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The influence of walking on risk factors associated with metabolic syndrome

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Abstract

Metabolic syndrome (MetS) represents a cluster of metabolic abnormalities, characterised by the presence of 3 or more of 1) abdominal obesity, 2) insulin resistance, 3) hypertension, 4) dyslipidaemia, and 5) emerging risk factors, such as pro-thrombotic and pro-inflammatory states, which are each independent cardiovascular disease (CVD) risk factors. This clustering of risk factors is reported to increase the odds ratio for cardiovascular and all-cause mortality above the risk associated with the individual components (Wilson, 2004). The precise aetiology of MetS is currently unknown, however an energy-dense diet, particularly high in carbohydrate, and an inactive lifestyle or low fitness may interact with a genetic susceptibility to contribute to the pathophysiology of MetS (Bouchard, 2007). Therefore the purpose of the studies included in this thesis were to determine whether accumulative brisk walking may improve risk factors associated with MetS and whether one single session of brisk walking at a moderate intensity may improve risk factors associated with MetS in middle-aged men at risk of MetS. Study one recruited 85 males aged 38-73 onto a 24-week randomised controlled trial with participants allocated to control (CON), single 30 minute daily brisk walking (SBW) or accumulative 30 minutes of daily brisk walking (ABW; 3×10 min or 2×15 min) groups. Measures included aerobic fitness ($\dot{V}O_{2max}$), body composition and selected blood variables. The main findings were that 24 weeks of accumulating 150 min·wk⁻¹ of brisk walking at ~65% HR_{max} significantly improved insulin sensitivity, which was associated with decreased abdominal adiposity, assessed by waist circumference, and was at least as effective as a single daily session of equal volume in middle-aged men at risk of MetS. Study two investigated the 24-hour effect of walking for 30 minutes at 50% $\dot{V}O_{2max}$ (30×50%), 30 minutes at 65% $\dot{V}O_{2max}$ (30×65%) and 60 minutes at 50% $\dot{V}O_{2max}$ (60×50%) compared to rest (CON) on cardiovascular control, resting metabolism and selected blood variables. The main findings were that a single 30 minute walking session at 50% $\dot{V}O_{2max}$ favourably improved cardiovascular control, indicated by decreased heart rate and systolic blood pressure, thus decreasing the workload of the heart, whereas increasing the intensity of the walk to 65% $\dot{V}O_{2max}$ attenuated this effect, while increasing the duration to 60 minutes had no additional effect compared to 30 minutes at 50% $\dot{V}O_{2max}$ in men at risk of MetS.

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Glossary of abbreviations

ABW – Accumulative brisk walking group refers to the group of participants who performed brisk walking in multiple sessions during the day

ACSM – American College of Sports Medicine is an international organisation for health-fitness professionals

AHA – American Heart Association is a charity that supports research related to heart disease

Apos A-I, A-II & B – Apolipoproteins A-I, A-II and B are the protein moieties of lipoproteins

ARIC – Atherosclerosis Risk in Communities is a large-scale, long-term prospective study that measures associations of established and suspected coronary heart disease risk factors

ATPIII – National Cholesterol Education Panel Adult Treatment Panel III published guideline criteria for diagnosing metabolic syndrome

Db – Body density is the ratio of mass to volume of the body and used in models to estimate body composition, which can be estimated by a number of methods

BF% - Body fat percentage is the percentage of the body composed of fat

BMI – Body mass index is an anthropometric estimation of body composition used in clinical practice by dividing body mass (kg) by height (cm)²

CAD – Coronary artery disease is a form of cardiovascular disease that relates specifically to the vessels supplying the heart with blood

CDC – Centers for Disease Control and Prevention is an organisation that provides health information in the United States

CETP(a) – Cholesteryl Ester Transfer Protein (activity) relates to the activity of a lipoprotein-bound enzyme that transfers cholesteryl esters and triacylglycerol between lipoproteins, which has implications for reverse cholesterol transport

CHD – Coronary heart disease is similar to coronary artery disease and relates to problems occurring from defects in the vessels supplying the heart with blood

CMO – Chief Medical Officer produced a report in 2004 to promote participation in physical activity

CON – Control refers to the sedentary control group within a trial

CRP – C-reactive protein is a marker of systemic inflammation

CVD – Cardiovascular disease relates to damage and impairments of the heart and blood vessels

DBP – Diastolic blood pressure is the residual pressure exerted by the vessels and blood in between contractions of the heart, measured in millimetres of mercury (mm Hg)

EE – Energy expenditure is energy used per unit of time and is used as a measure of physical activity

EGIR – European Group for the Study of Insulin Resistance is a European group of investigators studying insulin resistance and related disorders

EPOC – Elevated post-exercise oxygen consumption refers to the residual increase in metabolism related to a previous exercise bout

FXIIa – Factor XIIa is the active form of FXII which is involved in blood clotting

FFM – Fat-free mass relates to the proportion of the body that contains no fat, measured in kilograms

FM – Fat mass is the proportion of the body composed of fat, measured in kilograms

FRS – Framingham Risk Score is a 10-year risk of heart disease based on age, gender, TC, HDL-C, smoking status, SBP and prescribed cardiovascular medication use

GLUT-4 – Glucose transporter-4 is found in skeletal & cardiac muscle and liver tissue and translocates to the plasma membrane in response to increases in circulating insulin or glycogen depletion

HDL-C – High-density lipoprotein cholesterol is a micellular molecule involved in reverse cholesterol transport

HOMA – Homeostasis Model of Assessment is a model for expressing insulin resistance

HPA axis – Hypothalamic-pituitary adrenal axis controls reactions in the body through secretion of endocrine hormones into the circulation from endocrine organs in the brain and kidneys

HR_{max} – Maximum heart rate is the age predicted maximum heart rate based on $220 \text{ beats} \cdot \text{min}^{-1}$ minus age

HR_{peak} – Peak heart rate is peak heart rate attained during an exercise test

hrs·wk⁻¹ ≥4 METs - hours per week of PA at or above 4 METs and is a measure of the volume of PA at a sufficient intensity to enhance health

IDF – International Diabetes Federation provides guidance on matters relating to diabetes

IFG – Impaired fasting glucose is an elevation in fasting glucose and a risk factor for type II diabetes mellitus

IGT – Impaired glucose tolerance is a decreased ability to deal with an oral glucose load and a risk factor for type II diabetes mellitus

IL-6 – Interleukin-6 is a cytokine that is secreted by adipose tissue and acts as an inflammatory mediator

Kcal – kilocalories is measure of energy consumption and expenditure

LB – Long bout is a long exercise session in studies investigating the efficacy of accumulative short bouts of activity vs. longer sessions

LCAT(a) – Lecithin-cholesterol acyltransferase (activity) relates to the activity of a lipoprotein-bound enzyme that esterifies cholesteryl esters to be retained within lipoproteins as part of reverse cholesterol transport

LDL-C – Low-density lipoprotein cholesterol is a micellular molecule implicated in the pathogenesis of atherosclerosis

LPL(a) – Lipoprotein lipase (activity) relates to the activity of a capillary-bound enzyme that hydrolyses triacylglycerol-rich very-low-density lipoprotein

MAP – Mean arterial pressure is the mean pressure derived from systolic and diastolic blood pressures

MET – Metabolic equivalents are multiples of resting metabolic rate, specifically resting oxygen consumption, where $1 \text{ MET} = 3.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$

MetS – Metabolic syndrome is a cluster of cardiovascular disease risk factors

MI – Myocardial infarction is also known as a heart attack, where a section of heart tissue dies

Min·wk⁻¹ – Minutes per week of activity performed

mL·kg⁻¹·min⁻¹ - millilitres of oxygen consumed per kilogram of body mass per minute is a measure of oxygen consumption relative to body mass

MVO₂ – Myocardial oxygen demand is the volume of oxygen required by the heart per minute, an index of which can be estimated from heart rate and systolic blood pressure

NEFA – Non-esterified fatty acids are hydrocarbon chains mobilised from triacylglycerol stores in adipose tissue

NHANES II – Second National Health and Nutrition Survey is a national health survey from the United States conducted between 1976 and 1992

NHANES III – Third National Health and Nutrition Survey is a national health survey from the United States conducted between 1988 and 1994

NHANES 1999-2000 –National Health and Nutrition Survey is a national health survey from the United States conducted between 1999 and 2000

PA – Physical activity is any bodily movement produced by skeletal muscles that results in increased energy expenditure

PAI-1 – Plasminogen activator inhibitor-1 is an inhibitor of fibrinolysis and increases the risk of blood clotting

PLTP(a) – Phospholipid transfer protein (activity) relates to the activity of an enzyme that exchanges surface phospholipids from TAG-rich lipoproteins to HDL-C

PP – Pulse pressure is the additional pressure on diastolic blood pressure exerted by contractions of the heart and is the difference between SBP and DBP

Pre β ₁-HDL-C – Pre-beta-high-density lipoprotein cholesterol is the initial plasma acceptor of cell-derived cholesterol plasma

RCT – Randomised Controlled Trials are investigations where participants are randomly allocated to either a control group or the experimental group

RevCholT – Reverse cholesterol transport is the process where cholesterol is removed from peripheral tissues and returned to the liver to be re-processed

RMR – Resting metabolic rate refers to resting energy expenditure, measured in kcal

RPP – Rate-pressure product an index of myocardial workload, estimated from heart rate and systolic blood pressure

SB – Short bout refers to studies investigating the efficacy of accumulative short exercise sessions vs. longer sessions of exercise

SBP – Systolic blood pressure is the pressure exerted by the heart, blood and blood vessels when the heart contracts, measured in millimetres of mercury (mm Hg)

SBW – Single brisk walking group refers to those participants who performed brisk walking in one single daily brisk walking session

SeDS – Sedentary death syndrome describes a collection of physical inactivity-induced chronic diseases, such as type II diabetes mellitus

SNS – Sympathetic nervous system is the component of the autonomic nervous system generally responsible for excitation

TAG – Triacylglycerol is a fat source incorporating three fatty acids bound together by a glycerol molecule

TC – Total cholesterol is the term used for all of the micellular cholesterol-carrying molecules in the circulation

TC/HDL-C – Total cholesterol/High-density lipoprotein cholesterol ratio is a ratio of total cholesterol to ‘healthy’ cholesterol and provides an index of total cholesterol to ‘healthy’ cholesterol

tPA – Tissue plasminogen activator is involved in the the process of fibrinolysis which decreases the risk of blood clots

USGR – US Surgeon General Report is a report published in 1996 to promote participation in physical activity for public health

VLDL-C – Very-low density lipoprotein cholesterol carries triacylglycerol in the circulation

$\dot{V}O_{2max}$ – **Maximal oxygen uptake** is the maximal volume of oxygen that may be consumed during maximal intensity aerobic activity, measured in $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$

$\dot{V}O_{2peak}$ – **Peak oxygen uptake** is the peak volume of oxygen measured during an incremental test but without meeting the criteria for a maximal test, also measured in $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$

WC – Waist circumference is the circumference around the waist at the level of the umbilicus and is used to estimate abdominal adiposity

WHR - Waist:hip ratio is a measure of abdominal adiposity using hip circumference as a point of reference

WHO – World Health Organisation promotes health worldwide

30×50% - 30 minutes of walking performed at 50% $\dot{V}O_{2max}$

30×65% - 30 minutes of walking performed at 65% $\dot{V}O_{2max}$

60×50% - 60 minutes of walking performed at 50% $\dot{V}O_{2max}$

1.0 Introduction

The term metabolic syndrome (MetS) is used to classify a cluster of co-related cardiovascular disease risk factors that predict coronary heart disease and diabetes (Wannamethee *et al.*, 2005). Initially, Reaven (1988) used the term ‘syndrome X’ to describe a disorder characterised by insulin resistance, hypertension and dyslipidaemia, which are mediated through insulin resistance-induced compensatory hyperinsulinaemia, and abdominal obesity was later included to make the ‘deadly quartet’ of related CVD risk factors (Kaplan, 1989), where abdominal obesity appears to be more biochemically toxic to health than total adiposity (Moller & Kaufman, 2005). This clustering of risk factors is reported to increase the odds ratio for cardiovascular and all-cause mortality above the risk associated with the individual components (Wilson, 2004). Insulin resistance is a precursor for the more serious type II diabetes mellitus (T2D), where tissues of the body become resistant to the action of insulin, causing compensatory hyperinsulinaemia. Hypertension is a chronic elevation in systolic and diastolic blood pressure and hypertriglyceridaemia is a disorder of blood lipids marked by high levels of triacylglycerol (TAG). Due to the perturbations in TAG metabolism related to MetS, an atherogenic dyslipidaemic profile occurs, where low levels of the ‘healthy’ high-density lipoprotein cholesterol (HDL-C) and elevations in pathogenic total cholesterol (TC) and small dense low-density lipoprotein cholesterol (LDL-C) are common. Due to these related disorders of lipoprotein metabolism in MetS, agents involved in reverse cholesterol transport (RevCholT), which is the process by which cholesterol is transported from peripheral tissues to be processed by the liver, are disrupted (Moller & Kaufman, 2005). Pro-inflammatory and prothrombotic states have been implicated with MetS, including markers such as C-reactive protein (CRP) and plasminogen activator inhibitor-1 (PAI-1) (Reaven, 1994).

The precise aetiology of MetS is currently unknown, however an energy dense diet, particularly high in carbohydrate and fat, and an inactive lifestyle or low fitness may interact with a genetic susceptibility to contribute to the pathophysiology of MetS (Bouchard, 2007). Other purported precedents include gender (male), (Fox *et al.*, 2007), age (Schubert *et al.*, 2006), stress-neuroendocrine disorders (Kyrou *et al.*, 2006) and post-viral disorders (Vasilakopoulou & le Roux, 2007). Despite the aetiology of MetS still being debated, the future health outcomes are relatively clear: MetS may increase the risk of cardiovascular death 2-4 fold and reduce life expectancy by 5-10 years (Libby & Theroux, 2005), and using the National Cholesterol Education Panel Adult Treatment Panel III (ATPIII) definition of MetS the estimated worldwide prevalence of MetS may be ~56.7% and ~43.6% in men and women, respectively (Resnick, 2002). Due to the prevalence and potential costs of MetS, both financially and in terms of morbidity and mortality, those individuals diagnosed with the condition are encouraged to undergo lifestyle modification, with habitual physical activity (PA) (Carroll & Dudfield, 2004), aspects of diet (Mendoza *et al.*, 2007) and medication (Williams & Franklin, 2007) receiving most attention. Focusing on the impact of PA in particular, evidence demonstrates that long term participation in PA may improve aerobic fitness ($\dot{V}O_{2max}$) (Church *et al.*, 2007), obesity level (Jakicic, 2003), abdominal obesity (Kay & Fiatarone Singh, 2006), insulin resistance & glucose tolerance (Henriksen, 2002; Yates *et al.*, 2007), blood pressure (Pescatello, 2005), lipid and lipoprotein profile (Durstine *et al.*, 2001), markers of pro-inflammatory & pro-thrombotic states (Kasapis & Thompson, 2005; El-Sayed *et al.*, 2005) and markers of RevCholT (Seip *et al.*, 1993).

Current public health guidelines recommend that individuals perform at least 30 minutes per day ($\text{min}\cdot\text{d}^{-1}$) of moderate intensity PA on at least five days of the week ($\text{d}\cdot\text{wk}^{-1}$) or

vigorous intensity PA at least three $\text{d}\cdot\text{wk}^{-1}$ (American College of Sports Medicine (ACSM) & American Heart Association (AHA), 2007). These recommendations also support the accumulation of PA throughout the day in activities of daily living, such as walking, using stairs and household activities. Much of the research into the efficacy of accumulative PA has used walking as the mode of exercise, however many of these studies use changes in aerobic fitness ($\dot{V}\text{O}_{2\text{max}}$) as a surrogate measure of health outcomes. Therefore, the aims of study one in section 3.0 was to determine a) whether brisk walking performed for 30 $\text{min}\cdot\text{d}^{-1}$ at an intensity $>65\%$ of age-predicted maximum heart rate (HR_{max}) on five $\text{d}\cdot\text{wk}^{-1}$ for 24 weeks could improve risk factors associated with MetS and markers of RevCholT compared to a control group, and b) whether accumulating the same volume of brisk walking through the day in sessions of at least 10 minutes and less than 20 minutes would have a similar effect.

In addition to the long term adaptations associated with habitual PA, there are also immediate, although short-term, benefits that may be accrued from a single PA session. Evidence demonstrates that short-term improvements in independent MetS risk factors, such as blood glucose control (Englert *et al.*, 2006), blood pressure (Jones *et al.*, 2007), lipid and lipoprotein profile (Ferguson *et al.*, 1998), markers of pro-inflammatory (Murtagh *et al.*, 2005a) and pro-thrombotic states (Ivey *et al.*, 2003) may be gained from a single session of PA. Therefore, the aims of the follow-up study, (study two in Section 4.0), were to determine a) whether walking at $50\% \dot{V}\text{O}_{2\text{max}}$ for 30 minutes may have an effect on risk factors associated with MetS during the 24 hour post-exercise period compared to a control trial, and b) whether increasing the intensity of the walk to $65\% \dot{V}\text{O}_{2\text{max}}$ for 30 minutes or maintaining the intensity at $50\% \dot{V}\text{O}_{2\text{max}}$ and increasing the duration to 60 minutes would alter the magnitude or duration of these responses.

2.0 Review of literature

2.1 Metabolic syndrome: Definitions and mechanisms

The concept of metabolic syndrome has been noted for 50 years, where obesity was recognised as a predisposition to diabetes and atherosclerosis (Vague, 1956). However, it was not until Reaven's Banting Lecture in 1988 when insulin resistance was highlighted as a major public health problem associated with hypertension and hypertriglyceridaemia (Reaven, 1988). Subsequently, 'upper-body obesity', otherwise known as abdominal obesity, was added to the syndrome, which then became known as the 'deadly quartet' of insulin resistance, obesity, hypertension and hypertriglyceridaemia (Kaplan, 1989). Since then a number of related terms have been used to describe the syndrome, such as 'athero-thrombogenic syndrome', 'beer-belly syndrome' and 'chronic cardiovascular risk factor clustering syndrome', but for ease of reference in this thesis it will be termed *metabolic syndrome* (MetS) (Ford, 2004a). Insulin resistance is characterised by decreased insulin-mediated glucose clearance from the blood and abdominal obesity is an accumulation of adipose tissue particularly around the internal organs (viscera) and is a potent mediator of fatty acids (NEFA) and chemical messengers (adipokines) into the circulation (Moller & Kaufman, 2005). Hypertension is a chronic elevation in blood pressure that is mediated through increased sympathetic nervous system activity and impaired renal function and hypertriglyceridaemia relates to the elevations in blood triacylglycerol (TAG), which is associated with visceral obesity and is recognised today as dyslipidaemia, which also includes low levels of the 'healthy' high-density lipoprotein cholesterol (HDL-C) and elevations in pathogenic total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C). Pro-thrombotic and inflammatory markers have also since been implicated in the pathology of MetS (Reaven, 1994; Devaraj *et al.*, 2004).

2.1.1 Obesity

The purpose of adipose tissue is to store calories as TAG and release the energy in the form of NEFA when needed, but once the capacity of the adipocytes is exceeded, TAG accumulates in hepatocytes, myocytes and visceral adipocytes (Miranda *et al.*, 2005). Excess TAG in myocytes and abdominal adipose tissue appears to cause insulin resistance within these cells. Total body fat can be assessed using a number of techniques, such as body mass index (BMI), body fat percentage, using skin fold callipers or more elaborate techniques, such as underwater weighing (Wajchenberg, 2000). Furthermore, since abdominal adiposity is an indicator of metabolic health, simple techniques such as waist circumference and waist:hip ratio (WC divided by hip circumference; WHR) may be used to estimate abdominal adiposity in the absence of more elaborate techniques, such as computed tomography and magnetic resonance imaging. Waist circumference is significantly associated with MetS risk factors across white black and Hispanic Americans and can be used as a non-invasive clinical marker for the presence of MetS, and thus for assessing cardiovascular risk (Okosun *et al.*, 2000), and is one of the best predictors of MetS (odds ratio 1.7 per 11 cm) (Palaniappan *et al.*, 2004).

Positive energy balance and increased adiposity are capable of provoking other aspects of MetS, where the pathogenesis of visceral fat (fat accumulation around the internal organs) on metabolic processes may be mediated through the release of damaging molecules or the juxtaposition of visceral fat allowing direct provision of NEFA to the liver (Moller & Kaufman, 2005). Larger adipocytes (fat cells), particularly visceral adipocytes, are more metabolically active, with higher rates of lipolysis and visceral adiposity may drive the impact of NEFA on insulin resistance through enhanced release of NEFA due to their large number of β -adrenergic receptors. The greater presence of these receptors increases the

release of NEFA into the blood, thus increasing the risk of NEFA-mediated muscular insulin resistance and hepatic triacylglycerol-rich very low-density lipoprotein-cholesterol (TAG-rich VLDL-C) output (Björntorp, 1990). Data indicate that activities of lipoprotein lipase (LPLa) and hormone-sensitive lipase increase more in visceral adipose tissue with fat feeding relative to subcutaneous adipose tissue leading to enhanced flux of NEFA into the portal circulation of the liver (Bergman *et al.*, 2007). However, the high rates of lipolysis need to be matched with equally high rates of deposition through maintenance of positive energy balance, otherwise the visceral fat depot would disappear (Frayn, 2000).

As well as aspects of MetS being mediated through elevations in circulating NEFA related to visceral obesity, adipose tissue-derived increases in adipokines (fat hormones), such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), resistin and reduced adiponectin secretion may also mediate components of MetS (Bergman *et al.*, 2007). Increased adipocyte mass is also associated with increased expression of angiotensinogen, plasminogen activator inhibitor-1 (PAI-1) and leptin (Miranda *et al.*, 2005). Angiotensinogen is implicated with hypertension in the presence of obesity, PAI-1 is related to anti-fibrinolysis, leptin is involved in suppressing appetite and TNF- α and resistin are involved in obesity-mediated insulin resistance. When adipose tissue becomes resistant to the action of insulin, the anti-lipolytic effect of insulin on adipose tissue TAG lipolysis is lost and NEFA release continues despite no metabolic need and either causing or amplifying muscular and hepatic insulin resistance (Björntorp, 1994). However, over the course of the day plasma NEFA concentrations are highly variable, which generally precludes their practical use in clinical medicine (Miranda *et al.*, 2005).

2.1.2 Insulin resistance

Metabolic syndrome may manifest itself through impaired glucose tolerance (IGT) or insulin resistance. This results in hyperinsulinaemia to compensate for the decreases in insulin-mediated glucose uptake (Reaven, 1988; 2001) and insulin resistance, characterised by IGT or type II diabetes, is the feature most often suggested as the underlying cause of the symptoms of MetS (Robinson & Graham, 2004). Insulin resistance may be a secondary phenomenon of excess fat, where even normal weight individuals may be metabolically obese and present increased abdominal obesity (Moller & Kaufman, 2005). Visceral obesity in particular may contribute significantly to insulin resistance, because abdominal adipose tissue is capable of secreting adipokines, such as adiponectin and resistin, which may contribute to insulin resistance. Larger adipocytes secrete lower levels of adiponectin, an adipokine that enhances insulin sensitivity by reducing lipid accumulation, and secrete a greater amount of resistin, which induces insulin resistance, increases plasma glucose and enhances hepatic glucose production. Insulin resistant adipocytes continue to secrete NEFA, despite the hyperinsulinaemic state, due to the inability of insulin to suppress lipolysis (Miranda *et al.*, 2005). These NEFA are transported to the liver, which stimulates hepatic glucose output, and to muscle where they appear to inhibit insulin-mediated glucose disposal, thus impairing glucose clearance from the circulation.

Acute increases in NEFA inhibit muscular glucose oxidation through the glucose-fatty acid cycle, where elevations in NEFA cause preferential NEFA oxidation rather than glucose oxidation and the accumulation of muscular glycogen, mediated through increases in muscular glucose, decreases insulin-mediated glucose uptake through decreased presentation of glucose transporter-4 (GLUT-4) on the plasma membrane (Moller & Kaufman, 2005). Disruptions to muscular NEFA disposal may contribute to insulin

resistance, however evidence for this appears to be confined to only those with a BMI ≥ 35 kg·m⁻² and it is difficult to differentiate cause and effect between insulin resistance and obesity, since both influence glucose and NEFA clearance from the circulation (Blaak, 2005). Therefore, in lean individuals with impaired glucose tolerance, insulin resistance may be initiated through initial decreases in mitochondrial oxidative phosphorylation, where both fatty acids and glucose oxidation is insufficient, thus causing accumulation of these substrates within the muscle, which may be exhibited in lean and healthy individuals (Davidson & Yannicelli, 2006). Chronically, long-term obesity-induced elevations in circulating NEFA lead to increased intramuscular TAG, which also impairs insulin signalling and intracellular TAG may also accumulate in pancreatic islet β -cells and possibly impair insulin secretion (Moller & Kaufman, 2005). The maintenance of normal postprandial glucose homeostasis relies on pancreatic β -cells to secrete sufficient insulin to stimulate muscular glucose uptake, which is responsible for the disposal of 80-90% of the ingested glucose and suppress endogenous glucose production, of which over 80% derives from the liver (Miranda *et al.*, 2005). For a given level of obesity an individual may have normal glucose tolerance, impaired glucose tolerance or type II diabetes mellitus, where the degree of the impairment is related to insulin sensitivity and β -cell reserve capacity, and long term over-stimulation of pancreatic β -cells to maintain euglycaemia can lead to β -cell exhaustion and blood glucose levels begin to rise, eventually manifest as type II diabetes mellitus.

2.1.3 Dyslipidaemia

A triad of lipid abnormalities are associated with MetS, forming an atherogenic blood lipid profile, including hypertriglyceridaemia, low HDL-C and greater concentrations of small dense LDL particles, where hypertriglyceridaemia is the central metabolic defect leading to risk of atherosclerosis rather than the usual culprit, elevated LDL-C (Moller & Kaufman, 2005). Data indicate that both insulin resistance and obesity determine the lipoprotein profile, where those with insulin resistance have greater intra-abdominal fat, which are related to a higher cholesterol content in the VLDL, intermediate-density lipoproteins (IDL), small dense LDL particles and a lower cholesterol content in HDL particles (Nieves *et al.*, 2003). Plasma HDL-C concentrations are one of the best predictors of the incidence of MetS (odds ratio 0.6 per 0.38 mmol·L⁻¹) (Palaniappan *et al.*, 2004). Hypertriglyceridaemia was recognised as being related to insulin resistance and the accompanying hyperinsulinaemia 40 years ago (Reaven *et al.*, 1967) through increases in hepatic TAG-rich VLDL-C output, secondary to hepatic insulin resistance (Adiels *et al.*, 2006), and elevations in insulin and NEFA due to skeletal muscle and adipose tissue insulin resistance (Boden, 2006).

Circulating plasma insulin concentrations determine the hepatic TAG-rich VLDL-C output response to the hepatic NEFA input dose, where the higher the insulin concentration the greater the degree of NEFA that enter the liver and are synthesised into, and secreted as, TAG (Reaven, 2005). This is due to the maintenance of insulin sensitivity by the liver in the face of adipose tissue and muscular insulin resistance-mediated compensatory hyperglycaemia, and small dense LDL particles are associated with this condition. Hepatic lipase hydrolyses TAG-rich lipoproteins, including TAG-rich HDL, which serves as a potential mechanism by which insulin resistance-induced increases in plasma NEFA

increase hepatic TAG production, which is transferred from TAG-rich VLDL to HDL particles to form TAG-rich HDL by the action of cholesteryl ester transfer protein (CETP) (See section 2.3) (Moller & Kaufman, 2005). Furthermore, this same mechanism may be how LDL-C are transformed into small dense LDL particles in MetS, due to LDL-C being enriched with TAG by CETP and being cleared from the circulation by hepatic lipase, leaving only small dense LDL particles (Kathiresan *et al.*, 2006). Compared with individuals with no MetS risk factors, those individuals characterised with MetS have smaller mean LDL particle size, which are associated with intima media (artery wall) thickness and inversely related to plaque occurrence and size (Hulthe *et al.*, 2000).

2.1.4 Hypertension

More than 85% of individuals with MetS have elevated blood pressure or hypertension (Franklin, 2006) and data indicates that individuals with MetS and elevated blood pressure demonstrate greater carotid atherosclerosis compared with those who present aspects of MetS but not elevated blood pressure (Irace *et al.*, 2005). This illustrates the relative importance of the presence of hypertension in MetS as a predictor of CVD risk. Isolated systolic hypertension, without diastolic hypertension, is the most common hypertensive subtype in MetS, however isolated diastolic hypertension generally occurs at a younger age and exposes those to greater cardiovascular risk in the long term (Franks, 2006). Prospectively, baseline body mass predicts future increases in blood pressure, as does baseline blood pressure for future increases in body mass, i.e. elevated blood pressure at baseline indicates greater future risk of overweight/obesity (Julius *et al.*, 2000). It has been postulated that there is a two-way relationship between obesity and blood pressure through a primary increase in sympathetic tone, whereby increased energy consumption, leading to positive energy balance, may enhance sympathetic tone and conversely β -adrenergic responsiveness in adipocytes may become diminished due to the sympathetic overactivity in hypertension.

Mechanisms for the presence of elevated blood pressure in MetS include enhanced sympathetic nervous system (SNS) activity, insulin resistant blood vessels, insulin-mediated enhanced tubular sodium reabsorption, increased release of regulatory agents from enlarged adipocytes, such as angiotensinogen, and an association between NEFA and blood pressure. Sympathetic nervous system activity is elevated in individuals with insulin resistance (Reaven, 2001), where infused insulin can augment SNS activity and provides evidence for a link between insulin resistance and hypertension (Moller & Kaufman,

2005). Insulin normally mediates vasodilation, however vascular tissues may become insulin resistant and thus non-responsive. Insulin acutely increases renal sodium retention, a further example of an organ retaining sensitivity to insulin, despite muscular insulin resistance (Reaven, 2001). Increased adipocyte mass leads to increased angiotensinogen production and angiotensin-converting enzyme (ACE), which both predispose to elevations in blood pressure, through their effects on renal function (Miranda *et al.*, 2005). Systematic evidence suggests that circulating NEFA may also promote hypertension in insulin resistance, however the mechanism have not been elucidated, with suggestions including α_1 -adrenergic stimulation, endothelial dysfunction, increased oxidative stress or stimulation of vascular cell growth (Sarafidis & Bakris, 2007).

2.1.5 Emerging components

The metabolic CVD risk factors associated with MetS do not appear to fully explain the elevated CVD risk associated with the syndrome, therefore other abnormalities may contribute (Sakkinen *et al.*, 2000). Obesity leads to a pro-thrombotic and pro-inflammatory state that potentiates atherosclerosis (Moller & Kaufman, 2005). Elevations in plasma concentrations of fibrinogen and plasminogen activator inhibitor-1 (PAI-1) are associated with hypertriglyceridaemia and hypertension, and likely to be related to insulin resistance and compensatory hyperinsulinaemia (Reaven, 2001; Alberti *et al.*, 2006). PAI-1 inhibits tissue plasminogen activator, which is a key regulator of the endogenous fibrinolysis system and thus thrombus formation, and PAI-1 is highly correlated with all components of MetS (Mertens *et al.*, 2006). PAI-1 may even be an early marker of MetS and is secreted primarily by liver and endothelial cells, but also from adipose tissue, visceral adipose tissue in particular (Robinson & Graham, 2004). The greater the adipocyte and adipose tissue mass the greater the contribution to circulating PAI-1 because visceral adipose tissue has a greater capacity to synthesise PAI-1 than subcutaneous adipose tissue, which indicates the mechanism by which obesity may lead to impaired fibrinolysis (Skurk & Hauner, 2004). Where PAI-1 tends to cluster with BMI, fibrinogen, a strong risk factor for CHD (Yudkin *et al.*, 2000), clusters with markers of inflammation (C-reactive protein; CRP), suggesting that it is a marker of underlying inflammation rather than pro-coagulant potential (Sakkinen *et al.*, 2000).

Inflammation plays an important role in the pathogenesis of atherosclerosis, where excess adiposity is associated with the release of inflammatory adipokines and contributes to an increase in CRP, which is an inflammatory marker and a CVD risk factor (Moller & Kaufman, 2005). Increases in circulating adipokines may mediate the obesity-induced

increases in fibrinogen and PAI-1 levels (Moller & Kaufman, 2005). Inflammatory factors that may be predictors or markers of CVD risk include CRP, IL-6 and TNF- α , where CRP predicts future CVD risk even in apparently healthy individuals (Yudkin *et al.*, 2000). Associations between CRP with atherogenic dyslipidaemia in insulin resistance is mediated through increased fat mass and visceral adipose tissue accumulation, where CRP is significantly associated with fat mass and visceral adipose tissue and glucose tolerance, rather than blood lipid and lipoprotein profile in men with atherogenic dyslipidaemia (Lemieux *et al.*, 2001).

Microalbuminuria, hyperuricaemia and hyperhomocysteinemia are further parameters that have also been implicated with the presence of MetS. The involvement of microalbuminuria in MetS is considered by some to be controversial because there is no proven mechanistic evidence relating to an underlying cause for the elevated urinary albumin in MetS, however statistical associations have been found to suggest that microalbuminuria may be related to one of the observed manifestations of the syndrome (Alberti *et al.*, 2006). Uric acid has also been implicated MetS because there is an association between coronary heart disease (CHD) and uric acid, which is also present with insulin resistance (Reaven, 2001). However, the relationship between uric acid and CHD appears to be an epiphenomenon, where kidneys retain insulin sensitivity, even when other organs of the body are insulin resistant, and respond to the day long elevations in insulin by decreasing urinary uric acid clearance. Due to the effects of insulin on renal function, increased sodium retention (pre-cursor for high blood pressure) is also associated with decreased renal uric acid clearance. Thus, elevated uric acid concentrations are merely markers of insulin resistance mediated through hyperinsulinaemia and impaired renal function. Homocysteine is an amino acid that has been implicated in MetS because

moderate hyperhomocysteinemia is an independent risk factor for atherosclerotic and thrombotic morbidity and mortality, through altering vessel wall connective structure and enhancing sympathetic activity (Garcin *et al.*, 2006). However, it has only been weakly correlated with systolic & diastolic blood pressure and blood cholesterol & TAG, but not at all with BMI, WHR, blood glucose, insulin resistance, HDL-C and LDL-C. Therefore, despite hyperhomocysteinaemia being a useful risk marker in high-risk cardiovascular patients, it has little association with MetS.

2.1.6 Metabolic syndrome and cardiovascular disease

Coronary artery disease (CAD) is a form of CVD specifically related to the circulation of the heart, where blood flow becomes impaired by atherosclerosis, which is a “multi-focal, smouldering, immuno-inflammatory disease of medium-sized and large arteries fuelled by lipids” that decreases the lumen of coronary arteries (Falk, 2006). Atherosclerosis-induced CAD may lead to coronary heart disease (CHD), a precursor for conditions such as myocardial infarction (MI), which is where perfusion is limited enough to cause permanent ischaemia and results in the area of myocardium that it supplies becoming infarcted. Atherosclerosis *per se* is rarely fatal, it is the thrombosis that occurs that forms on a ruptured plaque that precipitates the fatal cardiovascular events, such as stroke and MI (Falk, 2006). Risk factors for thrombosis include elevated levels of fibrinogen and PAI-1, an inhibitor of anti-coagulation processes, and thus decreased fibrinolysis (Libby & Theroux, 2005). Elevated serum cholesterol is sufficient to drive the development of atherosclerosis in the absence of other risk factors, however these risk factors, such as hypertension, type II diabetes mellitus and inflammatory markers, appear to accelerate a disease driven by atherogenic lipoproteins, particularly LDL-C. The accelerating properties of these risk factors may include increasing the atherogenicity of the LDL-C, i.e. particle size, number or composition, or increase the susceptibility of the arterial wall (Falk, 2006). Indeed, among patients with MetS, the progression of atherosclerotic stenosis has been shown to be twice as fast in patients with aortic stenosis (Briand *et al.*, 2006). These risk factors are components of MetS, therefore it is clear that being diagnosed with MetS may be a strong indicator of CVD development, especially where MetS may increase the risk of cardiovascular death 2-4 fold and reduce life expectancy by 5-10 years (Libby & Theroux, 2005).

A meta-analysis involving >300,000 participants has demonstrated that the adverse effects of overweight on blood pressure and blood cholesterol may account for a 45% increased CHD risk, both independently and additively (Bogers *et al.*, 2007). Stroke is the second highest cause of mortality worldwide and MetS has been implicated with stroke as well as CHD (Bang, 2006). Analysis of data from NHANES III demonstrated that ATPIII-defined MetS was significantly associated with future myocardial infarction (MI)/stroke development, where insulin resistance, hypertension, hypertriglyceridaemia and low HDL-C were also independently related to MI/stroke (Ninomiya *et al.*, 2004). The WHO (1998) definition of MetS has been found to identify individuals with increased risk of cardiovascular morbidity and mortality (Isomaa *et al.*, 2001), as has the ATPIII definition, using participants involved in NHANES III (Alexander *et al.*, 2003), which has also been demonstrated to predict new-onset diabetes (Sattar *et al.*, 2003). However, evidence from a cross-ethnic population in west London comprising 2,346 Europeans, 1,711 South Asians and 803 African-Caribbeans suggests that the WHO (1998) and ATPIII (Cleeman, 2001) definitions of MetS provide an inconsistent picture of CVD risk across different ethnic groups and that ethnic-specific definitions should be developed (Tillin *et al.*, 2005). The WHO (1998) and ATPIII (Cleeman, 2001) definitions of MetS predicted CVD prevalence in European and South Asian men, however the associations were weaker for African-Caribbeans and inconsistent among European women.

2.1.7 Aetiology of metabolic syndrome - sedentariness

Metabolic syndrome is a multi-factorial condition and consequently there are a multitude of possible causes, where just as the diagnosis of MetS requires a combination of risk factors (hypertension, insulin resistance, abdominal obesity & dyslipidaemia) there are also different lifestyle and genetic factors that encourage the development of MetS. Possible determinants of MetS include modifiable factors such as sedentariness – an inactive lifestyle – by promoting positive energy balance, and nutrition, through elevated energy consumption or an imbalanced intake of macronutrients, such as carbohydrates, which may increase energy balance and/or alter metabolism. Non-modifiable determinants of MetS include a genetic predisposition, gender (male), age (\geq middle age), and more transient factors such as neuroendocrine imbalances and viral infections. Data from NHANES III suggests that when a combination of several low-risk lifestyle factors (physically active, non-smoking and moderate carbohydrate & fat consumption) are present in individuals with a BMI $<30 \text{ kg}\cdot\text{m}^2$ those individuals are at a lower risk of developing MetS (Zhu *et al.*, 2004). These determinants may act independently, collectively or contribute to each other in a knock on effect. Due to the nature of this thesis the contribution of physical (in)activity will be discussed here.

Sedentary behaviour impacts on MetS risk, where low levels of PA are associated with increased risk of obesity and its accompanying co-morbidities, such as hypertension, insulin resistance and dyslipidaemia, and performing less than $150 \text{ min}\cdot\text{wk}^{-1}$ of moderate-vigorous leisure time PA increases the risk of developing MetS two-fold (Ford *et al.*, 2005). Decreased energy expenditure is the likely mediator between physical inactivity and increased MetS risk, where objectively measured PA energy expenditure (EE) has been demonstrated to predict the progression towards MetS, independently of $\dot{V}\text{O}_{2\text{max}}$ and

obesity (Ekelund *et al.*, 2005). The relationship between PA and MetS risk appears to be unaffected by $\dot{V}O_{2\max}$ or adiposity, suggesting that improvements in $\dot{V}O_{2\max}$ or decreases in adiposity are not absolutely necessary to decrease MetS risk, providing that PAEE increases. However, further studies are needed to confirm these slightly controversial findings, since men with a $\dot{V}O_{2\max} \leq 29.1 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ are almost 7 times more likely to have MetS than those with a $\dot{V}O_{2\max} \geq 35.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (Lakka *et al.*, 2003). Middle-aged men performing less than $3 \text{ hrs}\cdot\text{wk}^{-1}$ of moderate-vigorous intensity leisure time PA are twice as likely to develop MetS compared to those performing more than $3 \text{ hrs}\cdot\text{wk}^{-1}$ of moderate intensity leisure time PA, and there is an even stronger inverse relationship between vigorous leisure time PA and MetS risk (Laaksonen *et al.*, 2002). Men in the upper third of $\dot{V}O_{2\max}$ classification are 75% less likely to develop MetS than those in the lowest third. A subsequent study from the same research group demonstrated that men engaging in less than $1 \text{ hr}\cdot\text{wk}^{-1}$ of at least moderate intensity PA were at a 60% greater risk of developing MetS than those performing more than $3 \text{ hr}\cdot\text{wk}^{-1}$ of at least moderate intensity PA (Lakka *et al.*, 2003). These findings are important because they indicate the crucial role of regular PA on decreasing future MetS risk and treating those at risk or with current MetS, which is the basis for this thesis.

2.1.8 Summary

The presence of MetS may be related in part to adiposity and fitness levels, but the overriding factor is likely to be of genetic origin, which predisposes individuals to some degree of impaired glucose tolerance, dyslipidaemia (\uparrow TAG & \downarrow HDL), essential hypertension and a pro-coagulant and pro-inflammatory state (Reaven, 2006). Although the exact pathogenesis of MetS is not yet certain, it is generally postulated that a metabolic susceptibility, such as genetic insulin resistance, racial/ethnic factors, endocrine dysfunction, increased abdominal fat, physical inactivity and/or advancing age, may interact with obesity to present components of MetS (Grundy, 2006). Each of the individual components of MetS are independently related to increased risk of CVD, however when present in combination these components may lead to even greater CVD risk than the sum of their parts (Wilson, 2004). Therefore, MetS may be considered as an intermediary between discrete CVD risk factors and those who develop diabetes and CVD. The diagnosis of MetS requires the individuals to present a number of CVD risk factors, however, the classification of MetS is controversial since the World Health Organisation (WHO) (Alberti & Zimmet, 1998), National Cholesterol Education Panel Adult Treatment Panel III (ATPIII) (Cleeman, 2001), European Group for the Study of Insulin Resistance (EGIR) (Balkau & Charles, 1999) and International Diabetes Federation (IDF) (Alberti *et al.*, 2006) have proposed subtly different classification criteria (Table 2.1). Generally, MetS represents a cluster of metabolic abnormalities, characterised by the presence of 3 or more of 1) abdominal obesity, 2) insulin resistance, 3) hypertension, 4) dyslipidaemia, and 5) emerging risk factors, such as pro-thrombotic and pro-inflammatory states, which are each independent cardiovascular disease (CVD) risk factors.

Table 2.1 Definitions of MetS

WHO (Alberti & Zimmet, 1998)	EGIR (Balkau & Charles, 1999)
<ul style="list-style-type: none"> • Impaired fasting glucose/impaired glucose tolerance/insulin resistance or diabetes, <u>and 2 or more of:</u> <ul style="list-style-type: none"> • Blood pressure – $\geq 160/90$ mmHg • Plasma TAG – ≥ 1.7 mmol·L⁻¹ and/or HDL-C < 0.9 mmol·L⁻¹ in men, and < 1.0 mmol·L⁻¹ in women • Waist:hip ratio – > 0.90 in men, > 0.85 in women, and/or BMI > 30 kg·m⁻² • Microalbuminuria – urinary albumin excretion rate ≥ 20 μg·min⁻¹ or albumin:creatinine ratio ≥ 30 mg·g⁻¹) 	<ul style="list-style-type: none"> • Hyperinsulinaemia – <i>fasting insulin above the upper quartile in non-diabetics, <u>and 2 or more of:</u> <ul style="list-style-type: none"> • Hyperglycaemia – <i>fasting plasma glucose ≥ 6.1 mmol·L⁻¹</i> • Hypertension – ≥ 140 and/or ≥ 90 mmHg, or treatment for hypertension • Dyslipidaemia – <i>plasma TAG ≥ 2.0 mmol·L⁻¹ and/or < 1.0 mmol·L⁻¹ or treatment for dyslipidaemia</i> • Central obesity – <i>waist circumference ≥ 94 cm for men, ≥ 0.80 cm for women</i> </i>
<p>ATPIII (Cleeman, 2001)</p> <ul style="list-style-type: none"> • <u>3 or more of:</u> <ul style="list-style-type: none"> • Waist circumference – > 102 cm in men, > 88 cm in women • Plasma TAG – ≥ 1.69 mmol·L⁻¹ • HDL-C – < 1.04 mmol·L⁻¹ in men, < 1.29 mmol·L⁻¹ in women • Blood pressure – $\geq 130/\geq 85$ mmHg • Fasting blood glucose – ≥ 6.1 mmol·L⁻¹ 	<p>IDF (Alberti <i>et al.</i>, 2006)</p> <ul style="list-style-type: none"> • Central obesity – <i>waist circumference ≥ 94cm for Europid men and ≥ 80cm for Europid women, <u>plus any two of the following four factors:</u></i> • Raised TAG – ≥ 1.7 mmol·L⁻¹, or treatment for dyslipidaemia • Reduced HDL-C – < 1.03 mmol·L⁻¹ in males and < 1.29 mmol·L⁻¹ in females, or treatment for dyslipidaemia • Raised blood pressure – <i>systolic BP ≥ 130 OR diastolic BP ≥ 85 mm Hg, or treatment of previously diagnosed hypertension</i> • Impaired fasting glucose – ≥ 5.6 mmol·L⁻¹, or previously diagnosed type 2 diabetes

WHO: World Health Organisation; EGIR: European Group for the study of Insulin Resistance; ATPIII: National Cholesterol Education Panel Adult Treatment Panel III; IDF: International Diabetes Federation.

The WHO criteria were developed for research, whereas the ATP III criteria are useful in clinical practice. However, joint statements from the American Diabetes Association and the European Association for the Study of Diabetes published simultaneously questioned the relevance of the ‘syndrome’ related to insulin resistance based on a lack of certainty regarding its pathogenesis and a doubt regarding its value as a marker of CVD risk (Kahn *et al.*, 2005a; 2005b). The basis of this argument was due to ambiguity of certain criteria,

i.e. whether to use systolic blood pressure and diastolic blood pressure, or either SBP and DBP, and whether this should be measured in the supine or seated position. Furthermore, waist circumference can be measured at a number of levels, therefore a specific protocol for measuring waist circumference should be specified. However, a recent prospective study has demonstrated that diagnosis of MetS by the WHO, ATP III or IDF defined criteria impart similar diabetes and CVD risk, particularly in men aged ≥ 45 and women aged ≥ 55 (Lorenzo *et al.*, 2007).

Explaining the national and worldwide epidemiology of MetS has proven difficult due to differences in study design, sample selection, the precise definition of MetS criteria used and the gender & ages of the studied samples (Cameron *et al.*, 2004). The overall prevalence of MetS is higher when using the WHO definition than with the ATP III or EGIR definitions (Ford & Giles, 2003; Cameron *et al.*, 2004). Using the ATP III definition, Native American females and males demonstrated the highest worldwide MetS prevalence of 56.7% and 43.6%, respectively (Resnick, 2002), whereas French females and males demonstrated the lowest prevalence at 7.0% and 10%, respectively, and the mean worldwide prevalence of ATP III-defined MetS is 27.9% for females and 22.8% for males (Cameron *et al.*, 2004). Studies using the EGIR definition of MetS place older Italian men with the highest worldwide prevalence at 24.6% and Danish females at 16.0%, whereas Mauritian men (9.0%) (Cameron *et al.*, 2003) and Dutch females (8.3%) (Balkau *et al.*, 2002) exhibit the lowest prevalence of EGIR-defined MetS, and the mean worldwide prevalence of EGIR-defined MetS is 10.9% for females and 16.1% for males (Cameron *et al.*, 2004). The WHO-defined worldwide prevalence of MetS is 18.7% for females and 28.2% for males (Cameron *et al.*, 2004), with English males and females aged 40-65 demonstrating the highest prevalence (>44.8 & 33.9%, respectively) and middle-aged

Italian males and females demonstrating the lowest prevalence (12.2 & 5.1%, respectively) (Balkau *et al.*, 2002). Combined data from the San Antonio Heart Study and the Framingham Offspring Study, using either the WHO (1998) and ATPIII (2001) criteria, indicate that MetS affects ~20-30% of middle-aged Americans, and these individuals are at increased risk of diabetes and CVD than those without MetS (Meigs *et al.*, 2003). Data from the Third National Health and Nutrition Survey 1988-1994 (NHANES III) show that the prevalence of MetS was 23.9% using the ATPIII definition and 25.1% using the WHO definition in 8,608 US adults and 86.2% of these were classified as having MetS under both definitions. Furthermore, the proportion of individuals in the US meeting the ATPIII MetS criteria increased from 24.1% reported in the NHANES III to 27.0% reported in the National Health and Nutrition Survey 1999-2000 (NHANES 1999-2000), primarily due to increases in waist circumference, hypertension and hypertriglyceridaemia, where 2000 consensus data indicate that ~47 million US residents have MetS (Ford *et al.*, 2002), and the increasing prevalence of MetS is likely to lead to future increases in diabetes and CVD (Ford *et al.*, 2004).

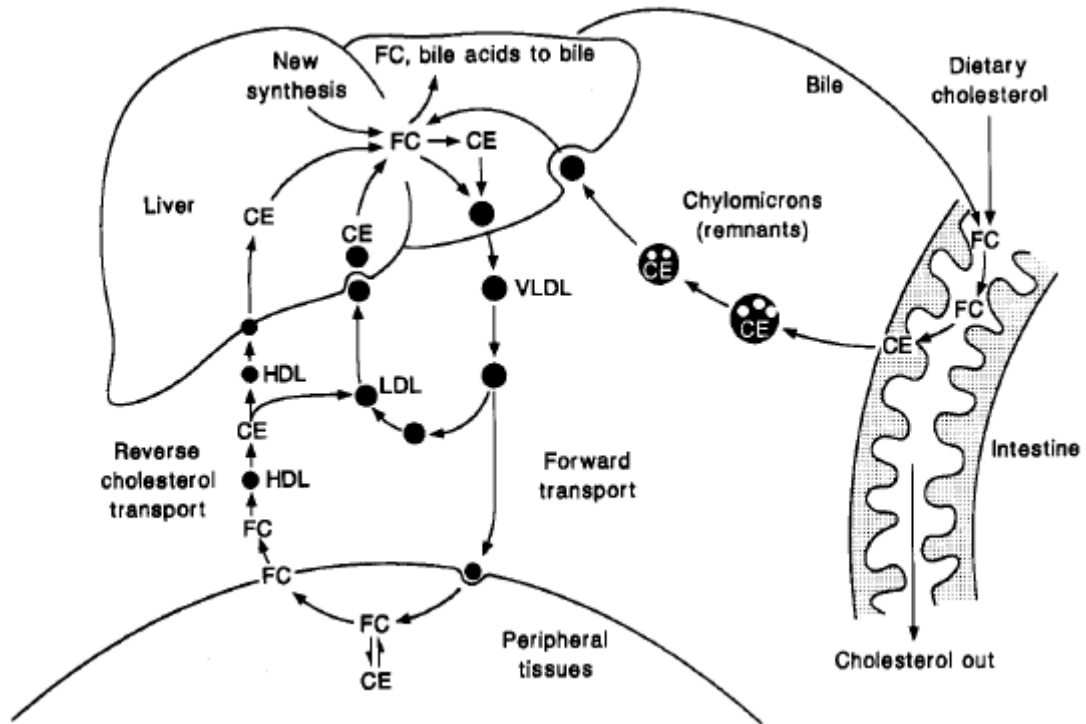
2.2 Reverse cholesterol transport & physical activity

2.2.1 Introduction

The concept of reverse cholesterol transport (RevCholT) was introduced 40 years ago by John Glomset (1968), who proposed that lecithin-cholesterol acyltransferase (LCAT) was involved in the transport of cholesterol from the peripheral tissues to the liver. RevCholT is included in this discussion because each component of MetS – central adiposity, insulin resistance, elevated plasma triacylglycerol and hypertension – is associated with low levels of HDL-C (Lewis & Rader, 2005), which is a primary agent in RevCholT and a component of dyslipidaemia (Barter, 2004). Dyslipidaemia is a derangement in lipid metabolism that manifests itself in elevated levels of serum TAG, TC and LDL-C and suppressed HDL-C. This is not a benign disorder because such changes in the blood lipid profile, especially small dense LDL-C and lower HDL-C, are associated with increased cardiovascular mortality (Zhang *et al.*, 2005; Clarke *et al.*, 2007).

Increased levels of LDL-C and decreased HDL-C create an environment that allows atherosclerotic plaques to form when LDL-C is oxidised into the tunica media of vessels, which increases the thickness of the arterial walls and narrows the lumen (Galle *et al.*, 2006). This can occur throughout the blood vessels of the body, however the greatest risks of atheroma are within the coronary and cerebral circulations, where decreased blood supply can increase the risk of ischaemia leading to myocardial infarction or cerebrovascular accident (Falk, 2006). Nevertheless, the body has the capability of reducing the risk of atheroma development through the process of RevCholT. This is the process by which cholesterol is removed from the circulation by HDL-C before it can be deposited in the artery walls (Figure 1) and also involves LCAT and cholesteryl ester transfer protein (CETP) (Fielding & Fielding, 1995). Other agents also involved include

lipoprotein lipase (LPL), phospholipid transfer protein (PLTP) and hepatic lipase, where increased LPL activity (LPLa) improves HDL-C concentration, whereas increased PLTP activity (PLTPa) and hepatic lipase activity impair HDL-C concentration.



Fielding & Fielding (1995)

Figure 2.1 RevCholT has five steps: 1) uptake of cholesterol from (cholesterol efflux), 2) esterification of free cholesterol (FC) within HDL-C by LCAT, 3) transfer of cholesteryl esters (CE) to the apoB-containing lipoproteins (cholesterol transfer), 4) remodelling of HDL-C by CETP & PLTP and (5) uptake of cholesterol from HDL-C by the liver (cholesterol uptake).

High-density lipoprotein (HDL-C) particles promote the efflux of excess cholesterol from peripheral tissues and return it to the liver for the production of bile in order to maintain cellular cholesterol homeostasis (Lewis & Rader, 2005). Apolipoprotein A-I (apoA-I) is secreted by the liver and intestines and represents ~70% of the apolipoprotein moiety of HDL-C and is present on the majority of HDL-C particles. When apoA-I is secreted it is lipid-poor and acquires cholesterol via lipid efflux of surface components of TAG-rich

lipoproteins in plasma. Low HDL-C is associated with insulin resistance, which causes hypertriglyceridaemia through increased hepatic production of very-low-density lipoprotein (VLDL) particles (Ji *et al.*, 2005). The relationship between elevated TAG and decreased HDL-C may be explained by hyperinsulinaemia-blunted decreases in LPLa, thus decreasing TAG clearance and impairing maturation of HDL-C by enhanced CETP-mediated exchange of TAG and cholesteryl esters between TAG-rich lipoproteins and HDL-C (Lewis & Rader, 2005). Combined with elevated hepatic lipase activity, the CETP-mediated remodelling of HDL-C may result in enhanced hepatic apoA-I clearance. Furthermore, when HDL-C particles are TAG-rich they are poor acceptors of cholesteryl esters from LCAT and thus TAG-rich HDL-C particles are poor donors of cholesteryl esters to the liver, which further inhibits RevCholT (Skeggs & Morton, 2002). Therefore interventions, such as PA, that are known to decrease serum lipids and increase HDL-C concentrations are important in improving HDL-C function (See section 2.5.4 and Kodama *et al.* 2007 for review).

Cholesterol ester transfer protein is an enzyme that impacts negatively on RevCholT, which circulates in plasma bound to lipoproteins and is produced by the liver and adipose tissue (Lewis & Rader, 2005). The main role of CETP is to redistribute hydrophobic lipids (cholesteryl esters & TAG) between HDL-C and apolipoprotein-B (apo-B) containing lipoproteins (intermediate-density lipoproteins (IDL), LDL-C, VLDL-C & chylomicrons). Cholesterol ester transfer protein enriches HDL-C with TAG and depletes them of cholesteryl esters, which reduces the size of the HDL-C particles (Skeggs & Morton, 2002). Cholesterol ester transfer protein is rate-limiting during the transfer of lipids between HDL-C and TAG-rich lipoproteins, but not for cholesteryl ester transfer between LDL-C and HDL-C. Increases in CETP activity (CETPa), when exchanging cholesteryl

esters out of HDL-C in return for TAG, have the ability to reduce circulating HDL-C (Lewis & Rader, 2005).

Lecithin-cholesterol acyltransferase is an enzyme that is bound to HDL-C particles and esterifies free cholesterol to form cholesteryl esters by transferring 2 acyl groups from lecithin to cholesterol. Cholesteryl esters are hydrophobic and thus remain within the HDL-C core, forming larger HDL-C particles (Sviridov & Nestel, 2002). However, the importance of LCAT in RevCholT has not been fully established because unesterified cholesterol can be directly transferred from HDL-C to the liver for excretion without the need for LCAT-mediated esterification (Lewis & Rader, 2005). Despite the equivocal importance of LCAT in RevCholT, it has been postulated that interventions to increase LCATa may be beneficial to health. Indeed, an *in vitro* study has demonstrated that increases in lipid-free apoA-I dissociation, such as decreased HDL-C particle density, was proportional to increased concentrations of VLDL-C, LDL-C and CETP, whereas HDL-C particle density returned to pre-intervention values when LCAT was introduced (Liang *et al.*, 1994).

Lipoprotein lipase is the enzyme principally responsible for TAG hydrolysis to form TAG-rich lipoproteins into smaller TAG-depleted remnant particles and is found on the luminal surface of endothelial cells, particularly in adipose and muscle tissues (Lewis & Rader, 2005). The process of TAG-rich lipoprotein hydrolysis releases surface lipids (free cholesterol and phospholipid) and apolipoproteins, which are added to HDL-C, thus increasing HDL-C and apoA-I concentrations. Decreases in LPLa may increase TAG-rich lipoproteins to such an extent that CETP-mediated lipid exchange of TAG to HDL-C would destabilise the particle, thus lowering HDL-C concentration and predisposing the

HDL-C to PLTP-mediated phospholipid exchange, creating lipid-poor apoA-I which can be cleared from plasma via hepatic lipase action in the liver. Phospholipid transfer protein exchanges surface phospholipids from TAG-rich lipoproteins to HDL-C and is responsible for the majority of phospholipid transfer in plasma (Lewis & Rader, 2005).

Phospholipid transfer proteins remodel HDL-C into larger particles by merging smaller HDL-C, which results in one large HDL-C and the release of lipid-poor apoA-I (Ji *et al.*, 2006). TAG-rich HDL-C is most prone to remodelling due to the destabilisation of apoA-I with increasing TAG saturation, thus decreasing plasma HDL-C. Plasma HDL-C (apoA-I) are removed from the circulation by hepatic lipase. Hepatic lipase is synthesised by hepatocytes and predominantly converts HDL-C particles to smaller HDL-C remnants, pre- β HDL-C (marker of HDL-C production and acceptor of cellular cholesterol) and lipid-poor apoA-I (Lewis & Rader, 2005). Increased CETP-mediated TAG-rich HDL-C enhances HDL-C remodelling by hepatic lipase, which commonly occurs in hypertriglyceridaemia because increased TAG concentrations destabilise HDL-C, causing the release of lipid-poor apoA-I which is excreted from the liver and reduces circulating HDL-C. Furthermore, plasma HDL-C is inversely proportional to hepatic lipase activity, and thus HDL-C is also a marker of dysfunctional lipid metabolism as well as having a direct role in RevCholT. Interventions to promote LPLa and to decrease the activity of PLTP and hepatic lipase could be beneficial to health because LPL facilitates RevCholT at the site of peripheral tissues, whereas PLTP and hepatic lipase inhibit the anti-atherogenic action of HDL-C (Ji *et al.*, 2006).

2.2.2 Physical activity and reverse cholesterol transport

Increasing physical activity (PA) has the potential to enhance RevCholT by decreasing CETPa, PLTPa and hepatic lipase activity, and increasing LPLa and LCATa, however there is little evidence for chronic adaptations in RevCholTran following PA intervention, despite evidence for increases in LPLa following a single exercise session (Gill *et al.*, 2003). The proposed association between systemic insulin resistance, hepatic insulin resistance and increased hepatic TAG-rich lipoprotein output provides a mechanism by which PA-induced changes may promote improvements in RevCholT. Insulin resistance is associated with obesity, particularly abdominal obesity, and PA of sufficient volume to decrease body fat should also affect insulin resistance. With the observed decreases in insulin resistance following PA (Katsanos, 2004) this would also decrease the hepatic lipase activity associated with insulin resistance. This is significant because hepatic lipase activity is partly responsible for the efflux of lipid-poor apoA-I from the plasma for biliary secretion, thus decreasing the plasma HDL-C, and this is a plausible mechanism by which increased PA may reduce HDL-C catabolism.

A further effect of PA on RevCholT is on LPLa, particularly muscular LPL (Gill *et al.*, 2003). Physical activity significantly increases metabolism in the active musculature and lipolysis increases proportionally to PA intensity from rest until a threshold of ~65-70% $\dot{V}O_{2max}$, with the increases in lipolysis following PA being proportional to the PA-induced decreases in intramuscular triacylglycerol and glycogen stores (Seip & Semenkovich, 1998). Following PA these must be restored, which results in increased uptake of TAG from the circulation. The increased need for blood-borne substrates increases LPLa, situated on the endothelium of the capillaries serving the active muscles, which is significant because LPL hydrolyses TAG-rich lipoproteins (LDL-C, VLDL-C &

chylomicrons). This is important for the maintenance of RevCholT because LPL removes TAG from the circulation before it can accumulate and destabilise HDL-C, resulting in the catabolism of HDL-C and its removal from the circulation.

Lipoprotein-bound CETP is involved in the transfer of cholesteryl esters from HDL-C to TAG-rich lipoproteins and TAG from TAG-rich lipoproteins to HDL-C (Tall, 1993). As previously discussed, overloading HDL-C with TAG results in the destabilisation of HDL-C with the net result of HDL-C catabolism. Therefore, if PA could influence CETPa, either through a direct effect or simply by lowering circulating TAG, then this provides a further mechanism for PA-induced increases in RevCholT. However, there is relatively limited literature describing the direct influence of PA/ $\dot{V}O_{2\max}$ on reverse cholesterol transport, despite there being a wealth of data on the relationship between increased PA/ $\dot{V}O_{2\max}$ and increased HDL-C concentrations, which is both an agent, and a rough marker, of RevCholT activity. Weight loss intervention programmes generally improve the blood lipid and lipoprotein profile (Purnell *et al.*, 2000), therefore if physical activity intervention are sufficient to improve body composition then it is likely that they would have an effect on RevCholT through two mechanisms: 1) by increasing muscular TAG uptake, and 2) by alleviating the insulin resistance-mediated effect of excess body fat on hepatic lipase activity and PLTPa, that remodel and help to remove HDL-C from the circulation.

The acute effects of exercise on factors relating to RevCholT have also been studied, such as HDL-C, LPLa, LCATa, CETPa and hepatic lipase activity (Zhang *et al.*, 2002; Grandjean *et al.*, 2000; Weise *et al.*, 2005). The consensus of these studies was that a single session can potentially improve RevCholT in the short-term. However, despite reported positive increases in HDL-C, LPLa and LCATa for 48 hours, weaknesses in these

studies were that they failed to include control procedures within their study designs, therefore it is difficult to interpret the results compared to what may have happened naturally during the same period.

The first published study investigating the influence of exercise training on plasma CETP was performed by Seip *et al.* (1993). The exercise training consisted of three to five sessions of supervised aerobic exercise (walking/jogging/stationary cycling) for 45-60 minutes per week at $\leq 85\%$ HR_{max} for 9-12 months. The main outcomes were that CETP concentrations fell and significantly in response to training, with the decreases in CETP concentration weakly associated with decreases in body fat. Pre-training CETP concentration predicted the training-induced increases in HDL-C, i.e. those with the lowest CETP concentrations gained the greatest increases in HDL-C and those with higher pre-training CETP concentrations achieved the smallest increases in HDL-C. This is likely due to the destabilising effect of CETP on HDL particles through increased transfer of TAG into the HDL particle.

In a cross sectional study of endurance athletes (33.6 ± 1.1 years; $\dot{V}O_{2max}$ 53.4 ± 1.2 $mL \cdot kg^{-1} \cdot min^{-1}$) versus moderately active controls (30.8 ± 1.0 years; $\dot{V}O_{2max}$ 38.8 ± 1.0 $mL \cdot kg^{-1} \cdot min^{-1}$), Olchawa *et al.* (2004) demonstrated that HDL-C, apoA-I, pre β_1 -HDL-C, LCAT activity and percentage cholesterol efflux from macrophages were each significantly greater in the athletes than the controls. However CETPa was similar. Furthermore, HDL-C, apoA-I and pre β_1 -HDL-C were significantly associated with $\dot{V}O_{2max}$ in both groups up to a threshold of $51 mL \cdot kg^{-1} \cdot min^{-1}$, which is consistent with other findings (Durstine *et al.*, 1987). In a similar study, Brites *et al.* (2004) studied RevCholT in well-trained soccer players compared to sedentary medical students. The main findings

were that cholesterol efflux was greater in the soccer players than controls ($20.5 \pm 0.4\%$ v $15.0 \pm 1.2\%$, respectively), which was directly associated with HDL-C, whereas LCAT and CETP activities were not influenced by PA level. Furthermore, cholesterol efflux was inversely correlated with waist:hip ratio (WHR) and LDL-C. However, while there is cross-sectional evidence demonstrating higher concentrations of LCAT in athletes compared to sedentary counterparts (Brites *et al.*, 2004; Olchawa *et al.*, 2004), there is no direct evidence that PA intervention can increase LCAT concentrations (Williams *et al.*, 1990) or LCATa (Bedgoni *et al.*, 2002), despite significant decreases in body mass and fat percentage in these studies.

In summary, the available evidence appears to suggest that PA has both an immediate and chronic impact on RevCholT, such as increased circulating HDL-C, mediated through decreases in circulating TAG. The immediate benefits of PA on RevCholT seem to be primarily exerted through increased LPL catabolism of TAG, whereas long term effects of PA may be mediated through decreasing hepatic insulin resistance, which decreases hepatic TAG-rich lipoprotein output, thus allowing HDL-C to transport cholesterol esters back to the liver more effectively without being saturated with TAG. Therefore, these mechanisms suggest the important role of PA in addressing dysfunctional blood lipid and lipoprotein profiles.

2.3 The management of metabolic syndrome with physical activity

2.3.1 Introduction

First-line therapies for the risk factors associated with the metabolic syndrome are weight reduction and increased PA (Cleeman *et al.*, 2001). It has been suggested that the chronic effect of exercise on metabolic co-morbidities may be largely, but not entirely, as a consequence of body fat losses, especially of abdominal adipose tissue (Carroll & Dudfield, 2004). Cross-sectional evidence suggests that the relationship between fitness and the presence of MetS is mediated through lower adipose tissue, where the relationship between body fat percentage, visceral fat, subcutaneous fat and MetS components are stronger than the association between fitness and MetS components, and the effects of fitness on individual MetS components is mostly attenuated after controlling for total and abdominal obesity (Boule *et al.*, 2005). Furthermore, evidence indicates that exercise does not significantly increase initial weight losses over and above that obtained with dietary intervention alone (Carroll & Dudfield, 2004). However, data substantiate the important role of exercise in the ‘mediation of a healthier bodyweight’ among overweight and obese adults without excessive energy deprivation, where the loss of only a few kilogrammes, particularly from visceral adipose tissue may lead to improvements in MetS components (Bussetto, 2001). Clinical trials have shown that modifying major components of the syndrome – atherogenic dyslipidaemia, hypertension and the prothrombotic state – will reduce CVD risk, yet few controlled studies have reported concurrently the effectiveness of exercise training alone for multiple metabolic risk factors (Carroll *et al.*, 2004).

Every 100 kcal·wk⁻¹ expended by men participating in vigorous PA (≥ 7.5 METs) is associated with mean decreases of -0.36 mmol·L⁻¹ in TC, -0.33 mmol·L⁻¹ in TAG, -3 mm

Hg in diastolic blood pressure, $-10 \text{ beats}\cdot\text{min}^{-1}$ in resting heart rate and $-1.1 \text{ kg}\cdot\text{m}^2$ in BMI, and increases in HDL-C of $0.17 \text{ mmol}\cdot\text{L}^{-1}$ (Mensink *et al.*, 1997). Furthermore, every PA session performed per week is associated with a decrease in TC of $-0.014 \text{ mmol}\cdot\text{L}^{-1}$, $-0.36 \text{ beats}\cdot\text{min}^{-1}$ in heart rate, $-0.093 \text{ kg}\cdot\text{m}^2$ in BMI, and serum lipids and BMI were more strongly associated with frequency than either intensity or duration of PA. Prospective evidence suggests that increasing PA routine after middle age even without active intervention is significantly associated with improvements in glucose control and HDL-C independently of changes in body mass compared to those who maintained their baseline PA levels over a 20 year follow-up period (Byberg *et al.*, 2001). Furthermore, increased PA was also associated with decreases in pulse rate and serum TAG when the health outcomes were analysed without adjusting for weight change. However, despite cross-sectional/prospective data to suggest that the presence of MetS is inversely associated with PA (Franks *et al.*, 2004), there is relatively sparse data to demonstrate the independent benefit of PA without additional dietary intervention on components associated with MetS. This is despite recent reviews presenting many studies concerning the potential for increased PA to favourably influence the individual components associated with MetS, such as levels of total & abdominal obesity (Jakicic, 2003; Kay & Fiatarone Singh, 2006), insulin resistance & glucose tolerance (Henriksen, 2002; Yates *et al.*, 2007), blood pressure (Pescatello, 2005), lipid and lipoprotein profile (Durstine *et al.*, 2001) and markers of pro-thrombotic & pro-inflammatory states (Imhof & Koenig, 2001; Kasapis & Thompson, 2005).

2.3.2 Physical activity and weight control

Obesity has classically been considered a problem of overeating, however persuasive evidence suggests that much obesity may be due to low energy expenditure rather than overeating (Prentice & Jebb, 1995). Excess fat deposition in the abdominal region, which is known to be independently associated with insulin resistance, glucose intolerance and dyslipidaemia, is a stronger predictor of CVD and T2D than overall adiposity (Abate, 2000; Bonora, 2000). Dietary energy restriction has been the principal intervention strategy in attempts to reduce intra-abdominal fat and most current knowledge is based on energy-restriction studies within obese adults, where it is apparent that for every kilogram of diet-induced weight-loss there is an approximate 2-5% reduction in visceral fat (Kay & Fiatarone Singh, 2006). However, caloric restriction is associated with a decrease in resting metabolic rate (RMR) and thermic effect of feeding (TEF), thus resisting weight reduction or even maintenance of achieved weight loss, whereas a combination of caloric restriction with exercise enhances the maintenance of RMR and thus improves long-term results of weight reduction programmes (Eriksson *et al.*, 1997). Resting energy expenditure in the obese is not related to BMI or insulin resistance, but is related to fat-free mass (FFM) (de Luis *et al.*, 2005) and PA preserves lean body mass, unlike dieting alone, which is crucial since the major determinant of the 24hr EE is the fat-free body mass, accounting for about 80% of the variance observed in 24hr EE between individuals (Pavlou *et al.*, 1985; Horton, 1986; Ravussin *et al.*, 1986). However, the usefulness of increasing PA in order to lose weight has been somewhat controversial, mainly because: 1) intense long-duration PA has been prescribed to obese individuals, which is unrealistic in this population, 2) when PA weight-loss interventions alone have been compared to dietary weight-loss interventions, the dietary weight-loss interventions have been assumed to be more effective, but this is mainly due to greater energy deficits from the diet groups than that being expended in PA

groups, and 3) obese individuals tend to overestimate the amount of exercise and underestimate the amount of consumed food (Eriksson *et al.*, 1997).

A study by Katznel *et al.* (1997) examined the effects of sequential interventions of 9 months of aerobic exercise training followed by weight loss (with continued training) on metabolic risk factors in obese middle-aged and older men, where PA increased maximal aerobic capacity by 14% but with no significant change in body weight or improvements in oral glucose tolerance responses, blood pressure and blood lipid & lipoprotein profile. However, evidence suggests that PA may have an independent effect on visceral and subcutaneous abdominal adipose tissue in overweight/obese individuals and an 8% weight reduction may reduce visceral fat mass by ~28%, and additional weight-loss intervention, without further fitness improvement, was associated with an 8% decrease in glucose, a 30% decrease in insulinaemia during an oral glucose tolerance test (OGTT) and improved blood lipid & lipoprotein profile (Ross *et al.*, 2000a; 2000b). Other studies have demonstrated that longer-term (≥ 12 months) PA, alone and combined with dietary intervention, has a significant impact on glucose-insulin homeostasis, blood lipid & lipoprotein profile and blood pressure in sedentary, overweight individuals at risk of MetS (Torjesen *et al.*, 1997; Anderssen *et al.*, 1998). The reductions in waist circumference (WC) significantly predict favourable changes in haemostatic parameters, blood lipid & lipoprotein profile and carbohydrate & lipid metabolism, independently of changes in physical fitness. In the Health Risk factors exercise Training And Genetics (HERITAGE) study including 621 sedentary African-American and Caucasian participants the prevalence of the ATP III-defined MetS was 16.9% and decreased to 11.8% following exercise training, where 30.5% of the 105 participants with MetS at baseline were no longer classified as having MetS and among these 32 participants, 28% decreased their

WC, 43% decreased TAG, 38% decreased blood pressure, 16% improved HDL-C and 9% improved fasting plasma glucose (Katzmarzyk *et al.*, 2003). Evidence demonstrates that PA-induced reductions in total and abdominal fat are more strongly associated with improvements in blood pressure, blood lipid & lipoprotein profile and insulin sensitivity than the improvements in fitness (Stewart *et al.*, 2005). However, both adiposity and fitness are important predictors of diabetes (Katzmarzyk *et al.*, 2007).

The frequency of PA is one of the most powerful determinants of successful weight maintenance because this facilitates the weekly accumulation of low-to-moderate intensity PA without the need for vigorous PA in order to achieve a satisfactory weekly EE and generally demonstrates good adherence to PA adoption (Jakicic, 2003). Data from 'Studies of Targeted Risk Reduction Intervention through Defined Exercise' (STRIDDE) demonstrate that six months of PA, consistent with CDC & ACSM guidelines (1995) (Section 2.8), was sufficient to prevent significant increases in visceral fat, however modestly increasing PA above these minimal recommendations ($\geq 150 \text{ min}\cdot\text{wk}^{-1}$ moderate PA) significantly decreases visceral and abdominal fat without decreasing energy consumption (Slentz *et al.*, 2005). The exercise prescription for improving body composition is related to creating a negative energy balance, whereby energy expenditure is greater than energy consumption, therefore daily moderate intensity PA sessions of at least 60 minutes may be required for this purpose in the obese (Pedersen & Saltin, 2006). The influence of increased PA on levels of obesity is created over a period of time, therefore the short-term benefits of PA on MetS risk factors associated with overweight/obesity may be mediated through mechanisms other than fat losses and these will be discussed within the subsequent sub-sections.

2.3.3 Physical activity and insulin resistance

In addition to those with obesity, insulin resistance is often present in individuals who are not overtly obese, such as first degree relatives of T2D patients, where these ‘metabolically obese normal-weight’ individuals appear to be quite common in the general population, and they could be an important target population for exercise therapy for the prevention of MetS (Carroll & Dudfield, 2004). To achieve maximum benefit from modification of multiple metabolic abnormalities the underlying insulin-resistant state must become a therapy target (Minehira & Tappy, 2002) and it is now well established that bodyweight reduction improves insulin resistance in those that are overweight (Kelley & Goodpaster, 1999; Ross *et al.*, 2000b). Three major RCTs conducted in diverse countries, settings and incorporating various ethnic groups have confirmed that lifestyle intervention, including regular structured exercise can prevent or delay progression to T2D among high-risk groups with impaired glucose regulation (Tuomilehto *et al.*, 2001; Knowler *et al.*, 2002; Li *et al.*, 2002).

When endurance-trained individuals stop training, their enhanced insulin sensitivity is rapidly reversed (King *et al.*, 1995), suggesting that this characteristic could partly be a consequence of the acute effects of their last bout of exercise. It has been demonstrated that acute exercise can normalise a defect in insulin-stimulated glucose transport-phosphorylation in insulin-resistant subjects (Perseghin *et al.*, 1996). A single bout of acute exercise enhances insulin-mediated glucose disposal in normal subjects, in insulin-resistant first degree relatives of T2D patients, in obese subjects with insulin resistance as well as in T2D patients. Exercising muscle may increase glucose clearance 7 to 20-fold (Wahren *et al.*, 1971), where following an acute bout of exercise, glucose uptake into skeletal muscle is enhanced, which is partly an insulin-independent contractile effect and

may persist for several hours after the cessation of exercise (Henriksen, 2002). The enhanced post-exercise insulin sensitivity is most likely due to the need to replenish muscle glycogen, where glycogen-depleting exercise results in increased non-oxidative glucose disposal as measured 12 h after exercise (Henriksen, 2002). However, when an untrained leg was subjected to a single exercise bout there was no effect on insulin action in the untrained muscle, suggesting that ‘the effect of training on insulin-mediated glucose disposal in muscle is a genuine adaptation to repeated exercise... but is short-lived’ (Dela *et al.*, 1992). Furthermore, in a study where one leg was trained over a period of 10 weeks and the other remaining untrained, GLUT-4 protein levels were 26% higher in the trained muscle, which was associated with an increase in insulin-stimulated glucose uptake in the trained leg (Dela *et al.*, 1993). In addition to the glycogen-depleting effect of PA, reduced NEFA availability due to aerobic exercise may improve insulin sensitivity and glucose metabolism, where six weeks of PA at 60-85% $\dot{V}O_{2max}$ for at least 60 minutes per week decreased circulating NEFA availability and improved insulin sensitivity, despite no changes in hepatic TAG or intramuscular TAG contents (Shojaee-Moradie *et al.*, 2007).

Data indicates that a threshold exercise intensity of 70% $\dot{V}O_{2max}$ may be needed in order for short-term post-exercise improvements in insulin sensitivity, possibly due to the greater depleting effect of vigorous intensity PA on muscular glycogen stores (Thompson *et al.*, 2001). Indeed, data demonstrates that exercise increases insulin-stimulated glucose disposal through increased glycogen synthesis without affecting rates of glucose oxidation in both type II diabetic and non-diabetic participants and may last for at least 24 hours (Christ-Roberts & Mandarino, 2004). Possible mechanisms by which PA may improve insulin sensitivity include increased mRNA expression, increased glycogen synthase activity, decreased release of adipose tissue-derived NEFA and enhanced clearance of

NEFA and increased delivery of glucose and insulin to muscles through increases in blood flow (Pedersen & Saltin, 2006). In order to enhance muscular metabolism of glucose and NEFA the PA prescription should be of at least moderate intensity with the aim of increasing total levels of energy expenditure and evidence suggests that if exercise is to be effective in promoting insulin sensitivity, it should be regular, frequent and of sufficient volume, in terms of energy expenditure to maintain desirable level of body fatness in the long term (Kriska *et al.*, 1994). This may be effectively achieved through the performance of 3-4 hours per week of moderate intensity PA, or 30 minutes of moderate intensity PA on a daily basis (Pedersen & Saltin, 2006).

2.3.4 Physical activity and dyslipidaemia

The combination of hypertriglyceridaemia, low levels of HDL-C and small dense LDL-C particles have been named the ‘atherogenic lipoprotein phenotype’, ‘atherogenic dyslipidaemia’ or lipid triad (Grundy, 1998), which are associated with the purported central components of MetS (abdominal fat accumulation and insulin resistance). Intra-abdominal fat is relatively insensitive to insulin and has a high lipolytic activity, partly due to its complement of adrenergic receptors (Björntorp, 1990) and hyperinsulinaemia and increased NEFA may affect several interconnected steps in lipoprotein-lipid metabolism (Carroll & Dudfield, 2004). Physical activity may improve the dyslipidaemic profile associated with MetS by increasing the ability of muscle tissue to take up and oxidize NEFA and increasing the activity of LPL in muscle (Pollare *et al.*, 1991). Reductions in TAG and elevations in HDL-C are commonly associated with increased PA, however TC and LDL-C are infrequently affected (Durstine *et al.*, 2001). Endurance-trained men and women demonstrate high rates of TAG clearance compared with sedentary controls, which may be related to the increased LPLa, the enzyme that hydrolyses TAG-rich lipoproteins (Kiens & Lithell, 1989). However, data suggests that the increases in TAG clearance associated with prior exercise are not related to improved insulin sensitivity (Gill *et al.*, 2002). This may benefit an atherogenic dyslipidaemic profile because circulating TAG concentrations are closely coupled with rates of HDL clearance and so an enhanced metabolic capacity for TAG may explain the high HDL-C levels in physically active people. In fact, in healthy trained men, a PA session expending 1,100 kcal may be sufficient to elevate HDL-C, however a lower PAEE threshold than this may be sufficient for sedentary individuals (Ferguson *et al.*, 1998). Physically active individuals have higher levels of HDL-C and HDL₂-C and lower levels of TAG, VLDL-C and small dense LDL-C compared to sedentary individuals (Haskell, 1984; Durstine & Haskell, 1994). Studies

concerning the effect of PA interventions on lipids and lipoproteins among subjects with the MetS remain scarce, partly due to the lack of precise definition of the syndrome. However, data indicates that over months, PA expending 1200-2200 kcal·wk⁻¹ results in increases in HDL-C, which are even greater when the exercise programme is accompanied by weight loss (Durstine *et al.*, 2001). Therefore, PA alone may not always be sufficient to normalise the atherogenic dyslipidaemia associated with MetS. Comparisons of two different interventions in sedentary overweight men and women – a hypo-caloric diet alone or the same diet plus PA (brisk walking and jogging) – showed that the addition of PA to the low-fat diet resulted in a greater reduction in WHR and more favourable changes in HDL-C than diet alone (Wood *et al.*, 1991).

The effects of acute PA on blood lipids are proportional to the energy expended, rather than meeting a specific EE threshold. However, despite those with the greatest TAG values having the potential for the greatest PA-induced reductions in blood TAG, unconditioned individuals may be unable to expend sufficient energy (Thompson *et al.*, 2001). Evidence also suggests that PA may acutely increase blood HDL-C values, where an PAEE of 350-400 kcal may be sufficient in moderately fit individuals. However, as with chronic training adaptations, changes in total cholesterol and LDL-C are more equivocal and potential favourable changes may be due to plasma volume expansion rather than changes in lipid metabolism (Thompson *et al.*, 2001). A suitable exercise prescription for improving an atherogenic blood lipid and lipoprotein profile may be to aim to cover ≥ 20 km·wk⁻¹ on foot (Pedersen & Saltin, 2006).

2.3.5 Physical activity and blood pressure

Among normotensive adults, blood pressure values are inversely associated with insulin sensitivity, i.e. lower BP is related to greater insulin sensitivity, particularly among the relatively lean (Sowers *et al.*, 2001). Furthermore, the insulin resistance of hypertension is often associated with a high rate of sodium/lithium counter-transport activity, salt sensitivity and microalbuminuria (Poppitt *et al.*, 2002). However, several RCTs incorporating exercise only intervention groups have not been consistent in demonstrating improvements in blood pressure and postprandial insulin responses (Dengel *et al.*, 1998; Blumenthal *et al.*, 2000). It is possible that these inconsistent findings may stem from heterogeneity in the association between insulin sensitivity and blood pressure, which may help to explain the inconsistent blood pressure responses observed across PA intervention studies (Eriksson *et al.*, 1997). Indeed, recent evidence from a cross-sectional study investigating the relationship between insulin sensitivity and hypertension suggests that adjusting for insulin sensitivity attenuates the relationship between habitual PA and hypertension in longitudinal exercise training studies (Foy *et al.*, 2006).

There may be certain subgroups of patients with hypertension who are more responsive to the blood pressure-lowering effects of PA, since the decrements in blood pressure associated with increased PA are not always sufficient to produce normotension in many studies (Eriksson *et al.*, 1997). Despite the potential disparity, randomised controlled trials (RCTs) among overweight middle-aged and older subjects have also demonstrated the effectiveness of PA to lower blood pressure, with or without weight loss (Dengel *et al.*, 1998; Blumenthal *et al.*, 2000). A 1999 meta-analysis of aerobic exercise training RCTs among overweight/obese participants, the blood pressure lowering effect of PA was small but statistically significant among normotensive study groups (baseline blood pressure

128/84 mm Hg) (Fagard, 1999) and a further meta-analysis showed modest weight loss (3-9%) was associated with a significant reduction in mean systolic and diastolic blood pressures of approximately 3 mm Hg compared with non-intervention controls (Hermansen, 2000). The overall results of the published studies indicate that blood pressure reductions may be apparent within 10 weeks of hypertensive patients beginning participation in PA, with recent evidence to suggest that significant reductions are possible within 4 weeks (Collier *et al.*, 2008) and blood pressure may continue to decline even further with prolonged maintenance of PA, which may occur independently of favourable changes in body composition (Hagberg *et al.*, 2000).

The reductions in blood pressure observed with regular PA may be due to the accumulative effects of single exercise bouts rather than to long term adaptations to PA (Kaufman *et al.*, 1987). The rationale for the exercise-induced decreases in blood pressure is that there is a marked dilation of blood vessels in active skeletal muscle decreasing resistance to flow during exercise, therefore the acute effects of exercise evident during recovery from individual bouts of exercise may be important, where the blood pressure of sedentary hypertensives may be reduced for up to 12 hours following a single exercise session (Pescatello *et al.*, 1991; Brown *et al.*, 1994). More recent data indicate that post-exercise hypotension may persist for up to 16 hours, thus PA may enable those with stage I hypertension to be normotensive for the majority of the day (Thompson *et al.*, 2001).

The immediacy by which post-exercise hypotension occurs suggests the hypotensive influence of regular PA may be partially an acute occurrence with the BP reductions accumulating as the training program continues (ACSM, 2004). Indeed, data from intervention studies suggest that longer training programmes may produce somewhat larger

reductions in blood pressure, suggesting that PA may impact on blood pressure control immediately and in the long term through separate mechanisms (Hardman, 1996). When the current ACSM recommendations for PA and hypertension were published the influence of the intensity and duration of PA sufficient to cause post-exercise hypotension was still unknown (ACSM, 2004). However, recent studies have demonstrated that although PAEE may be more important than either intensity or duration (Jones *et al.*, 2007), it is now known that 15 minutes of PA at 40% $\dot{V}O_{2\max}$ is sufficient to promote post-exercise hypotension (Guidry *et al.*, 2006). In order to achieve reductions in blood pressure, performing moderate intensity PA of at least 30 minutes on a daily basis should be sufficient to lower blood pressure, both in the immediate post-exercise period and for long-term adaptations.

2.3.6 Physical activity, coagulation and inflammation

The presence of MetS may predispose sufferers to an increased risk of thrombosis and inflammation, however both fitness (inversely) and fatness (directly) are associated with these disorders (Church *et al.*, 2002). Despite this evidence, due to the limited number of studies involving the potential improvements in thrombotic and inflammation risk associated with PA, the exact PA dose to promote a favourable response is currently unknown. Metabolic processes within skeletal muscles may drive the anti-thrombotic effects of PA, where data demonstrates that the fibrinolytic activities of tissue plasminogen activator (tPA), and its endogenous inhibitor (PAI-1), were favourably altered in skeletal muscle and serum following 9 months of exercise training expending 2000 kcal·wk⁻¹, as measured using the biopsy needle technique (Hittel *et al.*, 2003).

While TNF- α , IL-6 and PAI-1 have been the most studied inflammatory cytokines in lifestyle intervention studies to date (Bastard *et al.*, 2000; Mavri *et al.*, 2001; Bruun *et al.*, 2003), more recent attention has focused on adiponectin (Hulver *et al.*, 2002; Faraj *et al.*, 2003) and CRP, a potent inflammatory mediator (Esposito *et al.*, 2003). Analysis of data from the third National Health and Nutrition Examination Survey (NHANES III) appears to suggest that markers of inflammation (CRP, fibrinogen and white blood cell count) are decreased in association with increased participation in certain types of PA within the previous month, including jogging, swimming, cycling, aerobic dancing, callisthenics and resistance training compared to non-exercising controls (King *et al.*, 2003). However, activities such as gardening were not associated with decreased inflammation, and the investigators conceded that due to only the frequency of activities being recorded, and not the intensity and duration of these activities, it was difficult to evaluate the relative value of each type of PA in terms of dose-responses.

Modulation of cytokines and other inflammatory mediators is achievable through lifestyle modification leading to weight loss in obese subjects (Robinson and Graham, 2004). Furthermore, individuals could still be classified as obese at the end of a study yet, in most cases, subjects still demonstrate significantly favourable improvements in inflammation-related adipokines. Tumor necrosis factor- α is an inflammatory cytokine associated with obesity and Straczkowski *et al.* (2001) demonstrated a significant decrease in circulating TNF- α was not associated with insulin sensitivity after 12 weeks of exercise training in obese subjects, but the source of the TNF- α was not reported, and since fat mass and TNF- α decreased by 7.9% and 19.6%, respectively, the effect of physical activity on circulating TNF- α appeared to be additive and independent of changes in body composition. Due to the paucity of studies of PA intervention on coagulation and inflammation, further studies are needed to clarify the association between level of obesity, prothrombotic & inflammatory markers and future risk for insulin-resistant conditions such as MetS and CVD. In most cases, plasma or serum cytokine protein concentrations have been measured, while adipose tissue mRNA and protein expression have been less studied (Robinson and Graham, 2004). It is difficult to distinguish possible independent effects of dietary factors and/or PA *per se* from the impact of weight loss itself. This may be an important consideration since the effects of exercise and weight loss on inflammation-related cytokines implicated in insulin sensitivity/resistance may function via different mechanisms

2.3.7 Current physical activity recommendations

The current minimum PA recommendations are for 30 minutes of moderate intensity PA (at least 40/50% HRR) to be accumulated on 5 days of the week, 20 minutes of vigorous intensity PA (up to 85% HRR) to be performed on 3 days of the week, or a combination of the two sets of recommendations, such as 30 minutes of moderate intensity PA on two days of the week and a further two sessions of vigorous PA for 20 minutes (ACSM & AHA, 2007). In each of the PA recommendations since 1978 there has been an appreciation of the trade-off between the intensity and duration of PA sessions, however the greatest emphasis on the benefit of performing moderate intensity PA for at least 30 min arose following the CDC & ACSM (1995) recommendations and has been maintained since. Another significant recommendation from this particular report was that PA may be accumulated throughout the day in sessions of at least 10 minutes instead of a single prolonged session of equivalent duration. The essence of the PA recommendations for health-enhancing PA have remained the same since 1995, however there has been greater clarification on the interaction between PA intensity, duration and frequency to provide the appropriate PA volume, such as performing high intensity PA for at least 20 minutes on at least 3 days of the week, or moderate intensity PA for at least 30 minutes on at least 5 days of the week, or combinations of the two (ACSM & AHA, 2007).

Since the USGR (1996) was published, the ACSM (1998), CMO (2004) and ACSM & AHA (2007) have recommended that increasing PA volume through intensity, duration or frequency, or each, above the minimum recommendations may bring about greater reductions in the risk of CHD, diabetes, stroke, hypertension, obesity and certain cancers. The CMO report (2004) recommended that encouraging people to move from an inactive level to low to moderately active levels would produce the greatest reductions in risk and

that PA expenditures of 500-1000 kcals per week (~6-12 miles of walking for an average-weight individual) should be sufficient. Physical activities such as walking/hiking, running/jogging, bicycling, swimming, rowing, rope skipping, skating, cross-country skiing and endurance-based games, dancing, walking during a round of golf, standing & casting whilst fishing, housework and gardening have been recommended as sufficient to promote health if performed for the recommended volume on a weekly basis. Since the mid-90s the accumulation of PA throughout the day has been encouraged to gain health benefits, where any increases in PA are better than none, however sessions of at least 10 minutes per session adding up to at least 30 minutes per day are seen as a minimum target.

2.3.8 Summary

Since there is a clustering of risk factors in association with MetS the treatment chosen should attempt to aim at as many of the metabolic abnormalities as possible, and this opportunity is provided by regular PA (Hardman, 1996; Carroll & Dudfield, 2004). Even though performing PA on fewer than two sessions a week at less than 50% $\dot{V}O_{2\max}$ and for less than 10 min/day may to be inadequate for developing and maintaining fitness for healthy adults, lower intensities and volumes may still be capable of inducing favourable changes in energy expenditure, muscle LPLa and blood pressure in sedentary or low active individuals (Eriksson *et al.*, 1997). Evidence suggests that potential improvements in health outcomes related to MetS are inversely proportional to baseline health status, where those classified as ‘unhealthy’ are more likely to respond favourably than those classified as ‘healthy’ (Wilmore, 2001).

Although most of the beneficial changes are well correlated with the loss of body weight/body fat, i.e. glucose homeostasis (Yates *et al.*, 2007), increased PA may also stimulate metabolic improvements in the absence of weight loss, so failure to achieve this goal or improved fitness should not be interpreted to mean that no benefit has occurred (Ekelund *et al.*, 2007). Long-term PA, without weight reduction, modestly decreases abdominal adipose tissue and improves insulin action among overweight/obese adults. Longer-term PA in middle-aged and older overweight/obese adults, even in the absence of clinically significant weight loss, is associated with modest, but clinically relevant improvements in the dyslipidaemic profile. This effect is created by lowering TAG and raising HDL-C and the most positive changes are evident among overweight/obese individuals with hypertension (Carroll & Dudfield, 2004). Perhaps, due to the potential negative health outcomes associated with MetS, such as CHD, diabetes and premature

mortality, individuals at risk of MetS are prescribed medication to control the individual components, such as statins for hypercholesterolaemia, beta-blockers for hypertension and metformin for T2D. However, despite performing PA above current public health recommendations ($\geq 150 \text{ min}\cdot\text{wk}^{-1}$ moderate intensity PA) appearing to reduce such medication use (Williams & Franklin, 2007), there are currently insufficient data from non-randomised and randomised exercise trials to establish dose-response relationships between intensity and volume of exercise and multiple metabolic abnormalities.

The available data demonstrate a pattern between increased PA and decreased MetS risk, but well-designed RCTs are still required to establish the lower threshold for PA intensity and volume for favourable metabolic outcomes because there is presently limited evidence that lifestyle interventions, such as moderate intensity daily life physical activities, improve multiple metabolic abnormalities. Furthermore, the lower threshold of PA intensity and volume for promoting favourable acute responses in multiple metabolic abnormalities has yet to be established, therefore the influence of walking and accumulative PA on health outcomes will be discussed in the subsequent subsections 2.6 and 2.7.

2.4 Walking for health

2.4.1 Introduction

For most (>99.5%) of the last 100,000 years walking has been modern *homo sapiens*' main source of PA during ambulation and daily chores, however within the past 200 years daily PA levels have been systematically reduced by advancements in technology, such as cars and occupational instruments (Haskell & Torburn, 2006). In recognition that activities of daily living were decreasing, the notion of 10,000 steps per day originated from Japan in the 1960s and was exported to many countries in the late 1990s (Hatano, 1993; Haskell & Torburn, 2006). Very active individuals may still cover over 12,000 steps per day, with a sliding scale of active individuals ($\geq 10,000$ per day), somewhat active (7,500-9,999), low active (5,000-7,499) and sedentary individuals attaining less than 5,000 steps per day (Tudor-Locke & Bassett, 2004). Furthermore, studies evaluating how 10,000 steps per day equates to current public health recommendations ($\geq 150 \text{ min}\cdot\text{wk}^{-1}$) found that 73% of those who attained $\geq 10,000$ steps per day also reported performing 30 minutes of moderate activity per day (Welk *et al.*, 2000). However, a more recent study demonstrated that walking for 30 minutes only required $\sim 3,000$ steps, indicating a deficit of 7,000 steps that needed to be met to fulfil the 10,000 daily steps required (Tudor-Locke *et al.*, 2005a). Therefore, in order to meet the 10,000 steps per day target individuals must participate in other lifestyle activities on top of the structured $5 \times 30 \text{ min}$ sessions of brisk walking.

Despite evidence to the contrary, many people believe that PA must be of vigorous intensity to be beneficial to health-related fitness, which is perhaps grounded in the exercise training for sporting performance paradigm, where improved fitness is associated with aspects of health (Timperio *et al.*, 2000). However, even though vigorous PA is

beneficial to health, activities of daily life, such as walking, can be accumulated throughout the week to provide a large enough volume of PA to gain significant health benefits, evidence of which has been available for over 50 years (Morris *et al.*, 1953). Sporting activities and structured exercise programmes are rarely part of many peoples' lives due to age-related decreases in vigorous activity, the increase in sedentary occupations and lack of interest in participation in such activities (Haskell & Torburn, 2006). However 'active transport', which includes daily activities such as walking & cycling, and using stairs instead of escalators/lifts, has the potential to be a significant source of habitual PA for every able-bodied individual. The main premise of active transport is that travelling from A to B is a convenient way of increasing daily/weekly PA compared to automobile transportation and can also form part of leisure and recreational activities. This section will specifically refer to walking.

Public health recommendations (ACSM, 1978 through to ACSM & AHA, 2007) have included walking as an aerobic activity, because it is dynamic, rhythmical and uses large muscle groups for prolonged periods. Brisk walking exerts a greater metabolic load on those with low $\dot{V}O_{2max}$ and thus promotes improvements in $\dot{V}O_{2max}$ that are directly related to walking speed (Haskell & Torburn, 2006; Duncan *et al.*, 1991). Walking at $4.8 \text{ k}\cdot\text{hr}^{-1}$ on a level surface requires a $\dot{V}O_2$ of $\sim 13 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, which may be less than 30% $\dot{V}O_{2max}$ of a healthy 30-year-old man, whereas for the same individual at 75-years-old this could amount to over 60% $\dot{V}O_{2max}$ due to age-associated declines in $\dot{V}O_{2max}$ (Morris & Hardman, 1997). Walking at any pace expends energy and at the least has potential for weight control, however when individuals are instructed to walk briskly they automatically select a pace that elicits an intensity of $\sim 70\% \text{ HR}_{max}$ ($\sim 60\% \dot{V}O_{2max}$) and likely to improve cardiovascular fitness (Morris & Hardman, 1997; Murtagh *et al.*, 2002). Furthermore, a

previous study also reported that habitual walkers, $\dot{V}O_{2\max}$ $35.7 \pm 6.3 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, met the ACSM's recommendations for improving $\dot{V}O_{2\max}$ (Spelman *et al.*, 1993). Health risk factors that may be attenuated with regular moderate intensity PA, such as walking, include decreased arterial blood pressure, alteration in the lipoprotein profile, particularly decreases in TAG-rich particles and increases in HDL-C, enhanced glucose tolerance, lower tendency for thrombosis, decreased myocardial oxygen demand at rest and submaximal workloads and increased capacity of coronary circulation for vasodilation (Haskell & Torburn, 2006).

Those individuals that public health recommendations are aimed towards, such as sedentary obese individuals, may also benefit the most from increased walking activity because this population appears to be less energy efficient during walking (Foster *et al.*, 1995; Chen *et al.*, 2004), thus having greater implications for weight control, lessening the need for vigorous activity and also decreased risk of injury or sudden death. Furthermore, due to this reduction in movement economy there is an increased cardiovascular load, which means that the obese have greater potential to improve weight control and gain fitness from speeds equivalent to 'walking for pleasure' (Browning & Kram, 2005; Hills *et al.*, 2006). A study by Williams (2005) demonstrated that this is possible whereby those who walked the least were most obese and those individuals had the greatest potential for declines in weight.

Cross-sectional evidence has demonstrated that measures of obesity (BMI & fat%) are inversely related to ambulatory activity (Tudor-Locke *et al.*, 2001; Yoshioka *et al.*, 2005). In a relatively small-scale cross-sectional study of 150 older adults low rates of steps per day ($<3100 \text{ steps}\cdot\text{d}^{-1}$) were associated with increased risk of some components of MetS,

including high TAG and central adiposity, whereas steps·d⁻¹ were not predictive of the development of hypertension, impaired fasting glucose and low HDL-C (Strath *et al.*, 2007). However, overall in the Caucasian group, those accumulating <3100 steps·d⁻¹ were 96.8% more likely to have MetS than those accumulating >3100 steps·d⁻¹. In a further epidemiological study, investigating the relationship between steps per day and WC & BMI, increased daily steps were associated with a decline in measures of obesity (Dwyer *et al.*, 2007). Furthermore, an additional 2,000 steps per day was associated with a 2.8 cm decrease in waist circumference in those taking only 2,000 steps·d⁻¹, yet only a 0.7 cm reduction in WC in those already taking 10,000 steps·d⁻¹. Thus, the benefits of daily walking are logarithmic and those performing little habitual lifestyle PA have the greatest potential benefit from increased ambulation. Due to the cross-sectional nature of these studies causality of obesity due to ambulatory inactivity cannot be determined, therefore Williams (2007) performed a prospective study to investigate the role of 'leanness' in determining walking intensity and distance. The findings of the study were that although walking intensity and walking distance were both strongly associated with greater leanness the author concluded that distance was more causally related to leanness than intensity and should be encouraged for preventing/reversing weight gain. However, despite leanness being more causally associated with walking distance (Williams, 2007), when the impact of walking intensity and walking duration on all-cause mortality was investigated there was a significant inverse association between daily walking intensity and risk of death, whereas there was only a weak inverse association between daily walking duration and risk of death (Schnohr *et al.*, 2007).

Walking has been demonstrated to be at least as effective as equal volumes of more vigorous exercise in improving $\dot{V}O_{2max}$, where six months of six weekly 30 min sessions of

walking at 50% HR_{max} produced similar increases in $\dot{V}O_{2max}$ (2.5 v 2.9 $mL \cdot kg^{-1} \cdot min^{-1}$) compared to four 30 min sessions per week of jogging without significant changes in body composition or lipid profile (Suter *et al.*, 1994). Despite the improvements in $\dot{V}O_{2max}$ from both groups not being associated with improvements in body composition or lipoprotein profile in that study, a more recent prospective study involving 73,743 postmenopausal women demonstrated that walking and vigorous exercise were associated with similar cardiovascular risk reductions on follow-up and a faster walking pace also predicted lower risk (Manson *et al.*, 2002). Carrying on the theme that PA does not have to be vigorous to be effective, Duncan *et al.* (1991) demonstrated that although there was a dose-response effect between walking speed (4.8 v 6.4 v 8.0 $k \cdot hr^{-1}$) and improvement in $\dot{V}O_{2max}$ following the performance of 4.8 km of walking per day on 5 days of the week for 24 weeks, HDL-C was equally affected by the low- and vigorous intensity walks. Therefore, indicating that energy expended during the walks, as indicated by the distance walked in each group (24 $km \cdot wk^{-1}$), was key to the health improvements rather than walking speed.

Walking is an activity that is usually performed on a daily basis in activities of daily living rather than part of a structured exercise programme. Due to the nature of activities of daily living they are not performed in a neat and single three hour session, but are accumulated throughout the day in numerous discrete sessions, therefore the various studies that used walking as the mode of PA to investigate the health benefits of accumulative walking will be discussed in Section 2.7 and the next two sub-sections will deal with the short-term and long- term health outcomes of walking.

2.4.2 Short-term responses

Aside from the adaptations that take place following regular participation in walking activities, single walking sessions can have significant, although short-term, health benefits that may be maintained through regular PA. The potential effects of any form of PA intervention are limited in their short-term effects because not every health risk factor can respond immediately in the post-exercise period, i.e. reductions in body weight and its associated variables, therefore the majority of acute walking studies have focused on the influence of walking on postprandial lipaemia in particular, although some studies are available on the acute effects of walking on blood lipoproteins (Pronk *et al.*, 1995; Ainslie *et al.*, 2005), fibrinolysis (Ivey *et al.*, 2003), inflammation (Murtagh *et al.*, 2005a) and blood pressure (Park *et al.*, 2006). Walking at 50% $\dot{V}O_{2\text{peak}}$ for 40 minutes in a single session has been reported to decrease SBP by -5.6 ± 1.6 mm Hg and DBP by -3.1 ± 0.2 mm Hg for 7 hours in pre-hypertensive adults and this effect was augmented when walking was accumulated in 4×10 min bouts, which caused SBP to decrease by 5.4 ± 1.7 mm Hg for 11 hours and DBP by 3.4 ± 1.3 mm Hg for 10 hours (Park *et al.*, 2006).

In a sample of stroke patients walking at 60% heart rate reserve (HRR) for 20 minutes resulted in a 79% increase in tPA activity and decreased PAI-1 activity by 18% immediately post-walk and plasma tPA activity remained elevated by 43% and PAI-1 activity was depressed by 25% at least 1-hr post-walk (Ivey *et al.*, 2003). These findings are significant because tPA dissolves excess fibrin clots, which may otherwise increase risk of thrombosis and PAI-1 is the main physiological inhibitor of tPA. An extension of this study would be to measure the activities of tPA and PAI-1 beyond 1-hr post-walk to determine the possible duration of effects, however these must be interpreted with the sample in mind since post-stroke patients are likely to have greater potential for

improvement than the apparently healthy population due to their predisposition for thrombosis. Due to the emerging importance of inflammatory mediators and the fact that these may alter acutely following intense exercise, the influence of walking at 60-70% HR_{max} on the inflammatory response has been investigated (Murtagh *et al.*, 2005a). The main findings of a walking study on inflammation were that walking at 60-70% HR_{max} for 45 min tended to decrease IL-6 from baseline ($1.92 \pm 0.29 \text{ mg}\cdot\text{L}^{-1}$) to $1.73 \pm 0.18 \text{ mg}\cdot\text{L}^{-1}$ and $1.76 \pm 0.16 \text{ mg}\cdot\text{L}^{-1}$ immediately- and 1-hr post-walk (NS) respectively, however it was significantly decreased 24 hours post-walk ($1.37 \pm 0.14 \text{ mg}\cdot\text{L}^{-1}$). Samples were only collected up to 1-hr post-walk, therefore it was only possible to see changes in IL-6 at 24-hr post-walk, which may have been apparent earlier. Furthermore, the study only employed a single trial, whereas a control trial may have highlighted other patterns.

Plasma NEFA and glycerol concentrations can increase by ~31% and ~49%, respectively, following 1-hr of walking at 36% $\dot{V}O_{2peak}$ compared to resting values, indicating increased rates of adipose tissue lipolysis and muscular oxidation (Kaminsky *et al.*, 1986). Furthermore, plasma glycerol peaked at the end of exercise whereas plasma NEFA carried on increasing and peaked at 10 min post-exercise and plasma NEFA remained above resting values until at least 60 minutes post-exercise. Walking during the postprandial period has demonstrated decreases in alimentary lipaemia and especially during the post-walk period in response to a high-fat meal. Acute exercise studies of postprandial lipaemia have used a variety of timings of exercise prior to or following the high fat meal. The effect of walking on postprandial lipaemia has been investigated by participants walking up to 1.5 hours into the postprandial period (Hardman & Aldred, 1995), 12 hours before (Tsekouras *et al.*, 2007), 15 hours before (Aldred *et al.*, 1994; Tsetsonis *et al.*, 1996a; 1996b), 18 hours before (Gill *et al.*, 2003) and also over successive meals during the day

(Murphy *et al.*, 2000). When normolipaemic young adults walked at 40% $\dot{V}O_{2max}$ for 1.5 h following consumption of a high-fat test meal the 3-hour TAG area under the curve decreased by ~24% compared to resting control (Hardman & Aldred, 1995). The decrease in plasma triacylglycerol concentrations indicate increased alimentary TAG clearance, presumably through increased muscular TAG uptake, as previously mentioned in section 2.3.

The greatest decreases in postprandial lipaemia following walking occur from studies where walking is performed at least 12 hours prior to the high fat meal. Walking for 2 hours at only $30.9 \pm 1.6\%$ $\dot{V}O_{2max}$ 15 hours before the meal significantly decreased postprandial lipaemia (Aldred *et al.*, 1994). Furthermore, 3 hours of low intensity walking ($32 \pm 1\%$ $\dot{V}O_{2max}$) has been found to be as equally effective as 1.5 hours of moderate intensity walking ($63 \pm 1\%$ $\dot{V}O_{2max}$) in reducing postprandial lipaemia compared to control (Tsetsonis & Hardman, 1996a). Indeed, it appears that the energy expended during walking was the key because a further study from this group found that walking for 90 minutes at a moderate intensity ($61 \pm 1\%$ $\dot{V}O_{2max}$) was more effective than walking for the same duration at a low intensity ($31 \pm 1\%$ $\dot{V}O_{2max}$) (Tsetsonis & Hardman, 1996b). It has been demonstrated that increases in LPLa are responsible for some of the reductions in postprandial lipaemia resulting from moderate intensity activities, such as walking, however LPLa does not explain all of the effects (Gill *et al.*, 2003).

In terms of ecological validity it is unusual for people to consume a meal excessively high in fat, as with the high fat test meals, and then fast for at least 6 hours, as is the case with many postprandial lipaemic exercise studies. Therefore in variation to the usual exercise study design for investigating postprandial lipaemia the effect of single and accumulative

bouts of walking on the postprandial lipaemic response to successive meals was studied to replicate every day lifestyle rather than fasting for ~6 hours (Murphy *et al.*, 2000). The main findings of the study were that walking at 60% $\dot{V}O_{2max}$ for either 30 minutes at the start of the day or for 10 minutes prior to each of the three prepared meals equally decreased postprandial lipaemia compared to controls and this effect was augmented following successive meals. A further study on postprandial lipaemia and of similar real life relevance investigated the effect of walking at 50% $\dot{V}O_{2max}$ for 30, 60 and 90 minutes compared to control immediately followed by the ingestion of a mixed meal of moderate fat content, which was followed by another similar meal three hours later (Pfeiffer *et al.*, 2005). The main findings of the study were that although the 60 and 90 minute walking sessions reduced postprandial lipaemia by 14 & 15% respectively, these were not significant and indicate that either mixed meals are of insufficient fat content to overload fat uptake into the blood and thus provide walking intervention with limited potential for effect or that a longer timescale between walking and the meals was needed in order for adaptations to take place in the lipid handling processes, such as increased LPLa. Alternatively, it may have been that the walking was of insufficient volume (90 min at 50% $\dot{V}O_{2max}$) to promote an effect.

2.4.3 Long-term adaptations

Walking is the most common mode of exercise studied when investigating the efficacy of accumulative PA on factors of health-related fitness. However, physicians can sometimes be hesitant to recommend increasing lifestyle physical activities for improving health outcomes rather than more vigorous pursuits, which is not helped by the equivocal evidence from underpowered walking studies and those with small sample sizes. Therefore, a recent meta-analysis of 24 randomised controlled trials was performed to make use of the greater statistical power of multiple studies and provide evidence that regular brisk walking improves health outcomes in sedentary healthy individuals by significantly improving traditional cardiovascular risk factors; $\dot{V}O_{2\max}$, body weight, BMI, percent body fat and resting diastolic blood pressure (Murphy *et al.*, 2007). Eighteen of the studies included females only and four mixed, with a total of 193 men and 935 women with a drop-out rate of 20.2% and 12.4% from walking and control trials, respectively.

The mean characteristics of the walking interventions were 38.3 ± 14.4 mins·session⁻¹, on 4.4 d·wk⁻¹, giving a mean volume of 188.8 min·wk⁻¹, for 34.9 ± 4.9 weeks, at a mean intensity $70.1 \pm 9.1\%$ HR_{max} ($56.3 \pm 7.1\%$ $\dot{V}O_{2\max}$) reported from 20 of the studies, and the mean rate of adherence was $87.8 \pm 9.9\%$. Following the pooled walking interventions there was a mean increase in $\dot{V}O_{2\max}$ of 2.73 ± 0.35 mL·kg⁻¹·min⁻¹ (9%), mean decreases of -0.95 ± 0.61 kg (-1.4%) and -0.28 ± 0.20 kg·m⁻² (-1.1%) and $-0.63 \pm 0.66\%$ (-1.9%) in body mass, BMI and body fat percentage, respectively, compared to baseline. Each of these favourable changes were significant ($P \leq 0.035$) and although resting diastolic blood pressure was significantly decreased by -1.54 ± 0.79 mm Hg (-2%; $P=0.026$) systolic blood pressure was not favourably changed through walking intervention. However, relatively

few studies measured blood pressure pre & post (SBP=9; DBP= 6), thus decreasing the chances of a favourable outcome compared with the other measures.

In a meta-analysis of 25 randomised controlled walking trials involving 18 women only trials, 2 male only trials and 5 mixed sex trials, 1,176 participants were studied to investigate the influence of walking on blood lipids and lipoproteins (Kelley *et al.*, 2004). The walking studies were performed at $64.2 \pm 9.4\% \dot{V}O_{2\max}$ for $38.4 \pm 15.6 \text{ min}\cdot\text{session}^{-1}$ on $4.8 \pm 2.5 \text{ d}\cdot\text{wk}^{-1}$ for 23.2 ± 17.7 weeks and the rate of adherence was $83.4 \pm 18.0\%$. The main findings of the analysis was that walking can significantly reduce LDL-C by $\sim 5\%$ and TC/HDL-C ratio by $\sim 6\%$ independently of reductions in body mass, and although no significant changes in TC, HDL-C or TAG were observed, the direction of change tended to be favourable. A secondary finding of the study was an increase in $\dot{V}O_{2\max}$ of $3.6 \pm 0.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, which equated to a 15% increase compared to controls, but body mass, BMI, percent body fat and lean body mass were not significantly altered.

A study investigating similar health outcomes to those presented in section 3.0, such as measures of body composition and insulin resistance, demonstrated that by instructing sedentary obese Japanese participants to add 1,000 steps to daily walking activity at baseline for a 1-yr period, the increase in $\text{steps}\cdot\text{d}^{-1}$ was significantly correlated with reductions in visceral adipose tissue which was also associated with decreases in the homeostasis model assessment index (HOMA) ($P < 0.001$) (Miyatake *et al.*, 2002). Measures of changes in body composition also correlated significantly with decreases in HOMA index included body mass, BMI, WC, WHR, and subcutaneous & visceral fat area (all $P < 0.05$), however body fat percentage was not. A prospective study investigating the efficacy of walking on future risk of developing T2D in 70,102 female nurses aged 40-65

years found that walking produced similar magnitudes in risk reduction as vigorous activity of equivalent energy expenditure and that increased walking pace was independently associated with decreased risk during 8-year follow up (Hu *et al.*, 1999). These findings have significant health implications as all-cause mortality among US adults with diabetes was reported to be 39% lower in individuals who walk at least 2 h·wk⁻¹ and those who walked 3-4 h·wk⁻¹ had the lowest risk at 8-year follow up (Gregg *et al.*, 2003). Participants with T2D that were instructed to walk >10,000 steps·d⁻¹ on ≥5 d·wk⁻¹ for 6 weeks significantly increased HDL-C and resting energy expenditure, while significantly reducing PAI-1 activity compared to controls (Araiza *et al.*, 2006). One of the few studies to investigate the influence of walking on coagulation demonstrated that walking at 73.5 ± 7.2% HR_{max} for 18 weeks significantly decreased factor XIIa (FXIIa), the active form of FXII that is involved in coagulation, compared with sedentary controls, however there were no decreases in blood lipid profile and changes in $\dot{V}O_{2max}$ were not reported (Woolf-May *et al.*, 2000).

The total volume of PA is recognised as of greater importance for health benefits than intensity *per se* in epidemiological studies and reviews (Haskell, 1994), and to partly investigate this evidence in an intervention, the health benefits of different walking volumes were studied (Ready *et al.*, 1996). This was performed over a duration of 24 weeks and involved participants walking for 60 min·d⁻¹ at 60% $\dot{V}O_{2peak}$ for either 3 d·wk⁻¹ or 5 d·wk⁻¹, or remaining sedentary and the main findings were that walking on 3 and 5d·wk⁻¹ significantly improved $\dot{V}O_{2peak}$ by 12 & 14% and percent body fat by -1.1 & -1.3%, respectively, however neither walking volume was sufficient to favourably alter serum lipids. A more recent study went a step further to investigate whether different walking intensities (45% v 55% $\dot{V}O_{2max}$) at different walking volumes (200 v 300

kcal·session⁻¹) had variable effects on health outcomes (Asikainen *et al.*, 2003). Participants were randomised to walk for 30 min·d⁻¹ on 5 d·wk⁻¹, expending either 200 kcal·session⁻¹ at either 45% $\dot{V}O_{2max}$ or 55% $\dot{V}O_{2max}$ (both 1000 kcal·wk⁻¹) or expending 300 kcal·session⁻¹ at either 45% $\dot{V}O_{2max}$ or 55% $\dot{V}O_{2max}$ (both 1500 kcal·wk⁻¹) for 24 weeks. The main findings were that $\dot{V}O_{2max}$ improved irrespective of the walking intensity or volume compared to control, however none of the walking prescriptions significantly decreased body mass. Furthermore, it appears that despite the significant improvements in $\dot{V}O_{2max}$ these were also not accompanied by significant favourable changes in blood pressure, blood lipoprotein profile or glucose tolerance. The power calculations for the study were based on $\dot{V}O_{2max}$ changes, which may explain why this was the only health outcome to favourably change and that the study might have been underpowered.

A study involving overweight pre-menopausal women investigated whether supplementing daily diet limited to 5-5.8 MJ with 5 × 30 min or 5 × 60 min of walking would promote favourable changes in body composition, blood lipids, blood pressure, $\dot{V}O_{2max}$, compared to dietary intervention alone (Bond Brill *et al.*, 2002). The addition of walking to the dietary procedure did not enhance declines in body weight, body fat percentage, BMI, WHR, diastolic blood pressure TC, TAG and TC/HDL-C as all three programmes resulted in similar significant reductions, whereas WC, $\dot{V}O_{2max}$ and LDL-C demonstrated greater improvements than diet alone, with no differences between walking volumes. Due to the dietary procedures, this was a rather ambitious study and a little difficult to draw a firm conclusion. However, it appeared that the addition of walking to a dietary procedure did not augment improvements in every health variable, but only in WC, $\dot{V}O_{2max}$ and LDL-C. The improvements in $\dot{V}O_{2max}$ indicate that PAEE was significantly greater in the walking groups compared to the diet group, which is the most likely explanation for the greater

decreases in WC. The likely reason why there were no differences between exercise groups was the energy compensation in the 60 min walk group who consumed an extra 1300 kJ per day than the 30 min walk group. It may also be speculated that the additional decreases in LDL-C were due to the additional EE in the walking groups rather than improvements in $\dot{V}O_{2\max}$ as the decreases in LDL-C tended to be greater in 60 min than 30 min, despite a tendency for greater $\dot{V}O_{2\max}$ improvement in 30 min.

Studies of the effects of daily walking on cardiovascular health appear to have been equivocal, where 18 weeks of walking at $\sim 74\%$ HR_{\max} and expending ~ 900 kcal \cdot wk $^{-1}$ improved cardiovascular function by facilitating increases in the velocity of left ventricular relaxation and improved cardiovascular fitness in a study of 29 men and women (Woolf-May *et al.*, 1997), whereas 18 weeks of accumulative walking proved ineffectual in improving cardiac function in 64 men and women (Woolf-May *et al.*, 2003). However, in a more recent study similar to that presented in section 3.0, which investigated the effect of accumulative brisk walking on MetS risk, primary care patients took part in a randomised controlled walking trial investigating the benefits of accumulating 30 min \cdot d $^{-1}$ of brisk walking on 5 d \cdot wk $^{-1}$ for 12 weeks on body composition, blood pressure, functional capacity, blood lipoprotein profile and 10-year CVD risk (Tully *et al.*, 2005). The main findings of this study were that after the participants performed 27.72 ± 9.79 min \cdot d $^{-1}$ of walking for 12 weeks, systolic blood pressure, diastolic blood pressure, functional capacity and risk of stroke were favourably improved. However, there were no significant improvements in measures of body composition and lipoprotein profile. A further point to note was that the majority of the participants performed their walking in a single daily session and accumulative walking only accounted for 3.14% of the reported walking prescription.

The longest walking intervention included in this review investigated the influence of a 1-year programme of brisk walking on cardiorespiratory function, body composition and blood lipoprotein profile in previously sedentary middle-aged men (Stensel *et al.*, 1993; 1994). The participants walked at $1.95 \text{ m}\cdot\text{s}^{-1}$ ($68 \pm 1\% \text{ HR}_{\text{max}}$) for $28 \text{ min}\cdot\text{d}^{-1}$ and the main findings were that despite the long intervention period that required the participants to cover $\sim 1,168 \text{ km}$ in total, only cardiorespiratory function improved, whereas body composition and the blood lipoprotein profile were not favourably altered. However, the men participating in the study were relatively healthy, and thus had less potential gains to make from the intervention, and since dietary intake was only recorded for 7 days then there was also potential to compensation for energy expended during the walking activity through increased energy consumption. Evidence suggests that reductions in visceral fat are associated with improvements in $\dot{V}\text{O}_{2\text{max}}$, where sedentary obese women involved in a walking and weight loss programme who improved their $\dot{V}\text{O}_{2\text{max}}$ by $\sim 10\%$ reduced visceral fat by $\sim 20\%$, whereas those whose $\dot{V}\text{O}_{2\text{max}}$ did not improve only reduced their visceral fat by $\sim 10\%$ (Lynch *et al.*, 2001). However, despite dividing the study sample into quartiles based on $\dot{V}\text{O}_{2\text{max}}$ changes, PA level did not appear to be taken into consideration, which impacts both $\dot{V}\text{O}_{2\text{max}}$ and adiposity due to the direct relationship between PA and energy expenditure.

Exercise studies of postprandial lipaemia tend to use an acute design in order to determine the immediate (24 hr) effects of a single exercise session on blood TAG clearance (Section 2.6.2). A study that did investigate the ‘trainability’ of blood TAG clearance found that despite the experimental group walking at $1.76 \pm 0.02 \text{ m}\cdot\text{s}^{-1}$ for $21 \pm 1 \text{ min}\cdot\text{d}^{-1}$ for 12 weeks and improving $\dot{V}\text{O}_{2\text{max}}$, decreasing body fat and decreasing area under the insulin-time

curve compared with controls, postprandial lipaemia was unaffected (Aldred *et al.*, 1995). The rationale for endurance training to attenuate postprandial lipaemia is the training induced increases in capillarisation, which is significant because LPL, the enzyme responsible for hydrolysing TAG-rich lipoproteins, is located on the luminal surface of capillaries. Therefore, using this premise in order for exercise training to enhance TAG clearance then $\dot{V}O_{2\max}$ must be significantly improved and it appears that if this is true then the threshold $\dot{V}O_{2\max}$ for improving TAG clearance was not attained by the females in the study. The same research group also demonstrated that favourable exercise-induced changes, including cardiorespiratory function, body fat percentage and HDL-C, occurred following 12 weeks of brisk walking in sedentary females and that these changes were reversed following a further 12 week period of detraining. These findings demonstrate that walking can create significant health benefits and that walking needs to be maintained to retain the associated health benefits (Hardman & Hudson, 1994).

2.4.4 Summary

Evidence demonstrates that walking can promote favourable changes in CVD risk factors, both in the immediate post-exercise period and in response to long-term participation. However, the findings are equivocal at times, depending on the design of the study and the physiological variables being measured. In terms of the short-term responses to walking, blood pressure, fibrinolysis, inflammation and lipid metabolism may be favourably altered, while the health benefits that may be derived from long-term participation in walking include improvements in body composition (\downarrow body mass, \downarrow fat mass, \downarrow body fat percentage & \downarrow waist circumference), $\dot{V}O_{2\max}$, blood pressure, blood lipid and lipoprotein profile and decreased risk of blood clotting. Data from the available studies suggest that these changes may be attained by walking at $\sim 70\%$ HR_{\max} for $30 \text{ min}\cdot\text{d}^{-1}$ on $5 \text{ d}\cdot\text{wk}^{-1}$. This guidance provided the basis for the 24-week intervention described in section 3.0 investigating the influence of walking on a range of these risk factors, which characterise MetS. The shorter term effects of walking of different intensities and durations will also be assessed in section 4.0 because no published study has used this study design, particularly with MetS risk factors.

2.5 Physical activity accumulation

2.5.1 Introduction

Two common misconceptions regarding PA have been that it must be of vigorous intensity and performed in a single session to be of benefit to fitness and health. In order to address these misconceptions the joint statement by CDC & ACSM (1995) (See section 2.8.6) recommended that PA could be effectively accumulated in smaller bouts to produce similar improvements in health outcomes to single sessions of equal volume. Prior to these recommendations only two published studies had investigated the efficacy of accumulated PA on health. These studies investigated the influence of splitting running distance on cardiovascular endurance and blood lipids (Ebisu, 1985) and the effect of short vs. long bouts of exercise on $\dot{V}O_{2\max}$ (DeBusk *et al.*, 1990). Since the 1995 PA recommendations were published, there has been a rapid increase in the number of studies performed to determine the efficacy of accumulating PA on a range of health outcomes in both acute and long-term studies. The health outcomes investigated by acute PA studies include the immediate effect of exercise on elevated post-exercise oxygen consumption (EPOC)/energy expenditure (EE) (Kaminsky *et al.*, 1990; Fulton *et al.*, 2001; Peterson *et al.*, 2004), postprandial lipaemia (Murphy *et al.*, 2000), postprandial lipaemia & blood pressure (Miyashita *et al.*, 2006), blood lipid profile (Mestek *et al.*, 2006), blood pressure (Padilla *et al.*, 2005; Park *et al.*, 2006) and glucose control (Baynard *et al.*, 2005).

Although the immediate responses to PA could potentially be important for the maintenance of good health, the majority of studies have focused on the adaptations that occur from long-term participation in accumulative PA compared to controls and/or equal volumes of exercise performed in prolonged single sessions. The combinations of health outcomes studied by these investigations include cardiorespiratory fitness & weight loss

(Jakicic *et al.*, 1995; Schmidt *et al.*, 2001; Asikainen *et al.*, 2002), aerobic capacity, body composition, blood lipids & glucose control (Snyder *et al.*, 1997), $\dot{V}O_{2\max}$ & body composition (Murphy & Hardman, 1998; Osei-Tutu & Campagna, 2005), $\dot{V}O_{2\max}$ (Thomas *et al.*, 2001; Macfarlane *et al.*, 2006), $\dot{V}O_{2\max}$, blood lipids & body composition (Murphy *et al.*, 2002), weight control (Sykes *et al.*, 2003; Thompson *et al.*, 2004), exercise adherence (Jacobsen *et al.*, 2003), heart function (Woolf-May *et al.*, 2003), cardiorespiratory fitness & blood lipids (Woolf-May *et al.*, 1998; 1999; Boreham *et al.*, 2005), glucose control (Eriksen *et al.*, 2007; Venables & Jeukendrup, 2008), $\dot{V}O_{2\max}$ and HDL-C (Quinn *et al.*, 2006), aerobic capacity, blood pressure, blood lipids & glucose control (Asikainen *et al.*, 2003), body composition, blood pressure & blood lipids (Murtagh *et al.*, 2005b) and aerobic capacity, blood pressure, blood lipids and body composition (Kennedy *et al.*, 2007).

2.5.2 Long-term physical activity accumulation

In the first published study to challenge the dogmatic perception that PA must be performed in a single prolonged daily session in order to be effective, Ebisu (1985) demonstrated that splitting up running distance into smaller fractions had the same effect on cardiovascular function and blood lipids as performing the distance in a single bout. This study examined whether 10 weeks of progressive running had the same effect on the blood lipid profile and $\dot{V}O_{2\max}$ whether this was performed in one, two or three daily sessions compared to controls. The study employed an independent measures design where participants performed no activity, or ran for 3 miles·day⁻¹ in weeks 1-3, 4 miles·day⁻¹ in weeks 4-6 and 6 miles·day⁻¹ in weeks 7-10. There were three running groups who performed the running in a single session, 2 sessions or in 3 sessions per day, i.e. 1 × 3 miles, 2 × 1.5 miles and 3 × 1 mile, respectively during weeks 1-3 and so on. The main findings of this study were that 1.5 mile run time decreased and $\dot{V}O_{2\max}$ increased significantly following each pattern of running compared to control. However, HDL-C was only significantly increased following the pattern of splitting the running distance into three sessions during the day. A further finding of note was that there were no differences between running groups for any of the dependent variables, however no information was provided regarding adherence to the program, only that 53/60 completed, and no indication was given regarding the duration between the split sessions.

A slightly more high profile study investigated the effect of performing ‘jogging’ in 3 bouts of 10 minutes compared to 30 minutes on 5 days of the week at 65-75% peak treadmill HR (DeBusk *et al.*, 1990). This study differed to that by Ebisu (1985) because it identified the exercise frequency (5 d·wk⁻¹), exercise volume was prescribed as 150 min·wk⁻¹ rather than 3 miles·d⁻¹ and lasted 8 weeks, however it did not include a control

group and only monitored measures of functional capacity, but adherence to the programme was recorded and the duration between multiple bouts was stated (≥ 4 hr). The main conclusion to this study was that “multiple short bouts of moderate intensity physical exercise produced significant training effects” (DeBusk *et al.*, 1990). However, these significant training effects consisted of increases in $\dot{V}O_{2\max}$, exercise test duration and decreased HR at submaximal exercise compared to baseline following the exercise period. The authors failed to explain why, despite the post-intervention increases in exercise test duration and decreases in submaximal HR being similar between exercise patterns, the multiple bouts only improved $\dot{V}O_{2\max}$ by 57% of the improvement observed in the single bouts (7.6% v 13.9%).

A clear criticism of this study was that there was no control group, therefore single sessions of equal time were used as the benchmark for the efficacy of multiple bouts. A further unusual factor relating to the study was that participants with a mean baseline $\dot{V}O_{2\max}$ of $33 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ were reported to be jogging at only 70 & 71% HR_{peak} (54-56% $\dot{V}O_{2\max}$), however in this group running at 5 mph would require 8.6 METs (91% $\dot{V}O_{2\max}$) (LaFontaine & Robbins, 1991). This perhaps indicates that the participants performed brisk walking or jogged-walked during the sessions rather than jogged for the full duration of the sessions. It is important to bear in mind that, along with Ebisu (1985), it was the study that helped to break down the perception that PA had to be prolonged to be useful to health and provided the basis for PA recommendations for accumulating PA throughout the day in multiple shorter sessions (≥ 10 minutes) (CDC & ACSM, 1995) and prepared the groundwork for future studies in this area. In a similar study, Macfarlane *et al.* (2006) also investigated the efficacy of accumulating multiple smaller bouts of activity throughout the day, however these were ≤ 10 minutes. The study contained 45 participants (30 females)

who were randomly allocated to 30 min·d⁻¹ of PA on 3-4 d·wk⁻¹ (LB) or 6 min·session⁻¹ for 5 session·d⁻¹ on 4-5 d·wk⁻¹ (SB) for 8 weeks. Physical activity levels were 10-11 MET h·wk⁻¹ and estimated using daily activity logs, which included time of day, duration, mode and RPE, and the activities were calculated from published values (Ainsworth *et al.*, 1993), however no indication was provided regarding the nature of the PA, i.e. walking cycling. The main findings of the study were that $\dot{V}O_{2\max}$ improved significantly following both treatments (6.2% in LB & 4.1% in SB) with the improvements significantly higher in LB than SB, which were in agreement with the findings of Debusk *et al.* (1990).

In a study published at the time of the new recommendations (CDC & ACSM, 1995), the effect of multiple bouts of walking in 10 minute sessions (SB) on $\dot{V}O_{2\max}$ and weight loss was performed for 20 week by sedentary overweight females (Jakicic *et al.*, 1995). This study presented itself as a RCT, and has also been included in a list of RCTs (Asikainen *et al.*, 2002), however there were no control participants and 28 of the 56 participants were allocated to the separate walking groups. Therefore, the health outcomes were only compared to walking for equivalent durations in single long bouts (LB) instead of using a control group, which does not allow comparison to the effects that may naturally occur over the course of 20 weeks. Furthermore, participants' diets were restricted to 1200-1500 kcal·day⁻¹, thus clouding the discrete benefit of both patterns of walking on weight loss and consequently $\dot{V}O_{2\max}$. During the walking intervention the participants were instructed to accumulate 4 × 10 minutes of walking, however the accelerometers and diaries that were used to monitor compliance indicated that those in the SB group preferred to accumulate 3 × 15 minutes of walking instead. The main findings of that study were that walking performed in single or multiple sessions during the day produced similar increases in predicted $\dot{V}O_{2\text{peak}}$ (5.6% v 5.0% for LB and SB, respectively), but there was a trend for

weight loss to be greater in SB (-8.9 ± 5.3 kg) v LB (-6.4 ± 4.5 kg; $P=0.07$). It must be emphasised that both patterns of walking induced such weight losses predominantly due to the dietary intervention to promote weight loss. However, suggestions as to why there was a trend for SB to achieve greater weight losses are that SB tended to walk for a greater volume (223.8 ± 69.5 min·week⁻¹ vs 188.2 ± 58.4 min·week⁻¹; $P=0.08$). Participants reported exercising on more days than LB and it may be speculated that this was due to 10 minutes being easier to complete than 40 min and those in LB may not have attempted some sessions if they could not complete the entire 40 minutes. Furthermore, exercising in short bouts may influence calorie intake, i.e. reducing lunch hour or used to relieve boredom instead of food.

A further similar study performed two years later also investigated the influence of accumulative walking in 13 sedentary moderately obese females who were asked to perform 5 x 30 min (3 x 10 min) of walking ('walking briskly, yet comfortably' at 50-65% HRR) for 32 weeks (Snyder *et al.*, 1997). However, despite the long intervention period there were no significant changes in aerobic capacity, body composition, blood lipids, insulin or glucose. An issue with the design of this study was that there were no controls and not even a walking group performing the same volume of walking in single daily sessions, therefore no data was apparent to determine any relative changes, such as decreased fitness and increased body fat, which may have occurred naturally over the course of the 32 week period without the walking intervention.

There has been an increase in the RCTs performed to assess the efficacy of accumulative PA on health outcomes. In fact many of these studies not only contain a control group but also a group that performs a similar daily PA volume in a single session. The design of the

study in section 3.0, a RCT involving a sedentary control group and a long session brisk walking group, not only compares the benefits of accumulative PA compared to the outcomes that may occur as a result of sedentariness, but also allows the determination of the effect of PA accumulation compared to longer single PA sessions. These are essential when studying accumulative PA in order to investigate the relative efficacy of accumulative PA *per se*. The essence of the argument for promoting accumulative PA is that the energy expended in PA is more important than the frequency, intensity or duration in particular. In this respect, and in variation to the majority of studies that investigate daily accumulative sessions, Sykes *et al.* (2003) studied the effect of two different patterns of accumulating PA during the week rather than daily PA accumulation. Thirty Singaporean females were randomly allocated to one of two 8-wk programmes of cycling & treadmill walking at “a moderate, comfortable intensity”, and the programmes involved either 5 sessions·wk⁻¹, each expending 400 kcal·session⁻¹ (SB), or 2 sessions·wk⁻¹, each expending 1000 kcal·session⁻¹ (LB). Food intake was recorded in diaries. The main findings were that body mass (-2.03kg [-3.2%] & -1.90 kg [-3.0%]), BMI (-0.78 kg·m⁻² [3.2%] & -0.73 kg·m⁻² [3%]), body fat % (-1.37% [4.5%] & -1.19% [4.0%]) and WC (-2.62 cm [3.2%] & -2.34 cm [2.7%]) were significantly decreased in both SB & LB, whereas lean mass & HC remained the same. These are significant findings that moderate-intensity exercise, performed for only 8 weeks, and irrespective of the frequency and duration of PA, produces similar positive effects on body composition. However, a weakness of the study was a lack of control group, which would have strengthened the study.

In an earlier RCT, 34 women completed 10 weeks of brisk walking on 5 days of the week at 70-80% HR_{max} in either one 30 minute walk (LB) or three 10 minute walks per day (SB), or performed no walking as controls and participants were instructed to make no

dietary changes (Murphy & Hardman, 1998). The main findings of this study were that $\dot{V}O_{2\max}$ increased and sum of skin folds decreased significantly in both walking groups with no differences between walking groups relative to controls, however body mass and waist circumference decreased significantly only in the SB group. The authors also commented that body/fat mass gained by the controls contributed to the significant responses to the walking. Indeed this demonstrates the importance of including controls in such studies because although exercise intervention may not improve health outcomes, it can often prevent age-associated declines in health-related fitness, which are not necessarily demonstrated in non-controlled exercise trials.

A further RCT of accumulative walking studied 49 males and females, who completed an 18 week intervention where one group acted as non-exercising controls, one group performed single daily brisk walking sessions of 20-40 minutes (LB) and the other group accumulated the same duration of walking in 10-15 minute sessions throughout the day with ≥ 120 minutes between sessions (Woolf-May *et al.*, 1998). Participants were instructed to perform $60 \text{ min}\cdot\text{wk}^{-1}$ of walking in the first week, which increased to $200 \text{ min}\cdot\text{wk}^{-1}$ by week 12. The main outcomes of the study were that aerobic capacity significantly decreased and factor XIIIa, a marker of coagulation, significantly increased in the control group, however there were no significant changes in lipid profiles (TC, LDL-C, HDL-C and apos A-I, A-II & B) in the walking groups compared with controls. This was an important study because it was the first to compare the efficacy of accumulative walking sessions to walking of equal daily volume *and* to non-active controls, which fully examined the health benefits of accumulation *per se* relative to no activity and activity performed in single daily sessions. A further study by the same group performed a study of similar structure to that described above, however an additional group was added, in which

participants were instructed to perform brisk walking sessions in 5-10 minute bouts totalling 20-40 min·d⁻¹ (Woolf-May *et al.*, 1999). The main findings of this study were that each of the walking groups – long, intermediate and short duration – produced positive health outcomes compared to controls, however the long and intermediate groups demonstrated the more potent effects. Each of the walking groups promoted improvements in aerobic fitness, whereas only the intermediate and long sessions produced favourable improvements in the blood lipid profile compared to controls.

In a study of different patterns of walking (Murphy *et al.*, 2002), 21 participants (14 F) completed two different 6 week brisk walking programmes at 70-80% predicted HR_{max} in a cross-over design with 2 weeks in between. One programme involved brisk walking for 30 minutes in a single daily session on 5 days of the week (LB) and the other involved accumulating 30 minutes of brisk walking in 3 × 10 minute sessions on 5 days of the week (SB). The study did not include a 6 week control period. The main findings were that both programmes increased plasma HDL-C, and decreased TAG & total cholesterol, despite no changes in body mass. However, SoS, WC & HC were reduced, indicating that increases in lean body mass may have accounted for decreases in adiposity. Predicted $\dot{V}O_{2max}$ tended to increase following both programmes, but only significantly greater following SB. Despite the intervention being relatively short, the brisk walking interventions induced significant health benefits, which must be attributed to the high adherence to the programmes (88.2 ± 1.1% in SB & 91.3 ± 4.1% in LB) and also to the relatively high intensity of the walking (70-80% predicted HR_{max}).

In another RCT, 130 postmenopausal women completed single or double daily walking sessions, expending a total daily PA volume of 300 kcal·d⁻¹ at 65% $\dot{V}O_{2max}$, or remained

sedentary for 15 weeks (Asikainen *et al.*, 2002). The main outcomes of this study were that both single and multiple sessions of walking provided similar increases in $\dot{V}O_{2\max}$ (2.5 mL·kg⁻¹·min⁻¹ for both walking groups) and decreases in fat% (1.7% in SB & 21.1% in LB). An extension of this study, looking at blood pressure, serum lipoproteins, fasting blood glucose and plasma insulin, and also glucose tolerance, was published shortly afterwards (Asikainen *et al.*, 2003) and used the same study design and participants. The main findings of this study were that although diastolic blood pressure was not significantly reduced following walking in the discrete walking groups, when these were combined as a single group there was a mean decrease of -3 mmHg compared to controls. Furthermore, blood glucose was significantly reduced in both SB and LB and 2-hour glucose concentrations were decreased in both walking groups. However, despite 15 weeks of walking, neither the lipid profiles nor insulin concentrations changed. These data suggest that accumulative walking is at least as effective as walking in a prolonged single session of equal energy expenditure.

The study requiring the lowest weekly accumulative walking volume was a RCT by Murtagh *et al.* (2005b), involving brisk walking for a total of 20 min·d⁻¹ on 3 d·wk⁻¹ for 12 weeks, based on the absolute minimum PA recommendations, in 32 previously sedentary individuals. The three groups consisted of a control group, a group performing 20 min·d⁻¹ while another group performed 2 × 10 min·d⁻¹, however the recommended PA volume appeared to be insufficient to promote improvements in health, even in sedentary individuals. Body mass, adiposity, blood pressure, waist & hip circumferences and lipid profile were measured and there were no significant responses in any of these variables following either walking pattern relative to control. It must be emphasised that it was not accumulation *per se* that was ineffective for promoting positive health outcomes because

the LB group also failed to demonstrate improvements. In terms of PA recommendations, these are important findings because they suggest that performing PA according to previous PA recommendations, such as the minimum frequency (3 d), mode and intensity (walking) and duration (20 min), is insufficient to decrease certain CVD risk factors. Furthermore, a study involving only a four week training programme demonstrated that exercising for 30 min·d⁻¹ on 5 d·wk⁻¹ and increasing by 10 min·d⁻¹ until 60 min·d⁻¹ at an intensity designed to elicit maximal fat oxidation was more effective in improving fat oxidation and insulin sensitivity than isocaloric exercise accumulated in five minute bouts (Venables & Jeukendrup, 2008). Fat oxidation increased by 44% and insulin sensitivity increased by 27% following the LB programme, whereas no changes were apparent following SB, and these improvements occurred despite no changes in body mass, WHR or body fat percentage.

2.5.3 Summary

The results of studies investigating the efficacy of accumulating PA on health outcomes have been equivocal at times, however this is also true of exercise studies in general, and not specific to accumulated PA. The consensus from these studies is that, on the whole, the total PA volume is most important irrespective of the pattern of activity and this is why the most recent PA recommendations (ACSM & AHA, 2007), advocate that PA can be effectively accumulated throughout the day to promote good health. In the majority of the studies mentioned above, the common factor measured following accumulative PA was $\dot{V}O_{2max}$ with a range of other independent health risk factors also measured to varying degrees. However, $\dot{V}O_{2max}$ *per se* is not an actual risk factor and is only a marker for health based on the association between low $\dot{V}O_{2max}$ and increased presence of health risk factors, such as overweight/obesity, insulin resistance, dyslipidaemia & hypertension (Blair *et al.*, 1996). This means that studies that only measure $\dot{V}O_{2max}$ as the dependent variable simply infer improved health based on the relationship between $\dot{V}O_{2max}$ and CVD risk factors rather than demonstrated within the results. However, those studies that investigate the influence of accumulative PA on other CVD risk factors, such as blood pressure and blood lipid & lipoprotein profile, enhance the value of those studies that only study $\dot{V}O_{2max}$, which assume positive health outcomes by proxy. Unusually, few of the studies of accumulative PA have specifically used only males in their studies, where females only, a mix of males and females or post-menopausal women have been used as research participants. Therefore, the study in section 3.0 will investigate the efficacy of accumulative walking in middle-aged men

Interestingly, the majority of studies of accumulative PA have used walking as the PA medium of choice, but stair climbing appears to be gaining popularity (Boreham *et al.*,

2005; Kennedy *et al.*, 2007). However, this is most likely due to the target population being relatively sedentary and need a simple form of PA to encourage them to become more active on a daily basis. Furthermore, more vigorous forms of PA may be impractical because too much time during the day would be spent on washing and getting changed following this type of activity, thus missing the point of accumulative PA fitting neatly into daily living.

3.0 The influence of 24 weeks of accumulative brisk walking on risk factors associated with metabolic syndrome and reverse cholesterol transport

3.1 Introduction

As previously noted, MetS classifies the clustering of co-related cardiovascular disease risk factors, characterised by the presence of 1) abdominal obesity, 2) insulin resistance, 3) hypertension, 4) dyslipidaemia, and 5) emerging risk factors, such as pro-thrombotic and pro-inflammatory states (see section 2.1) (Wannamethee *et al.*, 2005). This clustering of risk factors is reported to increase the odds ratio for cardiovascular and all-cause mortality above the risk associated with the individual components, where MetS may increase the risk of cardiovascular death 2-4 fold and reduce life expectancy by 5-10 years (Libby & Theroux, 2005). There are numerous definitions of MetS from international organisations, such as WHO (Alberti & Zimmet, 1998), ATPIII (Cleeman, 2001), IDF (Balkau *et al.*, 1999) and EGIR (Balkau & Charles, 1999) and the ATPIII definition has been used to classify the presence of MetS in this study (see table 2.1, section 2.1.6). Using the WHO definition of MetS (Alberti & Zimmet, 1998), the worldwide prevalence of MetS is 18.7% for females and 28.2% for males (Cameron *et al.*, 2004), with English males and females aged 40-65 demonstrating the highest prevalence (>44.8 & 33.9%, respectively) (Balkau *et al.*, 2002).

Metabolic syndrome is a multi-factorial condition and consequently there are a multitude of possible causes, where lifestyle and genetic factors may predispose individuals to developing MetS. Possible determinants of MetS include modifiable factors such as sedentariness (Ford *et al.*, 2005; Ekelund *et al.*, 2005) and elevated energy consumption (Zhu *et al.*, 2004; Liese *et al.*, 2007) and when these lifestyle behaviours are combined

they may adversely affect vulnerabilities in the human genome (Bouchard, 2007), which are also influenced by gender (male) (Fox *et al.*, 2007) and age (\geq middle age) (Schubert *et al.*, 2006). Therefore, therapeutic interventions to prevent or ameliorate symptoms of MetS are aimed towards those determinants that may be modified. Many of the interventions to decrease MetS risk involve complementary treatments, which include both dietary and exercise therapy, however few have investigated the benefits of increasing PA alone. Clinical trials have shown that modifying major components of the syndrome – atherogenic dyslipidaemia, hypertension and the prothrombotic state – will reduce CVD risk, yet few controlled studies have reported the effectiveness of exercise training alone for multiple metabolic risk factors (Carroll & Dudfield, 2004). Recent reviews present many studies concerning the potential for increase PA to favourably influence the individual components associated with MetS, such as levels of obesity (Jakicic, 2003), abdominal obesity (Kay & Fiatarone Singh, 2006), insulin resistance (Henriksen, 2002) & glucose tolerance (Yates *et al.*, 2007), blood pressure (Pescatello, 2005), lipid and lipoprotein profile (Durstine *et al.*, 2001) and markers of pro-thrombotic (Imhof & Koenig, 2001) & pro-inflammatory states (Kasapis & Thompson, 2005) (see section 2.3).

Cross-sectional evidence suggests that the relationship between fitness and the presence of MetS may be mediated through lower adiposity, where the relationship between body fat percentage, visceral fat, subcutaneous fat and MetS components are stronger than the association between fitness and MetS components, and the effects of $\dot{V}O_{2\max}$ on individual MetS components is mostly attenuated after controlling for total and abdominal obesity (Boule *et al.*, 2005). In a 12 month intervention study involving 137 men with International Diabetes Federation (IDF)-defined MetS who were randomly allocated to control, diet, exercise or combined exercise/diet groups found that each intervention decreased the

presence of MetS symptoms compared to the control group (Anderssen *et al.*, 2007). Following the interventions 76.5% in the exercise group (supervised aerobic exercise; $3 \times 60 \text{ min} \cdot \text{wk}^{-1}$ at 60-80% HR_{peak}), 64.7% following 12 months in the diet group (dietary advice at 3, 6 & 9 months; energy restriction, reduced saturated fat & cholesterol and increased fish consumption) and 32.6% in the combined exercise and diet group still had MetS. An important aspect of MetS is the atherogenic blood & lipoprotein profile, however the body has an in-built capability to reduce some of the risk associated with dyslipidaemia through the process of reverse cholesterol transport (RevCholT), an emerging marker of MetS because each component of MetS – central adiposity, insulin resistance, elevated plasma triacylglycerol and hypertension – is associated with low levels of HDL-C (Lewis & Rader, 2005), which is a primary agent in RevCholT and a component of dyslipidaemia (Barter, 2004) (see section 2.2). However, there is relatively limited literature describing the direct influence of $\text{PA}/\dot{\text{V}}\text{O}_{2\text{max}}$ on reverse cholesterol transport, despite there being a wealth of data on the relationship between increased $\text{PA}/\dot{\text{V}}\text{O}_{2\text{max}}$ and increased HDL-C concentrations, which is both an agent, and a rough marker, of RevCholT activity (Kodama *et al.*, 2007). Despite the limited studies of PA and RevCholTran, there is cross-sectional (Brites *et al.*, 2004) and longitudinal (Seip *et al.*, 1993) evidence that PA may modulate improvements, with CETP and LCAT receiving the greatest attention.

Even though vigorous PA is beneficial to health, activities of daily life, such as walking can be accumulated throughout the week to provide a large enough volume of PA to gain significant health benefits, evidence of which has been available for over 50 years (Morris *et al.*, 1953). A recent meta-analysis of 24 randomised controlled trials found that walking for $188.8 \text{ min} \cdot \text{wk}^{-1}$ at a mean intensity of $70.1 \pm 9.1\% \text{ HR}_{\text{max}}$ ($56.3 \pm 7.1\% \dot{\text{V}}\text{O}_{2\text{max}}$)

improved $\dot{V}O_{2\max}$ by 9%, decreased body mass, body mass index (BMI) and body fat percentage by -1.4%, -1.1% and -1.9%, respectively, and decreased diastolic blood pressure by -2% compared to baseline (Murphy *et al.*, 2007) (see section 2.4). Walking is usually the PA medium of choice in the majority of studies of accumulative PA, and stair climbing appears to be gaining popularity (Boreham *et al.*, 2005; Kennedy *et al.*, 2007), which is most likely due to the target population being relatively sedentary and need a simple form of PA to encourage them to become more active on a daily basis. The results of studies investigating the efficacy of accumulating PA on health outcomes have been equivocal at times, however this is also true of exercise studies in general, and not specific to accumulated PA (see section 2.5). The consensus from these studies is that, on the whole, the total PA volume is most important irrespective of the pattern of activity (Woolf-May *et al.*, 1999; Murphy *et al.*, 2002) and this is why the Centers for Disease Control and Prevention and American College of Sports Medicine (CDC & ACSM, 1995), and the most recent public health recommendations (ACSM & American Heart Association (AHA), 2007), advocate that PA can be effectively accumulated throughout the day to promote good health (see section 2.3.7).

The current minimum PA recommendations are for at least 30 minutes of moderate intensity PA (at least 40/50% HRR) to be accumulated on 5 days of the week (ACSM & AHA, 2007). Therefore, the aims of the study were to determine whether walking briskly for 24 weeks on 5 days of the week and for 30 minutes each day could improve MetS risk factors and markers of RevCholTran compared to sedentary controls, and also whether accumulating the same volume of brisk walking during the day may be equally effective in improving risk factors associated with MetS.

3.2 Method

3.2.1 Introduction

Following a rolling programme of recruitment following a local media campaign involving editorials in newspapers, radio programmes and television news, 85 non-smoking sedentary males (age 54.0 ± 8.5 years), who were asymptomatic of cardiovascular disease and diabetes, volunteered and gave their informed consent to take part in the study, which was approved by the central NHS Research Ethics Committee (Appendix A.1; p242). Volunteers were recruited through a comprehensive and widespread advertising campaign (see 3.2.2). The volunteers were advised of the experimental procedures and possible discomforts (Appendix A.2; p275) and then signed the informed consent (Appendix A.3; p280), a pre-screening health questionnaire (Appendix C.1; p310), a physical activity questionnaire (Appendix C.2; p315) and gave permission for their General Practitioner to be contacted for approval of their entry onto the study (Appendix C.3.1; p319). The participants received written confirmation that they were under no obligation to begin or continue with the study and could withdraw at any time.

3.2.2 Participant recruitment

A priori power calculations based on change in serum CETP indicated that 156 participants were needed to complete the study, ie. 3 groups of 52 (see section 3.2.7). In May 2005 an editorial in a local newspaper and radio interview were used as the main methods for recruiting participants. However, this was only moderately successful and demonstrated that a large proportion of the suitable volunteers were prescribed cardiovascular medication and thus ineligible to partake according to the exclusion criteria at the out-set of the study (see section 3.2.3). With retrospect this was not surprising since MetS is recognised as being prevalent in those prescribed anti-hypertensive medication (Rantala *et*

al., 1999). The proportion of middle-aged men (40-59 years) with medication controlled hypertension *and* presenting symptoms of MetS was 35.3% for elevated BP & TAG, 26.8% for elevated BP, TAG and insulin resistance, and 16.9% for those presenting T2D and elevated BP & TAG (Rantala *et al.*, 1999). Furthermore, evidence has shown that even in middle-aged men who ran 0-15 km·wk⁻¹, 6.05% and 5.39% of were prescribed with anti-hypertensive and lipid-lowering medication, respectively (Williams & Franklin, 2007). Even still, this group of individuals would be too physically active/aerobically fit to meet the inclusion criteria of the present study, which were aimed toward non-smoking sedentary men presenting MetS. The randomised controlled aspect of the intervention was an additive methodological difficulty in recruiting participants because some potential participants did not want to volunteer in case they were randomised into the control group (personal communications, 2005).

In response to these occurrences, an application was made to the local NHS Research Ethics Committee in the summer of 2005 to allow volunteers prescribed with cardiovascular medication, ie. anti-hypertensive and lipid-lowering medication, onto the study and to allow participants allocated to the control group to enter their own 24-week walking intervention on completion of the study to optimise study numbers. Following the successful approval to the amendments to the study design, a further radio appeal was launched, emails were sent to local council personnel departments to be circulated to staff, web-links from the University of Kent & Canterbury Christ Church University homepages were created aswell as handing out flyers at Canterbury and Ashford International train stations. Adverts were placed in GP surgeries, businesses, schools and colleges, libraries, supermarkets, taxi companies and bowling clubs throughout Kent. This was a direct marketing technique aimed towards our target sample of middle-aged men. Despite all of

these efforts there were still insufficient individuals fitting the criteria that were volunteering for the study (n=18). This particular demographic is especially problematic to engage since health behaviours, such as sedentariness, poor dietary choices and smoking, tend to cluster (Villegas *et al.*, 2008). Figure 3.1 illustrates the withdrawal of participants based on medication and non-completion.

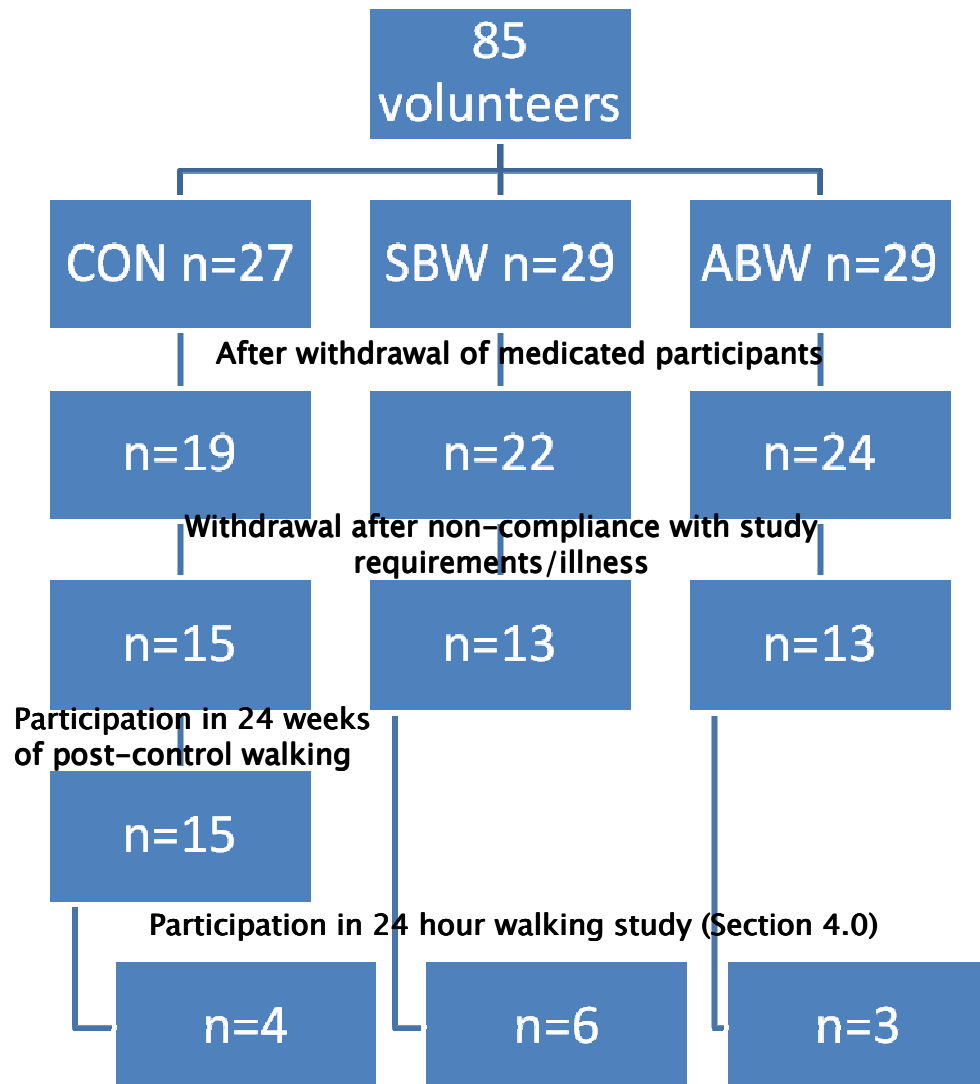


Figure 3.1 Participant exclusion/withdrawal flowchart

The penultimate recruitment drive occurred just after the New Year in January 2006, where a large editorial was placed in a local newspaper to take advantage of those with New Year's resolutions. This was by far the most successful avenue for recruiting, thus indicating that the timing of recruitment efforts may be more important than the type or

variety of methods used for attracting volunteers (n=70 overall). The final two recruitment efforts consisted of interviews on local radio stations in Kent and South East television news and in the match day programme at Kent County Cricket Club in April 2006. These efforts brought the final total of volunteers to 85 in 13 months.

In the light of evidence stretching back over 30 years, it is perhaps unsurprising that this range of recruitment methods was relatively unsuccessful in attracting middle-aged men to volunteer (Wilson & Webb, 1976). Those that refrain from participation tend to be older males from lower socioeconomic groups, whereas those who volunteer are generally younger, more highly educated and likely to report healthier lifestyle patterns (Jancey *et al.*, 2006; O'Neill *et al.*, 1995). Therefore, with the target volunteers being placed in the former, there were always going to be difficulties in recruiting and retaining 156 sedentary overweight middle-aged men onto a 24 week physical activity intervention. Indeed, a study examining the recruitment and retention of participants on a 6-month walking intervention demonstrated that out of 7,378 potential volunteers being invited to participate via a postcard only 734 individuals responded (Jancey *et al.*, 2006). Furthermore, out of this group 734 individuals only 573 completed a questionnaire at baseline (mean participation rate of 13.6%), 444 at 3 months and 413 at 6 months. This is despite the paper hailing itself as the “effective recruitment and retention of older adults in PA research” (Jancey *et al.*, 2006).

3.2.3 Experimental design

The study was a randomised controlled trial involving two exercise groups and a control group. Prior to the pre-exercise assessments, computer generated sequences (Clinstat, Martin Bland) were used to randomly allocate the participants into one of three groups:

- Normal lifestyle (unchanged diet or activity behaviour) (CON);
- Single bout of brisk walking for 30 minutes on 5 days of the week (SBW);
- Accumulative bouts of brisk walking for 30 minutes on 5 days of the week (ABW).

Prior to and following the 24 week walking intervention all of the participants had a pre- and post-intervention health and fitness assessments.

3.2.4 Pre-screening procedure

Potential participants were pre-screened using a health questionnaire and their general practitioner was asked for their approval prior to participation.

Inclusion criteria – Low active men ($<150 \text{ min} \cdot \text{wk}^{-1} > 4\text{METs}$) aged 40 to 65 years with a waist circumference of at least 102 cm (~ 40 inches) or waist:hip ratio of at least 0.9.

Exclusion criteria –

- a) Present cardiovascular disease
- b) Diabetes mellitus
- c) Tobacco smoking
- d) Waist circumference ≤ 102 cm (~ 40 inches) AND waist hip ratio of < 0.9
- e) Physically active, i.e. moderate intensity PA for 30 minutes per day on 5 days of the week
- f) Their general practitioner was unable to provide health clearance for them to participate, or
- g) The participant was unable to understand the nature of the study.

The original exclusion criteria included cardiovascular medication use, such as anti-hypertensive and lipid-lowering agents, however despite the exhaustive recruitment efforts insufficient volunteers were medication-free. Therefore, to boost participant numbers those participants prescribed such medication were accepted, even though they were later precluded from the final analyses in order to report full data ranges without excluding case-by-case on the basis of medication.

3.2.5 Health and fitness assessments

3.2.5.1 Height, body mass, resting heart rate and resting metabolic rate

After being allocated into a group, the participants arrived at the Sport and Exercise Science Laboratory following ~14 hour fast and received their health and fitness assessment, which included measures such as height, mass, body composition, blood pressure, blood test and aerobic fitness. Height was measured using a stadiometer to the nearest cm and mass to the nearest 100g using beam balance scales (both Seca, Hamburg, Germany). The participants then laid down in a supine position to rest for 5 minutes after which a 5 minutes expired air sample was collected using Douglas bags (Harvard Apparatus Ltd, Kent, UK) and resting heart rate (RHR) was also monitored during this period using a heart rate (HR) radio-telemetry system (Polar Beat, Polar Electro OY, Finland). The Douglas bags were then analysed (Servomex Series 1440, Servomex Group Ltd, Crowborough, UK) for oxygen and carbon dioxide concentrations and the volumes measured by a dry gas meter (Harvard Apparatus Ltd, Kent, UK). These values, plus ambient air temperature and barometric pressure were input into an MS Excel spreadsheet for the calculation of $\dot{V}O_2$ (Appendix F.2; p364). Resting metabolic rate (RMR) was calculated from oxygen consumption ($\dot{V}O_2$) by multiplying $\dot{V}O_2$ ($L \cdot \text{min}^{-1}$) by 5 (Montoye *et al.*, 1996) and resting fat oxidation rates were calculated using $f = 1.67 * \dot{V}O_2 - 1.67 * \dot{V}CO_2 - 1.92 * n$ [where f = fat and n = urinary nitrogen] (Frayn, 1983) (See Appendix G.2.2.1 for RMR reliability, p422).

3.2.5.2 Blood pressure and waist & hip circumferences

Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and pulse pressure (PP) were assessed after the participants had remained seated for at least 5 minutes in a chair with an arm rest. Three blood pressure measures were taken

from the participants' right arm using an aneroid sphygmomanometer (Accoson Limpet, A.C. Cossor & Son Ltd, London, UK) 30 seconds apart and the mean values were recorded. Mean arterial pressure was calculated using $(\text{SBP}-\text{DBP}) \times 0.33 + \text{DBP}$ from the mean of the SBP and DBP measures, and PP was calculated by the difference between the mean SBP and DBP values (See Appendix G.5.1 & G.6 for BP validity and reliability data, p474 & p495). The rate pressure product (RPP; index of myocardial workload) was calculated as RHR multiplied by SBP. The participants then stood in the anatomical neutral position and waist circumference (WC) was measured at the level of the umbilicus and hip circumference (HC) was measured at the largest circumference of the hips above the gluteal fold both to the nearest 1 mm using a self-tightening circumference tape measure (Seca, Hamburg, Germany). Three WC and three HC measures were taken and the mean values were recorded. The waist:hip ratio (WHR) was then derived from these measures (See Appendix G.4.1 for measures of body composition reliability, p454).

3.2.5.3 Body composition and blood samples

Body density (Db) was estimated from 7-site sum of skin fold thicknesses (chest, midaxillary, triceps, subscapular, abdomen, suprailiac and thigh (ACSM, 2000) and using skin fold callipers (Harpenden, British Indicators Ltd, Beds., UK). The participants stood in the anatomical neutral position whilst 3 skin fold measures were taken at each site, with the mean value being recorded. Body composition was then calculated using the Siri equation (1961) for white males aged 20-80 years $[495/\text{Db}-450]$ (ACSM, 2000) (See Appendix G.4.1 for measures of body composition reliability, p454). This calculation provided a value for body fat percentage (BF%), which was then applied to the body mass of the participant for the determination of absolute fat mass (FM). Blood samples were collected from an antecubital vein into vacutainers (BD Systems, Oxford, UK) whilst the participants were in a seated position, following an overnight fast (~14 hours), for the later

analysis of triacylglycerol (TAG), non-esterified fatty acids (NEFA), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), glucose, insulin, fibrinogen, cholesteryl ester transfer protein (CETP) and lecithin:cholesterol acyltransferase (LCAT).

3.2.5.4 Aerobic fitness

A predictive treadmill test was used to avoid complications regarding maximally testing sedentary participants. The participants were then allowed sufficient time to familiarise themselves with the treadmill (Mercury Med., HP Cosmos, Nussdorf-Traunstein, Germany) and gas analysis equipment (Oxycon Pro, Jäeger, Würzburg, Germany), which involved walking on the level treadmill at 3 mph with the use of their hands until they could walk comfortably unaided. After resting, the participants performed the Stanford sub-maximal walking test (ACSM, 2000). The test consisted of walking at 3 mph throughout the test at five different gradients for two minutes at each stage (Table 3.1) (See Appendix G.2.1.1 for walking test validity & reliability, p391).

Table 3.1 Graded treadmill test protocol

<i>Speed (mph)</i>	<i>Time (minutes)</i>	<i>Gradient (%)</i>
3.0	2	2.5
3.0	2	5.0
3.0	2	7.5
3.0	2	10.0
3.0	2	12.5

Expired air analysis ($\dot{V}O_2$) was performed using the online breath by breath gas analyser, HR was measured using a radio-telemetry system (Polar Beat, Polar Electro OY, Finland), which were both recorded every 5 sec. Ratings of perceived exertion (RPE; Borg 6-20 scale) were recorded at the end of each 2-minute stage and a blood lactate sample was taken at the end of the test (10 minutes) using a Softclix lancet gun (Accucheck, Roche Diagnostics GmbH, Germany) and collected into a 20 μ L capillary tube (Kunststoff-Kapillare, EKF Diagnostics, Germany). The sample was then stored in freezer (Revco Ultra, Asheville, NC, USA) at -70°C for later analysis (Biosen 5030L, Industrie-Elektronik

GmbH, Magdeburg, Germany). Samples were collected one at a time and analysed in series of ten once ten samples had been taken from 10 participants. Following the test, the HR- $\dot{V}O_2$ relationship was calculated and this relationship was extrapolated up to the participants age predicted maximum heart rate (age-220 beats·min⁻¹) to predict $\dot{V}O_{2max}$ (Maritz *et al.*, 1961). The HR- $\dot{V}O_2$ relationship was also used to estimate gross energy expenditure based on HRs recorded in the walking diaries using the heart rate flex method, where HRs recorded in the walking diaries were used to estimate $\dot{V}O_2$ during walking, from which PAEE was derived (Keytel *et al.*, 2005). Similar health and fitness assessments were performed by all participants following the 24-week walking intervention.

3.2.5.5 Validation and reliability procedures

The studies presented in this thesis required a range of techniques for measuring/estimating health indicators and were used within a particular demographic – middle-aged men. Therefore, it was felt prudent to perform tests of validity and reliability of these measures on samples of the target population. The validity tests were important to indicate the degree of confidence in the measures being a true description of health risk and the reliability data were collected because the studies utilised repeated measures designs, thus relying on a strong degree of reproduceability.

Physical activity is a behaviour that is not straight forward to measure due to its high variability and the available measurement tools. Walking diaries were the chosen recording medium in the 24 week walking intervention because they can be easily distributed at little expense, however they rely on the participants accurately recording their PA. Therefore, a comparison was performed between the walking diaries and walking activity recorded using an accelerometer during a seven-day period within the intervention. The main

findings of this study was that participants generally under-reported their walking activities in the diaries compared to that recorded by the accelerometer, with the greatest difference in those performing more than 150 min·wk⁻¹ in ABW (Appendix G.1; p365). Furthermore, there was greater agreement between methods for recording walking activity in SBW than ABW, where those in ABW under-measured their walking duration compared to the walking duration objectively measured using the accelerometers.

When measuring aerobic fitness the gold standard is the $\dot{V}O_{2max}$ test, which is designed to take the participant to exhaustion. However, other tests have been developed in order to predict $\dot{V}O_{2max}$ without taking sedentary/older individuals to exhaustion to avoid the potential risks associated with vigorous exercise (Raum *et al.*, 2007). A predictive $\dot{V}O_{2max}$ test was utilised for the studies in this thesis, therefore the validity and reliability of the submaximal walking fitness test was assessed, which demonstrated a high degree of reliability for measures of aerobic fitness with a slight over-estimation of $\dot{V}O_{2max}$ (Appendix G.2; p391). Resting metabolic rate was also assessed as part of both of the studies in this thesis in repeated measures designs. Therefore to test-retest reliability, RMR was assessed twice during the same day and repeated the next day using the Douglas bag technique, which indicated that both the inter- and intra-day reliability of RMR were relatively strong (Appendix G2.2.1; p422).

Indices of body composition formed a large proportion of the data collected in the 24-week study. Due to the nature of body composition analysis it needs to be estimated rather than directly measured. Sum of skin folds and body girths are commonly used methods to estimate body composition, however errors in validity and reliability of these measures can occur, thus adding error in a repeated measures design. Therefore, to test the validity of

body fat percentage, sum of skinfolds were compared to estimates using air displacement plethysmography, a surrogate for hydrostatic weighing (Gold standard) and reliability of body composition were estimated on two consecutive days to provide inter-day reliability (Appendix G.3; p433). Furthermore, waist and hip circumferences were assessed on two consecutive days to assess test-retest reliability of (Appendix G.4; p454). These studies found that despite separate principles of assessment the results obtained from seven-site sum of skin folds were similar to those using air displacement plethysmography. Furthermore, the reliability of measures of body fat percentage, waist circumference and waist:hip ratio were consistently high.

Blood pressure can be highly volatile, illustrated by 'white coat syndrome' where blood pressure can change suddenly usually by increasing without known cause (Gerin *et al.*, 2006). Therefore, measures of blood pressure were assessed in order to observe the degree of difference between different measurement techniques and the repeatability of aneroid sphygmomanometry. Automated oscillometry was used to compare to the aneroid sphygmomanometry measures because aortic catheterisation is the gold standard and too invasive for the purposes of the research being performed (Appendix G.5; p474). The study found that although blood pressure measures were similar between aneroid sphygmomanometry and automated oscillometry, particularly for systolic blood pressure, these methods should not be used interchangeably. Intra- and inter-day blood pressure reliability were similar and demonstrated that changes in blood pressure can be volatile even when measured only 30 minutes apart, thus possibly contributing to the moderate statistical power observed for the blood pressure measures in the studies included in Sections 3.0 and 4.0 (Appendix G.6; p495). The treadmill used in these studies was also

validated in Appendix G.7 (p503), which demonstrated that gradient, distance and speed were all highly valid with little variation between participants of various body mass.

3.2.6 Walking intervention

The participants were randomly assigned into their respective groups and the walking intervention required the participants in the walking groups to walk in their own time. The participants in the walking groups were advised about their heart rate training zone, heart rate palpation, walking briskly, and were also provided with walking diaries to record their daily walking activities (Appendices D.1.1 & D.1.2; pp321 & 331). The diaries were provided to motivate the participants and to monitor weekly walking activities. Participants in both of the walking groups (SBW & ABW) were instructed to walk for 30 minutes per day on five days of the week ($150 \text{ min}\cdot\text{wk}^{-1}$) at an intensity eliciting $>65\% \text{ HR}_{\text{max}}$ during the 24 week intervention, however the two groups differed in daily walking patterns. The SBW group were instructed to walk briskly for one single 30 minute daily bout, whereas the ABW group were instructed to accumulate 30 minutes of brisk walking in bouts of 3×10 minutes or 2×15 minutes (See Appendix G.1.1 for walking diary method comparison, p365). ABW participants were advised to leave at least 2 hours between each daily walking session and all participants were instructed not to change their diets.

To assess the efficacy of walking for 30 minutes on five days of the week, a group of participants were randomly allocated to the CON group so that differences over the 24 week walking intervention could be measured in addition to differences from baseline. The CON group were instructed not to change their usual lifestyle, i.e. physical activity and dietary behaviours, over the course of the intervention. Following this 24 week period, the participants returned to the laboratory, where a post-intervention health and fitness

assessment was performed to assess changes in health and fitness over the previous 24 weeks.

Prior to returning to the laboratory all participants were advised not to exert themselves or consume alcohol on the day before the 24 week re-assessment. Sixty five participants were accepted onto the study with 19 allocated into CON, 22 into SBW and 24 into ABW after removing participants from the study due to the confounding effects of their anti-hypertensive and/or lipid-lowering medication.

3.2.7 Blood sampling and analysis

Blood was dispensed into a collection tube to form serum, a tube containing 0.05 mm trisodium citrate at a ratio of 9 parts blood to 1 part anticoagulant and a tube containing fluoride oxalate, both for the formation of plasma. The samples were then centrifuged at room temperature (Eppendorf 5804, Hamburg, Germany) and stored in a freezer (Revco Ultra, Asheville, NC, USA) at -70°C for later analysis. The serum samples were analysed for concentrations of TAG, TC and HDL-C (all Horiba ABX Diagnostics, Cambridge) using enzymatic colorimetry, insulin was analysed using chemiluminescence (Bayer Advia Centaur, Bayer Healthcare, Diagnostics Division, Tarrytown, NY.), the serum samples were diluted 1:20 with assay buffer for the analysis of CETPa and LCATa using activity assays (Roar Biomedical Inc, New York) on a fluorimeter (Safire XFLUOR 4 Version 4.4, Tecan, UK) (See Appendix E for more detailed biochemistry procedures, p344). Insulin sensitivity was estimated using the homeostasis assessment model ratio formula (HOMA-IR; fasting serum insulin ($\mu\text{IU}\cdot\text{mL}^{-1}$) \times fasting plasma glucose ($\text{mmol}\cdot\text{L}^{-1}$))/22.5) and insulin secretion was estimated using: fasting serum insulin ($\mu\text{IU}\cdot\text{mL}^{-1}$) \times 20/fasting plasma glucose ($\text{mmol}\cdot\text{L}^{-1}$) $-$ 3.5 (HOMA- β cell), both described by Matthews *et al.* (1985) and LDL-C was derived from serum TC $-$ HDL-C $-$ (TAG/2.2) (Friedewald *et al.*, 1972). Insulin resistance and insulin secretion were estimated because measurement tests usually require an oral glucose tolerance test and invasive techniques such as hyperinsulinaemic clamping. The plasma collected in the fluoride oxalate tube was used to analyse glucose (Horiba ABX, Cambridge) and NEFA (Wako Ltd, Neuss, Germany) using enzymatic colorimetry and the plasma collected in the trisodium citrate tube was used for the analysis of fibrinogen using a modification of the Clauss clotting technique (Clauss, 1957) by adding prothrombin to the diluted plasma (PT-Fg technique) (Guideline, 2003). Pre- and post- samples were analysed next to each other within the same assay series to reduce

biochemical variability. The CVs for the assays, using low concentration quality control sera, were: glucose 2.24%; TC 3.82%; HDL-C 6.90%; TAG 8.04%; insulin 1.75%; fibrinogen 4.18%; CETP 8.2%; and LCAT 1.5%. [see Appendix F.1 for assay methods; p349].

3.2.8 Statistical analyses

Power calculations were performed using the mean and standard deviation from previous studies looking at changes following exercise intervention and the sample size was decided from the health outcome that required the greatest number of subjects (CETP; mean difference $0.35 \pm 0.55 \text{ ml}\cdot\text{L}^{-1}$). The results suggested that in order to have a 90% chance of finding significant differences, at an alpha of 0.05, a total sample size of 156 subjects would be required, including an estimated 20% subject drop out (controls n=52, ABW n=52 and SBW n=52). The results are presented as the mean \pm SD, with their respective post-hoc statistical power and were analysed using SPSS version 13 software, with significance being accepted at $P < 0.05$. Analysis of covariance (ANCOVA) was applied to the Pre- and Post-walking values using the Pre-walk values as the covariate, incorporating a Bonferroni correction, to determine differences in health outcomes between the groups. Baseline data were used as co-variates to overcome the slight heterogeneity within the groups. Non-normally distributed data were log-transformed and analysed using ANCOVA. If the data remained non-normally distributed the Kruskal-Wallis ANOVA was utilised and differences between the groups were analysed using Mann Whitney U tests. Post-hoc statistical power was also calculated. The data collected in the walking diaries were analysed parametrically using independent T-tests and data that could not be log-transformed were analysed using Mann Whitney U tests. Pearson's product moment correlation coefficients were used to explore relationships between factors.

3.3 Results

The characteristics of the 65 men accepted onto the study were: age 52.7 ± 8.7 years; height 1.78 ± 0.07 m; body mass 90.8 ± 12.2 kg; BMI 28.6 ± 3.6 kg·m⁻²; WC 102.5 ± 9.5 cm; WHR 0.98 ± 0.05 and $\dot{V}O_{2\max}$ 36.3 ± 6.9 mL·kg⁻¹·min⁻¹ (Table 3.2). Of the 65 non-medicated participants that were accepted onto the study 41 satisfactorily completed 24 weeks; 4 did not satisfactorily complete CON due to illness (2) and not understanding the requirements of the study (2), leaving 15 participants that completed the control procedure (78.9%); 9 did not satisfactorily complete SBW, leaving 13 participants that completed the intervention (59.1%); finally 11 did not satisfactorily complete ABW due to not adhering to the accumulative pattern of walking (3) and not completing 24 weeks of walking (8), leaving 13 participants that completed the intervention (54.2%). Therefore, group data are based on CON=15, SBW=13 and ABW=13, unless otherwise stated, where participants' data were omitted if there were problems with analysing the pre or post sample.

Table 3. 2 Pre-intervention participant characteristics; mean \pm SD

<i>Subject groups</i>	<i>n</i>	<i>Age (yrs)</i>	<i>Height (m)</i>	<i>Body mass (kg)</i>
CON	15	49.6 \pm 7.3	1.77 \pm 0.06	91.4 \pm 10.5
SBW	13	52.2 \pm 7.8	1.79 \pm 0.06	90.9 \pm 8.4
ABW	13	56.1 \pm 7.2	1.78 \pm 0.08	85.5 \pm 12.0

Pre-baseline physical activity levels were 0.75 ± 0.77 hrs·wk⁻¹ \geq 4 METs (CON 0.60 ± 0.89 hrs·wk⁻¹ \geq 4 METs, SBW 0.80 ± 0.84 hrs·wk⁻¹ \geq 4 METs, and ABW 0.81 ± 0.75 hrs·wk⁻¹ \geq 4 METs, as assessed using a 7-day PA questionnaire (Sallis *et al.*, 1985), and there were no differences for any characteristics between groups at baseline (P=0.693). According to the ATP III criteria (Cleeman, 2001), 2 participants in CON (13.3%) were classified as having MetS, 1 in SBW (7.7%) and 1 in ABW (7.7%) at baseline. At completion of the 24-week intervention the presence of MetS increased to 5 participants in CON (33.3%), 3 participants in SBW (23.1%) and the 1 participant remained in ABW (7.7%).

3.3.1 Walking data

Table 3.3 Walking diary data from the SBW and ABW groups; mean \pm SD

<i>Mean walking pattern characteristics</i>	<i>SBW n=13</i>	<i>ABW n=13</i>
Weeks walked	23.5 \pm 0.9	23.8 \pm 0.4
Total minutes walked	3623.0 \pm 687.8	3532.5 \pm 549.8
Mean minutes walked per week	153.7 \pm 27.9	148.3 \pm 23.0
Percentage of prescribed walking (%)	102.9 \pm 18.0	98.1 \pm 15.3
Walking intensity (%HR _{max})	64.5 \pm 5.8	66.5 \pm 8.0
Walking intensity (%VO _{2max})	49.2 \pm 7.2	50.7 \pm 11.4
Estimated walking gross energy expenditure (kcal·wk ⁻¹)	1196.2 \pm 214.5	1326.6 \pm 550.2
Total estimated walking gross energy expenditure (MJ)	120.6 \pm 26.4	132.8 \pm 55.5
Walking sessions per week **	4.7 \pm 0.3	9.4 \pm 1.7
Duration of each walking session (min) **	32.9 \pm 6.6	15.4 \pm 2.7

** Significantly different as determined by Mann Whitney U tests; $P < 0.001$.

3.3.1.1 Walking intensity

The allocated walking pattern did not influence either exercising HR or %HR_{max} recorded during the course of the walking intervention (Figure 3.2). The mean HR were 102.0 ± 18.8 beats·min⁻¹ and 107.4 ± 15.2 beats·min⁻¹ for SBW and ABW, respectively, and mean %HR_{max} were 67.0 ± 11.1% and 65.3 ± 8.7% for SBW and ABW, respectively.

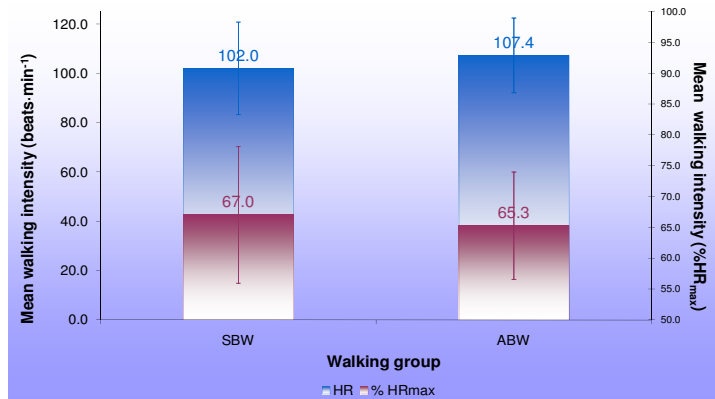


Figure 3.2 Mean walking intensity for SBW and ABW, expressed in absolute intensity (HR) and relative intensity (%HR_{max}). [No significant differences: No effect of walking pattern (ABW v SBW) on HR (F=0.404; P=0.839). No effect of walking pattern (ABW v SBW) on %HR_{max} (F=1.324; P=0.791)]. SBW=13; ABW=13.

3.3.1.2 Walking volume

The allocated walking pattern did not significantly change for either total mins completed or mean min per week recorded during the course of the walking intervention (Figure 3.3). The mean total min completed were 3623.2 ± 687.8 min and 3532.5 ± 549.8 min for SBW and ABW, respectively, and mean min per week were 153.7 ± 27.9 min·wk⁻¹ and 148.3 ± 23.0 min·wk⁻¹ for SBW and ABW, respectively. The mean estimated total gross energy expended during the walking programmes was 120.6 ± 26.4 MJ for SBW and 132.8 ± 55.5 MJ for ABW, however despite the apparent trend for ABW to expend a greater total energy expenditure during the 24 week intervention, these were not significantly different (P=0.496).

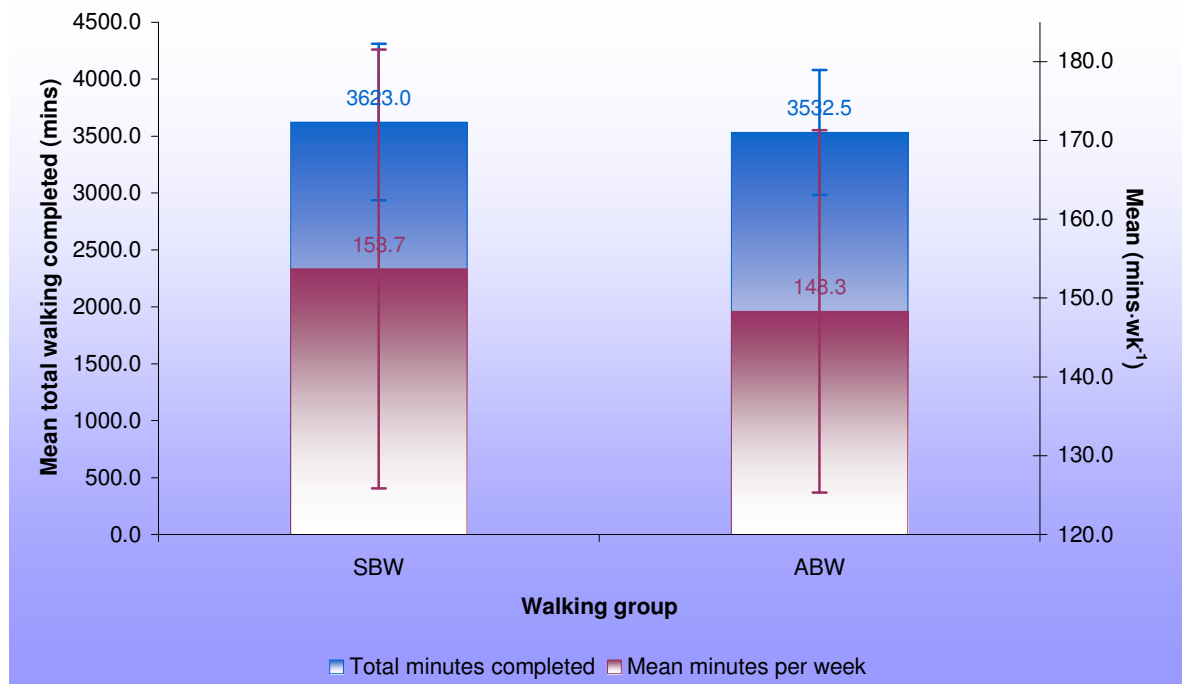


Figure 3.3 Mean walking volume for SBW and ABW, expressed in total mins completed and mean mins per week. [No significant differences: No effect of walking pattern (ABW v SBW) on total mins ($t=0.287$; $P=0.777$). No effect of walking pattern (ABW v SBW) on mean mins ($Z=-0.308$; $P=0.762$)]. SBW=13; ABW=13.

3.3.1.3 Walking frequency

The allocated walking pattern did not influence the mean weeks completed during the course of the walking intervention, however the mean sessions per week were significantly increased in ABW compared to SBW (Figure 3.4). The mean weeks completed were 23.5 ± 0.9 weeks and 23.7 ± 0.6 weeks for SBW and ABW, respectively, and mean sessions per week were 4.69 ± 0.33 sessions·wk⁻¹ and 9.74 ± 1.73 min·wk⁻¹ ($P < 0.001$) for SBW and ABW, respectively. Only one participant that completed ABW chose to perform three sessions of 10 minutes for the entire intervention, whereas the majority of the group chose two 15 minute walking sessions on each walking day.

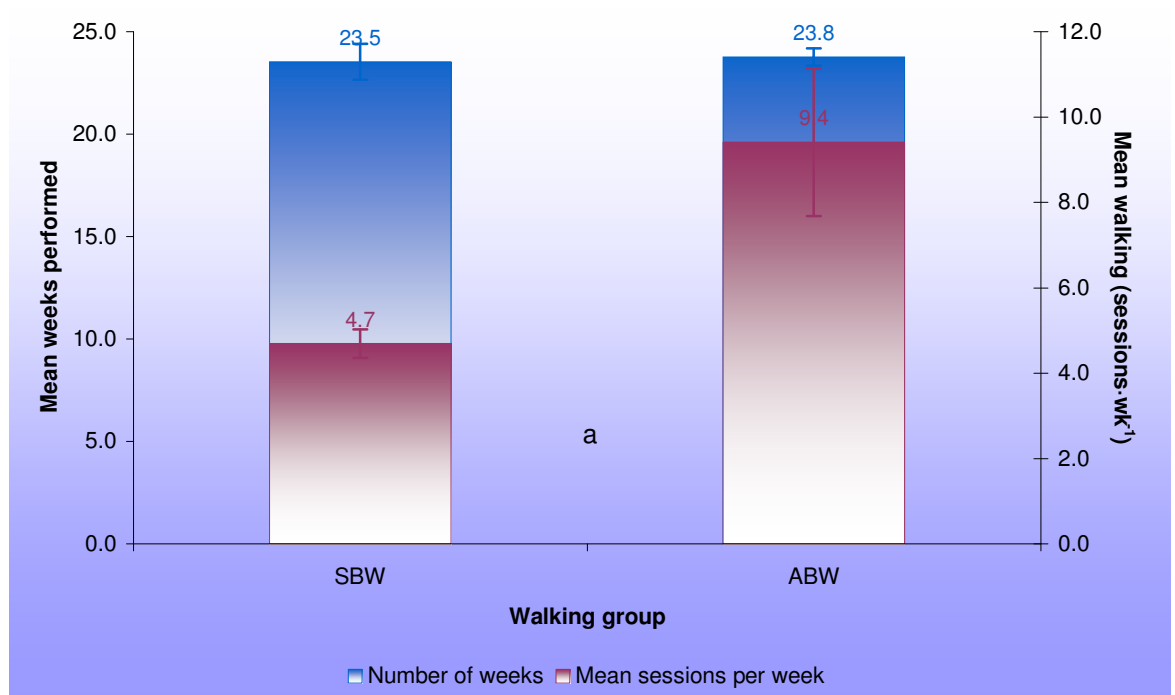


Figure 3.4 Mean walking frequency for SBW and ABW, expressed in total weeks completed and mean sessions per week. [Significant differences: a Effect of walking pattern (ABW v SBW) on mean sessions per week ($Z = -4.4334$; $P < 0.001$)]. [No significant differences: b No effect of walking pattern (ABW v SBW) on mean weeks performed ($Z = -0.429$; $P = 0.762$)]. SBW=13; ABW=13.

3.3.2 Anthropometric data

Table 3. 4 Pre- and post-intervention values for anthropometric measures; mean \pm SD

<i>Risk factor</i>	<i>CON n=15</i>		<i>SBW n=13</i>		<i>ABW n=13</i>		<i>Between group P value</i>
	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	
Body mass (kg)	91.4 \pm 10.5	92.6 \pm 10.6	90.9 \pm 8.4	90.5 \pm 8.7	85.5 \pm 12.03	84.4 \pm 12.2*	P=0.027
Body mass index (kg·m ²)	29.2 \pm 3.4	29.6 \pm 3.6	28.4 \pm 2.5	28.2 \pm 2.5	27.1 \pm 3.6	26.8 \pm 3.7	P=0.043
Waist circumference (cm)	101.6 \pm 9.5	103.8 \pm 10.1	103.7 \pm 7.3	102.6 \pm 7.3**	97.3 \pm 8.8	94.0 \pm 8.9**	P<0.001
Waist:hip ratio	0.96 \pm 0.06	0.99 \pm 0.07)	0.99 \pm 0.05	0.98 \pm 0.05**	0.94 \pm 0.04	0.92 \pm 0.04**	P<0.001
Body fat (%)	28.2 \pm 5.5	31.3 \pm 4.4	27.4 \pm 4.4	28.7 \pm 3.6	27.4 \pm 5.5	25.8 \pm 6.7**	P=0.001

Change in value compared to CON as determined by post-hoc Scheffe test or Mann Whitney U test ; * $P < 0.05$; ** $P < 0.01$.

3.3.2.1 Body mass

There was a main effect of group on body mass ($P=0.027$), where ABW significantly decreased body mass compared with CON ($P=0.033$; Figure 3.5). Body mass change in CON was 1.19 ± 2.75 kg, compared to losses of -0.46 ± 1.49 kg (NS) and -1.06 ± 1.77 kg in SBW and ABW, respectively. There was a significant effect of group on BMI ($P=0.043$), however neither ABW nor SBW significantly decreased BMI alone compared with CON (Table 3.4). BMI change in CON was 0.40 ± 0.87 $\text{kg}\cdot\text{m}^{-2}$, compared to decreases of -0.15 ± 0.48 $\text{kg}\cdot\text{m}^{-2}$ (NS) and -0.30 ± 0.56 $\text{kg}\cdot\text{m}^{-2}$ in SBW and ABW (NS), respectively.

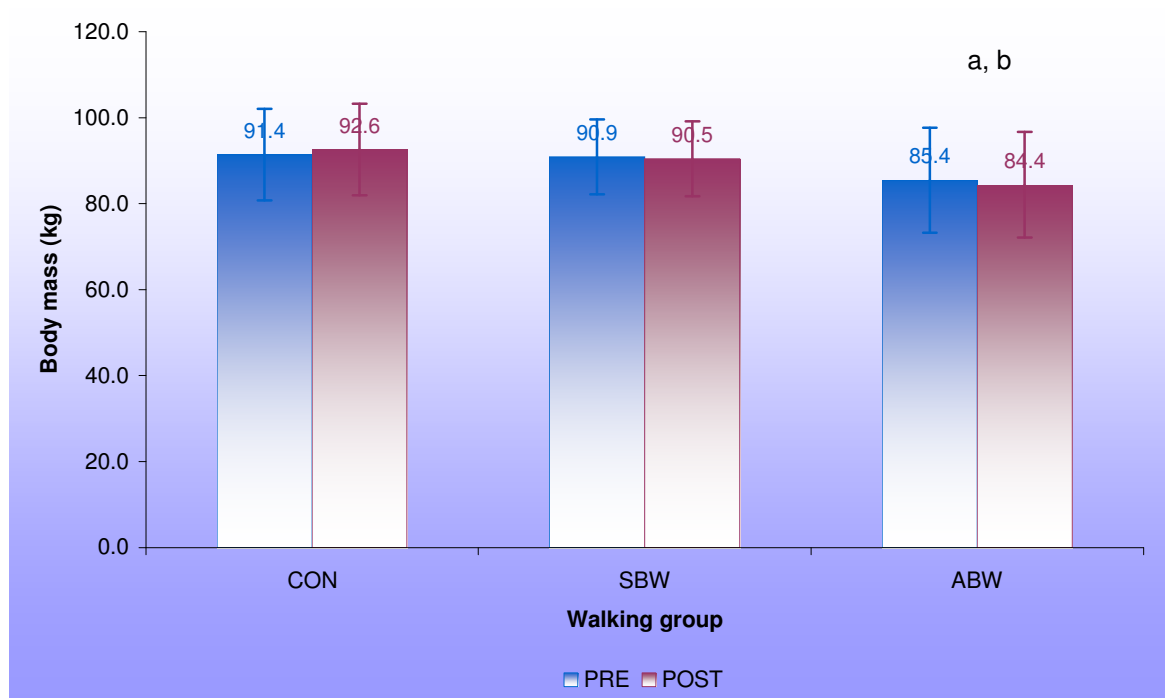


Figure 3.5 Mean pre- and post-intervention body mass values. [Significant differences: a Main of group on body mass ($F=4.006$; $P=0.027$). b Effect of ABW on body mass compared to CON ($P=0.033$)]. CON=15; SBW=13; ABW=13. Statistical power: 0.680.

3.3.2.2 Waist circumference

There was a main effect of group on WC ($P<0.001$), with WC being significantly reduced in both SBW and ABW compared to CON (Figure 3.6). WC change in CON was 2.19 ± 2.69 cm, compared to decreases of -1.12 ± 1.67 cm and -3.22 ± 3.38 cm in SBW ($P=0.009$) and ABW ($P<0.001$), respectively.

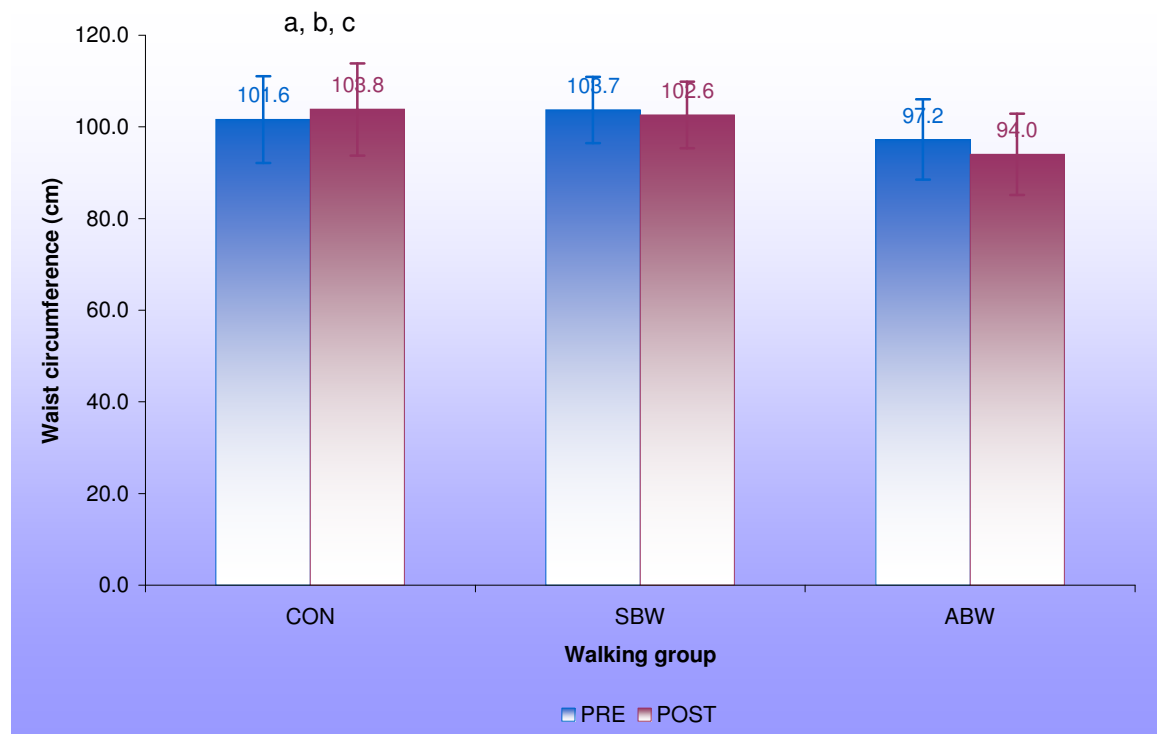


Figure 3.6 Mean pre- and post-intervention WC values. [Significant differences: a Main effect of group ($F=14.130$; $P<0.001$). b Effect of SBW on WC compared to CON ($P=0.009$). c Effect of ABW on WC compared to CON ($P<0.001$)]. CON=15; SBW=13; ABW=13. Statistical power: 0.997.

There was a main effect of group on WHR ($P<0.001$), with WHR being significantly reduced in both SBW and ABW compared to CON (Table 3.4). Waist:hip ratio change in CON was 0.03 ± 0.02 , compared to decreases of -0.01 ± 0.02 and -0.02 ± 0.03 in SBW ($P=0.005$) and ABW ($P<0.001$), respectively.

3.3.2.3 Body fat percentage

There was a main effect of group on BF% ($P=0.001$), with BF% being significantly decreased in ABW compared to CON (Figure 3.7). BF% change in CON was $3.08 \pm 2.40\%$, compared to an increase of $1.28 \pm 2.69\%$ in SBW and a decrease of $-1.56 \pm 5.13\%$ in ABW ($P=0.001$). There were no significant differences between walking groups on BF% ($P=0.558$).

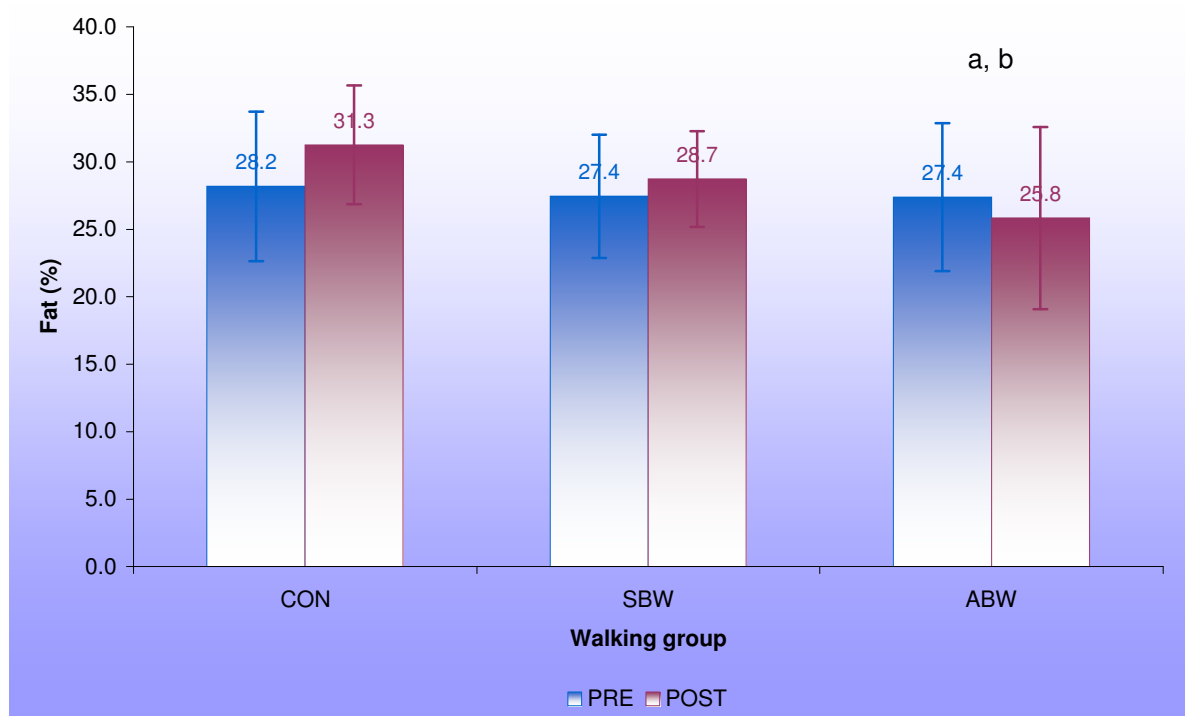


Figure 3.7 Mean pre- and post-intervention body fat percentage values. [Significant differences: a Main effect of group on fat% ($X^2=13.127$; $P=0.001$). b Effect of ABW on BF% compared to CON ($Z=-3.386$; $P=0.001$)]. CON=15; SBW=13; ABW=13. Statistical power: 0.959.

3.3.3 Cardiovascular data

Table 3.5 Pre- and post-intervention values for fitness parameters, resting heart rate and resting metabolism; mean \pm SD

<i>Risk factor</i>	<i>CON n=15</i>		<i>SBW n=13</i>		<i>ABW n=13</i>		<i>Between group P value</i>
	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	
VO _{2max} (mL·kg ⁻¹ ·min ⁻¹)	36.8 \pm 7.4	36.6 \pm 6.4	35.7 \pm 4.6	39.4 \pm 5.6*	38.9 \pm 7.8	40.3 \pm 5.9	P=0.005
[La ⁻] (mmol·L ⁻¹)	4.6 \pm 2.3	4.3 \pm 2.0	4.4 \pm 1.9	4.35 \pm 1.6	6.2 \pm 1.6	3.5 \pm 1.3*#	P=0.003
CON=12, SBW=13, ABW=7							
SBP (mm Hg)	132.0 \pm 17.1	131.4 \pm 15.8	130.0 \pm 15.5	124.6 \pm 12.7	126.9 \pm 13.7	121.3 \pm 15.7	P=0.173
DBP (mm Hg)	88.5 \pm 8.6	89.2 \pm 10.4	84.1 \pm 8.8	83.5 \pm 8.8	84.5 \pm 10.3	83.1 \pm 10.3	P=0.494
MAP (mm Hg)	102.9 \pm 10.8	102.3 \pm 12.4	99.3 \pm 10.6	97.1 \pm 8.9	98.5 \pm 11.0	95.7 \pm 11.3	P=0.469
PP (mm Hg)	43.4 \pm 11.7	42.2 \pm 10.9	45.9 \pm 9.5	41.1 \pm 10.5	42.4 \pm 7.2	38.18 \pm 10.9	P=0.974
Resting heart rate (beats·min ⁻¹)	61.5 \pm 10.2	61.2 \pm 8.2	57.4 \pm 9.2	53.0 \pm 6.5	61.0 \pm 5.5	58.4 \pm 6.5	P=0.132
CON=11, SBW=7, ABW=11							
Rate-pressure product (RHR \times SBP)	8043 \pm 1900	8056 \pm 1656	7568 \pm 1132	6635 \pm 1231	7929 \pm 1097	7013 \pm 1147*	P=0.019
CON=11, SBW=7, ABW=11							

*Change in value compared to CON as determined by post-hoc Scheffe test; $P < 0.05$; # compared to SBW; $P < 0.05$.

3.3.3.1 Aerobic fitness

There was a main effect of group on $\dot{V}O_{2\max}$ ($P=0.005$), where it was significantly increased following SBW compared to CON ($P=0.004$) (Figure 3.8). Change in $\dot{V}O_{2\max}$ following CON was $-0.21 \pm 2.54 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, compared to increases of $3.74 \pm 3.18 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and $1.41 \pm 3.65 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in SBW and ABW (NS; $P=0.180$), respectively. The improvements in $\dot{V}O_{2\max}$ were not significantly different between ABW and SBW ($P=0.459$).

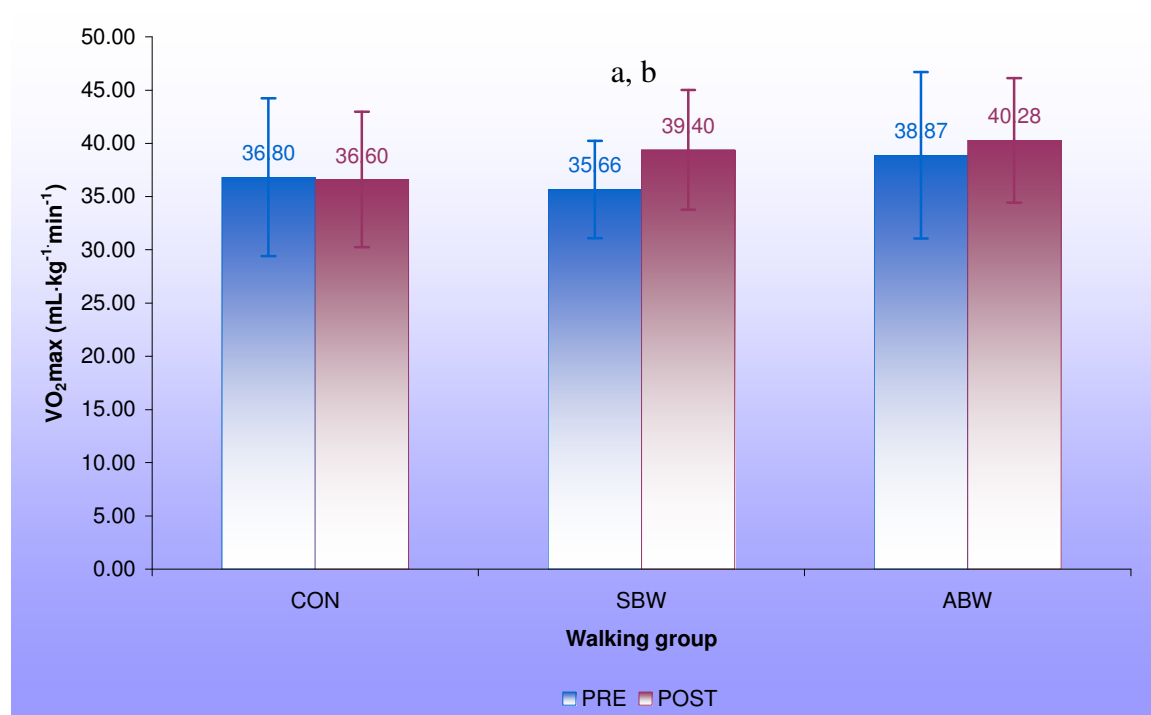


Figure 3.8 Mean pre- and post-intervention $\dot{V}O_{2\max}$ values. [Significant differences: a Main effect of group on $\dot{V}O_{2\max}$ ($F=6.161$; $P=0.005$). b Effect of SBW on $\dot{V}O_{2\max}$ compared to CON ($P=0.004$)]. CON=15; SBW=13; ABW=13. Statistical power: 0.864.

3.3.3.2 Blood lactate

There was a main effect of group on $[La^-]$ ($P=0.022$), which significantly decreased following ABW compared to CON ($P=0.044$) and SBW ($P=0.028$; Figure 3.9). Change in $\dot{V}O_{2max}$ following CON was $-0.25 \pm 1.48 \text{ mmol}\cdot\text{L}^{-1}$, compared to decreases of $-0.06 \pm 1.80 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$ and $-2.72 \pm 1.20 \text{ mmol}\cdot\text{L}^{-1}$ in SBW and ABW, respectively.

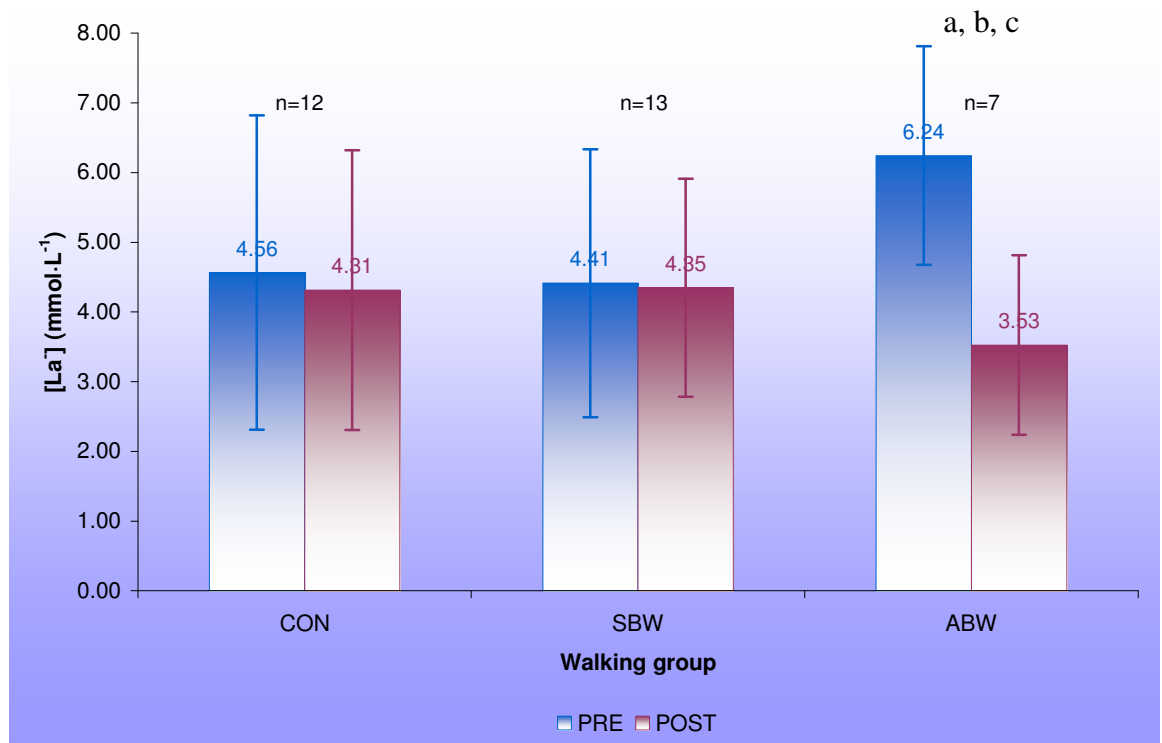


Figure 3.9 Mean pre- and post-intervention $[La^-]$ values. [Significant differences: a Main effect of group on $[La^-]$ ($F=4.362$; $P=0.022$). b Effect of ABW on $[La^-]$ compared to CON ($P=0.044$). c Effect of ABW on $[La^-]$ compared to SBW ($P=0.028$). Statistical power: 0.707.

3.3.3.3 Systolic blood pressure

Systolic blood pressure did not appear to change following the intervention period, with non-significant differences between groups ($P=0.173$; Figure 3.10). However, there was a trend for decreases in SBP following the SBW (-5.44 ± 7.59 mm Hg; $P=0.427$) and ABW (-5.62 ± 13.61 mm Hg; $P=0.260$) compared to a smaller decrease following CON (-0.58 ± 8.35 mm Hg).

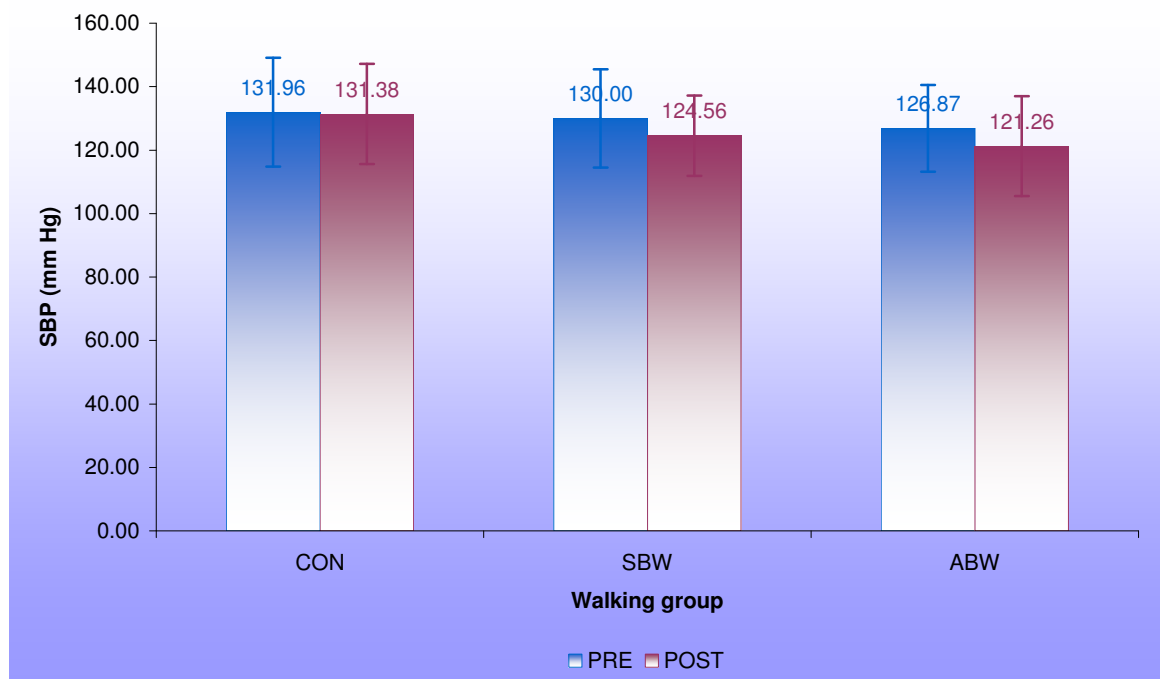


Figure 3. 10 Mean pre- and post-intervention SBP values. [No significant differences: No effect of group on SBP ($F=1.843$; $P=0.173$)]. CON=15; SBW=13; ABW=13. Statistical power: 0.359.

3.3.3.4 Diastolic blood pressure

Diastolic blood pressure did not appear to change following the intervention period, with non-significant differences between groups ($P=0.494$; Figure 3.11). However, there was a trend for decreases in DBP following SBW (-0.62 ± 5.36 mm Hg; $P=1.000$) and ABW (-1.44 ± 6.18 mm Hg; $P=0.752$) compared to CON (0.62 ± 5.22 mm Hg).

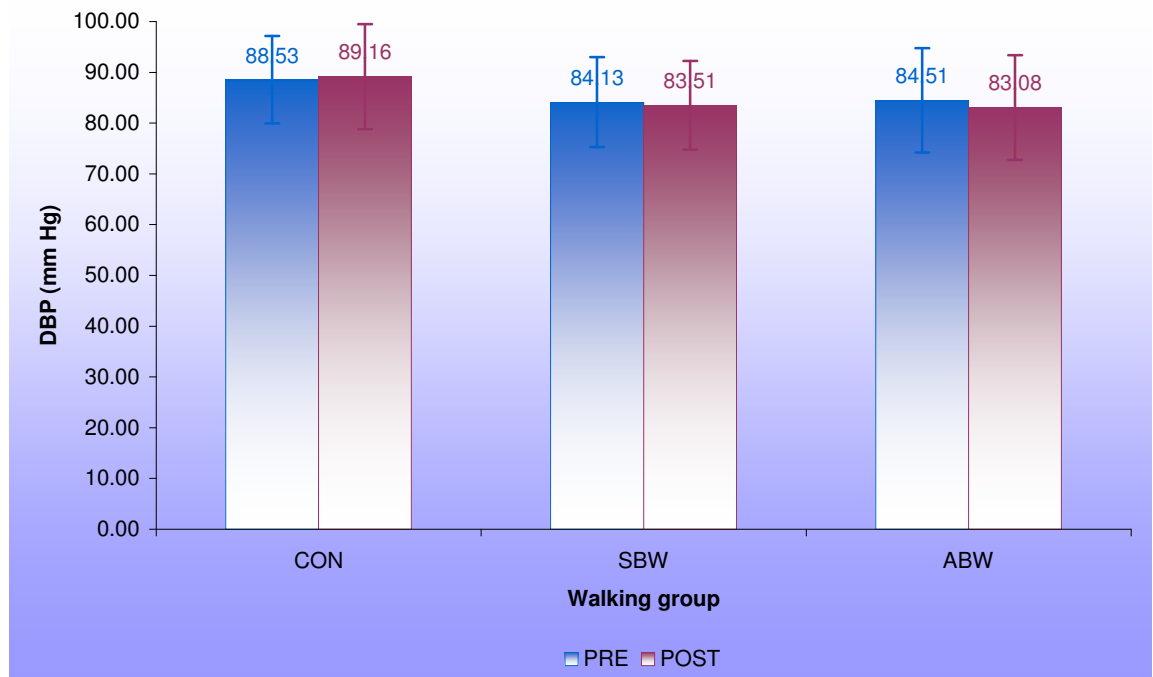


Figure 3.11 Mean pre- and post-intervention DBP values. [No significant differences: No effect of group on DBP ($F=0.485$; $P=0.494$)]. CON=15; SBW=13; ABW=13. Statistical power: 0.162.

Mean arterial pressure did not appear to change following the intervention period, with non-significant differences between groups ($P=0.469$) (Table 3.5). However, the trend was that the decreases in MAP following SBW (-2.21 ± 4.87 mm Hg; $P=1.000$) and ABW (-2.82 ± 7.53 mm Hg; $P=0.731$) were slightly greater when compared to CON (-0.57 ± 7.12 mm Hg).

There were no effects of group on PP ($P=0.974$) with fairly uniform mean decreases in PP following CON (-3.60 ± 10.97 mm Hg), SBW (-4.82 ± 8.12 mm Hg; $P=1.000$) and ABW (-4.18 ± 11.64 mm Hg; $P=1.000$) (Table 3.5).

3.3.3.5 Rate-pressure product

Resting heart rate was not recorded from the beginning of the study, therefore the first 12 participants' RHR were not included in the analyses (CON 4; SBW 6; ABW 2). There was no effect of group on RHR ($P=0.132$), where changes in RHR following CON were -0.27 ± 6.96 beats·min⁻¹, compared to decreases of -4.43 ± 5.47 beats·min⁻¹ and -2.64 ± 4.99 beats·min⁻¹ in SBW and ABW, respectively (all NS) (Table 3.5). Resting heart rate was not recorded from the beginning of the study, therefore the first 12 participants' RPP could not be calculated and were not included in the analyses (CON 4; SBW 6; ABW 2). There was a significant effect of group on RPP ($P=0.019$; Figure 3.12), where change in RPP following CON was 13 ± 51 , compared to decreases of -933 ± 1503 and -916 ± 756 in SBW and ABW ($P=0.031$), respectively.

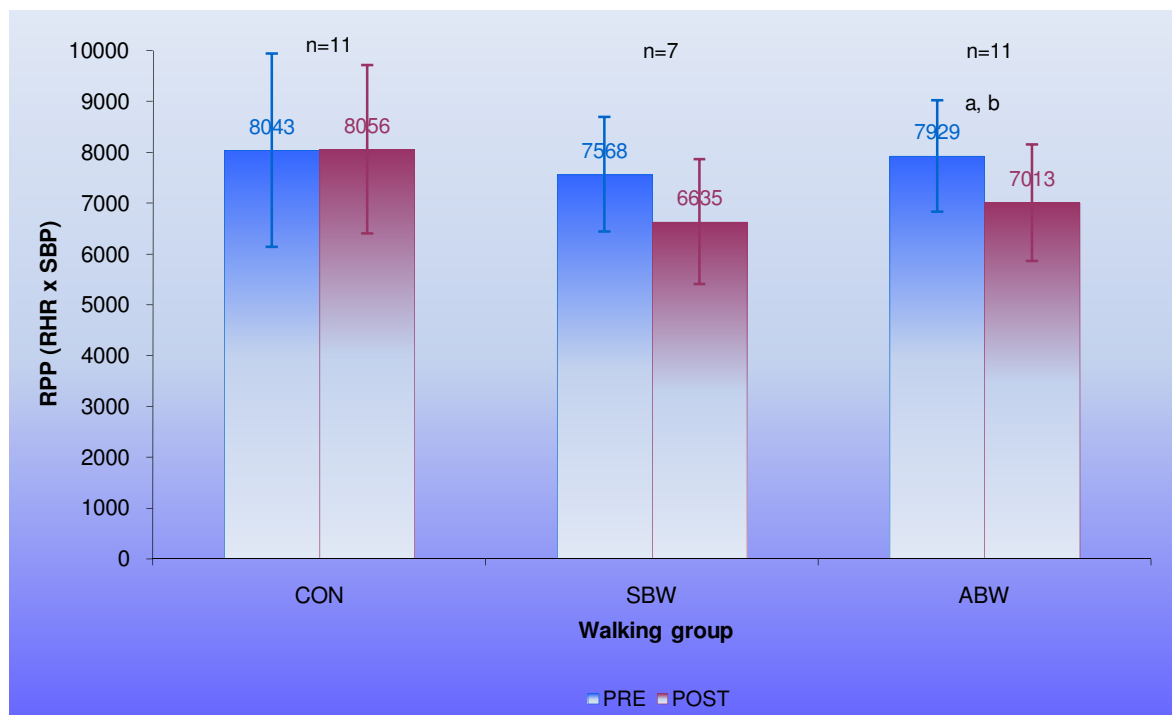


Figure 3.12 Mean pre- and post-intervention RPP values. [Significant differences: a Main effect of group on RPP ($F=4.681$; $P=0.019$). b Effect of ABW compared to CON ($P=0.031$)]. Statistical power: 0.734.

3.3.4 Metabolic data

Table 3. 6 Pre- and post-intervention values for fitness parameters, resting heart rate and resting metabolism; mean \pm SD

<i>Risk factor</i>	<i>CON n=15</i>		<i>SBW n=13</i>		<i>ABW n=13</i>		<i>Between group P value</i>
	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	
Resting metabolic rate (kcal·day ⁻¹) CON=11, SBW=7, ABW=10	2025 \pm 318	1651 \pm 438	2078 \pm 360	1900 \pm 197	1951 \pm 320	1704 \pm 392	P=0.366
Resting fat metabolism (kcal·day ⁻¹) CON=11, SBW=7, ABW=10	898 \pm 401	472 \pm 345	418 \pm 781	870 \pm 567	648 \pm 839	581 \pm 729	P=0.060
Resting percentage fat utilisation (%) CON=11, SBW=7, ABW=10	43.3 \pm 16.9	28.4 \pm 20.0	15.9 \pm 41.7	44.6 \pm 28.0*	36.3 \pm 37.4	30.4 \pm 34.7	P=0.046

Change in value compared to CON as determined by Mann Whitney U test; * $P < 0.05$.

3.3.4.3 Resting percentage fat utilisation

Resting metabolic rate was not measured at the beginning of the study, therefore this data is not included for the first 12 participants (CON 4; SBW 6; ABW 2) (Table 3.6). There was a trend for RMR to decrease in each group over the course of the study, however there was no main effect of group ($P=0.366$). Mean change in fat oxidation rate following CON was $-374.40 \pm 377.71 \text{ kcal}\cdot\text{day}^{-1}$, compared to decreases of $-177.94 \pm 267.17 \text{ kcal}\cdot\text{day}^{-1}$ and $-247.68 \pm 271.30 \text{ kcal}\cdot\text{day}^{-1}$ in SBW and ABW, respectively (all NS).

Resting fat metabolism was not measured at the beginning of the study, therefore this data is not included for the first 12 participants (CON 4; SBW 6; ABW 2). There was a trend for resting fat metabolism to decrease in CON and ABW, however there was no main effect of group ($P=0.060$) (Table 3.6). Mean change in fat metabolism following CON was $-426.58 \pm 401.03 \text{ kcal}\cdot\text{day}^{-1}$, compared to an increase of $452.47 \pm 670.21 \text{ kcal}\cdot\text{day}^{-1}$ in SBW and a decrease of $-67.18 \pm 887.65 \text{ kcal}\cdot\text{day}^{-1}$ in ABW (all NS).

Resting percentage fat oxidation was not measured at the beginning of the study, therefore this data is not included for the first 12 participants (CON 4; SBW 6; ABW 2). There was a trend for resting percentage fat oxidation to decrease in CON and ABW, and there was a main effect of group on resting percentage fat oxidation ($P=0.046$; Figure 3.13). Furthermore, rates were significantly increased in SBW compared to CON ($P=0.012$). Mean change in fat metabolism following CON was $-14.98 \pm 23.71 \%$, compared to an increase of $28.67 \pm 33.19 \%$ in SBW ($P=0.012$) and a decrease of $-5.94 \pm 42.21 \%$ in ABW (NS).

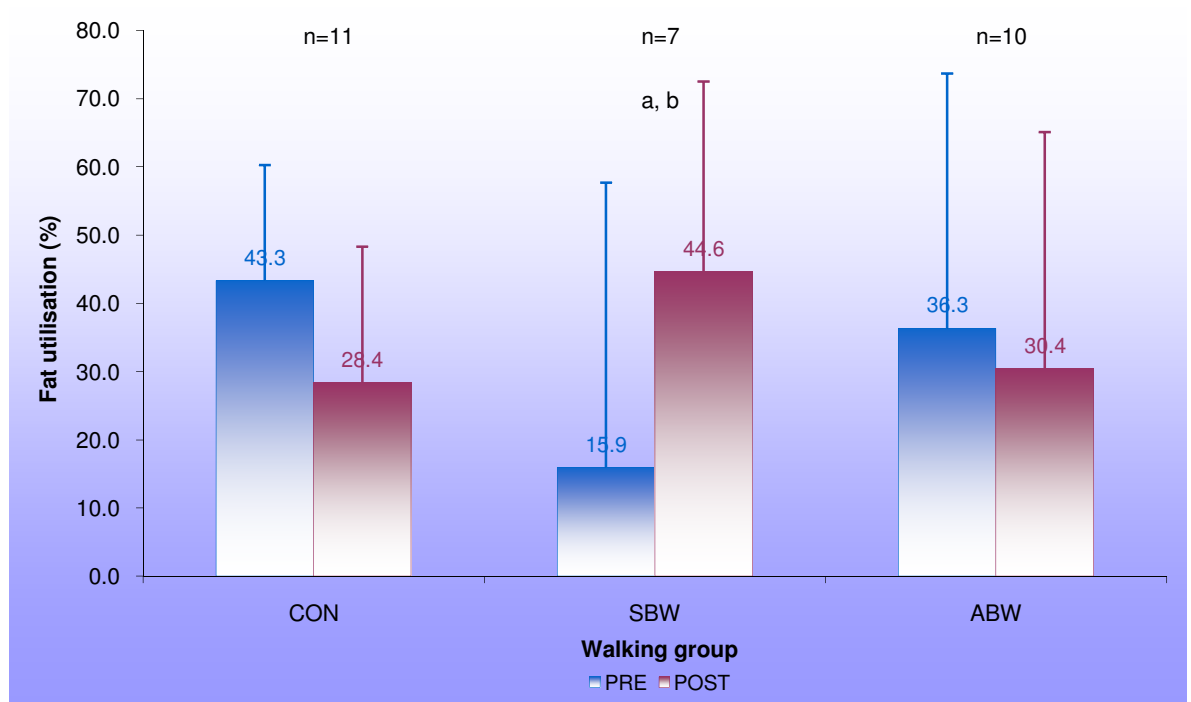


Figure 3.13 Mean pre- and post-intervention percentage fat utilisation. [Significant differences: a Significant differences between groups ($X^2=6.161$; $P=0.046$). b Significant difference between SBW and CON ($Z=-2.672$; $P=0.012$)]. Statistical power: 0.635.

3.3.5 Blood data

Table 3.7 Pre- and post-intervention measures of blood MetS and markers of RevCholTran; mean \pm SD

<i>Risk factor</i>	<i>CON n=15</i>		<i>SBW n=13</i>		<i>ABW n=13</i>		<i>Between group P value</i>
	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	
Fibrinogen (g·L ⁻¹) CON n=13, SBW n=11, ABW n=12	2.93 \pm 1.01	3.10 \pm 0.72	3.07 \pm 0.44	2.91 \pm 0.38	2.96 \pm 0.45	2.78 \pm 0.68	P=0.249
Insulin (μ IU·mL ⁻¹) SBW n=12, ABW n=12	13.07 \pm 6.16	16.06 \pm 4.72	11.36 \pm 3.84	11.06 \pm 3.80	11.35 \pm 8.35	8.74 \pm 6.09*	P<0.001
HOMA-IR (HOMA units) SBW n=12, ABW n=12	2.92 \pm 1.21	3.97 \pm 1.21	2.67 \pm 1.01	2.76 \pm 1.06	2.87 \pm 2.29	2.18 \pm 1.68**	P<0.001
HOMA- β (%) SBW n=12, ABW n=12	41.5 \pm 20.5	52.9 \pm 18.6	39.7 \pm 13.5	36.1 \pm 12.6	36.9 \pm 27.9	27.9 \pm 19.8*	P=0.001
Glucose (mmol·l ⁻¹) CON n=14, SBW n=12, ABW n=12	5.45 \pm 0.65	5.68 \pm 0.48	5.25 \pm 0.43	5.57 \pm 0.34	5.56 \pm 0.61	5.43 \pm 0.53	P=0.072
TAG (mmol·L ⁻¹) SBW n=12	1.38 \pm 0.59	1.68 \pm 0.90	1.13 \pm 0.36	1.22 \pm 0.75	1.25 \pm 0.52	1.24 \pm 0.60	P=0.425
NEFA (mmol·L ⁻¹) CON n=13, SBW n=12	0.33 \pm 0.17	0.25 \pm 0.14	0.29 \pm 0.10	0.29 \pm 0.13	0.29 \pm 0.14	0.24 \pm 0.10	P=0.271
TC (mmol·L ⁻¹) SBW n=12	5.67 \pm 0.94	5.51 \pm 0.98	5.21 \pm 0.84	4.80 \pm 0.73	5.53 \pm 0.82	5.42 \pm 0.82	P=0.544
HDL-C (mmol·L ⁻¹) SBW n=12	1.38 \pm 0.35	1.30 \pm 0.39	1.27 \pm 0.33	1.19 \pm 0.31	1.37 \pm 0.35	1.47 \pm .44	P=0.165
TC/HDL ratio SBW n=12	4.33 \pm 1.33	4.54 \pm 1.36	4.23 \pm 0.85	4.18 \pm 0.84	4.19 \pm 0.88	4.06 \pm 1.09	P=0.154
LDL-C (mmol·L ⁻¹) SBW n=12	3.66 \pm 0.85	3.45 \pm 0.79	3.46 \pm 0.73	3.06 \pm 0.73	3.59 \pm 0.67	3.39 \pm 0.67	P=0.821
CETP (pmol·hr ⁻¹) SBW n=12	156.3 \pm 70.0	146.5 \pm 70.9	171.3 \pm 99.3	151.3 \pm 88.9	176.2 \pm 94.5	180.5 \pm 74.5	P=0.371
LCAT (ratio 470/390nm of emission intensity) SBW n=12	0.880 \pm 0.024	0.880 \pm 0.036	0.880 \pm 0.035	0.880 \pm 0.034	0.881 \pm 0.034	0.887 \pm 0.034	P=0.814

Change in value compared to CON as determined by *post-hoc Scheffe test*; * $P<0.05$, ** $P<0.001$.

3.3.5.1 Plasma fibrinogen

There was no main effect of group on plasma fibrinogen ($P=0.249$; Figure 3.14), where mean change following CON was $0.17 \pm 0.69 \text{ g}\cdot\text{L}^{-1}$, compared to decreases of $-0.16 \pm 0.41 \text{ g}\cdot\text{L}^{-1}$ and $-0.18 \pm 0.68 \text{ g}\cdot\text{L}^{-1}$ in SBW and ABW, respectively.

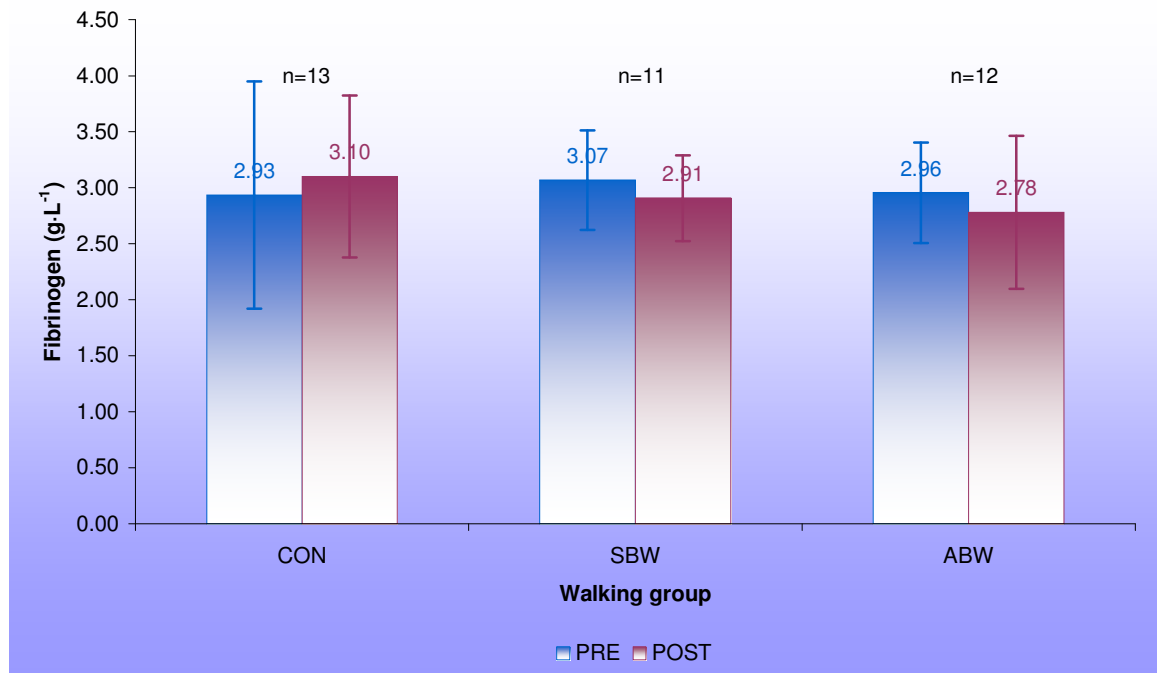


Figure 3. 14 Mean pre- and post-intervention plasma fibrinogen concentrations. [No significant differences: No effect of group on plasma fibrinogen ($F=1.454$; $P=0.249$)]. Statistical power: 0.288.

3.3.5.2 Insulin resistance

Insulin resistance was estimated using the HOMA ratio formula and there was a main effect of group ($P < 0.001$; Figure 3.15). Change in insulin resistance following CON was 1.05 ± 1.29 , compared to an increase of 0.09 ± 0.84 and a decrease of -0.69 ± 0.78 in SBW and ABW, respectively. There was a significant improvement in insulin resistance following ABW ($P < 0.001$) compared to CON, and despite a slight increase in insulin resistance following SBW this was significantly less than following CON ($P = 0.012$) and ABW and SBW were not significantly different ($P = 0.161$).

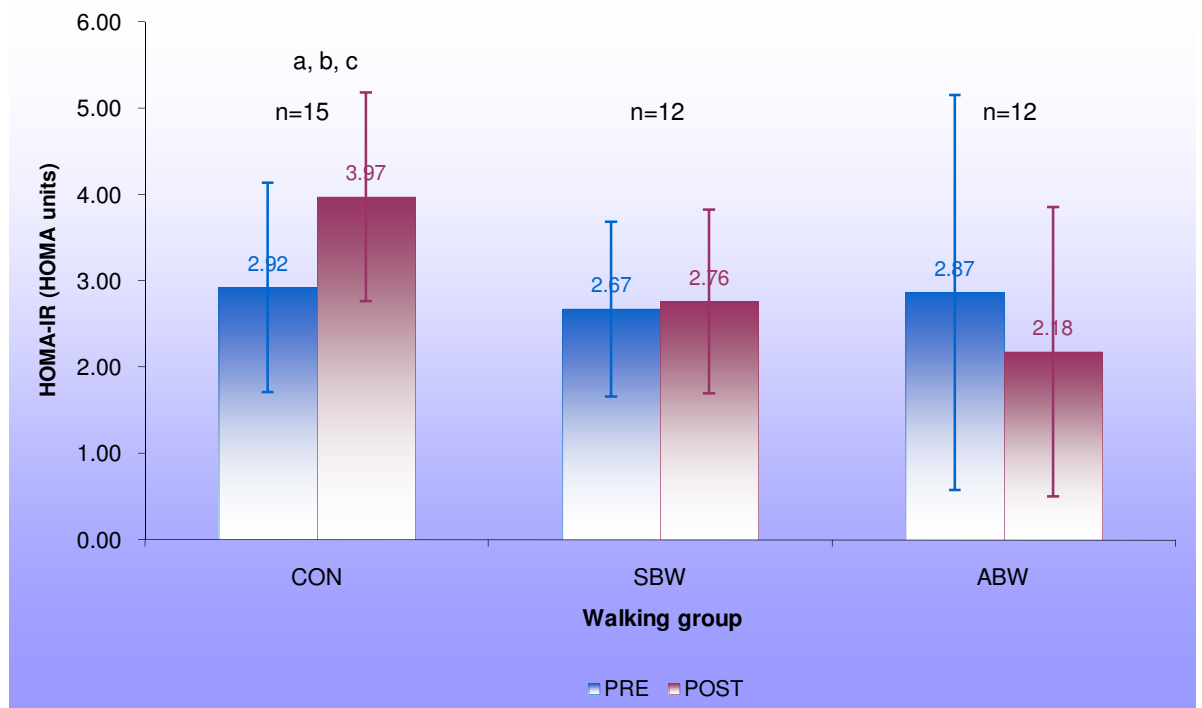


Figure 3. 15 Mean pre- and post-intervention insulin resistance values (HOMA-IR). [Significant differences: a Main effect of group on insulin resistance ($F = 13.670$; $P < 0.001$). b Effect of SBW on insulin resistance compared to CON ($P = 0.012$). c Effect of ABW on insulin resistance compared to CON ($P < 0.001$).]. Statistical power: 0.996.

There was a main effect of group on serum insulin ($P < 0.001$), where change in serum insulin following CON was $2.99 \pm 6.32 \mu\text{IU} \cdot \text{mL}^{-1}$, compared to decreases of $-0.30 \pm 3.30 \mu\text{IU} \cdot \text{mL}^{-1}$ and $-2.61 \pm 3.04 \mu\text{IU} \cdot \text{mL}^{-1}$ in SBW ($P = 0.020$) and ABW ($P = 0.001$), respectively, and the decreases in serum insulin were not significantly different between walking groups ($P = 0.384$) (Table 3.7).

3.3.5.3 Insulin secretion rates

Insulin secretion rates were estimated using the HOMA- β formula and there was a main effect of group ($P=0.001$; Figure 3.16). Change in insulin secretion following CON was $11.41 \pm 25.55\%$, compared to decreases of $-3.64 \pm 12.09\%$ ($P=0.024$) and $-9.01 \pm 11.79\%$ ($P=0.001$) in SBW and ABW, respectively.

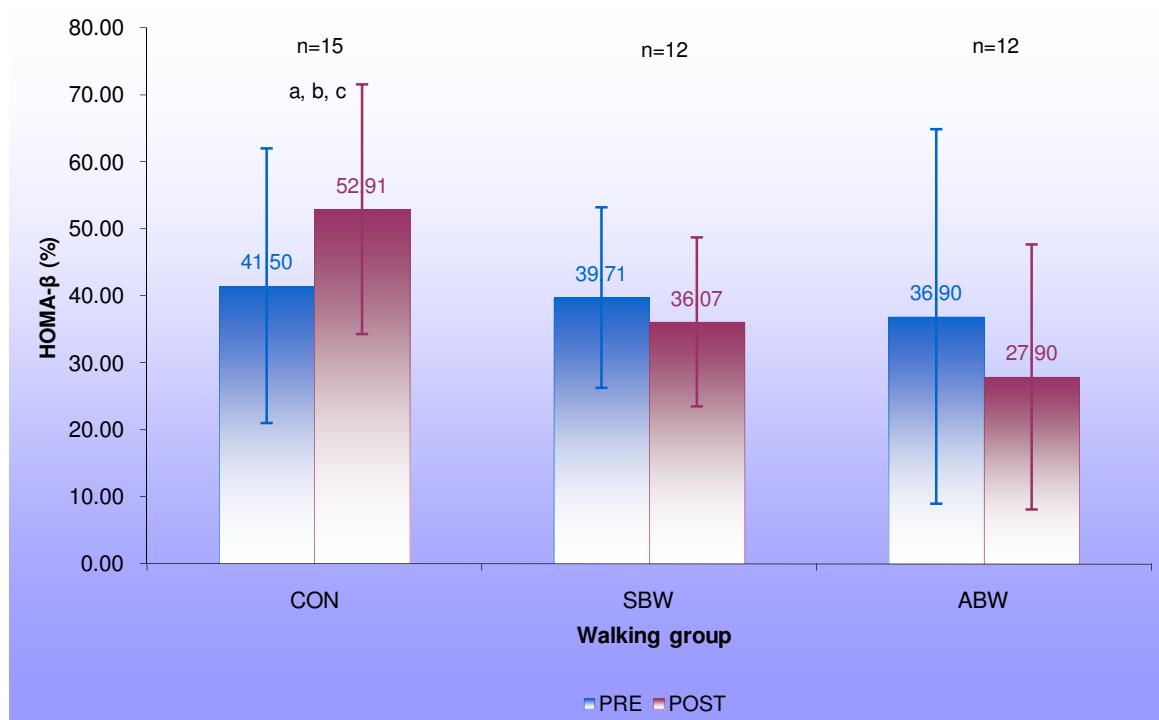


Figure 3. 16 Mean pre- and post-intervention β -cell insulin secretion rate (HOMA- β). [Significant differences: a Main effect of group on β -cell insulin secretion ($F=8.652$; $P=0.001$). b Effect of SBW on β -cell insulin secretion compared to CON ($P=0.024$). c Effect of ABW on β -cell insulin secretion compared to CON ($P=0.001$)]. Statistical power: 0.954.

There was no effect of group on plasma glucose ($P=0.072$), with only a slight increase in CON ($0.23 \pm 0.59 \text{ mmol}\cdot\text{L}^{-1}$), compared to an increase following SBW ($0.33 \pm 0.32 \text{ mmol}\cdot\text{L}^{-1}$) and a decrease following ABW ($-0.13 \pm 0.39 \text{ mmol}\cdot\text{L}^{-1}$) (Table 3.7).

3.3.5.4 Blood Lipids

Plasma NEFA was not significantly changed following walking compared to CON ($P=0.271$), where changes in plasma NEFA following CON were $-0.08 \pm 0.11 \text{ mmol}\cdot\text{L}^{-1}$, compared to an increase of 0.00 ± 0.11 and decrease of $-0.05 \pm 0.13 \text{ mmol}\cdot\text{L}^{-1}$ in SBW and ABW $\text{mmol}\cdot\text{L}^{-1}$, respectively. The changes in NEFA were not significantly different between walking groups ($P=0.543$) (Table 3.7).

Serum TAG was not significantly altered between groups ($P=0.425$), however there was an increase following CON ($0.30 \pm 0.74 \text{ mmol}\cdot\text{L}^{-1}$), compared to a slight increase in SBW ($0.08 \pm 0.76 \text{ mmol}\cdot\text{L}^{-1}$) and a slight decrease following ABW ($0.01 \pm 0.42 \text{ mmol}\cdot\text{L}^{-1}$), all NS (Table 3.7).

Serum TC concentrations were not significantly altered between groups following the intervention period ($P=0.544$). There was a general trend for serum TC to decrease in each group following the intervention, where there was a slight decrease in CON ($-0.15 \pm 0.79 \text{ mmol}\cdot\text{L}^{-1}$), compared to decreases of $-0.41 \pm 0.63 \text{ mmol}\cdot\text{L}^{-1}$ and $-0.10 \pm 0.72 \text{ mmol}\cdot\text{L}^{-1}$ in SBW and ABW, respectively (Table 3.7).

Serum HDL-C concentrations were not significantly altered between groups following the intervention period ($P=0.165$). Trends in HDL-C following the intervention were a slight decrease in CON ($-0.08 \pm 0.17 \text{ mmol}\cdot\text{L}^{-1}$), compared to a decrease of $-0.09 \pm 0.19 \text{ mmol}\cdot\text{L}^{-1}$ ($P=1.000$) and an increase of $0.10 \pm 0.32 \text{ mmol}\cdot\text{L}^{-1}$ ($P=0.144$) in SBW and ABW, respectively (Table 3.7).

3.3.5.5 Serum TC/HDL-C ratio

Serum TC/HDL-C concentrations were not significantly altered between groups following the intervention period ($P=0.154$; Figure 3.17). Trends in HDL-C following the intervention were a slight increase in CON ($0.21 \pm 0.50 \text{ mmol}\cdot\text{L}^{-1}$), compared to a mean increase of $-0.05 \pm 0.43 \text{ mmol}\cdot\text{L}^{-1}$ in SBW ($P=0.434$) and a decrease of $-0.27 \pm 0.69 \text{ mmol}\cdot\text{L}^{-1}$ ($P=0.080$) in ABW.

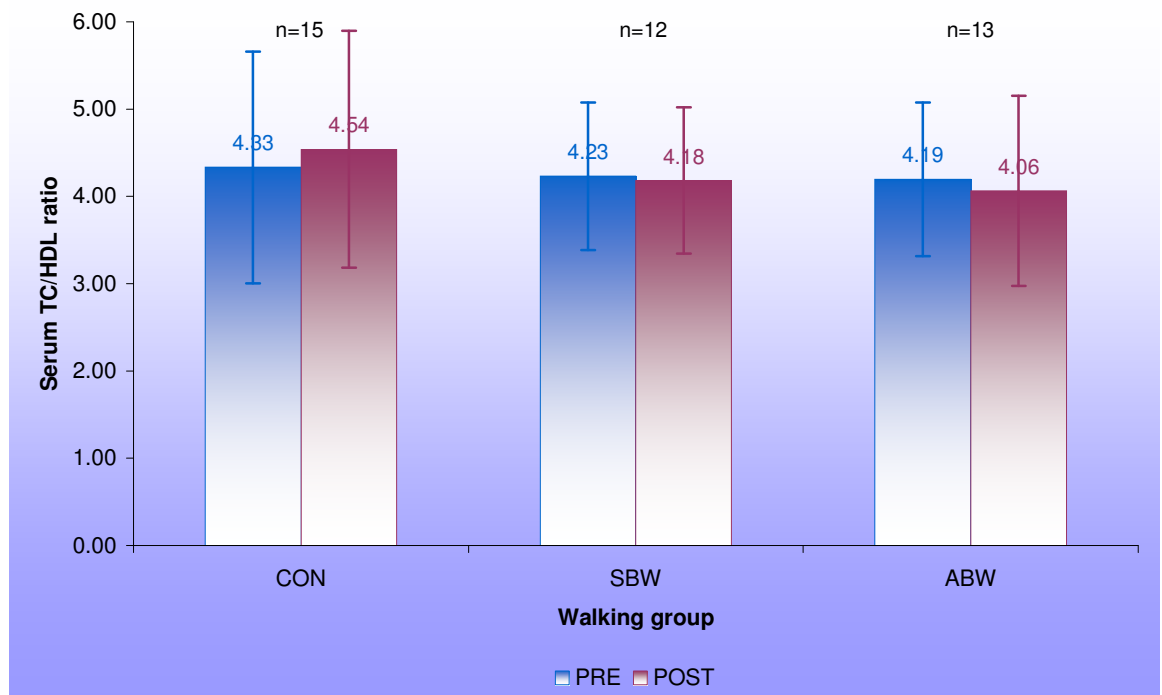


Figure 3.17 Mean pre- and post-intervention serum TC/HDL-C ratios. [No significant group differences ($X^2=3.740$; $P=0.154$)]. Statistical power: 0.493.

Serum LDL-C concentrations were not significantly altered between groups following the intervention period ($P=0.821$) (Table 3.7). There was a general trend for LDL-C to decrease during the study, where there was a slight decrease in CON ($-0.21 \pm 0.62 \text{ mmol}\cdot\text{L}^{-1}$), compared to a mean increase of $-0.40 \pm 0.77 \text{ mmol}\cdot\text{L}^{-1}$ ($P=0.683$) and a decrease of $-0.20 \pm 0.46 \text{ mmol}\cdot\text{L}^{-1}$ ($P=0.751$) in SBW and ABW, respectively.

3.3.6 Reverse cholesterol transport activity

Serum CETP activity was not significantly altered between groups following the intervention period ($P=0.371$; Figure 3.31). Trends in serum CETP activity following the intervention were a slight decrease in CON ($-9.80 \pm 43.61 \text{ pmol}\cdot\text{hr}^{-1}$), compared to a mean decrease in SBW ($-20.00 \pm 96.69 \text{ pmol}\cdot\text{hr}^{-1}$; $P=1.000$) and an increase in ABW ($4.31 \pm 53.94 \text{ pmol}\cdot\text{hr}^{-1}$; $P=0.156$).

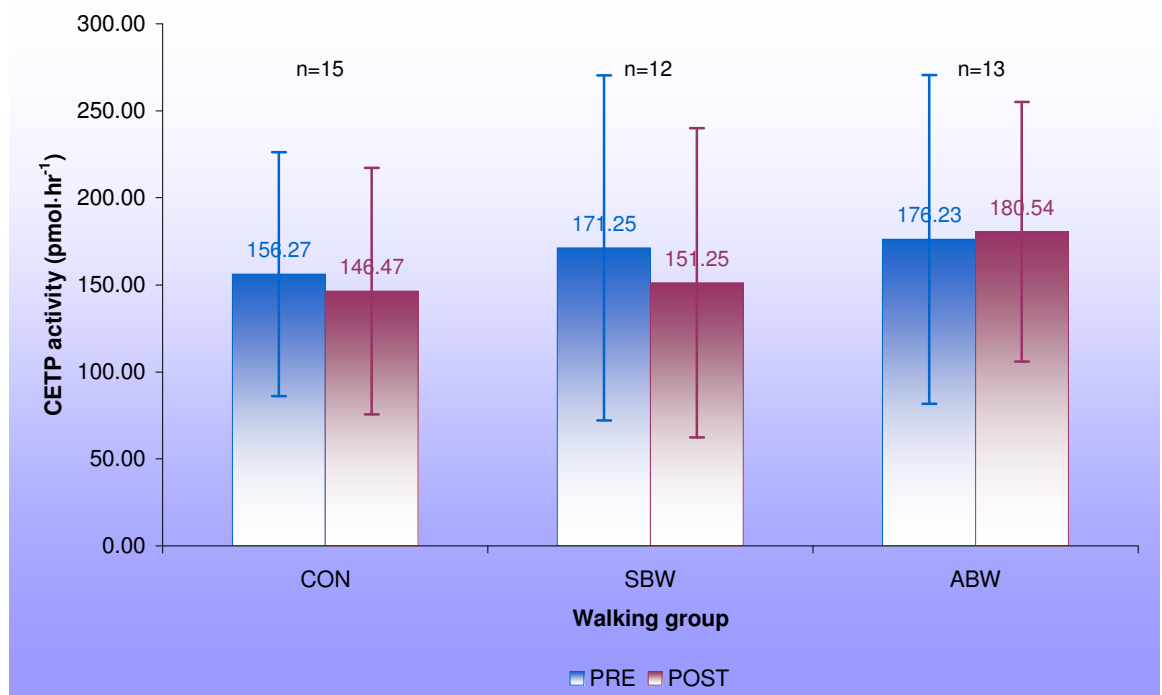


Figure 3.18 Mean pre- and post-intervention serum CETP activity. [No significant group differences ($X^2=1.980$; $P=0.371$)]. Statistical power: 0.113

Serum LCAT activity was not significantly altered between groups following the intervention period ($P=0.814$; Table 3.7). Trends in serum LCAT activity following the intervention were a slight decrease in CON (-0.0002 ± 0.0298 470/390nm ratio), compared to a mean decrease in SBW (-0.0001 ± 0.0160 470/390nm ratio; $P=1.000$) and an increase in ABW (0.0053 ± 0.0305 470/390nm ratio; $P=1.000$).

3.4 Discussion

The aims of the study were to determine whether walking briskly for 24 weeks on 5 days of the week and for 30 minutes each day could improve MetS risk factors and markers of RevCholTran compared to sedentary controls, and also whether accumulating the same volume of brisk walking during the day may be equally effective. Of the 65 non-medicated participants that began the study 41 satisfactorily completed 24 weeks; 4 did not satisfactorily complete CON due to illness (2) and not understanding the requirements of the study (2), leaving 15 participants that completed the control procedure (78.9%); 9 did not satisfactorily complete SBW, leaving 13 participants that completed the intervention (59.1%); finally 11 did not satisfactorily complete ABW due to not adhering to the accumulative pattern of walking (3) and not completing 24 weeks of walking (8), leaving 13 participants that completed the intervention (54.2%) (see Figure 3.1). These are greater than mean values from previous randomised walking trials, where mean percentage drop out rates were 20.2% among walkers and 12.4% for controls (Murphy *et al.*, 2007) compared to a mean drop-out rate of 43.4% in ABW and SBW combined and 21.1% in CON in the present study. However, this was still within the range of those studies included in the meta-analysis: 0–53.8% and 0–38% among walkers and controls, respectively.

The study recruited sedentary middle-aged men at risk of MetS and use of such medications is common in these groups (Rantala *et al.*, 1999; Williams & Franklin, 2007). Following the recruitment period there were a number of participants that were excluded from the study due to their use of anti-hypertensive and/or lipid-lowering medication (8 CON; 5 SBW; 5 ABW) in order to remove the confounding effects of these medications on the health outcomes being examined. In those who completed the study, the presence of

MetS increased in CON from 3 participants to 5 and in SBW from 1 participant to 3, whereas the participant with MetS in ABW remained in the MetS category as defined by ATPIII criteria (Cleeman, 2001). Considering that few of the participants began the study diagnosed with MetS, after exclusion of those using medication, it is conceivable that the relative good health of the participants precluded improvements in MetS components due to the walking, as those with greater health are known to derive less benefit from exercise than those of lower health status (Wilmore, 2001). At baseline the participants' mean values for serum lipids and lipoproteins, RevCholTran enzymes, plasma fibrinogen, plasma glucose and serum insulin were within the normal ranges, while the participants' mean aerobic capacity was in the 'good' classification for males of this age. However, mean blood pressure measures, WC and subsequent WHR values were towards high normal values. Therefore, in general, the participants could only have been considered to be pre-obese and borderline MetS at pre-intervention and perhaps not as likely as less healthy individuals to respond to PA treatment due to having less potential for improvement (Wilmore, 2001; Cleeman, 2001). However, exercise-only interventions have proven effective in reducing presence of MetS. Indeed, a 12 month intervention study involving 137 men with International Diabetes Federation (IDF)-defined MetS who were randomly allocated to control, diet, exercise or combined exercise/diet groups, found that each intervention decreased the presence of MetS symptoms (Anderssen *et al.*, 2007). Following 12 months of the interventions 76.5% in the exercise group (supervised aerobic exercise; $3 \times 60 \text{ min} \cdot \text{wk}^{-1}$ @ 60-80% HR_{peak}), 64.7% following 12 months in the diet group (dietary advice at 3, 6 & 9 months; energy restriction, reduced saturated fat & cholesterol and increased fish consumption) and 32.6% in the combined exercise and diet group still had MetS.

In the present study the CON group were instructed to maintain their pre-intervention low levels of PA throughout the duration of the study and those in SBW and ABW were instructed to record their walking activities within diaries. These diaries indicated that there were no significant differences in the intensity of brisk walking in terms of mean HR_{max} and $\%HR_{max}$ (Figure 3.2) and volume of brisk walking in terms of minutes per week, total minutes performed or total estimated energy expenditure during the study (Figure 3.3 & Table 3.3). There were no significant differences between the walking groups for the number of weeks completed, however as would be expected ABW performed a significantly greater number of walking sessions during the course of the study than SBW (Figure 3.4). Compared to a meta-analysis of 20 randomised controlled walking trials (Murphy *et al.*, 2007) the characteristics of the walking intervention in the present study were generally of lower duration (32.9 ± 5.8 mins·session⁻¹ & 15.6 ± 3.0 mins·session⁻¹ for SBW & ABW, respectively), lower mean weekly volume (150.6 ± 25.1 min·wk⁻¹), shorter intervention period (23.6 ± 0.8 weeks) and at a lower relative intensity ($66.2 \pm 9.9\%$ HR_{max}). However, walking frequency was similar to the pooled data in the meta-analysis (4.69 ± 0.33 sessions·wk⁻¹ & 9.74 ± 1.73 min·wk⁻¹ for SBW & ABW, respectively). The mean characteristics of the randomised controlled walking trials included in the meta-analysis were 38.3 ± 14.4 mins·session⁻¹, on 4.4 d·wk⁻¹, giving a mean volume of 188.8 min·wk⁻¹, for 34.9 ± 4.9 weeks, at a mean intensity of $70.1 \pm 9.1\%$ HR_{max} .

The accumulative brisk walking group (ABW) was associated with significant improvements in the majority of the anthropometric measures of obesity including body mass, WC, WHR and %BF compared to CON, however SBW only improved WC and W:H compared to CON (Table 3.4 & Figures 3.5-3.7). Blood [La⁻] was collected on completion of the submaximal walking test as an additional measure of cardiorespiratory

fitness to predicted $\dot{V}O_{2\max}$ as an indicator of metabolic changes due to walking. Interestingly, SBW improved $\dot{V}O_{2\max}$ compared to CON, whereas although there appeared to be a tendency for ABW to improve $\dot{V}O_{2\max}$ compared to CON, this did not attain statistical significance ($P=0.180$). However, the opposite was true for $[La^-]$, measured on completion of the submaximal walking test, where ABW decreased $[La^-]$ compared to both CON ($P=0.044$) and SBW ($P=0.028$) (Figures 3.8-3.9). It may be speculated that this difference was due to the pattern of walking where the longer duration bouts in SBW may have stimulated greater central cardiorespiratory adaptation, whereas the repetitive nature of the walks in ABW may have affected $[La^-]$ buffering capacity by the musculature of the legs being re-stimulated on multiple occasions on the same day and promoting peripheral metabolic adaptation. Change in $\dot{V}O_{2\max}$ appeared to be unrelated to changes in any of the health outcomes that were investigated. Indeed, evidence demonstrates that PA-induced reductions in total and abdominal fat are more strongly associated with improvements in blood pressure, blood lipid & lipoprotein profile and insulin sensitivity than the improvements in fitness (Stewart *et al.*, 2005).

Long-term adherence to PA may lower blood pressure independently of weight loss and may be related to increased vasodilation due to enhanced vagal tone and reductions in insulin resistance/hyperinsulinaemia-induced mechanisms, such as enhanced tubular sodium reabsorption, decreases in insulin resistant blood vessels, increased capillarisation and decreased secretion of vasoactive substances from adipose tissue (Pedersen & Saltin, 2006). However, there may be certain subgroups of patients with hypertension who are more responsive to the blood pressure-lowering effects of PA, since the decrements in blood pressure associated with increased PA are not always sufficient to produce normotension in many studies (Eriksson *et al.*, 1997). Despite the apparent disparity, RCTs

among overweight middle-aged and older subjects demonstrate the effectiveness of PA to lower blood pressure, with or without weight loss (Dengel *et al.*, 1998; Blumenthal *et al.*, 2000). A 1999 meta-analysis of aerobic exercise training RCTs among overweight/obese participants, the blood pressure lowering effect of PA was small but statistically significant among normotensive study groups (baseline blood pressure 128/84 mm Hg) (Fagard, 1999) and a further meta-analysis showed modest weight loss (3-9%) was associated with a significant reduction in mean systolic and diastolic blood pressures of approximately 3 mm Hg compared with non-intervention controls (Hermansen, 2000).

The overall results of the published studies indicate that blood pressure reductions may be apparent within 10 weeks of hypertensive patients beginning participation in PA, with recent evidence to suggest that significant reductions are possible within 4 weeks (Collier *et al.*, 2008) and blood pressure may continue to decline even further with prolonged maintenance of PA, which may occur independently of favourable changes in body composition (Hagberg *et al.*, 2000). In the present study, blood pressure tended to decrease slightly in each group throughout the duration of the study however it was not significantly augmented in either of the walking groups (Figures 3.10-3.11). Despite favourable changes in blood pressure, a primary MetS marker, these were not statistically significant, which was possibly at least partly due to the low statistical power, where changes of a similar magnitude have been reported in other exercise intervention studies and found to be significant (Wilmore, 2001). Therefore, it may be reasonable to state that both walking interventions appeared to be equally as effective as other interventions in reducing blood pressure, if non-significant. However, the trends for both BP (Figures 3.10-3.11) and resting heart rate (Table 3.5) to decrease during ABW and SBW compared to CON, resulted in significant differences in rate-pressure product following the 24 weeks of brisk

walking (Figure 3.12). This effect was evident for ABW compared to CON, however there was only a trend for the favourable effect of SBW compared to CON ($P=0.077$) and the favourable trends in SBP and RHR appear to have been additive to decrease RPP, in which changes were significantly associated with changes in WHR ($P=0.018$).

There was an apparent trend for RMR to decrease in each group over the course of the 24 weeks, however although the increase in resting fat oxidation in $\text{kcal}\cdot\text{d}^{-1}$ following SBW was not significant (Table 3.6), when resting fat oxidation was expressed proportionally to RMR, percentage resting fat utilisation was significantly increased following SBW compared to CON (Figure 3.13). However, this did not impact significantly upon body mass losses following SBW. Change in body mass was associated with change in fasting serum insulin ($P=0.007$), a relationship that was also apparent between change in body mass and change in HOMA-IR ($P=0.015$) and HOMA- β ($P=0.018$). Change in body mass was also associated with change in DBP ($P=0.014$), BF% ($P=0.013$) and WC ($P<0.001$). However, change in WC was more strongly associated with $[\text{La}^-]$ ($P=0.025$), DBP ($P=0.007$) and fasting serum insulin ($P=0.005$). The changes in HOMA-IR and HOMA- β associated with changes in body mass appear to be mediated predominantly through changes in WC for HOMA-IR ($P=0.001$) and HOMA- β ($P=0.013$), even though change in BF% remained strongly associated with change in HOMA-IR ($P=0.005$). Improvements in glucose homeostasis occurred following both walking interventions compared to CON. Both fasting insulin secretion (Figure 3.16) and serum insulin (Table 3.7) tended to decrease compared to baseline in SBW and ABW, however the tendency for these measures to increase during CON increased the difference. Accumulative walking demonstrated a decrease in insulin resistance compared to baseline, whereas there was a trend for insulin resistance to increase slightly following SBW (Figure 3.15). However,

since serum insulin and insulin secretion were significantly lower following the walking intervention, this phenomenon is likely due to the slight increase in fasting plasma glucose that occurred following SBW (Table 3.7). These findings were similar to those of Miyatake *et al.* (2002) and showed greater improvement than the findings of Snyder *et al.* (1997). It is debatable whether hyperinsulinaemia is the primary defect that causes obesity or actually a response, however it is clear that reductions in abdominal adiposity and hyperinsulinaemia are related to increased PA and that increases in non-oxidative glucose disposal following chronic PA is likely to be responsible (Ross *et al.*, 2000b). These favourable changes occur even in the absence of body mass losses, which indicates that improvements in muscle metabolism decreases abdominal adiposity and enhances insulin-stimulated insulin uptake, however significant decreases in body fat may augment this effect (Kelley & Goodpaster, 1999; Ross *et al.*, 2000b).

Intra-abdominal fat is relatively insensitive to insulin and has a high lipolytic activity, partly due to its complement of adrenergic receptors (Björntorp, 1990) and hyperinsulinaemia and increased NEFA may affect several interconnected steps in lipoprotein-lipid metabolism (Carroll & Dudfield, 2004). Physical activity may improve the dyslipidaemic profile associated with MetS by increasing the ability of muscle tissue to take up and oxidize NEFA and increasing the activity of LPL in muscle (Pollare *et al.*, 1991). Reductions in TAG and elevations in HDL-C are commonly associated with increased PA, however TC and LDL-C are infrequently affected (Durstine *et al.*, 2001). Endurance-trained men and women demonstrate high rates of TAG clearance compared with sedentary controls, which may be related to the increased LPLa, the enzyme that hydrolyses TAG-rich lipoproteins (Kiens & Lithell, 1989). This may benefit an atherogenic dyslipidaemic profile because circulating TAG concentrations are closely

coupled with rates of HDL clearance and so an enhanced metabolic capacity for TAG may explain the high HDL-C levels in physically active people. However, there was no apparent effect of brisk walking on blood lipid and lipoproteins, with plasma NEFA, serum TAG, serum total cholesterol, serum HDL-C, TC/HDL-C ratio and serum LDL-C remaining unchanged compared to CON following SBW and ABW (Table 3.7 & figure 3.17). Changes in fasting serum TAG were associated with change in HOMA-IR ($P=0.020$) and HOMA- β ($P=0.020$), and change in plasma NEFA were also associated with HOMA-IR ($P=0.022$), while change in serum TAG was also associated with change in the TC/HDL-C ratio ($P<0.001$), with much of this effect being mediated through the relationship between change in serum TAG and serum HDL-C ($P=0.031$) rather than changes in serum TC ($P=0.106$).

Two of the primary markers of MetS, TAG and HDL-C, were unchanged as a result of the intervention, which may have been due to insufficient PAEE. Evidence suggests that there is a weekly PAEE threshold of 1200-2200 kcal·wk⁻¹ that needs to be met for increases in HDL-C to occur (Durstine *et al.*, 2001) and although the mean estimated weekly PAEE in the present study was just over 1200 kcal·wk⁻¹, the lack of change in serum HDL-C may have been due to the mean baseline blood lipid and lipoprotein profiles being in the fairly good category leaving little potential for favourable change (Wilmore *et al.*, 2001). Furthermore, the participants in the walking groups only walked at ~50% $\dot{V}O_{2\max}$, which is considerably lower than 75% $\dot{V}O_2$ max that has been shown to cause changes in blood lipid and lipoprotein profile (Durstine *et al.*, 2001). In a meta-analysis of 25 randomised controlled walking trials, 1,176 participants were studied to investigate the influence of walking on blood lipids and lipoproteins (Kelley *et al.*, 2004). The walking studies were performed at $64.2 \pm 9.4\%$ $\dot{V}O_{2\max}$ for 38.4 ± 15.6 min·session⁻¹ on 4.8 ± 2.5 d·wk⁻¹ for 23.2

± 17.7 weeks and the rate of adherence was $83.4 \pm 18.0\%$. The main findings of the analysis was that walking can significantly reduce LDL-C by $\sim 5\%$ and TC/HDL-C ratio by $\sim 6\%$ independently of reductions in body mass, and although no significant changes in mean TC, HDL-C or TAG were observed, the direction of change tended to be favourable. A secondary finding of the meta-analysis was an increase in $\dot{V}O_{2\max}$ of $3.6 \pm 0.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, which equated to a 15% increase compared to controls, but body mass, BMI, percent body fat and lean body mass were not significantly altered. These findings are contradictory to the present study, where although the walking interventions in the present study were similar to the mean characteristics of those included in the meta-analysis, those studies generally demonstrated decreases in serum TC/HDL-C ratio and LDL-C without associated improvements in body composition. Furthermore, even though significant improvements in body composition were observed in the present study, these were not associated with significant decreases in serum LDL-C and TC/HDL-C ratio, despite favourable trends. However, even following meta-analysis, these were the only components of the lipid & lipoprotein profile that walking intervention significantly improved (Kelley *et al.*, 2004).

Serum CETPa and LCATa were measured to indicate mechanisms for potential changes in TAG-HDL-C dynamics as a result of the walking interventions. However, as with serum TAG and HDL-C concentrations, CETPa and LCATa remained unchanged, indicating that not only did blood lipid and lipoprotein profiles not favourably respond compared to CON following the brisk walking, despite significant reductions in anthropometric measures of abdominal adiposity in SBW and ABW, measures of RevCholTran remained unchanged (Table 3.7 & Figure 3.18). Therefore, aside from TC/HDL-C ratio there was no clear pattern for SBW or ABW to favourably influence the blood lipid and lipoprotein profile,

thus indicating that both walking interventions may have been collectively ineffective in improving this profile.

Studies concerning the effect of PA interventions on lipids and lipoproteins among individuals with MetS remain scarce, partly due to the lack of precise definition of the syndrome. However, data indicates that over months, PA expending 1200-2200 kcal·wk⁻¹ results in increases in HDL-C, which are even greater when the exercise programme is accompanied by weight loss (Durstine *et al.*, 2001). Therefore, PA alone may not always be sufficient to normalise the atherogenic dyslipidaemia associated with MetS without significant weight loss. Low HDL-C is associated with insulin resistance, which causes hypertriglyceridaemia through increased hepatic production of VLDL particles (Ji *et al.*, 2005). Hyperinsulinaemia-blunted decreases in LPLa have also been suggested to explain the relationship between elevated TAG and decreased HDL-C, thus decreasing TAG clearance and impairing maturation of HDL-C by enhanced CETP-mediated exchange of TAG and cholesteryl esters between TAG-rich lipoproteins and HDL-C (Lewis & Rader, 2005). However, neither serum TAG, HDL-C or CETPa were favourably influenced following the walking interventions, despite increases in insulin sensitivity and decreases in serum insulin, particularly ABW. Cholesterol ester transfer protein enriches HDL-C with TAG and depletes them of cholesteryl esters, which reduces the size of the HDL-C particles (Skeggs & Morton, 2002). Reductions in CETPa have the ability to elevate circulating HDL-C through reductions in TAG-rich HDL-C (Lewis & Rader, 2005). The first published study investigating the influence of exercise training on plasma CETP was performed by Seip *et al.* (1993). The exercise training consisted of three to five sessions of supervised aerobic exercise (walking/jogging/stationary cycling) for 45-60 minutes per week at $\leq 85\%$ HR_{max} for 9-12 months. The main outcomes were that CETP concentrations

fell and significantly in response to training, with the decreases in CETP concentration weakly associated with decreases in body fat. Pre-training CETP concentration predicted the training-induced increases in HDL-C, i.e. those with the lowest CETP concentrations gained the greatest increases in HDL-C and those with higher pre-training CETP concentrations achieved the smallest increases in HDL-C. As with the mean characteristics of randomised controlled walking trials mentioned above, the study by Seip *et al.*, (1993) involved a greater PA volume than the present study, in terms of PA session duration, intensity and total intervention period, indicating that the present intervention may have been insufficient to promote favourable responses rather than being underpowered *per se*. Therefore, increasing PA intensity, session duration or total intervention period may have resulted in increases in CETPa and concomitant increases in HDL-C.

Lecithin-cholesterol acyltransferase is an enzyme that is bound to HDL particles and esterifies free cholesterol to form cholesteryl esters by transferring 2 acyl groups from lecithin to cholesterol. While there is cross-sectional evidence demonstrating higher concentrations of LCAT in athletes compared to sedentary counterparts (Brites *et al.*, 2004; Olchawa *et al.*, 2004), there is no direct evidence that PA intervention can increase LCAT concentrations (Williams *et al.*, 1990) or LCATa (Bedgoni *et al.*, 2002), despite significant decreases in body mass and fat percentage in these studies. Therefore, it may be reasonable to speculate that the lack of change in LCATa may have been due to an insufficient PA volume. Furthermore, the importance of LCAT in RevCholT has not been fully established because unesterified cholesterol can be directly transferred from HDL-C to the liver for excretion without the need for LCAT-mediated esterification (Lewis & Rader, 2005). A combination of the equivocal importance of LCAT in RevCholTran and the lack of change in LCAT or LCATa following PA intervention substantiates the present findings that

LCATa remained similar between groups during the present study, despite greater concentrations in athletes compared to sedentary individuals (Brites *et al.*, 2004; Olchawa *et al.*, 2004).

Neither ABW nor SBW appeared to significantly improve plasma fibrinogen concentrations, despite favourable trends in both walking groups compared to CON (Figure 3.14). However, due to the limited number of studies of the potential improvements in thrombotic risk associated with PA, the exact PA dose to promote a favourable response is currently unknown. Metabolic processes within skeletal muscles may drive the anti-thrombotic effects of PA, where data from the longitudinal ‘Studies of Targeted Risk Reduction Intervention through Defined Exercise’ (STRIDDE) demonstrated that the fibrinolytic activities of tissue plasminogen activator (tPA), and its endogenous inhibitor (PAI-1), were favourably altered in skeletal muscle and serum following 9 months of exercise training expending 2000 kcal·wk⁻¹, as measured using the biopsy needle technique (Hittel *et al.*, 2003). In the present study mean weekly estimated PAEE was only 1345.3 kcal·wk⁻¹ in ABW and 1211.5 kcal·wk⁻¹ in SBW over the course of ~6 months, therefore well below the PAEE threshold achieved by STRIDDE. Therefore, since the exact PA dose required to improve fibrinolytic activity is yet to be defined, it is seems that the PAEE achieved in the current study was insufficient to significantly decrease plasma fibrinogen concentrations.

A limitation of the outcome of the present study was that blood pressure and blood lipid & lipoprotein profile tended to be underpowered in contrast to anthropometric and aerobic fitness measures (>0.70), particularly for WC, WHR and BF%, with WC, WHR, $\dot{V}O_{2max}$ and glucose homeostasis being the only health outcomes that were favourably improved

following SBW compared to CON. Despite relatively low statistical power for some of the health outcomes in this study it is possible that the walking interventions (walking intensity and volume) were insufficient to improve some of the health outcomes. Although walking at any pace expends energy, usually when individuals are instructed to walk briskly they automatically select a pace that elicits an intensity of $\sim 70\%$ HR_{max} ($\sim 60\%$ $\dot{V}O_{2max}$), which likely to improve cardiovascular fitness (Morris & Hardman, 1997; Murtagh *et al.*, 2002). Walking at $4.8 \text{ k}\cdot\text{hr}^{-1}$ on a level surface requires a $\dot{V}O_2$ of $\sim 13 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, which could amount to over 60% $\dot{V}O_{2max}$ for a 75-year-old due to age-associated declines in $\dot{V}O_{2max}$, but may be less than 30% $\dot{V}O_{2max}$ of a healthy 30-year-old man (Morris & Hardman, 1997). A study from the early 1990s reported that habitual exercise walkers, $\dot{V}O_{2max} 35.7 \pm 6.3 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, met the ACSM's recommended PA intensity for improving $\dot{V}O_{2max}$ ($\geq 70\%$ HR_{max} ; $\geq 60\%$ $\dot{V}O_{2max}$) (Spelman *et al.*, 1993). However, due to the mean age (52.71 ± 8.73 years) and mean $\dot{V}O_{2max}$ ($36.30 \pm 6.84 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) of the participants in this study being categorised as fairly fit for their age, it is likely that the mean walking intensity ($66.2 \pm 9.9 \text{ HR}_{max}$), although generally sufficient to improve $\dot{V}O_{2max}$ in the walking groups compared to CON, was of insufficient intensity to improve primary health outcomes, such as blood pressure and blood lipid and lipoprotein profiles. Furthermore, the characteristics of the walking interventions were generally lower than the mean values for those reported in the recent meta-analysis on the impact of walking on body composition, fitness and blood pressure (Murphy *et al.*, 2007).

Data from the available randomised controlled walking trials suggest that favourable changes in body composition, $\dot{V}O_{2max}$, blood pressure, blood lipid and lipoprotein profile and blood clotting may be attained by walking at $\sim 70\%$ HR_{max} for $30 \text{ min}\cdot\text{d}^{-1}$ on $5 \text{ d}\cdot\text{wk}^{-1}$ (Woolf-May *et al.*, 1998; Murphy & Hardman, 1998). Mean data pooled in a recent meta-

analysis of randomised controlled walking interventions demonstrated mean increases in $\dot{V}O_{2\max}$ of $2.73 \pm 0.35 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (9%), mean decreases of $-0.95 \pm 0.61 \text{ kg}$ (-1.4%) and $-0.28 \pm 0.20 \text{ kg}\cdot\text{m}^{-2}$ (-1.1%) and $-0.63 \pm 0.66\%$ (-1.9%) in body mass, BMI and body fat percentage, respectively, compared to baseline (Murphy *et al.*, 2007). Each of these favourable changes were significant ($P \leq 0.035$) and although resting diastolic blood pressure was significantly decreased by $-1.54 \pm 0.79 \text{ mm Hg}$ (-2%; $P=0.026$) systolic blood pressure was not favourably changed through walking intervention, however relatively few of the walking studies measured blood pressure pre & post (SBP=9; DBP= 6), thus decreasing the chances of a favourable outcome compared with the other measures. These findings clearly suggest that walking may have its limitations for enhancing certain risk factors, where even in meta-analysis there are non-significant findings. Furthermore, despite including data for 116 participants between four walking groups, walking did not significantly improve body composition, blood pressure, blood lipoprotein profile or glucose tolerance despite increases in $\dot{V}O_{2\max}$, which also suggested a lack of power Asikainen *et al.* (2003).

Prior to the original PA recommendations for accumulating PA being published (CDC & ACSM, 1995) only two empirical studies had investigated the efficacy of accumulated PA on health. These studies investigated the influence of splitting running distance on cardiovascular endurance and blood lipids (Ebisu, 1985) and the effect of short vs. long bouts of exercise on $\dot{V}O_{2\max}$ (DeBusk *et al.*, 1990). Subsequently, in a more eloquent RCT, the efficacy an 18 week intervention of accumulative walking was investigated incorporating a CON group, a single daily brisk walking involving 20-40 minute sessions (LB) and another group accumulated the same duration of walking in 10-15 minute sessions throughout the day with ≥ 120 minutes between sessions (Woolf-May *et al.*, 1998).

The main outcomes of the study were that aerobic capacity significantly decreased and factor XIIa, a marker of coagulation, significantly increased in the control group, however there were no significant changes in lipid profiles (TC, LDL-C, HDL-C and apos A-I, A-II & B) in the walking groups compared with controls. This was an important study because it was the first study to compare the efficacy of accumulative walking sessions to walking of equal daily volume *and* to non-active controls, which more comprehensively examined the health benefits of accumulation *per se* relative to no activity and activity performed in single daily sessions, in comparison to the more simplistic studies of Ebisu (1985) and DeBusk *et al.* (1990).

In close chronological proximity a RCT, involving 10 weeks of brisk walking on 5 days of the week at 70-80% HR_{max} in either one 30 minute walk (LB) or three 10 minute walks per day (SB), or performing no walking as controls, found that $\dot{V}O_{2max}$ increased and sum of skin folds decreased significantly in both walking groups with no differences between walking groups, however body mass and waist circumference decreased significantly only in the SB group (Murphy & Hardman, 1998). The authors also commented that body/fat mass gained by the controls contributed to the significant responses to the walking, whereby the control participants decline in health parameters contributed to the effect of walking as much as the walking improved the health in the walking group. These findings are in slight contrast to the present study, where $\dot{V}O_{2max}$ was only significantly increased following SBW and BF% was only significantly reduced following ABW, with both of these variables presenting relatively strong statistical power. These differences are despite the present intervention being of longer duration and of similar frequency, however the walks were performed at a greater mean relative intensity.

A further study from Woolf-May *et al.* (1999) used a similar design to their previous study (Woolf-May *et al.*, 1998), but with an additional accumulative walking group, in which participants were instructed to perform brisk walking sessions in 5-10 minute bouts totalling 20-40 min·d⁻¹. The main findings of this study were that each of the walking groups – long, intermediate and short duration – produced positive health outcomes compared to controls, however the long and intermediate groups demonstrated the more potent effects. Each of the walking groups promoted improvements in aerobic fitness, whereas only the intermediate and long sessions produced favourable improvements in the blood lipid profile compared to controls. These studies both agree and disagree with the findings of the present study, where $\dot{V}O_{2max}$ tended to increase in this study too, yet blood lipid profile improved in the long and intermediate sessions in the second study by Woolf-May *et al.* (1999), despite both interventions being shorter than the present study. However, a telling difference may be the intensity of the walking interventions, ~74% HR_{max}, compared to only ~66% in the present study.

The influence of brisk walking on the risk factors associated with MetS has been slightly equivocal in the present study, with measures of body composition and insulin resistance responding positively, whereas certain other health outcomes, such as blood pressure and blood lipid and lipoprotein profile, did not appear responsive. However, in conclusion, the findings of this study clearly demonstrate that current recommendations for the accumulation of 30 minutes of physical activity throughout the day on at least 5 days of the week (ACSM & AHA, 2007), performed at an easily attainable walking intensity, was effective in reducing certain risk factors associated with MetS, particularly BMI, WC, WHR, BF%, fasting serum insulin and insulin resistance. These findings are particularly significant and provide a mechanism by which walking >2 h·wk⁻¹ is related to a 39% reduction in mortality (Gregg *et al.*, 2003). Furthermore, accumulative walking also

showed favourable trends toward decreasing blood pressure, plasma fibrinogen and an increase in $\dot{V}O_{2\max}$ in men at risk of MetS, therefore this would be a useful strategy for reducing symptoms of MetS in those at risk. Future research may wish to re-investigate these health outcomes using participants of lower health status but still without medication or using a greater walking volume. Research should also be performed to investigate the short-term impact of single walking sessions on similar health outcomes to those studied here. Due to the apparent limited effect of the 24 weeks of walking on certain health outcomes it would be of interest to determine whether discrete walking sessions may have an effect and if so whether the intensity or duration of walking session may influence the magnitude or duration of any effects.

4.0 The 24 hour effect of different intensities and durations of brisk walking on risk factors associated with metabolic syndrome

4.1 Introduction

In follow-up to the study in the previous section and in slight contrast, the short term effects of walking on MetS risk factors was studied to discern whether the same health outcomes respond immediately or only following adaptation and if the non-responding health outcomes to chronic walking may be affected in the short-term.

Sedentary behaviour impacts on MetS risk, where low levels of PA are associated with increased risk of obesity and its accompanying co-morbidities, such as hypertension, insulin resistance and dyslipidaemia, where performing less than 150 min-wk⁻¹ of moderate-vigorous leisure time PA increases the risk of developing MetS two-fold (Ford *et al.*, 2005). Decreased energy expenditure is the likely mediator between inactivity and increased MetS risk, where objectively measured PAEE has been demonstrated to predict the progression towards MetS independently of $\dot{V}O_{2max}$ and obesity (Ekelund *et al.*, 2005). The relationship between PA and MetS risk appears to be unaffected by $\dot{V}O_{2max}$ or adiposity, suggesting that improvements in $\dot{V}O_{2max}$ or decreases in adiposity are not absolutely necessary to decrease MetS risk, providing that PAEE increases.

Public health problems occur in the least active 20-30% of the population (Hamilton *et al.*, 2004), where bed-rest models of physical inactivity demonstrate that as the stimulus for muscular protein synthesis decreases there are associated increases in insulin resistance and insulin secretion, which are associated with components of MetS (Biolo *et al.*, 2005). Participation in regular PA is now promoted as one of the 'best buys' in the prevention and

management of physiological disorders, such as hypertension, dyslipidaemia, overweight and obesity, diabetes and heart disease. Indeed, long-term increases in PA alone have been shown to decrease the prevalence of MetS, which are further enhanced by combining dietary intervention with PA (Anderssen *et al.*, 2007) (see section 2.3). It may also be possible that the benefits associated with PA may be partly as a consequence of the acute effects of the last bout of exercise rather than from favourable adaptations to chronic PA (Thompson *et al.*, 2001). However, no published study appears to have investigated the potential for PA to impact on MetS risk factors in the immediate post-exercise period, despite evidence demonstrating short-term improvements in independent MetS risk factors, such as blood glucose control (Englert *et al.*, 2006), blood pressure (Jones *et al.*, 2007), lipid and lipoprotein profile (Ferguson *et al.*, 1998), markers of pro-thrombotic (Ivey *et al.*, 2003) and pro-inflammatory states (Murtagh *et al.*, 2005a) that may be gained from a single PA session. These are important questions both physiologically and in terms of PA promotion, since many individuals appear to like a ‘quick fix’.

When the impact of walking intensity and walking duration on all-cause mortality has been investigated there was a significant inverse association between daily walking intensity and risk of death, whereas there was only a weak inverse association between daily walking duration and risk of death (Schnohr *et al.*, 2007). Furthermore, data demonstrates that those whose walking speed exceeds $2.1 \text{ m}\cdot\text{s}^{-1}$ have 64% lower odds for anti-diabetic, 50% lower odds for anti-hypertensive and 47% lower odds for LDL-C lowering medication use compared with those whose walking speed is less than $1.2 \text{ m}\cdot\text{s}^{-1}$ (Williams, 2008). The current minimum PA recommendations are for 30 minutes of moderate intensity PA ($\geq 40/50\%$ HRR) to be accumulated on 5 days of the week or 20 minutes of vigorous intensity PA (up to 85% HRR) to be performed on 3 days of the week, or a combination of

these two sets of recommendations (ACSM & AHA, 2007). However, these recommendations are based on long-term adherence to these guidelines rather on the immediate health-enhancing effects that may be gained from a single session (see section 2.3.7). Furthermore, the minimum volume of PA for affecting each aspect of MetS in a single session is currently unknown.

Therefore the purpose of this study was to determine a) whether walking at 50% $\dot{V}O_{2max}$ for 30 minutes may effect favourable changes in risk factors associated with MetS during the 24 hour post-exercise period compared to a control trial, and b) whether increasing the intensity of the walk to 65% $\dot{V}O_{2max}$ for 30 minutes or maintaining the intensity at 50% $\dot{V}O_{2max}$ and increasing the duration to 60 minutes would alter the magnitude or duration of these responses.

4.2 Method

4.2.1 Introduction

The study included 13 non-smoking middle-aged males (age 59.92 ± 6.64 years; height 1.79 ± 0.05 cm; mass 94.1 ± 11.2 kg; BMI 29.37 ± 4.69 kg·m²; waist 104.0 ± 10.4 cm; waist:hip ratio 0.98 ± 0.05 ; $\dot{V}O_{2\max}$ 37.1 ± 6.1 mL·kg⁻¹·min⁻¹). The participants were recruited from the previous study (section 3.0) following its completion and were asymptomatic of cardiovascular disease and diabetes, and not prescribed cardiovascular medication (see Figure 3.1). There were at least 4 weeks between completing the 24 week walking study and commencing this 24-hour study. The study was approved by the central NHS Research Ethics Committee (Appendix B.1; p281) and the participants were advised of the experimental procedures and possible discomforts (Appendix B.2; p299) and then signed the informed consent form (Appendix B.3; p304), a pre-screening health questionnaire (Appendix C.1; p310), and gave permission for their General Practitioner to be contacted for approval of their entry onto the study (Appendix C.3.2; p320). The participants received written confirmation that they were under no obligation to begin or continue with the study and could withdraw at any time.

4.2.2 Pre-screening procedure

Potential participants were pre-screened using a health questionnaire and their general practitioner was asked for their approval prior to participation.

Exclusion criteria –

- a) Symptomatic of cardiovascular disease
- b) Diabetes mellitus
- c) Tobacco smoking
- d) Prescribed cardiovascular or diabetic medication
- e) Their general practitioner was unable to provide health clearance for them to participate, or
- f) The participant was unable to understand the nature of the study.

4.2.3 Experimental design

The study employed a repeated measures design, where participants took part in all four trials and acted as their own controls. The main trials took place in a randomly assigned order and included:

- Trial A: No exercise (CON) – 30 min lying in a semi-supine position on the couch
- Trial B: 30 minute walk at 50% $\dot{V}O_{2max}$ (30×50%)
- Trial C: 30 minute walk at 65% $\dot{V}O_{2max}$ (30×65%)
- Trial D: 60 minute walk at 50% $\dot{V}O_{2max}$ (60×50%)

4.2.4 Initial assessments

The initial assessment included height, mass, waist circumference (WC) and hip circumference (HC) measurements, and a sub-maximal walking test was performed to calculate the correct walking intensities during the brisk walking sessions. Initial assessments in this study were performed as part of the post-intervention assessments in the study in section 3.0.

4.2.4.1 Height, body mass and waist & hip circumferences

Height was measured using a stadiometer to the nearest cm and mass to the nearest 100g using beam balance scales (both Seca, Hamburg, Germany). The participants then stood in the anatomical neutral position and waist circumference was measured at the level of the umbilicus and hip circumference was measured at the largest circumference of the hips above the gluteal fold both to the nearest 1 mm using a self-tightening circumference tape measure (Seca, Hamburg, Germany). Three WC and three HC measures were taken and the mean values were recorded. The WHR was then calculated from these measures.

4.2.4.2 Submaximal walking test

The test consisted of walking at 3 mph throughout the test at five different gradients for two minutes at each stage (Table 4.1).

Table 4.1 Graded treadmill test protocol

<i>Speed (mph)</i>	<i>Time (minutes)</i>	<i>Gradient (%)</i>
3.0	2	2.5
3.0	2	5.0
3.0	2	7.5
3.0	2	10.0
3.0	2	12.5

To assess aerobic fitness the participants performed the Stanford sub-maximal walking test (ACSM, 2000) on a motorised treadmill (Mercury Med., HP Cosmos, Nussdorf-Traunstein, Germany) while oxygen consumption ($\dot{V}O_2$) was analysed using an online breath by breath gas analyser (Oxycon Pro, Jäeger, Würzburg, Germany). Heart rate (HR) was also recorded using a radio-telemetry system (Polar Beat, Polar Electro OY, Finland), which was recorded every 5 seconds with oxygen consumption. Ratings of perceived exertion (RPE; Borg 6-20 scale) were recorded at the end of each 2-min stage. Following the test, the HR- $\dot{V}O_2$ relationship was calculated and this relationship was extrapolated up to the participants age predicted maximum heart rate ($220 - \text{age}$) to predict $\dot{V}O_{2\text{max}}$ (Maritz *et al.*, 1961). The treadmill gradient required to elicit 50% and 65% $\dot{V}O_{2\text{max}}$ was interpolated from the HR- $\dot{V}O_2$ relationship.

4.2.5 Pre-trial dietary records and health assessments

On the day prior to each trial, the participants recorded the food they consumed in a food diary (Appendix D.2; p341) so that this same diet could be replicated on the first day of the subsequent trials. This was in order to reduce the confounding effect of diet. The data from the diaries were analysed using DietMaster Pro (Version 6 Software, Lifestyles Technologies Inc, Valencia, CA). Following this the participants fasted for at least 14

hours (i.e. from 7.00 PM until after the post-1 hr assessment the following morning) and again for the same period the following night, before the 24 hour post-walk assessment on the second morning. All assessments included a blood test, blood pressure, resting metabolic rate/fat oxidation and resting heart rate.

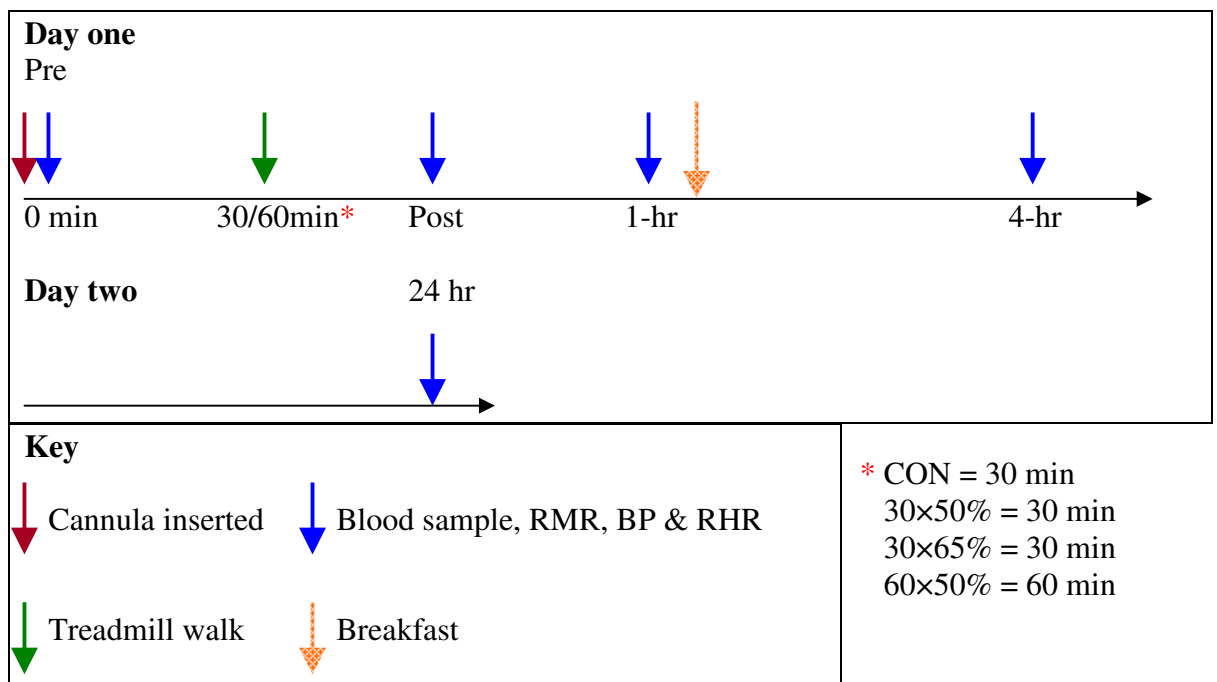


Figure 4. 1 Timescale for each trial

These assessments were performed immediately prior to walk (Pre), immediately post-walk (post), 1 hour post-walk (1-hr), 4 hours post-walk (4-hr) and 24 hours post-walk (24-hr) (Figure 4.1). The participants were required to fast until after the 1-hr assessment when breakfast was provided (See section 4.2.6). Following the 4 hour post-walk assessment on the morning of the first test the participants recorded the food they consumed for the duration of the day in the food diary.

4.2.5.1 Blood sampling, blood pressure, resting heart rate and resting metabolic rate

On arrival in the Sport and Exercise Science Laboratory the participants lay in a semi-supine position and a cannula (Venflon, BD, Oxford) was inserted into a forearm vein of

the participants to allow the series of blood samples to be collected and was kept patent using saline solution (0.9% w/w, BD, Oxford). Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and pulse pressure (PP) were assessed after the participants had remained in the semi-supine position for 5 minutes. Three blood pressure measures were taken using an aneroid sphygmomanometer (Accoson Limpet, A.C. Cossor & Son Ltd, London, UK) each 30 seconds apart from the opposite arm to where the cannula was inserted and the mean values were recorded. MAP was calculated using $(SBP-DBP)*0.33 + DBP$, and PP was calculated by the difference between the mean SBP and DBP values.

For the assessment of resting metabolic rate (RMR) and fat oxidation rates, the participants remained in a supine position while a five minute expired air sample was collected using Douglas bags (Harvard Apparatus Ltd, Kent, UK) and resting heart rate (RHR) was also monitored during this period using a heart rate radio-telemetry system (Polar Beat, Polar Electro OY, Finland). Rate-pressure product (RPP) was calculated from RHR and SBP measures. The Douglas bags were then analysed (Servomex Series 1440, Servomex Group Ltd, Crowborough, UK) for oxygen and carbon dioxide concentrations and the volumes were measured using a dry gas meter (Harvard Apparatus Ltd, Kent, UK). These values, plus ambient air temperature and barometric pressure were input into an MS Excel spreadsheet for the calculation of $\dot{V}O_2$ (Appendix F.2; p364). Resting metabolic rate was calculated from $\dot{V}O_2$ by multiplying $L \cdot \text{min}^{-1}$ by 5 (Montoye et al. 1996) and resting fat oxidation rates were calculated using $f = 1.67 * \dot{V}O_2 - 1.67 * \dot{V}CO_2 - 1.92 * n$ [where f = fat and n = urinary nitrogen] (Frayn, 1983).

4.2.6 Treadmill walk and breakfast

Prior to the walking trials, the participants performed a standard warm up on the treadmill to prepare them for the walking session ahead (Table 4.2). When the participants performed the main test, the speed of the treadmill remained at 3 mph throughout, with the intensity being dictated by the gradient. The gradient of the treadmill was set to elicit either 50% or 65% $\dot{V}O_{2max}$, depending on the trial, and the treadmill walks lasted for either 30 or 60 minutes. Exercising HR and ratings of perceived exertion (RPE; 6-20) were monitored at five minute intervals, while $\dot{V}O_2$ was measured during the final 5 minutes of each treadmill walk using the online gas analysis equipment to validate the actual walking intensity and to allow the calculation of energy expenditure (EE) by multiplying $\dot{V}O_2$ ($L \cdot min^{-1}$) by 5 (Montoye *et al.*, 1996). Oxygen consumption was also measured using the online system during the 30 min CON period for the purpose of calculating EE during CON.

Table 4.2 Standard warm up

Time (mins)	Speed (mph)	Gradient (%)
2	3	0.0
2	3	2.5
1	3	5

Breakfast was supplied to the participants relative to body mass, consisting of muesli, milk and orange juice, each provided at $1g \cdot kg^{-1}BM$, which were measured to the nearest 1g using electronic scales (Ohaus LS500, Ohaus Scale Corporation, NJ). The mean energy content of the meal was 541.4 ± 62.6 kcal (protein 53.2 ± 8.1 kcal; carbohydrate 438.8 ± 45.5 kcal; fat 49.3 ± 9.0 kcal) and water was consumed *ad libitum* following the 1-hr assessment.


4.2.7 Blood sampling and analysis

Blood was dispensed into a collection tube to form serum and a tube containing fluoride oxalate for the formation of plasma. The samples were then centrifuged at room temperature (Eppendorf 5804, Hamburg, Germany) and stored in a freezer (Revco Ultra, Asheville, NC, USA) at -70°C for later analysis. The serum was analysed for concentrations of TAG, TC and HDL-C using enzymatic colorimetry (all Horiba ABX, Cambridge), and insulin by chemiluminescence (Bayer Advia Centaur, Bayer Healthcare, Diagnostics Division, Tarrytown, NY.). The plasma collected in the fluoride oxalate tube was used to analyse glucose (Horiba ABX, Cambridge) and NEFA (Wako Ltd, Neuss, Germany) using enzymatic colorimetry (See Appendix E for more detailed biochemistry procedures, p344). Insulin resistance was estimated using the homeostasis assessment model ratio formula (HOMA-IR): $\text{fasting serum insulin } (\mu\text{IU}\cdot\text{mL}^{-1}) \times \text{fasting plasma glucose } (\text{mmol}\cdot\text{L}^{-1}) / 22.5$ and insulin secretion (HOMA- β cell) was estimated using: $\text{fasting serum insulin } (\mu\text{IU}\cdot\text{mL}^{-1}) \times 20 / \text{fasting plasma glucose } (\text{mmol}\cdot\text{L}^{-1}) - 3.5$, both described by Matthews *et al.* (1985) and LDL-C was derived from the Friedewald *et al.* (1972) equation using the TC, HDL-C and TAG values. The samples were analysed within the same assay series to reduce variability within the biochemical analytical procedures and were then corrected for changes in plasma volume (Dill & Costill, 1974). Changes in plasma volume were calculated from finger prick haemoglobin and haematocrit samples (see Appendices F.1.13 and F.1.14; pp 462-3). The coefficients of variations for the assays, using low concentration quality control sera, were: glucose 0.25%; NEFA 2.40%; TC 5.81%; HDL-C 8.24%; TAG 7.09%; insulin 4.61%; (See Appendix F.1 for the Assay principles, p349).

4.2.8 Statistical analyses

The number of research participants to be recruited was calculated using data from Tsetsonis & Hardman (1996b), which was similarly structured to the present study. The mean differences and SD for serum TAG were input into a programme (Clinstat, Martin Bland) for the calculation of sample size with an alpha of 0.05 at 80% power, which indicated that eight participants were required to avoid a type II error. However, that study utilised 12 participants, therefore the aim was to recruit at least 12 participants onto the study. Post-hoc power was also calculated for each health outcome between treatments. The descriptive data are presented as the mean \pm SD and differences between treatments were analysed using two-way ANOVA with a Bonferroni correction (See Appendix B.4 for tabulated mean data, p305). The data was analysed using SPSS version 13 and significance was accepted at $P < 0.05$. Non-normally distributed data were log-transformed and analysed using two-way ANOVA. The energy expended during the intervention sessions were analysed using one-way ANOVA and the HR & %HR_{max} data recorded during the exercise sessions were analysed using two-way ANOVA. Friedman's ANOVA for ranks was used to analyse the intensity of the walks (% $\dot{V}O_{2max}$). Friedman's two-way ANOVA for ranks was used to analyse the RPE data recorded during the exercise sessions because it remained non-normally distributed after log-transformation. The data recorded from the food diaries was analysed using one-way ANOVA. Friedman's ANOVA for ranks were employed if the data remained non-normally distributed. Pearson's product moment correlation coefficients were used to explore relationships between factors, with an alpha of 0.05 level of statistical significance.

4.3 Results

The mean intensity of each session was $49.1 \pm 3.6\% \dot{V}O_{2\max}$ during 30×50%, $65.2 \pm 3.6\% \dot{V}O_{2\max}$ during 30×65% and $49.0 \pm 3.6\% \dot{V}O_{2\max}$ during 60×50%. The higher intensity walk was at a significantly greater intensity than both of the lower intensity walks ($X^2=15.200$; $P=0.001$), whereas both of the lower intensity walks were of similar intensity ($Z=-0.941$; $P=0.347$). The arrow on the graphs  indicates when breakfast was consumed following 1-hr. Breakfast was initially not included in the proposed study, but it was a stipulation by the research ethics committee, and unfortunately influenced some data, particularly the metabolism, glycaemic and lipaemic data

4.3.1 Walking intensity

4.3.1.1 Sessional energy expenditure

There were significant differences in EE between CON and each of the walking sessions ($P < 0.001$) (Figure 4.2). Energy expenditure during CON was just 47.63 ± 1.97 kcal·session⁻¹, compared to 248.80 ± 11.34 kcal·session⁻¹ during 30×50% and 335.51 ± 14.67 kcal·session⁻¹ during 30×65%, with the greatest expenditure of 501.57 ± 18.02 kcal·session⁻¹ in 60×50%.

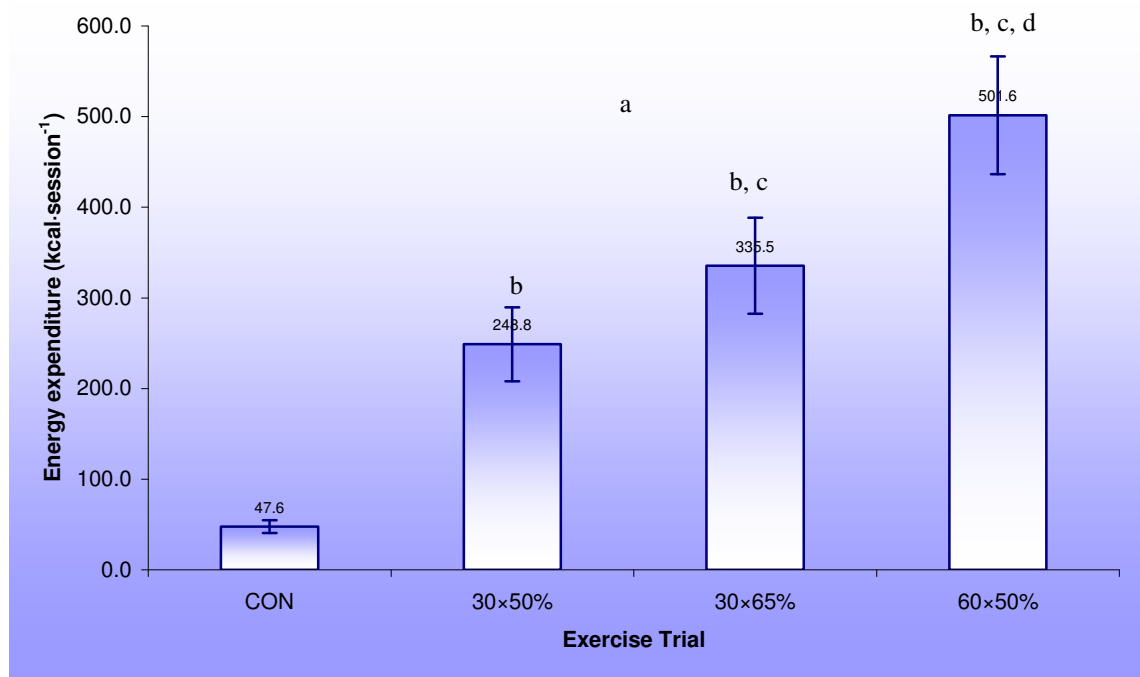


Figure 4.2 Mean energy expenditure of each exercise trial. [Significant differences: a Main effect of walking session on EE ($F=212.471$; $P < 0.001$). b EE significantly higher than CON ($P < 0.001$). c EE significantly higher than 30×50% ($P < 0.001$) d EE significantly higher than 30×65% ($P < 0.001$)].

4.3.1.2 Heart rate, Percentage HR_{max} and Perceived Exertion

Exercising heart rate was significantly different between walking sessions ($P < 0.001$). HR in 30×65% was significantly higher than 30×50% and during both the 1st and 2nd 30 min of 60×50% ($P < 0.001$), furthermore HR in the 2nd 30 min of 60×50% was significantly higher than 30×50% ($P = 0.003$) and the 1st 30 min of 60×50% ($P < 0.001$). HR during 30×50% and 1st 30 min of 60×50% were similar ($P = 0.519$). Mean final HR during 30×50% was 99.2 ± 10.0 beats·min⁻¹, 127.8 ± 11.8 beats·min⁻¹ during 30×65%, 97.9 ± 9.9 beats·min⁻¹ at 30 min during 60×50% and 103.3 ± 9.8 beats·min⁻¹ at 60 min of 60×50%.

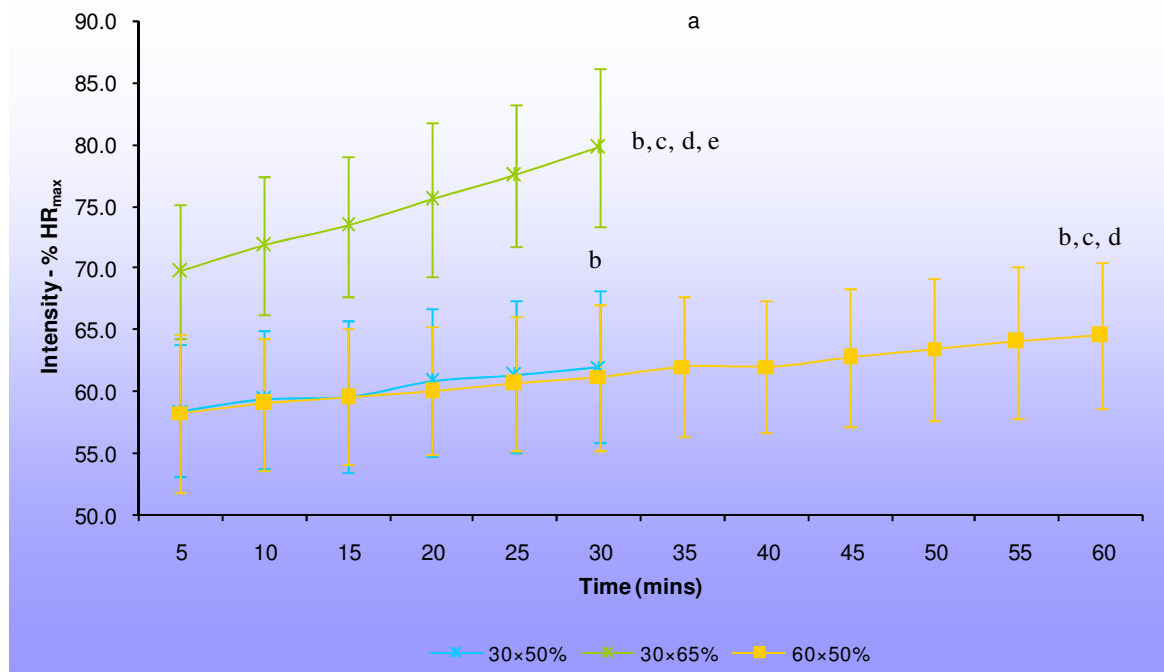


Figure 4.3 Mean %HR_{max} values recorded during each trial. [Significant differences: a Main effect of intensity × time ($F = 13.317$; $P < 0.001$). b %HR_{max} significantly higher between 5 & 30 mins, and 35 & 60 mins ($F = 55.741$; $P \leq 0.012$). c %HR_{max} significantly higher than 30×50% ($F = 74.403$, $P \leq 0.015$). d %HR_{max} significantly higher than 60×50% (1st 30 min) ($F = 74.403$; $P < 0.001$). e %HR_{max} significantly higher than 60×50% (2nd 30 min) ($F = 74.403$; $P < 0.001$)].

Exercising %HR_{max} was significantly different between walking sessions over time ($P < 0.001$; Figure 4.3). %HR_{max} in 30×65% was significantly higher than 30×50% and 60×50% ($P < 0.001$), furthermore %HR_{max} in the 2nd 30 min of 60×50% was significantly

higher than 30×50% (P=0.002) and the 1st 30 min of 60×50% (P<0.001). %HR_{max} during 30×50% and 1st 30 min of 60×50% were similar (P=0.509). Mean final %HR_{max} during 30×50% was 62.0 ± 6.2 %, 79.8 ± 6.4 % during 30×65%, 61.2 ± 5.9 % at 30 min during 60×50% and 64.6 ± 5.9 % at 60 min of 60×50%.

RPE was significantly different between walking sessions over time (P<0.001). RPE in 30×65% was significantly higher than 30×50% and 60×50% (P<0.05), furthermore RPE in the 2nd 30 min of 60×50% was significantly higher than 30×50% (P<0.05) and the 1st 30 min of 60×50% (P<0.05). %HR_{max} during 30×50% and 1st 30 min of 60×50% were similar (P=0.161). Mean final %HR_{max} during 30×50% was 11.46 ± 1.90, 14.08 ± 1.50 during 30×65%, 11.00 ± 1.68 at 30 min during 60×50% and 12.31 ± 1.75 at 60 min of 60×50%.

4.3.2 Dietary records

4.3.2.1 Macronutrient intake prior to day 1

Food intake was recorded on the day prior to each trial and there were no significant differences in energy consumption, carbohydrate, fat or protein (each kcal·day⁻¹) between trials (Figure 4.4).

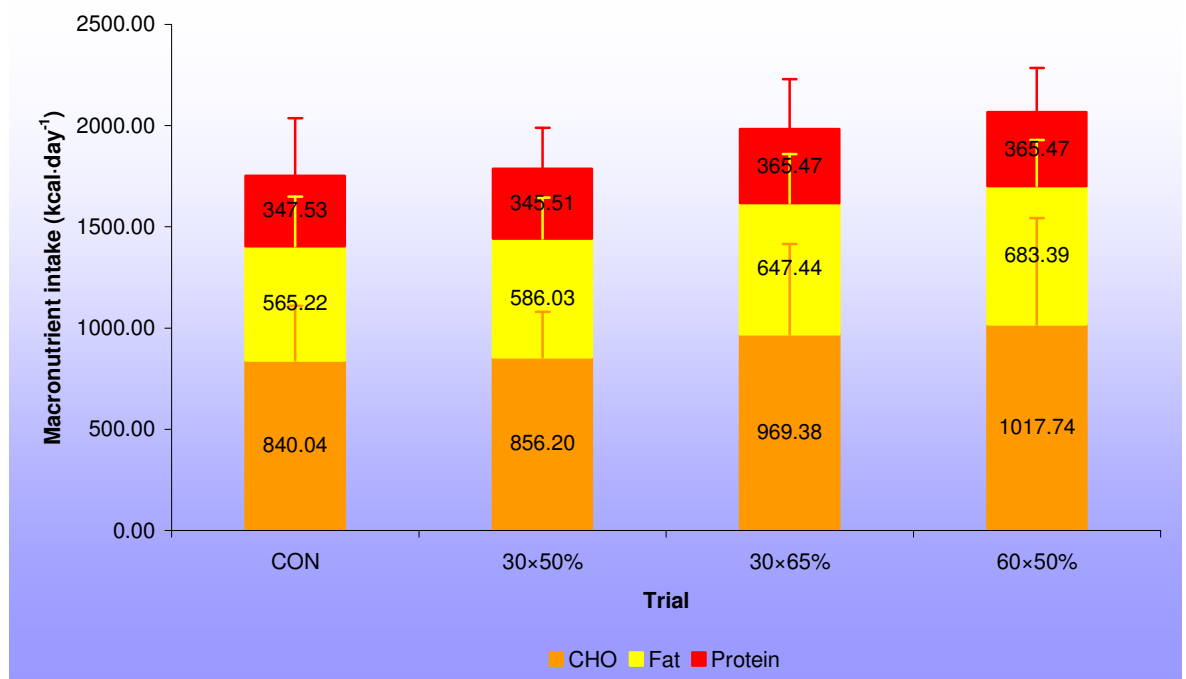


Figure 4.4 Mean macronutrient intake during the day prior to day 1 of each trial. [No significant differences in macronutrient energy intakes prior to day 1 between trials; CHO: F=0.551; P=0.651, Fat: F=0.619; P=0.617, Protein: $\chi^2=4.080$; P=0.253, & Total Energy: F=0.702; P=0.557].

4.3.2.2 Macronutrient intake prior to day 2

Food intake was recorded for the rest of the first day of each trial prior to the second day, and there were no significant differences in energy consumption, carbohydrate, fat or protein (each kcal·day⁻¹) between trials (Figure 4.5). There were no significant differences in energy consumption between day 1 and day 2 across the four trials (P=0.551).

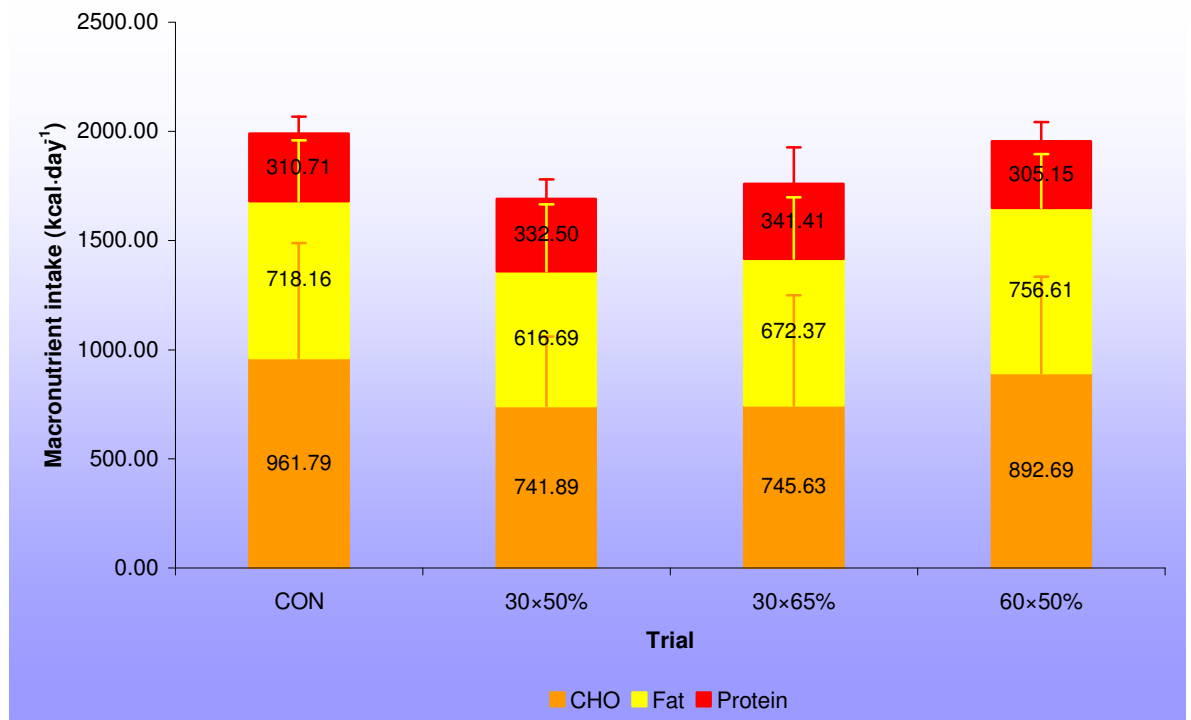


Figure 4.5 Mean macronutrient intake during the day prior to day 2 of each trial. [No significant differences in macronutrient energy intakes prior to day 1 between trials; CHO: F=0.838; P=0.481, Fat: F=0.489; P=0.692, Protein: F=0.160; P=0.922, & Total Energy: F=0.628; P=0.602].

4.3.3 Cardiovascular responses

4.3.3.1 Systolic blood pressure

SBP decreased significantly for ≥ 4 hours following 30×50% ($P \leq 0.023$), SBP was only significantly lowered at 1 hour post-walk following 30×65% ($P = 0.032$) and not significantly different to Pre values by 4 hours, and SBP was lowered for at least 1 hour but less than 4 hours following 60×50% compared to Pre ($P \leq 0.006$; Figure 4.6). Mean SBP increased by up to 9.2 ± 13.4 mm Hg (at 4-hr) following CON, compared to significant decreases of $\geq -8.7 \pm 7.7$ mm Hg following 30×50%, $\geq -11.0 \pm 10.8$ mm Hg following 30×65% and $\geq -10.4 \pm 8.0$ mm Hg following 60×50%. Moreover, SBP was significantly lower following 30×65% and 60×50% than CON at Post-walk ($P \leq 0.026$), significantly lower in 30×50%, 30×65% and 60×50% than CON at 1-hr ($P \leq 0.043$) and significantly lower in 30×50% & 60×50% than CON at 4-hr ($P \leq 0.007$), but there were no significant differences between 30×50%, 30×65% and 60×50% throughout.

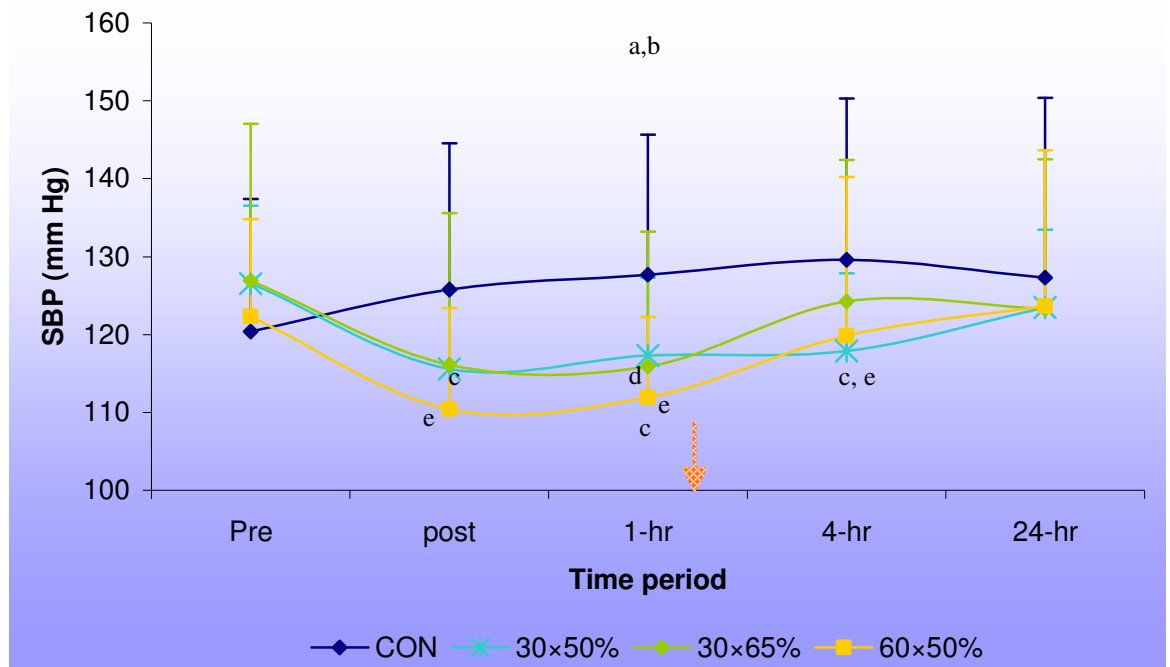


Figure 4.6 Mean systolic blood pressure values during each trial. [Significant differences: a Main effect of intensity \times time on SBP ($F = 3.620$; $P = 0.006$). b Main effect of intensity compared to CON [30×50% & 60×50%] ($P \leq 0.005$). c 30×50% significantly lower than CON ($P \leq 0.007$). d Effect of time following 30×65% only at 1 hr ($P = 0.032$). e 60×50% significantly lower than CON ($P \leq 0.006$). Statistical power: 0.991]

4.3.3.2 Diastolic blood pressure

There was a significant effect of time on DBP during the trials, however there was no direct effect of intensity between walking groups compared to CON (Figure 4.7). This effect occurred immediately post-walk and is likely due to the additive effect of the three walking intensities compared to CON ($P=0.038$). However, there was a trend for DBP to be lowered for at least 1 hour post-walk following 30×50% & 60×50%, and immediately post-walk following 30×65% (each NS).

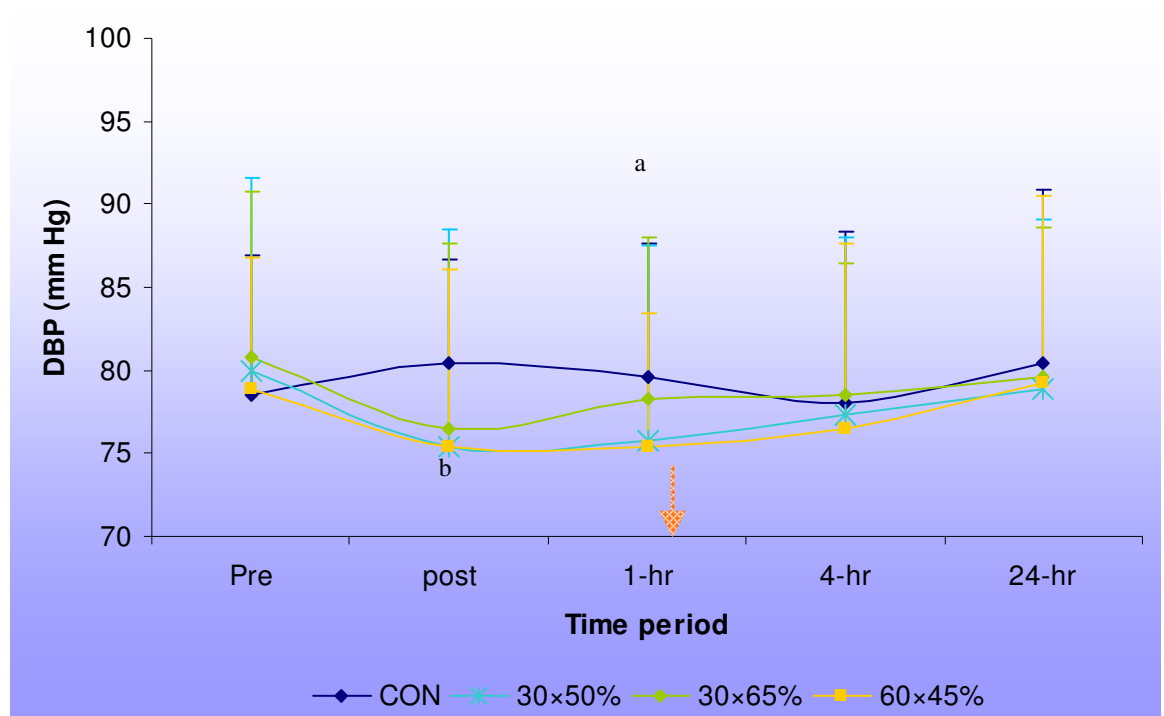


Figure 4.7 Mean diastolic blood pressure values during each trial. [Significant differences: a Main effect of time on DBP ($F=4.399$; $P=0.010$). b Main effect of time: Post-walk significantly lower than Pre-walk ($P=0.038$)]. Statistical power: 0.443.

There was an interaction between walking intensity over time over the course of the 24 hour period, with 60×50% significantly lowering MAP for at least 4 hours post-walk ($P=0.007$; Figure 4.10), whereas this effect was not apparent following the more intense 30×65%. MAP decreased significantly by at least -5.9 ± 4.4 mm Hg for at least 1 hour following 30×50% ($P \leq 0.018$) but for less than 4 hours (-4.7 ± 5.0 mm Hg; $P=0.052$) and by at least -5.7 ± 6.1 mm Hg, but for less than 1 hour following 60×50% ($P=0.022$). SBP did not significantly change over the 24 hour period following CON. MAP returned to Pre values by 4-hr in all trials.

There was an interaction of intensity and time on PP ($P=0.015$) and a main effect of intensity on PP ($P < 0.05$). PP appeared to rise slightly until at least 4-hr (9.6 ± 13.0 mm Hg; NS), whereas each of the walking trials appeared to slightly reduce PP ($\leq 8.5 \pm 9.4$ mm Hg). PP was reduced at 1-hr following 30×65% & 60×50%, which lasted until 4-hr in 60×50% only, by which time MAP was also decreased following 30×50%. PP at 4-hr during CON was significantly higher than post (6.2 ± 6.2 mm Hg; $P=0.036$), whereas PP significantly lower at 4-hr than pre in 30×50% (-6.0 ± 5.9 mm Hg; $P=0.043$). PP returned to Pre values at 24-hr for each treatment.

4.3.3.6 Rate-pressure product

RHR was highly variable throughout the treatments, with the post-walk RHR creating the greatest source of variation ($\geq 7.2 \pm 4.3$ beats·min⁻¹; $P \leq 0.046$) and the elevations in post-walk RHR were apparent for >1-hr, particularly in 30×65% & 60×50% ($\geq 4.9 \pm 3.5$ beats·min⁻¹; $P \leq 0.005$; Figure 4.12). RHR was also elevated above Pre at 4-hr in CON, therefore there may have been a post-prandial or circadian effect because RHR was not different between treatments at 4-hr. RHR returned to Pre values at 24-hr. Rate-pressure product remained relatively stable during the course of the study between trials, most

particularly so in 30×50% where there was only a marginal increase in RPP post-walk (260.3 ± 815.0) (Figure 4.8). Neither 30×50% nor 60×50% significantly influenced RPP throughout the 24-hr period, however 30×50% prevented the spike in RPP experienced in the other three trials, where 30×65% was significantly greater than 30×50% at 4-hr (7980 ± 1851 v 7156 ± 1916 ; $P=0.033$). The largest fluctuations in RPP arose post-walk in 30×65% (1354.9 ± 806.7 ; $P=0.001$) and RPP also increased at 4-hr in CON compared to post & 1-hr ($\geq 695.8 \pm 572.9$; $P \leq 0.005$). All values returned to Pre values at 24-hr.

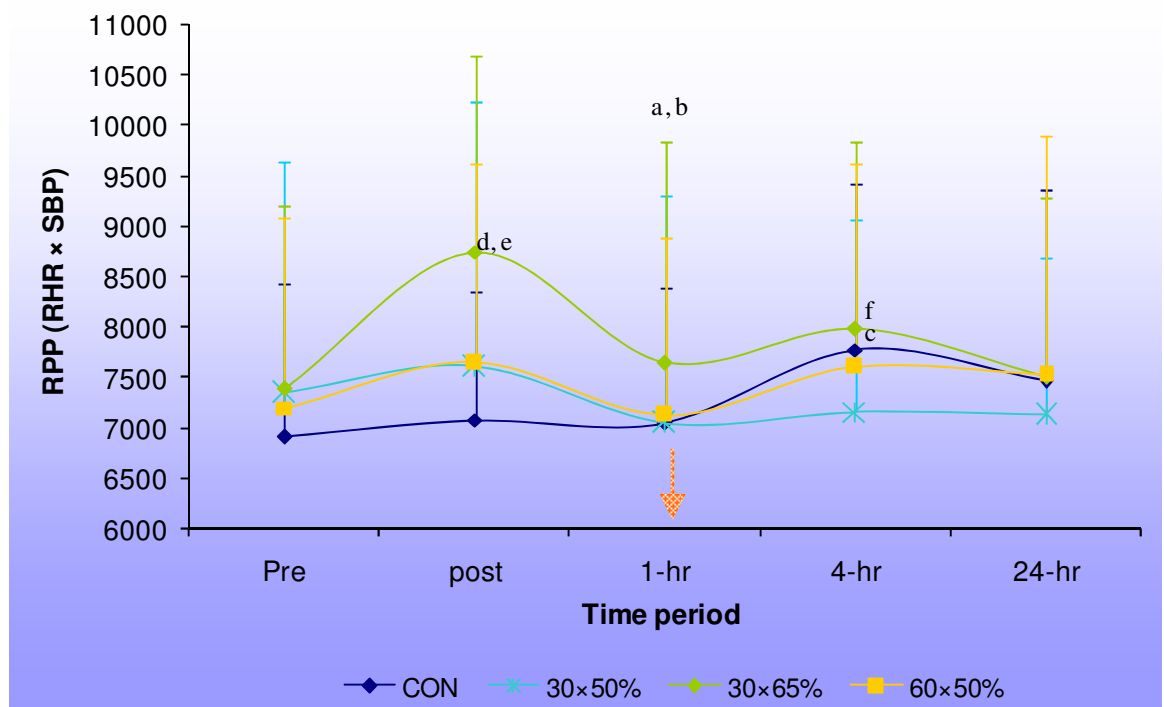


Figure 4.8 Mean rate-pressure product values during each trial. [Significant differences: a Main effect of intensity × time on RPP ($F=3.135$; $P=0.011$). b Main effect of intensity on RPP in 30×65% compared to 30×50% ($P=0.045$). c RPP in CON significantly higher than Post & 1-hr ($P \leq 0.005$). d RPP in 30×65% significantly higher than pre, 1-hr & 24-hr ($P \leq 0.010$). e RPP in 30×65% significantly higher than CON, 30×50% & 60×50% ($P \leq 0.020$). f RPP in 30×65% significantly higher than 30×50% ($P=0.033$)]. Statistical power: 0.680.

4.3.4 Metabolic responses

4.3.4.1 Resting metabolic rate

There was a main effect of intensity and time on RMR over the 24-hr period with the greatest fluctuations post-walk ($\geq 160.6 \pm 181.6$ kcal·day⁻¹; $P \leq 0.025$) and 4-hr ($\geq 132.9 \pm 369.3$ kcal·day⁻¹; $P \leq 0.005$) (Figure 4.9). RMR was only significantly different at 4-hr in CON, whereas RMR was significantly greater in post than pre & 1-hr in 30×65% & 60×50%. Furthermore, RMR was greater in 30×50% than CON and was also significantly greater in 30×50% than CON and was also significantly greater in 30×65% than 30×50%. RMR returned to Pre values at 24-hr.

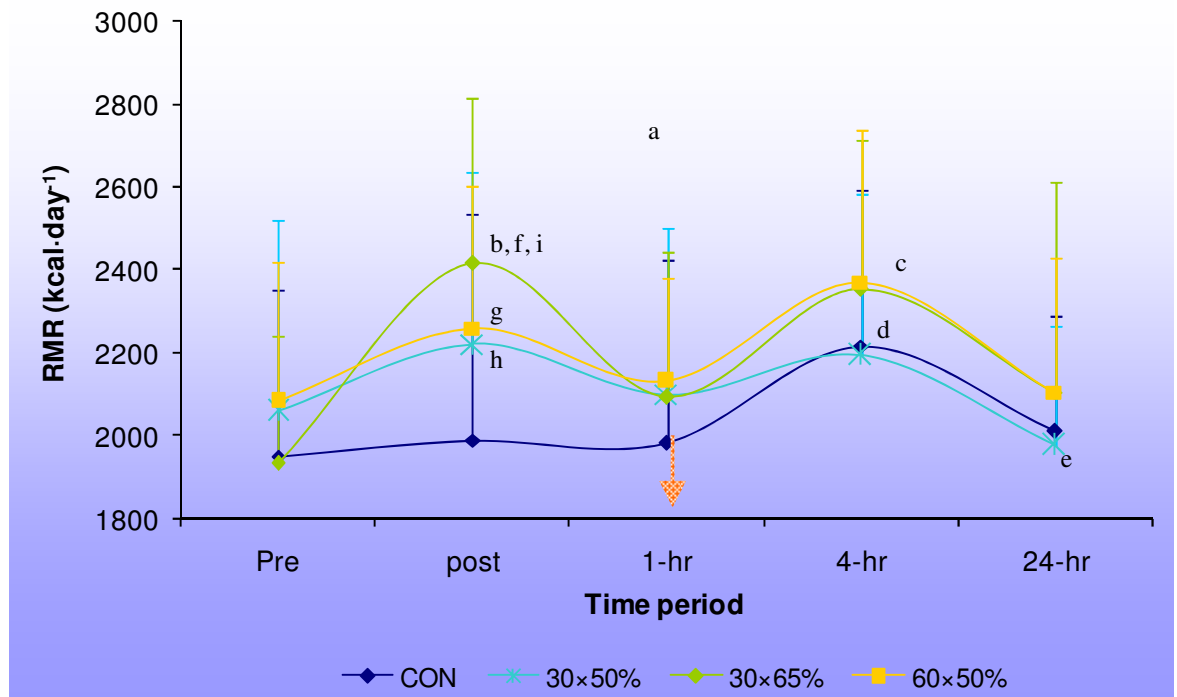


Figure 4.9 Mean resting metabolic rate values during each trial. [Significant differences: a Main effect intensity × time on RMR ($F=2.542$; $P=0.043$). b Main effect of time on RMR ($P \leq 0.025$). c Main effect of time on RMR ($P \leq 0.005$). d RMR in CON significantly higher than pre, 1-hr & 24-hr ($P \leq 0.05$). e RMR in 30×50% significantly lower than post & 4-hr ($P \leq 0.041$). f RMR in 30×65% significantly higher than pre & 1-hr ($P \leq 0.005$). g RMR in 60×50% significantly higher than pre ($P=0.020$). h RMR in 30×50% & 30×65% significantly higher than CON ($P \leq 0.031$). i RMR in 30×65% significantly higher than 30×50% ($P=0.044$)]. Statistical power: 0.484.

4.3.4.2 Resting rate of fat oxidation

The pattern of resting fat oxidation throughout the 24-hr period was relatively uniform throughout, except for the spikes in oxidation rates following 30×50% & 60×50% at 1-hr (NS). There was a main effect of time on resting fat oxidation rate, with the main effect taking place at 4-hr. Rates of fat oxidation following the 30×50% & 30×65% were significantly decreased at 4-hr compared to post-walk & 1-hr, whereas rates were only significantly lower than post-walk in 60×50%. There were no significant differences between treatments at any stage and resting fat oxidation rates returned to Pre values at 24-hr.

4.3.4.3 Percentage fat utilisation

There was no interaction of intensity and time on percentage fat oxidation, however intensity ($P=0.034$) and time ($P=0.001$) each had discrete effects, where there was a main effect of intensity between 30×65% & 60×50% and CON ($P\leq 0.049$; Figure 4.10). The greatest effect of walking appeared to occur at 1-hr following 30×65% ($P\leq 0.027$), however the walking sessions could not counteract the decrease in percentage fat oxidation that occurred at 4-hr. Percentage fat oxidation rates were not significantly different between pre and 24-hr.

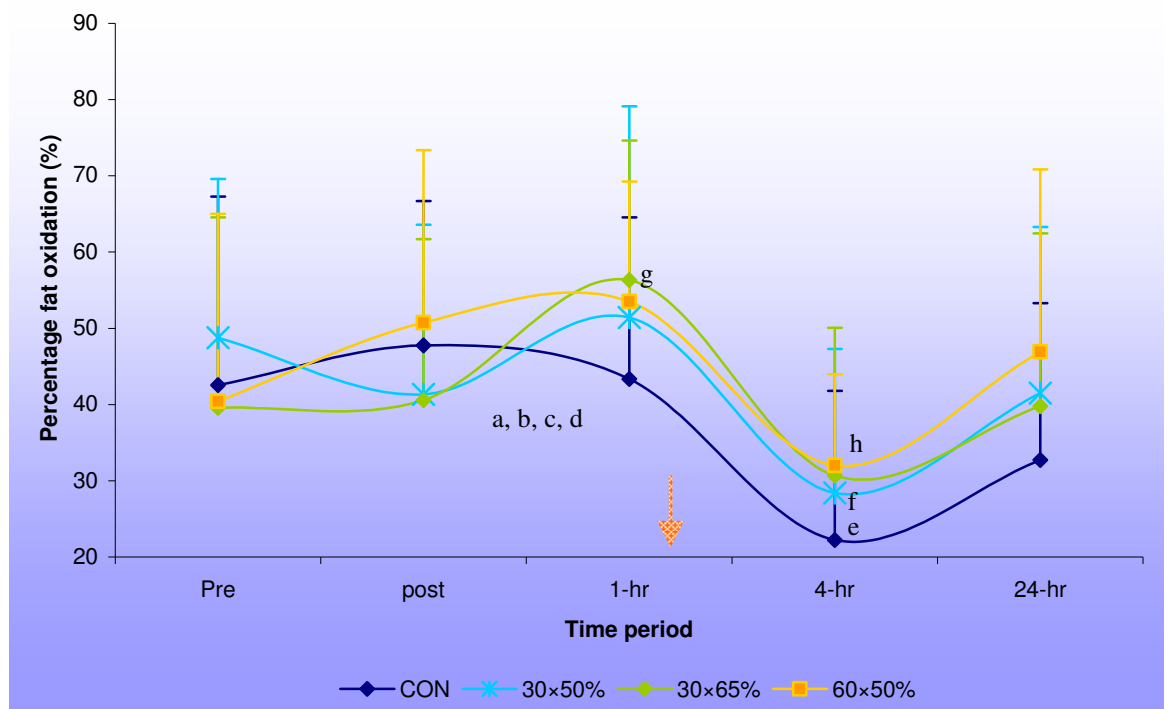


Figure 4. 10 Mean percentage fat oxidation values during each trial. [Significant differences: a Main effect of intensity on percentage fat oxidation ($F=4.102$; $P=0.034$). b Main effect of time on percentage fat oxidation ($F= 24.189$, $P<0.001$). c Main effect of intensity between 30×65% & CON ($P=0.049$). d Main effect of intensity between 60×50% & CON ($P=0.046$). e Percentage fat oxidation in CON significantly lower at 4-hr than post & 1-hr ($P\leq 0.035$). f Percentage fat oxidation significantly lower in 30×50% at 4-hr than pre, post & 1-hr ($P\leq 0.011$). g Percentage fat oxidation in 30×65% significantly higher than post & 4-hr ($P\leq 0.027$). h Percentage fat oxidation in 60×50% significantly lower than post, 1-hr & 24-hr ($P\leq 0.017$)]. Statistical power: 0.260

4.3.5 Blood responses

Hydration status remained relatively stable between walking sessions and over the duration of each test, with the exception of a slight and non-significant rise in the post-walk assessment in CON.

4.3.5.1 Plasma glucose

There was no significant effect of treatment or time on plasma glucose during the study, with glucose remaining stable throughout (Figure 4.11). The only interesting pattern to note was the non-significant spikes in glucose at 4-hr in the 30×65% & 60×50% treatments, which required the greatest energy expenditures during the walks.

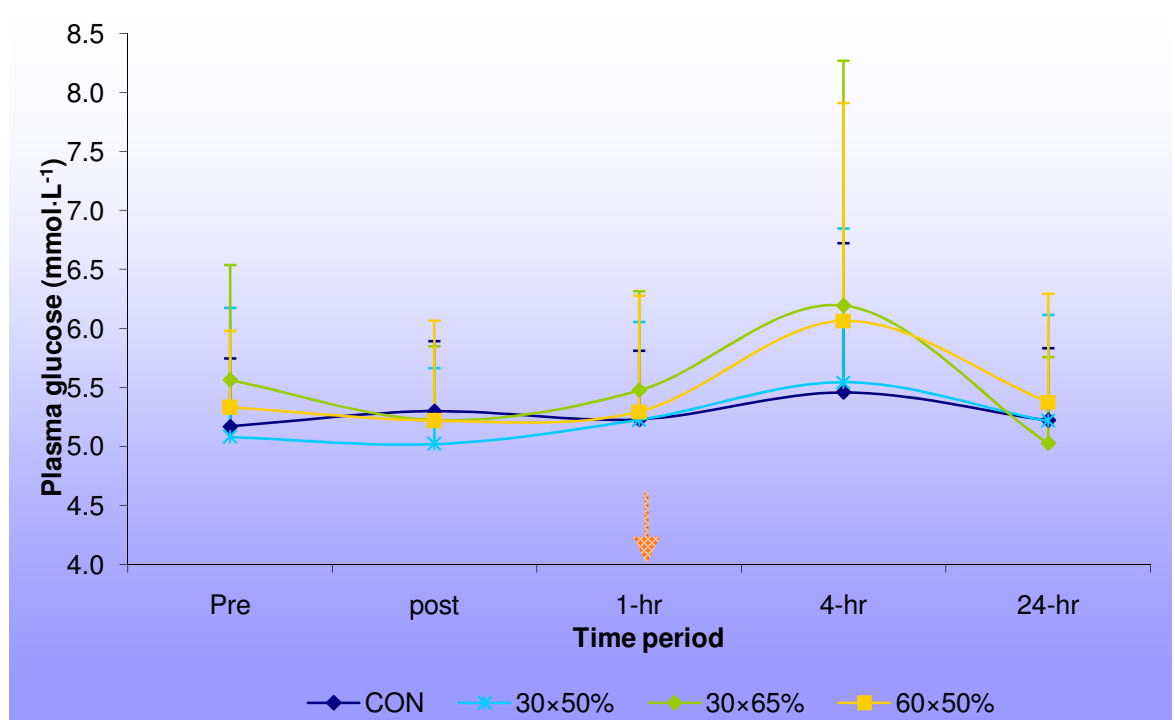


Figure 4.11 Mean plasma glucose values during each trial. [No significant differences between walking sessions over time ($F=1.255$; $P=0.295$)]. Statistical power: 0.111.

4.3.5.2 Serum insulin dynamics

There was a main effect of time on serum insulin, where it remained elevated at 4-hr following the post-1-hr meal ($P \leq 0.004$) and there was no effect of treatment at any point. Elevations in serum insulin at 4-hr were $44.2 \pm 47.5 \mu\text{IU}\cdot\text{mL}^{-1}$ in CON, $45.4 \pm 78.7 \mu\text{IU}\cdot\text{mL}^{-1}$ in 30×50%, $48.2 \pm 59.9 \mu\text{IU}\cdot\text{mL}^{-1}$ in 30×65% and $52.2 \pm 63.7 \mu\text{IU}\cdot\text{mL}^{-1}$ in 60×50%. There was a main effect of time on insulin resistance, which fluctuated to a similar extent as serum insulin ($P \leq 0.001$). The pattern in CON was for insulin resistance to steadily increase until 1-hr, whereas there was a non-significant trend for insulin sensitivity to decrease between immediately post-walk, following each walking intensity, before reaching parity with CON at 1-hr. Insulin resistance increased significantly at 4-hr following the post-1-hr meal ($P \leq 0.003$), however there was no effect of walking compared to CON. Increases in insulin resistance at 4-hr compared to 1-hr were -11.3 ± 12.7 in CON, 14.7 ± 30.2 in 30×50%, 17.0 ± 24.7 in 30×65% and 17.7 ± 25.5 in 60×50%.

4.3.5.3 Insulin secretion

There was a main effect of time on β -cell insulin secretion rates, where insulin secretion mirrored serum insulin ($P \leq 0.001$; Figure 4.12). The pattern in CON was for insulin secretion to steadily increase until 1-hr, whereas there was a non-significant trend for insulin secretion to decrease between immediately post-walk, following each walking intensity, before reaching parity with CON at 1-hr. Insulin secretion increased significantly at 4-hr following the post-1-hr meal ($P \leq 0.003$), however there was no effect of walking compared to CON. Increases in insulin secretion at 4-hr were 157.7 ± 159.5 in CON, 127.9 ± 181.8 in $30 \times 50\%$, 128.7 ± 130.3 in $30 \times 65\%$ and 144.4 ± 141.8 in $60 \times 50\%$.

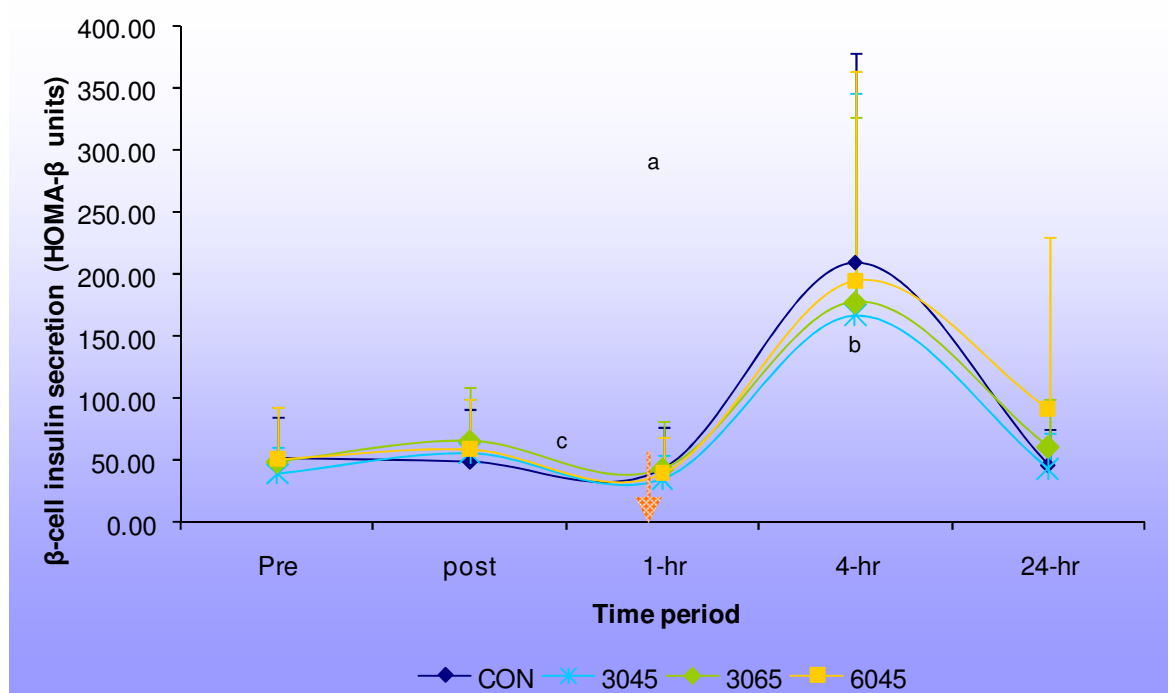


Figure 4. 12 Mean β -cell insulin secretion rates (HOMA- β) during each trial. [Significant differences: a Main effect of time on insulin secretion ($F=36.529$; $P < 0.001$). b Effect of meal (4-hr) on insulin resistance compared to pre, post, 1-hr & 24-hr in CON, $30 \times 50\%$, $30 \times 65\%$ & $60 \times 50\%$ ($P \leq 0.001$). c Effect of time on insulin secretion between post-walk & 1-hr ($P=0.022$)]. Statistical power: 0.270.

4.3.5.6 Plasma non-esterified fatty acids

There was an effect of intensity over time on plasma NEFA ($P=0.011$) and also an effect of walking compared to CON post-walk ($P\leq 0.05$; Figure 4.13). There was a tendency for NEFA to increase post-walk compared to Pre and this effect was proportional to the energy expended during the walk with 60×50% exhibiting the most significant increase of $0.5 \pm 0.4 \text{ mmol}\cdot\text{L}^{-1}$ ($P=0.001$) and a non-significant increase in 30×50% of $0.2 \pm 0.2 \text{ mmol}\cdot\text{L}^{-1}$ ($P=1.000$). Plasma NEFA were back to Pre levels at 1-hr and significantly decreased in all treatments at 4-hr following the post-1-hr meal ($P\leq 0.003$). There were no significant differences between walking sessions.

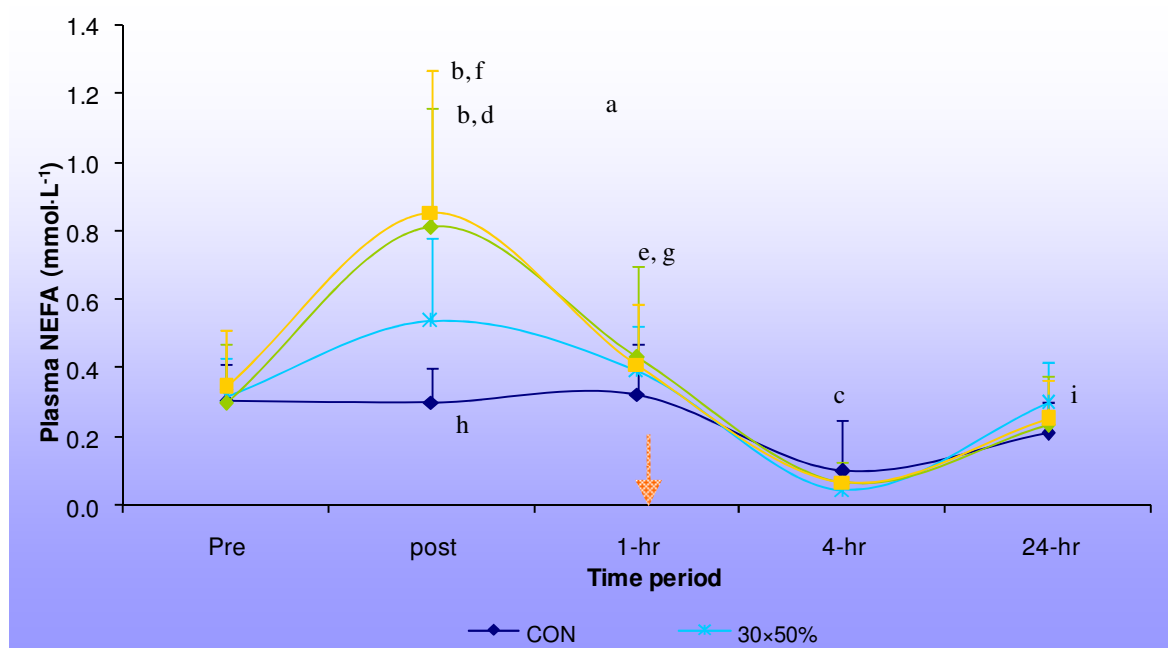


Figure 4.13 Mean plasma NEFA values during each trial. [Significant differences: a Main effect of intensity × time on plasma NEFA ($F=3.856$; $P=0.011$). b Main effect of walking sessions compared to CON ($P\leq 0.05$). c Plasma NEFA significantly lower than pre, post, 1-hr & 24-hr in all treatments ($P\leq 0.003$). d. Effect of 30×65% on plasma NEFA compared to pre & 1-hr ($P\leq 0.006$). e Effect of 30×65% on plasma NEFA compared to post & 4-hr ($P<0.001$). f Effect of 60×50% on plasma NEFA compared to pre, 1-hr, 4-hr & 24-hr ($P=0.001$). g Effect of 60×50% on plasma NEFA compared to post, 4-hr & 24-hr ($P\leq 0.005$). h plasma NEFA significantly greater following 30×65% & 60×50% than CON ($P<0.001$). i plasma NEFA significantly greater following 30×50% than CON ($P<0.041$)]. Statistical power: 0.837.

4.3.5.7 Serum triacylglycerol

There was no effect of treatment or time on serum TAG, where the only pattern was that it remained relatively similar throughout between treatments (Figure 4.14). The apparent interaction of treatment and time appears to have been due to the stable pattern in TAG following 30×50% compared to the fluctuations in TAG in CON, 30×65% & 60×50%. This effect is most apparent at 4-hr where TAG remained stable during 30×50%, whereas the other three treatments demonstrate a spike, with the mean CON value peaking the greatest at $1.4 \pm 0.6 \text{ mmol}\cdot\text{L}^{-1}$ ($P<0.050$). The mean difference between CON and 30×50% at 4-hr was $-0.3 \pm 0.5 \text{ mmol}\cdot\text{L}^{-1}$ ($P=0.017$).

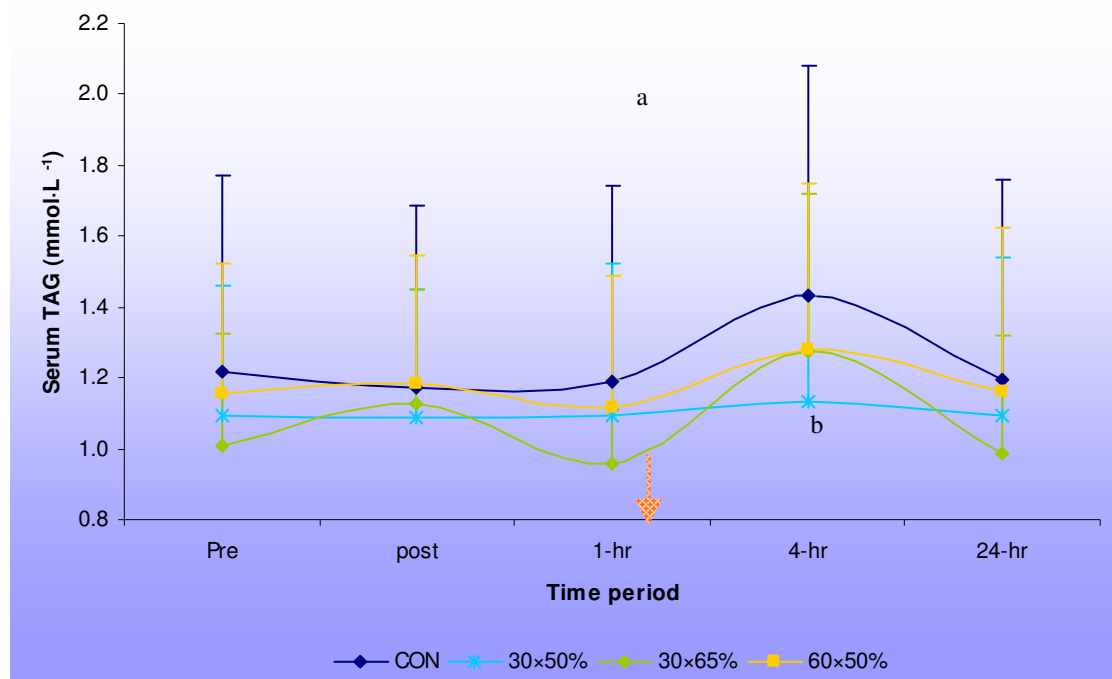


Figure 4. 14 Mean serum TAG values during each trial. [Significant differences: a Main effect of intensity × time on serum TAG ($X^2=40.651$; $P=0.003$). b Effect of 30×50% compared to CON ($P=0.017$)]. Statistical power: 0.256.

4.3.5.8 Serum lipids

There was no effect of treatment or time on serum TC, where the only pattern was that it remained relatively similar throughout between treatments. There was no effect of treatment or time on serum HDL-C, where it was similar to serum TC and remained relatively similar throughout between treatments. There was no effect of treatment or time on the TC/HDL-C ratio, where this measurement mirrored that of serum TC and HDL-C, where it remained relatively similar throughout between treatments. There was no effect of walking or time on serum LDL-C during the study, which was unaffected by either walking, or at 4-hr following the morning meal. There was no significant change despite there appearing to be a depression in LDL-C at 1-hr in 30×65% compared with the other treatments (P=1.000).

4.4 Discussion

The purpose of this study was to determine a) whether walking at 50% $\dot{V}O_{2\max}$ for 30 minutes (30×50%) may produce favourable changes in risk factors associated with MetS during the 24 hour post-exercise period compared to a control trial, and b) whether increasing the intensity of the walk to 65% $\dot{V}O_{2\max}$ for 30 minutes (30×65%) or maintaining the intensity at 50% $\dot{V}O_{2\max}$ and increasing the duration to 60 minutes (60×50%) would alter the magnitude or duration of these responses. The mean intensity of each session was $49.1 \pm 3.6\%$ $\dot{V}O_{2\max}$ during 30×50%, $65.2 \pm 3.6\%$ $\dot{V}O_{2\max}$ during 30×65% and $49.0 \pm 3.6\%$ $\dot{V}O_{2\max}$ during 60×50% and EE during CON was just 47.63 ± 1.97 kcal·session⁻¹ compared to 248.80 ± 11.34 kcal·session⁻¹ during 30×50% and 335.51 ± 14.67 kcal·session⁻¹ during 30×65%, with the greatest expenditure of 501.57 ± 18.02 kcal·session⁻¹ in 60×50% (Figure 4.2). Despite the intensity of the treadmill walks remaining constant throughout, where 30×65% was significantly greater than both 30×50% and 60×50%, mean HR rose significantly in a linear fashion between 5 minutes and completion of the walks (Figure 4.3). Exercising HR increased by 5.7 ± 3.3 beats·min⁻¹ from 5 to 30 minutes in 30×50%, by 16.7 ± 5.9 beats·min⁻¹ in 30×65% and by 10.0 ± 3.9 beats·min⁻¹ in between 5 to 60 minutes 60×50%, where the final HR in 60×50% was significantly greater than the final HR in 30×50%. The rise in HR during 30×65% was significantly greater than 30×50% ($P<0.001$) and 60×50% ($P=0.004$), while 60×50% tended to induce a greater increment in HR than 30×50% ($P=0.059$). The rise in participants self-rated RPE also increased in a similar fashion to HR, with RPE significantly greater during 30×65% than both 30×50% and 60×50% and RPE at 60 minutes in 60×50% was significantly greater than 30 minutes in 30×50% ($P<0.001$). These data demonstrate that irrespective of walking intensity, both HR and RPE rise significantly for the duration of each of the walks, thus highlighting that using HR or RPE to monitor

walking and/or exercise intensity might be dependent on the intensity and duration of the session, ie. is the prescription of walking intensity based on HR/RPE at the beginning or towards the end of the session.

Even though the energy cost of habitual PA rarely accounts for more than 20% of the total, it is the only way in which energy expenditure can be increased voluntarily, yet to expend the energy stored in 1 kg of adipose tissue would mean walking or running 93 miles or 146 miles for individuals with a body mass of 80 kg or 50 kg. This makes PA appear to be an unattractive strategy for weight loss, however there is an alternative way of looking at this relationship, where walking one extra mile every day for a year may expend approximately 2.5-4 kg of adipose tissue (Hardman, 1996). The energy stored in 1 kg of adipose tissue equates to ~7700 kcals, therefore for this group of men to lose 1 kg of fat the mean number of sessions required would be 30.9 sessions of 30×50%, 23.0 sessions of 30×65% and 15.4 sessions of 60×50%. If these were performed according to current PA recommendations (ACSM & AHA, 2007; 30 min·d⁻¹ on 5 d·wk⁻¹) the mean number of weeks required to lose 1 kg would be 6.18 weeks in 30×50%, 4.6 weeks in 30×65% and 3.08 weeks in 60×50%. In gross terms, not taking dietary practices, additional activities, improvements in $\dot{V}O_{2max}$ and decreases in body mass into consideration, this would produce annual gross body mass losses of 8.4 kg in 30×50%, 11.3 kg in 30×65% and 16.9 kg in 60×50%. Furthermore, for every kilogram of weight-loss there is an approximate 2-5% reduction in visceral fat (Kay & Fiatarone Singh, 2006), therefore visceral adipose tissue losses of 16.8-42%, 22.6-56.5% and 33.8-85.5% may mathematically be expected by performing 30×50%, 30×65% and 60×50%, respectively according to the recommended pattern for a year. Indeed, data from the STRRIDE study demonstrate that six months of PA, consistent with CDC & ACSM guidelines (1995), was sufficient to prevent significant increases in visceral fat, and

modestly increasing PA above these minimal recommendations ($\geq 150 \text{ min}\cdot\text{wk}^{-1}$ moderate PA) significantly decreases visceral and abdominal fat without decreasing energy consumption, which is of benefit to those at risk of MetS (Slentz *et al.*, 2005).

Data from the two-day food record diaries indicated that there were no significant differences between dietary intake between CON, 30×50%, 30×65% and 60×50% for total energy, carbohydrate, protein or fat consumption during the days prior to days one or day two of each trial, therefore dietary intake should not have been a confounding factor during the study (Figure 4.4 & 4.5). Statistical power ranged from 0.071 for TC/HDL-C ratio to 0.991 for SBP. Favourable changes in SBP, DBP, MAP, PP, RHR, RPP, RMR, fat oxidation, percentage fat utilisation, plasma NEFA, and serum TAG were detected, however there appeared to be no effect of walking on glucose homeostasis or blood lipoprotein profile.

More than 85% of individuals with MetS have elevated blood pressure or hypertension (Franklin, 2006) and data indicates that individuals with MetS and elevated blood pressure demonstrate greater carotid atherosclerosis compared with those who present aspects of MetS but not elevated blood pressure (Irace *et al.*, 2005). There was a main effect of walking on blood pressure, with this effect most evident on SBP, where each of the walking trials exerted an effect (Figure 4.6). However, the more vigorous 30×65% only exerted a lowering effect on SBP for less than 4 hours, whereas both lower intensity walks (30×50% & 60×50%) exerted a SBP-lowering effect for at least 4 hours compared to CON, with 60×50% tending to exert a greater magnitude of SBP reduction than 30×50% (NS). However, the most interesting finding was that only 30×50%, the lowest walking volume (intensity × duration), decreased SBP compared to Pre-walk at 4-hr. A limitation in the

measurement periods within this study was the large gap between 4-hr and 24-hr, due to the unavailability of ambulatory blood pressure monitors, therefore the exact duration of the SBP-lowering effect of the lower intensity walks could not be established. Despite the favourable responses of SBP to the walking trials compared to CON, the effect of walking on DBP appeared to be less substantial. The statistical analyses demonstrated a main effect of time on DBP, where there was a tendency for DBP to be decreased Post-walk compared to Pre-walk following each of the walking trials, whereas DBP tended to rise slightly during this period during CON (NS) (Figure 4.7). However, the walking-induced reductions in DBP were not as dramatic as those observed for SBP and MAP, where DBP tended to remain lower at 1-hr following the two lower intensity walks (30×50% & 60×50%) compared to CON and Pre-walk. Due to the modest effects of the walks on DBP and the more substantial SBP response there was a main effect of the walks on MAP, which remained relatively constant throughout CON, but was significantly decreased Post-walk compared to Pre and CON in each of the walking trials and this reduction was maintained at 1-hr in the two lower intensity walks (30×50% & 60×50%). However, although there was a tendency for MAP to be reduced at 4-hr in 30×50% and 60×50% compared to Pre and CON, these were non-significant. Walking also exerted an effect on PP, which was significantly reduced at 1-hr and 4-hr in 60×50%, but only at 1-hr following 30×65% and at 4-hr following 30×50% compared to CON. These are clinically significant findings because a 1 mm Hg reduction in blood pressure may reduce MI risk by 2-3% (Libby & Theroux, 2005).

These results extend the work of a previous study, which found that walking at 50% $\dot{V}O_{2peak}$ for 40 minutes in a single session has been reported to decrease SBP by -5.6 ± 1.6 mm Hg and DBP by -3.1 ± 0.2 mm Hg for 7 hours in pre-hypertensive adults and this

effect was augmented when walking was accumulated in 4×10 min bouts, which caused SBP to decrease by 5.4 ± 1.7 mm Hg for 11 hours and DBP by 3.4 ± 1.3 mm Hg for 10 hours (Park *et al.*, 2006). When the current ACSM recommendations were published (ACSM, 2004) the influence of the intensity and duration of PA sufficient to cause post-exercise hypotension was still unknown. However, recent studies have demonstrated that although PAEE is more important than either intensity or duration (Jones *et al.*, 2007), it is now known that a minimum of 15 minutes at $40\% \dot{V}O_{2\max}$ is sufficient to promote post-exercise hypotension (Guidry *et al.*, 2006). During the 90 minute post-exercise period following cycling for 45 minutes at 30%, 50% and 75% $\dot{V}O_{2\text{peak}}$, only the 50% and 75% $\dot{V}O_{2\text{peak}}$ sessions significantly decreased blood pressure in normotensives compared to the control session, and were associated with a decrease in systemic vascular resistance and increased cardiac output (Forjaz *et al.*, 2004). However, a similarly designed study, that recorded for a longer post-exercise period (9 hours) and used hypertensive participants, demonstrated that reductions in systolic blood pressure were similar between cycling for 15 minutes at 40% or 60% $\dot{V}O_{2\max}$ or for 30 minutes at 40% or 60% $\dot{V}O_{2\max}$ (Guidry *et al.*, 2006). Thus, reinforcing the benefits of performing short bouts of PA at a low intensity, however the exact PA dose needed to lower blood pressure remains to be identified. Furthermore, it may be possible that a threshold intensity of only $40\% \dot{V}O_{2\max}$ may be required to elicit a hypotensive effect and only three training sessions for this effect to become chronic (Thompson *et al.*, 2001).

Further data appears to support the significance of PAEE over PA intensity for the mediation of post-exercise hypotension, where isoenergetic PA sessions at either 40% or 70% $\dot{V}O_{2\text{peak}}$ produced 'clinically similar' reductions in blood pressure during the 20min post-exercise period (Jones *et al.*, 2007). The immediacy by which post-exercise

hypotension occurs suggests the hypotensive influence of regular PA may be partially an acute occurrence with the BP reductions accumulating as the training program continues (ACSM, 2004). Indeed, data from intervention studies suggest that longer training programmes may produce somewhat larger reductions in blood pressure, suggesting that PA may impact on blood pressure control immediately and in the long term through separate mechanisms (Hardman, 1996). The rationale for the exercise-induced decreases in blood pressure is that there is a marked dilation of blood vessels in active skeletal muscle that decreases resistance to flow during exercise, therefore the acute effects of exercise evident during recovery from individual bouts of exercise may be important, where the blood pressure of sedentary hypertensives may be reduced for up to 12 hours following a single exercise session (Pescatello *et al.*, 1991; Brown *et al.*, 1994). Post-exercise hypotension may persist for up to 16 hours, thus PA may enable those with stage I hypertension to be normotensive for the majority of the day (Thompson *et al.*, 2001). The mechanisms by which PA may acutely modulate post-exercise hypotension include sympathoinhibition, where sympathetic nerve fibre activity-induced vasoconstriction of the musculature becomes inhibited, and increases in circulating vasodilator substances, such as nitric oxide, which attenuate the vasoconstrictor response to α -adrenergic receptor stimulation, and these responses are not fully offset by increases in cardiac output during post-exercise hypotension (Halliwell, 2001). It may be speculated that the increases in SNSa associated with more vigorous exercise may have overcome the increases in vagal tone that may have been responsible for some of the effects of the lower intensity walks on post-exercise hypotension. Furthermore, the increases in HR associated with 30x65% compared to 30x50% and 60x50% may have been sufficient to overcome the walking-induced vasodilation that may have occurred during the post-walk period.

Despite the favourable effects of the walking trials on blood pressure, significant increases in resting (recovery) HR were present following the walking sessions compared to CON. These effects were particularly evident following the higher intensity 30×65% and longer duration 60×50%, which elevated HR ≥ 1 -hr post-walk compared to CON and for ≥ 4 -hr post-walk compared to Pre, whereas HR was only elevated above CON and Pre immediately Post-walk following 30×50%, which was significantly lower than HR following 30×65% immediately Post-walk. However, the magnitude and duration of the decreases in SBP associated with walking were sufficient to offset some of the walking-induced elevations in HR to produce similar levels of RPP compared to CON (Figure 4.8). An exception to this pattern was the higher intensity 30×65%, which increased RPP significantly above the lower intensity 30×50%, particularly immediately Post-walk and at 4-hr following the breakfast meal. Furthermore, although RPP was not significantly different between the lower intensity 60×50% and 30×50% there was a pattern for RPP to remain reduced following 30×50% compared to the more energetic walks at 4-hr and 24-hr. These findings have great significance because increased myocardial workload, estimated by RPP, often precedes cardiac events (Mittleman *et al.*, 1993; Willich *et al.*, 1993; Raum *et al.*, 2007) therefore low-moderate intensity walking may be recommended to reduce the risk of acute coronary events in apparently healthy populations both during walking and in the post-walking period. Perhaps more crucially these findings may have significant implications for the promotion of regular walking from healthcare professionals to cardiac patients, such as angina and post-myocardial infarction patients, and this area must now be further researched.

Resting metabolic rate was not significantly different between treatments at Pre, 1-hr, 4-hr and 24-hr, however RMR in 30×65% and 60×50% were significantly elevated compared to

CON, with RMR in 30×65% also significantly elevated above 30×50% Post-walk (Figure 4.9). There were main effects of time on RMR, such as Post-walk in each of the walking trials and at 4-hr in each treatment following the breakfast meal, which was consumed immediately following the measures at 1-hr, demonstrating the thermic effect of food. However, there was no effect of walking on changes in postprandial RMR, which was significantly higher at 24-hr following 30×65% than following 30×50%. In contrast to RMR there was relatively little change in fat oxidation rates, where only the consumption of the breakfast meal significantly effected fat oxidation, which was significantly lower in each treatment at 4-hr, with none of the walking treatments exerting a significant effect on absolute fat oxidation. However, when absolute fat oxidation rates were expressed relative to RMR there was a significant effect of 30×65% and 30×50% compared to CON on percentage fat utilisation (Figure 4.10). It may be speculated that these differences may have occurred at 1-hr, 4-hr and 24-hr, where there was a tendency for percentage fat utilisation to be depressed during CON relative to each of the walking trials (NS).

Acute exercise also has the potential to reduce plasma volume, which may impact upon blood biochemistry values (Kargotich *et al.*, 1998), therefore despite no significant changes in hydration status throughout the duration of the study the plasma and serum samples were corrected for any changes in plasma volume that may have occurred. The walking trials did not significantly affect plasma glucose compared to CON (Figure 4.11). Despite concentrations remaining relatively constant following CON and 30×50%, plasma glucose tended to be augmented at 4-hr following the breakfast meal, particularly in 30×65% and 60×50%, which required the greatest energy expenditures during the walks. However, these were only tendencies rather than significant differences. Serum insulin concentrations remained relatively stable throughout the study, with the exception of 4-hr

in each treatment where serum insulin spiked significantly compared to Pre, Post, 1-hr and 24-hr due to control the glycaemic load consumed following the 1-hr measurements. The homeostasis assessment models (HOMA-IR & HOMA- β) were used to predict insulin resistance from fasting glucose and insulin concentrations (Matthews *et al.*, 1985). All measures within this study were from fasting blood samples, with the exception of those taken at 4-hr, which were collected following a ~2.5 hr fast. Due to this, those HOMA measures recorded at 4-hr are not valid and appear to demonstrate increased fasting insulin, whereas this is due to the normal hyperinsulinaemia that occurs in response to meals containing a moderate volume of carbohydrate. Therefore, non-surprisingly there was an apparent increase in 'insulin resistance' as determined by HOMA and this phenomenon tended to be increased following 30 \times 65% and 60 \times 50%, but less so following 30 \times 50% and even less during CON (NS). There was a trend for insulin secretion to decrease slightly from Pre to Post during CON (NS), and to increase from Pre to Post during each of the walking sessions (NS) (Figure 4.12). Consequently, insulin secretion decreased significantly, predominantly following the walking sessions between Post and 1-hr. The rise in glycaemia between and 1-hr and 4-hr stimulated increases in β -cell insulin secretion following each treatment, however there was a non-significant trend for the walking sessions to effect a decreased insulin secretion relative to CON.

When endurance-trained individuals stop training, their enhanced insulin sensitivity is rapidly reversed (King *et al.*, 1995), suggesting that this characteristic could partly be a consequence of the acute effects of their last bout of exercise. It has been demonstrated that acute exercise can normalise a defect in insulin-stimulated glucose transport-phosphorylation in insulin-resistant subjects (Perseghin *et al.*, 1996). A single bout of acute exercise enhances insulin-mediated glucose disposal in normal subjects, in insulin-

resistant first degree relatives of T2D patients, in obese subjects with insulin resistance as well as in T2D patients, where exercising muscle may increase glucose clearance 7 to 20-fold (Wahren *et al.*, 1971). Following an acute bout of exercise, glucose uptake into skeletal muscle is enhanced, which is partly an insulin-independent contractile effect and may persist for several hours after the cessation of exercise (Henriksen, 2002). The enhanced post-exercise insulin sensitivity is most likely due to the need to replenish muscle glycogen, where glycogen-depleting exercise results in increased non-oxidative glucose disposal as measured 12 hours after exercise. However, when an untrained leg was subjected to a single exercise bout there was no effect on insulin action in the untrained muscle, suggesting that ‘the effect of training on insulin-mediated glucose disposal in muscle is a genuine adaptation to repeated exercise ... but is short-lived’ (Dela *et al.*, 1992).

Possible explanations for the lack of effect of the walks on insulin sensitivity include insufficient exercise stimulus and the predominant use of fasting blood samples rather than post-exercise oral glucose tolerance tests (OGTTs). Data indicates that a threshold exercise intensity of 70% $\dot{V}O_{2max}$ may be needed in order for short-term post-exercise improvements in insulin sensitivity, possibly due to the greater depleting effect of vigorous intensity PA on muscular glycogen stores (Thompson *et al.*, 2001). Indeed, data demonstrates that exercise increases insulin-stimulated glucose disposal through increased glycogen synthesis without affecting rates of glucose oxidation in both type II diabetic and non-diabetic participants and may last for at least 24 hours (Christ-Roberts & Mandarino, 2004). Possible mechanisms by which PA may improve insulin sensitivity include increased mRNA expression, increased glycogen synthase activity, decreased release of adipose tissue-derived NEFA and enhanced clearance of NEFA and increased delivery of

glucose and insulin to muscles through increases in blood flow (Pedersen & Saltin, 2006). Furthermore, the observation of the response to a glucose load may provide greater insights into the effects of prior exercise on glucose homeostasis rather than measuring static measures of glucose metabolism in the fasting state, as was the case in the present study.

Unfavourable metabolic changes in sedentary individuals may be partly mediated by inactivity-induced down-regulations in LPLa (Hamilton *et al.*, 2004), which is bound to the capillaries of muscles and responsible for the hydrolysis of TAG-rich lipoproteins and thus decreases in LPLa may promote an atherogenic blood lipid and lipoprotein profile, primarily through elevations in TAG-rich lipoproteins. However, studies demonstrate that low muscular LPLa can be reversed through a single session of walking at a moderate intensity, thus suggesting that the maintenance of the residual effects of the previous bout of lifestyle PA, such as walking, may enhance metabolic health (Bey & Hamilton, 2003). Plasma NEFA and glycerol concentrations have been shown to increase by ~31% and ~49%, respectively, following 1-hr of walking at 36% $\dot{V}O_{2peak}$ compared to resting values, indicating increased rates of adipose tissue lipolysis and muscular oxidation (Kaminsky *et al.*, 1986). Furthermore, plasma glycerol peaked at the end of exercise whereas plasma NEFA carried on increasing and peaked at 10 min post-exercise and plasma NEFA remained above resting values until at least 60 min post-exercise. In the present study, plasma NEFA concentrations demonstrated much greater fluctuations following the walking sessions compared to glucose, where each of the walking sessions exerted an effect on plasma NEFA compared to CON, despite only small statistical power (0.256). Plasma NEFA increased significantly following 30×65% and 60×50% compared to Pre, 1-hr and CON, however despite plasma NEFA being slightly raised compared to CON following 30×50% this was non-significant (Figure 4.13). Plasma NEFA returned to Pre

values by 1-hr in each treatment and were significantly decreased at 4-hr compared to all other time points (Pre, Post, 1-hr and 24-hr) following the breakfast meal that was consumed immediately after the measurements were collected at 1-hr and there was no effect of walking on this depression in plasma NEFA. Despite plasma NEFA being unaffected by 30×50% during day one it was significantly higher than CON at 24-hr.

The greatest fluctuations in serum TAG occurred at 4-hr, after the breakfast meal, following CON, 30×65% and 60×50% but not following 30×50% (Figure 4.14). Mean serum TAG remained relatively uniform throughout the study, particularly following 30×50%, where even following the breakfast meal serum TAG remained unchanged at 4-hr compared to CON, which increased significantly compared to 30×50%, and 30×65% and 60×50% also tended to increase at 4-hr (both NS). However, the Bonferroni corrected analyses and low statistical power (0.072) may explain why the postprandial elevations in serum TAG following 30×65% and 60×50% did not attain statistical significance. The blood lipoprotein profile (TC, HDL-C, TC/HDL-C ratio & LDL-C) appeared to be unaffected by the walking trials compared to CON, where not only were there no significant differences between the treatments there were no clear changes throughout the 24 hour intervention in any trial (Figures 4.24-4.27). Despite minor fluctuations, particularly TC/HDL-C ratio, the blood lipoprotein profile remained steady throughout the four trials. There appeared to be a trend for 30×65% to decrease TC and LDL-C, and increase HDL-C, highlighted in the greater fluctuations in TC/HDL-C ratio. Data from other studies indicates a dose-response for PAEE in reducing postprandial lipaemia because walking for 90 minutes at a moderate intensity ($61 \pm 1\% \dot{V}O_{2max}$) was more effective than walking for the same duration at a low intensity ($31 \pm 1\% \dot{V}O_{2max}$) (Tsetsonis & Hardman, 1996b). It has been demonstrated that increases in LPLa are

responsible for some of the reductions in postprandial lipaemia resulting from moderate intensity activities, such as walking, however LPLa does not explain all of the effects (Gill *et al.*, 2003). However, an oral fat challenge was consumed in the above studies, whereas in the present study the majority of the blood samples were in the fasted state (except 4-hr). As with OGTTs, oral fat loads pose a greater challenge to the body in the rested state and allow a greater demonstration of the effects of prior exercise on metabolism than in the fasted state.

Other walking studies have investigated the effect of walking intensity on the blood lipid and lipoprotein profile. During the 48-hr post-exercise period, 11 pre-menopausal and 10 post-menopausal women performed 2 isocaloric walking trials expending 350 kcal at 50% $\dot{V}O_{2max}$ and 70% $\dot{V}O_{2max}$ (Pronk *et al.*, 1995). The author reported that serum LDL-C decreased immediately following walking at 70% $\dot{V}O_{2max}$ in both the menopausal and post-menopausal women, indicating that there was a different response between the two walking intensities, despite both intensities appearing to slightly raise serum LDL-C, and from looking at the data there appeared to be little favourable improvement in TC, HDL-C (including HDL₂-C & HDL₃-C), LDL-C or TAG. More recently, a 10-day walking intervention was performed by a group of young (24 ± 3 years) and a group of older (56 ± 3 years) participants (Ainslie *et al.*, 2005). The main findings of the study were that TC and LDL-C decreased and HDL-C increased significantly greater in the older group compared to the young group.

Physical activity may improve the dyslipidaemic profile associated with MetS by increasing the ability of muscle tissue to take up and oxidize NEFA and increasing the activity of LPL in muscle (Pollare *et al.*, 1991). The effects of acute PA on blood lipids are

proportional to the PAEE, rather than meeting a specific EE threshold. However, despite those with the greatest TAG values having the potential for the greatest PA-induced reductions in blood TAG, unconditioned individuals may be unable to expend sufficient energy to do so (Thompson *et al.*, 2001). In healthy trained men, a PA session expending 1,100 kcal may be sufficient to elevate HDL-C, however a lower EE threshold than this may be sufficient for sedentary individuals (Ferguson *et al.*, 1998). Indeed, evidence suggests that a PAEE of 350-400 kcal may be sufficient to acutely increase blood HDL-C values in moderately fit individuals. Furthermore, changes in total cholesterol and LDL-C are more equivocal and potential favourable changes may be due to plasma volume expansion rather than changes in lipid metabolism (Thompson *et al.*, 2001), which were corrected for this factor in the present study.

Despite moderate or vigorous PA apparently being initially advocated by ACSM (1978), in the next recommendations (ACSM, 1990) there was an important distinction made between PA for health and exercise training for fitness, where the exercise prescriptions to enhance fitness were not absolutely necessary to improve health. Subsequently, the more heralded recommendations by CDC & ACSM (1995) and USGR (1996) were published, which along with the findings in the present study, are highly important to health-fitness professionals and the sedentary public because the perceived intensity and mode of exercise necessary to enhance health is often a barrier to exercise adoption for sedentary individuals who mistakenly believe that exercise must be vigorous to be of benefit (Buckworth & Dishman, 2002). However, sedentary individuals are more likely to choose and adhere to lower intensity leisure activities, such as walking programs than more vigorous exercise (Dishman & Buckworth, 1996).

In conclusion, the main findings of this study were that a single 30 minute walking session at 50% $\dot{V}O_{2max}$ was sufficient to significantly reduce systolic blood pressure, mean arterial pressure and the rate-pressure product for at least four hours post-exercise, however there did not appear to be any significant effects on glucose homeostasis or the blood lipid and lipoprotein profile. Furthermore, an interesting and perhaps important finding is that increasing the walking intensity to 65% $\dot{V}O_{2max}$ or the duration to 60 minutes failed to significantly augment the physiological responses to the 30 minute walk at 50% $\dot{V}O_{2max}$. These findings are significant because demonstrating that low volumes of PA are capable of enhancing certain aspects of health may encourage sedentary individuals to increase habitual leisure time activities and discourage the assumption that exercise must be vigorous to be of benefit to health.

5.0 The acute and chronic effects of walking on risk factors associated with metabolic syndrome

Metabolic syndrome has been associated with future CVD during 11-year follow-up, with elevations in BP and low HDL-C independently associated with CVD development in the Atherosclerosis Risk in Communities (ARIC) study, however MetS was less predictive than Framingham Risk Score (FRS) (McNeill *et al.*, 2005). Evidence suggests that although the presence of ATPIII-defined MetS is a significant predictor of CVD and type II diabetes, it is a stronger indicator of type II diabetes, but not as predictive of CHD as FRS (Wannamethee *et al.*, 2005). However, when three or more components are present, MetS is a strong predictor of CVD and type II diabetes in Britain (Wannamethee, 2008). Despite the majority of this risk being already predicted by FRS it is possible that identification of those with MetS may provide earlier opportunities to intervene with individuals predisposed to diabetes and/or CVD, where measures of abdominal obesity, inflammatory markers, IFG, and prothrombotic activity are included in the ATPIII MetS definition, but not FRS (Wilson, 2004). Evidence suggests that CRP independently predicts CVD, which is more common in those with MetS who have elevated CRP, and therefore stratification based on CRP may add further prognostic value in CVD prediction in those with MetS (Malik *et al.*, 2005).

The WHO-defined prevalence of MetS is >44.8% and 33.9% in English males and females aged 40-65, respectively, demonstrating the highest worldwide prevalence using these criteria, whereas the mean worldwide prevalence of ATPIII-defined MetS, which was used to diagnose MetS in this thesis, is 27.9% for females and 22.8% for males (Cameron *et al.*, 2004). There are different lifestyle and genetic factors that encourage the development of MetS, however when a combination of several low-risk lifestyle factors (physically active,

non-smoking and moderate carbohydrate & fat consumption) are present in individuals with a BMI $<30 \text{ kg}\cdot\text{m}^2$ those individuals are at a lower risk of developing MetS (Zhu *et al.*, 2004). In the UK alone, the direct costs of obesity are at least 500 million per year, with costs to the individual and industry ~£2 billion per year (Bourn, 2001) and CHD costs the UK health care system £1.73 billion (Liu *et al.*, 2002), which can both be addressed through lifestyle modification, particularly PA. Data from the Health Survey for England, covering 95,342 individuals in 1991-4, 1997-9 and 2003-4, demonstrated that despite occupational PA being on the decline there was a significantly consistent upward trend in regular sporting participation and an overall increase in time spent performing PA and the percentage of adults meeting public health recommendations for PA (Stamatakis *et al.*, 2007). Therefore, within this context the initial aim of the work included in this thesis was to recruit individuals with MetS to demonstrate the influence of accumulative walking on ameliorating the components of MetS, particularly abdominal obesity, elevated fasting blood glucose, dyslipidaemia and hypertension, and the associated elevated risk for a pro-thrombotic and pro-inflammatory state.

There are at least four definitions used to diagnose MetS, however the definition proposed by ATP III (Cleeman, 2001) was used to diagnose MetS within this thesis because the criteria most closely match the health outcomes recorded during the pre- and post-walking assessments. This definition requires 3 or more of: WC $>102 \text{ cm}$, plasma TAG $\geq 1.69 \text{ mmol}\cdot\text{L}^{-1}$, HDL-C $<1.04 \text{ mmol}\cdot\text{L}^{-1}$, blood pressure $\geq 130/\geq 85 \text{ mmHg}$ and fasting blood glucose $\geq 6.1 \text{ mmol}\cdot\text{L}^{-1}$. Despite many attempts trying to recruit individuals with MetS, due to potential participants being required to volunteer for the studies, these attempts were relatively unsuccessful. Shown below is the list of methods that were employed to attract volunteers to take part in the research:

- Adverts in GP surgeries throughout Kent
- Adverts in Schools and Colleges throughout Kent
- Notice letters to Kent businesses
- Notice letters to Primary Care Trusts
- Notice letters to and leaflets Taxi companies
- Notice letters to and leaflets Bowling clubs
- Advert in Kent County Cricket Club match-day programme
- Circular emails to local councils
- Link on University of Kent homepage
- Own webpage on Canterbury Christ Church University homepage
- Email to Sport Science, Tourism & Leisure students
- Notice on staff and student PC desktop alerts at Canterbury Christ Church University
- Adverts in supermarkets
- Adverts in libraries
- Handing out flyers at Canterbury train stations and Ashford International
- Editorial in local newspapers
- Interview on local radio stations in Kent – twice
- Interview on South East television news

Additionally, many of the volunteers that participated in the 24 week study were using anti-hypertensive and/or lipid-lowering medication. Due to the nature of the required participants (see Mets criteria above) and that these individuals were low active, many of the volunteers for the 24-week intervention study were likely to be prescribed hypertensive or lipid-lowering medications (Rantala *et al.*, 1999; Williams & Franklin, 2007). However, despite medication use being a confounding factor to the impact of walking interventions on MetS risk factors, these participants were allowed onto the study in order to reduce the presence or risk of their developing MetS. Eventually, medicated participants' results were not included in the final analyses of the study in order to present full results for each component associated with MetS, rather than using different participants' data for different parameters based on whether they were medicated or not. Therefore, lack of volunteers and

ruling out participants using certain medications led to relatively low numbers of participants in each of the groups: CON (15), SBW (13) and ABW (13). A small selection of participants that took part in the 24 week intervention study also took part in the 24 hour study in order to avoid having to re-recruit for participants to take part in a study that potentially offered less personal reward than the 24 week study. All of the participants that were not prescribed medication were contacted to volunteer for the study and a small selection did volunteer. There was also an intention to investigate the influence of the 24-week and 24-hour walking interventions on CRP, a marker of inflammation and strong predictor of future CVD for those with MetS (Malik *et al.*, 2005). However, there were calibration problems with the CRP assay resulting in insufficient numbers of the samples being assayed to be included in the results of either study.

The 24 week intervention study within this thesis demonstrates that walking can influence risk factors associated with MetS. In contrast to the common misconception that exercise must be vigorous and sport-related to be of benefit to health (Dishman & Buckworth, 1996), both accumulative brisk walking during the day and a single walking session at no more than a moderate intensity ($50\% \dot{V}O_{2max}$) are capable of reducing risk factor associated with MetS. Furthermore, the evidence in this thesis suggests that brisk walking in multiple smaller sessions during the day or a short walk (30 minutes) at a moderate intensity are at least as effective for improving risk factors associated with MetS than walking sessions of greater duration or intensity. Indeed, despite a trend for the walking intervention requiring $150 \text{ min}\cdot\text{wk}^{-1}$ to be performed in 5×30 minute sessions per week (SBW) to decrease waist circumference and its associated insulin resistance, only when this volume of walking was accumulated in smaller sessions during the day (ABW) were these effects significant. Conversely, in a study investigating similar health outcomes to those presented in section

3.0 demonstrated that by instructing sedentary obese Japanese participants to add 1,000 steps to daily walking activity at baseline for 1 year the increase in steps·d⁻¹ significantly reduced visceral adiposity and decreases in insulin resistance assessed using HOMA (Miyatake *et al.*, 2002). Furthermore, a prospective study investigating the efficacy of walking on future risk of developing in 70,102 female nurses aged 40-65 years found that walking produced similar magnitudes in T2D risk reduction as vigorous activity of equivalent energy expenditure and that increased walking pace was independently associated with decreased risk during 8-year follow up (Hu *et al.*, 1999). Together these findings have significant health implications since all-cause mortality has been reported to be 39% lower in individuals who walk at least 2 h·wk⁻¹ and those who walked 3-4 h·wk⁻¹ had the lowest risk at 8-year follow up (Gregg *et al.*, 2003).

Similarly to the study in section 3.0, the response of various health outcomes to accumulative walking has been variable, but generally positive. In one of the earliest RCTs, investigating 10 weeks of accumulative brisk walking at 70-80% HR_{max} in either one 30 minute walk (LB) or three 10 minute walks per day (SB), body mass and waist circumference decreased significantly only in the SB group. A further 18 week RCT of accumulative walking studied 49 males and females, involving either single daily brisk walking sessions of 20-40 minutes (LB) or accumulating the same volume of walking in 10-15 minute daily sessions found that aerobic capacity significantly decreased and factor XIIa, a marker of coagulation, significantly increased in the control group, however there were no significant changes in lipid profiles (TC, LDL-C, HDL-C and apos A-I, A-II & B) in the walking groups compared with controls (Woolf-May *et al.*, 1998). A further study by the same group added an additional group, in which participants were instructed to perform brisk walking sessions in 5-10 minute bouts totalling 20-40 min·d⁻¹, which found

that although each of the walking groups produced improvements in aerobic fitness the long and intermediate groups also demonstrated favourable improvements in the blood lipid profile (Woolf-May *et al.*, 1999).

Despite not attaining statistical significance, there were favourable improvements in blood pressure following both patterns of brisk walking (SBW & ABW) compared to control, which is a similar finding to the majority of walking interventions (Murphy *et al.*, 2007). The outcome for plasma fibrinogen, a marker of blood clotting risk, was similar, where despite favourable decreases in plasma fibrinogen compared to control in both walking patterns these were statistically non-significant. Conversely, one of the few previous studies to investigate the influence of walking on coagulation demonstrated that walking at a higher intensity at $73.5 \pm 7.2\%$ HR_{max} for 18 weeks significantly decreased factor XIIa (FXIIa), the active form of FXII that is involved in coagulation however there were no decreases in blood lipid profile (Woolf-May *et al.*, 2000). Similarly, in the study in section 3.0 apart from TC/HDL-C ratio and serum LDL-C, there appeared to be no pattern for both SBW and ABW to improve the blood lipid and lipoprotein profile, which is a common finding in the majority of other published walking interventions (Kelley *et al.*, 2004). Despite a 24 week walking intervention requiring $60 \text{ min}\cdot\text{d}^{-1}$ at 60% $\dot{V}O_{2peak}$ on $5 \text{ d}\cdot\text{wk}^{-1}$ significantly improving $\dot{V}O_{2peak}$ 14% and percent body fat by -1.3%, this was still insufficient to favourably alter serum lipids. (Ready *et al.*, 1996). Furthermore, an RCT, 130 postmenopausal women completed single or double daily walking sessions, expending a total daily PA volume of $300 \text{ kcal}\cdot\text{d}^{-1}$ at 65% $\dot{V}O_{2max}$ with both single and multiple sessions of walking providing similar increases in $\dot{V}O_{2max}$ ($2.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for both walking groups) and decreases in BF% (1.7% in SB & 21.1% in LB) (Asikainen *et al.*, 2002). An extension of this study, looking at a wider array of risk factors found that

diastolic blood pressure was not significantly reduced when the walking groups were analysed separately and only when these were combined as a single group that a mean decrease of -3 mmHg was demonstrated compared to controls and despite 15 weeks of walking, neither the lipid profiles nor insulin concentrations changed (Asikainen *et al.*, 2003).

The independent association between fitness and components of MetS may be relatively small and benefits associated with PA may be partly as a consequence of the acute effects of the last bout of exercise rather than from favourable adaptations to chronic PA (Thompson *et al.*, 2001). However, no published study appears to have investigated the potential for PA to impact on MetS risk factors (blood pressure, dyslipidaemia, glucose control & haemostatic factors) in the immediate post-exercise period, that may be maintained through regular PA, despite evidence demonstrating short-term improvements in independent MetS risk factors, such as blood glucose control (Englert *et al.*, 2006), blood pressure (Jones *et al.*, 2007), lipid and lipoprotein profile (Ferguson *et al.*, 1998), markers of pro-thrombotic (Ivey *et al.*, 2003) and pro-inflammatory states (Murtagh *et al.*, 2005a) may be gained from a single PA session. Therefore, since there appears to be no published studies to have investigated MetS risk factors collectively in acute PA interventions in general, the findings are important. In contrast to the influence of 24 weeks of brisk walking on risk factors associated with MetS, a single session of brisk walking affects different risk factors. Systolic blood pressure in particular significantly responds to a 30 minute walking session at 50% $\dot{V}O_{2max}$ for at least four hours, which was at least as effective as a 60 minute walking session at a similar intensity and more effective than a 30 minute walking session of higher intensity (65% $\dot{V}O_{2max}$). Combined with the reduced increments in post-walk heart rate associated with walking at 50% $\dot{V}O_{2max}$, the lower

intensity walk significantly reduced rate-pressure product, an index of myocardial workload, for at least four hours compared to CON and generally resulted in lower perturbations in rate-pressure product than either 30×50% or 30×65%, with RPP following 30×65% significantly higher than the other walking trials as well as CON.

Patterns in response to the walking sessions could only be assessed within the design of the 24-hour study, ie. measured BP, therefore mechanisms can only be postulated. The mechanisms by which PA may acutely modulate post-exercise hypotension include sympathoinhibition, where sympathetic nerve fibre activity-induced vasoconstriction of the musculature becomes inhibited, and increases in circulating vasodilator substances, such as nitric oxide, which attenuate the vasoconstrictor response to α -adrenergic receptor stimulation, and these responses are not fully offset by increases in cardiac output during post-exercise hypotension (Halliwill, 2001). In a study of the influence of PA intensity on blood pressure, during the 90 minute post-exercise period following cycling for 45 minutes at 30%, 50% and 75% $\dot{V}O_{2peak}$, only the 50% and 75% $\dot{V}O_{2peak}$ sessions significantly decreased blood pressure in normotensives compared to the control session, and were associated with a decrease in systemic vascular resistance (Forjaz *et al.*, 2004). However, a similarly designed study, but recording for a longer post-exercise period (9 hours) and using hypertensive participants, demonstrated that reductions in systolic blood pressure were similar between cycling for 15 minutes at 40% or 60% $\dot{V}O_{2max}$ or for 30 minutes at 40% or 60% $\dot{V}O_{2max}$ (Guidry *et al.*, 2006). Thus, reinforcing the benefits of performing short bouts of PA at a low intensity, and even though the exact PA dose needed to lower blood pressure remains to be identified it may be possible that a threshold intensity of only 40% $\dot{V}O_{2max}$ may be required to elicit a hypotensive effect and only three training sessions for this effect to become chronic (Thompson *et al.*, 2001). Data appears to demonstrate that

PAEE is more significant than PA intensity for the mediation of post-exercise hypotension, where isoenergetic PA sessions at either 40% or 70% $\dot{V}O_{2peak}$ produced 'clinically similar' reductions in blood pressure during the 20 minute post-exercise period (Jones *et al.*, 2007).

None of the short-term walking treatments produced significant favourable improvements in insulin sensitivity or blood lipoprotein profile compared to CON. However, both of the walking interventions that required the greatest PAEE produced significant increases in plasma NEFA immediately post-walk, but only 30x50% induced significant decreases in serum TAG at 4-hours after the mid morning breakfast. To investigate the effect of walking intensity on the blood lipoprotein profile during the 48-hr post-exercise period, 11 pre-menopausal and 10 post-menopausal women performed 2 isocaloric walking trials expending 350 kcal at 50% $\dot{V}O_{2max}$ and 70% $\dot{V}O_{2max}$ (Pronk *et al.*, 1995). The author reported that serum LDL-C decreased immediately following walking at 70% $\dot{V}O_{2max}$ in both the menopausal and post-menopausal women, indicating that there was a different response between the two walking intensities, despite both intensities appearing to slightly raise serum LDL-C and from looking at the data there appears to be little favourable improvement in TC, HDL-C, LDL-C or TAG.

Certain health outcomes responded positively to the walking interventions, such as adaptations in body composition and insulin resistance following the 24 week study, and short term blood pressure and cardiac responses to the acute walking sessions. However, other health outcomes, such as blood pressure and blood lipid and lipoprotein profile in the 24-week walking intervention and blood lipid and lipoprotein profile in the 24-hour walking study appeared relatively unchanged. These are in line with current data, where even meta-analyses have been unable to demonstrate favourable changes following

walking interventions and SBP (Murphy et al., 2007), serum TAG, TC and HDL-C (Kelley et al., 2004) have not been found to respond significantly. Indeed, the findings in this thesis contribute to current knowledge because to the author's understanding this is the first published walking intervention to demonstrate improvements in glucose homeostasis associated with changes in abdominal adiposity, which is especially novel because the accumulation of walking was particularly effective. Furthermore, although favourable adaptations in blood pressure were not present following 24 weeks of walking, short-term favourable responses occurred in SBP and MAP following walking at 50% $\dot{V}O_{2max}$ for at least 4 hours, which is significant for PA promotion because this appeared to be more effective than walking at 65% $\dot{V}O_{2max}$ and this phenomenon has also not been demonstrated before. Additionally, rate-pressure product, an index of myocardial workload, was influenced by both chronic and acute bouts of walking. Most notably, despite a trend for SBW to decrease RPP, this was only significantly decreased following ABW, and RPP was also most beneficially effected following the shorter duration and lower intensity 30x50% walking session. These findings suggest that low-moderate activities of daily living, such as walking should be recommended to decrease the risk of cardiac events during activity and future development of heart conditions. Consequently, if this type of activity can decrease RPP in an apparently healthy population then it may be speculated that this type of activity should also be recommended to participants with current heart conditions, such as angina and post-myocardial infarction. Therefore, this area warrants further thorough research.

The findings from both of the main studies in this thesis (sections 3.0 and 4.0) are significant for promoting health through activities of daily living. The studies clearly suggest that a lifestyle activity, such as walking, at only moderate intensities can promote

significant improvements in health outcomes both in the short and long term. This thesis therefore has important implications for guiding the current public health policies for promoting lifestyle activities. The Chief Medical Officer's report (2004) is a 'high level Department of Health document' aimed towards public health and Primary Care Trusts to encourage the promotion of physical activity and *active travel* to achieve health gains. This thesis is crucial to help these aims because walking exemplifies the most accessible mode of active travel and also illustrates the potential health outcomes, particularly the immediate optimisation of blood pressure and long term decreases in abdominal adiposity and decreased insulin resistance.

The findings in this thesis add particular weight to the fight to promote PA to those in need (the sedentary) in the face of others (the highly active) who believe that promoting vigorous PA will improve the health status of the wider population. A recent article has been written describing the need to promote vigorous intensity PA because of the increasing perception that moderate intensity PA is more beneficial to health than vigorous PA (O'Donovan & Shave, 2006). However, although the PA-health dose-response relationship clearly demonstrates that with increasing PA volume there are greater health benefits, this is logarithmic rather than linear (Paffenbarger *et al.*, 1986). Furthermore, findings from the same group demonstrate that only LDL-cholesterol and apo-B concentrations were lower in lean exercisers than in lean sedentary men, indicating that energy balance is more of a key to health than PA intensity per se (O'Donovan *et al.*, 2005). The key to this debate is PA promotion, at any intensity, rather than promoting the most effective PA intensity because frequency and adherence to PA are much more important for achieving the required PA volume (American College of Sports Medicine & American Heart Association, 2007). This is precisely why, despite health responses to

moderate intensity PA may be lesser compared to those attained by adhering to vigorous PA, moderate intensity PA is promoted: because it can be attained by the masses rather than being a preserve for those who already demonstrate high 'performance-related fitness'. The problem with these groups advocating vigorous intensity PA is that the perceived intensity and mode of exercise necessary to enhance health is often a barrier to exercise adoption for sedentary individuals who mistakenly believe that PA must be vigorous to be of benefit (Buckworth & Dishman, 2002). However, sedentary individuals are more likely to choose and adhere to lower intensity leisure activities, such as walking, than more vigorous PA (Dishman & Buckworth, 1996). Both the lower intensity walks and the accumulative walking groups in this thesis support the assertions that lifestyle activities can be beneficial to health.

This work supports the governments aims to increase adults PA to at least 30 minutes per day on at least 5 days per week, which was initially developed in 2004 (Department of Health, 2004). Indeed, this thesis contributes to each aspect of the '*be active, be healthy*' strategy for increasing PA, where walking forms part of each activity category: 'everyday activities', 'active recreation' and 'sport' (Department of Health, 2009). This thesis clearly demonstrates that walking should be at the forefront of PA promotion policies due to the weight of physiological evidence (see sections 3.0 & 4.0), and especially considering it should have no cost to the participant. This message appears to have been missed during the past 200 years since daily PA levels have been systematically reduced by advancements in technology, such as cars and occupational instruments, despite walking being modern *homo sapiens*' main source of PA during ambulation and daily chores during the previous 100,000 years (Haskell & Torburn, 2006). Due to the rise in sedentary occupations, the 'Work place health promotion strategy' advises employers to create

policies to encourage their staff to use other modes of transport involving physical activity and this thesis could contribute to information on how to be more physically active and on the health benefits of such activity (National Institute for Health and Clinical Excellence (NICE), 2008). Therefore, this work supports their recommendations to ‘move around more at work’, such as walking to external meetings, using the stairs rather than lifts, providing information about walking routes and encouraging walks during work breaks. The work in this thesis complements evidence from a prospective study of 6,017 normotensive Japanese men aged 35-60 by demonstrating that despite BP not being significantly lowered, RPP, WC and measures of insulin resistance were significantly decreased. That study found that walking to work and walking during leisure time for more than 20 min·d⁻¹ was associated with a 0.71 relative risk of developing hypertension in compared to those who walked less than 10 min·d⁻¹ (Hayashi *et al.*, 1999).

In 2006 a NICE report also stated that there were still gaps in current knowledge relating to the efficacy and cost effectiveness of PA interventions, therefore this thesis presents evidence that physical benefits can occur when accumulating walking according to national PA recommendations (5×30 min·wk⁻¹) and that no costs accompany such activities. Therefore this information will be valuable to professionals working in the NHS, local authorities and the voluntary sector, such as directors of public health, public health advisers, commissioners of services, general practitioners (GPs), other primary healthcare professionals, those working in Healthy Living Centres, leisure service managers, walking and cycling officers, exercise and leisure professionals and community development workers and those developing and delivering walking schemes or pedometer loan schemes (NICE, 2006).

This research is also timely considering that the 2012 Olympic Games in London legacy plans are currently being formulated (Weed *et al.*, 2009; Department of Health, 2009). Indeed, given that a recent systematic review found that “there was no usable evidence returned in relation to the direct leveraging of public health... or of ‘active living’ or incidental physical activity from Olympic Games” (Weed *et al.*, 2009); it may be suggested that publicising work from this thesis may promote the development of such evidence in the run up to and post-2012. Crucially, evidence suggests that only if individuals become physically active before the Games that London 2012 and be sustained rather than stimulate sedentary individuals to become more active once the Games have finished (Weed *et al.*, 2009). Therefore, widespread publicity of effective health-enhancing PA initiatives prior to the Games combined with the event may be an effective health promotion tool in order to develop pre-contemplators into preparers for PA and ready for ‘action’.

With regards to sport the studies in this thesis lend weight to using golf as a health promotion tool because due to the distances covered whilst walking with a golf bag during a round there is potential for significant health benefits. During a 20-week trial 55 male golfers playing 2-3 full rounds of golf per week improved their time to fatigue on an exercise test by 36 seconds, WC decreased by 2.2 cm and abdominal skin folds also decreased by 2.2 cm, and HDL-C were increased (Parkkari *et al.*, 2000). Furthermore, recent evidence demonstrates that walking is associated with reduced medication use for MetS components such as diabetes, hypertension and hypercholesterolaemia, where weekly walking distance, the longest distance walked and walking intensity were inversely related to anti-diabetic, anti-hypertensive and LDL-C lowering medication use in 32,683 females and 8,112 males in the National Walkers’ Health Study (Williams, 2008). Those

whose walking speed exceeded $2.1 \text{ m}\cdot\text{s}^{-1}$ had 64% lower odds for anti-diabetic medication use, 50% lower odds for anti-hypertensive medication use and 47% lower odds for LDL-C lowering medication use compared with those whose walking speed was $<1.2 \text{ m}\cdot\text{s}^{-1}$. This is of significance to those with MetS since many, as was a complication of the studies in this thesis, that many individuals suffering from MetS are also prescribed medication (Rantala *et al.*, 1999).

5.1 Conclusion

Metabolic syndrome is a strong predictor of future CVD, diabetes and increased all-cause mortality (Wannamethee *et al.*, 2005; Lakka *et al.*, 2002), however lifestyle modification appears to prevent and/or treat symptoms associated with MetS (Irwin *et al.*, 2002; Rennie *et al.*, 2003; Anderssen *et al.*, 2007). Indeed, the research included in this thesis demonstrates that walking may attenuate some of the risk factors associated with MetS. These effects are even possible following a single walking session and are independent of the favourable adaptations that are possible following long term adherence to a walking programme.

Data from the study in Section 4.0 has demonstrated that not only does a single 30 minute session of walking at 50% $\dot{V}O_{2max}$ optimise systolic blood pressure, mean arterial pressure and myocardial workload more than walking for 30 minutes at 65% $\dot{V}O_{2max}$, for at least four hours. Furthermore, increasing the duration of the walking session to 60 minutes at 50% $\dot{V}O_{2max}$ had no additional effect on blood pressure and myocardial workload. However, there were no significant effects of walking on blood glucose homeostasis and blood lipoprotein profile from any of the walking trials.

Conversely, in the study presented in Section 3, accumulating 30 minutes of brisk walking per day in 10-15 minute sessions on five days of the week for 24 weeks was equally as effective as performing the same volume of walking performed in single daily sessions in significantly decreasing waist circumference, which was also associated with significant reductions in insulin resistance and circulating insulin. However, there were no adaptations in blood pressure or blood lipid and lipoprotein profile associated with 24 weeks of brisk walking.

5.2 Limitations

Limitations are apparent in both studies. In the 24 week training study, the majority of the participants accepted onto the study were relatively healthy, which precluded their being diagnosed with MetS. This meant that the population were only at risk of MetS rather than actually being diagnosed with MetS, which lessened their potential for positive gains from the walking intervention (Wilmore, 2001). Furthermore, other participants using anti-hypertensive and lipid-lowering medications that volunteered for the study were excluded to ensure that these individuals did not confound the findings. The use of medication in the target population is non-surprising since sedentary individuals at risk of MetS are known to use such medications (Williams & Franklin, 2007) and are included in the diagnostic criteria in MetS (Alberti & Zimmet, 1998; Cleeman, 2001). Added to the relatively low number of participants that volunteered for the study, the ramifications of these events possibly resulted in the low statistical power that limited the significance of the walking intervention on blood pressure, plasma fibrinogen, TC/HDL-C ratio and serum LDL-C.

The main limitation of the 24-hour study, investigating the short-term effects of walking on MetS risk factors, was the large 'window' between the measurements at 4-hours post-walk and 24-hours post-walk. This was particularly apparent for the measurements of systolic blood pressure, which appeared to carry on increasing from Pre to 4-hr in CON and remained lowered in 30×50% and 60×50%. This limitation in the study was unfortunate because the exact duration of the blood pressure-lowering effect of the two lower intensity walking trials could not be discerned and it could only be concluded that 30×50% was at least as effective as 60×50% for at least 4 hours.

5.3 Future research

Although the studies included in this thesis answered some compelling questions regarding the efficacy of single and chronic walking sessions for improving risk factors associated with MetS, some uncertainty remained. In the 24-week study, future research could recruit more non-medicated participants to the study groups who fulfil the diagnostic criteria for MetS, in an effort to remove the confounding effects of the low statistical power experienced for certain measures. In terms of the 24-hour study, extending the blood pressure measurements by using 24-hr ambulatory blood pressure monitoring rather than ending at 4-hours post-walk may help to determine the exact end-point of the effects of the walking sessions. Additional measures may also be included, such as measures of fibrinolytic activity and cardiovascular control. Analysing the plasma samples for fibrinogen concentrations and its metabolic agents – tPA & PAI-1 – would shed light on the influence of acute PA, particularly different intensities and durations of walking, on the risk of blood clotting and the mediating thrombotic pathways. Furthermore, although cardiovascular function, i.e. blood pressure and resting heart rate, were measured in this study and the efficacy of 30×50% for modifying these variables was established, the exact mechanisms by which this was possible could not be elucidated, particularly why 30×50% was more effective in this respect than 30×65%. Therefore, future studies may wish to make use of other measurement techniques to assess the underlying physiological processes that mediate these effects, such as heart rate variability and baroreflex reactivity. Furthermore, due to the problems in analysing the serum samples for CRP, these could be analysed to gauge the effect of a single session and 24 weeks of walking on chronic low-grade inflammation.

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