MetSyn[144], hypertension[145], obesity[146], and hypercholesterolemia[141]. A cause and effect relationship showing that low levels of HDL-C result in reduced endothelial-mediated microvascular vasodilation has not been established.

The best evidence to date of a relationship between HDL-C level and endothelial function comes from a study showing that endothelium-dependent vasodilation with higher levels of HDL-C in young and middle aged women[147]. In that study, a small cohort of men (n=13) and women (n=21) had microvascular skin vessel reactivity to ACh, sodium nitroprusside (SNP), and isoprenaline assessed by laser Doppler perfusion imaging. The skin vessel reactivity was analysed in the context of serum lipids and lipoproteins. The results of that study suggested that it was primarily HDL-C that influenced skin vessel reactivity in healthy women and that HDL-C may also affect function of the vascular smooth muscle as well as the endothelium. No relationship was found in males although the authors suggested that this may be due to the small number of male participants in that study (male n = 13). However, significant questions still remain on what role, if any, HDL-C has on endothelial function.

6.1.1 **HDL-C, Oxidative Stress and Endothelial Dysfunction**

The endothelium covers the luminal surface of the arteries, arterioles, venules, and veins. In the smallest vessels (i.e., capillaries) the entire vessel is composed of a single layer of endothelial cells. In addition, a specialized group of endothelial cells line all four chambers of the heart. The endothelium produces and releases several vasoactive compounds in response to extracellular signalling molecules (i.e., bradykinin) and physical stimuli (i.e., shear stress)[148]. The vasoactive compounds alter vascular smooth muscle contractility that in turn alters luminal diameter and blood flow. This regulatory control of blood flow is altered by endothelial

dysfunction ultimately contributing to a loss of tissue function (i.e., loss of contractility and or ischemic pain in the myocardium).

Endothelial dysfunction may occur for a variety of reasons. A general model for development of endothelial dysfunction is centred on an increase in reactive oxygen species (ROS) development without a concomitant increase in anti-oxidant production. ROS are normal by-products of aerobic metabolism, and also produced for cellular communication[149]. For example, NO is produced by endothelial cells in blood vessels that promotes relaxation of arterial smooth muscle. In normal physiological conditions ROS are neutralized by anti-oxidant compounds but when there is a shift towards increased ROS development (e.g., low HDL-C) without a concomitant increase in anti-oxidant production, oxidative stress develops. ROS molecules by nature are highly reactive with other molecules. In the endothelium, a ROS such as super oxide (O_2^-) which can arise from oxidative metabolism can combine with NO. This "quenches" or removes NO diminishing its biological effect. Unfortunately when O_2 and NO combine they form peroxynitrite that penetrates cell membranes causing significant cell damage and endothelial inflammation further reducing endothelial function[14,150]. The result is an increase in adhesion molecules, growth factors, inflammation, and oxidant activity in the endothelium, which promotes the development of CVD.

HDL may play a role in attenuating and maintaining the balance between production of ROS and antioxidants. HDL functions as an anti-oxidant due to the presence of paraoxanase isomers that are bound to the apo-lipoproteins A-1 and J on the HDL molecule[151]. Furthermore, HDL may bind transition metals and thereby limit the oxidative properties of these compounds[14]. HDL also has other vascular protective effects and binds to toxic phospholipids (lipids generated by oxidation of LDL-C) that helps protect vascular smooth muscle[151]. HDL also attenuates the reduced vasodilation capacity associated with CVD and exposure to oxidized

LDL-C[151]. Therefore, people with low HDL-C (i.e., the HDL component of MetSyn) may have a reduction of many of HDL's vascular protective properties leading to an increased oxidative state that contributes to endothelial dysfunction and altered microvascular functioning.

The role of HDL-C in mitigating development and or the action of ROS within the endothelium provides a reasonable foundation on which to develop a model where low HDL-C in young adults could precipitate microvascular dysfunction in the microvasculature before development of over symptoms of CVD. If low HDL-C failed to control the production and action of ROS, it would be expected that the endothelium would be less capable of producing NO, or that the NO produced would be less effective. This would impair vasodilation within various microvascular beds which can be measured and quantified to determine how much function has been lost. Therefore, the current study investigated microvascular reactivity in young adults with and without the low HDL-C component of MetSyn. The primary hypothesis is that young adults with the low HDL-C component will have reduced endothelial-mediated microvascular reactivity.

6.2. Methods

Participants reported to the laboratory (maintained between 22.8 ±1.4°C) in the morning after an overnight fast. Participants were instructed to avoid alcohol the night prior, and refrain from heavy exertion 48 hours prior to testing. Participants were investigated for MetSyn using the procedures detailed in Chapter Four, section 4.2.3. Participants were considered to have low HDL-C if HDL-C $\leq$ 1.29 mmol·L^{-1} for females or $\leq$ 1.03 mmol·L^{-1} for males as per the harmonized guidelines for identifying MetSyn[3].

The protocol for this study was approved by the University of Auckland Human Participants Ethics Committee (protocol number: 016500). Written informed consent was obtained from all participants after being provided a participant information sheet (Appendix D) and afforded

the opportunity to ask and receive information regarding any relevant questions regarding involvement in the study.

6.2.1 **Assessment of Skin Blood Flow**

Skin is composed of the epidermis, dermis, and subcutaneous layers that are supported by a microvascular network that can be imaged using minimally invasive techniques such as laser Doppler flowmetry (LDF). The skin microvasculature is increasingly being viewed as a suitable surrogate for investigating the mechanisms of microvascular dysfunction present in other vital tissues such as the myocardium[139]. The epidermis is avascular whilst the dermis is supplied by microvascular blood vessels (diameter < 200μm) comprised of arterioles, capillaries, and venules ranging in diameter from 10 to 35 μm[148]. SkBF is tightly regulated and largely determined by the input of neural mechanisms with mediation from local humoral factors. Vasoactive compounds that promote vasodilation (e.g., NO) or vasoconstriction (e.g., endothelin) are released by the endothelium and interact with membrane receptors, channel proteins, protein carrier molecules, or penetrate into vascular smooth muscle. When the balance between the vasodilating and vasoconstricting compounds is tilted towards vasodilation, an increase in the luminal diameter of the blood vessel occurs[149]. Therefore, SkBF is the result of the competing contributions of vasoconstriction and vasodilation signals. LDF is a commonly used procedure for the direct assessment of SkBF in conjunction with iontophoresis to assess microvascular endothelial function[148].

6.2.2 **Laser Doppler Flowmetry**

In the current study, SkBF was assessed by LDF. LDF is based on the principle of the Doppler shift that occurs when light is reflected off moving objects[148]. A probe composed of an emitting probe and a receiving probe is attached to the skin. A beam of laser light with a known wavelength is emitted directly into the skin and the light is dispersed through the underlying

tissue. A portion of this light is reflected back to the receiving probe. If the reflected light has interacted with tissue that is stationary, the wave length of the light does not change. However, if the reflected light has interacted with a moving structure (i.e., a red blood cell), the wave length will shift (Doppler shift). The amount and wave length of reflected light measured at the probe reflects the number and velocity of blood cells in the volume of skin tissue illuminated directly beneath the probe. A proprietary algorithm is used to calculate a measure of SkBF from this information. The calculated unit is called a "perfusion unit (PU)". A PU is not a true measure of flow (i.e., a unit of volume per unit of time) but it has been shown to change in proportion to measured blood flow[152]. The absolute blood flow underneath the probe is not able to be determined via LDF. Rather, the flux signal attained by LDF is proportional to the blood flow and it is common to provide some form of provocation (e.g., hyperaemic, thermoregulatory, pharmaceutical) to assess changes in SkBF with LDF. LDF typically measures SkBF to a depth of 1 – 1.5 mm from the epidermis[153] which is likely to represent the blood flow in the sub-papillary arterial and venous plexuses[148].

6.2.3 **Iontophoresis**

In the current study, SkBF changes were induced by iontophoresis of ACh and SNP. Iontophoresis is a technique that uses electro-repulsion to "push" similar-charged particles into the skin directly beneath the drug delivery electrode. A simple electrical circuit is created on the skin using two electrodes (e.g., with one acting as a drug reservoir) and a DC power source. In solution ACh dissociates into a positive acetylcholine and negative chlorine ion species. When current is applied to the circuit it will travel from the cathode (negative charge) to the anode (positive charge). By designating the drug delivery electrode containing ACh as the anode it will attract negatively charged ions and repel positive charged ions. Since ACh is a positively charged ion, it is repelled by the anode creating the force that facilitates penetration of ACh into the skin. Iontophoresis allows controlled drug delivery to a very small section of

skin (1 mm^3) which minimizes systemic drug exposure and non-target effects that could confound measurement of SkBF.

ACh is commonly used in iontophoresis and is hypothesised to act primarily on the endothelium when used in physiologic concentrations to promote vasodilation primarily through cyclooxygenase (COX), NO, prostaglandin, and endothelium-derived hyperpolarizing factor (EDHF) dependent pathways[154]. Iontophoresis of ACh allows for evaluation of endothelial function in the skin[155] because of the endothelial-dependent nature of ACh action[156]. It should be noted that this method does not allow delineation of the individual pathways (i.e., COX, NO, prostaglandin, EDHF).

A solution of SNP will spontaneously degrade and release NO[157]. SNP iontophoresis is undertaken to provide an endothelial cell independent source of NO to measure the responsiveness of the vascular smooth muscle to NO. The maximum vasodilatory response to SNP is considered the maximum vasodilation attainable for the area being measured[157]. Thus, SNP is commonly used to assess the total vasodilatory capacity of the vascular bed being investigated.

6.2.4 **LDF and Iontophoresis Protocol**

Resting SkBF and the change in SkBF in response to two iontophoresis challenges were measured via LDF. Participants removed shoes and socks and lay supine on a comfortable bed. The plantar surface of both feet were inspected for abrasion, calluses and cuts before potential electrode sites were cleaned, lightly abraded, and swabbed lightly with alcohol. The plantar surface of the foot was used because it is covered with glabrous skin (i.e., non-hairy) that has a dense dermal microvasculature that when interrogated with laser Doppler produces a robust signal for clear measurement. One drug delivery electrode (PF 383, Perimed AB, Stockholm, Sweden) was attached to the plantar surface of each foot, posterior to the 1st and 2nd metatarsal

heads. Before application of the electrodes, the drug delivery reservoir was filled with 150 µL of a 1% ACh or SNP solution. Both compounds were dissolved in a solution of 0.9% sodium chloride. A sodium chloride solution is a more effective solvent than deionized water because it produces fewer non-specific vasodilatory effects[156]. Each foot was treated with only one drug. The electrodes were connected to a thermostatic probe (PF 481) that enables temperature control of the skin electrode surface. The skin electrode surface was set to 33°C. The probes were connected to the LDF unit that emits a laser wavelength of 780nm sampling at 32Hz (Periflux 5000, Perimed AB, Stockholm, Sweden).

The Periflux 5000 system accuracy was verified weekly using a liquid motility solution (PF1001) and re-calibrated if necessary according to manufacturer's instructions (PF1000 Calibration Device, Perimed AB, Stockholm, Sweden).

To reduce any confounding effects of sympathetic nervous system activity on SkBF, room lights were dimmed and participants were discouraged from any activities that may promote increases in SkBF (e.g., animated conversation, reading social media etc.). After a period of no less than ten minutes of continuous measurement of supine rest to determine resting SkBF, BP was measured using an automated sphygmomanometer (Lifebrand, Toronto).

6.2.5 **Iontophoresis**

To minimize potential non-specific vasodilatory responses in SkBF, iontophoresis of ACh or SNP was delivered via discontinuous doses of electrical current interspersed with a rest period of one minute. The details of each protocol including charge density and current density for each pulse are outlined in Table 6.1. An image of the LDF and iontophoresis setup on one foot is provided as Figure 6.1.

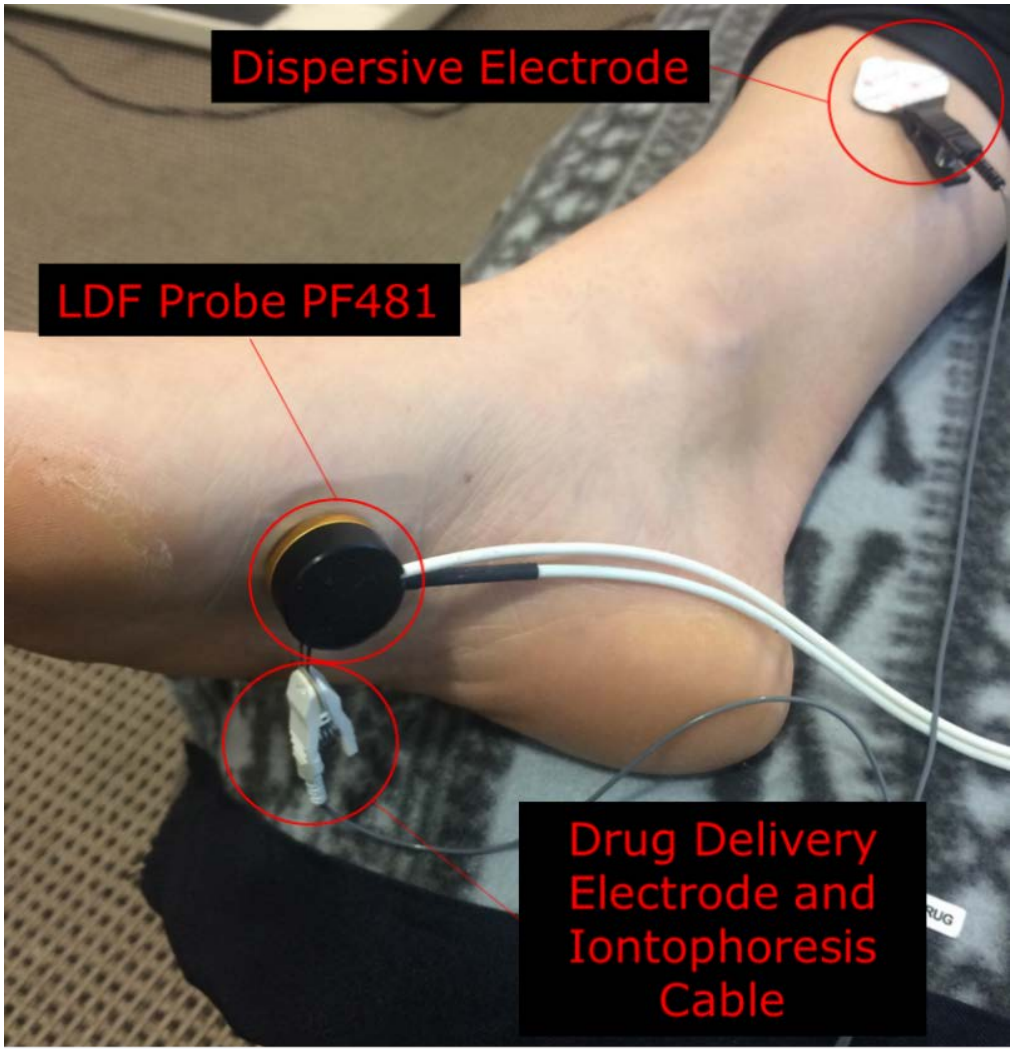


Figure 6.1. Laser Doppler Flowmetry and Iontophoresis Setup

The iontophoresis protocol was halted when no further increase in PUs was apparent. The drug delivery leads were then placed on the other foot (i.e., the remaining drug delivery electrode and dispersive electrode) and the second drug was delivered according to the iontophoresis protocol listed in Table 6.1. The iontophoresis protocol was repeated until no further increases in PU were apparent. ACh and SNP were randomly assigned to different feet and iontophoresis protocols were conducted in a random order.

Table 6.1. Iontophoresis Protocols for LDF Assessment

	ACh			SNP		
Pulse	**Current Density ($mA \cdot cm^{-2}$)**	**Charge Density ($mC \cdot cm^{-2}$)**	**Pulse Duration (s)**	**Current Density ($mA \cdot cm^{-2}$)**	**Charge Density ($mC \cdot cm^{-2}$)**	**Pulse Duration (s)**
1	0.05	5.71	120	0.19	16.00	120
2	0.05	5.71	120	0.19	16.00	120
3	0.11	13.71	120	0.19	16.00	120
4	0.11	13.71	120	0.19	16.00	120
5	0.11	13.71	120	0.19	16.00	120
6	0.19	11.43	60	0.19	16.00	120
7	0.19	11.43	60	0.19	16.00	120
8	0.19	11.43	60	0.19	16.00	120
9	0.24	14.29	60			

6.2.6 **Cutaneous Vascular Conductance**

ACh and SNP iontophoresis (PU) were analysed for baseline and peak response values from the continuous traces. The average of a two minute period of continuous data prior to the electrical current being applied was used as the baseline value for each drug challenge. This was performed for each channel. The PU of the highest 30 second average for both ACh and SNP iontophoresis was determined as the peak response to that drug.

MAP was calculated by the following formula, where SBP and DBP were the data recorded lying supine on the bed prior to iontophoresis.

$$MAP = \frac{(2 \times DBP)+SBP}{3}$$

PU were converted to cutaneous vascular conductance (CVC) to account for the effect of mean arterial pressure on perfusion. Thus changes in CVC were likely to be due to a change in lumen diameter rather than a change in MAP.

$$CVC = \frac{PU}{MAP}$$

6.2.7 **Statistical analyses**

6.2.8 **Primary analyses – Response to Iontophoresis**

Data from the response to each iontophoresis protocol were analysed via a two-way mixed ANOVA to determine SkBF within subject, between subject, and interaction effects, in participants with low and normal levels of HDL-C. A Shapiro-Wilk test was performed to assess the assumption of normality. When data were not distributed normally, data were transformed by a factor of Log_{10}. Outliers were determined as ±3 S.D. of the studentized residuals. Homogeneity of variances was assessed by Levene's test of homogeneity. Homogeneity of covariance was assessed via Box's M test. The change in CVC for the ACh and SNP protocols were analysed via a one-way ANOVA.

6.2.9 **Participant Characteristics – Mann Whitney U test**

Due to outliers in various parameters being present (Boxplot procedure), multiple breaches of the assumption of normality (Shapiro-Wilk test), and non-homogenic equality of variances (Levene's test) in multiple parameters, a Mann Whitney U test was performed to determine whether group differences exist between the low and normal HDL-C groups.

6.2.10 **Reliability Analysis – Intraclass Coefficients (ICCs)**

To determine the reliability of the LDF and iontophoresis protocols, 14 participants returned on a separate day for repeat assessment of SkBF response to iontophoresis. All procedures were

conducted in the same manner as described for the initial assessment. The initial test and repeat test were analysed to calculate ICCs using a two-way mixed absolute agreement model for the resting ACh, resting SNP, Peak ACh, and Peak SNP CVC conditions. ICCs were considered poor if <0.40, fair to good if 0.40 – 0.75, and excellent if >0.75 as recommended for LDF[158].

Statistical significance was set at α=0.05 for all analyses. All statistical analyses were performed using IBM SPSS Statistics version 23 (IBM Corporation, New York, USA). All figures were created using Prism software (Graphpad Software Inc., California, USA).

6.3. Results

6.3.1 **Baseline Characteristics**

Table 6.2 displays resting HR, BP, anthropometric, lipid, and blood glucose data (median (median ±95% confidence interval). There were 15 participants (8 male) in the low HDL-C group and 28 participants (7 male) in the normal HDL-C group. The distribution of data within each parameter was similar for both groups as assessed by visual inspection. Median SBP, DBP, HDL-C, and LDL-C were significantly different between groups; however, the median values for SBP, DBP, and LDL-C, were all below conventional CVD risk factor thresholds for both groups.

6.3.2 **Cutaneous Vascular Conductance at Baseline and Peak**

Table 6.3 displays the resting, peak and change in CVC under the different iontophoresis protocols. CVC was similar at baseline in both feet in the control and low HDL-C groups. There was a slightly larger change in CVC with SNP iontophoresis compared to ACh iontophoresis which was expected and is consistent with reports from other research groups[159] although no differences in CVC or percentage change due to iontophoresis were statistically significant (all p>0.05) (Table 6.3).

Table 6.2. Cohort and HDL-C Group Characteristics

	Entire Cohort (n=43)	**Low HDL-C** (n=15)	**Control** (n=28)	**p-value**
Age (years)	22 (21 - 23)	22 (20 – 23)	22 (22 – 23)	0.374
SBP (mmHg)	115 (110 – 121)	123 (116 – 126)	112 (108 – 117)	0.012*
DBP(mmHg)	72 (68 – 76.5)	80 (68 – 83)	71 (67 – 74)	0.024*
RHR (bpm)	64 (58 – 68)	63 (55 – 70)	64 (57 – 68)	0.990
BMI ($kg \cdot m^{-2}$)	23.2 (22.1 – 24.5)	23.2 (21.5 – 26.7)	23.2 (22.0 – 24.5)	0.646
WC (cm)	72.9 (71.0 – 76.8)	76.8 (71.1 – 83.1)	72.5 (68.5 – 75.5)	0.081
TGs ($mmol \cdot L^{-1}$)	0.82 (0.67 – 0.92)	0.89 (0.75 – 1.51)	0.74 (0.59 – 0.89)	0.076
HDL-C ($mmol \cdot L^{-1}$)	1.38 (1.17 – 1.57)	1.14 (0.92 – 1.14)	1.58 (1.46 – 1.76)	<0.0005*
LDL-C ($mmol \cdot L^{-1}$)	2.06 (1.8 – 2.39)	2.60 (2.29 – 2.74)	1.84 (1.63 – 2.20)	0.001*
TOTAL ($mmol \cdot L^{-1}$)	4.00 (3.79 – 4.33)	4.03 (3.77 – 4.49)	3.93 (3.47 – 4.33)	0.532
FBG ($mmol \cdot L^{-1}$)	4.75 (4.52 – 4.95)	4.89 (4.33 – 5.05)	4.75 (4.52 – 4.95)	0.909

***Statistically significant difference between low HDL-C and Control (normal HDL-C) groups.**

Table 6.3. Cutaneous Vascular Conductance Baseline and Peak Response to Acetylcholine Chloride and Sodium Nitroprusside Iontophoresis in the Plantar Surface of the Foot

	Entire Cohort (n=43)	**Low HDL-C** (n=15)	**Control** (n=28)	**p value**
ACh Baseline (PU/mmHg)	0.104 (0.010)	0.103 (0.018)	0.104 (0.011)	0.451
ACh Peak (PU/mmHg)	1.161 (0.101)	1.01 (0.158)	1.24 (0.130)	0.340
SNP Baseline (PU/mmHg)	0.091 (0.007)	0.102 (0.016)	0.085 (0.007)	0.514
SNP Peak (PU/mmHg)	1.140 (0.084)	1.240 (0.188)	1.087 (0.083)	0.530
ACh increase (%)	1150 (109)	1014 (145)	1223 (148)	0.366
SNP increase (%)	1259 (83)	1180 (134)	1300 (106)	0.495

Data displayed as mean (±S.E.M)
ACh –Acetylcholine Chloride, SNP – Sodium Nitroprusside
p value – comparison of low HDL-C group with Control group

6.3.3 **Response to ACh Iontophoresis**

There was no significant difference between low and normal HDL-C groups in the response to iontophoresis of ACh; $F(1, 41) = 0.933$, $p=0.340$, partial $\eta^2 = 0.022$. The main effect of ACh iontophoresis showed a significant increase in CVC; $F(1, 41) = 568.53$, $p<0.0005$, partial $\eta^2 = 0.933$ (Figure 6.2). There was no significant difference in resting or peak CVC between the low HDL-C and normal HDL-C groups; $F(1, 41) = 0.579$, $p = 0.451$, partial $\eta^2 = 0.014$ (Table 6.4, Figure 6.2).

6.3.4 **Response to SNP Iontophoresis**

Homogeneity of variance was not present in the SNP peak group ($p=0.027$). There was no significant difference between low and normal HDL-C groups in the response to iontophoresis of SNP; $F(1, 41) = 0.404$, $p=0.53$, partial $\eta^2 = 0.010$. The main effect of SNP iontophoresis showed a significant increase in CVC in response to SNP iontophoresis; $F(1, 41) = 1302.40$, $p<0.0005$, partial $\eta^2 = 0.969$ (Figure 6.2). There was no significant difference in resting or peak

CVC between the low HDL-C and normal HDL-C groups; $F(1, 41) = 0.514$, $p = 0.514$, partial $\eta^2 = 0.010$ (Table 6.4, Figure 6.2).

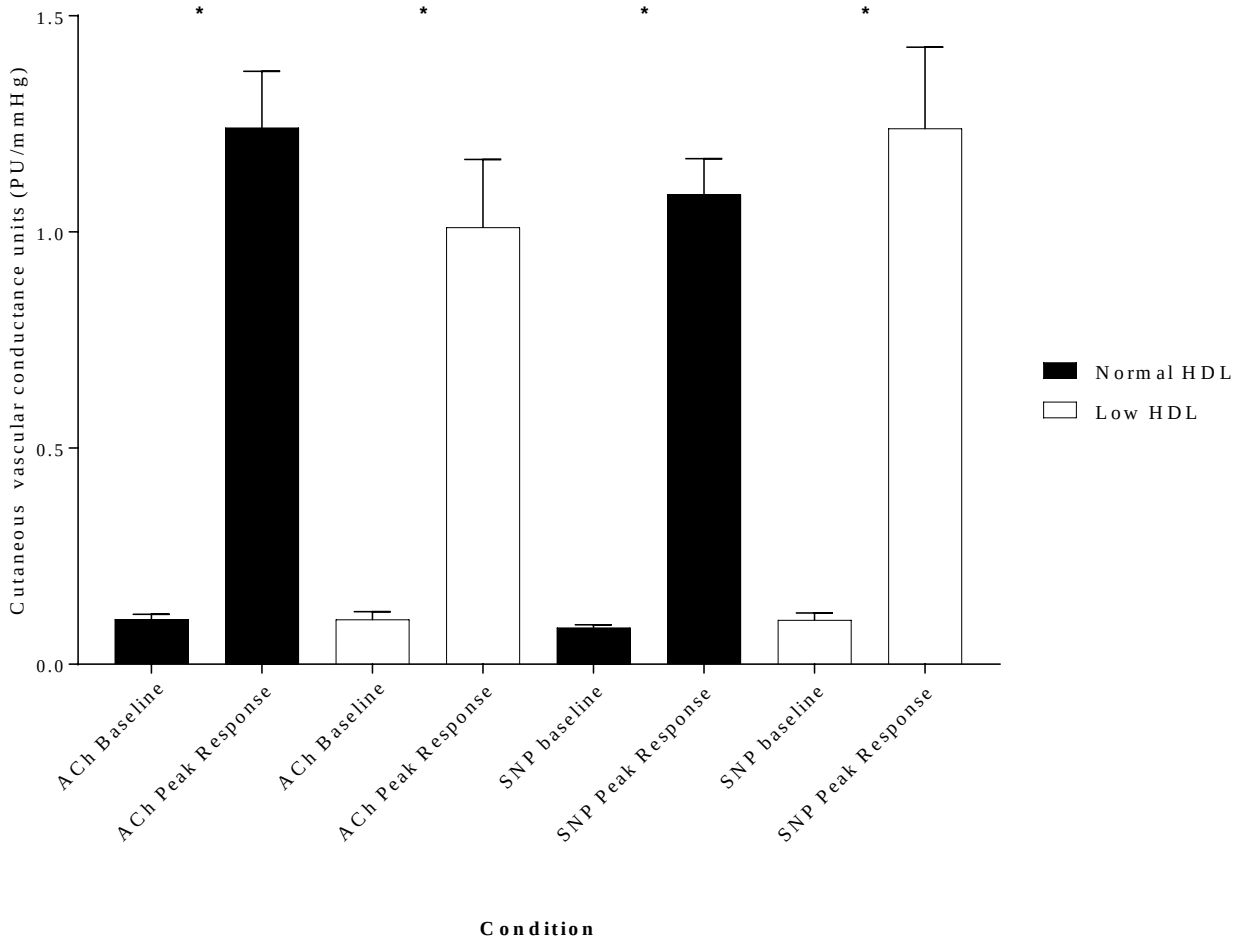


Figure 6.2. Skin Blood Flow in Response to Iontophoresis of Acetylcholine Chloride and Sodium Nitroprusside on the Plantar Surface of the Foot in Young Adults with Normal (black bars) and Low HDL-C (white bars).

All data displayed as mean ± S.E.M; n=43. (*) identifies within group significant differences. There were no differences between groups.

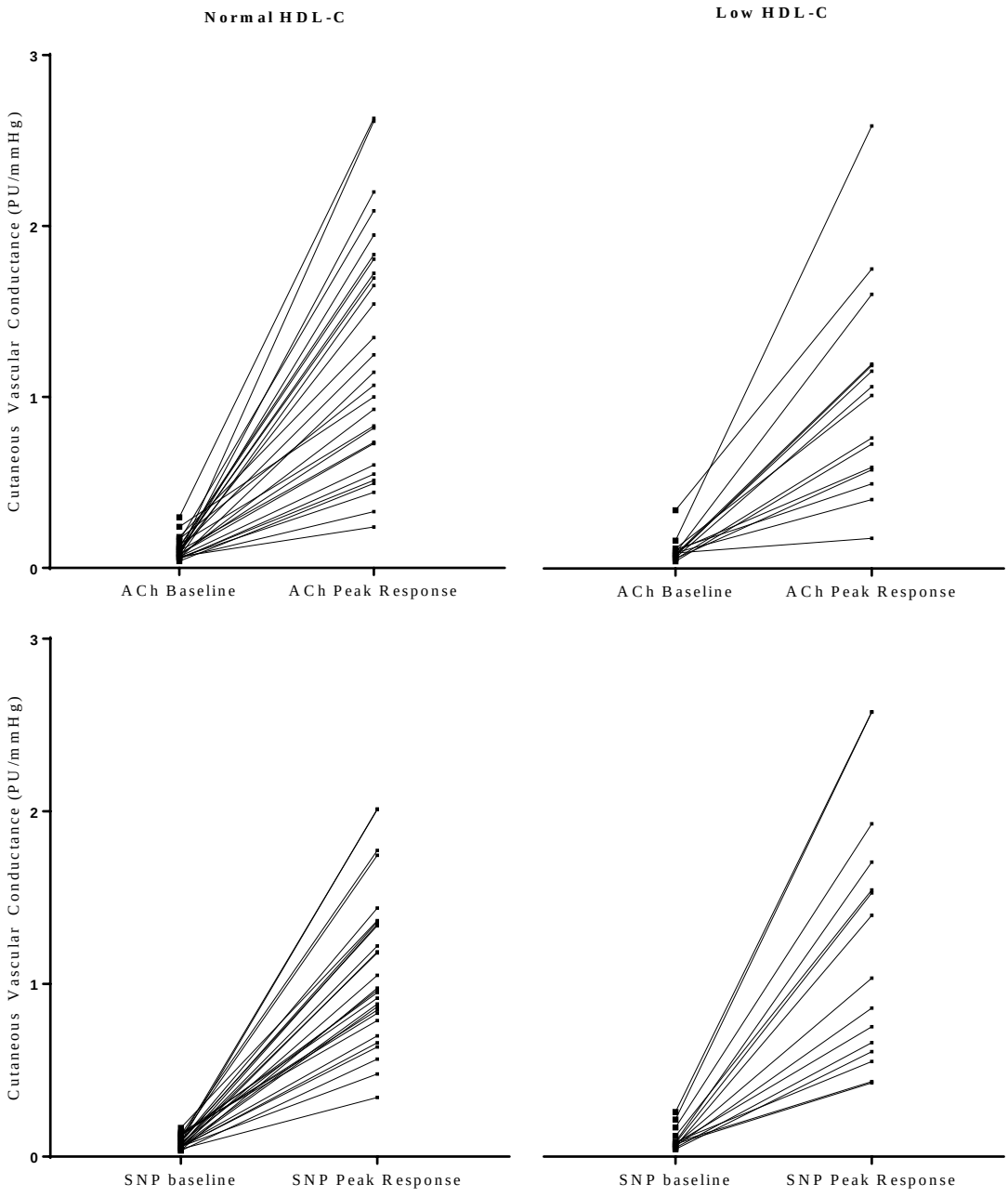


Figure 6.3. Individual Skin Blood Flow Response to Acetylcholine Chloride and Sodium Nitroprusside Iontophoresis in Young Adults with Normal and Low levels of HDL-C.

6.3.5 **IntraClass Coefficient Results**

The ICC for ACh rest (0.686), SNP rest (0.638), and ACh peak (0.589) indicated fair to good reliability. The ICC for SNP peak (0.117) indicated poor reliability for this protocol.

6.4. Discussion

The main finding of this study is that there were no between-group differences in SkBF at rest or in response to ACh and SNP iontophoresis in young adults with low and normal levels of HDL-C. This finding indicates that young adults with the low HDL-C component of MetSyn do not appear to have an impairment in the production of endothelial vasodilatory compounds or the ability of the smooth muscle to respond to NO. This result does not support the current hypothesis that endothelial-mediated microvascular dysfunction will be present in individuals with the low HDL-C component of MetSyn.

6.4.1 **Validity of Laser Doppler Flowmetry and Iontophoresis Protocols**

All participants had an increase in SkBF in response to ACh and SNP but there was large individual variability in SkBF response (Figure 6.3). The mean group increase (%) in SkBF in response to ACh was slightly less than the SkBF response to SNP response (Table 6.4). The relative increases were expected as the response to SNP is considered the maximal amount of vasodilation possible and therefore the SkBF response to ACh (i.e., endothelial-mediated) should be less than the response to SNP. Furthermore, the standard error of the mean (S.E.M.) (Table 6.4) for each protocol was also consistent with previous reports. For example, the S.E.M. (relative to the change in SkBF) for the increase in response is similar to Walther et al.[159], and less than Puissant et al.[158] (S.E.M. (% of change) = 9.5% (109/1150), Walther et al = 8.6%, Puissant et al. = 33.8%). Therefore, the response to ACh and SNP, and the individual variability in response to ACh and SNP, is consistent with previous research using similar protocols.

The calculated ICCs based on repeat testing of a small number of individuals (n = 14) indicated good reliability in the response to ACh (0.589) and both resting conditions (0.686, 0.638) but

poor reliability (ICC <0.40)[158] in the response to iontophoresis of SNP (ICC = 0.117). Previous work has also reported poor reliability with SNP iontophoresis (coefficient of variation 66-160%)[160]. Moreover, it has been suggested that examination of SkBF in glabrous skin may result in increased dermal clearance of SNP due to the dense dermal vascular network[160] leading to an increase in unreliability of the response. Other potential reasons for the poor reliability are spatial variability in SkBF response, skin resistance, and non-specific galvanic effects of the iontophoresis protocol[160]. However, in the current study the skin site was carefully prepared in all participants to reduce skin resistance via light abrasion and alcohol swab, and a very low, intermittent current was applied using a sodium chloride solution as the diluent to minimize galvanic effects of the iontophoresis current[156]. Therefore, the exact reason for the poor reliability of the SNP protocol remains elusive.

Fortunately, the poor reliability in the response to SNP does not affect the analysis of the main hypothesis regarding HDL-C and endothelial-mediated vasodilation response. In the current study, iontophoresis of ACh (ICC = 0.589) had good reliability (0.40-0.70) compared with previous reports. Puissant et al.[158] reported much lower ICCs for ACh iontophoresis (0.16) although different sites were examined. Furthermore the ICCs reported here for the ACh protocol are similar to a very recent report (0.59)[156]. Therefore, the reliability of the ACh iontophoresis protocol was consistent with other studies using similar techniques and therefore the finding that young adults with low HDL-C do not appear to have dysfunction in endothelial-mediated microvascular reactivity is most likely correct. However, possible reasons why there was no difference is worth examining in some detail.

There are three plausible explanations for the current finding. First, while HDL-C levels are an independent marker of CVD risk[16,142], low HDL-C *per se* may not be involved in the series of events that leads to dysfunction of microvascular endothelial-mediated vasodilation. It may be

that the other MetSyn components and the interaction between multiple MetSyn components may be necessary for endothelial-mediated vasodilation dysfunction to occur in young adults. Second, HDL-C concentration is a very crude indicator of HDL function (defined as the ability to promote reverse cholesterol transport (RVCT) and the efflux capacity of HDL) and therefore HDL-C may not be a reliable indicator of the effects that the HDL molecule has on the endothelium. Third, current recommended thresholds for the HDL-C component of MetSyn may not reflect increased and decreased risk of clinical disease adequately in young adults. Therefore, it is possible that dysfunction of endothelial-mediated vasodilation is not present at the current thresholds set for the HDL component of MetSyn. These three plausible explanations for the current findings will be discussed in more depth in the following sections.

6.4.2 **Low HDL-C as an Isolated Component of Metabolic Syndrome**

While the current study showed no difference in microvascular response to ACh and SNP, Walther et al[159] showed a reduced response to ACh in people with MetSyn and T2DM (n=53, 59 ± 5 years) and people with MetSyn without T2DM (n=25, 60 ± 5 years). Similarly, Shimabukuro et al. reported a reduced ACh-induced maximal forearm blood flow impairment in males with MetSyn (n=18, 51 ± 9 years). Therefore it appears that older people with MetSyn, have reduced endothelial-mediated microvascular reactivity.

However, Shimbukoro et al.'s secondary analyses of the individual MetSyn components and response to ACh showed there was a poor correlation (r = 0.098) between HDL-C level and the ACh-induced maximal forearm blood flow[155]. Therefore, it could be speculated that if low HDL-C does not have any effect in regards to endothelial function when MetSyn is present[155], it is more likely the other components of MetSyn play a larger role in the development of microvascular dysfunction. Moreover it appears that low HDL-C on its own is not sufficient to result in microvascular dysfunction and the other components of MetSyn may be required.

Previous research has reported that the other components of MetSyn: hypertension[145], obesity[146], and hypercholesterolemia[141]; are associated with endothelial dysfunction. In the current study, the participants in the low HDL-C group did not have MetSyn and median SBP, DBP, FBG, TG, and WC, were all within a normal healthy range. Therefore, microvascular dysfunction may require multiple components of MetSyn to be present before low HDL-C manifests as a reduced microvascular endothelial function.

6.4.3 **HDL-C is not a Marker of HDL Function**

HDL plays a role in removing cholesterol from the vascular wall as well as improving endothelial function, promoting endothelial repair, and increasing insulin sensitivity[161]. Recent research has highlighted the uncertainty surrounding the role of HDL-C *per se* in the development of CVD. Genome-wide association studies on people with mutations in genes that result in very low HDL-C concentrations, hypothesised that premature CVD rates would be increased due to the loss of HDL-C cardiovascular protective effects. However, it appears that there is a minimal increase in premature CVD in these people despite very low levels of HDL-C[162].

Furthermore, pharmacological studies that have been successful in raising HDL-C levels have shown little or no effect in the prevention of premature CVD[162]. While research in this area is still ongoing, raising HDL-C or lifelong exposure to low HDL-C does not appear to alter rates of CVD. Therefore, while HDL-C remains a very strong independent CVD risk factor[142], HDL-C itself may not play a role in the progression of CVD. Rather, the relative make up of HDL subclasses or the cholesterol efflux capacity of HDL may be more important in regards to the prevention of CVD[162].

There are five different HDL sub-particle types ranging from HDL-very small to HDL-very large. Depending on the size of the HDL sub class and the composition of proteins on the HDL,

HDL-C concentration will vary. For example, smaller HDL-particles (HDL-P) are more efficient at binding to ATP-binding Cassette Transporter A1 to extract cholesterol from the walls of arteries while larger HDL-P are more efficient at interacting with hepatic scavenger receptor type BI (SR-BI) receptors to deliver cholesterol to the liver[138]. Therefore, HDL-C will depend on the number of HDL-P, the relative composition of proteins on each HDL-P, the amount of HDL-P of each different particle size, and the amount of HDL-C per HDL-P. As a recent review states, in regards to HDL-C *"…it is essential to emphasize that these are crude measures and do not reflect the quality and function of HDL, including RVCT efficiency, inflammation, redox conditions, or the proteomic cargo of HDL"* (pg. 206-207)[163]. Therefore, HDL-C, on its own, may not reflect HDL function *per se* and it is possible that it is the function of HDL rather than the concentration of HDL-C that is more important in the prevention of CVD.

Previous reports of an enhancement in endothelium and NO dependent relaxation with HDL in the aorta of wild mice but not in SR-BI knockout mice[164] may indicate that the interaction of specific HDL proteins that interact with SR-BI receptors are important for endothelial function. Furthermore, Yuhanna et al. report that apolipoprotein A-I (a HDL bound protein) binds to SR-BI resulting in stimulation of endothelial nitric oxide synthase (eNOS) in cultured endothelial cells[165]. Therefore, many of HDL's effects on endothelial function may be independent of the cholesterol component of the HDL molecule.

HDL may also directly affect insulin sensitivity. HDL has been suggested to exert a positive effect on insulin secretion and beta cell survival, resulting in potential improvements in insulin sensitivity in adipose tissue, muscle and liver[166]. This is primarily attributed to HDL's role in regulating cholesterol homeostasis in the pancreas. Apolipoprotein A-I has also been shown to inhibit gluconeogenesis as well as increasing uptake of glucose into muscle cells[166]. Therefore,

the relative makeup, and therefore function, of the HDL molecules may play an important role in the development and consequence of MetSyn rather than just the HDL-C aspect. Unfortunately, due to resource constraints only HDL-C was measured in the current study and therefore substantiated conclusions of the HDL makeup or function of HDL in the current study cannot be made.

6.4.4 **HDL-C Threshold for Metabolic Syndrome**

Another possible, and perhaps controversial explanation for the lack of difference in endothelial-mediated vasodilation, could be in the HDL-C thresholds suggested for MetSyn. Low levels of HDL-C have always been included in the various definitions of MetSyn and are currently proposed at <1.03 mmol·L^{-1} for males and <1.29 mmol·L^{-1} for females[3]. Dr Reaven included low HDL-C as part of the MetSyn criteria due to the hyperinsulinemic and hyperglycaemic response to an oral glucose tolerance test in adult males with low HDL-C[31]. However, Dr Reaven provided no specific threshold where the risk of clinical disease was increased or decreased, nor were sex differences in HDL-C levels provided as the study cited in Dr Reaven's lecture included middle to older aged males only (n=40, 45-72 years)[167]. He also cited no evidence regarding the relationship between HDL-C and CVD in this lecture although major reports surrounding HDL-C and CVD were present at the time. However, HDL-C was not a primary focus of Dr Reaven's lecture, which was instead insulin resistance. Regardless, the reasons for why low HDL-C thresholds for MetSyn are determined at the current levels are still not clear.

Presumably, the original report from the Framingham Heart study by Gordon in 1977 was the basis for the original WHO definition of MetSyn determining the low HDL-C component of MetSyn as <35 mg·dL^{-1} (<0.90 mmol·L^{-1}) for males[33]. Unfortunately, no references were provided in the WHO report indicating why the HDL-C threshold was set at this level for males.

The threshold for low HDL-C in MetSyn was revised to <40mg·dL^{-1} for males in 2001 by the NCEP/ATPIII[29] although no rationale was provided for the change except a brief sentence stating *"...because the latter is a better measure of a depressed HDL."*(Table 1, pg. 2487)[29]. The HDL-C threshold has remained at <40 mg·dL^{-1} for males in every subsequent MetSyn definition. Therefore, a degree of speculation is required to determine why the HDL-C thresholds are set at the current levels.

Presumably, the HDL-C thresholds originate from the major epidemiological studies from the 1950s to 1980s. Perhaps the most famous study, the Framingham Heart study, initially reported that men and women with HDL-C under 35mg·dL^{-1} (~0.90 mmol·L^{-1}) had increased risk of developing CHD[137]. A follow up report on Framingham by Castelli et al. 12 years later, used 10 mg·dL^{-1} (~0.26 mmol·L^{-1}) increments starting at <40 mg·dL^{-1} (1.03 mmol·L^{-1}) when examining the relationship between incidence of CHD and each level of HDL-C[168]. This study is notable for three main points relevant to the current study on young adults. First, the majority of CHD occurred in the HDL-C group <40 mg·dL^{-1} regardless of total cholesterol level, indicating HDL-C to be an independent risk factor for CHD. Second, while <40 mg·dL^{-1} (~1.03 mmol·L^{-1}) is the MetSyn threshold for males currently, male and female data were analysed together in Castelli et al.'s analysis. Therefore, it is unclear why the female specific threshold exists based on Castelli et al.'s study. Third, the mean age of males was 61 years and females 62 years at the initial assessment (follow up time 12 years) is vastly different from the current study's age group.

The female specific threshold for low HDL-C was originally set at <39 mg·dL^{-1} (<1.0 mmol·L^{-1}) in the original WHO report before being revised to <50 mg·dL^{-1} (<1.29 mmol·L^{-1}) in the 2001 NCEP/ATPIII definition with no clear indication of why either threshold was included specifically for females. However, it is notable that low HDL-C was defined as <40 mg·dL^{-1}

for males and <50 mg·dL^{-1} for females in a major paper released in 1989 analysing four major American studies reporting on HDL-C and heart disease[169]. Perhaps it is because of the findings from this study that there are male and female HDL-C thresholds. Regardless of the specific thresholds, low HDL-C has always been a diagnostic feature in the detection of MetSyn for nearly 20 years despite uncertain origins for the inclusion and thresholds used in various definitions of MetSyn.

Therefore the significance of the low HDL-C component of MetSyn and future CVD risk specifically in younger adults remains unclear. Furthermore, the reason(s) for a female specific threshold for HDL-C also remain unclear. In regards to the current study, the low HDL-C group did not have endothelial-mediated microvascular dysfunction which is proposed as one of the earliest signs of CVD[139]. Therefore, the HDL-C threshold that endothelial-mediated microvascular dysfunction may occur remains uncertain.

6.4.5 **Limitations**

While some limitations in this study have already been acknowledged in the discussion (e.g., HDL function measures were not made, no quantitative measure of the different HDL proteins), there are other limitations in this study that are important to acknowledge. The current study hypothesised that low HDL-C may result in a shift towards an increased oxidative state due to lower levels of paraoxanase, a reduced ability to bind transition metals, and reduction in clearance of toxic phospholipids. However, no measure of oxidative state was made in the current study so confirmation that low HDL-C results in increased oxidation and subsequent endothelial dysfunction cannot be made. Furthermore, no measurement of inflammation (e.g., CRP) was made that may have helped to confirm that the low HDL-C group had an increased inflammatory state that also promotes endothelial dysfunction. However, the primary purpose of this study was to examine the relationship of the *low HDL-C MetSyn component* and

endothelial-mediated vasodilation in the microvasculature. Oxidative and inflammation status are potential explanations as to why HDL-C may contribute to endothelial dysfunction but were not variables of interest. Therefore, while measures of oxidation or inflammation would have been nice to have, these measures would not change the conclusion of this study in regards to HDL-C and young adults.

In the current study iontophoresis of ACh was used as the provocation test to assess endothelial-mediated vasodilation. Other provocation tests of the endothelial-mediated vasodilation response such as post-occlusive hyperaemia may have helped to confirm the findings of this study. However, post-occlusive hyperaemia provocation would have required a further testing session for each participant and was not feasible.

Lastly, the gender balance (male/female) between groups was different, with 53.3% being male in the low HDL-C group, while this was only 25% in the normal HDL-C group. To date, there have been no reports that have directly examined any potential gender-related differences in endothelial-mediated vasodilation in the microvasculature; therefore, it is difficult to conclude whether differences in gender balance contributed to the current finding.

6.5. Summary

SkBF response to iontophoresis of ACh and SNP was not different in young adults with and without the low HDL-C component of MetSyn. This result indicates that endothelial-mediated vasodilation is not reduced in the microvasculature of young adults with low HDL-C. Therefore, a key early step in the process of CVD is not present in young adults with the low HDL-C component of MetSyn. There are numerous possible explanations for the null result and further research is required to ascertain the significance, if any, of the low HDL-C component of MetSyn in young adults. This is critical work as low HDL-C is the most prevalent component of MetSyn in young adults.

Chapter 7. **Conclusion**

This thesis described an investigation into apparently healthy young adults who were free of diagnosed disease, and whose metabolic health characteristics were within what would be considered an appropriate range. This thesis reveals that even in apparently healthy young adults, 40% have MetSyn components with a significant proportion having three or more MetSyn components. MetSyn was prevalent in 14% of the young adults in the first study (Chapters Four and Five), but overall global prevalence appears to be 5-7% in young adults (Chapter Three). Simple, and easily assessed measures of body size, PA, CRF, and sitting time, are all predictors of MetSyn in young adults. Substitution of sitting time for walking or moderate PA may be a very simple way of reducing the odds of having MetSyn as a young adult.

The second part of the thesis describes the implication of currently having low HDL-C in isolation in young adults. Low HDL-C was consistently the most prevalent MetSyn component in young adults. However, despite the high prevalence of the low HDL-C component of MetSyn, endothelial function in the skin microvasculature was not different in young adults with and without low HDL-C. This finding raises larger questions regarding the role of HDL-C in the progression of vascular disease and therefore usefulness of HDL-C in the MetSyn definition.

7.1. Metabolic Syndrome Prevalence in Young Adults

MetSyn is prevalent in 5-7% of apparently healthy young adults[13]. It is important to highlight that the young adults that were sampled in the current study and the pooled analysis in Chapter Three, were apparently healthy (i.e., did not have any diagnosed underlying metabolic disease) and were not sampled based on any anthropometric criteria (e.g., obese). Therefore, in young

adults who are otherwise healthy, 5 - 7% will have MetSyn with approximately 30-40% having at least one component of MetSyn. Young adults identified as having MetSyn stand to benefit greatly by adopting a healthy lifestyle that will reduce the odds of having MetSyn and future disease. Furthermore, in a public health context, significant reductions in future CVD, T2DM and other vascular-related diseases may be achieved with identification and intervention in young adults with MetSyn components and young adults with the lifestyle characteristics associated with an increase in the odds of having MetSyn. The primary message from Chapters Three to Five is clear – young adults need to be cognisant of their lifestyle because it has a significant impact on the odds of having MetSyn and its components.

7.2. Metabolic Syndrome Component Prevalence is High in Apparently Healthy Young Adults

Approximately 40% of young adults had one or more components of MetSyn despite not having any diagnosed metabolic disease. The mean group characteristics such as SBP, BMI etc., were within what would be considered a normal and healthy range. Therefore these participants are considered apparently healthy. Furthermore, we did not have a large number of overweight or obese participants compared to population estimates (particularly in the NZ cohort) which may have resulted in an underestimation of MetSyn component prevalence. Therefore, it was surprising such a high prevalence of MetSyn components were present in such an apparently healthy group. Furthermore, people with increased numbers of MetSyn components had close to universally less-optimal health parameters (SBP, LDL-C etc.). Speculatively, these small changes may result in an increased risk of developing future clinical disease.

There is a growing body of evidence suggesting that maintaining optimal CVD risk factor profiles into middle age is cardiovascular protective[20,22]. Furthermore, many of the MetSyn

predictors highlighted in the current thesis (BMI, CRF, PA, sitting time) have been shown to worsen with increasing age. The findings observed in the current thesis suggest that many young adults already do not have optimal CVD risk factor profiles, as shown by the high prevalence of low HDL-C and the raised BP components of MetSyn. Moreover, previous research indicates that most young adults are not aware of their current CVD risk and are unlikely to change behaviours established during early adulthood[18]. However, the findings from Chapter Five indicate that increasing PA, improving CRF, reducing BMI, and reducing sitting would be effective in reducing MetSyn component and MetSyn prevalence in young adults whose CVD risk factor profiles are already less than optimal. Furthermore, either an improvement[21] or a maintenance[170] of healthy lifestyle practice as a young adult is associated with significantly less subclinical disease in middle age. An increase in the public health effort to promote the maintenance of a healthy BMI, increasing CRF, increasing PA, and reducing sitting time, is crucial to help prevent future disease in the face of the increasing obesity and physical inactivity epidemic.

7.3. Lifestyle is Associated with Metabolic Syndrome in Young Adults

Chapter Five highlights that participation in regular moderate PA, limiting daily sitting time, maintaining a good level of CRF, and maintaining a healthy BMI, are all important lifestyle behaviours that young adults should adhere to as much as possible to reduce the odds of having MetSyn components. Further, the isotemporal substitution model (Chapter Five) predicted a reduction in the odds of MetSyn when young adults replaced 30 minutes of sitting with 30 minutes of walking or moderate PA. Simply, young adults need to be encouraged to move their body more and sit less. Whilst not a primary focus of the current research, it is prudent to also encourage dietary practice to ensure adequate intake of nutrients while maintaining optimal levels of energy intake. Substituting sitting time for PA is an important message for young

adults and potentially should be encouraged in workplaces and educational institutions where a significant portion of young adult's weekly time is spent. Further research is required to test the substitution model.

7.4. Low HDL-C is not Associated with Endothelial Dysfunction in Young Adults

The last part of this thesis describes the SkBF response to iontophoresis of ACh and SNP in young adults with the low HDL-C component of MetSyn. The results were unequivocal and indicate that SkBF response to ACh and SNP is not different in young adults with low or normal levels of HDL-C. This finding indicated that endothelial-mediated microvascular vasodilation was similar despite differences in HDL-C levels. The finding did not support the experimental hypothesis, that was based on previous work showing reduced endothelial function in people with MetSyn[144], T2DM[143], and a range of other comorbidities[141,145-146]. Given the high prevalence of low HDL-C in young adults, further investigation is required to understand the role, if any, that low HDL-C may play in the development of MetSyn. It is critically important to determine whether low HDL-C is just an indicator of underlying metabolic disturbances rather than HDL-C being involved in the development of MetSyn *per se*.

7.5. Future Directions

7.5.1 **Improving Young Adults Lifestyle Behaviour is Important**

It could be speculated that a reduction in MetSyn in young adults will lead to a reduction in future clinical disease. Improvements or maintenance of BMI[76] and a healthy lifestyle[171] from young adulthood have previously been shown to reduce the risk of developing clinical disease and the primary prevention interventions for the treatment of MetSyn. This is a positive public health message as the lifestyle changes required to prevent MetSyn are entirely achievable and

scalable. There is no need for special equipment, complicated diets, or inaccessible or expensive interventions. Simply, there is a need to educate, encourage, and inspire young adults to maintain or adopt a healthy lifestyle that includes increased PA, reducing sitting time, and maintaining a healthy BMI. However, there has been a failure of translating research into effective interventions to promote healthy lifestyles over the past three or four decades as indicated by the increasing obesity rates, and declining PA rates in Western and developing countries. Therefore, there is a need to re-examine how to reverse the decline in PA rates and increase in BMI in young adults.

While not specifically investigated as part of this thesis, social media, mobile connectivity, and smart wear (technology enabled clothing/watches), are all media with great potential to connect research and interventions that promote awareness and adoption of a healthy lifestyle. Young adults are part of the so-called “digital native generation” and embrace new technologies quite readily given their familiarity with digital devices. Optimising the use of such technology to improve behaviours associated with disease (including MetSyn) in young adults is a potential strategy to improve young adult’s lifestyle behaviours. Rather than viewing technology as part of the problem of physical inactivity etc., technology could form a large part of the solution. Key questions remain on how to best use technology to promote lifelong healthy lifestyles and reduce future clinical disease.

7.5.2 **Metabolic Syndrome Definition Sensitivity**

Throughout this thesis, MetSyn has been used to identify young adults who may have increased risk of developing future clinical disease. The research is clear that MetSyn is useful for this purpose but requires further development. Age-specific, gender-specific, and ethnic-specific, thresholds for all MetSyn components may improve the sensitivity, and therefore usefulness, of identifying people that have increased lifetime risk for future clinical disease. The WC

component is one MetSyn component where this work has already been done. Ethnic and gender specific guidelines exist and have been presumably set at the recommended thresholds due to the relationship of the component with future disease. Given the high prevalence of low HDL-C in young adults, the low HDL-C component thresholds for gender, age, and ethnicity, is worth revisiting.

7.5.3 **The Cause of Metabolic Syndrome**

The final study of this thesis showed there was no difference in endothelial-mediated microvascular vasodilation or smooth muscle cell response to NO in young adults with and without the low HDL-C component. It is important to remember that low HDL-C is supposed to occur due to the presence of high TGs. However, TG prevalence was not increased in the same magnitude as the low HDL-C component in the pooled analysis (Chapter Three) or in the first study (Chapter Four). Therefore, the exact reasons why so many young adults had low HDL-C are not clear but low HDL-C occurred without raised TGs in a large proportion of low HDL-C cases. Considering the lack of difference in endothelial function and the low prevalence of raised TGs, three conclusions can be made. First, the HDL-C threshold may not be adequately calibrated for young adults; second, low HDL-C may be attributed to other mechanisms not well understood or still to be discovered; third, low HDL-C may be a biomarker for metabolic dysfunction in a general sense but may have only a modest direct role in changes in vascular health. Regardless of the specific reasons, all three conclusions would be worthy of further investigation due to the high prevalence of low HDL-C in young adults.

7.5.4 **Investigation along the Continuum of Disease in Young Adults**

Investigation of young adults along the continuum of clinical disease (e.g., no MetSyn components, components present, MetSyn present, demonstrable subclinical disease, and clinical disease) would be useful to determine how MetSyn components progress to MetSyn

(three or more components) and subsequently clinical disease (T2DM, CVD). For example, a cross sectional study investigating microvascular endothelial-mediated vasodilation in a group of young adults with zero MetSyn components, one or two MetSyn components, MetSyn (three or more components), and a group of young adults with T2DM, would be insightful in determining the key mechanistic differences in microvascular endothelial-mediated vasodilation in young adults with varying levels of subclinical and clinical disease. The next step would be to intervene in people with impaired endothelial-mediated vasodilation and evaluate how the intervention affected the key mechanisms involved in the progression of MetSyn over a period of time.

7.5.5 **Summary Statement**

MetSyn and MetSyn components are present in apparently healthy young adults and having MetSyn and its components is linked to increased BMI, lower CRF, low levels of PA, and increased sitting time. Promotion of a healthy lifestyle during young adulthood is important in reducing the lifetime risk of clinical disease. Low HDL-C is the most prevalent MetSyn component in young adults. Microvascular endothelial-mediated vasodilation and smooth muscle response to NO is not affected by the low HDL-C component in young adults. However, low HDL-C is a potent CVD risk factor and further research is required to examine the significance of having low HDL-C as a young adult.

Chapter 8. Appendices

8.1. Appendix A: Manual of Procedures for Study One

PhD Experiment 1

Prevalence of Metabolic Syndrome Components in Undergraduate University Students

Manual of Procedures

Abbreviations

MetS	Metabolic syndrome
CRF	Cardiorespiratory fitness
UoA	the University of Auckland
WSCU	Western State Colorado University
PIS	Participant information sheet
EFTS	Equivalent full-time student
IPAQ	International Physical Activity Questionnaire
BP	Blood pressure
HR	Heart rate
LDL	Low density lipoprotein
HDL	High density lipoprotein
TGs	Triglycerides
BMI	Body Mass Index

Introduction

This study is the first of four studies in Mr Paul Nolan's PhD. This study will use 2 sites – UoA and WSCU. Although UoA is the preferred text used in this document, WSCU should be substituted for researchers using this manual at WSCU.

The design of this study is cross-sectional.

There is no grant currently associated with this study.

Participants (n=150) will be recruited from students currently attending the University of Auckland. WSCU will endeavour to recruit a similar number of participants.

Contact List and Structure

Mr Paul Nolan will be responsible for the day to day aspects of the study. However, if the need should arise to contact another member of the team, the following contacts are to be used.

Dr Jim Stinear (UoA)	j.stinear@auckland.ac.nz	373 7599 ext 82378
Dr Graeme Carrick-Ranson	g.ranson@auckland.ac.nz	3737 7599 ext 86849
Dr Lance Dalleck (WSCU)	ldalleck@western.edu	970 943 7132
Mr Paul Nolan	p.nolan@auckland.ac.nz	373 7599 ext 86630

Study Centre Locations

The UoA study centre location will be located at:

The Health and Performance Centre
The University of Auckland Clinics
Building 750a
71 Merton Road
Glen Innes
Auckland
1142

The Clinical Research Centre
University of Auckland Grafton Campus
Level 4 Building 502
FMHS
85 Park Rd
Grafton

High Altitude Performance Laboratory
Western State Colorado University

600 N Adams Street
Gunnison Colorado USA 812341

Due to a high number of enrolled students spending most of their time at the UoA city campus, alternative arrangements may be made to have a testing room on the city campus.

Ethical Requirements

Prior to commencement of this study, ethical approval was obtained from approved by the University of Auckland Human Participants Ethics Committee on 26/08/2014 for 3 years, Reference Number 012554.

Space and Equipment Requirements

Each study centre will require the following equipment:

- Clean, quiet, well ventilated room with a desk and chairs
- Cholestech cholesterol measuring device and cassettes
- Lancets, capillary tubes, swabs, plasters (i.e., band aids), alcohol wipes
- Fridge for storage of cholestech cassettes
- 41.3cm step, stopwatch and HR monitor
- BP sphygmomanometer and stethoscope
- Stadiometer, scales, cloth measuring tape
- Handgrip dynamometer
- Pen, paper and questionnaires

Recruitment

To be eligible, participants will:

- Be aged 17-24 years old[1]
- Be currently enrolled for at least part time undergraduate study (0.5 EFTS) at UoA

[1] WSCU will enrol 18-24 year olds only

- Not have one of the following:
    - BMI ≤ 18.5
    - Pregnant or lactating
    - Documented underlying metabolic disease at the time of assessment including:
        - Hyperaldosteronism
        - Type 1 diabetes Mellitus
        - Diagnosed polycystic ovary syndrome
        - Systemic Lupus Erythematosus
        - HIV/Aids
        - Hepatitis C
    - Documented Familial Hypercholesterolemia
    - Documented Familial Hypertension

Participants will be recruited via posted advertisements around the UoA city and Tamaki campuses. Course coordinators of large undergraduate classes will be informed about the study in the hope they will highlight this to their students at the start (or end) of their lectures. The general education coordinators (e.g., SPORTSCI 100G) will be sought in the first instance.

After initial contact from the participant, contact details will be recorded and an appointment for the assessment will be made. Initial contact may occur face to face, email or via phone. A quick verbal check will be made to ensure the participant is eligible before an appointment time, date and location is confirmed. A standard email text also containing the PIS (appendix D) and consent form (appendix E) will be sent following the initial contact confirming the appointment time, date and location. This will also include procedures for fasting 9-12 hours before the appointment time.

Assessment

Informed consent will be obtained at the baseline assessment although participants will have the opportunity to read the PIS and consent form ahead of the appointment. The participant **must** be asked if they have any questions and/or concerns and must be addressed before proceeding. The consent form **must** be signed by the participant and witnessed by the researcher before the assessment proceeds.

See next section for specific details on assessment protocols.

Participants will undergo the following procedures:

- Explanation of the study
- Completion or collection of completed informed consent (appendix F)
- Completion of Par-Q (appendix G)

- Completion of IPAQ (appendix A)
- BP and HR recording
- Height, weight and waist circumference
- TG's, HDL, LDL and Blood Glucose
- Muscular strength test
- Queen's College step test

At the end of the assessment, participants will be offered their results if they wish to know them. Additional information will be available regarding the MetS and associated components for those participants who wish to learn more.

General Structure of Assessment

Measurement	Cumulative Time
PIS, informed consent (seated at this time)	0
Questionnaires	5
Resting BP and HR measured	15
Resting BP and HR repeated	16
Weight measured	17
Height measured	18
Weight repeated	19
Height repeated	20
Waist circumference measured	21
Waist circumference repeated	22
Dynanometer test	24
At point blood test	25
Submaximal exercise test	35
End of Assessment	60 minutes

Please note, an extra 30 minutes allowed for any procedural issues and data recording into computer spreadsheet (NZ only).

Measurements

IPAQ

The short form IPAQ is to be self-administered according to standardised instructions available at the website below. The IPAQ is attached in appendix A.

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

BP and Heart rate

1. The participant should be seated for approximately 5 minutes in a relaxed state. They may speak or complete paperwork but refrain from physical work or stress prior to this measurement.
2. BP must be measured against the skin therefore clothing needs to allow access to a bare upper arm.
3. **The forearm should rest relaxed on a support such as a table.**
4. Wrap BP cuff around upper arm and align with the brachial artery and ensure Velcro has good adhesion.
5. Start the BP machine.
6. Two measurements should be taken for each measurement.

- **FOR BP:** A third measure should be taken where the second measure is **not within 5 mmHg for** systolic and diastolic blood pressure.
- **FOR HEART RATE:** If the readings **differ by more than 4 bpm**, take a third measure and record.
- The mean (average) value is used in any further calculations if two measurements are taken, and the median value is used if three measurements are taken.

Height and weight

Height and weight should be measured as alternate measures and repeated, that is,

1. Measure height

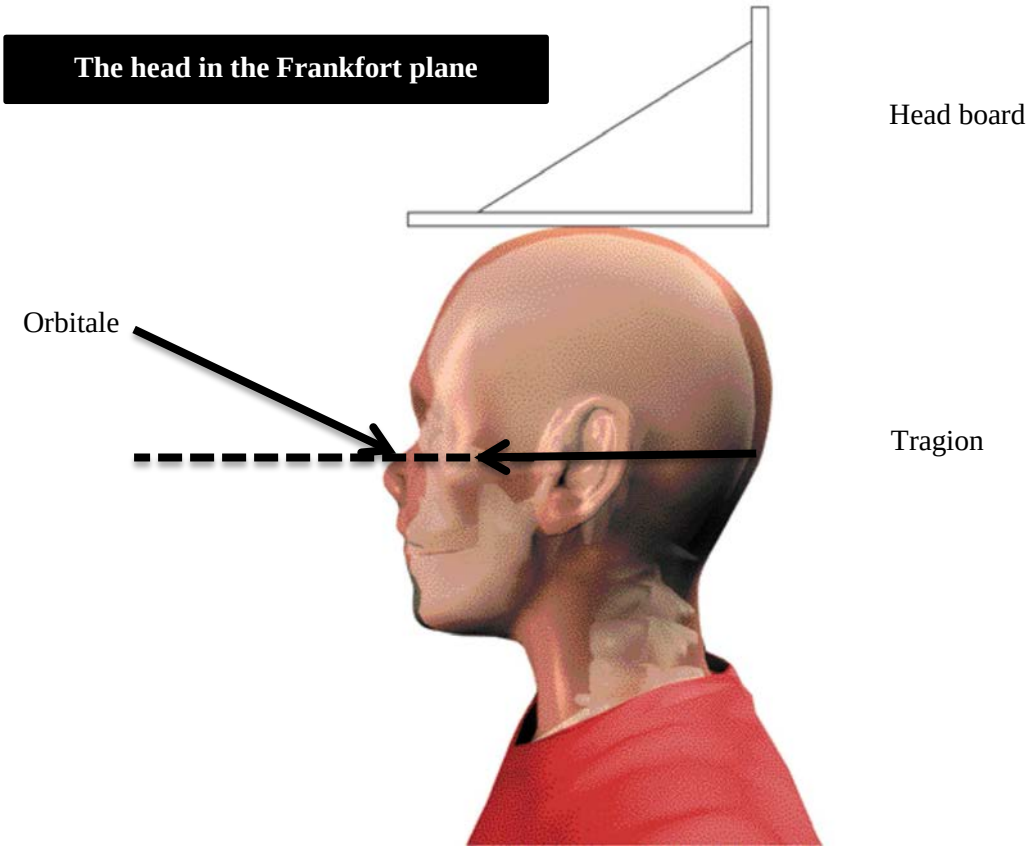
2. Measure weight

3. Measure weight again

4. Measure height again

Height is the perpendicular distance between the transverse planes of the Vertex and the inferior aspects of the feet. The Vertex is the most superior point on the skull when the head is positioned in the Frankfort plane. The Frankfort plane is the alignment of the head when the Orbitale (the lower bony margin of the eye socket) is in the same horizontal plane as the Tragion (the notch superior to the tragus or flap of the ear).

The head in the Frankfort plane



1. Have the participant stand with their back flush against the wall or stadiometer pole.
2. Ensure the head is in the Frankfort plane.
3. Having positioned the head in the Frankfort plane, the participant is instructed to take and hold a deep breath and while keeping the head still. The tester places the headboard firmly down on the top of the head, compressing the hair as much as possible.
4. Measurement is taken before the subject breathes out
5. When reading the value, read directly next to the red arrows

6. Round the value to the nearest centimetre using Swedish rounding system (5 or more round up, 4 or less round down)
7. Record the reading and ask the participant to step away from the stadiometer.

- Two measurements should be taken separately. **A third measure should be taken where the second measure is not within 1.0cm.**
- The mean (average) value is used in any further calculations if two measurements are taken, and median if a third measure is required.

Weight

1. Mass (weight) should be measured in light indoor clothing. Shoes, coats and jumpers should be removed
2. Check that the scale is placed on a hard, even surface (avoid carpet)
3. The participant stands still on the centre of the scales without support and with the weight distributed evenly on both feet
4. Record the reading and ask the participant to step off the scales and to step on again.
5. Record the reading. **If the reading differs by more than 0.2kg (200g), take a third measure** and record

- The mean (average) value is used in any further calculations if two measurements are taken, and the median value is used if three measurements are taken.

Waist circumference

1. Stand on the left side of the participant
2. Ask the participant to stand with feet together
3. Place tape in the narrowest spot between the xiphoid process and umbilicus
4. Pull tape taught but you should not compress the skin
5. Ensure the tape is horizontal
6. Take the 0 end of the tape and cross it over the opposite end of the tape and record the measurement.
7. Two measurements should be taken separately. **A third measure should be taken where the second measure is not within 1.0cm.**

- The mean (average) value is used in any further calculations if two measurements are taken, and the median value is used if three measurements are taken.

Blood measures

Please read appendix H before proceeding with measuring blood glucose and cholesterol.

- TG's, HDL, LDL and blood glucose measurements will be performed via the Cholestech device.

IMPORTANT - Before the test

- The participant will be instructed to report to the site in a fasted state. That is, no food and minimal fluids in the preceding 8-12 hours. This **must** be queried upon arrival at the testing site to confirm the fasted state.
- Calibrate and check the Cholestech device as per manufacturer's instructions.

Data Collection

1. Wash your hands with warm soapy water
2. Wipe the finger site to be tested with alcohol and wait for surface to dry.
3. Place lancet device firmly against the finger and click until a puncture has been established.
4. Wipe away the first drop of blood.
5. Let the blood come out from the puncture and hold a capillary tube horizontal to collect the blood. You may have to gently squeeze the finger to get the sample but do not squeeze too hard. Capillary tube should be filled within 10 seconds
6. Dispense the collected blood onto a new cassette.
7. Insert cassette into device and run the analysis.
8. Print the results AND write down the result.

Physical measures

Please note, before physical measures are to be performed a PAR-Q (Appendix G) must be filled in prior to the start of the tests. If the PAR-Q is failed, the participant will not be assessed further.

Muscular strength

Muscular strength is to be assessed using a handgrip dynamometer. Ensure the dynamometer is calibrated according to manufacturer's instructions before performing the test in the following manner:

1. Ask the participant to stand.
2. Adjust the grip bar so that the second joint of the fingers will be bent to grip the handle of the dynamometer.
3. Ask the participant to hold the handgrip dynamometer parallel to the side of the body with the elbow flexed at 90 degrees.
4. Ask the participant to squeeze the dynamometer as hard as possible without holding their breath.
5. Record the highest sustained recording and repeat the measurement using the opposite arm.

- This test should be done 3 times per side. Sum the highest recorded measurements from each side for comparison to normative data.

Queen's College Step Test

1. Check for radial pule on the participant using your index and middle fingers – **do not use your thumb**. It may be preferable to put a small mark where the pulse is most strongly felt. **It is imperative that the HR measurement is as accurate as possible.**
2. Instruct the subject to stand close to the step.
3. Depending on the gender of the subject, set the metronome to operate at the following cadence:
    - Males – 24 step cycles per minute (one cycle is stepping up and back down again)
    - Females – 22 step cycles per minute (one cycle is stepping up and back down again)
4. On your signal, instruct the subject to step up onto the step using their left foot, and step down with their right foot. Continue leading with the left foot for 1.5 minutes, then swap so that the subject is leading with their right foot to step onto the step and their left foot to step down to the ground.
5. Ensure the participant steps into the middle of the box.
6. Monitor the participant for adverse signs and symptoms.

7. Continue the test for 3 minutes. After 3 minutes has passed, instruct the subject to stop.

8. **<u>5 seconds after the test is complete, start counting the radial pulse for 15 seconds.</u>**

    - Start counting from zero when you start the timer if you start right on a pulse.

    - Start counting from one when you start the timer if you start in between two pulses.

- Write down this number and multiply by 4. This will be used in the estimated VO_2max formulae.

- The participant's VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$) will be estimated based on the following formula:

    - Males = 111.33 – (0.42 x HR)

    - Females = 65.81 – (0.1847 x HR)

<u>NOTE: Please note any problems/difficulties with the test. For example, if the person was unable to switch the leading legs halfway through or were poor in coordination with the metronome.</u>

IPAQ

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe

somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

Par-Q

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

GUIDELINES FOR HANDLING UNFIXED HUMAN TISSUE, BLOOD AND BODY FLUIDS IN RESEARCH

A. Introduction

While risks associated with exposure to blood and tissues contaminated by Hepatitis B can be mitigated by vaccination of laboratory workers, the potential risk of infection by other agents such as Hepatitis C, HIV and CJD can only be reduced by following prudent safety measures when handling specimens. Therefore, human material must be handled within the confines of appropriate laboratory facilities and the following precautions adhered to, regardless of lack of evidence of infection by any of these agents.

B. Standard Precautions for Handling Unfixed Human Tissue, Blood and Body Fluids

1. Treat all human blood, tissue and body fluids as potentially infectious.
2. Absolutely no eating, drinking or applying cosmetics in the laboratory. Food or drink should not be stored in laboratories. Hand/mouth contact should be kept to a minimum.
3. Laboratory coats or appropriate protective gowns must be worn in the laboratory and fastened properly. Laboratory coats/Protective gowns must be removed when leaving the laboratory area to go to tea-rooms, offices, toilets or seminar rooms.
4. Gloves must be worn when handling:
5. human blood, tissue and body fluids
6. Infectious, or potentially infectious materials
7. Hazardous chemicals.
8. Care must be taken to prevent contaminated gloves coming in contact with laboratory furniture, door handles, telephones and the like.
9. All disposable equipment, tissues and gloves must be disposed as medical waste.
10. Hands must be washed and dried after removing gloves and before leaving the laboratory/blood collection area.
11. All open cuts and abrasions must be covered.
12. Any spills of infectious (or potentially infectious) material on floors, benches or equipment must be cleaned up immediately with disinfectant (see below).

13. All samples must be properly labelled. Because the outside of the tube may be contaminated, tubes should be handled with care. Samples must be stored in an appropriate labelled, designated refrigerator or portion of the refrigerator or freezer.

14. All sample tubes must be placed within a leak-proof container with a secure lid.

15. Glass containers, vacutainers, used gloves, scalpels, needles and syringes must be placed in sharps bins. Sharps bins must never be overfilled.

16. Do not attempt to separate needles from syringes. Discard both together. Do not attempt to recap a needle.

17. Avoid techniques with a high potential for creating aerosol (sonication, vortexing, blowing out pipette contents).

18. All accidents must be reported immediately to the Exercise Physiology Technical or Teaching Fellow and an accident/incident form filled out.

C. Guidelines for Working with Human Blood, Unfixed Tissue and Body Fluids

Venous blood must only be taken by suitably trained staff. Such staff may be doctors or nurses or those who have under gone training in phlebotomy.

1. Ensure adequate consent has been obtained and that Faculty privacy protocols are followed.

2. All laboratory personnel must have their Hepatitis B antibody checked (and be immunised, if necessary) before handling human blood, tissue or body fluids.

3. Wherever possible blood or tissue that has been shown not to be contaminated by Hepatitis B, Hepatitis C or HIV should be used.

4. Never use cells from staff or their relatives to transform cell lines, due to the higher risk of re-exposure to histocompatible cell lines.

5. All work with human blood in the laboratories must be performed in a certified Class 2 Biohazard cabinet.

6. Where blood is being collected with minimal processing (i.e., when serum is being isolated), work may be conducted outside a certified Class 2 Biohazard cabinet, provided centrifuges are fitted with sealed rotors and Standard Precautions for Handling Blood are observed.

7. Use disposable equipment wherever possible and discard into medical waste. Double-bag any material that might potentially puncture medical waste bag. All high risk material should be discarded into a sharps bin.

8. Do not use vacuum aspiration. Pipette supernatants to a disposable tube and then autoclave/chemically sterilise the waste supernatant.
9. Use sealed tubes for centrifuging blood samples. Use sealed rotors to minimise contamination in the event of tube failure. In the event of a failure of tubes the centrifuge rotor and bowls should be disinfected with 1% Virkon solutions.
10. Laboratory benches and hood surfaces where blood has been handled must be cleaned and decontaminated at the completion of work. Use swab impregnated in an intermediate disinfectant such as 0.05% sodium hypochlorite or per oxygen biocide such as 1%Virkon.
11. Report any accident or spillage of infectious material to the Exercise Physiology Technician or Teaching Fellow immediately.

D. Cleaning and Disinfecting Equipment

Disposable equipment

Discard disposables (e.g., pipettes, needles and syringes) into sharps bins. Sharps bins must never be overfilled. Ensure all lids are very secure before placing sharps bin for collection.

Reusable equipment

Soak glass in 0.05% sodium hypochlorite or a proprietary disinfectant such as 1% Virkon for at least 30 minutes. The action of many disinfectants is severely hampered by the presence of protein. Where possible, remove proteinaceous material before soaking.

Metal will be corroded by sodium hypochlorite, use 1% Virkon solutions to disinfect centrifuge rotors, centrifuge bowls and other metal equipment.

E. Disposal of Waste Specimens

Discard disposables (e.g., pipettes, needles and syringes) that have come into contact with blood into sharps bins.

Blood or tissue must be decontaminated, preferably by autoclaving (for large volumes). Where this is impractical, material should be chemically disinfected with hypochlorite and sent out for incineration.

F. Blood Accidents

1. Wear gloves throughout the clean-up procedure.
2. Spills can be decontaminated with Sodium hypochlorite (1:100 final concentration of household bleach or 5% solution).

3. Surfaces can be decontaminated with a wipes impregnated with0.05% sodium hypochlorite.

After clean up, dispose of gloves in medical waste and wash hands.

G. 'Needle Stick' Injury

1. When a person sustains a needle stick or similar injury involving blood or body fluid they must report the injury. Assistance of Student Health Service must be sought immediately. Do not assume that because blood has been drawn from a colleague that it is safe, or that you have sufficient levels of antibody against Hepatitis B virus because you have been received Hepatitis B vaccine.

2. Wherever possible contact Student Health Services (Ext 87681 or 87682) to provide advance notice of ‘needle-stick injury’.

3. Student Health Service will provide appropriate testing and treatment for persons having sustained a needle stick injury. If the Student Health Service is closed then the Auckland Hospital Emergency Department will provide care. It is very important that such accidents are reported and the assistance of the Student Health Service is sought immediately.

4. The Student Health Service or the Auckland Hospital Emergency Department may need to arrange tests to detect Hepatitis B, Hepatitis C and HIV infection in the donor.

5. If Student Health Services is not available for any reason, the injured person should seek treatment from Emergency Services at Auckland Hospital. Wherever possible, the person who was the source of the blood should also attend.

8.2. Appendix B: Participant Information Sheet – Study One

DEPARTMENT OF SPORT AND EXERCISE SCIENCE
Faculty of Science

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
Te Whare Wānanga o Tāmaki Makaurau

Department of Sport and Exercise Science
Building 734.321 261 Morrin Road,
Glen Innes, Auckland, New Zealand
Telephone 64 9 373 7599 ext.86887
Facsimile 64 9 373 7043
Email: sportsscience@auckland.ac.nz
www.ses.auckland.ac.nz

The University of Auckland
Private Bag 92019
Auckland, New Zealand

Participant Information Sheet
For research participant

Project title: **Prevalence of Metabolic Syndrome Components in Undergraduate University Students**

Primary Investigator: Dr James Stinear
Co-Investigators: Dr Lance Dalleck, Dr Graeme Carrick-Ranson
Student Researcher: Paul Nolan

Researchers Introduction

My name is Paul Nolan and I am a Doctoral (PhD) student in the Department of Sport and Exercise Science at the University of Auckland. Dr James Stinear is a Senior Lecturer and Dr Graeme Carrick-Ranson is a Lecturer in the Department of Sport and Exercise Science at the University of Auckland. Dr Lance Dalleck is an Assistant Professor in the Recreation, Exercise and Sport Science Department at Western State Colorado University, USA.

Project description and invitations

I am inviting you to participate in this multi-centre cross sectional study investigating the prevalence of metabolic syndrome components in university students. You must be a current student at the University of Auckland enrolled in at least part time (0.5 EFTS) study and aged between 17 and 24 years old. Students from Western State Colorado University are also being invited to participate.

Your participation in this study is voluntary and you have the right to withdraw from the study at any time you desire, without giving a reason. All information obtained in this study will be kept confidential.

Rationale

Heart disease rates have fallen dramatically over the past few decades. However, there has been a rise in overweight and obesity rates in young New Zealanders and Americans.

Additionally, evidence has emerged linking birth weight, gestational age and birth order to the future development of obesity. People who are obese or overweight are at higher risk of developing metabolic syndrome and metabolic disease. This confers a significant lifetime risk for heart disease and diabetes.

Metabolic syndrome is a clustering of different risk factors (high blood pressure, large waist circumference, impaired blood glucose, and dyslipidemia (abnormal levels of some fats in the blood). These risk factors can create an inflammatory state in the body. This promotes favourable conditions for depositing cholesterol in the arteries of the heart (atherosclerosis). Therefore, identification of people with components of metabolic syndrome is an important step in prevention of heart disease in the face of increasing obesity.

International research indicates that approximately 10% of University students have metabolic syndrome and approximately 50% have at least one component of metabolic syndrome. However, there are large differences between geographical locations, and the current prevalence of metabolic syndrome and its components in NZ University students is unknown. Additionally there have been no studies comparing the prevalence of metabolic syndrome and its components in University students from different countries.

Project procedure

At your appointment, you will be afforded the opportunity to ask any questions you may have regarding the study. Once this has occurred, you will be invited to sign the Consent Form.

Eligibility criteria

Students must be aged 17-24 years old and currently enrolled for at least part time undergraduate study (0.5 EFTS) at the University of Auckland or Western State Colorado University.

Students who have any of the following may be excluded:

- Underweight (BMI ≤ 18.5 kg/m^2)
- Pregnant or lactating
- Known metabolic disease (e.g., Type 1 Diabetes Mellitus) at the time of assessment

Please note: You will be asked whether you have certain medical conditions (e.g., Hepatitis C, AIDs) that may influence the likelihood of having metabolic syndrome. The presence of such conditions may preclude you from participating in this study.

Please contact Paul Nolan if you have any of the aforementioned conditions so that the researchers can determine if you are eligible to participate in this study.

Baseline assessment

All assessment will take place in a private, quiet room on University of Auckland property.

The 3 main campuses of the University of Auckland are located at:

Tamaki Campus, 71 Merton Road, Glen Innes, Auckland.

Grafton Campus, 85 Park Rd, Grafton, Auckland

City Campus, Auckland 1010.

The baseline assessment may take up to **1.5 hours** of your time.

All testing will take place in the morning. You will be required to **fast for 9-12 hours** prior to testing. You also need to refrain from consuming caffeine, food, or beverages with caloric value, as well as refrain from any form of strenuous exercise prior to testing. You will be allowed to consume water prior to testing.

Additionally, you will be asked questions regarding your birth weight, gestational age and birth order. It would be helpful if you could **bring your Plunket book to the assessment** or gather this information prior to assessment.

You will be required to wear clothing and footwear that is suitable and comfortable for exercise. When you attend, you will have the opportunity to ask any questions before completing the informed consent form. You will first be asked to fill in a Physical Activity Readiness Questionnaire and then asked to fill in a short questionnaire detailing some background of your lifestyle and upbringing and your current residential, physical activity, alcohol consumption and dietary status. Your resting blood pressure, heart rate, weight, height and waist circumference will then be measured and a finger prick blood test will be performed to measure your blood glucose and cholesterol levels. Lastly, a short handgrip strength test and a 3 minute step test to measure your fitness level will be performed.

If you would like a copy of your results, please indicate on the informed consent form.

Risks

Every measure is taken to minimise the risk of injury during the assessment. All staff are trained in first aid and resuscitation (Level 5) and a direct phone line is present near the assessment room. During the exercise test you may experience some discomfort and the exercise may cause dizziness, muscular fatigue and/or shortness of breath. By following the researcher’s instructions you will minimise the risk of developing these symptoms.

Incidental Findings

It is possible we find that you have one or more components of metabolic syndrome, or you may provide other information that could also compromise your health. If either of these happen, you will be informed (as our duty of care) and provided with some general information on what you can do to help curb these risk factors, and we may also encourage you to consult with your General Practitioner (GP) for further advice. Additionally, in the unlikely event that you have any unusual symptoms (e.g.,, chest pain) during the exercise test, you will be informed and encouraged to consult with your GP. If you do not wish to be informed of these findings you should not agree to participate in this study.

Storage of data

All data obtained from this study will be stored until December 2020 and may be used as part of Paul Nolan's PhD thesis. Data obtained from the study will be processed by computer software and analysed. All participants will be assigned a unique code for all documentation and data files. A list will be kept separate to allow researchers to match documentation with participants but no information allowing you to be identified directly will be on any documentation.
All data and participant information from Auckland will be stored securely in a locked filing cabinet at the University of Auckland at 261 Morrin Road, Glen Innes, Auckland and only the researchers will have access to this.

All data will be encrypted with a password.

All electronic data will be stored on a password secure USB memory stick which will be saved and backed-up on a secure password protected University of Auckland server. All data and participant information collected for the purpose of the study will be securely shredded and destroyed after a period of no less than 6 years; any electronic data will be destroyed using formatting software.

Right to withdraw from participation

Your participation within this study is voluntary. Your decision to participate or not participate in this study will not affect your University of Auckland academic standing. If you feel that this assurance has been breached in any way, you should contact your Head of Department. You have the right to withdraw from participating in this study at any time without explanation. You also have the right to withdraw your data from the study up to 1st December 2015.

Confidentiality

Your identity throughout the study will be kept confidential throughout the study. Please note that no research participant will be able to see or access any personal information. Any data collected and displayed in results will be displayed in a way which will not disclose your identity. Only the student researcher and his supervisors (named above) will have access to the identity of the subjects. Consent forms will be kept in a secure filing cabinet at the University of Auckland, 261 Morrin Road, Glen Innes, Auckland.

Compensation

There is no cost to you for taking part in this study, and all exercise protocols and procedures are free of charge. There is no compensation or reimbursement for your participation. However, as part of the study you will be screened for metabolic syndrome, a valuable health screening that could prove beneficial for your long term health.

In the unlikely event of injury as a direct result of your involvement in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. ACC cover is not guaranteed and will be evaluated by ACC. If your claim by ACC is accepted, you still may not receive compensation. ACC generally only provides partial reimbursement of costs and expenses and there may be no lump sum compensation available. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. More information is available from http://www.acc.co.nz or speak with the researchers if you have any questions.

Contact details

Should you have any questions about this form or the study, please contact one of the people below and we will happily answer them for you.

Researcher: Paul Nolan
Email: p.nolan@auckland.ac.nz
Phone: (09) 373 7599 ext: 86630
Address: The University of Auckland, 71 Merton Road, Glen Innes, Auckland 1072

Supervisor: Dr James Stinear
Email: j.stinear@auckland.ac.nz
Phone: (09) 373 7599 ext: 82378
Address: The University of Auckland, 261 Morrin Road, Glen Innes, Auckland 1072

Head of Department: Associate Professor Greg Anson
Email: g.anson@auckland.ac.nz
Phone: (09) 373 7599 ext: 82975
Address: The University of Auckland, 261 Morrin Road, Glen Innes, Auckland

For any queries regarding ethical concerns you may contact the Chair, The University of Auckland Human Participants Ethics Committee, The University of Auckland, Office of the Vice Chancellor, Private Bag 92019, Auckland 1142.

Phone: (09) 373-7599 ext: 83771

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON 26/08/2014 for 3 years, Reference Number 012554.

8.3. Appendix C: Western State Colorado University Consent Form Study One

Revised Date 01/27/2014

Consent to Participate in Biomedical Research
Information to Consider About this Research

Prevalence and Prevention of Metabolic Syndrome Components in University Individuals From Indigenous and European Cultures

Principal Investigator: Dr. Lance Dalleck

Department: Recreation, Exercise, and Sport Science

Contact Information: ldalleck@western.edu

What is the purpose of this research?

The purpose of this research is two-fold:

1) to investigate the prevalence of metabolic syndrome and its components in University students; and
2) to determine lifestyle behaviors and social factors that are associated with components of the metabolic syndrome in University students.

Why am I being invited to take part in this research?

You are an 18-24 year old student (of any gender or ethnicity) undertaking full time undergraduate University study. Your participation in this study is completely voluntary and you have the right to withdrawal from the study at any time you desire, with no questions asked. All information obtained in this study will be treated with complete confidentiality; it is anticipated that collected data will be submitted for publication.

What will I be asked to do?

- You will complete one single session. Each session will take place in the High Altitude Performance Laboratory between 6:00am and 11:00am during weekdays and will take approximately 60 minutes.
- Upon being scheduled for your testing session you will be provided (either via email or a hard copy) the following instructions:
    - *You are required to fast for 9-12 hours prior to testing. You also need to refrain from caffeine, food, beverages with caloric value as well as refrain from any form of strenuous exercise prior to testing. You will be allowed to consume water prior to testing.*
- Upon arriving for the testing sessions you will fill out a consent form, physical activity readiness questionnaire, and a general health form concerning daily habits, diet, and physical

activity levels. Next, resting heart rate, resting blood pressure, anthropometric data, fasting blood glucose, and fasting lipids will be measured. A stress questionnaire, sitting questionnaire, and other information questionnaire will also be given to you during this time. Fasting blood glucose and lipids will be measured with a finger prick, collecting 40 microliters of blood. Blood samples will be dispensed onto an available Cholestech LDX Lipid Profile GLU cassette for analysis. The Cholestech LDX analyzer measures total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides and fasting blood glucose. Following the finger prick, you will undergo a three minute step test to assess fitness and estimate VO_2max. Heart rate will be recorded immediately following the step test.

What are possible harms or discomforts that I might experience during the research?

As with any physical exercise there is possible risk of episodes of transient light headedness, fainting and nausea with the Step test. These risks are similar to those regularly encountered during physical training sessions. Short-term mild to moderate discomfort may be experienced during the step test. You may also be exposed to a very slight risk of infection or cross-contamination due to blood sampling. This risk is minimal and standard laboratory procedures are in place to further minimize the risks associated with exposure to bodily fluids (sweat, saliva, blood) during normal physiological testing.

What are possible benefits of this research?

You will be informed about your cholesterol, blood glucose levels, blood pressure, fitness and strength levels which you may not have an awareness of. These are all factors relating to cardiovascular health that are not immediately apparent without a test

Will I be paid for taking part in the research?

We will not pay you for the time you volunteer while being in this study.

How will you keep my private information confidential?

Your information will be combined with information from other people taking part in the study. When we write up the study to share it with other researchers, we will write about the combined information. You will not be identified in any published or presented materials. To ensure that your information is kept confidential, identification numbers but not names will be used on all documents. All data entry and analysis will be conducted with statistical programs using identification. Your files will be stored in Dr. Lance Dalleck's office under lock and key and identifiable information will be deleted after one year.

Whom can I contact if I have a question?

The people conducting this study will be available to answer any questions concerning this research, now or in the future. You may contact the Principal Investigator at 970-943-7132. If you have

questions about your rights as someone taking part in research, contact the Western State Colorado University Human Research Committee Chair Lance Dalleck via email at ldalleck@western.edu.

Do I have to participate?

☐ Your participation in this research is completely voluntary. If you choose not to volunteer, there is no penalty or consequence. If you decide to take part in the study you can still decide at any time that you no longer want to participate. You will not lose any benefits or rights you would normally have if you do not participate in the study.

This research project **[HRC2013-02-61R3]** has been approved on **30 September 2014** by the Institutional Review Board (IRB) at Western State Colorado University. This approval will expire on **30 September 2015** unless the IRB renews the approval of this research.

I have decided I want to take part in this research. What should I do now?

If you have read this form, had the opportunity to ask questions about the research and received satisfactory answers, and want to participate, then sign the consent form and keep a copy for your records.

Participant's Name (PRINT) Signature Date

8.4. Appendix D: Participant Information Sheet – Study Two

DEPARTMENT OF SPORT AND EXERCISE SCIENCE
Faculty of Science

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
Te Whare Wānanga o Tāmaki Makaurau

Department of Sport and Exercise Science
Building 734.321 261 Morrin Road,
Glen Innes, Auckland, New Zealand
Telephone 64 9 373 7599 ext.86887
Facsimile 64 9 373 7043
Email: sportsscience@auckland.ac.nz
www.ses.auckland.ac.nz

The University of Auckland
Private Bag 92019
Auckland, New Zealand

Participant Information Sheet
For research participant

Project title: **Prevalence of Metabolic Syndrome Components in Undergraduate University Students**

Primary Investigator: Dr James Stinear
Co-Investigators: Dr Lance Dalleck, Dr Graeme Carrick-Ranson
Student Researcher: Paul Nolan

Researchers Introduction

My name is Paul Nolan and I am a Doctoral (PhD) student in the Department of Sport and Exercise Science at the University of Auckland. Dr James Stinear is a Senior Lecturer and Dr Graeme Carrick-Ranson is a Lecturer in the Department of Sport and Exercise Science at the University of Auckland. Dr Lance Dalleck is an Assistant Professor in the Recreation, Exercise and Sport Science Department at Western State Colorado University, USA.

Project description and invitations

I am inviting you to participate in this multi-centre cross sectional study investigating the prevalence of metabolic syndrome components in university students. You must be a current student at the University of Auckland enrolled in at least part time (0.5 EFTS) study and aged between 17 and 24 years old. Students from Western State Colorado University are also being invited to participate.

Your participation in this study is voluntary and you have the right to withdraw from the study at any time you desire, without giving a reason. All information obtained in this study will be kept confidential.

Rationale

Heart disease rates have fallen dramatically over the past few decades. However, there has been a rise in overweight and obesity rates in young New Zealanders and Americans.

Additionally, evidence has emerged linking birth weight, gestational age and birth order to the future development of obesity. People who are obese or overweight are at higher risk of developing metabolic syndrome and metabolic disease. This confers a significant lifetime risk for heart disease and diabetes.

Metabolic syndrome is a clustering of different risk factors (high blood pressure, large waist circumference, impaired blood glucose, and dyslipidemia (abnormal levels of some fats in the blood). These risk factors can create an inflammatory state in the body. This promotes favourable conditions for depositing cholesterol in the arteries of the heart (atherosclerosis). Therefore, identification of people with components of metabolic syndrome is an important step in prevention of heart disease in the face of increasing obesity.

International research indicates that approximately 10% of University students have metabolic syndrome and approximately 50% have at least one component of metabolic syndrome. However, there are large differences between geographical locations, and the current prevalence of metabolic syndrome and its components in NZ University students is unknown. Additionally there have been no studies comparing the prevalence of metabolic syndrome and its components in University students from different countries.

Project procedure

At your appointment, you will be afforded the opportunity to ask any questions you may have regarding the study. Once this has occurred, you will be invited to sign the Consent Form.

Eligibility criteria

Students must be aged 17-24 years old and currently enrolled for at least part time undergraduate study (0.5 EFTS) at the University of Auckland or Western State Colorado University.

Students who have any of the following may be excluded:

- Underweight (BMI $\leq$ 18.5 kg/m^2)
- Pregnant or lactating
- Known metabolic disease (e.g., Type 1 Diabetes Mellitus) at the time of assessment

Please note: You will be asked whether you have certain medical conditions (e.g., Hepatitis C, AIDs) that may influence the likelihood of having metabolic syndrome. The presence of such conditions may preclude you from participating in this study.

Please contact Paul Nolan if you have any of the aforementioned conditions so that the researchers can determine if you are eligible to participate in this study.

Baseline assessment

All assessment will take place in a private, quiet room on University of Auckland property.

The 3 main campuses of the University of Auckland are located at:

Tamaki Campus, 71 Merton Road, Glen Innes, Auckland.

Grafton Campus, 85 Park Rd, Grafton, Auckland

City Campus, Auckland 1010.

The baseline assessment may take up to **1.5 hours** of your time.

All testing will take place in the morning. You will be required to **fast for 9-12 hours** prior to testing. You also need to refrain from consuming caffeine, food, or beverages with caloric value, as well as refrain from any form of strenuous exercise prior to testing. You will be allowed to consume water prior to testing.

Additionally, you will be asked questions regarding your birth weight, gestational age and birth order. It would be helpful if you could **bring your Plunket book to the assessment** or gather this information prior to assessment.

You will be required to wear clothing and footwear that is suitable and comfortable for exercise. When you attend, you will have the opportunity to ask any questions before completing the informed consent form. You will first be asked to fill in a Physical Activity Readiness Questionnaire and then asked to fill in a short questionnaire detailing some background of your lifestyle and upbringing and your current residential, physical activity, alcohol consumption and dietary status. Your resting blood pressure, heart rate, weight, height and waist circumference will then be measured and a finger prick blood test will be performed to measure your blood glucose and cholesterol levels. Lastly, a short handgrip strength test and a 3 minute step test to measure your fitness level will be performed.

If you would like a copy of your results, please indicate on the informed consent form.

Risks

Every measure is taken to minimise the risk of injury during the assessment. All staff are trained in first aid and resuscitation (Level 5) and a direct phone line is present near the assessment room. During the exercise test you may experience some discomfort and the exercise may cause dizziness, muscular fatigue and/or shortness of breath. By following the researcher’s instructions you will minimise the risk of developing these symptoms.

Incidental Findings

It is possible we find that you have one or more components of metabolic syndrome, or you may provide other information that could also compromise your health. If either of these happen, you will be informed (as our duty of care) and provided with some general information on what you can do to help curb these risk factors, and we may also encourage you to consult with your General Practitioner (GP) for further advice. Additionally, in the unlikely event that you have any unusual symptoms (e.g.,, chest pain) during the exercise test, you will be informed and encouraged to consult with your GP. If you do not wish to be informed of these findings you should not agree to participate in this study.

Storage of data

All data obtained from this study will be stored until December 2020 and may be used as part of Paul Nolan's PhD thesis. Data obtained from the study will be processed by computer software and analysed. All participants will be assigned a unique code for all documentation and data files. A list will be kept separate to allow researchers to match documentation with participants but no information allowing you to be identified directly will be on any documentation.
All data and participant information from Auckland will be stored securely in a locked filing cabinet at the University of Auckland at 261 Morrin Road, Glen Innes, Auckland and only the researchers will have access to this.

All data will be encrypted with a password.

All electronic data will be stored on a password secure USB memory stick which will be saved and backed-up on a secure password protected University of Auckland server. All data and participant information collected for the purpose of the study will be securely shredded and destroyed after a period of no less than 6 years; any electronic data will be destroyed using formatting software.

Right to withdraw from participation

Your participation within this study is voluntary. Your decision to participate or not participate in this study will not affect your University of Auckland academic standing. If you feel that this assurance has been breached in any way, you should contact your Head of Department. You have the right to withdraw from participating in this study at any time without explanation. You also have the right to withdraw your data from the study up to 1st December 2015.

Confidentiality

Your identity throughout the study will be kept confidential throughout the study. Please note that no research participant will be able to see or access any personal information. Any data collected and displayed in results will be displayed in a way which will not disclose your identity. Only the student researcher and his supervisors (named above) will have access to the identity of the subjects. Consent forms will be kept in a secure filing cabinet at the University of Auckland, 261 Morrin Road, Glen Innes, Auckland.

Compensation

There is no cost to you for taking part in this study, and all exercise protocols and procedures are free of charge. There is no compensation or reimbursement for your participation. However, as part of the study you will be screened for metabolic syndrome, a valuable health screening that could prove beneficial for your long term health.

In the unlikely event of injury as a direct result of your involvement in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. ACC cover is not guaranteed and will be evaluated by ACC. If your claim by ACC is accepted, you still may not receive compensation. ACC generally only provides partial reimbursement of costs and expenses and there may be no lump sum compensation available. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. More information is available from http://www.acc.co.nz or speak with the researchers if you have any questions.

Contact details

Should you have any questions about this form or the study, please contact one of the people below and we will happily answer them for you.

Researcher: Paul Nolan
Email: p.nolan@auckland.ac.nz
Phone: (09) 373 7599 ext: 86630
Address: The University of Auckland, 71 Merton Road, Glen Innes, Auckland 1072

Supervisor: Dr James Stinear
Email: j.stinear@auckland.ac.nz
Phone: (09) 373 7599 ext: 82378
Address: The University of Auckland, 261 Morrin Road, Glen Innes, Auckland 1072

Head of Department: Associate Professor Greg Anson
Email: g.anson@auckland.ac.nz
Phone: (09) 373 7599 ext: 82975
Address: The University of Auckland, 261 Morrin Road, Glen Innes, Auckland

For any queries regarding ethical concerns you may contact the Chair, The University of Auckland Human Participants Ethics Committee, The University of Auckland, Office of the Vice Chancellor, Private Bag 92019, Auckland 1142.

Phone: (09) 373-7599 ext: 83771

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON 26/08/2014 for 3 years, Reference Number 012554.

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