ADAPTATIONS TO SPRINT INTERVAL TRAINING AND COMPARISONS OF GENDER RESPONSE

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Contents

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"The pursuit of natural knowledge, the investigation of the world - mental and material - in which we live, is not a dull and spiritless affair: rather is it a voyage of adventure of the human mind"

Archibald V. Hill

(Nobel Media AB, 2014)

Dissemination of Study Findings

Published Articles

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Changes in body composition and rates of fat oxidation following 12 weeks high intensity exercise L. Bagley, M. Slevin, N. Al-Shanti, M. Piasecki, G. Morrissey, H. Foulkes, M. Carroll, C. Murgatroyd, D. Liu, W. S. Gilmore, J. S. McPhee. **Proc 37th IUPS** (2013) – International Union of Physiological Sciences 2013, 21-26 July 2013, Birmingham, UK

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List of Abbreviations

%S: %Sensitivity

ACC: acetyl-CoA carboxylase

ACSM: American College of Sport Medicine

ADP: Adenosine Di-Phosphate

AMPK: Adenosine Mono-Phosphate Kinase

ATP: Adenosine Tri-Phosphate

BPM: Beats per minute

Ca^{2+}: Calcium

CaMK: Calmodulin-Dependent Kinase

CO_2: Carbon Dioxide

CoASH: Co Enzyme A

CPT1: Carnitine Palmitoyltransferase 1

CPT2: Carnitine Palmitoyltransferase 2

CSA: Cross Sectional Area

DEXA: Dual Energy X-Ray Absorptiometry

DoH: Department of Health

FABPpm: Plasma Membrane Fatty Acid Binding Protein

FAD: Flavin Adenin Dinucleotide

FAT/CD36: Fatty Acid Translocase

FATmax: Maximal Rate of Fatty Acid Oxidation

GLUT-4: Glucose Transporter Molecule 4

H+: Hydrogen

H_2O: Water

mTOR: Mammalian Target of Rapamycin

MVC: Maximum Voluntary Contraction

Na+: Sodium

NAD+: Nicotinamide Adenine Dinucleotide

NHS: UK National Health Service

NICE: UK National Institute of Health and Care Excellence

Nmol: Nanomole

Nm: Newton Metre

Nm/Cm^2: Newton Metre/Centimetre Squared

O_2: Oxygen

P38 MAPK: P38 Mitogen-Activated Protein Kinase

PAI-1: Plasminogen Activator Inhibitor 1

PAV: Proportional Assist Ventilator

Pg/ml: Picogram/Millilitre

PGC-1α: Peroxisome Proliferator-Activated Receptor- Gamma Coactivator 1α

Pi: Inorganic Phosphate

PI3K: Phosphoinositide 3-Kinase

RER: Respiratory Exchange Ratio

RPE: Rate of Perceived Exertion

RPM: Revolutions per Minute

SIT: Sprint Interval Training

SPSS: Statistics Program for the Social Sciences

T2D: Type 2 Diabetes

HDL: High Density Lipoprotein

HIIT: High Intensity Interval Training

HOMA: Homeostatic Model of Assessment

HR: Heart Rate

HRmax: Maximal Age Related Heart Rate

IFNγ: Interferon Gamma

IL: Interleukin

K+: Potassium

Kg: Kilogram

l/min: Litres per Minute

LDL: Low Density Lipoprotein

M: Metres

ml/min/kg: millilitres/minute/kilogram

Mmol: Millimole

MRI: Magnetic Resonance Imaging

TC: Total Cholestrol

TNFα: Tumor Necrosis Factor Alpha

UK: United Kingdom

VCO_2: Volume of Carbon Dioxide

VO_2: Volume of Oxygen

VO_2max: Maximal Rate of Oxygen Uptake

VO_2peak: Peak Rate of Oxygen Uptake

W: Watts

W/Kg: Watts/Kilogram

WHO: World Health Organization

µg/ml: Microgram/Millilitre

µSv: Micro Sievert

µU/ml: Microunits/Millilitre

Abstract

Physical activity helps maintain health, promotes adaptations of the cardiovascular and neuromuscular systems to increase uptake, transport and utilisation of oxygen for aerobic energy production and improve fatty acid metabolism. Emerging evidence suggests sprint interval training (SIT) may be as effective as endurance exercise. The overall aim was to measure physiological effects of SIT in males and females from the general population. The first objective was to recruit males and females from the general population to complete 12 weeks cycling SIT and monitor changes relating to health and physiological function. The primary outcomes were changes in body fat mass, VO_2max, FATmax, knee extensor muscle size, strength, power, fatigue resistance, circulating concentrations of lipoproteins and inflammatory markers. The secondary outcome was a comparison of results between males and females. The second objective was to recruit Masters sprint and endurance runners to complete measurements of health and physiological function. The primary outcomes were peak power output and VO_2peak in one and two-leg cycling. After 12 weeks SIT, females showed higher increases (18.7%) in VO_2max (ml/kg/min) than males (6%) (gender comparison: p=0.009), males exhibited greater body fat (%) reductions (1.5%) than females (0.1%) (gender comparison: p=0.015). Males and females had similar increases in knee extensor fatigue resistance (4.0% and 8.9% respectively, gender comparison: p=0.221) and muscle cross sectional area (CSA) (cm^2) (4.1% and 5.8% respectively, gender comparison: p=0.895). Neither gender showed changes in circulating inflammatory proteins, but LDL decreased in males (7.8%) and females (3.7%) (gender comparison: p=0.161) and the ratio of cholesterol:HDL improved in females (13.1%) and males (19.6%) (gender comparison: p=0.523). Master sprint athletes had 22% higher peak power output (W/Kg) than endurance athletes (discipline difference: p=0.045), but endurance master athletes have 17% higher VO_2peak (ml/kg/min) (p=0.012) and 30% higher FATmax (mg/kg/min) (discipline difference: p=0.041). The inverse relationship between VO_2peak, FATmax and peak power with age was similar (10-12% per decade) for sprint and endurance athletes. It is concluded that males and females adapt positively to SIT, although gender differences in VO_2max and changes to body fat were found. Despite differences between masters endurance and sprint athletes in FATmax, VO_2peak and peak power, age related decline is similar in both disciplines.

Chapter 1

Sprint Interval Training: An Introduction

This section sets out the context for the original research studies that are presented in later chapters and were designed to examine the physiological effects of sprint interval training (SIT) in males and females. The balance of evidence from the research literature suggests that SIT can improve health and physiological capability.

1.1 Introduction To Sprint Interval Training (SIT)

Endurance (aerobic) and resistance (strength) training feature in the United Kingdom Department of Health recommendations which state that for an adult, “activity should add up to at least 150 minutes of moderate intensity activity in bouts of 10 minutes or more” (UK Department of Health, 2011). Additionally that “comparable benefits can be achieved through 75 minutes of vigorous intensity activity”, this suggests that higher intensity activities of shorter duration are equally beneficial as higher volume, lower intensity activity. However, the physical activity recommendations stop short of specifically recommending very high intensity sprinting activities that are inevitably much shorter duration due to the onset of fatigue.

In the past few years there has been a resurgence of scientific interest in sprint interval training (SIT) or very high intensity interval training (HIIT) (these terms have been used interchangeably in the scientific literature). These training modalities consist of very low volume, but very high intensity bursts of activity, such as sprinting. Typical training sessions include around 4-5 repeated sprints lasting around 20-60 sec each. Thus, weekly training volumes are around 5-10 mins. This type of training is different from the “75 min vigorous activities” mentioned in the UK physical activity recommendations. Yet, SIT seems to elicit similar health and fitness benefits to longer-duration endurance training protocols that are around 30-fold greater volume (Gillen and Gibala, 2014).

SIT has been around for many years in one form or another, and has been used for decades by athletes and those wanting to improve performance (Cicioni-Kolsky et al., 2013; Buchheit and Laursen, 2013). SIT alternates between extremely intense exercise at a high workload for a set amount of time followed by a period of lower intensity or cessation of exercise completely. One of the best known pioneers in this area was Professor Izumi Tabata, who studied SIT on various populations and noted significantly improved anaerobic capacity of skeletal muscle and increased VO_2 max. In Tabata’s original studies (Tabata et al., 1996), he used a model based on an ergonomic stationary cycle at 200% of VO_2max, which is very high intensity, although this study recruited participants with an already high cardiorespiratory fitness to begin with (VO_2max ~53ml/min/kg). Studies since then have used the Wingate test or some variation of this on an ergonomic stationary bike (Whyte et al., 2010; Burgomaster et al., 2005). This involves 4 to 6 repeats of ‘all-out’ maximal efforts around 30 seconds in

duration followed by a rest period of low intensity cycling to avoid venous pooling of blood in the legs, then repeated a prescribed amount of times (Bar-Or, 1987). Session time is usually around 10 minutes in length, including the 'rest' period between sprints (Burgomaster et al., 2008; Rakobowchuk et al., 2008; Little et al., 2010). Ergonomic stationary cycles have the advantage over running because they limit the effect that body mass has upon the individuals' workload, making the type of training possible for overweight and obese people. One problem with using cycles, however, is that they can be expensive, so a more applied version of this training mode is to complete hill-sprints or a treadmill sprint (Macpherson et al., 2011).

Research has started to identify the molecular regulators of skeletal muscle adaptations to SIT (Gibala et al., 2012; Gibala and McGee, 2008; Bartlett et al., 2012; Rakobowchuk et al., 2008; Babraj et al., 2009). Little et al found that NAD-dependent deacetylase sirtuin-1 (SIRT1), an activator of PGC-1α which in turn has been shown to increase mitochondrial biogenesis, was increased by ~56% after a 2 week HIIT intervention in 7 males (mean age: 21±0.7 years, VO_2peak= 46±2 ml/kg/min) (Little et al., 2010). In the same paper, nuclear abundance of PGC-1α was increased ~25% after training, suggesting that high intensity training is a potent activator of mitochondrial biogenesis and therefore muscle oxidative capacity. Later research from the same group suggests that AMPk activation leads to this increased PGC-1α concentration after just one bout of SIT cycling. Observing that after 4 30 second maximal cycling sprints, in 8 males (mean age:24±1 years, VO_2peak: 45±4 ml/kg/min), acetyl-CoA carboxylase (ACC) phosphorylation (a marker of AMPK activation) was significantly increased immediately post exercise, with a sequential increase in nuclear PGC-1α 3 hours later (Little et al., 2011a).

There are very similar metabolic and muscular adaptations following SIT as those that occur after much higher volume endurance type training (Bartlett et al., 2012; Burgomaster et al., 2008). Babraj et al. saw after only 6 sessions of SIT (an average of 15 minutes of exercise) on an ergonomic cycle, increased insulin sensitivity by ~23% (Babraj et al., 2009). This was one of the first papers to link SIT with an increase in insulin sensitivity, thus identifying SIT as an effective strategy for reducing the metabolic risk factors in sedentary populations. Little et al took this model one step further and recruited Type 2 Diabetes patients, finding a ~369% increase in protein GLUT4 content after only 2 weeks of SIT, as well as improved glucose control in all participants (Little et al., 2011a). Trapp et al also took SIT and applied a 15

week intervention to 15 females aged 18-30 years, finding that SIT gave a 33% increase in insulin sensitivity compared to steady state exercise controls (11%) (Trapp et al., 2008), consistently showing an improvement of metabolic disease symptoms, in the short term and longer term (15 weeks). Gillen et al. measured postprandial hyperglycaemia in T2D patients after just one session of SIT (approximately 10 minutes in length), finding that the time spent in hyperglycaemia experienced by these patients after a meal was reduced by 65% following one session of SIT (Gillen et al., 2012). These areas of research suggest that SIT is a viable and practical strategy for improving glycaemic control.

High body subcutaneous and visceral fat is associated with development of the metabolic syndrome (Miyazaki et al., 2002; Miyazaki and DeFronzo, 2009), so it is of benefit to maintain adipose tissue mass at relatively low levels. In this respect, it is interesting to note that the direct calorific expenditure during SIT sessions is very low (Babraj et al., 2009) compared with conventional endurance training (Burgomaster et al., 2008), but evidence is emerging that fat oxidation and fat loss are increased by a larger margin than that of traditional endurance type training (Shepherd et al., 2010). Tremblay and colleagues were amongst the first to principally study the effect SIT has on fat loss, finding that SIT had a much higher reduction in skinfold thickness than steady state prolonged exercise (Tremblay et al., 1994). Several other studies since then have reported large fat mass losses through SIT compared to steady state exercise controls using more sophisticated methods such as Dual Energy X-Ray Absorptiometry (DEXA) and Magnetic Resonance Imaging (MRI) (Trapp et al., 2008; Boudou et al., 2003), reporting abdominal fat losses of ~15% over 8 to 15 weeks of training.

This consistent finding in fat loss by participants in these sprint interventions is backed up by many studies whose results show significantly increased muscular adaptations and capacity to oxidise fat (highlighted in a review from Boutcher, (2011)). Talanian et al. observed an increase in whole body fat oxidation rates of 36%, as well as increasing maximal activities of mitochondrial oxidation enzymes β-HAD and Citrate Synthase by 32% and 20% respectively (Talanian et al., 2007). There was also a 25% increase in plasma membrane fatty acid binding protein (FABPpm), showing fatty acid transport is also improved, promoting increased oxidation of fatty acids.

The maintenance of fatty acid oxidation is crucial to healthy ageing and metabolism, with the avoidance of the onset of metabolic syndrome and chronic obesity related inflammation

(Blaak et al., 2001; Zakrzewski and Tolfrey, 2011). Maintaining good metabolic fitness in terms of fatty acid oxidation has also been shown as key in weight management and improving body composition (Folch et al., 2003; Smith et al., 2000). However, the weight loss after a period of SIT is not only due to the direct fatty acid oxidation, but might also be due to increased sensations of satiety. King et al. observed participants completing SIT taking a longer period of time to reach a volitional onset of eating than those of a control group or matched work low intensity groups (King et al., 1994).

It is clear that exercise of any sort affects muscle function and morphology (Flück and Hoppeler, 2003; Coffey and Hawley, 2007). However, specific modalities of exercise are traditionally thought to promote very different adaptations within skeletal muscle. Endurance type training primarily increases muscle oxidative capacity. However, it should be noted that in previously untrained people endurance training also has a significant effect on muscle size (Harber et al., 2012), fatigue resistance (Holloszy and Coyle, 1984) and to a certain extent, strength (Bell et al., 2000). Resistance training promotes muscle mass, strength and power (Folland and Williams, 2007) in most populations, including an ageing population (Ferri et al., 2003) and has little effect on VO_2max or cardiorespiratory fitness (Campos et al., 2002). SIT includes very high force and power contractions. It seems to promote muscle adaptations synonymous with endurance training, but little is known about whether or not SIT promotes adaptations synonymous with resistance training. It has been identified that sprint athletes, both young and old seem to benefit from the neuromuscular adaptations that both endurance and strength athletes benefit from (Stensvold et al., 2010; Boudou et al., 2003). Boudou et al (2003) showed a 24% increase in thigh muscle cross sectional area after 8 weeks of high intensity interval cycling at 85% VO_2peak in Type 2 diabetic males (mean age 45±7). However in terms of the effects of high intensity training (bouts of exercise eliciting workload ≥100%VO_2max (Weston et al., 2014)) on muscle CSA and force output as a consequence of this, evidence is scarce. Astorino et al. observed no change to isokinetic force production (peak torque at 60°sec^{-1}) in 20 males and females (aged 23±3 years, $p>0.05$) after 2 weeks of sprint interval training (Astorino et al., 2012). The effects of a longer sprint interval training intervention (>2 weeks) on peak muscular force is lacking.

Thus, as long as people would be willing to complete the very strenuous exercise (and assuming that it is safe), SIT could help to overcome one of the most common perceived

barriers in the population for not meeting the UK Department of Health guidelines of 150 minutes of physical activity, which is a lack of time (Stutts, 2002). To this end, Bartlett et al. (2011) found that his participants had significantly increased enjoyment levels after SIT running compared to continuous running. Further evidence from McRae et al. found that enjoyment and intention to engage in exercise was improved significantly with the SIT group (McRae et al., 2012). This suggests that more people may adhere to SIT than traditional endurance type training, which is especially important in clinical settings of exercise prescription, where lifestyle changes are the first treatment option for metabolic syndrome.

However, the major limitation to the majority of studies into SIT is that the participants were recruited from amongst male university students (most likely Sports Science students), or were recruited specifically as obese/overweight populations and were given a very strict, supervised training programme, or the training programmes were relatively short (between 2-6 weeks). It therefore remains unclear whether people from the general population or older people would experience the same health-related adaptations to SIT, whether gender-differences exist in these training responses and whether the adaptations that are evident from the first few weeks of training remain after much longer-term training after the initial "stress" of the new training regimen has subsided.

1.2 The rationale for Sprint Interval Training

SIT has received a great deal of attention in recent years as an effective and time efficient modality of exercise training, which is accessible to a large variety of populations and patients (Guiraud et al., 2010). Due to the common conception that exercise "takes too much time" (Stutts, 2002), SIT may present an effective method to improve public health. This exercise methodology could potentially be employed to improve the symptoms of Type 2 Diabetes (Babraj et al., 2009), reduce the risk factors for cardiovascular disease (Levinger et al., 2015) and significantly reduce body fat mass (Boutcher, 2011). The reasons for the omission of SIT from the UK Department of Health physical activity recommendations are not entirely clear, but are likely to be related to the fact that very intense exercise may not be well tolerated by some middle-aged or older people in whom risks, or fears, of adverse events may be greater. Moreover, the vast majority of research into SIT has been undertaken using young males as research participants. Therefore, it remains unclear how effective SIT would be when prescribed to the general population, unclear whether health

benefits would be experienced equally for males and females and unclear whether people who followed sprint-type training into older age would attenuate the usual age-related decline in physiological profile and health.

1.3 Aims, Objectives and Outcomes of the Original Research Presented in this Thesis

The overall aim of the work presented in this thesis was to measure the physiological effects of sprint interval training (SIT) in males and females recruited from the general population of Manchester, UK. This was achieved by addressing two main objectives, each with distinct primary and secondary outcomes:

Objective 1: To recruit males and females from the general population to complete 12 weeks cycling SIT and monitor changes relating to health and physiological function. The results were split into three sections based on the primary outcomes, but all were addressed from the data available from the single training study:

I. ***Body composition, maximal oxygen uptake and rates of fatty acid oxidation during exercise: effects of 12 weeks sprint interval training and gender comparisons***. The primary outcomes were the changes in body fat mass, VO_2max and FATmax after 12 weeks training. The secondary outcome was the comparison between responses of males and females in order to assess gender responses. This work is presented in Chapter 3.

   Hypothesis: It was expected that VO_2max and maximal rates of fat oxidation would be higher after training and that body fat mass would be lower after training. Based on the limited available evidence, it was also expected that females would increase FATmax more than males after training and lose more fat mass.

II. ***Knee extensor size, torque-velocity relationship and fatigue resistance: effects of 12 weeks sprint interval training and gender comparisons***. The primary outcomes were the changes in knee extensor muscle size, strength, power and fatigue resistance after 12 weeks SIT. The secondary outcome was the comparison between

responses of males and females in order to assess gender responses. This work is presented in Chapter 4.

Hypothesis: It was expected that muscle size, strength, power and fatigue resistance would be higher after training. Based on the limited available evidence, it was also expected that females would increase fatigue resistance more than males.

III. ***Circulating lipoproteins, adipokines and cytokines: responses to 12 weeks sprint interval training and their association with VO_2max and fat mass***. The primary outcomes were the changes in circulating concentrations of lipoproteins, adipokines and inflammatory markers. The secondary outcome was whether these circulating concentrations were associated with VO_2max and body fat % in 13 males and 7 females. This work is presented in Chapter 5.

Hypothesis: It was expected that lipoproteins, adipokines and cytokines would be responsive to SIT training and their levels would be associated with the VO_2max and body composition (as indicators of health status).

Objective 2: To recruit Masters Athlete sprint and endurance runners to complete measurements of health and physiological function. There were three primary outcomes, all of which are presented in Chapter 6, entitled: ***Aerobic And Anaerobic Power In Sprint And Endurance Master Athletes Aged 38-90 Years***:

I. Compare peak power output measured by jumping mechanography between sprint and endurance trained athletes. The secondary outcome was to compare the slope of decline with increasing age between the differently trained athletes.

Hypothesis: It was expected that sprint athletes would have higher peak power output than endurance athletes of the same age, however the slope of decline with increasing age would be similar to endurance athletes.

II. Compare the peak rate of oxygen uptake during single-leg and two-leg incremental cycling between sprint and endurance trained masters runners. The

secondary outcome was to compare the slope of decline with increasing age between the differently trained athletes.

Hypothesis: It was expected that endurance trained athletes would have higher aerobic capacity than sprint athletes, but the slope of decline with increasing age was expected to be similar between sprint and endurance athletes.

III. Compare the peak rate of fatty acid oxidation during exercise (FATmax) between sprint and endurance athletes. The secondary outcome was to compare the slope of decline with increasing age between the differently trained athletes.

Hypothesis: It was expected that FATmax would be higher in endurance athletes compared with controls and that the slope of decline with increasing age would be similar in endurance and sprint-trained individuals.

Chapter 2

Literature Review

Chapter One provided a brief overview of SIT and the associated health benefits. This chapter will provide greater detail relating to the methodology used in past research into SIT and to explain the physiological basis of adaptations to SIT.

2.1 Introduction

In the animal kingdom, physical activities including speed, agility and muscular strength are tools for both defence and attack, but rarely are these traits required in 21st century humans to protect against predators. However, it has long been suggested that we require high physical activity levels in order to live longer, healthier and higher quality lives (Blair et al., 1989). This protection afforded by physical activity in humans is now reasonably well understood, yet advice to increase or even maintain our levels of movement and physical activity are seldom heeded, with the majority of the population failing to reach government recommended, evidence-based weekly targets (UK Department of Health, 2011). The prevailing low adherence to exercise programmes through a "lack of time" (Grubbs and Carter, 2002) has led to the research and development of alternative and often lower volume methods of gaining a similar protection against disease and mortality to that offered by longer and higher volume activities.

This literature review examines current thinking and proposed mechanisms in the studies of physical activity and health.

Physical activity and health

The notion that regular physical activity has beneficial health effects is not a new one, the classic philosophers of ancient Greece such as Plato and Hippocrates, emphasised the importance of physical activity and the maintenance of health. This suggests that it is important to maintain movements and minimise the time that we sit still. Sedentary living has become a large problem in western societies in the past half century and has been highlighted by numerous studies in the past few decades (Owen et al., 2010; Katzmarzyk et al., 2009). Sedentary living refers not only to the lack of participation in sports and exercise in leisure time, but is also linked to work-related physical activity. In the 1950s, a group led by Jerry Morris published a series of papers investigating the link between sedentary jobs and the increased incidence of cardiovascular disease (Morris et al., 1953). Morris studied the incidence of cardiovascular disease in workers of the London bus service and Royal Mail employees. It was found that those in the more sedentary roles such as bus driving, clerks and telephonists, had a significantly higher risk of cardiovascular disease than those in active roles such as the bus conductor or those who delivered mail by bicycle or foot. The

increased physical activity on a daily basis in the participant's occupation was described as the major determinant of the cardiovascular disease risk.

The UK physical activity guidelines are based on evidence that regular, prolonged physical activity can help to increase physical fitness, control body weight and improve metabolic health (UK Department of Health, 2011). For instance, regular endurance exercise increases muscle capillarisation as well as muscle metabolic enzymes involved in oxidation of fatty acids and glucose (Boule et al., 2005; Despres et al., 1991; Holloszy, 2005; Howald et al., 1985). These adaptations alongside the direct energy expenditure of physical activity contribute to the control of body fat mass and risk factors for Type two Diabetes Mellitus and cardiovascular disease (Joyner and Green, 2009; Khan et al., 2012; Powell et al., 2011). To be effective, moderate intensity endurance-training sessions should be high volume, meaning that prolonged exercise should be completed on most days of the week. However, the reality is that around 60% of males and 72% of females in England do not meet the minimum recommended physical activity levels (UK Department of Health, 2011). The most common reason cited for not completing regular exercise is "lack of time" (Grubbs and Carter, 2002). Thus, the majority of adults in Western, developed countries follow sedentary lifestyles associated with higher risk of developing major debilitating and life-threatening diseases, such as cardiovascular disease and Type 2 Diabetes Mellitus (World Health Organisation, 2010; Khan et al., 2012).

The UK government recognises the need for physical activity and its relationship with improved cardiovascular, general physical health and indeed, mental health as well as for economic reasons (UK Department of Health, 2011). Recent evidence has suggested that an increase in sedentary behaviours such as total sitting time and TV viewing time leads to an increase in the risk of premature mortality with an estimated 9% of deaths worldwide attributable to physical inactivity (Lee et al., 2012). However, a recent meta-analysis of around 1 million males and females indicated that 60-75 minutes of moderate intensity physical activity a day can eliminate the ill effects of sitting for >8 hours per day as well as having a lower risk of death than those who sat for <4 hours per day but were physically inactive (Ekelund et al., 2016). This observation suggests that despite sedentary behaviour promoting poor health prospects, physical activity and exercise has effects that can ameliorate the ill effects of high volume sedentary time. Sedentary behaviours and the

associated disease conditions cost the National Health Service (NHS) approximately £1.06bn per year (Allender et al., 2007). Because of these conditions and diseases, approximately £6.6bn was lost in business production in 2011, therefore this issue has a real world application to the socioeconomics of countries worldwide.

Due to the considerable healthcare and economic costs associated with treating obesity and sedentary lifestyle related illness and diseases, the UK Department of Health has built upon the original recommendations given by the American College of Sports Medicine in 1978 (Garber et al., 2011; UK Department of Health, 2011). The older recommendations advised health practitioners of how much exercise is beneficial for the health of their patients based on the current knowledge in the field of exercise physiology. These recommendations were:

> "A minimum of 3x20 continuous minutes of moderate to vigorous intensity exercise per week"

- 60-90% HRmax (Maximal Heart Rate, estimated from: 220 *minus* age in years)
  OR
- 50-85% VO_2max (Maximal Oxygen Uptake)
  OR
- 40-85% HRreserve (Heart Rate Reserve) (The % difference between resting HR and HRmax)
  OR
- 12-16 on Rate of Perceived Exertion (RPE) scale

The recommendations were revised in 1990, 1998 and again in 2011 due to updates and advances in knowledge. These recommendations are supported by primary research elucidating the health benefits from physical activity. However, this was difficult to understand for most people in the general population who had little understanding of the concept of 50-85% of VO_2max. It also became evident from recent research that splitting up exercise bouts into multiple short sessions is as effective in improving health as one continuous bout of equal length. Miyashita et al. utilised two day trials separated by one week, studying 10 healthy young males (ages 21-32) after resting, carrying out 30 min treadmill running in either one 30 min bout or 3 x 10 min bouts at the corresponding participants' 70% VO_2max workload. The following day, participants consumed high fat

controlled breakfasts and lunches. It was observed that the males who walked (similarly in continuous or intermittent conditions) had significantly reduced plasma triacylglycerol and resting systolic blood pressure than the males who rested (Miyashita et al., 2006). Findings of this nature were key in the UK Department of Health releasing their recommendations in 2011, which are:

- Be active every day in bouts of >10 minutes moderate exercise, achieving >150 minutes per week
- Alternatively; 75 minutes of vigorous activity per week, or combine vigorous with moderate activities
- Include activities to increase muscular strength at least twice per week
- Minimise sedentary activities

This new advice from the UK Department of Health makes it easy for the general public to understand that physical activity can be completed every day in a variety of contexts.

The first recommendation, suggesting that being active every day in bouts of at least 10 minutes, is based on studies that demonstrated similar health-related benefits of regular, brief moderate intensity activities each day, as compared with single prolonged spells of physical activities. The National Institute for Health and Care Excellence recognize the importance of prolonged, low intensity activities through published guidance on walking and cycling for the public (NICE, 2012). Achieving >150 min/week moderate-intensity aerobic exercise, such as walking, is associated with at least 30% lower risk of morbidity, mortality and functional dependence (Paterson and Warburton, 2010; Chou et al., 2014). Walking 5 – 7 days per week was associated with 50 – 80% lower risk of mobility impairments (Clark, 1996; Roh and Park, 2013) and improves longevity by around 4 yrs and disability-free life expectancy by around two years (Ferrucci et al., 1999).

An attraction of advocating regular short spells of activity (of more than 10 min) is that it seems more achievable and might help to overcome the problem that the majority of people do not take part in any physical activities most days of the week due to their perceived lack of time (Stutts, 2002). Stutts interviewed 137 people from various groups; the responses revealed that low engagement in physical activity was due to a perceived “lack of time”.

Miyshita et al. examined 15 healthy young males (aged 18-28) and provided three separate walking tasks, each separated by one week: in one, the participants rested; in another, the participants exercised continuously for 30 min; and in a third, the participants exercised three separate 10 min bouts which matched work done in the 30 min continuous trial. The day after each trial, the participants rested and ate controlled high fat meals. Both the continuous and the 3x10 min bouts of exercise reduced post prandial plasma triglycerides by 16%, whereas the rest condition did not (Miyashita et al., 2008). This suggests that sedentary people who are unable to adjust to prolonged activities can improve their health and decrease risk for cardiovascular disease by completing regular brief activity periods of as little as 1 min (Healy et al., 2008) or preferably at least 10 min bouts (Powell et al., 2011) to break-up prolonged periods of sedentary sitting or lying.

The second recommendation implies that higher intensity exercise performed for shorter periods can give similar gains to prolonged low intensity activities. Vigorous exercise is associated with lower overall risk of developing cardiovascular disease compared with regular moderate intensity exercise (Swain and Franklin, 2006). Bartlett et al. studied 10 active males on two separate trials, in one trial they ran continuously for 50 minutes at 70% of their VO_2max, and in the second they ran 6x3 min bouts at 90% VO_2max separated by 3 min recovery periods at 50% VO_2max (Bartlett et al., 2012). Bartlett et al. showed that skeletal muscle markers of mitochondrial biogenesis such as p38 MAPK and AMPK were up-regulated to similar levels after both continuous and high intensity trials. This increase in markers of mitochondrial biogenesis after high intensity training to a similar or higher degree than continuous training is a common observation in the literature (Gibala and McGee, 2008) and is advantageous because mitochondria is the site of aerobic respiration and oxidation of fuel substrates and higher concentrations of mitochondria are associated with lower risk of metabolic disease (Ritov et al., 2010). This therefore suggests that higher intensity physical activity is having a similar benefit on skeletal muscle as lower intensity but higher volume exercise modes and thereby influencing its positive effects on health (Burgomaster et al., 2008).

The third recommendation in the guidelines was to “include activities to increase muscular strength at least twice per week”. This advice will help to combat the problem of muscle weakness in old age. There is an increasing life expectancy in the UK and a larger proportion of the population is aged over 65 years, with the average life expectancy now at 78.9 years

for males and 82.7 for females (Office of National Statistics UK, 2012). This 'ageing population' comes with a number of health related concerns, one of which is sarcopenia, which refers to the age related atrophy of muscle mass. This sarcopenic state seems to be only improved by regular physical activity to maintain muscle mass (Fitzgerald et al., 1997; Degens et al., 2013; Rogers et al., 1990), such as resistance exercise interventions (Johnston et al., 2008; Dunstan et al., 2002; Morley et al., 2001). There is a dose-response relationship, meaning that higher intensity resistance training tends to lead to greater gains in muscle mass, strength and power (Steib et al., 2010). However, it is important to avoid very strenuous exercise for people who were recently sedentary (Powell et al., 2011).

Reducing the amount of time spent sedentary has a direct link with longevity and health (Tremblay et al., 2010; Blair et al., 1989; Owen et al., 2010). The demands of modern urban living, in combination with technological and work or social developments, is a main factor in sedentary lifestyles (Morris et al., 1990). A longitudinal study of 8071 females aged 45-55 years in Australia found that time spent sedentary (sitting time) was a significant marker of weight gain over 5 years, even after this was adjusted for physical activity and food intake, further suggesting that small changes in lifestyle and reduction of sitting time prevents this weight gain (Brown et al., 2005). Not only is sedentary behaviour linked to weight gain, it is strongly linked to the onset of metabolic syndrome and Type 2 Diabetes (Dunstan et al., 2004). In one study, sedentary time was positively associated with plasma glucose concentration 2 hours after consuming a bolus of glucose in 173 middle aged Australians (mean age 53.4), whereas light intensity physical activity and moderate to vigorous intensity physical activity was inversely associated with plasma glucose concentration 2 hours after consuming a bolus of glucose (Healy et al., 2007). These studies therefore add evidence to the argument that sedentary behaviours increase the risk of metabolic and cardiovascular disease.

2.2 Skeletal Muscle

"A muscle is… an engine, capable of converting chemical energy into mechanical energy. It is quite unique in nature, for there has been no artificial engine devised with the great versatility of living muscle"

-Modern College Physiology (Stacy and Santolucito, 1966)

Physical activity, or exercise requires contraction of skeletal muscles. Skeletal muscle is primarily for locomotion, or movements *per se*. Skeletal muscles are made up of individual muscle fibres, which are long, cylindrical cells with a large number of nuclei on its surface (up to several hundred in some cells due to cell fusion during growth). The primary contractile proteins are actin and myosin, for which the sliding filament structure and function describes the molecular mechanisms of muscular contraction (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954).

Skeletal muscle can adapt to periods of increased use or disuse. The stimulus for adaptation is linked to various metabolic and mechanical stimuli that occur as a consequence of muscle contraction. This is a complex process triggered by arrival of the action potential from motor neurons that stimulates calcium release from the sarcoplasmic reticulum into the muscle fibre cytosol. The calcium binds with troponin and causes a conformational change of tropomyosin that exposes the myosin-binding site on actin. Myosin binds with actin and splitting of ATP into ADP + Pi initiates the 'power stroke' whereby the myosin pulls the actin molecule. ADP and the liberated Pi are released and a new molecule of ATP takes its place on the myosin to cause detachment of the myosin head from actin. The recurring process of millions of actin-myosin interactions governs the muscle contraction, albeit the overall regulation is the arrival of an action potential to trigger release of calcium into the cytosol.

Skeletal muscle fibres can be classified into three Types: Type I, IIa and IIb, which have distinct characteristics and contractile properties. Broadly, Type I fibres have a high oxidative capacity, slow velocity of shortening and are fatigue-resistant (Essén et al., 1975); Type II muscle fibres have a faster shortening velocity, larger cross sectional area giving higher force than Type I fibres and up to 10-times greater power, but they are more fatigable than Type 1 fibres due to a lower oxidative capacity and greater rate of ATP turnover.

Endurance athletes tend to have a high Type I muscle phenotype (Costill et al., 1976; Gollnick et al., 1973) with high rates of fatty acid oxidation due to their oxidative nature (Kiens et al., 1993) as well as a high capillary density (Andersen and Henriksson, 1977) and mitochondrial density (Holloszy and Coyle, 1984). Sprinters on the other hand have a high proportion of fast twitch Type II muscle fibres, meaning a higher maximum running speed in sprinters (Mero et al., 1981), but a lower oxidative capacity than endurance athletes (Bergh et al., 1978; Costill et al., 1976). It is also possible that the larger Type II muscle fibres have a higher basal metabolic rate than Type I (Tzankoff and Norris, 1978), which may reduce metabolic risk factors such as obesity and dyslipidaemia (Hurley et al., 1988), although this is a complex issue related to the balance of energy intake and expenditure, as well as physical activity levels.

Muscle fibre-type composition is highly variable between people (Simoneau and Bouchard, 1989) and there is a large heritable or genetic component (Bouchard et al., 1986; Simoneau and Bouchard, 1995). It is likely that specialist-trained high-performance athletes had heritable predispositions towards high proportions of particular fibre types that were advantageous for their athletic performance. Nevertheless, muscle fibre cross-sectional areas and metabolic features are highly adaptable with training and can improve performance to better cope with the demands of the exercise most regularly performed. However, it is not entirely clear whether muscle fibre types can be easily altered through training (Pette and Staron, 1997). For example, 19 weeks of resistance training was unable to elicit a shift in muscle fibre type (Adams et al., 1993), while the opposite has been seen for Type I fibres, with endurance type training leading to increases in Type I fibre percentage (Howald et al., 1985).

The energy turnover during skeletal muscle contractions are the key to the prevention of chronic disease and metabolic syndrome (Wolfe, 2006). Energy to fuel muscle contraction is produced aerobically and/or anaerobically, mainly from glucose or fatty acids (Krogh and Lindhard, 1920; Hill, 1924), depending primarily on the intensity of the exercise performed as well as if the individual is trained or not (Romijn et al., 1993; van Loon et al., 1999). Higher intensity activities rely upon a greater contribution from anaerobic glycolysis (Dyck et al., 1993; van Loon et al., 2001), most likely due to inhibition of fatty acid transport and oxidation (described below). The threshold of the shift from aerobic to increasingly anaerobic metabolism is termed the ventilatory threshold or anaerobic threshold, and can

be seen during progressive incremental exercise by the increased production of CO_2 that stimulates faster breathing rates and expiration of the CO_2 at rates in excess of the increase in VO_2. In non-athletes, the ventilatory threshold is reached at approximately 55% of VO_2 max and in athletes it can be above 80%, although it is highly variable between people and ranges from around 34% to up to 83% in general populations (Gaskill et al., 2001). Fatty acid oxidation seems to peak at approximately 50% VO_2max in most non-athletes, but again, there is large variance between people, ranging from approximately 35% up to 65% VO_2max (Venables et al., 2005).

2.3 Maximal Rate of Oxygen Uptake (VO_2max)

Fitness and health status are generally proportional to the maximal rate of oxygen uptake, known as VO_2max, and thus, VO_2max is considered a 'gold-standard' assessment of the health and fitness of an individual. It is therefore important to understand the physiology of VO_2max.

A standard test to assess VO_2max requires the participant to complete incremental exercise in which the workload begins low and then is increased incrementally until the participant can no longer continue to exercise due to fatigue. The oxygen and carbon dioxide in the inspired and expired gasses are measured and the oxygen uptake (VO_2) and carbon dioxide production (VCO_2) are estimated. The upper limit in oxygen uptake is defined by there being no change in oxygen uptake despite an increase in workload (Hill and Lupton, 1923). Realistically however, this peak is rarely actually reached during incremental exercise because participants normally voluntarily stop exercising due to severe fatigue (Rossiter et al., 2006; Bassett and Howley, 2000). Therefore, other indirect indicators of a maximal effort are commonly used, such as the maximum age-adjusted heart rate (commonly calculated as 220 minus age in years) or a respiratory exchange ratio (RER) of 1.1 and over.

VO_2max is a very good example of integrative systems physiology since it involves numerous systems and processes working together under stressful conditions (intense exercise). VO_2max is often described in the science and medical literature from the USA as a measurement of 'cardiopulmonary fitness' and indicator of health (Kodama et al., 2009; LaMonte et al., 2005). Epidemiological studies have demonstrated a relationship between relatively lower VO_2max and a lower physical function and health, leading to increased

mortality (Grundy et al., 2012; Blair et al., 1989). However, VO_2max is more than simply a measurement of cardiovascular and pulmonary systems, it is a measurement of the effectiveness of oxygen uptake: from the inhalation of oxygen from the atmosphere (pulmonary system), transportation of oxygen through the body (cardiovascular system), to the use of oxygen in respiration at the site of working muscle (musculoskeletal system).

In young, healthy individuals undertaking maximal aerobic exercise such as running or cycling, a large body of evidence suggests VO_2max is limited by the capacity of the cardiovascular system to supply oxygen-rich blood to the working muscles, rather than the capacity of the muscles to use the available oxygen (Richardson et al., 1999; Saltin and Calbet, 2006). Several factors contribute to a limitation of cardiac output, with each component of the oxygen supply chain contributing a little to the overall limitation (di Prampero, 1985). Despite this, diffusion of gases in the lungs is not usually thought to be limiting, however this may be the case in some elite endurance athletes where a large cardiac output during intense exercise causes blood to be forced through capillaries around the alveoli at high rates and reduces the time for oxygen to be diffused into the blood (Dempsey et al., 1984). However, this does not seem a likely limiting factor in most people, whether trained or not, as most healthy individuals have near-maximal blood oxygen saturation levels, even during a bout of high intensity exercise (Powers et al., 1989). Nevertheless, changing the capacity to supply oxygen to working muscles, without changing the capacity of the muscles to utilise the available oxygen, can cause VO_2max to change in the same direction. As an example, animal studies that surgically removed the pericardium show an increased stroke volume (and thus, cardiac output) and therefore VO_2max was significantly increased (Hammond et al., 1992; Stray-Gundersen et al., 1986). On the contrary, supplementation with beta-blocker drugs caused a reduction in cardiac output and therefore VO_2max during exercise (Tesch, 1985). It has also been observed that despite a lack of history of training, a high blood volume is often accompanied by a high VO_2max (Martino et al., 2002), suggesting that an increase in blood volume and therefore oxygen carrying capacity and cardiac output is indicative of higher VO_2max. Furthermore, infusion of whole blood to increase blood volume has also increased total oxygen carrying capacity and cardiac output and therefore increased VO_2max (Ekblom et al., 1972). It appears that a "critical point" occurs during exercise whereby the amount of muscle that is metabolically active, and therefore requiring high levels of oxygen supply, cannot be met by the

cardiovascular system which is unable to provide the adequate volume of blood to working muscles as well as carefully regulating blood flow to the brain and to maintain mean arterial pressure (Saltin, 2007). The careful regulation of leg blood flow during intense exercise is exemplified by studies that examined limb blood flow during two leg exercise, compared with two-leg combined with two-arm exercise. During the exercise of all four limbs, blood flow to the legs was 20-30% lower than during exercise that used the legs only, however, maximal oxygen uptake was similar in both conditions (*i.e*. similar in two leg exercise compared with four-limb exercise), even though four limb exercise used much larger muscle mass (Secher et al., 1977). This suggests that blood delivery during higher intensities of whole body exercise restricts the maximal value for oxygen uptake.

It has been demonstrated that peripheral vasoconstriction reduces blood flow to working muscles during high intensities in exercise (Harms et al., 1997), and that the demand for oxygen and blood flow by the respiratory muscles during intense exercise will reduce the flow to limb muscles (Harms, 2000) and that ~10% of total VO_2 is required by respiratory muscles in moderately fit individuals (Aaron et al., 1992). The Harms et al. papers further suggested that the fatigue of the respiratory muscles will cause a large sympathetic 'outflow' to peripheral muscle in order to improve respiratory muscle blood flow and limit peripheral muscle supply. To test this hypothesis, Harms et al. utilised mechanical proportional assist ventilators (PAV) in order to reduce the work of breathing in trained cyclists. The cyclists were able to exercise for longer at a near VO_2max workload (W) as well as achieving a higher VO_2max (l/min) with ventilation assistance from PAV (Harms et al., 1997; Romer et al., 2006).

Other studies utilising a method with hyperoxic inhalation have seen VO_2max increase during exercise, suggesting this higher percentage of oxygen may be required to overcome issues of diffusion capacity and utilisation (Ekblom et al., 1975; Knight et al., 1993). This finding also gives the impression that diffusion capacity and not mitochondrial oxygen utilisation is limiting VO_2max, as mitochondria seem to have the capacity to deal with more oxygen than what is available during normoxic conditions (Richardson et al., 1999; Richardson, 2000). Adding to this evidence in favour of an 'oxygen supply' limitation to VO_2max, studies that exercised just a single leg and, therefore, a smaller amount of muscle mass that is well within the capacity of the cardiovascular system to deliver blood to the working muscles, found oxygen uptake per unit active muscle mass was much higher in

single-leg compared with two-legged exercise (Andersen and Saltin, 1985; Davies and Sargeant, 1974; Richardson et al., 1999). It has also been shown that improving the muscle mitochondrial concentrations by one legged cycle training did not equate to an increased two-legged cycling VO_2max (Davies and Sargeant, 1975).

These studies provide evidence pointing toward a cardiovascular limitation to VO_2max in healthy adults (Saltin and Calbet, 2006). However, Professor Timothy Noakes proposed an alternative hypothesis, suggesting that the central nervous system is also responsible for limiting maximal oxygen uptake (termed the "central governor model"), by regulation of neural recruitment in skeletal muscle in order to avoid "catastrophic failure of homeostasis" (Noakes, 1997). This central governor model proposes that the cessation of maximal exercise at VO_2max has its cause in the increase of sympathetic activity, causing a reduction in blood flow to exercising limbs through vasoconstriction as well as through a reduction in motor unit firing to reduce muscle activity, thereby reducing exercise intensity in order to prevent myocardial ischemia (Noakes, 2012; Noakes and Marino, 2007). However, there is little experimental evidence to support this.

The improvement of VO_2max with exercise training or the decrease in VO_2max with sedentary living might theoretically include changes to lung capacity, efficiency of oxygen diffusion capacity at the individual alveoli, to oxygen carriage by haemoglobin, to efficiency of the cardiovascular system (including stroke volume and vascular reactivity). At the muscle level, this could include adaptations such as improved oxygen extraction and uptake, as well as use of oxygen by the working muscle, and finally by the reverse of this entire process and exhalation of waste gases and prevent metabolic shifts that might induce fatigue. However, the evidence thus far suggests that the sum of changes to 'supply' of oxygen to the working muscles is likely to be the main factor in the limitations to VO_2max (Bassett and Howley, 2000).

2.4 Fatty Acid Oxidation and Weight Loss

Weight loss and the ability to oxidise fatty acids at higher rates is a key outcome goal for the general population as well as elite endurance athletes. In a survey of 107,804 people in 1999, it was found that 29% of males and 44% of females in the USA were trying to lose weight, and out of those, only ~40% were attempting to do so via physical activity, whilst

even fewer (~20%) were trying to achieve this through physical activity and reduction in caloric intake (Serdula et al., 1999). This study highlights that a large proportion of the general public are attempting weight loss, but are not able to ascertain the best way to go about this, due to an even spread of individuals across a number of weight loss strategies such as calorie restriction, reduced fat intake, physical activity or a combination of these. In order to facilitate this weight loss, it is important to understand just how fatty acids are oxidised.

Adipose tissue is primarily used to store energy in the form of lipid droplets or triacylglycerides, and it has a much higher energy yield than carbohydrate or protein per gram. Metabolism of fat, or more particularly its constituent fatty acids, is a complex molecular process, which takes place in the cytosol and the mitochondria of cells. Firstly, triglycerides in the cytosol are hydrolysed by lipase enzymes into three fatty acids and glycerol, the latter being utilised through glycolysis with the end product of pyruvate and is used in the same manner as glucose. The former, the fatty acids, are then transported into the mitochondria for metabolism into usable energy in the form of ATP. This requires transport molecules such as fatty acid translocase (FAT/CD36) and plasma membrane bound fatty acid binding protein ($FABP_{pm}$), which transport fatty acids across the muscle cell membrane. Therefore, the concentration of these molecules that regulate fatty acid metabolism is important and it is worthy to note that exercise training increases concentration of these molecules (McPhee et al., 2011).

The transportation and oxidation of fatty acids is a slow process and can only take place when energy and oxygen are plentiful, *i.e*. in an aerobic state. Once inside the mitochondrial outer membrane, fatty acids undergo transport that begins with the addition of co-enzyme A (CoA), which then forms it into acyl-CoA. Carnitine palmitoyltransferase 1 (CPT1), which is located on the outside of the inner mitochondrial membrane, esterifies the acyl-CoA into acyl-carnitine that is transportable across the inner mitochondrial membrane by the reaction of carnitine translocase (CAT) that exchanges carnitine for acyl-carnitine over the inner mitochondrial membrane. Once over the membrane, acyl-carnitine is re-esterified back into its original form of fatty acyl-CoA, this time by carnitine palmitoyltransferase 2 (CPT2). It is at this point that beta-oxidation can take place, which is a four-phase reaction and involves a number of processes (Schulz, 2008):

- Firstly, Acyl-CoA dehydrogenase (there are individual isoforms of this enzyme for long, medium and short chain fatty acids) removes two hydrogen atoms (H^+) from Acyl-CoA and donates them to Flavin Adenine Dinucleotide (FAD) to produce $FADH_2$. This means Acyl-CoA is now 2-trans-enoyl CoA. The $FADH_2$ is then entered into the Electron Transport Chain.
- Secondly, this 2-trans-enoyl CoA is hydrated in a reaction catalysed by enoyl CoA hydratase. The end product of this reaction is L-3-hydroxy acyl CoA.
- Nicotinamide adenine dinucleotide (NAD+) is then added, cleaving two H^+ ions away from L-3-hydroxy acyl CoA giving NADH in a reaction catalysed by β-Hydroxyacyl CoA dehydrogenase (β-HAD). Again this NADH is entered into the Electron Transport Chain for ATP production. This forms 3-ketoacyl CoA.
- Finally, two carbon molecules are cleaved from 3-ketoacyl CoA to form acetyl CoA, in a reaction catalysed by ketothiolase. This acetyl CoA can then enter the citric acid cycle. This final cleaving of two carbon molecules also yields acetyl CoA, less two carbon molecules, so therefore can re-enter the beta-oxidation cycle until the entire fatty acid is oxidised.

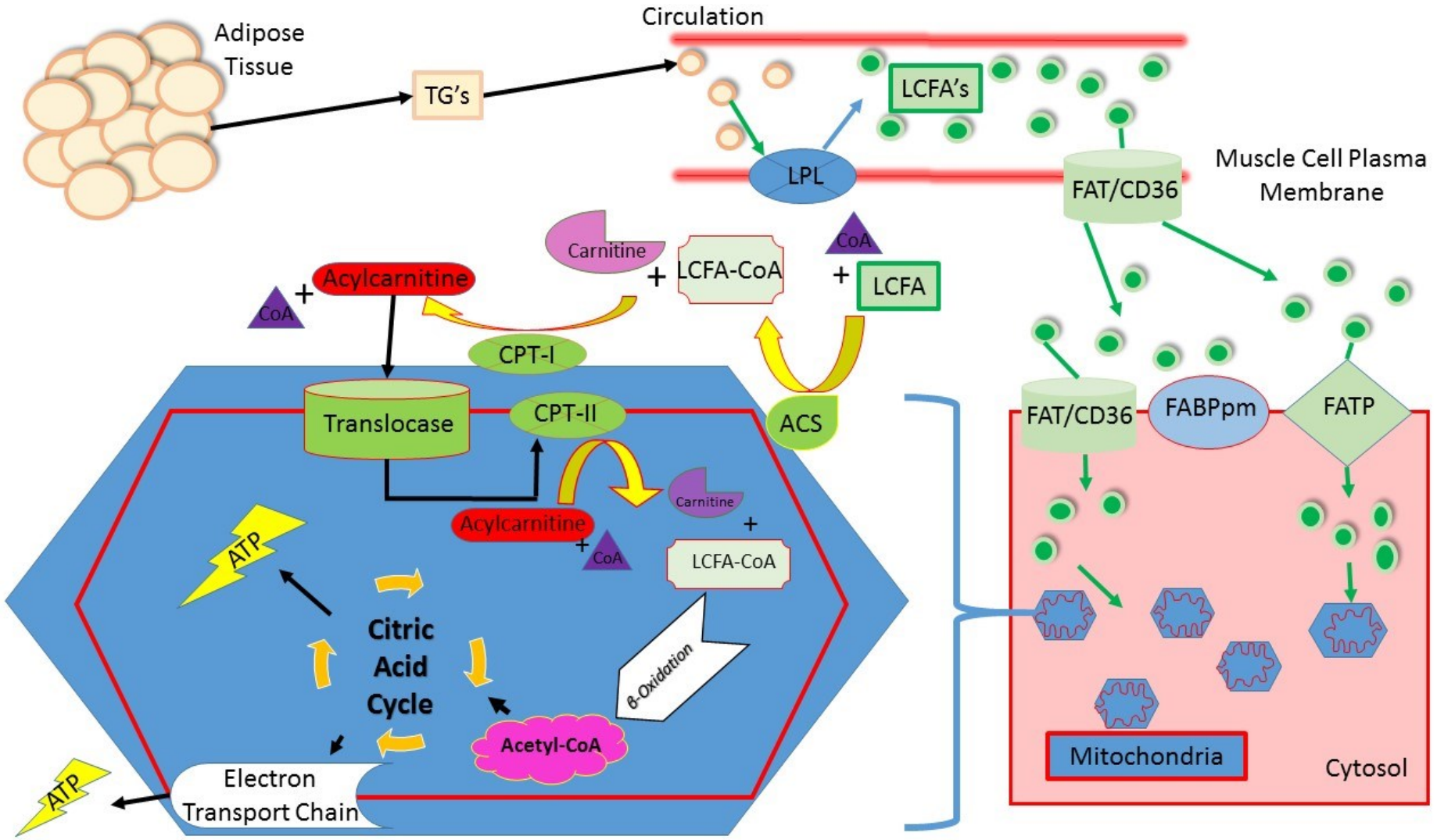

Figure 1: Adipose to ATP: An Overview Of Fat Oxidation

TG, Triglyceride; LPL, Lipoprotein Lipase; LCFA, Long Chain Fatty Acid; FAT/CD36, Fatty Acid Translocase; FATP, Fatty Acid Transfer Protein; FABPpm, Fatty Acid Binding Protein (Plasma Membrane); CoA, Enzyme CoA; ACS, AcylCoA-Synthetase; CPT-I, Carnitine Palmitoyltransferase 1;

In states of very high intensity exercise, fatty acid oxidation is not possible and the working muscle relies upon anaerobic glycolysis for energy maintenance (Jeppesen and Kiens, 2012). The reason this process stops at higher intensities of exercise is firstly due to the reduced availability of free carnitine (Roepstorff et al., 2005). Carnitine is involved in the transport of long chain fatty acids across the mitochondrial inner membrane into the mitochondrial matrix (as described on page 23). CPT-I transports fatty acyl CoA into the mitochondrial membrane by binding to carnitine on the inside of the outer mitochondrial membrane. After transport, carnitine binds to the fatty acyl CoA to form fatty acyl-carnitine, whilst simultaneously transporting Co Enzyme A (CoA) out of the mitochondrial outer membrane via CPT-I. The newly formed fatty acyl-carnitine is then diffused to the inner mitochondrial membrane, and binds to a second transport enzyme called carnitine palmitoyltransferase 2 (CPT-II), which transports fatty acyl-carnitine into the mitochondrial matrix. In order to do this, CoA binds on the mitochondrial matrix side to CPT-II, at the same time, acyl-carnitine binds to CPT-II in the inter-membrane space. This reaction cleaves carnitine from acyl-carnitine and binds to form fatty acyl-CoA and a free carnitine, the free carnitine is transported out of the mitochondrial matrix whilst fatty acyl-CoA can then be utilised in beta-oxidation in the mitochondrial matrix. During exercise or states of altered lipid metabolism, high concentrations of acyl-carnitine in the inner mitochondrial matrix reduces the amount of free carnitine available to transport FFA into the mitochondria (Stephens et al., 2007). In 8 trained male cyclists (mean age; 22±0.7 years, VO_2max~74ml/kg/min), van Loon et al. found that exercising for 30 mins at 55% of the individual's maximal exercise workload (Wmax), 25% of the cyclists' energy expenditure was derived from free fatty acids with a muscle tissue concentration of free carnitine of 10.29±0.84 mmol/kg. However, when the intensity of exercise was increased to 75% Wmax, only 15% of total energy expenditure was derived from free fatty acids and free carnitine in the muscle tissue had reduced by 45% (5.61±0.76 mmol/kg) (van Loon et al., 2001). This observation and similar observations, point heavily toward a free carnitine limited process of fatty acid oxidation during exercise (Roepstorff et al., 2005; Stephens et al., 2006). Also, a reduced pH due to the intensity of exercise (caused by an increase of lactic acid concentration from skeletal muscle contraction), makes CPT-I less efficient in converting LCFA-CoA and free carnitine into acylcarnitine (McGarry and Brown, 1997). Due to this reduction in fatty acid transport and therefore oxidation, the skeletal muscle relies more on glycolysis in these situations

(Jeppesen and Kiens, 2012). This principle of fatty acid transport being the limiting factor to oxidation of fat was investigated by Romijn et al (1995), whereby 6 endurance trained cyclists (mean age= 28±2 years, VO_2max= 64±3 ml/kg/min) cycled at 85% VO_2max for 30 minutes then repeated the exercise after a plasma lipid infusion. The cyclists saw an increase in fatty acid oxidation from 26.7±2.6 µmol/kg/min to 34.0±4.4 µmol/kg/min (~27%, $p<0.05$) after infusion with free fatty acids, despite rate of appearance of free fatty acids increasing from 12.4±1.7 µmol/kg/min to 61.0±10.6 µmol/kg/min (~390%, $p<0.05$) (Romijn et al., 1995). Demonstrating that fatty acid oxidation hits a plateau, despite a massively increased availability of free fatty acids, pointing clearly toward a skeletal muscle transport limitation to fat oxidation.

Exercise training attenuates this limitation in rates of fatty acid transport. Kiens et al. (1993) saw that after 8 weeks of 1 legged extension exercise at 65%VO_2max in 7 healthy young males (age 21-26 years), the fatty acid delivery to skeletal muscle in the untrained leg was similar to that of the trained leg during a 120 minute one legged extension exercise bout ($p>0.05$). However, uptake of free fatty acid into the working muscle significantly increased in the trained leg at 10, 60 and 110 minutes into the exercise bout ($p<0.05$), however did not increase in the untrained leg ($p>0.05$). This suggests that despite a similar free fatty acid release and delivery in trained and untrained muscle, the subsequent transport (and therefore utilization) into the working muscle is increased in trained skeletal muscle (Kiens et al., 1993).

An unexplored area of fat oxidation rates is the differences in training responses between males and females, although it has been observed that the peak rate of fatty acid oxidation occurs at higher exercise intensity in females than in males (Venables et al., 2005). Conversely, however, a recent review by Henderson indicated that males utilize relatively more fatty acids after exercise than females (Henderson, 2014). However, whether any gender dimorphism exists for exercise training induced increases in rates of fat oxidation during subsequent exercise remains unclear, particularly after sprint exercise (Talanian et al., 2007; Perry et al., 2008).

The estimation of fat oxidation during exercise by indirect calorimetry is well described and widely used in the literature (Venables et al., 2005; Achten et al., 2002; Kiens et al., 1993). The majority of these studies utilize the stoichiometric equation described by Frayn, as: 1.67 x VO_2 – 1.67 x VCO_2 (providing that urinary nitrogen excretion is negligible) (Frayn, 1983).

This assumption of urinary nitrogen being negligible is based upon the notion that the primary source of the energy utilized during graded exercise consists of fat and carbohydrate. Protein is metabolized into amino acids via proteases in the stomach and small intestine, which may then be utilised during exercise in skeletal muscle metabolism (Goodman, 2010). These amino acids are then deaminated by addition of NAD and H_2O to produce Ammonia (NH_3), which is excreted as urea (hence the measurement of urinary nitrogen) and an organic acid (Silverthorn, 2010). The organic acid that is yielded by this reaction is dependent upon the amino acid utilised in the reaction. Glucogenic amino acids (*eg*, alanine) produce pyruvate, which can then be used during gluconeogenesis in the liver for glucose, or a ketogenic amino acid (*eg*, leucine), which produces acetyl CoA, and can subsequently be added to the citric acid cycle for oxidative metabolism (Berg et al., 2002). The evidence thus far suggests that protein metabolism during an endurance exercise bout of around 90 minutes in duration, accounts for around only 3-6% of oxidative metabolism (Hargreaves and Snow, 2001; Calles-Escandon et al., 1984; Lemon and Mullin, 1980) and is negligible at exercise intensities ≤70% VO_2max (Wagenmakers, 1998). McKenzie et al. (2000) saw in healthy, young males (n=6, Mean age=26.9±3.4 years, VO_2peak=45.9±4.4 ml/kg/min) that in the final 30 minutes of a 90 minute endurance cycling bout using an isotope tracer technique (~60% VO_2peak), 85.2±21.2 µmol/kg/hour (or ~1.42 µmol/kg/min) of leucine was oxidized. Similarly, in the same study with the same experimental protocol described, young, healthy females (n=6, Mean age=23.7±1.8 years, VO_2peak=37.7±6.1 ml/kg/min), only 38.8±24.7 µmol/kg/hour (or ~0.65 µmol/kg/min) of leucine was oxidized (McKenzie et al., 2000). To put this in perspective, and in line with fatty acid oxidation data available, this equates to ~0.19 mg/kg/min of leucine oxidation in the males studied in McKenzie et al. (2000) (calculation based on Leucine having a molecular mass of 131 g/mol (PubChem, 2004)). Compare this to Achten and Jeukendrup (2003), who studied 26 young healthy males (VO_2max=58.6±5.2 ml/kg/min) and saw that at the exercise intensity of 62.5% VO_2max, the rate of fatty acid oxidation was ~6.43mg/kg/min. This evidence suggests that fatty acid oxidation is occurring to a much higher and much more significant degree than protein oxidation as a source of energy to fund exercise bouts. Therefore, the assumption in the stoichiometric equation that urinary nitrogen is negligible is a limitation of the calculation, however it is a minor limitation due to the oxidative contribution of proteins being around 3-6% of 90 minute endurance exercise bouts (Hargreaves and Snow, 2001)

and ~3% of the rates of fatty acid oxidation at a similar exercise intensity (utilizing example above).

This method is highly repeatable, although exercise at a higher intensity may reduce the efficacy of this estimate by a shift in the bicarbonate pool as the equation assumes that VCO_2 comes directly from the CO_2 produced in the working tissues (Jeukendrup and Wallis, 2005). A number of studies have examined the variance in the measurement of FATmax, however evidence is contradictory as a number of methods are used and results are interpreted in a number of different ways. For example, Achten and Jeukendrup (2003) studied 10 healthy young males (Mean age=24±6 years, VO_2max=60.1±0.3 ml/kg/min) by the participants completing 3 separate graded exercise bouts, during which breath by breath gas analysis and estimation of FATmax was carried out using the stoichiometric equations (Frayn, 1983). Between these three individual assessments in the same participants, the co-efficient of variation (CV%) was 9.6%, which equates to a variation in heart rate of 9 (range: 7-14) beats per min or in VO_2 of 0.23 (range: 0.17-0.34) l/min (Achten and Jeukendrup, 2003). Pérez-Martin et al. (2001) similarly studied 10 healthy males (Mean age=32.8±4.2 years, BMI=22.7±0.4 kg/m^2) and observed a CV% of 11.6% between two separate assessments performed within one week. Despite a markedly higher CV% for FATmax than variables such as VO_2 (~3.4% (Achten and Jeukendrup, 2003; Croci et al., 2014)), similar methods of measurement of rate of FATmax and fatty acid oxidation *in vivo* give similar CV% and results within 90% similarity on different days. Therefore, this method of indirect calorimetry and determination of fatty acid oxidation rates and FATmax should be considered valid with a large study cohort and interpreted accordingly (Croci et al., 2014).

Another more complex method of assessment is via stable isotope infusion, however this method has the same issue as indirect calorimetry in that VCO_2 is interfered with by the shift in acid-base balance, causing lactate accumulation and increased [H^+], which is in turn buffered by bicarbonate. This results in an increased CO_2 production and therefore an increase in VCO_2 (Ferrannini, 1988). Romijn et al. studied an alternative method of stable isotope infusion whereby VCO_2 can be circumvented by measurement of expired absolute $^{13}C/^{12}C$ ratio. No statistically significant difference in rates of fat oxidation was seen between methods, suggesting indirect calorimetry is a valid method suitable to an intensity of exercise ~85% VO_2max (Romijn et al., 1992).

2.5 Steroid Hormone effects on oxidative metabolism

A key difference between males and females is the concentrations of circulating sex steroid hormones, primarily oestrogen concentration being higher in females, with males having a higher concentration of circulatory testosterone (Simpson et al., 2005).

Steroid hormones, including oestrogen, may influence the molecular adaptations to exercise stress and it therefore follows that the differences between males and females in circulating concentrations of oestrogen can influence training responses (Campbell and Febbraio, 2001b). Of the few human studies on this topic, Venables et al. (2005) studied 300 participants (143 females; mean age= 32±12, VO_2max= 41±1 and 157 males; mean age= 30±11, VO_2max=51±1) and found that maximal fat oxidation measured by indirect calorimetry was significantly higher in females than in males. Evidence suggests that upregulation via oestrogen receptors of Peroxisome proliferation activator receptors (PPAR), leading to increased activity of LCFA transport proteins such as FAT/CD36, FATP and CPT-I is the reason behind the higher fatty acid oxidation in females than males (Oosthuyse and Bosch, 2010). Oestrogen supplemented Sprague-Dawley rats rapidly increased the phosphorylation of AMPK in the rat soleus musle *in vivo* (Rogers et al., 2009), which could potentially explain increased mitochondrial proteins involved with fat transport and oxidation (see Chapter 2.6). Furthermore, Campbell et al. (2003) observed that oestrogen supplementation in ovariectomized female Sprague-Dawley rats produced a 7-fold increase in CPT-1 mRNA and a 23-fold increase in Pyruvate Dehydrogenase Kinase-4 (PDK-4; a key enzyme responsible for phosphorylation and deactivation of the pyruvate dehydrogenase complex, which is involved with increased glycolytic flux. Thus PDK-4 decreases oxidative glucose metabolism (Sugden et al., 2000)). These findings suggest that oestrogens are increasing the oxidative capacity for fatty acids in females over that of males, as ovariectomy significantly reduced activities of enzymes involved in fat oxidation such as β-HAD and CPT-1, but is restored with supplementation of oestrogen (Campbell and Febbraio, 2001a).

The female menstrual cycle also has the effect of altering the circulatory concentrations of sex steroid hormones, being split into two distinct phases, the follicular and luteal phases, each lasting around 10—14 days. The increase in oestrogen occurs throughout the follicular phase, building to ovulation at around 14 days, then steadily declines throughout the luteal

phase (Isacco et al., 2012). Due to this variable level of oestrogen throughout the menstrual cycle, it has been hypothesised that females may oxidize fat during exercise bouts at different rates during the follicular or luteal phase (Oosthuyse and Bosch, 2010). Despite this reasonable assumption, this does not seem to be the case in the literature thus far. Jacobs et al. (2005) studied 8 healthy, eumenorrheic females (mean age= 25±1, VO_2max= 42±2) in the luteal and follicular phases of menses. Rates of fatty acid oxidation as measured using isotopic tracers were not different between menstrual phases at rest (p=0.09), or during exercise at 45% VO_2peak (p=0.33) and 65% VO_2peak (p=0.49). Similarly in 13 healthy, eumenorrheic females (mean age=29±5 years, VO_2max=40±6), Glycerol and fatty acid turnover were similar between all menstrual phases during a cycling exercise bout at 50%VO_2max for 90 minutes (Horton et al., 2006). The majority of evidence seems to agree that the effect of oestrogen is to increase fat oxidation during rest and exercise, however the studies thus far seem to have little variation between menstrual phase circulatory oestrogen concentration, as well as being highly individualistic. Which suggests why the menstrual phase has not affected rates of fat oxidation during exercise (Oosthuyse and Bosch, 2010). Another factor for consideration is the use of oral contraceptives (OC) by participants when studying gender differences, as the supplementation of oestrogen may have an effect on fat utilization during exercise. Bemben et al. (1992) studied 16 females, 8 of whom had used OC for >9 months (mean age= 24±1, VO_2max= 42.8±1.3) and 8 of whom who did not use OC served as controls (mean age= 26±1, VO_2max= 44.0±1.9) during 90 minutes of treadmill exercise at 50% VO_2max. During the exercise bout, total carbohydrate utilization (estimated using indirect calorimetry) was significantly lower in the OC group (OC: 0.63±0.05 g/kg, Control: 0.82±0.07 g/kg, $p<0.05$), however fat utilization in both groups was similar (OC: 0.67±0.04 g/kg, Control: 0.63±0.06 g/kg, $p>0.05$). Going further, Casazza et al. (2004) studied 8 eumenorrheic females using isotopic glycerol tracers measuring appearance rates during the follicular and luteal phases, as well as after 4 months of OC supplementation during 60 minutes of cycle exercise at 45% and 65% VO_2peak. Rates of glycerol appearance were not different between the follicular and luteal phases ($p>0.05$), however after 4 months of OC use, the rate of glycerol appearance increased by 24% during 45% VO_2peak cycling (OC use: 7.7±1.1 µmol/kg/min, Before OC use: 6.2±0.2 µmol/kg/min, $p<0.05$). As exercise intensity increased to 65% VO_2peak, rate of glycerol appearance decreased (consistent with the literature, see Chapter 2.4), however it was still >20% higher

than before OC use ($p<0.05$). Data from the same cohort of females observed no significant difference in fat oxidation rates (from indirect calorimetry) between the menstrual phases, or after OC use ($p>0.05$) (Suh et al., 2003). Taken together, this suggests that these mobilized fatty acids were reesterified as opposed to oxidized. Therefore the results from studies investigating the effects of OC on lipid utilization is reasonably consistent in their findings that lipid mobilization is increased during OC use in bouts of exercise, however reesterification as opposed to oxidation is the likely fate of the extra lipids.

Despite oestrogen's observed effects in skeletal muscle, only one human study has examined the effects of regular exercise on oestrogen receptor concentration in skeletal muscle. Wiik et al. (2005) saw a 3-5 times lower level of oestrogen receptor mRNA in 10 moderately active males (mean age= 24±3, VO_2max= 46±6) compared to 10 highly trained endurance athlete males (mean age= 22±3, VO_2max= 73±5) ($p<0.01$), suggesting that oestrogen activity may be upregulated due to increased levels of physical training (Wiik et al., 2005). Nevertheless, no studies have examined gender effects of SIT interventions in terms of substrate utilization during exercise and this therefore requires further investigation.

2.6 Mitochondrial Biogenesis

As described in section 2.4, the site of fatty acid oxidation and subsequent energy transfer into ATP occurs primarily in the mitochondria of the skeletal muscle cell (*Figure 1*). A primary adaptation of endurance exercise training is mitochondrial biogenesis (formation of new mitochondria), which in turn is correlated with increased exercise performance and in turn, with increased independence and quality of life through the lifespan (Irrcher et al., 2003). An increase in muscle mitochondrial content therefore increases the potential for fatty acid transport (Talanian et al., 2010), oxidation (Bruce et al., 2006) and total muscle oxidative capacity (Jacobs et al., 2013). On the contrary, type 2 diabetes patients have a lower mitochondrial size, content and therefore function (Ritov et al., 2010; Kelley et al., 2002), suggesting that the content, function and capacity of skeletal muscle mitochondria are vital for improved health outcomes.

A higher mitochondrial content is often brought about by a number of molecular signaling mechanisms as a result of an exercise bout or repeated training (Hood, 2001). Primarily,

current knowledge suggests that ATP turnover and Ca^{2+} flux promote downstream phosphorylation and activation of a number of transcription factors, such as PGC-1α (Zhang et al., 2014; Adhihetty et al., 2003). ATP turnover increases during contractile activity in order to fund the energy requirements of the working muscle, reducing cellular ATP and phosphocreatine (PCr) stores, thus increasing AMP and decreasing PCr concentrations in muscle, this in turn leads to the activation of AMP-activated protein kinase (AMPK) (Ponticos et al., 1998). A mouse study showed that an increased AMPK activation after feeding with β-guanadinopropionic acid (β-GPA) (a potent activator of AMPK), led to increased PGC-1α, which did not occur in AMPK knockout mice (Zong et al., 2002). Likewise in rats fed 5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside (AICAR, a known activator of AMPK) for 14 days, there was an increase in AMPK concentration alongside a concurrent increase in PGC-1α concentration in soleus and extensor digitorum longus muscles of the rat legs, as well as a significant increase of ~24% in the β-oxidation enzyme, β-HAD, in skeletal muscle biopsy (Suwa et al., 2003).

With increasing age, a loss of skeletal muscle mass and strength is observed (further discussed in section 2.8). Alongside these events, is a significant loss of skeletal muscle "quality" as we age, further reducing the skeletal muscle function, which includes the loss of mitochondrial function with increasing age (Peterson et al., 2012). In a study of 14 young, healthy males and females (7 males, 7 females. Mean age=27±1) and 15 elderly males and females (7 males, 8 females. Mean age=76±1), mitochondrial capacity (measured by maximal ATP output) was 17% lower in the elderly group, despite there being no difference in citrate synthase activity between groups (Johannsen et al., 2012). Furthermore, Petersen et al. (2003) saw similar results in 16 elderly males and females (8 male, 8 female. Mean age=70±2) and 13 young males and females (6 males, 7 females. Mean age=27±2), whereby the elderly participants had a ~40% lower mitochondrial oxidative and phosphorylation activity than young participants. This evidence suggests that there is a reduction in the ATP generating capacity from skeletal muscle mitochondria with increasing age. This leads to the question as to whether this decline in mitochondrial function can be averted by exercise, as seen in animal studies, and does this lead to better health outcomes.

In early exercise training studies, it was observed that 5 well trained males (VO_2max=76.1 ml/kg/min) had a ~3x higher mitochondrial content than 9 untrained males (VO_2max=61.3 ml/kg/min), despite both groups being arguably well trained, indicated by their high VO_2max

(Hoppeler et al., 1973). Similar to studies utilizing rodent models, mitochondrial proteins such as cytochrome c oxidase (COX), citrate synthase and β-HAD increase in activity and concentration after endurance exercise training (Spina et al., 1996; Kiens, 1997; Chesley et al., 1996; Gibala et al., 2006). These increases in mitochondrial function and content after training contribute to the increases in metabolic health, in terms of increases in oxidative capacity (Holloszy and Coyle, 1984) and insulin sensitivity (Goodpaster et al., 2003). The training stimulus to mitochondrial biogenesis is most reliant on exercise volume and intensity, with large, immediate increases in mitochondrial content and/or function being observed if either of these variables are increased (Bishop et al., 2014). Hoppeler et al. (1985) observed a 40% increase in mitochondrial volume in the vastus lateralis muscle of 5 recreationally active males (mean age= 31±5 years, VO_2max=47.0±6.2 ml/kg/min) and 5 recreationally active females (mean age= 28±5 years, VO_2max=39.4±4.0 ml/kg/min) after 6 weeks cycling training (30 minutes at 75% VO_2max, 5 times per week). This increase in mitochondrial volume after training seen by Hoppeler et al (1985) is also observed in all muscle fibre types (in the same participants and training intervention), indicating that the training effect of increased mitochondrial volume is seen across the entirety of the muscle, despite potential differences between fibre metabolic properties (Howald et al., 1985). Similarly, Russell et al. (2003) saw a 72% increase in cytochrome c oxidase (COX, a key mitochondrial content indicator as the last enzyme in the electron transport chain) content. As well as a 2.7x increase in PGC-1α expression, both after 6 weeks of high intensity endurance running (up to 80% VO_2max at 6 weeks, three times per week) in 7 healthy males (mean age=34±5 years, VO_2max=54±4 ml/kg/min) (Russell et al., 2003). The potential for SIT to increase mitochondrial protein concentration and biogenesis is alluded to in Chapter 1, with short, intense bouts of sprinting exercise leading to large increases (~56%) of the mitochondrial content master regulator molecule, PGC-1α in healthy and type 2 diabetic patients (Little et al., 2010; Little et al., 2011a; Little et al., 2011b). These findings add compelling evidence to the case for low volume, time efficient SIT, in a number of populations and patient groups, to improve metabolic health outcomes through increased mitochondrial capacity and function (Petersen et al., 2003).

2.7 Exercise and Inflammation

Health and fitness may be quantifiable in terms of the maximal oxygen uptake or maximal fatty acid oxidation, as described above, but there are also 'biomarkers' of health and fitness circulating in blood during the rested state. The best-characterised biomarkers are cholesterol, glucose and insulin, which are used by the World Health Organisation (WHO) as indicators for the risk of cardiovascular disease, insulin resistance and Type 2 Diabetes or 'metabolic syndrome' (which is the combined risk of cardiovascular disease with insulin resistance). However, concentrations of cytokines indicative of inflammation have emerged as novel biomarkers of health.

In healthy people, an inflammatory process occurs as a result of cellular stress, such as damage, in which cells release growth factors and cytokines (for example interleukins (ILs) and tumour necrosis factor-α (TNF-α)) that in turn draw neutrophils and macrophages to the site of injury (Tidball, 2005). These macrophages conduct phagocytosis and secrete further growth factors and cytokines that augment tissue repair (Tidball, 2011). This results in acute inflammation characterised by high levels of circulating cytokines and in more serious conditions it is also evident as swelling around the injured area. In this respect, the inflammation plays an important role in tissue repair and regeneration. In relation to exercise, mild tissue injury is a common outcome of exercise training, and the localised inflammation plays an important role in skeletal muscle adaptation to training (Suzuki et al., 1999).

In addition to the acute inflammation, a secondary state of inflammation also exists, known as 'chronic, low-grade systemic inflammation'. This is characterised by chronic circulating levels of cytokines that are higher than those found in healthy people, but lower than levels that occur after tissue injury. For example, chronic, low-grade systemic inflammation is a feature of insulin resistance (Dandona et al., 2004; Barzilay et al., 2001) and cardiovascular disease (Volpato et al., 2001) as well as advancing old age (Paolisso et al., 1998), smoking (van der Vaart et al., 2005) and obesity (Hotamisligil et al., 1993).

There is a suggestion that chronic low grade systemic inflammation is the result of a number of exposures over the lifetime to "mini hits" to the immune and other systems, resulting in damage to proteins and DNA (Degens, 2010). This state of altered regulation of cytokine production and macrophage response in old age, or "inflammageing" as it has been termed

(Franceschi et al., 2000), leads to a chronic low grade inflammatory response when compared to younger people (Przybyla et al., 2006). A significant body of work in rodents suggests that the inflammatory response with old age is associated with reductions in muscle mass and function (Hardin et al., 2008; Langen et al., 2004).

It has been observed that the extent of chronic low-grade inflammation can be alleviated by regular physical activity, which is partly due to adaptations within skeletal muscle that alter the types or concentrations of cytokines and growth factors that are released by either skeletal muscle or adipose tissue and improve muscle tissue to reduce the infiltration by immune cells (Drenth et al., 1995; Ostrowski et al., 1998). The concept of 'skeletal muscle as an endocrine organ' has received attention in the past few years after observations that muscle releases molecules after strenuous exercise (Petersen and Pedersen, 2005), which subsequently affect the metabolic adaptation to exercise training (Pedersen and Febbraio, 2012). For example, skeletal muscles can release very high levels of cytokines (such as IL-6) into the circulation after intense exercise, but the cytokine concentrations return back to normal within around 24-48 hrs. Thus, it is important to distinguish between the acute inflammatory responses to exercise, which are widely seen as positive and important for muscle adaptation, from chronic low-grade systemic inflammation, which is regarded as a negative consequence of ageing or disease indicating chronic activation of immune cells or tissue damage.

Interleukin 6 (IL-6) has been proposed as a key indicator of the acute inflammatory response to exercise, as it is secreted from adipose, skeletal muscle and immune cells (Lyngsø et al., 2002; Steensberg et al., 2000). Although IL-6 seems to be involved in the early stages of exercise adaptations, IL-6 has been seen to have chronic anti-inflammatory effects, through the inhibition of TNFα and IL-1 (Schindler et al., 1990). The evidence thus far suggests that circulating IL-6 after a bout of intense exercise markedly increases, sometimes up to 100-fold (Pedersen and Fischer, 2007), however it is dependent upon exercise duration (Reihmane et al., 2013), intensity (Ostrowski et al., 2000) and the size and type of muscle mass recruited (Helge et al., 2011). IL-6 also has links to increased lipolysis and increased fat oxidation (Bruce and Dyck, 2004), whilst also increasing glucose production during muscle contraction (Febbraio et al. 2004).

As well as skeletal muscle being identified as a key endocrine organ, releasing key cytokines such as IL-6 described above, adipose tissue also contribute to the chronic inflammatory

state (Tilg and Moschen, 2006) and release other molecules, known as adipokines. The described effects of circulatory adipokines thus far is wide ranging, they have been associated with insulin resistance (Steppan et al., 2001), insulin sensitivity (Tschritter et al., 2003), rates of fatty acid metabolism (Dyck et al., 2006) and severity of obesity (Considine et al., 1996). Among these adipokines, adiponectin has received a lot of research interest. Adiponectin is released from white adipose tissue and circulating adiponectin concentrations are lower in people who are obese or have metabolic syndrome or cardiovascular disease. Conversely, intense exercise has been shown to increase circulatory adiponectin concentrations (Simpson and Singh, 2008). Another adipokine, Adipsin, was the first adipokine described (Cook et al., 1987) and is modestly increased in circulation of obese participants (Cianflone et al., 2003). The effect of adipsin is to increase the rate of fatty acid re-esterification, thereby increasing body fat stores (Van Harmelen et al., 1999; Maslowska et al., 1999). Despite this, there is little evidence to suggest that circulating adipsin concentration is altered after acute or chronic exercise; however, those who experience weight loss have been seen to reduce adipsin concentration, therefore adipsin levels seem to be driven by levels of adiposity (Napolitano et al., 1994). Resistin is also an adipokine that has been strongly linked to insulin resistance, hence “resistin” (Steppan et al., 2001), with its effects believed to be mediated through the interference of glucose uptake at the cellular level (Moon et al., 2003). Acute exercise has not been seen to reduce circulatory resistin levels, however chronic exercise training has been seen to reduce circulatory resistin levels in diabetic patients (Monzillo et al., 2003).

It is possible that exercise interventions that decrease body fat mass have an effect on the circulating concentration of adipokines (Bouassida et al., 2010). As yet, it remains unclear as to whether circulating adipokine concentration could be related to cardiorespiratory fitness as a marker of health.

2.8 Endurance and Resistance Training

It is common knowledge that exercise is beneficial for health and has even been portrayed as a ‘medicine’ by some clinical practitioners (Sallis, 2009) and by ACSM health initiatives, but there are different types of exercise. Exercise training can be broadly placed into two categories; these are endurance type training activities, and resistance training activities.

Endurance Type Training

Endurance type training is normally centred on physical activities that are aerobic in nature, such as steady state running or cycling and for the most part, it is aimed at improvement of VO_2max and the oxidative capacity of skeletal muscle (Hawley, 2002). Repeated bouts of endurance training promote physiological adaptations to cardiopulmonary and neuromuscular systems. These adaptations include increased muscle oxidative enzymes and capillarisation, altered substrate utilisation towards greater rates of fatty acid oxidation while also increasing storage of muscle glycogen, and adaptations within the cardiovascular system including increased total blood volume and erythrocyte numbers that together increase cardiac output and supply of oxygen to working muscles, in turn increasing VO_2max.

Within muscle, the molecular regulators and some by-products of muscle contraction and energy turnover are sensed, and this leads to changes in gene expression that promote the adaptations synonymous with endurance exercise, such as mitochondrial adaptations (Gollnick et al., 1973; Dudley et al., 1982). Turnover of Adenosine Tri-Phosphate (ATP) during continued muscle contractions causes increases in Adenosine Di-Phosphate (ADP) and Adenosine Mono-Phosphate (AMP) (Martini and Nath, 2009). There are also increases to intracellular Hydrogen (H+), Sodium (Na+), Calcium (Ca_2+) and Carbon Dioxide (CO_2), as well as decreases in potassium (K+) and its increased accumulation in t-tubules that contribute to cellular stress and to fatigue. These metabolite changes occur as the utilisation of glucose and fatty acids increases to regenerate ATP through glycolysis (glucose) and beta-oxidation in the mitochondria (fatty acids). In particular, the transient influxes of Ca+ activate calcium-sensitive signalling molecules, such as CaM-kinases, and the AMP activates AMPk and these molecules influence subsequent gene expression of proteins involved in oxidative phosphorylation (Atherton et al., 2005). Many studies have seen that peroxisome proliferator-activated receptor-gamma coactivator alpha (PGC-1α) is the key regulator of this improved substrate metabolism and a shift toward fatty acid oxidation from carbohydrate, making it a key molecular regulator of lipid metabolism (Liang and Ward, 2006). An increase in PGC-1α is thought to be due to a number of signalling pathways promoting gene expression, from p38 mitogen activated protein kinase (p38 MAPK) (Akimoto et al., 2005) to Calmodulin-Dependent Kinase (CaMK) (Wu et al., 2002) or AMP kinase (AMPk) (Coffey and Hawley, 2007).

The mitochondrial biogenesis associated with endurance training improves energy supply to the working muscle to delay the onset of fatigue during intense exercise (Phillips et al., 1996; Holloszy and Coyle, 1984). This increased mitochondria also facilitates oxidation of fatty acids, which provides a high amount of energy for a longer period of time, giving significant performance and endurance benefits but also improved health by reduction of free fatty acids and their metabolites and intermediates, such as diacyl-glycerol (DAG), which can interfere with the insulin signalling cascade and cause insulin resistance (Koya and King, 1998; Isacco et al., 2013; Helge et al., 2001). With endurance exercise, this effect is believed to be mediated by increased ATP turnover in exercise, leading to higher levels of ADP and AMP, where the latter activates AMPk. This in turn increases the concentration and activation of Glucose Transporter Type 4 (GLUT4) (Torjesen et al., 1997; Dela et al., 1992; Devlin et al., 1987), which is the primary method of Glucose uptake in skeletal muscle, thereby increasing insulin sensitivity by increasing glucose uptake. Even a single bout of endurance training has observed increased insulin sensitivity (Greiwe et al., 1999; Devlin et al., 1987).

Resistance Type Training

Resistance type training involves brief, high force anaerobic type muscle contraction and normally aims to bring about muscle hypertrophy (growth). The training modality involved tends to be weight lifting or other high-resistance exercises. This type of training results in a number of key adaptations including a higher basal metabolic rate, increase in bone mineral density and a maintenance of physical function into older age (Kraemer et al., 1996).

The most obvious and common adaptation to resistance training is an increase in muscle size and cross sectional area (CSA), with this adaptation being almost universal regardless of gender, age and training status (Abe et al., 2000; Tracy et al., 1999). This muscle hypertrophy is believed to come about in the form of satellite cell proliferation, which then secrete muscle specific proteins to increase fibre size (Darr and Schultz, 1987) after myofibrillar growth. Petrella et al. studied 66 participants (35 males and 31 females aged 20-75) and classified them by their muscular content of satellite cells, with those having the highest concentration of satellite cells in muscle showing the highest amount of hypertrophy as a result of 16 weeks of knee extensor exercise (Petrella et al., 2008). This finding adds evidence to the case of satellite cell recruitment as a mechanism of skeletal

muscle hypertrophy *in vivo*. The high mechanical loading stimulus on skeletal muscle in heavy resistance training therefore brings about large amounts of hypertrophy as a main adaptation to the exercise. A complex molecular signalling pathway also accompanies this process, with much of the attention in the literature being focussed on mammalian target of rapamycin (mTOR). The mTOR is activated by Insulin like growth factor 1 (IGF1) as well as other intramuscular signals and is the best characterised of the muscle hypertrophy promoting factors, with significant increases in local IGF1 concentrations being directly related to muscle growth (Musarò et al., 2001). IGF1 binding at its cell-membrane receptor eventually activates Phosphoinositide 3-Kinase (PI3K) within the cytosol, which in turn activates Protein Kinase B (AKT). AKT is a key regulator of mTOR and therefore protein synthesis for muscle hypertrophy, with studies showing increased expression of AKT leading to increasing fibre size (Bodine et al., 2001; Pallafacchina et al., 2002; Lai et al., 2004). mTOR in this process seems to have emerged as a main regulator of protein synthesis and therefore muscle growth (Hay and Sonenberg, 2004) and concomitant strength gains (Terzis et al., 2008) and is controlled by a number of factors such as nutrient availability, upstream growth factors and energy turnover (AMPK as described previously). mTOR has its effect by recruitment of ribosomes for mRNA translation for protein synthesis (Hannan et al., 2003).

Protein synthesis is the key response to any kind of resistance training through the pathways listed above. This protein synthesis leads to hypertrophy of the muscle cells, with the result being an increase in fibre cross sectional area (Chesley et al., 1992; Breen and Phillips, 2012).

It may be assumed that combining endurance and resistance training would give maximum health benefit and the adaptations of both modes of exercise. However, there is conflicting literature as to whether this is true, and it has been suggested that concurrent training in both endurance and resistance types, muscular adaptation is blunted, particularly skeletal muscle hypertrophy (Bell et al., 2000; Sale et al., 1990). Although a lot of evidence suggests that there is an interference effect of concurrent training, there is little known about the molecular pathways that determine the response, as well as the interference effect only being seen with long term concurrent training, with only positive effects being seen in short term interventions (Fyfe et al., 2014). It is likely that concurrent endurance and resistance training will promote both mitochondrial adaptations and muscle size, but these specific

adaptations are not as extensive if either endurance training alone (mitochondrial) or resistance training alone (hypertrophy) had been completed.

2.9 Exercise, Ageing and the Master Athlete

Between 2001 and 2031, the number of people aged over 65 is predicted to increase by 53%, increasing the workload and financial strain on the National Health Service due to an increase in age related illness (Majeed and Aylin, 2005) and more specifically, an increased risk of falls in the elderly (Rubenstein, 2006). Falls are a major issue to an ageing population, causing debilitation and social isolation on the elderly, which leads to not only physical impairment but also has an impact on mental health and a decline in confidence (Clemson et al., 2012).

An often overlooked element to the decrease in skeletal muscle mass with age (sarcopenia), is a significant reduction in physical activity as we advance in years which might be an important factor in atrophy of muscle fibres, particularly Type II fibres (Lexell et al., 1988). This reduction in fibre size however has been reversed in some cases by exercise training, and primarily, resistive training is yielding similar percentage increases in fibre size and rates of hypertrophy to younger participants (Häkkinen et al., 1985; Ferri et al., 2003). Studies of this type suggest that elderly muscle is still responsive to exercise stimuli, maintaining its cross sectional area, as well as its contractile quality (Frontera et al., 1991). Exercise has been shown as a successful and effective strategy to reduce the risk of falls in the elderly and maintain or even improve health. However, it is not clear what type of exercise would be most beneficial, although the latest recommendations are that older people should be active every day and perform resistance and balance training (Sherrington et al., 2008). Robertson and colleagues studied 1,016 males and females aged 65 to 97 years with 612 of these taking part in an exercise intervention of individually prescribed muscle strengthening and balance training programme (Robertson et al., 2002). The number of falls in the exercise group was 35% lower compared to a non-exercising control group, as well as fewer injuries being recorded from falls in the exercising group compared to the non-exercising controls.

It is therefore clear that high physical activity levels into older age is associated with better health and mobility (Hirvensalo et al., 2000). To this end, it is instructive to study Master Athletes, who are competitive athletes aged 35+ yrs. These athletes reach high levels of physical performance despite advancing age and have been described as a “model of

successful ageing" (Hawkins et al., 2003). For example a winning time of 12s was recorded in the 100m sprint at the first modern Olympics in 1896, however this has now been surpassed by a 61 year old male with a time of 11.7s (Tanaka and Seals, 2008). Similarly in the marathon, in 1896 the winning time was 2h:58m:50s, the master athletics record is now 2h:54m:05s set by a 73 year old male (Tanaka and Seals, 2008).

Cross sectional studies have reported improved muscle structure (Korhonen et al., 2006) and metabolic capabilities (Trappe et al., 2013) in master athletes, regardless of athletic discipline, over age matched controls. Korhonen et al. observed a ~31% higher maximal force output and ~47% higher maximal rate of force development in sprint master athletes (aged 70-84 years) when compared to 70 year old non athletes in a study carried out by the same laboratory (Häkkinen et al., 1998; Korhonen et al., 2006). Therefore, maintenance of physical activity into old age is paramount to healthy ageing, to protect against the effects of age related muscle wasting (sarcopenia) (Drey et al., 2014; Genton et al., 2011), the concomitant increase in fat accumulation (Hughes et al., 2002) and a heightened inflammatory state (Petersen and Pedersen, 2005). Although age related declines in muscle power are seemingly inevitable, regardless of high training volume, there is a large "saving effect" in Master athletes, showing a higher muscle power output over all age matched participants (Michaelis et al., 2008). There also seems to be an athletic event-specific effect for this sparing of muscle, with both male and female master sprint athletes showing much higher muscle power maintenance through the lifespan than endurance athletes or age matched participants (Michaelis et al., 2008; Runge et al., 2004).

As discussed in section 2.5 of this chapter, mitochondrial function and capacity reduces with increasing age (Petersen et al., 2003; Peterson et al., 2012), however a potential sparing effect is observed when individuals participate in high volumes and intensities of exercise into older age. Citrate synthase activities from vastus lateralis biopsies were similar in a young, endurance trained group (6 males, 5 females. Mean age= 26±1 years, VO_2peak=66.7±1.7 ml/kgFFM/min) and an older, endurance trained group (6 males, 4 females. Mean age=65±2 years, VO_2peak=54.0±3.6 ml/kgFFM/min) (Lanza et al., 2008). Furthermore, in the same study by Lanza et al. (2008), an older sedentary group (6 males, 4 females. Mean age=65±2 years, VO_2peak=36.5±1.6 ml/kgFFM/min) had a significantly lower PGC-1α protein expression in muscle than older endurance trained participants. These data suggest that endurance training impacts positively on the mitochondrial capacity and

function of older muscle, improving the health span of individuals by ways of maintenance of metabolic potential in skeletal muscle with increased age, however little is known as to whether this capacity may differ between differing athletic events or muscle loading (Joseph et al., 2015).

VO_2max is suggested to decline from the age of 25-30 years of age in most adults, with an even more pronounced decline in older age of both males and females (Tanaka et al., 1997; Eskurza et al., 2002; Pimentel et al., 2003). This decline of maximal oxygen uptake is illustrated in *Figure 2*, combining the findings of a number of studies on both ageing athletes or highly active individuals (Pollock et al., 1974; Tanaka et al., 1997) and healthy age matched control participants (Jackson et al., 1996; Jackson et al., 1995).

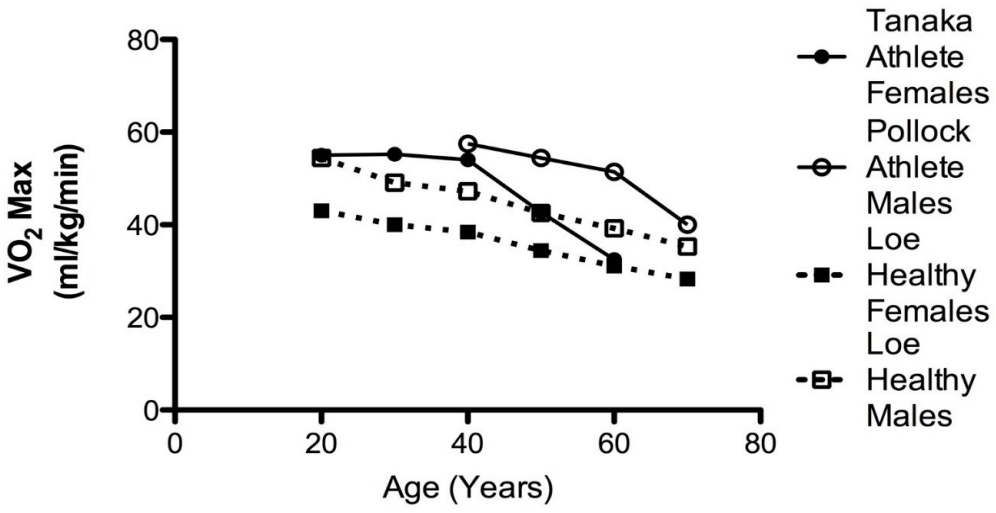

Figure 2: Comparison of a number of studies comparing the decline of VO_2max (ml/kg/min) over lifespan in trained and untrained participants.

Adapted from data in "Physiological Characteristics of Champion American Track Athletes 40 to 75 Years of Age" (Pollock et al., 1974), "Greater rate of decline in maximal aerobic capacity with age in physically active vs. sedentary healthy women" (Tanaka et al., 1997) and "Aerobic Capacity Reference Data in 3816 Healthy Men and Women 20-90 years" (Loe et al., 2013)

Ranges of this decline have varied through a number of studies, but classically are approximately 10% per decade (ml/min/kg) in both sedentary and non-athlete, healthy populations (Hossack and Bruce, 1982; Jackson et al., 1995; Jackson et al., 1996; Tanaka et al., 1997).

Despite a larger training volume, endurance trained participants have a tendency to have a similar percentage decrease in VO_2max over their lifespan of approximately 10% (Pollock et al., 1974; Tanaka et al., 1997) and 15% in longitudinal studies (Pollock et al., 1997). Other evidence suggests that this decline in VO_2max in highly trained participants is even higher, with a study reporting 29% decline per decade in 42 male athletes (Katzel et al., 2001) and another reporting 24.5% declines per decade in 86 male athletes (Hawkins et al., 2001). This lack of consistency in evidence suggests that there is still some confusion as to the effect of exercise and training status on VO_2max and its maintenance through the lifespan, which is likely explained by cessation of training, or a significant reduction in training volume over the age of 60 years of age (Buskirk and Hodgson, 1987). The effect that exercise training has into our old age is therefore still an open question, with an inevitable decline seeming certain, but to what extent SIT or endurance training can attenuate this decline remains a subject of debate. To date, no studies have directly compared the sprint-trained masters athletes with endurance trained masters athletes for the combination of physiological characteristics including: maximal muscle power, VO_2max and rates of fatty acid oxidation in cross-sectional studies so that their relative 'declines' with advancing age can be compared between the two training modalities. Studying Masters Athletes who have trained for many years will also provide some insights into the extent to which the short-term gains following SIT in previously sedentary people are maintained with very long term training. For instance, SIT in young males increases VO_2max (in ml/kg/min) to a similar extent as conventional endurance training (Gibala et al., 2006; Burgomaster et al., 2008). It therefore follows that sprint and endurance athletes might have similar VO_2max.

In conclusion, there is strong evidence to suggest a causal link between increased physical activity and improved health outcomes. Individuals with increased levels of physical fitness and activity are significantly less likely to develop adverse health issues (LaMonte et al., 2005) as well as maintain a higher level of metabolic and physical fitness throughout their lifespan (Tarpenning et al., 2004; Trappe et al., 2013). This improvement and maintenance of health through physical activity poses the question, can very low volume but extremely intense exercise bring about these substantial health and performance-related benefits in a cohort recruited from the general population?

Chapter 3

Body Fat Mass, VO_2max And The Rates Of Fatty Acid Oxidation During Exercise: The Effects Of 12 weeks Sprint Interval Training And Gender Comparisons

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Chapters One and Two suggest that SIT is an effective method of improving cardiorespiratory fitness and fatty acid oxidation. This chapter examines the possibility of gender dimorphism in the response to SIT, as well as the feasibility of introducing this training method to the general population.

The findings of this chapter are published as:

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3.1 Introduction

Aerobic-type training of between 225 – 420 min/week is recommended to those who wish to lose fat mass by increasing physical activity levels (Swift et al., 2014), but very high intensity, short duration training might also be effective (Trapp et al., 2008; Shepherd et al., 2010). 'Sprint interval training' (SIT) sessions require participants to perform repeated short duration (often ~30-60 sec) bouts at very high or maximal power output, each separated by a recovery period at very low intensity exercise (Babraj et al., 2009; Little et al., 2011b). This exercise differs from conventional aerobic training that typically involves prolonged and continuous exercise, which during cycling is equivalent to around 25% of the peak leg power output (Zoladz et al., 2000) that is met principally by the aerobic energy systems (Talanian et al., 2007; Garber et al., 2011).

Despite the intensity, duration and energetic differences between short-duration, high intensity activities and endurance activities lasting around 60 min per session, they promote similar physiological adaptations (Gist et al., 2014). This includes improvements to the maximal rate of oxygen uptake (VO_2max) (Gist et al., 2014; Sloth et al., 2013) which occurs in association with improvements to skeletal muscle capillarisation (Jensen et al., 2004; Iaia et al., 2009), enzymes of fat metabolism (Burgomaster et al., 2008; Talanian et al., 2007; Little et al., 2011a; Little et al., 2011b; Perry et al., 2008; Whyte et al., 2010; Stensvold et al., 2010; Trapp et al., 2008; Tremblay et al., 1994) and improved insulin sensitivity (Babraj et al., 2009; Little et al., 2011b; Shepherd et al., 2013; Gibala et al., 2012). All of which contribute to better health-status and physical performance.

There are also reports that very high intensity, short duration training can lead to loss of total body fat mass (Macpherson et al., 2011; Hazell et al., 2014; Gillen et al., 2013; Trapp et al., 2008). These studies used weekly durations of around 9 min (Macpherson et al., 2011; Hazell et al., 2014; Trapp et al., 2008) and 30 min (Gillen et al., 2013) without controlling food intake. Whether even shorter duration exercise will be effective at reducing fat mass in males and females remains unknown.

Compared with males, females have lower relative muscle mass and higher relative fat mass (Bijlsma et al., 2013), slower skeletal muscle contractile properties (McPhee et al., 2014; Simoneau et al., 1985b), oxidise fatty acids at higher relative exercise intensity (Venables et al., 2005) and have less reliance on muscle glycogen stores following bouts of repeated

sprinting (Esbjörnsson-Liljedahl et al., 1999). These differences may be partly a result of differences between males and females in concentrations of circulatory steroid hormones (see chapter 2.5), with some evidence suggesting oestrogen signalling is a mechanism behind a higher rate of fat oxidation in females over males (Oosthuyse and Bosch, 2010). Wiik et al. (2005) saw a 3-5 times lower level of oestrogen receptor mRNA in 10 moderately active males (mean age= 24±3, VO_2max= 46±6) compared to 10 highly trained endurance athlete males (mean age= 22±3, VO_2max= 73±5) ($p<0.01$), suggesting that oestrogen activity may be upregulated due to increased levels of physical training. Interestingly, the endurance trained males also showed a correlation between VEGF mRNA (a potent angiogenic mediator, suggesting potential effects on oxygen uptake) and oestrogen receptor mRNA ($r=0.54$, $p<0.05$) (Wiik et al., 2005). Remarkably, there is limited evidence in humans available to examine if eumenorrheic females respond in the same manner as males in terms of oestrogen receptor upregulation after exercise. Despite the potential for oestrogen to be upregulated by exercise training, fluctuating physiological levels of oestrogen in females throughout the menstrual cycle do not seem to impact substrate utilisation during exercise, with human studies suggesting no difference between menstrual phases after moderate intensity exercise bouts performed at different stages of the menstrual cycle in the same females (Jacobs et al., 2005; Horton et al., 2002). Therefore, it remains unknown as to whether males and females show similar adaptations to fatty acid metabolism and body composition after SIT, but such gender differences remain unknown because the overwhelming majority of research into SIT included only young males (usually university students) who trained for between 2-6 weeks.

The primary aim of this study was to recruit males and females from the general population to complete 12 weeks cycling SIT and monitor changes relating to health and physiological function. The primary outcomes were the changes in body fat mass, VO_2max and FATmax after 12 weeks training. The secondary outcome was the comparison between responses of males and females in order to assess possible gender differences in these responses. It was hypothesised that VO_2max and maximal rates of fat oxidation would be higher after training and that body fat mass would be lower after training. Based on the limited available evidence, it was also expected that females would increase FATmax more than males after training and lose more fat mass.

3.2 Method

Participants

The study conformed to the latest revisions of the Declaration of Helsinki (World Medical Association, 2013) and was approved by the Faculty of Science and Engineering Ethics Committee at Manchester Metropolitan University (application number: SE111216). Volunteers provided written informed consent prior to participation. Those with a history of cardiovascular, neuromuscular or metabolic disease were excluded as well as people whom had suffered a leg fracture within the past two years. The aim of recruitment was to include a large sample of males and females in order to reduce the risk of selection bias that is inherent to small sample studies. This was particularly important for addressing the secondary aim to compare gender training responses and necessary due to the decision to recruit from the general public (rather than university student population), where we anticipated variability in terms of age, activity levels, socioeconomic status and other factors that may influence baseline fitness and training responses. This recruitment strategy differs from previous studies that almost all included single gender cohorts of n~10. The target was to include similar numbers of males and females of similar in order to more directly compare the effects of the SIT on the participants.

The purpose of the secondary outcome (compare gender in training responses) was not to identify mechanistic causes of any possible gender differences, rather it was to assess evidence of the existence, as no previous studies have provided that evidence. Therefore, inclusion of as many participants as possible within the inclusion window timeframe took precedence over the possibility to restrict the point of inclusion for females to specific menstrual phase. Information relating to menstrual cycle phase and contraceptive use of the female participants was not available in this study. However, the 12 week training programme ensured that female participants attended pre- and post-training assessments in the same menstrual phase and the training programme was sufficient duration for any cyclic hormonal changes to have gone through three full phases (assuming an average 28 day menstrual cycle). Participants are categorised by gender and referred to as males and females, in accordance with the American Physiological society recommendations (The American Physiological Society, 2012). The categorisation of gender is due to the

participants in this study self-selecting male or female in a questionnaire and no physiological assessment of sex being undertaken in this protocol.

Participants were recruited from university and a local health club's notice boards as well as a national newspaper story on the study for a period of around 2 months before the study began. A total of 91 participants (51 males and 40 females) were eligible and began the training study at baseline, however, a total of 41 participants (24 males and 17 females) successfully completed the 12-week SIT intervention and post-training measurements. The participants that did not complete the training (27 males and 23 females) commonly stated the reason for withdrawal from the study was a lack of time to complete the training due to other commitments, but no further follow up of these participants was undertaken because it was beyond the scope of the study to monitor motivations and drop-outs. Completing participant characteristics are shown in *Table 2*.

Design

Participants attended the research laboratory in the morning following a 12 hr overnight fast and having avoided strenuous activity and alcohol in the 24 hours prior to the testing. After providing informed consent and resting for at least 15 min, a 10 mL blood sample was collected into EDTA collection tubes from a vein in the forearm and then a second sample during the same laboratory visit was collected 20 min after completion of the physiological testing. Blood was immediately centrifuged at 1500g for 10 minutes at 4^{o}C, the plasma separated and stored at -80^{o}C until analysis. This protocol was repeated on the second visit after training (12 weeks) to the laboratory. Each laboratory session included no more than 4 participants.

Body fatness was assessed from a total-body dual-energy x-ray absorptiometry (DEXA) scan using a Lunar Prodigy Advance (GE Healthcare; EnCore version 10.50.086). Total body fat, trunk fat and leg fat mass were measured on initial visit at baseline, then again after training at 12 weeks. Participants were scanned in the supine position with both hands by their sides and placed within scanning limits of the equipment, with the participant's feet secured via a manufacturer provided strap to ensure consistency. The participant was scanned by an experienced and authorised user of the DEXA scanning equipment and placed in the same posture for each scan, taking place between 09:00am to 10:00am on testing days.

Participants wore light, gym style clothing and removed all removable metal objects from their person before scanning. The analysis was carried out in EnCore Software (GE Healthcare, Madison, WI, USA) by adjusting each individuals scanned image to the regions of interest in the software package to ensure all scans consistent. The author only throughout the study carried out this analysis. An experienced operator calibrated the equipment daily before use using the manufacturers (GE Healthcare) provided spinal block. The variance in measurements in a similar device (Lunar DPX-L densitometer, deemed to be similar by the manufacturer, GE Healthcare) after scanning the same healthy participants (n=20) over 4 consecutive days yielded a 1% variance for fat free mass and 2% variance for fat mass (Kiebzak et al., 2000). DEXA scanning was carried out pre (0 weeks) and post (12 weeks) training before any exercise during laboratory sessions. The time needed to scan each participant was variable from each individual dependent upon the individual's height, however the manufacturer claims the total body scan programme, used in this study, has a typical duration of 4min 55sec, which exposed each participant to a total effective radiation dose of 0.4 µSv (GE Healthcare, 2009).

Maximal rates of oxygen uptake and fatty acid oxidation were measured during cycle ergometry (Jaeger Ergocycle, Germany) with VO_2, VCO_2 and heart rate (HR) measurements collated by a breath by breath metabolic cart (Cortex Biophysik, Germany). The metabolic cart was switched on approximately 30minutes before calibration, which was carried out each morning of laboratory testing by the author. No more than 4 participants were measured per calibration. Calibration included input of air temperature and atmospheric pressure obtained from a barometer, volume calibration with an air syringe and gas sampling with known concentrations of 16% O_2 and 5% CO_2 with an additional ambient air sample consisting of concentrations of 20.93% O_2 and 0.03% CO_2. In order to avoid variations in VO_2 due to circadian rhythms, all data collection was carried out at the same time each morning before and after the SIT program. This protocol and analysis has been well established in the literature, with Meyer et al reporting only a 0.3 l/min variation between test and retest in 15 healthy individuals, with a correlation co-efficient of 0.969 for VO_2, suggesting a very strong reliability (Meyer et al., 2001).

The seat height was adjusted on the cycle ergometer for each individual participant due to variance in height. With the participant standing, seat height was adjusted to approximately

hip level, so that when seated and feet secured to the pedals with hips and torso facing to the front of the cycle, the leg was almost fully extended at the lowest position in the cycling exercise. The participant rested on the cycle for 2 minutes with the mask worn before beginning the protocol in order to acclimatise to the equipment being used. Workload began at 50 W and a cadence of 70 rpm was maintained throughout. Workload was increased in increments of 50 W for males and 30 W for females every 3 minutes until the respiratory exchange ratio (calculated as VCO_2/VO_2) was higher than 1.0 for at least 1 min. From this point onwards, increments of 20 W were given every minute until volitional exhaustion. The VO_2max was then calculated from the mean VO_2 value of the 30 seconds data collection phase that gave the highest value, which in all participants occurred during the final minute of the exercise test.

Estimation of rates of fatty acid oxidation were carried out by stoichiometric equation as described by Frayn et al. (1983) (with the assumption that urinary nitrogen excretion rate was negligible):

FATmax (g/min) = $(1.67 \times VO_2) - (1.67 \times VCO_2)$

In addition, maximal rate of fatty acid oxidation (FATmax) was calculated as the highest data point situated on a polynomial trend line after plotting all fat oxidation data points (g/min) from each individual as a function of exercise intensity (%VO_2max). The highest value on the polynomial line of best fit of calculated rates of fatty acid oxidation (plotted from 50 W through to the point at which RER was higher than 1.0) was used as an individual's FATmax result. This method has been utilised several times in the primary research literature (Achten et al., 2002; Croci et al., 2013; Venables et al., 2005) and has been reported to have good reliability (Croci et al., 2014).

Fasting blood lipoprotein profile, insulin and glucose

Four fasting plasma samples were collected as described on page 39, 1) at baseline in the fasted and rested state; 2) at baseline in the fasted state but 20 min after completion of the physiological testing session; 3) after the 12 week exercise intervention in the fasted and rested state; 4) after the 12 week exercise intervention in the fasted state but 20 min after completion of the physiological testing session.

Biochemical markers were determined from fasting plasma samples using the RX Daytona auto analyser (Randox Laboratories, UK). High-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (CHOD-PAP), triglycerides (GPO-PAP) and glucose (GOD-PAP) concentrations were determined in duplicate and an average value calculated by the analyser.

The clinical chemical analyser (Randox Daytona, Randox Laboratories, UK) utilises a photometric technique via measurement of the intensity of dye produced during reagent reactions with the target marker in plasma. In the LDL and HDL concentration determination, cholesterol esterase, cholesterol oxidase and catalase are added sequentially in order to eliminate HDL/LDL (depending on kit), V-LDL and chylomicron from the serum sample. HDL/LDL is then released after addition of specific detergents in the reagents and quinone dye is produced, which is directly proportional to the concentration of HDL/LDL and read by the chemical analyser at 600 nm light intensity. The determination of total cholesterol utilised an enzymatic end point method, in which cholesterol esterase is added to hydrolyse cholesterol esters into cholesterol and fatty acids, then oxidation by cholesterol oxidase cholestene-3-one and peroxide (H_2O_2), the latter of which then reacts with added peroxidase to form the marker quinoneimine which is then determined by absorbance. Triglycerides were determined by an enzymatic hydrolysis by lipases into glycerol and fatty acids, followed by glycerol and ATP forming glycerol-3-phosphate and ADP catalysed by glycerol-kinase. Glycerol-3-phosphate and oxygen is then hydrolysed to form dihydroxyacetone, phosphate and peroxide, the latter of which is then oxidised by peroxidase to again form the marker quinoneimine, following the same method as cholesterol determination. Similarly, determination of glucose is by quinoneimine absorbance, by oxidation of peroxide formed from oxidation of glucose by glucose oxidase to form peroxide and gluconic acid.

Circulatory insulin concentration measured using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) (EZHI-14K Human Insulin ELISA, Merck Millipore, Darmstadt, Germany). This procedure included an overnight incubation period in order to improve the sensitivity of the assay. Assay plates were analysed using a 96 well spectrophotometer (MultiSkan GO, Thermo Fisher Scientific, MA, USA) and concentration calculated from a standard curve generated by absorbency readings provided by the spectrophotometer and

relevant concentrations corresponding to absorbency by the manufacturer of the assay (Merck Millipore, Darmstadt, Germany). Samples were run in duplicate and an average value calculated.

Precision and CV(%) for these assays as provided by the manufacturer are supplied in *Table 1*.

Table 1: Sensitivity characteristics of analytes

Analyte	Minimum Detectable Concentration (pg/ml)	Intra-Assay (%CV)	Inter-Assay (%CV)
Glucose (mmol/L)	0.335	1.96	5.87
Insulin (µU/ml)*	1.000	5.96	10.3
Triglycerides (mmol/L)	0.134	1.55	2.58
Total Cholesterol (mmol/L)	0.865	1.67	1.00
High Density Lipoprotein (mmol/L)	0.189	1.45	2.40
Low Density Lipoprotein (mmol/L)	0.189	1.14	2.21

**Insulin concentration was determined utilising an ELISA technique with sensitivity characteristics provided by the manufacturer (Merck Millipore, Darmstadt, Germany) from 6 assays involving 8 serum samples each ran in duplicate. All other values were obtained from a clinical chemical analyser (Randox Daytona). Sensitivity characteristics are those provided by the manufacturer (Randox Laboratories, UK). Intra and Inter-Assay %CV is calculated from 20 results for each analyte.*

Insulin sensitivity was estimated using the Homeostatic Model of Assessment (HOMA) as described by Mathews et al. (1985) which was calculated as:

Fasting Plasma (Glucose [nMol/L] x Insulin [µMol/L]) ÷ 22.5

Protocol

Laboratory sessions

All laboratory sessions took place in the labs at Manchester Metropolitan University. All participants arrived at 9am on the day of their allocated session, whereby the study was explained to each participant, the measurements that would be taken, as well as any risks associated with their involvement in the study. The participants then all gave informed consent to take part in the study. A baseline blood sample was taken from the ante cubital vein of the forearm from each participant. MRI and DEXA imaging measurements were taken first in order to avoid any exercise induced muscle volume changes. After satisfactory

imaging, the participant carried out the VO_2max protocol described previously, after which they rested for approximately 10-15 minutes. Knee extensor dynamometry measurements were also carried out, for which the protocol can be found in Chapter 4. After a short rest again of 10-15 minutes, the participant then carried out a familiarisation session of SIT, whereby an investigator guided the participant through the set up and operation of the cycle ergometers used during training. This also included discussion of health and safety elements and to stop exercise immediately if injury or serious illness occurred. The participants were then allowed to rest for around 10-15 minutes, before giving a second blood sample as described previously. The participant was then advised to train 3 times per week for 12 weeks until a return session at the laboratory was organised and carried out.

Sprint Interval Training

Participants were asked to maintain their usual dietary and exercise habits throughout the intervention. SIT was completed on cycle ergometers (Cateye, Japan). The training consisted of a 2 min warm-up at a self-selected moderate intensity. This was followed by four bouts of 20 seconds 'maximal effort' sprints at a workload that was set at 175% of the workload attained in the VO_2max test. Each of these intervals was separated by 2 min of very low intensity cycling (a workload of approximately 20% of that attained at VO_2max). Thus, each training session lasted less than 10 min and only 80 seconds was completed at an intensity that would be expected to improve physical fitness.

The first training session for each participant was fully supervised in the research laboratory and participants also received clear instructions on the use of the cycle ergometers and the training regimen. Participants trained 3 times per week for 12 weeks using the ergonomic cycles that were provided. The training workload was increased by 5% every two weeks. Gym staff were fully informed of the research and training protocols, they logged the training session and were available to offer advice to research participants if needed during training sessions. Participants maintained a training-log to record workloads during training sessions.

Statistical analysis

Data was analysed using SPSS (v.20 IBM). Initially, the data was tested for normal distribution utilising Kolmogorov-Smirnov and Shapiro-Wilk testing. All data was found to be

normally distributed, therefore data are presented as mean± Standard Error of Mean (SEM), with SEM values utilised due to the cross population nature of the data presented between males and females. Two-Factor repeated measures ANOVA was used to assess adaptations to training and gender comparisons, which also assessed the interaction between participant gender and the effect of training. This gives an estimate of the effect of gender on training adaptations to SIT. Sphericity assumed values were used for p-values generated, however Greenhouse-Geisser correction values were utilised when Mauchly's test of sphericity was violated ($p<0.05$). Greenhouse-Geisser correction values were utilised if the epsilon value was <0.75, if >0.75 Huynh-Feldt correction values were utilised. Pearson's Product Correlation was used to examine relationships between variables. Statistical significance was accepted at $p<0.05$.

3.3 Results

Body composition

Changes to body composition are shown in *Table 2* for males and females. Body fatness (%) decreased after training by 1.2% ($p<0.001$), with males losing more total body (%) and trunk fat (kg) than females (*Table 2*). Total body lean mass increased by 1.2% in males and 0.03% in females after training, but there was no statistically significant gender x training interaction ($p=0.162$), indicating that males and females showed similar changes to total lean mass after training (*Table 2*).

Table 2: Body composition in males and females before and after 12 weeks SIT

	Males (Pre)	Males (Post)	Females (Pre)	Females (Post)	Training effect	Gender effect	Gender x Training
Age (yrs)	38 (2.7)		41 (3.2)				
Height (m)	1.77 (0.16)		1.65 (0.12)				
Total body mass (kg)	80.0 (2.2)	79.2 (2.1)	62.5 (2.6)	62.1 (2.5)	0.070	**<0.001**	0.542
Body Mass Index (kg/m^2)	26.3 (0.8)	26.0 (0.7)	22.2 (0.7)	22.1 (0.6)	0.081	**0.001**	0.554
Total body fat (kg)	17.9 (1.3)	16.6 (1.3)	18.6 (1.4)	18.0 (1.2)	**<0.001**	0.556	0.052
Total Body Fat (%)	22.7 (1.7)	21.2 (1.4)	31.2 (1.7)	31.3 (1.6)	**<0.001**	**<0.001**	**0.015**
Trunk fat (kg)	9.7 (0.8)	9.0 (0.9)	8.1 (0.8)	8.0 (0.7)	**0.014**	0.313	**0.046**
2-Leg fat (kg)	5.7 (0.4)	5.4 (0.4)	7.8 (0.6)	7.0 (0.5)	**<0.001**	**0.001**	0.208
Total body lean mass (kg)	60.4 (1.6)	61.1 (1.6)	39.1 (0.9)	39.2 (1.0)	**0.016**	**<0.001**	0.162
2-Leg lean mass (kg)	21.6 (0.7)	21.0 (0.7)	13.6 (0.3)	13.0 (0.4)	0.185	**<0.001**	0.821

Data are shown as mean (SEM).

Maximal oxygen uptake and fatty acid oxidation

Table 3 shows data for VO_2max and FATmax in males and females. In the untrained state, males were able to utilise an absolute higher amount of fatty acids (in g/min) compared with females (p=0.027) and FATmax (g/min) occurred at a higher absolute workload (W) (p<0.001). Lean mass (kg) was correlated with FATmax (g/min) at baseline (r=0.366; p=0.019), however when separated into males and females, neither males nor females showed a correlation between fat oxidation (g/min) and total body lean mass (kg) (*Figure 3a+b*). The training related change in total body lean mass (kg) was not statistically significantly correlated to training-related change in FATmax (g/min) (r=0.100, p=0.536) or when normalised to body mass (mg/kg/min) (r=0.065, p=0.688).

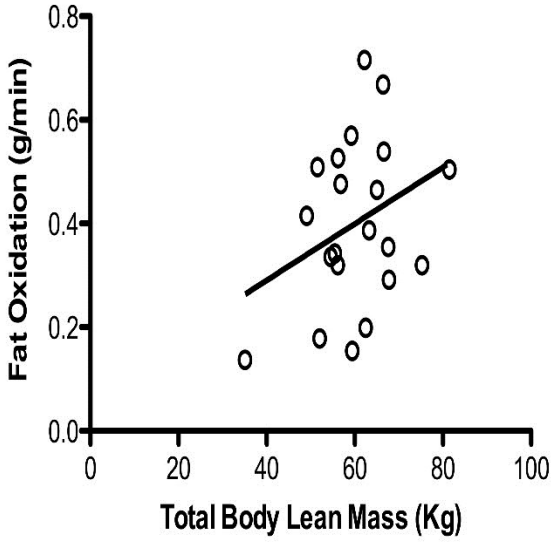

Figure 3a: Total body lean mass is not correlated with FATmax (g/min) at baseline in males (r=0.266, p=0.256)

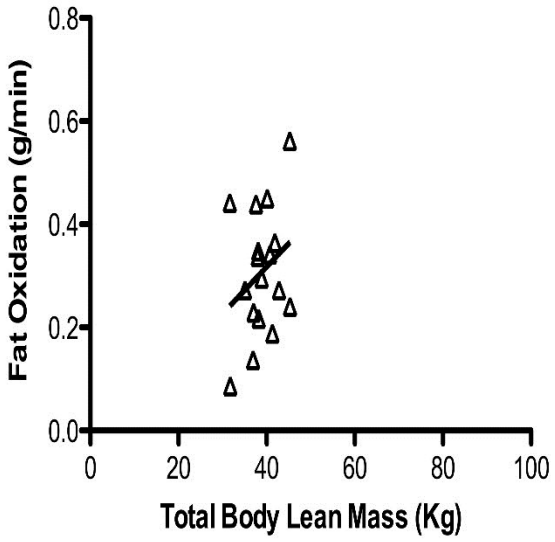

Figure 3b: Total body lean mass is not correlated with FATmax (g/min) at baseline in females (r=0.367, p=0.147)

When normalised to total body mass and to lean mass (kg) FATmax did not differ between males and females (p=0.985 and 0.287, respectively). FATmax (g/min) occurred at a lower %VO_2max in males than females (p=0.023).

After 12 weeks SIT, males and females increased VO_2max (ml/kg/min), with the percentage gains being 18.7% in females and 6.0% in males (gender x training interaction: p=0.009). Males and females showed similar gains in FATmax after training (*Table 3*).

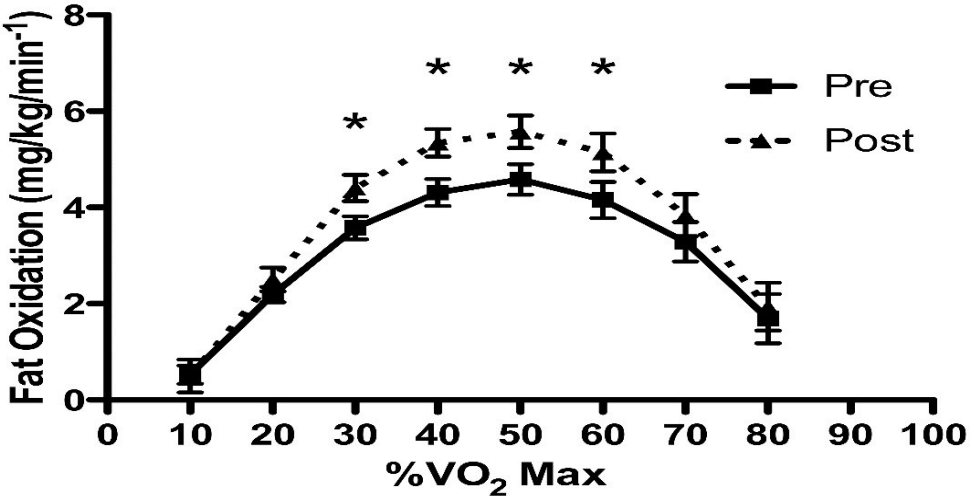

Figure 4: Fat oxidation rates before and after 12 weeks SIT in males and females.

Rates of fat oxidation were higher after training at all intensities ranging from 30 – 60% VO_2max: * indicates significant difference from pre- to post-training (p≤0.05). The highest rate of fat oxidation occurred at 50% VO_2max before and after training.

The gender x training interaction revealed that FATmax occurred at a statistically significantly lower %VO_2max in females after training but slightly higher %VO_2max in males after training (*Table 3)*.

Table 3: Maximal oxygen uptake and rates of fat oxidation measured during exercise in males and females before and after 12 weeks SIT.

	Males (Pre)	Males (Post)	Females (Pre)	Females (Post)	Training effect	Gender effect	Gender x Training
VO_2 max (L/min)	3.49 (0.13)	3.68 (0.15)	1.99 (0.11)	2.37 (0.12)	**0.001**	**<0.001**	**0.049**
VO_2 max (ml/kg/min)	42.91 (1.84)	45.49 (1.48)	33.56 (2.29)	39.84 (2.44)	**<0.001**	**0.005**	**0.009**
HR_{max} (bpm)	177 (3)	178 (3)	175 (4)	174 (4)	0.645	0.977	0.268
RER_{max}	1.21 (0.01)	1.18 (0.02)	1.20 (0.02)	1.21 (0.02)	0.430	0.763	0.214
FATmax (g/min)	0.41 (0.03)	0.49 (0.04)	0.31 (0.03)	0.34 (0.02)	**0.032**	**0.004**	0.465
FATmax (mg/kg/min)	5.12 (0.39)	6.04 (0.40)	5.14 (0.52)	5.77 (0.41)	**0.033**	0.815	0.690
FATmax (mg/kg lean mass/min)	6.90 (0.50)	8.10 (0.62)	7.90 (0.76)	8.87 (0.65)	**0.041**	0.272	0.824
Workload at FATmax (W)	111.6 (9.81)	112.80 (7.56)	62.94 (5.43)	62.73 (6.76)	0.961	**<0.001**	0.834
FATmax (as %VO_2max)	51.43 (1.88)	51.83 (1.65)	60.26 (3.61)	53.25 (2.18)	0.229	0.066	**0.047**
Heart Rate at FATmax (bpm)	123 (5)	115 (3)	120 (4)	112 (4)	**0.038**	0.601	0.465

Data are shown as mean (SEM). HRmax: maximal heart rate; RERmax: maximal respiratory exchange ratio; FATmax; maximal rate of fat oxidation. All measurements were recorded during an incremental cycling test.

Fasting plasma glucose, insulin and lipids

There were no statistically significant training effects for fasting plasma glucose (p=0.496), insulin (p=0.708), HOMA (p=0.426), total triglyceride (p=0.702), total cholesterol (p=0.129) or HDL (p=0.332). Training decreased LDL by 8% (p=0.028) and the HDL:Total Cholesterol ratio improved by 6% (p=0.005). There were no statistically significant gender x training interaction effects (all p>0.05; *Table 4*).

The responses to the acute mixed-exercise session were analysed by comparing the rested samples with those taken 20 min after exercise both at baseline and after the 12 week SIT. Total triglycerides, total cholesterol and LDL did not change in concentration between time points 1 and 2, or 3 and 4 (all $p>0.05$). HDL increased by 4% from time point 1 to 2 ($p=0.001$), but did not differ between 3 and 4 ($p=0.318$). Total cholesterol:HDL ratio decreased by 2% from time point 1 to 2 ($p=0.002$) and by 1% between time points 3 and 4 ($p=0.013$). There were no statistically significant gender x time point interactions, indicating similar responses for males and females.

Table 4: Glucose and Insulin concentrations in serum of males and females before and after 12 weeks SIT

	Males (Pre)	Males (Post)	Females (Pre)	Females (Post)	Training effect	Gender effect	Gender x training
Glucose (mmol/L)	5.10 (0.16)	5.06 (0.05)	4.99 (0.11)	4.99 (0.05)	0.496	0.634	0.663
Insulin (µU/ml)	3.50 (0.06)	3.48 (0.05)	3.35 (0.08)	3.44 (0.08)	0.708	0.995	0.241
HOMA (%S)	76 (2)	77 (1)	71 (2)	75 (2)	0.426	0.880	0.897
Triglycerides (mmol/L)	1.14 (0.27)	0.83 (0.07)	0.85 (0.06)	0.84 (0.08)	0.702	0.899	0.661
Total Cholesterol (mmol/L)	4.97 (0.24)	5.18 (0.20)	4.68 (0.20)	5.09 (0.19)	0.129	0.307	0.456
High Density Lipoprotein (mmol/L)	1.39 (0.06)	1.81 (0.14)	1.43 (0.07)	1.83 (0.14)	0.332	**0.008**	0.811
Low Density Lipoprotein (mmol/L)	2.75 (0.18)	2.55 (0.20)	2.51 (0.13)	2.42 (0.21)	**0.042**	0.562	0.523
Total Cholesterol:HDL ratio	3.60 (0.16)	3.01 (0.21)	3.29 (0.09)	2.91 (0.21)	**0.010**	**0.039**	0.161

Data are shown as mean (SEM). HOMA: homeostatic model of assessment.

Training completion

Of 91 participants (51 males and 40 females) who began the training study at baseline, a total of 41 participants (24 males and 17 females) successfully completed (SC) the 12-week SIT intervention and post-training measurements. This means that 49 participants (26 males and 23 females) did not finish (DNF) the 12 week SIT intervention. The characteristics of these participants are shown in *Table 5.*

Table 5: Physiological characteristics of participants who did not complete the full 12 weeks SIT programme

	DNF (n=49; 26 males, 23 females)	SC (n=41; 24 males, 17 females)	Sig. (p-value)
Age (years)	33 (1.46)	39 (2.12)	**0.010**
Total Body Mass (Kg)	74.0 (2.11)	72.8 (2.14)	0.683
Body Mass Index (kg/m^2)	23.8 (0.74)	24.5 (0.57)	0.465
Body Fat (%)	27.5 (1.55)	26.5 (1.25)	0.631
VO_2max (ml/kg/min)	38.32 (1.37)	39.06 (1.45)	0.713
FATmax (mg/kg/min)	6.03 (0.30)	8.06 (0.42)	**<0.001**

Data are shown as mean (SEM). DNF: Did not finish 12 week training programme; SC: Successfully completed 12 week training programme.

The participants who did not complete the training were younger than those who completed the training as well as a lower FATmax (mg/kg/min) than those who completed the training (*Table 5*). However, VO_2max (ml/kg/min), BMI (kg/m^2) and body fat (%) was similar between groups (*Table 5*).

3.4 Discussion

The present study showed that the sprint-interval training programme of just 4 min per week for 12 weeks decreased total body fat mass in males but not females, while females improved VO_2max more than males. Improvements to the rates of fatty acid oxidation during submaximal exercise after the 12-week training programme were similar in males and females. These results highlight statistically significant gender-differences in physiological responses to very high intensity training in the participants of this study who were recruited from the general population.

Body composition

The total body lean mass was statistically significantly increased after 12 weeks SIT (1.2% in males, 0.03% in females). This small increase to lean mass is in line with expectations (discussed in more detail in Chapter 4), but it is close to the previously reported error range for test re-test values in fat free mass (1%) (Kiebzak et al., 2000). A shorter SIT intervention found no change in lean mass after 2 weeks of SIT cycling in 48 young males and females (35 males, 13 females; mean age= 24±3 years, VO_2max= 47±7; p>0.430) (Hazell et al., 2010). A 6 week SIT running intervention, however, did cause a significant increase in total body lean mass of 0.6 kg (1%, p=0.037) in 10 young males and females (mean age= 24±3 years, VO_2max= 47±5 ml/kg/min) (Macpherson et al., 2011). These studies both utilised air displacement via a BodPod® system, which are less accurate than DEXA (Noreen and Lemon, 2006). This suggests that SIT may be an effective strategy to modestly increase total body lean mass, however only in longer interventions (>6 weeks), with the majority of participants in this large sample size changing in a similar manner and attaining statistical significance (p=0.016). This question is addressed in more detail in Chapter 4.

Body fat levels were within normal ranges for participants' ages at baseline (Kelly et al., 2009) and lower body fat than their BMI related cut offs (Heo et al., 2012). Just 80 seconds of very intense sprint exercise per session, equal to 48 min exercise over 12 weeks, resulted in statistically significant reductions to body fat mass. As far as the author is aware, the training duration of 4 min per week is the shortest reported to effectively cause fat loss without additionally restricting food intake. These results build upon previous high-intensity training studies that also reported fat loss (Macpherson et al., 2011; Hazell et al., 2014;

Gillen et al., 2013; Trapp et al., 2008), although they utilised lower intensity and longer duration intervals than this training programme.

The fat loss was principally due to changes seen in males, since females did not change their total body composition despite losing ~800 g fat from their legs. This study is the first to show gender-comparisons in the changes to body composition after SIT and are in line with previous work indicating that males generally lose more fat than females do after endurance training (Donnelly and Smith, 2005). The reasons for the gender differences in training responses of body fatness are not clear (Devries, 2015), however a review by Tarnopolsky (2008) suggests that gender-differences in body fatness and oxidation rates are linked to physiological actions of sex steroid hormones, primarily oestrogen (Tarnopolsky, 2008). Oestrogen has been closely linked to Peroxisome proliferation activator receptors (PPAR) and is upregulated in females compared to males (Fu et al., 2009). In turn, PPARα may induce transcription of CPTI mRNA, a key enzyme in mitochondrial fatty acid transport (Mascaró et al., 1998). Additionally, Kiens et al (2004) observed a 49% higher protein level of FAT/CD36 and 160% higher Lipoprotein Lipase (LPL) mRNA in females than males (n=24 females and 22 males, mean age 23-27 years) (Kiens et al., 2004). Despite this gender difference in hormonal and enzymatic characteristics, when energy balance was more closely regulated throughout an endurance training programme, overweight and obese males and females had similar fat loss (Caudwell et al., 2013). The gender differences in fat loss might also be related to the gender differences in relative muscle mass (Bijlsma et al., 2013), skeletal muscle contractile (McPhee et al., 2014; Simoneau et al., 1985b) and metabolic characteristics (Esbjörnsson-Liljedahl et al., 1999; Venables et al., 2005).

SIT sessions have very low energy consumption, of around 200 kJ/week, compared with endurance exercise of around 2000 kJ/week (Burgomaster et al., 2008). The direct energy expenditure therefore cannot explain the fat loss occurring after 12 weeks SIT. Other contributing factors might include an increase in post-exercise energy expenditure or overall shift towards greater fatty acid oxidation during habitual activities throughout the day, as occurs after endurance training (Henderson and Alderman, 2014): the results showing increased fatty acid oxidation during low and moderate intensity activity are consistent with this. It is also possible that metabolic rate and lipid oxidation remain elevated after each training session and there are reports that such effects are pronounced in males and

negligible in females (Henderson and Alderman, 2014; Henderson, 2014) which might help to explain the observed loss of fat in males but not females. Females may also have a lower energy expenditure during exercise due to the lower absolute workloads, despite working to similar relative exercise intensities, allowing males to reduce fat mass more than females after training (Donnelly et al., 2003). Treuth et al. (1996) found that resting metabolic rate remained around 15% higher after an acute session of SIT compared to those who completed endurance training. Higher intensity exercise is also associated with prolonged suppression of appetite and hunger (Thompson et al., 1988). However, others have reported increased energy intake after high intensity training compared to non-exercising controls and lower-intensity exercise (Pomerleau et al., 2004). The combination of a shift towards greater fatty acid oxidation and elevated resting energy expenditure could lead to a negative calorie balance and thus loss of body fat.

Maximal rate of oxygen uptake

This data showed an overall 9% increase in VO_2max after 12 weeks SIT. This increase is similar to previous reports from younger participants after SIT (Gist et al., 2014) and of similar magnitude to VO_2max gains after conventional endurance training programmes in which training sessions lasted around 1 hr (Burgomaster et al., 2008; Tremblay et al., 1994). Both males and females improved VO_2max, but the gains in females were greater than those in males. It is not clear why disparity between genders would occur in VO_2max SIT responses. Males have been shown to have higher gains in VO_2max following conventional endurance exercise (Bouchard et al., 1999), but results from SIT studies are mixed. Scalzo et al. (2014) showed young females had similar gains in VO_2max to young males, but other studies reported that males did not increase VO_2max (Allemeier et al., 1994) whilst females showed large increases after SIT (Talanian et al., 2010).

VO_2max is an indicator of overall cardiopulmonary fitness and dependent on the transportation of oxygen through the respiratory, cardiovascular and muscle systems to supply oxygen to the mitochondria for oxidative metabolism. The supply of oxygen to the working skeletal muscles is thought to be a limiting factor in VO_2max (Saltin and Calbet, 2006), so the higher VO_2max response in females might point to higher adaptations of oxygen supply than males following SIT, but more focussed studies examining cardiac output, blood volume, haematocrit and blood flow distribution are needed to clarify this

finding. Conversely, after regular endurance training, males had higher gains in VO_2max compared with females (Bouchard et al., 1999). It is possible that the training volume (higher in endurance) and the training intensity (higher in SIT) lead to disparate adaptations between males and females in the oxygen carrying capacity of blood (e.g. total blood volume, haemoglobin or cardiac output) or local vasculature, but physiological mechanisms driving such responses are unclear.

Rates of fatty acid oxidation

In the untrained state, females have higher relative oxidative capacity than males (Maughan et al., 1986) and higher relative rates of fatty acid oxidation during prolonged exercise (Carter et al., 2001), slower muscle phenotype with proportionally more Type I fibres (Simoneau et al., 1985a) and lower mitochondrial fractional synthetic rate after training (Karakelides et al., 2010). Despite these gender differences in muscle metabolism and contractile characteristics, males and females in the present study showed similar gains in rates of fatty acid oxidation at all workloads from 30 – 60% VO_2max after training (*Figure 4*). This metabolic adaptation occurred independently of changes to VO_2max and was not associated with the changes to body composition. This was the first study to compare genders FATmax responses after SIT, but increased fat oxidation has previously been reported (Shepherd et al., 2010; Trapp et al., 2008; Whyte et al., 2010) and could be related to improved muscle oxidative capacity and enzymes of fatty acid oxidation (Gollnick et al., 1973; Holloszy and Coyle, 1984; Hoppeler et al., 1985). The underlying metabolic pathways coordinating these adaptations seem to be similar in SIT and conventional endurance exercise; with increased metabolic enzymes and capillarisation (Burgomaster et al., 2008; Talanian et al., 2007; Shepherd et al., 2013) regulated in part by PGC-1α, AMPk and CAMk (Gibala et al., 2012; Burgomaster et al., 2008). Elevated post exercise oxygen consumption may reflect increased rates of fat utilization after high intensity exercise (Børsheim and Bahr, 2003; LaForgia et al., 2006). The elevated rates of fatty acid oxidation are associated with improved muscle metabolism (Jeukendrup et al., 1998) alongside changes to blood lipid profiles, cardiopulmonary fitness and body composition; it is a defining feature of health status (Wolfe, 2006).

The females in this study trained for 12 weeks, equating to approximately 3 menstrual cycles, meaning the stage of menstruation should be similar between entry and exit from

the study, therefore having relative estimations of any observed change after training. FATmax (mg/kg/min) was similar at baseline between males and females (p=0.815) and similarly increased after SIT in the present study (gender x training interaction; p=0.690), alluding to a mechanism outside that of the interaction of oestrogen and metabolism. The effect of oestrogen in skeletal muscle is primarily linked to a metabolic shift in muscle toward glycogen sparing and increase in fatty acid oxidation (Oosthuyse and Bosch, 2010). However, exercise studies in females do not suggest this is the case between menstrual phases, with females of all stages of the menstrual cycle unchanging in their substrate metabolism during moderate intensity exercise (Horton et al., 2002; Jacobs et al., 2005). Taken together, this suggests that sex hormones are not the full explanation for the differences between males and females in absolute rates of fatty acid oxidation.

Although total body lean mass was correlated to fat oxidation rates in this cohort, when separated into males and females, there was no correlation between these variables in males or females (*Figure 3a+b*), which is an indication that rates of fat oxidation are not dependent solely on the overall lean mass. This may have been related to circulatory sex steroid hormones in the females in this study as these can vary dependent on age and menstrual cycle phase (Isacco et al., 2012). Despite this, the regulation of fat oxidation in females seems to be much more multi factorial than in males with the impact of oestrogen on mitochondrial biogenesis and fatty acid transport (Oosthuyse and Bosch, 2010). Therefore more carefully controlled molecular data collection is required in order to fully understand the mechanism of this gender difference in muscle metabolism.

Fasting plasma glucose, insulin and lipid profiles

Levels of circulating total triglycerides in fasting plasma samples remained unchanged after training, while LDL and the cholesterol:HDL ratio reduced (both by 7%, $p<0.05$). A reduction of total circulating triglycerides has been associated with regular aerobic exercise (Plaisance et al., 2008; Durstine et al., 2001; Crouse et al., 1997), although it is not a consistent finding in high-intensity training programmes (Tsekouras et al., 2008; Bellou et al., 2013), as noted in a recent review (Kessler et al., 2012). HDL increased after the first mixed exercise session, but did not change over the 12-week training. Fasting plasma glucose, insulin and the insulin sensitivity estimated using HOMA (Matthews et al., 1985) did not change significantly with training. No change in fasting plasma glucose and insulin was also reported in a study of 16

healthy males after 6 SIT sessions, although those participants did improve glucose tolerance when measured using an oral glucose tolerance test (Babraj et al., 2009). Hood et al. reported 35% improvement in HOMA in seven middle-aged males (n=4) and females (n=3) after six interval training sessions performed at lower intensity than that used in the present study (Hood et al., 2011). Whyte et al. observed improved insulin sensitivity (23.3%, p=0.027) in ten sedentary males (mean age= 32 years) after six SIT sessions (Whyte et al., 2010). These previous reports outlining positive effects of SIT on glucose metabolism contrast with those from the present study. It is possible that the benefits in glucose homeostasis observed by others after short-term interventions are not extended longer-term as people adjust their lives and physical activity/nutrition habits to the new training regimen. It is also possible that the benefits of SIT on glucose homeostasis are transient, as Whyte et al showed improvements 24 hr after the final SIT session, but benefits had diminished after 72 hr.

Training completion

From baseline to the end of the training period, 46% (n=49) of the originally recruited participants withdrew from the study. Further analysis of these participants at baseline *(Table 5)* shows that the participants who did not complete the 12 weeks training were younger (p=0.010) and had a lower FATmax (mg/kg/min) (p<0.001) than those who did complete the 12 weeks of training. However, VO_2max and body fat were similar between the groups at baseline (p>0.05), suggesting that cardiorespiratory fitness and body composition upon entry into the study did not play an important role in the reason for not completing an arduous SIT programme. In coronary artery bypass patients (mean age: 60±7, VO_2peak: 27±5), 30% did not complete 6 months of aerobic intense interval walking (Moholdt et al., 2009). Whereas only 8% of metabolic syndrome patients (mean age: 55±13.2, VO_2max: 34±3 ml/kg/min) completing 16 weeks of aerobic intense interval running, did not complete the allocated training intervention (Tjønna et al., 2008). However, the studies that report the rates of completion are of a much lower intensity of the training programme employed here. It is possible that the lower completion rate could be due to the high intensity that participants are asked to work at. Bartlett et al. on the other hand suggested that an acute bout of high intensity interval running was perceived as more enjoyable than moderate intensity continuous running in 8 healthy, active males (mean age:

25±5 years, VO_2max: 57±4 ml/kg/min) (Bartlett et al., 2011). Although not assessed in the present study, participants commonly report feelings of nausea, light headedness and muscle soreness after high intensity exercise interventions (Richards et al., 2010). This could be a reason for the high dropout rate. The present study was not designed to determine reasons for dropout, so very little follow-up of these participants was undertaken. From those participants who provided explanation for their reasons for discontinuing training, the most commonly occurring explanation was that of a lack of time to complete the training sessions, which is consistent with reasons for failing to complete adequate exercise into the daily routine (Stutts, 2002).

Limitations

The semi-supervised design of the training programme gave exercise volunteers more control and although this is the case in real-life situations, it may confer less commitment or obligation to training compared with typical fully supervised laboratory-based programmes.

Menstrual cycle variations or use of oral contraceptives were not controlled for in this study. Not controlling the menstrual phase upon entry to the study or measuring sex hormones limits the possibility to gain mechanistic insights into sex hormone associations with the training responses. However, before looking for mechanisms underlying gender differences it is first important to establish that gender differences do indeed exist. The present study was adequately designed to address this fundamental question because the 12 week training programme ensured females would be at the same phase of the menstrual cycle before and after completion of training and was of sufficient duration to ensure females had experienced on average three full menstrual cycles. The reasons for the gender differences observed in body fat and VO_2max training responses should be the focus of further work to understand whether lifestyle choices, e.g. nutrition or physical activity levels, or biological variation in, e.g. sex hormones, are the cause.

It was not possible to control for physical activities outside of the training programme and dietary intake was not monitored throughout the training programme. Instead, participants were asked to maintain their usual patterns of food and drink consumption. The interpretation of the rates of fat oxidation must therefore be considered when a high fat

diet is consumed that fat oxidation may be increased (Coyle et al., 2001), likewise a high carbohydrate intake may reduce the rates of fat oxidation (Cameron-Smith et al., 2003).

Conclusion

A total of 4 min exercise per week over 12 weeks improved cardiorespiratory (9% increase of VO_2max (ml/kg/min)), metabolic (15.2% increase of FATmax (mg/kg/min)) and body composition profiles (1.2% reduction in body fat) of males and females recruited from the general population. When genders were compared, males lost more fat mass than females, and females showing higher VO_2max adaptations than males. Furthermore, 46% of the study group did not complete the 12-week SIT intervention. Care should be taken if prescribing SIT to a population group as adherence to an extremely high intensity of exercise may not be suited to all.

Chapter 4
Knee Extensor Muscle Size, Torque-Velocity Relationship And Fatigue Resistance: The Effects Of 12 Weeks Sprint Interval Training And Gender Comparisons

Chapter Three reported significantly different responses in males and females to SIT in terms of VO_2max (ml/kg/min) and body fat mass (kg). This chapter will explore changes in muscle size, strength and fatigue resistance, which occur due to SIT and any divergence in response between males and females.

4.1 Introduction

Males have higher maximal skeletal muscle strength and power compared with females (McPhee et al., 2013; Stenroth et al., 2015) and higher maximal power output during sprinting (Esbjörnsson-Liljedahl et al., 2002; Esbjörnsson et al., 1993; Froese and Houston, 1987). However, the superior performance does not lie entirely with males, since males fatigue more quickly than females during controlled isometric contractions of single muscle groups (Mcphee et al., 2014; Hunter, 2014) and during sprinting, while females recover faster during short rest periods between repeated sprinting bouts (Esbjörnsson et al., 1993; Esbjörnsson-Liljedahl et al., 2002; Froese and Houston, 1987).

The male advantage when producing maximal muscle force and power is largely due to the higher relative muscle mass (Bijlsma et al., 2013; Janssen et al., 2000), larger muscles (Janssen et al., 2000) and larger fibre cross-sectional areas in males than females (Simoneau et al., 1985b). Any comparisons between the genders for muscle force and power should therefore normalise values to muscle size ('normalised' force and power). However it is not only muscle mass that differs between the genders, but also the contractile and metabolic characteristics. For instance, males have higher concentrations of glycolytic enzymes and faster rates of contraction and relaxation than females (McPhee et al., 2014; Simoneau et al., 1985b; Esbjörnsson et al., 1993; Jaworowski et al., 2002). Males use relatively more carbohydrates during sub-maximal aerobic exercise than females (Venables et al., 2005), shift to anaerobic metabolism at lower relative intensity during incremental exercise (Russ and Kent-Braun, 2003) and during maximal sprinting males have higher glycolytic contributions than females (Esbjörnsson-Liljedahl et al., 1999; Gratas-Delamarche et al., 1994). Skeletal muscle characteristics such as these that affect energetics may also confer gender differences in force and fatigability, even after normalisation to muscle size, and may influence adaptations to exercise training.

In Chapter 3, genders were compared for VO_2max and body fat loss responses to sprint interval training (SIT). Extending these findings to maximal power output, Esbjörnsson-Liljedahl et al (1996) showed that females increased their power output more than males after 4 weeks SIT, whereas males and females showed similar gains in power after a very short training programme of just 6 sessions of SIT (Astorino et al., 2011).

Increased power output could in theory be due to changes to neural activation, but the results from Esbjörnsson-Liljedahl et al (1996) allude to proportionally larger gains in fibre cross sectional area (particularly Type 2x) in females than males being a mechanism. This would suggest that females have a greater hypertrophic response to SIT than males, but there is little evidence to this effect because hypertrophy and strength gains have been largely overlooked in studies of SIT, but could be expected due to the very high power contractions. Hypertrophy follows a net increase in anabolic signalling over time, and in this regard it is interesting to note that a recent study found no differences between males and females in their anabolic responses to a single SIT session (Fuentes et al., 2012). Another study, however, reported higher rates of muscle protein synthesis in males than females (Scalzo et al., 2014), which conflicts with the report of higher increases in Type 2x muscle fibre CSA in females than males (Esbjörnsson Liljedahl et al., 1996). Irrespective of the conflicting reports, neither of these cross-sectional studies of acute training responses examined changes to muscle size after a period of training.

Thus, the aim of this study was to recruit males and females from the general population to complete 12 weeks cycling SIT and monitor changes relating to health and physiological function. The primary outcomes were changes in knee extensor muscle size, strength, power and fatigue resistance after 12 weeks SIT. The secondary outcome was the comparison between responses of males and females in order to compare genders in these responses. It was hypothesised that muscle size, strength, power and fatigue resistance would be higher after training. Based on the limited available evidence, it was also expected that females would increase fatigue resistance more than males.

4.2 Method

Participants

The study conformed to the latest revisions of the Declaration of Helsinki (World Medical Association, 2013) and was approved by the Faculty of Science and Engineering Ethics Committee at Manchester Metropolitan University (application number: SE111216). Volunteers provided written informed consent prior to participation. Those with a history of cardiovascular, neuromuscular or metabolic disease were excluded as well as people whom had suffered a leg fracture within the past two years. The aim of recruitment was to recruit

a large number of participants from the general population to complete 12 weeks of cycling SIT with the same recruitment objectives as stated in Chapter 3. This is due to previous studies utilising largely single gender cohorts of n~10. Equal numbers of males and females of similar ages were specifically recruited in order to more directly compare the effects of the SIT on the participants. Participants are categorised by gender and referred to as males and females, in accordance with the American Physiological society recommendations (The American Physiological Society, 2012). The categorisation of gender is due to the participants in this study self-selecting male or female in a questionnaire and no physiological assessment of sex being undertaken in this protocol.

The participants recruited are from the same cohort as in Chapter 3. A total of 31 participants who completed the 12-week SIT intervention and post-training measurements were utilised in this chapter, due to these participants having data values for both pre and post SIT (except for MRI which was collected in 16 males and 10 females only due to reduced access to equipment during a planned maintenance period). Participant characteristics are shown in *Table 6*.

Design

Total body and regional lean mass were assessed by dual-energy x-ray absorptiometry (DEXA: Lunar Prodigy Advance; GE Medical; EnCore version 10.50.086) using the same procedures as those reported in Chapter 3.

Magnetic resonance imaging (MRI) was used to measure peak quadriceps cross-sectional area using a T1-weighted turbo 3D sequence (256 x 256 matrix, Repetition Time 40 ms, Echo Time 16 ms) on a 0.25-T MRI scanning machine (G-scan, Esaote, Genoa, Italy). The scanning coil was positioned over the thigh of the dominant leg and continuous transverse-plane slices of 6 mm thickness were collected with no gap between slices. Images were analysed using Osirix imaging software (OsiriX medical imaging, OsiriX, Atlanta, USA) by manually tracing the quadriceps muscles and avoiding any obvious fat deposits in the muscle. Slices 24 mm apart were analysed (every 4th slice) and the slice with the highest quadriceps cross-sectional area was recorded, all analysis was carried out by the same user (LB). Previous research from our research group using the same equipment, protocols and training have observed a co-efficient of variation of 0.43, 0.35, 0.30 and 0.31% for measurement of *Vastus*

Lateralis, *Rectus Femoris*, *Vastus Medialis* and *Vastus Intermedius* muscles respectively (de Boer et al., 2007). These scanning procedures and analysis were repeated after completion of the 12 weeks SIT in 16 males and 10 females.

Knee extensor torque-velocity relationship and fatigue were assessed in 31 participants (16 males and 15 females) on a Cybex Norm Dynamometer (Cybex, division of Lumex Inc, Ronkonkoma, New York, USA). Participants were seated upright (hip angle of 85^{o}) with straps secured firmly around the upper body and the hips. The torque lever was strapped just above the ankle malleolus of the dominant leg and the femur lateral condyle was aligned with the point of rotation. A brief warm up included six isokinetic contractions at 180^{o} sec^{-1} using approximately 60-70% of maximal effort. The maximal voluntary isometric torque (MVC) was assessed three times at a knee angle of 90° and the highest value in Newton Metres (Nm) recorded. Following a 3 min rest, isokinetic torque was assessed at 60, 120, 180, 240, 300, 360 and 420° sec^{-1}. Each trial started with the leg flexed as far as possible and participants made two maximal efforts at each velocity through the full range of movement until the leg was almost fully extended. A rest of around 30 seconds was given between maximal efforts and strong verbal encouragement was given throughout. The reliability of isometric concentric knee extension measurements is very high. For example, in 20 healthy females (mean age=25±5), a correlation coefficient of 0.97 and 0.86 was seen in two separate observers respectively, assessed from 3 contractions on 3 separate occasions (Molczyk et al., 1991).

Knee extensor fatigue resistance was assessed after a 3 min rest. The test started with the knee flexed as far as possible and participants performed 60 maximal-effort isokinetic contractions over 2 minutes (one every 2 seconds, as timed by a metronome) at a velocity of 120° sec^{-1}. The fatigue index was calculated using the formula:

$$\text{Fatigue Index} = (\text{Torque Produced in final contraction (Nm)} \div \text{Torque Produced in First Contraction (Nm)}) \times 100$$

In this instance, a higher value would indicate greater fatigue resistance, *i.e*. the percentage of muscle torque output maintained as a proportion of the first contraction.

At velocities of 120° sec^{-1}, dynamometry has been shown to be an accurate and repeatable method of muscle torque measurement. Li et al. (1996) studied 18 males (mean age= 27±7

years) and 12 females (mean age= 26±6 years) using a Cybex 6000 isokinetic dynamometer and found peak knee extensor torque at 120° sec^{-1} had a high interclass correlation co-efficient (males: 0.86, females: 0.83; $p<0.05$).

Protocol

Sprint Interval Training

Participants were asked to maintain their usual dietary and exercise habits throughout the intervention. SIT was completed on cycle ergometers (Cateye, Japan). The training consisted of a 2 min warm-up at a self-selected moderate intensity. This was followed by four bouts of 20 seconds 'maximal effort' sprints at a workload that was set at 175% of the workload attained in the VO_2max test. Each of these intervals was separated by 2 min of very low intensity cycling (a workload of approximately 20% of that attained at VO_2max). Thus, each training session lasted less than 10 min and only 80 seconds was completed at an intensity that would be expected to improve physical fitness.

The first training session for each participant was fully supervised in the research laboratory and participants also received clear instructions on the use of the cycle ergometers and the training regimen. Participants trained 3 times per week for 12 weeks using the ergonomic cycles that were provided. The training workload was increased by 5% every two weeks. Gym staff were fully informed of the research and training protocols, they logged the training session and were available to offer advice to research participants if needed during training sessions. Participants maintained a training-log to record workloads during training sessions.

Statistical analysis

The data collected in this chapters study is analysed in the same fashion as chapter 3, however in brief; data were analysed using SPSS (v.20 IBM). Test of data normality was carried out using Kolmogorov-Smirnov and Shapiro-Wilk tests. Two-Factor repeated measures ANOVA were used to assess adaptations to training and gender comparisons, using Greenhouse-Geisser or Huynh-Feldt correction values were utilised when sphericity was violated (Mauchly's test of sphericity: $p<0.05$). Relationships between variables were examined using Pearson's Product Moment Correlation Coefficients. Statistical significance was accepted at $p<0.05$. Data are presented as mean ± SEM.

4.3 Results

Body composition and knee extensor muscle size

Table 6 shows indices of skeletal muscle size in males and females. Total body lean mass increased significantly by around 1% after training (p=0.016). The more detailed analysis of knee extensor cross-sectional area from MRI showed 4.1% increases in males (p=0.033) and 5.8% increases in females (p=0.011), with no gender-differences in this response (gender * training interaction p=0.895).

Table 6: Skeletal muscle size in males (n=16) and females (n=15) before and after 12 weeks SIT

	Males (Pre)	Males (Post)	Females (Pre)	Females (Post)	Training effect	Gender effect	Gender x Training interaction
Age (yrs)	40.8 (3.2)		40.9 (3.9)				
Total body mass (kg)	80.0 (2.2)	79.2 (2.1)	62.5 (2.6)	62.1 (2.5)	0.070	**<0.001**	0.542
Total body lean mass (kg)	60.4 (1.6)	61.1 (1.6)	39.1 (0.9)	39.2 (1.0)	**0.016**	**<0.001**	0.162
Leg lean mass (kg)	21.6 (0.7)	21.0 (0.7)	13.6 (0.3)	13.0 (0.4)	0.185	**<0.001**	0.821
Right thigh lean mass (kg)	5.0 (0.2)	5.1 (0.2)	3.4 (0.1)	3.5 (0.2)	0.064	**<0.001**	0.719
Knee Extensor CSA (cm^2)	86.2 (3.3)	89.7 (3.1)	56.7 (1.9)	60.0 (1.6)	**0.001**	**<0.001**	0.895

Data are shown as mean (SEM). Knee Extensor CSA (cm^2) was measured in 16 males and 10 females.

Knee extensor torque-velocity relationship and fatigue resistance

Figure 5 shows the knee extensor isometric MVC (Nm) as a function of quadriceps CSA (cm^2) for males and females at baseline. Males and females fitted along a similar regression line, indicative of similar 'normalised' forces (males: r=0.630, p=0.009, females: r=0.784, p=0.007, combined: r=0.635, p=0.001).

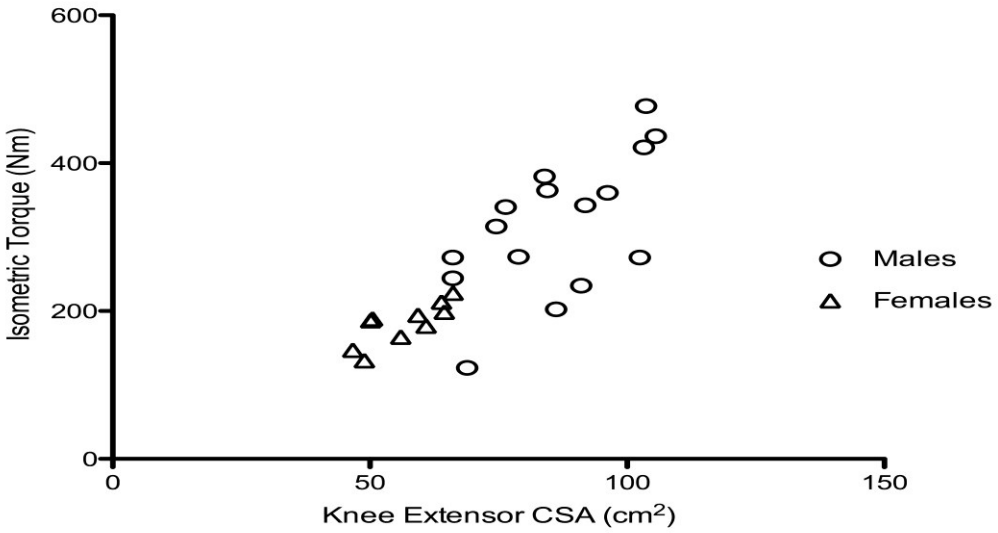

Figure 5: Knee extensor MVC Torque (Nm) as a function of peak knee extensor CSA (cm^2) in males (n=16) and females (n=15) at baseline

Figure 6a shows the decline in torque as velocity increased in both males and females, while *Figure 6b* illustrates this decline as a percentage of maximal isometric torque (MVC) in order to normalise the torque-velocity relationship between males and females to more accurately compare genders. Males and females showed similar declines in torque as velocity increased (p=0.813). *Figure 7* compares the males and females for the normalised torque measured at different velocities. Males had higher normalised torque than females at 180, 240, 300 & 360 °/sec^{-1} (all $p<0.05$). There were no significant increases in torque after training at any velocities with the exception of 120 °/sec^{-1}, which increased by 12% in females (p=0.012). There was no divergent response to training in torque-velocity relationships after training between males and females (all velocities, $p>0.05$).

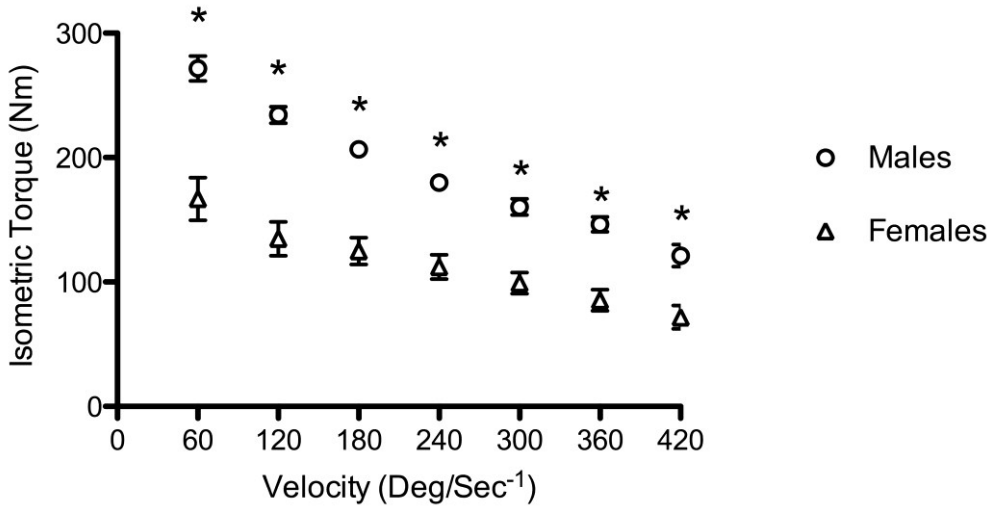

Figure 6a: Isokinetic knee extensor torque at increasing velocity in males (n=16) and females (n=15)

****indicates statistically significant difference between males and females (p<0.05)***

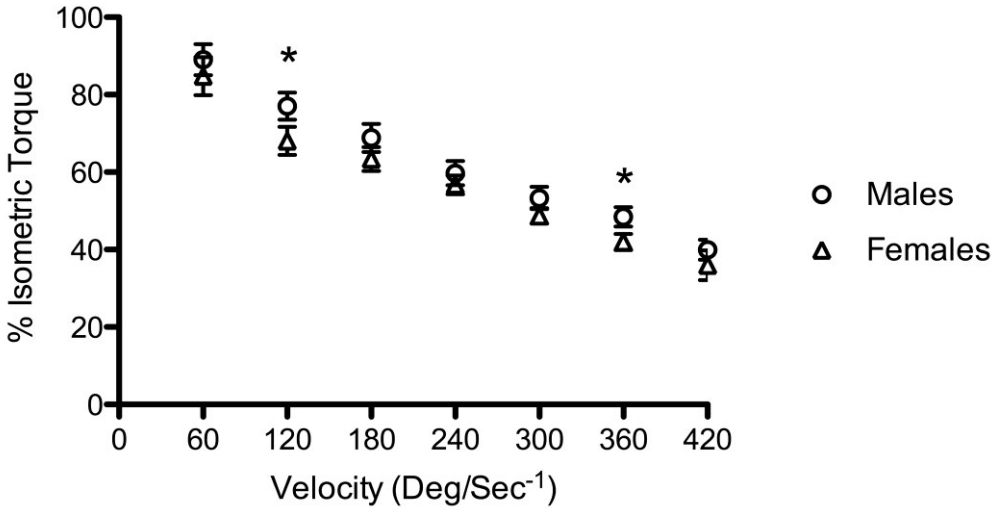

Figure 6b: Isokinetic knee extensor torque as a percentage of isometric MVC at increasing velocity in males (n=16) and females (n=15)

****indicates statistically significant difference between males and females (p<0.05)***

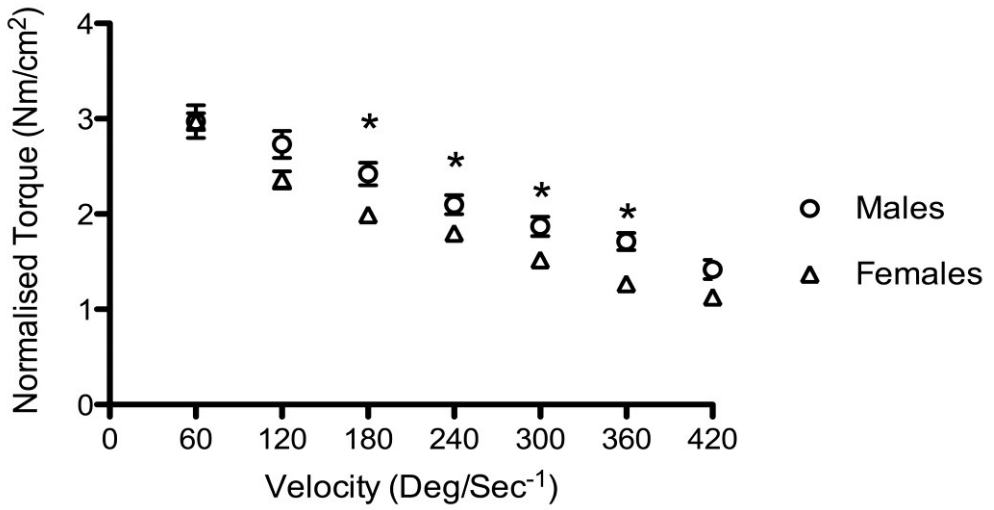

Figure 7: Isokinetic knee extensor torque normalised to knee extensor cross sectional area at increasing velocity (deg/sec) in males (n=16) and females (n=15)

****indicates statistically significant difference between males and females (p<0.05)***

Table 7: Skeletal muscle torque and fatigue index in males (n=16) and females (n=15) before and after 12 weeks SIT

	Males (Pre)	Males (Post)	Females (Pre)	Females (Post)	Training effect	Gender effect	Gender x Training interaction
Isometric MVC (Nm)	314.32 (21.86)	322.98 (23.10)	193.33 (9.43)	204.20 (9.65)	0.249	**<0.001**	0.895
Isometric Normalised Torque (Nm/cm^2)	3.65 (0.21)	3.57 (0.24)	3.23 (0.09)	3.23 (0.11)	0.712	0.200	0.705
Fatigue Index (%)	52.40 (2.52)	56.42 (3.27)	55.92 (3.56)	64.80 (2.26)	**0.003**	0.153	0.221

Data are shown as mean (SEM)

The fatigue index (percentage of power that remained after 60 maximal-effort concentric contractions) was similar between males and females at baseline (p=0.459). Fatigue index improved 4.8% after training (pre-training: 54.03% ±2.12 vs post-training: 60.31% ±2.14; p=0.003), with no gender differences in this response (p=0.153) (*Table 7*).

4.4 Discussion

The higher values in males compared with females across the torque-velocity relationship are not only due to the larger muscles in males, the torque per muscle cross-sectional area was also higher in males than in females. Torque decreased linearly with increasing velocity of shortening at similar rates in males and females. Twelve weeks SIT did not increase torque, but lean mass, quadriceps CSA and fatigue resistance all improved. Both genders responded similarly to training.

Females were previously reported to have lower anabolic response to exercise compared with males (Scalzo et al., 2014; Fuentes et al., 2012), leading to the hypothesis that females would have a lower hypertrophic response compared with males after 12 weeks SIT. However, this was not the case, as the males and females had similar increases of 4.1 and 5.8%, respectively, in quadriceps CSA, which are the main agonist muscles during cycling (Bijker et al., 2002). The similar increase in quadriceps CSA for males and females might seem to contradict the findings of (Esbjörnsson Liljedahl et al., 1996), who reported that females increased Type IIx fibre CSA more than males after 4 weeks of SIT. However, Type IIx fibres make up only a very small fraction of fibre types in the quadriceps muscles (Staron et al., 2000), so whether or not gender differences in Type IIx fibre hypertrophy occurred, it is unlikely to impact greatly on the overall hypertrophy of the quadriceps.

A review of the literature indicated that the extent of hypertrophy may depend on the length of the training programme: training 6-weeks or less did not cause significant changes to fibre cross-sectional areas (Ross and Leveritt, 2001). However, longer term training of 7-weeks or more generally increased fibre cross-sectional areas, however all studies that were reviewed had only small sample sizes (n=8 – 13) and none of them compared adaptations between the genders (Ross and Leveritt, 2001). For example, a six-week SIT protocol caused non-significant 6-12% fibre hypertrophy in 11 untrained males (mean age= 23±5 years) (Allemeier et al., 1994). However, 8 months SIT increased fibre CSA by 8-16% in 13 male and female athletes (mean age= 17±0.63 years) (gender comparisons were not possible due to small sample sizes and training programmes differed between the genders) (Cadefau et al., 1990).

Total body lean mass measured by DEXA increased by around 1%, with no significant gender difference in this response. However, this observed change could be due to the small amount of error involved in the DEXA scan itself. Kiebzak et al. observed a 1% variance in lean mass in repeated DEXA scans (utilising similar, but not the same equipment as this study) on the same participants over consecutive days (Kiebzak et al., 2000). The leg lean mass and thigh lean mass, both measured using DEXA, showed no significant changes after training. It is not clear why the DEXA did not detect significant changes with training that were evident using MRI, but disparity between these two techniques for measuring muscle size has been reported previously. For instance, DEXA failed to reveal the full extent of muscle loss with ageing (Maden-Wilkinson et al., 2013; MacDonald et al., 2011), so it is possible that the DEXA is not suitable for detecting small, but physiologically-relevant changes to muscle tissue.

The increase in muscle size in the present study did not lead to substantial gains in knee extension torque in either males or females. A previous study that measured torque before and after SIT (9 females and 11 males completed repeated Wingate tests over 3 weeks) also found no significant changes to knee extensor strength in males or females (Astorino et al., 2012). It is possible that the mode of exercise training (cycling) might not have trained the neural control needed for isolated knee extensions, as was suggested when the converse was observed when knee extension training did not increase cycling power output (Erskine et al., 2011).

The majority of studies comparing gender in muscle fatigue during controlled exercise of individual muscle groups utilised isometric contractions (Hunter, 2014; Hunter, 2015) and the results from such studies generally indicate that females have superior fatigue resistance compared with males (McPhee et al., 2014; Senefeld et al., 2013). Two previous studies showed that during concentric contractions, males fatigued more quickly than females after repeated knee extensions at 180 °/sec^{-1} (Pincivero et al., 2003) and concentric elbow flexions at 60º/sec (Yoon et al., 2015). However, the results of the present study did not find such a gender difference. Fatigability, measured in the present study as the decline in torque after 60 maximal-effort unilateral moderate-velocity concentric knee extensions, was similar in males and females at baseline, with torque during the 60th contraction dropping to around 55% of the first contraction. Other studies that utilised maximal velocity

contractions at a load equal to 20% of the participant's MVC also reported similar fatigue in males and females during knee extension (Senefeld et al., 2013).

There are reports that fatigue characteristics of individual muscle groups are unaffected by sprint training in males (Harridge et al., 1998; Zhou et al., 1996), but no previous studies compared training responses of males and females. Fatigue resistance improved after 12-weeks SIT in the present study. The increase of 15.9% in females tended to be greater than the 7.7% increase seen in males, but this difference was not statistically significant due to the large inter-individual variability in training responses. The physiological mechanisms underlying the training-induced improvement to fatigue cannot be identified from the present study, but are likely to be associated with increases in mitochondrial concentrations (Burgomaster et al., 2005; Gibala et al., 2006; Little et al., 2010) and capillary density (Cocks et al., 2013; Daussin et al., 2008) that have been found after SIT and collectively improve muscle oxidative energy recovery during the brief rest intervals between contractions.

Limitations

Similar limitations in the interpretations exist for this study as they do for the results presented in Chapter 3. In brief, the semi-supervised design of the training programme gave exercise volunteers more control and although this is the case in real-life situations, it may confer less commitment or obligation to training compared with typical fully supervised laboratory-based programmes. It was not possible to control for physical activities outside of the training programme and dietary intake was not monitored throughout the training programme. Instead, participants were asked to maintain their usual patterns of food and drink consumption. Finally, menstrual cycle variations were not controlled for, which will limit the ability to understand possible physiological mechanisms underpinning gender differences in physiological function or training responses (which is discussed in chapter 2.5).

Conclusion

Contrary to the proposed hypothesis, the findings of this study suggest that SIT cycling has no impact on the primary outcomes of knee extensor strength or power of healthy males and females ($p>0.05$). However, increases were seen in the exercising muscle resistance to fatigue (4.8%, $p=0.003$) and knee extensor size (3.7%, $p=0.001$) in both males and females. The knee extensor torque in males was higher than for females at a wide range of velocities ($p<0.05$) due to both higher muscle mass ($p<0.001$) and higher isokinetic torque per unit muscle cross-sectional area ($p<0.05$). This difference in muscle characteristics between males and females builds upon the results presented in Chapter 3 in suggesting there may be a difference in muscle morphology in response to SIT.

Chapter 5

Circulating Lipoproteins, Adipokines And Cytokines: Responses To 12 Weeks Sprint Interval Training And Their Associations With VO_2max And Body Fat Mass

Chapters Three and Four have reported the significant physiological adaptations as a result of SIT in this cohort. However, it remains to be seen whether there is an alteration of inflammatory protein profiles as a result of SIT. Also, are these inflammatory proteins related to the subsequent adaptation of physiological outcomes? This chapter will explore these issues and outline the potential for an area of future research.

5.1 Introduction

The circulating blood carries lipoproteins, adipokines and cytokines to tissues around the body. Their concentrations can be an indicator of the person's health status (Cesari et al., 2003).

Lipoproteins are small molecules that transport cholesterol and fatty acids. There are several types, including high density lipoprotein (HDL) which are commonly described as 'good' because they transport cholesterol and triglycerides from cells to be stored or processed by the liver, and low density lipoprotein (LDL) which are often described as 'bad' because they carry cholesterol and triglycerides around the body for longer periods. In healthy people, the concentrations of HDL, LDL and total cholesterol are >2.5, <5.5 and <11.1 mmol/L, respectively (Birtcher and Ballantyne, 2004). Any changes to these concentrations can be a sign of poor metabolic homeostasis that is indicative of underlying diseases.

Adipokines are cell signalling molecules that are categorized as being released by adipocytes in white adipose tissue and have either pro or anti-inflammatory effects elsewhere in the body and even modulating lipid and glucose metabolism and atherosclerosis (Kwon and Pessin, 2013; Rabe et al., 2008). Circulating adipokine concentration has been linked to the risk of metabolic syndrome with a lower circulatory adiponectin concentration being significantly related to a higher risk of metabolic dysregulation and a lower maximal oxygen uptake in non-diabetic offspring of a Type 2 diabetic parent (Salmenniemi et al., 2004).

A review by Simpson and Singh (2008) on exercise and its effects on circulatory adiponectin levels examined evidence from a number of acute and chronic exercise studies, from elite athletes to general populations. They concluded that exercise training can increase circulatory adiponectin levels, but requires a high intensity of exercise in order to bring about the largest increases in circulatory adiponectin concentration (Simpson and Singh, 2008).

Cytokines, such as interleukins and chemokines, act in a similar way to hormones to regulate cell-cell signalling. In the rested state, the cytokines studied in this chapter are found in relatively low concentrations (~1-500pg/ml (Biancotto et al., 2013)) in the circulation and are derived mainly from immune cells, although cytokines are also released from a variety of other tissue types. Cells and tissues that are under stress can release much higher

concentrations of cytokines or attract immune cells that infiltrate the stressed tissue and release cytokines in an effort to orchestrate the repair process (Tidball, 2005). In older people and individuals with some disease states that have an inflammatory component (such as rheumatoid arthritis), there is a chronic elevation of cytokines in the circulating blood which is termed 'chronic low-grade systemic inflammation' (Petersen and Pedersen, 2005). A chronic low-grade inflammatory state has been linked to a number of diseases, such as cardiovascular disease and insulin resistance (Barzilay et al., 2001; Festa et al., 2000; Pradhan et al., 2001; Freeman et al., 2002; Mishima et al., 2001; Winkler et al., 1998). This chronic low-grade inflammation differs from the acute inflammation occurring due to localised tissue damage (e.g. a swollen ankle). It is also different from intense exercise after which skeletal muscle can increase expression of some cytokines by around 100-fold and thereby cause substantial increases in circulating cytokine concentrations, although within just a few hours the circulating concentrations can return to normal as long as lasting tissue damage did not occur (Ostrowski et al., 1999).

Cytokines can be broadly classified as pro- or anti-inflammatory (*see Table 8*). For example, the Tumor Necrosis Factor alpha (TNFα) is a pro-inflammatory cytokine and high levels are associated with cell catabolic states (Beutler and Cerami, 1986). It is released by immune cells, but synthesis in the adipose tissue and skeletal muscle of obese participants was associated with insulin resistance (Hotamisligil et al., 1996; Kern et al., 1995; Saghizadeh et al., 1996). On the other hand, IL-1 receptor antagonist (IL-1ra) is an anti-inflammatory inhibitor of pro inflammatory IL-1, modulating inflammatory pathways (Dinarello, 1996). IL-6 is also a commonly studied cytokine with both pro- and anti-inflammatory properties and is associated with a number of positive health outcomes, such as increased rates of fatty acid oxidation and insulin sensitisation (Febbraio and Pedersen, 2002; van Hall et al., 2003).

The chronic inflammation in the metabolic syndrome has often been linked to an increased adiposity, with many of the cytokines involved in this state being synthesised in adipose tissue or by immune cells that infiltrate adipose tissue (Hotamisligil et al., 1993; Ouchi et al., 2003). Obese people who lose weight normally also reduce the systemic inflammation, suggesting a causal link between the levels of body fatness and the chronic inflammatory state (Lambert et al., 2008; Christiansen et al., 2010).

Since the circulating levels of lipoproteins, adipokines and cytokines are associated with health status, it is possible that they may be correlated with the physiological

measurements of VO_2max, FATmax and body composition which are also indicators of health and fitness, and associated with responses to training. These physiological measurements were shown to be sensitive to training (reported in previous chapters), so it is expected that the lipoproteins, adipokines and cytokines might also be responsive to a period of exercise training. Kullo et al. reported an inverse correlation between markers of systemic inflammation, including IL-6, and VO_2 max in asymptomatic 172 middle aged males (mean age= 51±9 years) (Kullo et al., 2007). However, no association between inflammatory profile and maximal oxygen uptake was observed in 152 sedentary overweight and obese females (mean age= 57.5 years) (Lavoie et al., 2010).

Exercise training has been shown to reduce the extent of chronic low-grade systemic inflammation (Petersen and Pedersen, 2005). The majority of evidence so far has focused on endurance type exercise of around 60-80% VO_2 max and has seen significant inflammatory cytokine response after long-term exercise training and acute high volume exercise bouts (Mattusch et al., 2000; Stewart et al., 2007; Ostrowski et al., 1998; Ostrowski et al., 1999). However, there is little information on the inflammatory response to low volume, higher intensity exercise training programmes. Primarily, the acute inflammatory response to high volume endurance exercise has been described by the substantial increase of circulatory IL-6 concentration (Steensberg et al., 2002; Febbraio et al., 2004).

Sprint interval training (SIT) has become a viable and accessible form of exercise and data suggests IL-6 is increased after an acute SIT session, potentially to an equal degree as that of acute moderate intensity exercise (Leggate et al., 2010; Croft et al., 2009). However, the systemic inflammatory response to SIT programmes yields some contradictory evidence. Zwetsloot et al. saw no change in resting IL-6, IL-8, IL-10 or TNFα after two weeks of high intensity sprint cycling in young, healthy males (mean age= 22±2 years) (Zwetsloot et al., 2014). However, Munk et al. saw a significant decrease in circulatory IL-6 and IL-8 whilst the anti-inflammatory cytokine IL-10 increased significantly in 20 stable angina patients after 6 months of high intensity interval training (Munk et al., 2011). This may suggest that longer SIT interventions (>2 weeks) are required to induce a reduction in systemic inflammatory profile.

The overall aim of this study was to recruit males and females from the general population to complete 12 weeks cycling SIT and monitor changes relating to health and physiological function. The primary outcomes were the changes in circulating concentrations of

lipoproteins, adipokines and inflammatory markers. The secondary outcome was whether these circulating concentrations were associated with VO_2max and body fat % in 13 males and 7 females. It was expected that lipoproteins, adipokines and cytokines would be responsive to SIT training and their levels would be associated with the VO_2max and body composition (as indicators of health status).

5.2 Method

Participants

The study conformed to the latest revisions of the Declaration of Helsinki and was approved by the local research ethics committee. Volunteers provided written informed consent prior to participation. Those with a history of cardiovascular, neuromuscular or metabolic disease were excluded as well as people whom had suffered a leg fracture within the past two years. A total of 20 participants (13 males, 7 females) completed the measurements. Participant characteristics are shown in *Table 10.*

Design

Participants attended the research laboratory in the morning following a 12 hr overnight fast and having avoided strenuous activity and alcohol in the 24 hours prior to the testing. After providing informed consent and resting for at least 15 min, a 10 mL blood sample was collected into EDTA collection tubes from a vein in the forearm and then a second sample during the same laboratory visit was collected 20 min after completion of the physiological testing. Blood was immediately centrifuged at 4^{o}C, the plasma separated and stored at -80^{o}C until analysis.

Body fatness was assessed from a total-body dual-energy x-ray absorptiometry (DEXA) scan using a Lunar Prodigy Advance (GE Healthcare; EnCore version 10.50.086). Total body fat, trunk fat and leg fat mass were analysed as previously reported in Chapter 3.

Maximal rates of oxygen uptake was measured during cycle ergometry (Jaeger Ergocycle, Germany) with VO_2, VCO_2 and heart rate (HR) measurements (Cortex Biophysik, Germany). Workload began at 50 W and a cadence of 70 rpm was maintained throughout. Workload was increased in increments of 50 W for males and 30 W for females every 3 minutes until the respiratory exchange ratio (calculated as VCO_2/VO_2) was higher than 1.0 for at least 1

min. From this point onwards, increments of 20 W were given every minute until volitional exhaustion.

Cytokine and adipokine profiles

Fasted plasma samples were collected as described on page 49. The cytokine and adipokine profiles were measured using commercially available Multiplex immunoassay kits (Milliplex MAP Human Adipocyte Magnetic Bead Panel for Adipokine concentrations and Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel for cytokine concentrations, Merck Millipore, Darmstadt, Germany). This method allowed simultaneous quantification of all cytokines and adipokines on their respective immunoassay plate in a 96 well format. This procedure included an overnight incubation period in order to improve the sensitivity of the assays. The manufacturers claim no detectable or negligible cross reactivity between analyte antibodies with an accuracy of 87-107% (Merck Millipore, 2013a). The sensitivities of each analyte are shown in *Table 7*. Assay plates were analysed using a Luminex 200 Bioanalyser (Merck Millipore, Darmstadt, Germany) and protein concentrations were calculated and analysed with the xPONENT software (Luminex, v.3.1.871) in duplicate and an average value calculated.

Table 7: Sensitivity characteristics of analytes using multiplex ELISA

Cytokine	Minimum Detectable Concentration (pg/ml)	Inter-Assay (%CV)	***Adipokine***	Minimum Detectable Concentration (pg/ml)	Inter-Assay (%CV)
TNFα	0.7	13.0	Adipsin	4.8	6.0
IL-2	1	6.3	Adiponectin	11	10.0
IL-8	0.4	3.5	Resistin	2.2	14.0
IL-1β	0.8	6.7	Lipocalin	1.7	12.0
IFNγ	0.8	12.0	PAI-1	4.1	14.0
IL-1ra	8.3	10.7			
IL-6	0.9	18.3			
IL-4	4.5	14.2			
IL-10	1.1	16.8			

**Values provided by manufacturer (Merck Millipore, 2013a+b). Inter-Assay %CV for cytokine data is calculated from 4 reportable results at 2 concentrations across 6 assays, for adipokines, %CV is calculated from 2 concentrations across 8 assays.*

Table 8: Pro- and Anti-Inflammatory cytokines studied and their physiological effects

Cytokine	***Metabolic Effect***
TNFα	Pro-Inflammatory cytokine, increased circulatory concentration in obesity, may reduce insulin sensitivity and increase lipolysis
IL-2	Pro-Inflammatory cytokine, potential role in muscle damage and modulation of lymphocytes
IL-8	Pro inflammatory cytokine, increased expression in insulin resistant participants
IL-1β	Pro inflammatory cytokine, pro artherogenic, increased in CVD patients
IFNγ	Pro inflammatory cytokine, pro artherogenic, increases plasma lipid accumulation
IL-1ra	Anti inflammatory cytokine, modulates the effect of pro inflammatory IL-1 through antagonist effects on IL-1 receptor
IL-6	Anti inflammatory cytokine, multi functional, released from adipose tissue to increase fat oxidation, released from skeletal muscle in exercise to act as an energy sensor
IL-4	Anti inflammatory cytokine, beta cell protective, inhibition of TNFα
IL-10	Anti inflammatory cytokine, inhibits the production of TNFα and IL-1b whilst increasing production of IL-1ra

Table 9: Adipokines studied and their physiological effects

Adipokines	***Metabolic Effect***
Adipsin	Increased in obesity, inhibits lipolysis and stimulates triglyceride storage
Adiponectin	Reduced in obesity and significantly improves insulin sensitivity
Resistin	Increased in T2D patients, significantly associated with insulin resistance and obesity
Lipocalin	Increased in obesity and T2D, associated with increased circulatory triglycerides and hyperglycaemia
Plasminogen Activator Inhibitor-1 (PAI-1)	Pro inflammatory adipokine, associated with an increased risk of cardiovascular risk and Diabetes

Fasting blood lipoprotein profile, insulin and glucose

Fasting plasma samples were collected as described above. Insulin sensitivity was estimated using the Homeostatic Model of Assessment (HOMA) as described by Mathews et al. (Matthews et al., 1985) which was calculated as:

Fasting Plasma (Glucose [nMol/L] x Insulin [µMol/L]) ÷ 22.5

Biochemical markers were determined from fasting plasma samples using the RX Daytona auto analyser (Randox Laboratories, UK). High-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (CHOD-PAP), triglycerides (GPO-PAP) and glucose (GOD-PAP) concentrations were determined in duplicate and an average value calculated.

Statistical analysis

The data collected in this chapters study is analysed in a similar fashion as chapter 3, however in brief; data were analysed using SPSS (v.20 IBM). Normal distribution of data was tested using Kolmogorov-Smirnov and Shapiro-Wilk testing with normality being accepted at $p>0.05$. Data are presented as mean ±SEM for normally distributed data and median (inter quartile range) for non-normally distributed data. Two-Factor repeated measures ANOVA were used to assess adaptations to training and gender comparisons, using Greenhouse-Geisser or Huynh-Feldt correction values were utilised when sphericity was violated (Mauchly's test of sphericity: $p<0.05$). Non-parametric data was normalised using $\log^{10}$ and the same process applied to the analysis of the data, however the non-parametric data presented in this study is the original data point values and has not been normalised. Relationships between variables were examined using Pearson's Product Moment Correlation Coefficients for normally distributed data and Spearman rho correlations for non-normally distributed data. Statistical significance was accepted at $p<0.05$. All correlations are the results of the partial correlations (*i.e*. after controlling for gender effects).

5.3 Results

Blood samples were available from 7 females and 13 males (characteristics shown in *Table 10*). Initial analyses compared genders in baseline values and only adiponectin was expressed significantly differently between males and females (Males: 13.19 (±1.88) vs Females: 24.25 (±5.87), $p=0.006$). Next, the gender*training interactions were examined and

no significant differences were observed between males and females ($p>0.05$). Since the vast majority of proteins were found in similar concentrations in males and females and responded in a similar way to training, all the data were combined in order to determine the overall response to training of the different proteins and their associations with the training responses of VO_2max (ml/kg/min) and total body fat (%). Nevertheless, gender was used as a covariate during correlation analysis.

Table 10: Participant characteristics at baseline (n=20, 13 Males, 7 Females)

Age	40.25 (±3.42)
Height (m)	1.73 (±0.02)
Body Mass (kg)	72.24 (±2.64)
BMI (kg/m^2)	24.01 (±0.78)
VO_2 max (L/min)	2.97 (±0.20)
VO_2 max (ml/kg/min)	40.63 (±1.97)
Body Fat (%)	25.18 (±1.46)
Body Fat (kg)	17.33 (±1.08)

Data are shown as mean (SEM)

12 weeks of sprint interval training had a significant and positive effect on physiological factors such as body fat percentage and maximal oxygen uptake in males and females (Chapter 3). However, this did not translate to any changes in the systemic inflammatory protein or adipokine concentrations, but the circulating concentrations of LDL decreased and the ratio of cholesterol:HDL also decreased after 12 weeks training (*Tables 11+12*).

Table 11: Fasting glucose, insulin, triglycerides and lipoprotein concentrations before and after 12 weeks sprint interval training

	Males (Pre)	Males (Post)	Females (Pre)	Females (Post)	Training effect	Gender effect	Gender x training
Glucose (mmol/L)	5.10 (0.16)	5.06 (0.05)	4.99 (0.11)	4.99 (0.05)	0.496	0.634	0.663
Insulin (µU/ml)	3.50 (0.06)	3.48 (0.05)	3.35 (0.08)	3.44 (0.08)	0.708	0.995	0.241
HOMA (%S)	76 (2)	77 (1)	71 (2)	75 (2)	0.426	0.880	0.897
Triglycerides (mmol/L)	1.14 (0.27)	0.83 (0.07)	0.85 (0.06)	0.84 (0.08)	0.702	0.899	0.661
Total Cholesterol (mmol/L)	4.97 (0.24)	5.18 (0.20)	4.68 (0.20)	5.09 (0.19)	0.129	0.307	0.456
High Density Lipoprotein (mmol/L)	1.39 (0.06)	1.81 (0.14)	1.43 (0.07)	1.83 (0.14)	0.332	0.008	0.811
Low Density Lipoprotein (mmol/L)	2.75 (0.18)	2.55 (0.20)	2.51 (0.13)	2.42 (0.21)	**0.042**	0.562	0.523
Total Cholesterol:HDL ratio	3.60 (0.16)	3.01 (0.21)	3.29 (0.09)	2.91 (0.21)	**0.010**	**0.039**	0.161

Data are shown as mean (SEM). HOMA: homeostatic model of assessment.

Table 12: Cytokine and adipokine concentrations (pg/ml unless stated otherwise) before and after 12 weeks sprint interval training

	Pre	Post	Training Effect	Gender Effect	Gender*Training Interaction
Adipokines					
Adipsin (µg/ml)*	3.51 (2.40-5.97)	3.12 (2.29-4.27)	0.271	0.073	0.268
Adiponectin (µg/ml)*	15.39 (8.91-19.37)	16.15 (9.78-21.69)	0.579	**0.006**	0.508
Resistin	170.61 (28.15)	213.91 (22.72)	0.094	0.146	0.091
Lipocalin	662.59 (105.57)	799.16 (82.27)	0.150	0.208	0.166
PAI-1	298.07 (58.46)	310.89 (47.01)	0.686	0.390	0.365
Pro Inflammatory Cytokines					
TNFα	9.92 (0.56)	11.26 (0.83)	0.996	0.107	0.164
IL-2*	5.69 (2.97-8.56)	6.84 (4.91-12.92)	0.846	0.401	0.337
IL-8*	7.63 (6.40-9.80)	7.69 (4.41-12.16)	0.961	0.229	0.099
IL-1β*	5.48 (3.20-8.44)	4.98 (3.50-13.17)	0.913	0.298	0.258
IFNγ	54.26 (6.99)	74.65 (16.98)	0.379	0.227	0.227
Anti Inflammatory Cytokines					
IL-1ra	86.29 (7.01)	87.13 (8.75)	0.762	0.072	0.543
IL-6*	6.26 (3.81-11.69)	6.59 (2.65-9.41)	0.286	0.151	0.224
IL-4*	28.02 (13.20-66.26)	28.02 (14.04-52.13)	0.244	0.176	0.414
IL-10*	10.53 (2.92-13.28)	8.88 (4.67-25.59)	0.509	0.214	0.161

**Data are shown as median (inter quartile range), otherwise as mean (SEM)*

Relationships between baseline measurements of circulating analytes and the baseline measurements of VO_2max and body fatness

Of all of the blood analytes measured at baseline, only the circulating adipokine concentrations of lipocalin and PAI-1 were significantly inversely correlated with absolute VO_2max (l/min), but not weight normalised VO_2max (ml/kg/min). Similarly, only adipsin was significantly positively associated with body fat (kg) at baseline (*Table 13*).

Table 13: Fasting blood analyte concentrations and their relationship (correlation co-efficient) to VO_2 max and body fat at baseline. Partial correlations controlled for gender.

	VO_2 max (ml/min/kg)	VO_2 max (l/min)	Body Fat (%)	Body fat (kg)
Glucose	0.049 (0.852)	0.224 (0.388)	0.211 (0.416)	0.238 (0.358)
Insulin	-0.270 (0.294)	-0.279 (0.278)	0.298 (0.245)	0.324 (0.204)
HOMA (%S)	-0.070 (0.790)	0.063 (0.811)	0.316 (0.216)	0.342 (0.179)
Triglycerides	-0.133 (0.610)	0.251 (0.332)	0.333 (0.192)	0.427 (0.088)
Cholesterol	-0.076 (0.764)	0.025 (0.921)	0.221 (0.378)	0.121 (0.634)
HDL	0.089 (0.725)	-0.210 (0.403)	0.074 (0.769)	0.145 (0.567)
LDL	-0.192 (0.445)	-0.009 (0.971)	0.159 (0.529)	-0.073 (0.773)
Cholesterol:HDL ratio	-0.049 (0.848)	0.408 (0.092)	0.062 (0.808)	-0.045 (0.859)
Adipokines				
Adipsin*	0.261 (0.296)	0.275 (0.269)	-0.015 (0.953)	**0.682 (0.002)**
Adiponectin*	0.212 (0.399)	0.239 (0.339)	0.028 (0.102)	-0.238 (0.341)
Resistin	-0.194 (0.441)	-0.454 (0.059)	0.479 (0.306)	0.103 (0.684)
Lipocalin	-0.267 (0.285)	**-0.494 (0.037)**	0.400 (0.100)	0.271 (0.278)
PAI-1	-0.330 (0.181)	**-0.480 (0.044)**	0.335 (0.174)	0.327 (0.185)
Pro Inflammatory Cytokines				
TNFα	-0.277 (0.266)	-0.128 (0.614)	0.142 (0.575)	0.135 (0.594)
IL-2*	-0.013 (0.959)	0.293 (0.238)	-0.036 (0.887)	0.088 (0.730)
IL-8*	0.236 (0.345)	0.334 (0.175)	0.127 (0.615)	0.139 (0.583)
IL-1β*	0.014 (0.957)	0.369 (0.132)	-0.055 (0.828)	0.027 (0.915)
IFNγ	-0.151 (0.549)	-0.131 (0.604)	0.057 (0.824)	0.325 (0.188)
Anti Inflammatory Cytokines				
IL-1ra	-0.027 (0.914)	-0.093 (0.715)	-0.313 (0.206)	-0.073 (0.772)
IL-6*	-0.134 (0.595)	0.128 (0.611)	0.057 (0.823)	0.186 (0.460)
IL-4*	-0.013 (0.960)	0.309 (0.212)	0.031 (0.902)	0.148 (0.557)
IL-10*	-0.014 (0.956)	0.319 (0.196)	-0.010 (0.969)	0.144 (0.569)

**Data are shown as Spearman Rho (p-value), otherwise as Pearson's Product Moment (p-value)*

Relationships between baseline measurements of circulating analytes and the training-induced changes to VO_2max and body fatness

Significant positive correlations were observed between changes in body fat and baseline insulin, adiponectin (*Figure 8a*), resistin (*Figure 8b*) and lipocalin concentrations (*Figure 8c*) (*Table 14*).

Significant positive correlations were observed between training-induced changes in VO_2max and baseline HDL (*Figure 9a*), resistin, lipocalin (*Figure 9b*) and PAI-1 (*Figure 9c*).

Table 14: Fasting blood analyte concentrations and their relationship (correlation co-efficient) to training-induced changes to VO_2max and body fat. Partial correlations controlled for gender.

	VO_2 max (ml/min/kg)	VO_2 max (l/min)	Body Fat (%)	Body Fat (kg)
Glucose	-0.117 (0.655)	-0.184 (0.480)	0.089 (0.735)	0.002 (0.994)
Insulin	-0.164 (0.530)	-0.164 (0.528)	0.583 (0.014)	0.349 (0.169)
HOMA (%S)	-0.204 (0.433)	-0.260 (0.314)	0.316 (0.216)	0.342 (0.179)
TGs	0.107 (0.684)	0.036 (0.890)	-0.266 (0.301)	-0.476 (0.054)
Cholesterol	0.319 (0.196)	0.273 (0.274)	0.137 (0.588)	0.060 (0.813)
HDL	0.534 (0.022)	0.553 (0.022)	0.225 (0.368)	0.240 (0.338)
LDL	0.101 (0.689)	0.107 (0.672)	0.101 (0.691)	0.042 (0.869)
Cholesterol:HDL ratio	-0.385 (0.115)	-0.451 (0.060)	-0.197 (0.433)	-0.273 (0.274)
Adipokines				
Adipsin	0.261 (0.296)	0.275 (0.269)	0.089 (0.724)	0.054 (0.832)
Adiponectin	0.212 (0.399)	0.239 (0.339)	0.398 (0.102)	0.736 (<0.001)
Resistin	0.442 (0.066)	0.475 (0.046)	0.479 (0.044)	0.550 (0.018)
Lipocalin	0.590 (0.010)	0.623 (0.006)	0.468 (0.050)	0.509 (0.031)
PAI-1	0.699 (0.001)	0.720 (0.001)	0.412 (0.089)	0.331 (0.180)
Pro Inflammatory Cytokines				
TNFα	-0.196 (0.435)	-0.196 (0.435)	0.382 (0.118)	0.208 (0.408)
IL-2	0.259 (0.299)	0.249 (0.319)	0.247 (0.323)	0.147 (0.559)
IL-8	0.088 (0.728)	0.113 (0.657)	0.041 (0.873)	0.018 (0.942)
IFNγ	0.175 (0.488)	0.203 (0.419)	-0.102 (0.687)	-0.101 (0.689)
IL-1β	0.168 (0.505)	0.155 (0.539)	0.197 (0.433)	0.105 (0.679)
Anti Inflammatory Cytokines				
IL-1ra	0.106 (0.675)	0.123 (0.626)	0.135 (0.594)	0.139 (0.583)
IL-6	0.287 (0.248)	0.282 (0.256)	0.231 (0.356)	0.093 (0.714)
IL-4	0.259 (0.300)	0.249 (0.320)	0.186 (0.459)	0.052 (0.839)
IL-10	0.250 (0.317)	0.246 (0.326)	0.161 (0.524)	0.056 (0.825)

**Data are shown as Spearman Rho (p-value), otherwise as Pearson's Product Moment (p-value)*

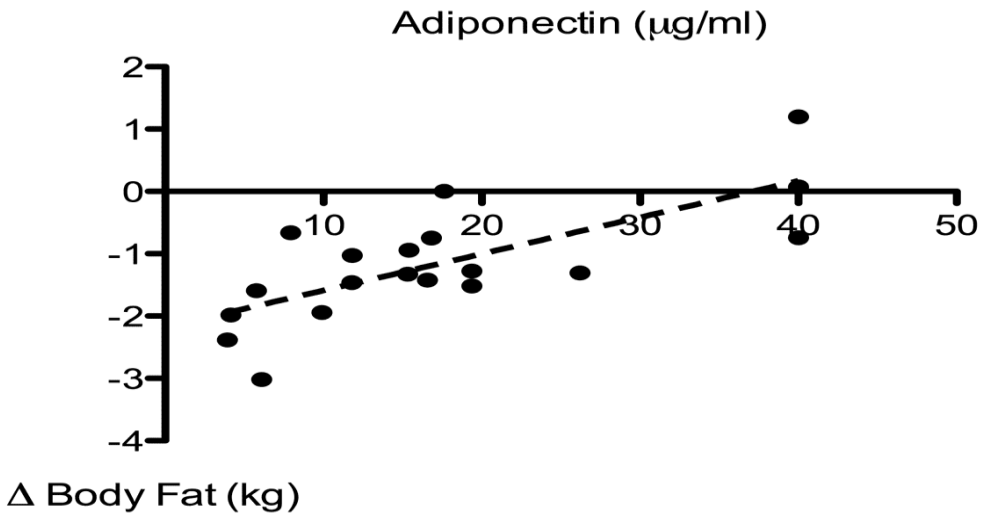

Figure 8a: Circulatory Adiponectin (µg/ml) is positively correlated with training induced change in body fat (kg) (r=0.736, p<0.001)

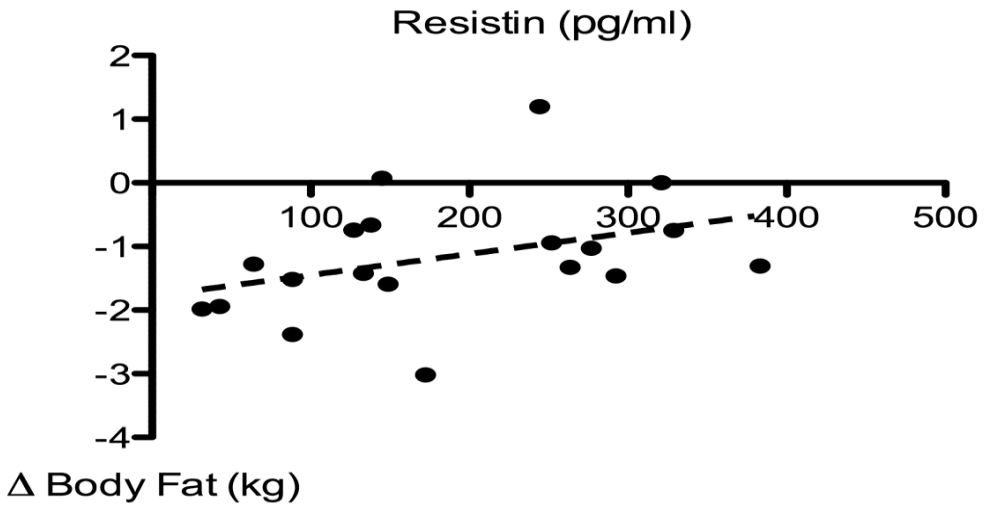

Figure 8b: Circulatory Resistin (pg/ml) is positively correlated with training induced change in body fat (kg) (r=0.550, p=0.018)

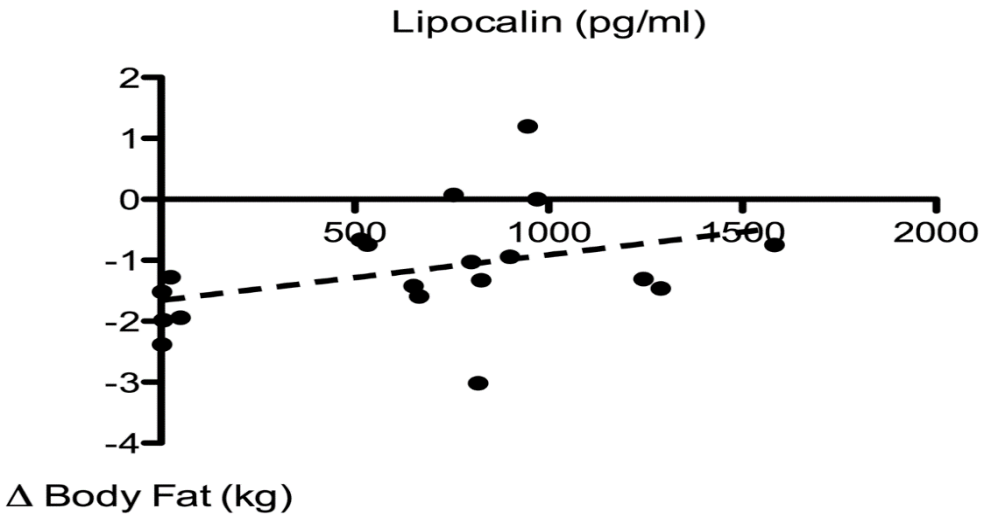

Figure 8c: Circulatory Lipocalin (pg/ml) is positively correlated with training induced change in body fat (kg) (r=0.509, p=0.031)

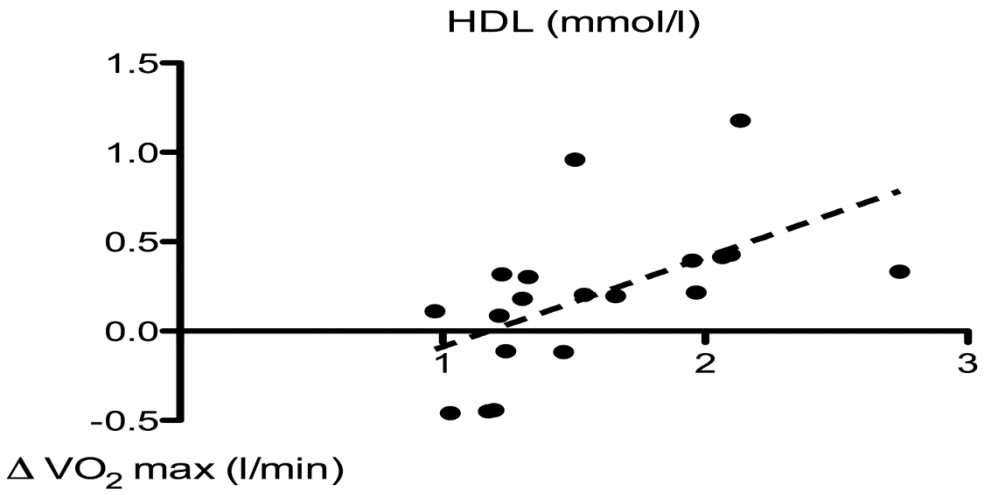

Figure 9a: Circulatory HDL (mmol/l) is positively correlated with training induced change in VO_2 max (l/min) (r=0.553, p=0.022)

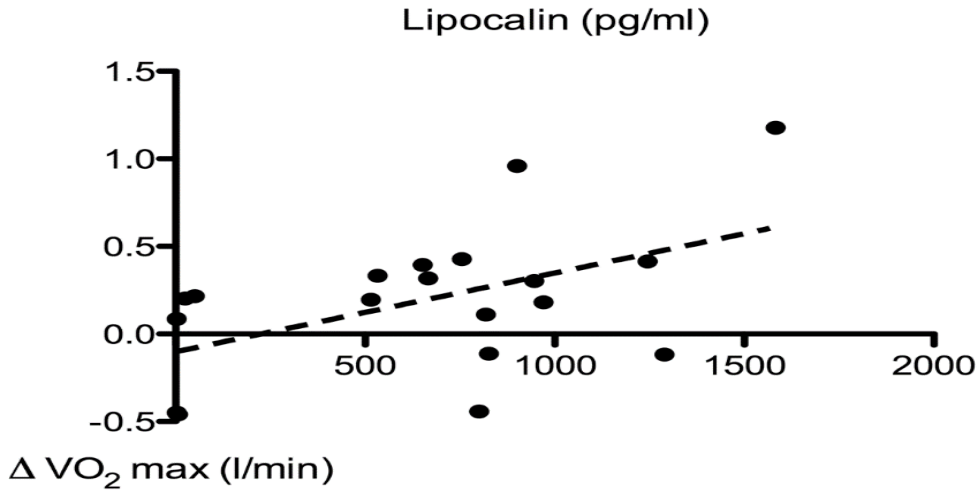

Figure 9b: Circulatory Lipocalin (pg/ml) is positively correlated with training induced change in VO_2 max (l/min) (r=0.623, p=0.006)

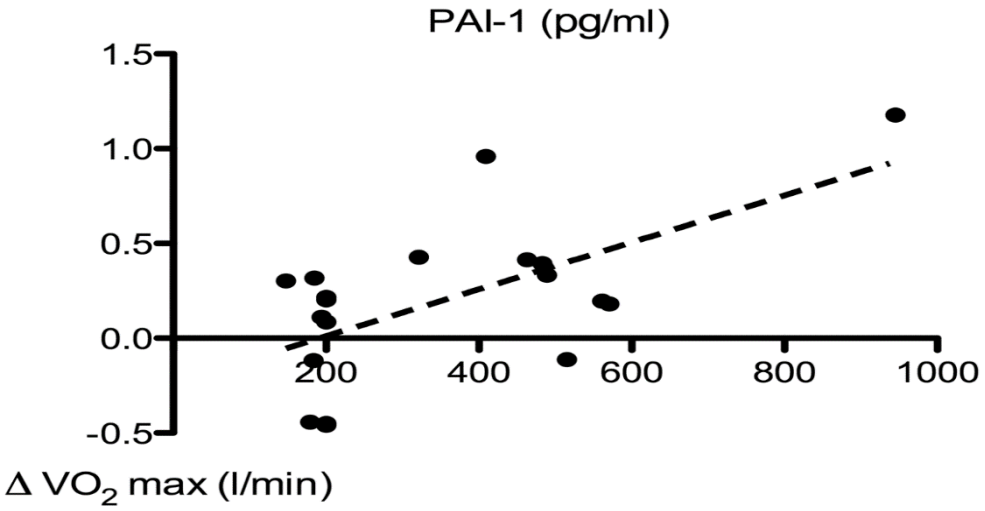

Figure 9c: Circulatory PAI-1 (pg/ml) is positively correlated with training induced change in VO_2 max (l/min) (r=0.720, p=0.001)

5.4 Discussion

SIT was shown in the previous chapters to have a significant, positive effect on physiological markers of health and fitness, including the VO_2max and body fat. The results of the present study build upon these findings to show significant improvements to the circulating concentrations of LDL and the ratio of total cholesterol:HDL after 12 weeks SIT. This study went further to explore potential associations between physiological outcomes such as training induced changes in VO_2max and body fat mass with circulatory concentrations of adipokines and cytokines. The circulating concentrations of adipokines and cytokines showed little change after training. However, the concentrations of adipokines in the untrained state were associated of the response of VO_2max and body fat after 12 weeks SIT.

The participants were middle-aged and were not training or competing regularly in sports prior to their involvement in this study. The concentrations of circulating cholesterol, TGs, lipoproteins, glucose, insulin and cytokines were not correlated with baseline VO_2max or body fat mass, but lipocalin and PAI-1 were inversely correlated with absolute VO_2max (L/min). These results indicate that people with higher fitness have lower circulating concentrations of lipocalin and PAI-1. This is consistent with previous studies that showed lipocalin to be elevated in Type 2 Diabetes patients along with elevated TGs, hyperglycaemia and insulin resistance (Wang et al., 2007), and PAI-1, a pro-inflammatory adipokine, was elevated in patients with cardiovascular and metabolic disease (Trayhurn and Wood, 2004; Alessi et al., 2011). Some studies have shown PAI-1 to be responsive to training while others showed that it was not (Stratton et al., 1991; Bodary et al., 2003; Svendsen et al., 1996), it is not clear why discrepancy exists between studies with respect to the training responses of adipokines, but it may be linked to the differences in participant characteristics. This chapters results also showed that adipsin was elevated in individuals with higher body fat. This is in agreement with previous findings showing that adipsin is higher in people who are obese and alters lipolysis and TG storage (Maslowska et al., 1999). Together, these results indicate that low circulating lipocalin, adipsin and PAI-1 are associated with higher fitness, and that the loss of metabolic homeostasis in patients with metabolic syndrome increases the expression of these adipokines.

The 12 week SIT programme had no effect on circulating adipokines, cytokines or HDL. A review by Kodama et al. suggests that HDL is not easily moderated by training, but higher

volume is most beneficial, however SIT was not adequately covered in this review (Kodama et al., 2007). Circulatory concentrations of Adipokines in response to SIT however have been little studied. Shing et al. saw a significant increase in circulatory adiponectin after a single bout of high intensity rowing after 4 weeks high intensity interval training in athletic rowers, however resting concentration after training was unchanged (n=5 males and 2 females, mean age= 19±1.2 years) (Shing et al., 2013). In relation to cytokines, Leggate et al. observed no change in circulatory IL-6, IL-10 or TNFα after two weeks of high intensity sprint cycling in overweight and obese males (Leggate et al., 2012). These results indicate that SIT has little effect on rested, fasted concentrations of adipokines and cytokines in people who are already relatively healthy. Similarly, the TGs, glucose and insulin concentrations, which are commonly used indicators of health status, did not change after 12 weeks SIT. A reduction in TGs with regular aerobic exercise is a common finding (Plaisance et al., 2008; Durstine et al., 2001; Crouse et al., 1997), but previous high-intensity training programmes have showed mixed results (Tsekouras et al., 2008; Bellou et al., 2013), as noted in a recent review (Kessler et al., 2012). No change in fasting plasma glucose and insulin was also reported in a study of 16 healthy males after 6 SIT sessions, although those participants did improve glucose tolerance when measured using an oral glucose tolerance test (Babraj et al., 2009). Hood et al. (Hood et al., 2011) reported 35% improvement in HOMA in seven middle-aged males (n=4) and females (n=3) after six interval training sessions performed at lower intensity than that used in the present study. Whyte et al. showed improved insulin sensitivity in ten young, sedentary males (mean age= 32 years) after six SIT sessions (Whyte et al., 2010). These previous reports outlining positive effects of SIT on glucose metabolism contrast with those from the present study. It is possible that the benefits in glucose homeostasis observed by others after short-term interventions are not extended longer-term as people adjust their lives and physical activity/nutrition habits to the new training regimen. It is also possible that the benefits of SIT on glucose homeostasis are transient, as Whyte et al showed improvements 24 hr after the final SIT session, but benefits had diminished after 72 hours.

The main novel finding in the present study was that the baseline concentrations of some analytes, and in particular the adipokines, was significantly associated with the VO_2max and body fat responses to 12 weeks SIT. The individuals with higher concentrations of HDL,

resistin, lipocalin and PAI-1 showed the greatest gains in VO_2max, both when expressed as percentage changes to absolute as well as weight-normalised VO_2max. Sunami et al (Sunami et al., 1999) observed no correlation between training induced change in VO_2 max and HDL concentrations after 5 months of moderate intensity training, although they did not report whether correlations existed between the baseline HDL and the training response of VO_2max. Like lipocalin and PAI-1, resistin is normally elevated in obese individuals (Gharibeh et al., 2010; Steppan et al., 2001).

Moreover, the resistin and lipocalin concentrations at baseline as well as adiponectin and insulin were also associated with of the training-induced changes to body fat measures. No previous SIT studies have identified baseline adipokine concentrations are associated with training responses in body fat variables. However, Kadoglou et al. reported significant reductions in circulating resistin in overweight and obese Type 2 diabetic patients after 16 weeks moderate intensity exercise (50-85% VO_2 max, cycling, running and calisthenics), but there was no reduction in body weight (Kadoglou et al., 2007). Another study found that 4 weeks of moderate intensity endurance training resulted in a significant reduction in body fat (kg) and increase in serum adiponectin concentration (Blüher et al., 2006). Unlike resistin, lipocalin and PAI-1, higher levels of adiponectin are generally associated with better health status (Chandran et al., 2003). All of these studies indicate that an association exists between higher circulating adiponectin concentrations and greater fat loss (kg).

Conclusion:

In contradiction to the hypothesis that 12 weeks SIT would change the primary outcomes of circulatory concentrations of inflammatory markers and adipokines, no such effect was seen. However, these results suggest that HDL, insulin and adipokines that have known metabolic effects, are associated with VO_2max and body fat training responses to SIT. However, the mechanisms of these interactions are not yet clear, with a large amount of variation in the circulatory concentrations of these inflammatory proteins, this issue will need to be the focus of future research. Furthermore, only adiponectin was significantly different between males and females. This difference between males and females in terms of circulatory adipokines and any difference in training response also warrants further

investigation due to the potential association with SIT induced loss of body fat and improvement of VO_2max as identified by this study.

Limitations:

The limitations to this study are similar to those reported in Chapters 3 and 4 due to the participants being sampled from the same training study. In brief, the semi-supervised design of the training programme gave exercise volunteers more control over their training loads and duration and although this is the case in real-life situations, it may confer less commitment or obligation to training compared with typical fully supervised laboratory-based programmes. It was not possible to control for physical activities outside of the training programme and dietary intake was not monitored throughout the training programme. Instead, participants were asked to maintain their usual patterns of food and drink consumption. There was no control for menstrual cycle variations, which are potentially limiting the interpretation of data in terms of metabolic outcomes (which is discussed in chapter 2.5). Furthermore, the variations in circulatory cytokines and adipokines between individuals may affect interpretation of this data and a larger sample size may be needed in future studies to confirm the observations made here. The total number of participants was relatively small for inflammatory association studies, the significant time demands of data collection and analysis meant it was not possible to include a higher number of participants.

Many of the inflammatory proteins examined in this thesis have a timescale and cascade type release, requiring previous cytokines to be released in order to activate further inflammatory signalling, particularly in response to exercise training (Febbraio and Pedersen, 2002). Previous literature suggests that there is a distinct inflammatory response observed in plasma after an acute about of SIT exercise, with a direct effect of exercise intensity and length (Peake et al., 2005; Ostrowski et al., 1999). This suggests there may be a link between acute change to inflammatory response to exercise and a longer term training response seen in chapter 4. Again, the scope of this thesis did not allow this analysis to take place, however, this area therefore requires significantly more research into the molecular mechanisms of these inflammatory proteins in order to ascertain their response to SIT in males and females as identified in this exploratory study.

Chapter 6
Aerobic And Anaerobic Power In Sprint And Endurance Master Athletes Aged 38-90 Years

Chapters Three and Four outlined the potential for SIT to be an effective and time efficient mode of exercise in the general population, while Chapter five reported circulating lipoprotein and cytokines responses in males and females. However it remains to be seen as to whether training in this modality over a long period of time (years) compares with long term endurance training. To address this question, Master Athletes at a major European track and field competition were studied, giving insight into the differences and similarities between sprint and endurance trained Master Athletes. This data is of use to future guidelines for public physical activity and health.

6.1 Introduction

The previous chapters reported results from 12 week SIT and highlighted key physiological adaptations, such as improved VO_2max, increased rates of fatty acid oxidation (Chapter 3) and improved resistance to fatiguing muscle contractions (Chapter 4). Although 12 weeks of training is considerably longer than the majority of previous SIT studies, it is still only a very short time period of an individual's life and it therefore remains unclear if or how training adaptations are maintained over a lifespan.

Endurance type training is completed at a much lower intensity than SIT (~65% VO_2max as opposed to ≥100% VO_2max in SIT (Weston et al., 2014; Garber et al., 2011)), but much higher volume in terms of time commitment to training (≥30 min per training bout as opposed to 10 min per training bout in the previous chapters (but only around 2 min being intense physical effort), (Garber et al., 2011)). These different physiological stressors may lead to divergent adaptations when performed over the lifespan and might even influence the rate of declining function in later life. Longitudinal studies lasting many years are very difficult to achieve due to funding issues and difficulties maintaining equipment and laboratory access, so most studies into the effects of lifelong exercise recruit older athletes into cross-sectional study designs. The study of master athlete populations may give some indication as to the potential divergent decline between sprint and endurance type training over long periods of time and lifespan.

Low cardiopulmonary fitness, neuromuscular function and high body fatness are common features of ageing and risk factors for disability and all-cause mortality (Blair et al., 1989; Rogers et al., 1990; Evans and Campbell, 1993; Harper et al., 2004). Studies of individuals who maintain exceptionally high physical activity levels throughout their adult lives invariably show that they have better physiological function (Faulkner et al., 2008; Cobley et al., 2012), longer lifespan and better quality of life when compared to sedentary people (Pollock et al., 2015). This provides some evidence that regular exercise can help to combat the effects of ageing.

Very athletic older people, or 'Master Athletes', who remained highly competitive in sports have exceptional physical performance for their age (Rittweger et al., 2009). Their performance is associated with high peak rates of oxygen uptake (VO_2peak) (Tanaka and Seals, 2008), muscle strength (Tarpenning et al., 2004), fibre size (Korhonen et al., 2006),

power (Trappe et al., 2013) and metabolic enzyme activity (Coggan et al., 1990) when compared with non-athletic controls. Nevertheless, studies invariably show that these physiological characteristics decline with ageing, even in masters athletes.

However, it is not clear whether peak anaerobic power and aerobic capacity decline at similar rates in sprint and endurance athletes because no previous studies have measured anaerobic and aerobic performance in the same athletes.

One legged cycling has previously been used to test the hypothesis of the limitations in VO_2max (see chapter 2.3). However, it is also a useful tool in the determination of aerobic power of skeletal muscle, due to the participant not reaching maximal heart rate during a single leg bout so central oxygen supply is not the limiting factor. The one legged test therefore indicates the potential of the working muscle to uptake oxygen in order to carry out aerobic metabolism in the presence of sufficient oxygen supply (Davies and Sargeant, 1975; McPhee et al., 2009). The evidence suggests that SIT and endurance training increase the levels of metabolic enzyme activity to a similar degree (Burgomaster et al., 2008). However it is unknown as to whether chronic (years) athletic training will maintain this metabolic power of the working muscle for a longer period into older age. More specifically, it is currently unknown as to whether there is a difference in metabolic power between training type (sprint vs endurance) over years in masters athletes.

In terms of anaerobic power, Michealis et al. showed higher power in sprinters than endurance runners in a cross-sectional study, which also demonstrated that muscle power was inversely related to age in both types of masters athletes (Michaelis et al., 2008). The rate of decline was around 13% per decade (Michaelis et al., 2008). Rittweger et al. analysed world records to demonstrate an inverse relationship between athletic performance and age that was evident in both power and endurance events (Rittweger et al., 2009). The rates of decline were similar for endurance and sprint events, being around 7% per decade. However, these different types of athletes complete discipline-specific conditioning that will favour adaptations of the anaerobic or the aerobic systems that diverge from one-another. Therefore, it cannot be discerned from these results (Rittweger et al., 2009) whether the characteristics that determine sprint performance, such as muscle size and fibre types, decline with ageing at similar or different rates from those that determine endurance performance, such as cardiopulmonary fitness and muscle oxidative potential (assuming discipline-specific conditioning and skill are equal in the two types of athletes).

The objective of this study was to recruit Masters Athlete sprint and endurance runners to complete measurements of health and physiological function. The primary outcomes of this study were peak power output measured by jumping mechanography, the peak rate of oxygen uptake during single and two-leg incremental cycling and peak rates of fatty acid oxidation during exercise. The main purpose was to compare the results between sprint and endurance masters runners. The secondary outcome was to compare the slope of decline in each of the primary outcomes with increasing age between the differently trained athletes. It was hypothesised that sprint athletes will have higher peak power but lower peak rate of oxygen uptake and fatty acid oxidation than endurance masters runners. However, due to "lifelong" training, the decline with age in these outcomes was expected to be similar between disciplines.

6.2 Method

Participants

The study conformed to the latest revisions of the Declaration of Helsinki (World Medical Association, 2013) and was approved by the local research ethics committee. Volunteers were recruited and assessed at the 18th European Veterans Athletics Championships (EVAC) at Weinau Stadium, Zittau, Germany between the 16-25 August 2012. VO_2peak measurements were performed using a MetaLyzer 3B - R2 (Cortex BioPhysik GmbH, Leipzig, Germany) and cycle ergometer (Excalibur Sport, Lode B.V, Groningen, Netherlands).

Volunteers provided written informed consent prior to participation. Participants are categorised by gender and referred to as males and females, in accordance with the American Physiological society recommendations (The American Physiological Society, 2012). The categorisation of gender is due to the participants in this study self-selecting male or female in a questionnaire and no physiological assessment of sex being undertaken in this protocol. Those with a history of cardiovascular, neuromuscular or metabolic disease were excluded as well as people whom had suffered a leg fracture within the past 2 years. Participant characteristics are shown in *Table 15*.

Design

Participants were recruited during the 18th EVAC (Zittau, Germany). After providing informed consent, assessments of jumping power and VO_2max were completed.

VO_2peak and FATmax determination: VO_2peak determination was carried out as per the method described in Chapter 3, with the exception of the assessment being terminated when a participant reached age-related maximal heart rate (220-participant age (years). This decision was taken by a cardiologist (medical doctor) due to safety concerns with the increased mean age of the participants in this study *(Table 15)*. In brief, the VO_2peak assessment was carried out on a cycle ergometer (Jaeger Ergocycle) with a MetaLyzer 3B - R2 (Cortex BioPhysik GmbH, Leipzig, Germany) to measure VO_2 and VCO_2. Workload began at 50 Watts, with a cadence of 70 rpm. Heart rate was measured every minute using a Polar monitor (Polar, Oy, Finland). Workload was increased in increments of 50 Watts for males and 30 Watts for females every 3 minutes until the respiratory exchange ratio was higher than 1.0 for at least 1 min. From this point onwards, increments of 20 Watts were given every minute until age related HR*max* (220-participant age (years)) was reached. The assessment was followed by a 5-minute cool down at low cadence (~40rpm) and workload (25-75 Watts).

One legged cycling ergometry and determination of one legged VO_2peak was also carried out in these participants using the same equipment and calibrations as the two legged VO_2peak assessment described previously. The participant's dominant exercising leg was secured to the corresponding pedal on the cycle ergometer, with the other leg resting on a central platform on the cycle and the participant was asked to restrict movements of their upper body during the exercise. The non-exercising leg was positioned so that it would not interfere or contribute to the cycling power of the exercising leg. Cycling workload began at 20 W at 70 rpm for the first two minutes of the test, after which the workload was increased to 50 W for one minute. After this minute at 50 W, cycling workload was increased by 10 W per minute until volitional exhaustion or a cadence of 70 rpm could not be maintained. The one legged VO_2peak value (l/min) was determined by the average of the last 30 seconds of the exercise, as per the two legged protocol described in Chapter 3.

Further analysis was completed to estimate rates of fat oxidation during exercise as described by Frayn (1983) and maximal rate of fat oxidation (FATmax) as described by Venables et al. (2005):

$$FATmax\ (g/min) = (1.67 \times VO_2) - (1.67 \times VCO_2)$$

Estimation of peak jumping power: Jumping mechanography assessed locomotive peak power. Participants made several jumps to acquaint themselves with the procedure and the highest of three two legged jumps was identified as the peak value. Jumping was performed with hands free to move, with the head and trunk being raised as far as possible. The jumping assessment was made on a Leonardo force platform system (Novotec Medical, Pforzheim, Germany). The system computed the participants vertical velocity from the ground reaction force as described by Cavagna (1975). Instantaneous power was calculated as the product of force and velocity (Power= Torque x Angular Velocity). This equipment and method has been extensively studied and provides excellent reliability. Matheson et al. (2013) observed a coefficient of variation of 0.3% of the maximal power produced in a 2 legged jump between three separate investigators and a total of 9 jumps in 10 healthy males and females (age range 19-35 years, 6 males and 4 females). Similarly in a study with a similar population to that used in the present work, 36 males and females aged 24-88 years (mean age= 61±19 years, 14 males and 22 females), a 3.6% short-term error was reported in measurements taken 2 weeks apart, with a test-retest correlation coefficient of r=0.99. Taken together this suggests that this method is a highly repeatable test which is capable of detecting difference between participants (Rittweger et al., 2004).

Statistical analysis

The data collected in this chapters study is analysed in a similar fashion as chapter 3, however in brief; data were analysed using SPSS (v.20 IBM). Normal distribution of data was tested using Kolmogorov-Smirnov and Shapiro-Wilk testing with normality being accepted at $p>0.05$. Data are presented as mean ±SEM for all data as all data is normally distributed in this study. Two-Factor repeated measures ANOVA were used to compare genders and differences between athletic disciplines, using Greenhouse-Geisser or Huynh-Feldt correction values were utilised when sphericity was violated (Mauchly's test of sphericity: $p<0.05$). Independent samples t-test was used to examine difference between athlete athletic event discipline (ie, sprint athlete and endurance athlete differences. Relationships between variables were examined using Pearson's Product Moment Correlation Coefficients. Statistical significance was accepted at $p<0.05$. All correlations are the results of the partial correlations (*i.e*. after controlling for gender effects).

Some data (*Figure 11a+b and Figure 13c*) was also normalised to that of a 35 year old athlete in order to properly assess the time course of physiological variable change through lifespan. This was carried out by plotting the variable against the age of the participant then utilising the formula of the regression line, to ascertain the mean value of a 35 year old athlete. This normalised value was calculated for both males and females. Normalised data was analysed alongside absolute values so as to gain a more specific insight into the decline of physiological variables with increasing age, providing a comparison to the mean values of a 35 year old master athlete throughout lifespan. This gives some indication of a longitudinal effect on the investigated variables by study of the reduction from the age of 35 years.

6.3 Results

Participant characteristics are shown in *Table 15.* There were no significant differences between the endurance and sprint athlete groups for the numbers of males and females, age or height. Sprinters had significantly higher body mass and higher BMI than endurance runners.

Table 15: Characteristics of participants separated by running discipline.

	N	Age (years)	Height (m)	Weight (Kg)	BMI (kg/m^2)
Endurance	8Males, 10Females	58.8 (10.7)	1.68 (0.11)	60.3 (9.5)	21.3 (2.0)
Sprint	11Males, 11Females	59.8 (15.8)	1.71 (0.11)	70.5 (11.7)	23.9 (1.9)
p-value	0.416	0.841	0.385	0.006	<0.0005

Data are shown as mean (SEM)

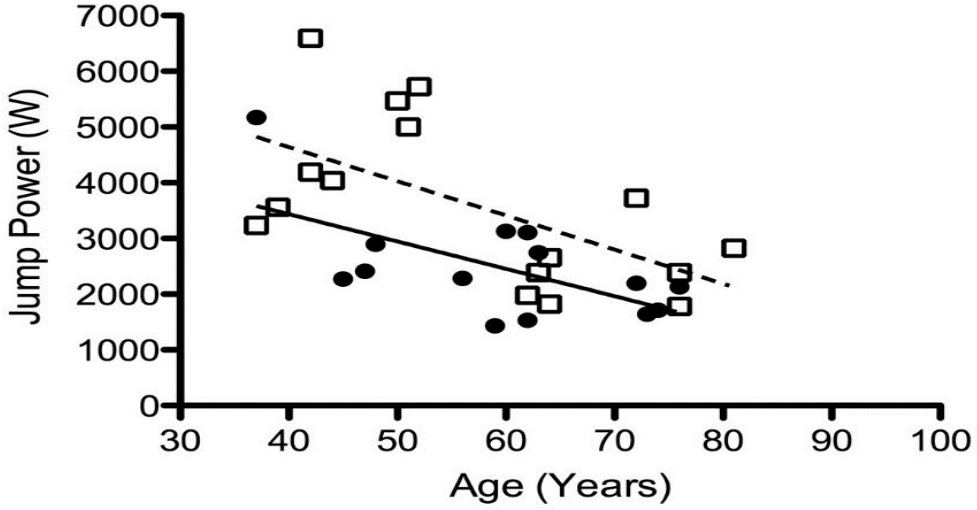

Figure 10a: Peak anaerobic power: absolute values for power output.
Sprinters (open squares) and endurance runners (shaded circles).

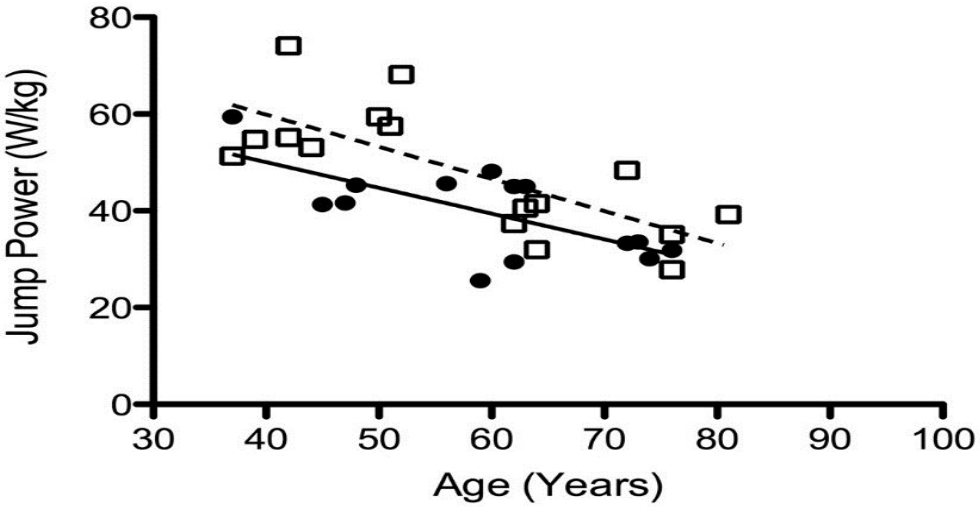

Figure 10b: Peak anaerobic power: power normalised to total body mass (W/kg).
Sprinters (open squares) and endurance runners (shaded circles).

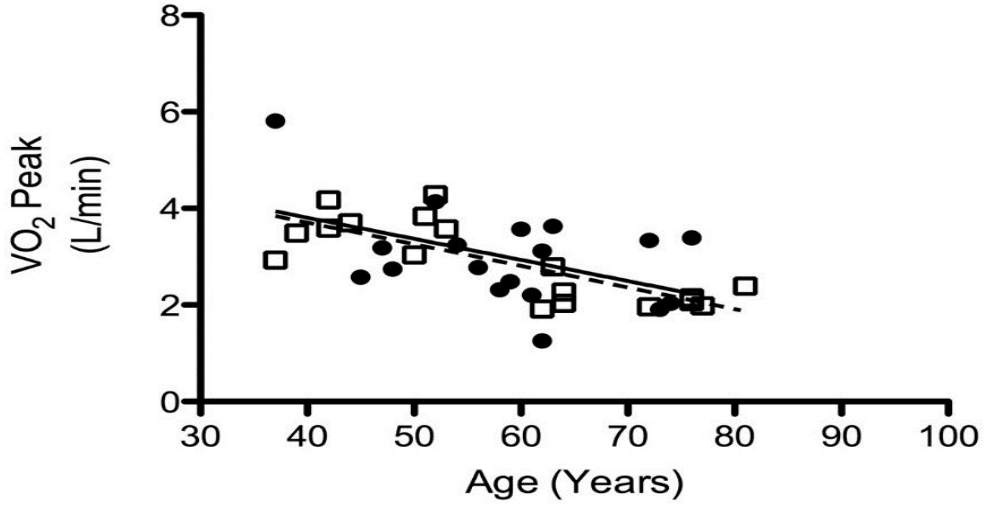

Figure 10c: Peak oxygen uptake: absolute values for peak oxygen uptake (L/min).

Sprinters (open squares) and endurance runners (shaded circles).

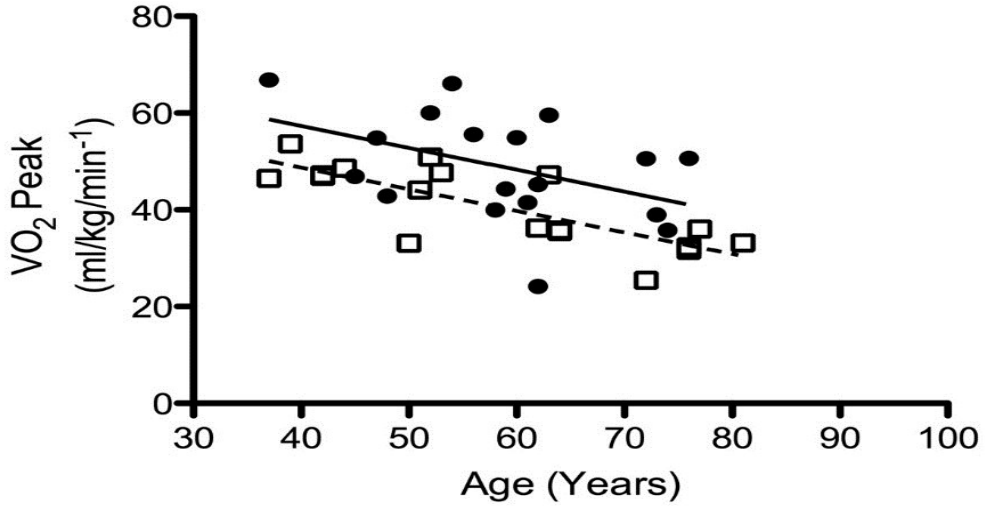

Figure 10d: Peak oxygen uptake: peak oxygen uptake normalised to total body mass (ml/kg/min^{-1}).

Sprinters (open squares) and endurance runners (shaded circles).

Sprinters achieved 45% higher power during vertical jumps compared with long distance runners (Fig 10a; p=0.024) and 22% higher power than distance runners when normalised to total body mass (Fig 10b; p=0.045). Conversely, the VO_2peak achieved during two-legged cycling was 17% lower in sprinters compared with long distance runners when normalised to total body mass (Fig 10d; p=0.012), but did not differ between groups when expressed in L/min (Fig 10c; p=0.700).

Power but not VO_2peak was significantly inversely related to age in long distance runners (jump power (W): r=-0.618, p=0.024; VO_2peak (L/min): r=-0.489, p=0.090). However both VO_2peak and power were significantly inversely related to age in sprinters (jump power (W): r=-0.779, p=0.001; VO_2peak (L/min): r=-0.699, p=0.004). The rates of decline in power and in VO_2peak with increasing age were very similar in sprinters and long distance runners (Fig 11a and 11b). These cross-sectional data indicate that both power and VO_2peak decreased by around 12% per decade.

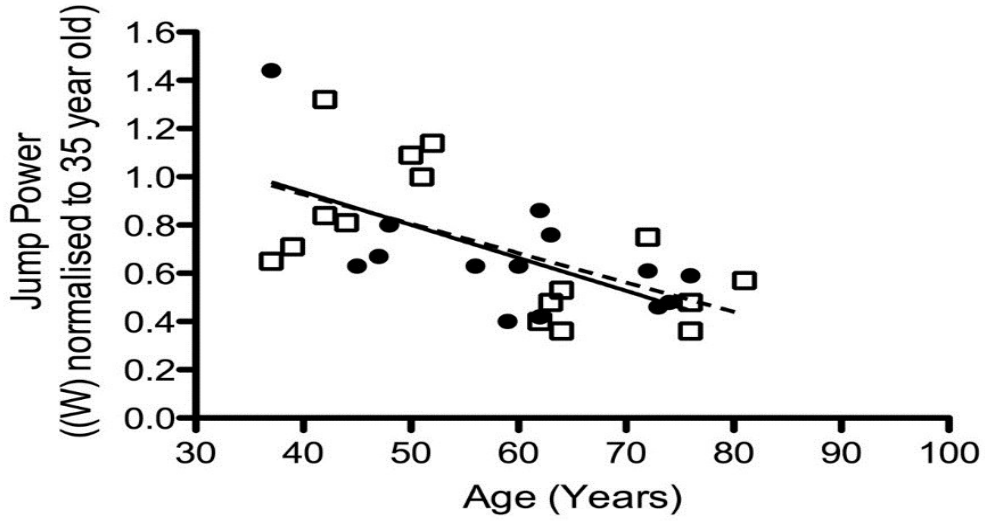

Figure 11a: Normalised Peak anaerobic power as a function of age (years).
The values for sprinters (open squares) and long distance runners (shaded circles) decreased linearly with increasing age at a rate of around 12% per decade in power (W).

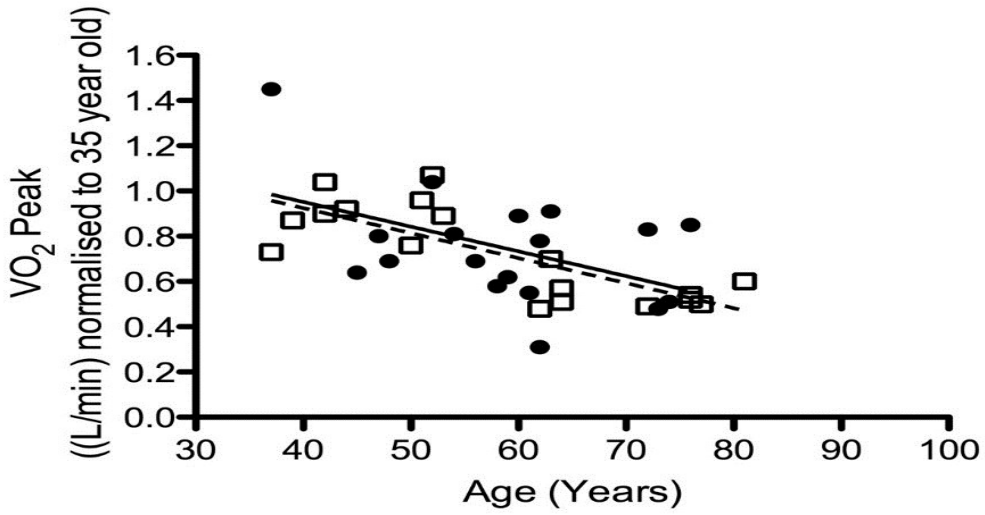

Figure 11b: Normalised Peak oxygen uptake as a function of age (years).
The values for sprinters (open squares) and long distance runners (shaded circles) decreased linearly with increasing age at a rate of around 12% per decade in VO_2Peak (L/min).

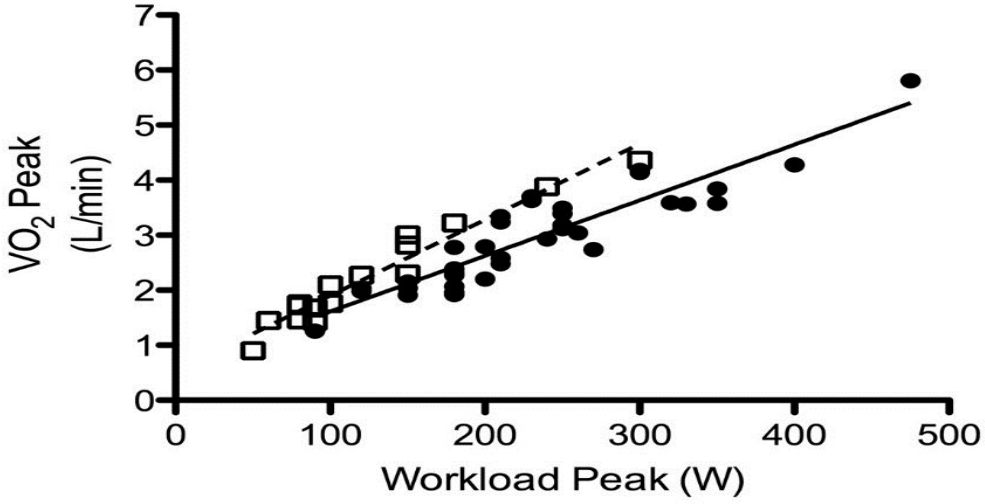

Figure 12: VO_2peak expressed as a function of peak workload.
The VO_2/WL regression was significantly higher during single-leg (open squares) than two-leg cycling (shaded circles).

Figure 12 shows the VO_2peak achieved during single-leg and two-leg cycling trials as a function of the peak workload. During the single-leg cycling tests, HR*peak* reached 87% (±2) and 92% (±1) (p=0.343) of the values achieved during two-leg cycling for sprinters and long

distance runners, respectively. In sprinters and long distance runners, the single-leg VO_2peak (L/min) values were 75% (±3) and 83% (±2) (p=0.171), respectively, of the two-leg cycling VO_2peak. The peak workload achieved during single leg cycling relative to two-leg cycling was significantly higher in long distance runners than sprinters, with the values being 49% (±2) and 61% (±1), respectively (p=0.005). The ratio of one-leg to two-leg VO_2peak was not correlated with age.

The peak VO_2/WL relationship did not differ significantly between sprinters and endurance athletes for single-leg (p=0.067) or two-leg cycling (p=0.307), but was significantly higher during single-leg cycling than two-leg cycling (Fig 12; p<0.0005).

During steady-state two-leg cycling, the long distance runners oxidised fatty acids at significantly higher rates than sprinters across a wide range of submaximal exercise intensities (Fig 13d). The peak rate of fatty acid oxidation was 30% higher in long distance runners than sprinters (Figs 13b and 13c; 8.26±0.69 mg/kg/min vs 6.34±0.59 mg/kg/min for long distance vs sprint runners respectively). Which occurred at relatively higher heart rate (117 (±5) vs 105 (±4) bpm for distance vs sprint runners, respectively, p=0.043) and at relatively higher percentage of the VO_2peak (57% (±2) vs 48%(±2) for long distance vs sprint runners, respectively, p=0.043; Fig 13a). The peak rate of fatty acid oxidation decreased by around 8% per decade in sprinters and 13% per decade in long distance runners (p=0.385; Fig 13d). However, there was a very large inter-individual variation in these measurements, particularly amongst the long-distance runners.

The aerobic power was only a small fraction of the peak anaerobic power, demonstrating a very large anaerobic reserve (*Figure 14*). The power (W) at VO_2peak was 9.5% (±0.5) of that achieved during a vertical jump in long distance runners, and in sprinters it was significantly lower (p=0.001) at 6.9% (±0.3) of the peak jump power. The power (W) at peak fat oxidation was 4.4% (±0.3) of power achieved during a vertical jump in long distance runners, and in sprinters it was significantly lower (p<0.0005) at 2.6% (±0.2) of the peak jump power. These measurements of aerobic and anaerobic reserves expressed proportionally to the anaerobic potential did not change significantly with increasing age (*Figure 14*).

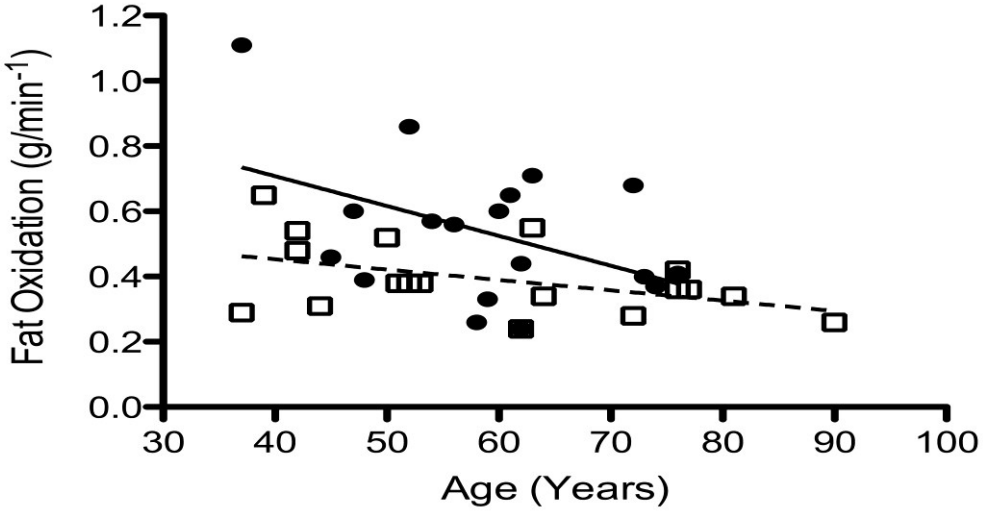

Figure 13a: Rates of fatty acid oxidation during submaximal intensity two-legged cycling: plotted against age (years).
Sprinters (open squares) and long distance runners (shaded circles).

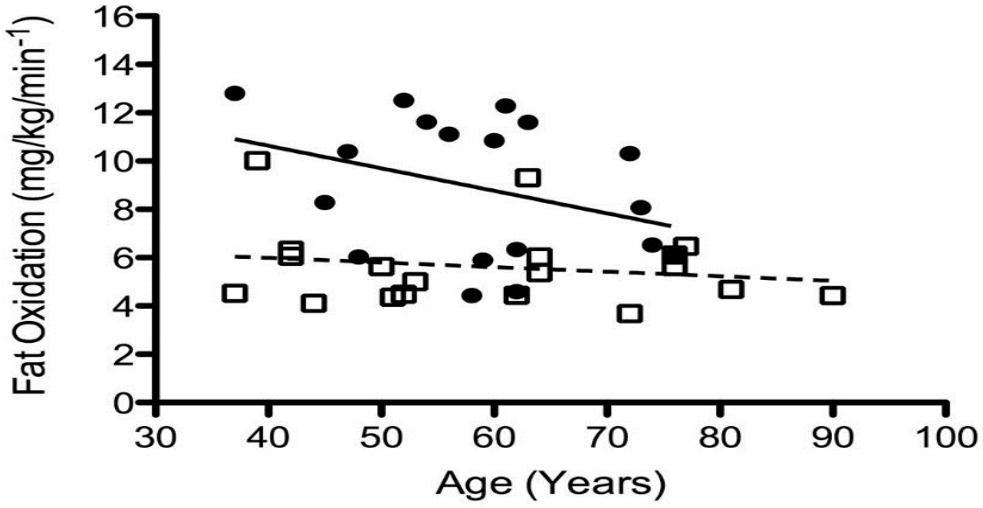

Figure 13b: Rates of fatty acid oxidation during submaximal intensity two-legged cycling: normalised to total body mass and plotted against age (years).
Sprinters (open squares) and long distance runners (shaded circles).

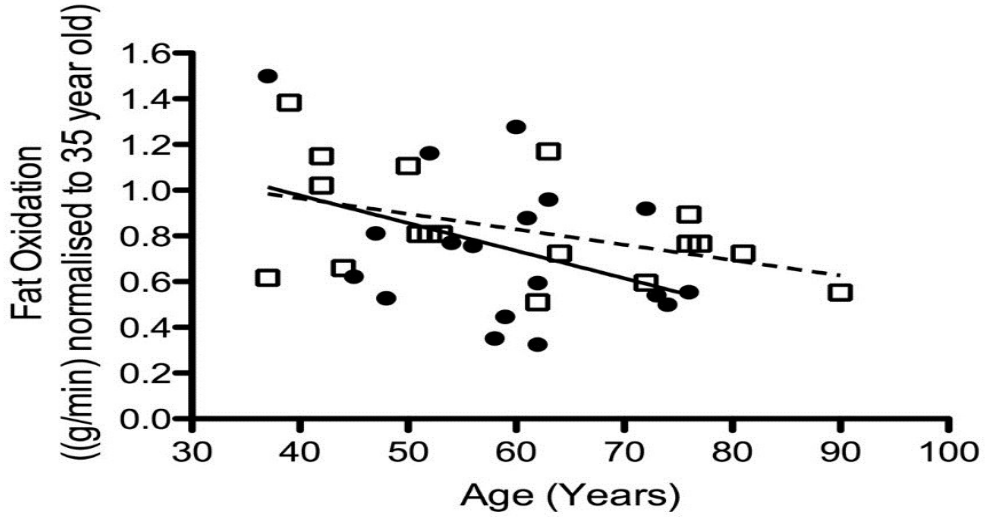

Figure 13c: Rates of fatty acid oxidation during submaximal intensity two-legged cycling: normalised to values for 35 year olds and plotted against age (years).

Sprinters (open squares) and long distance runners (shaded circles).

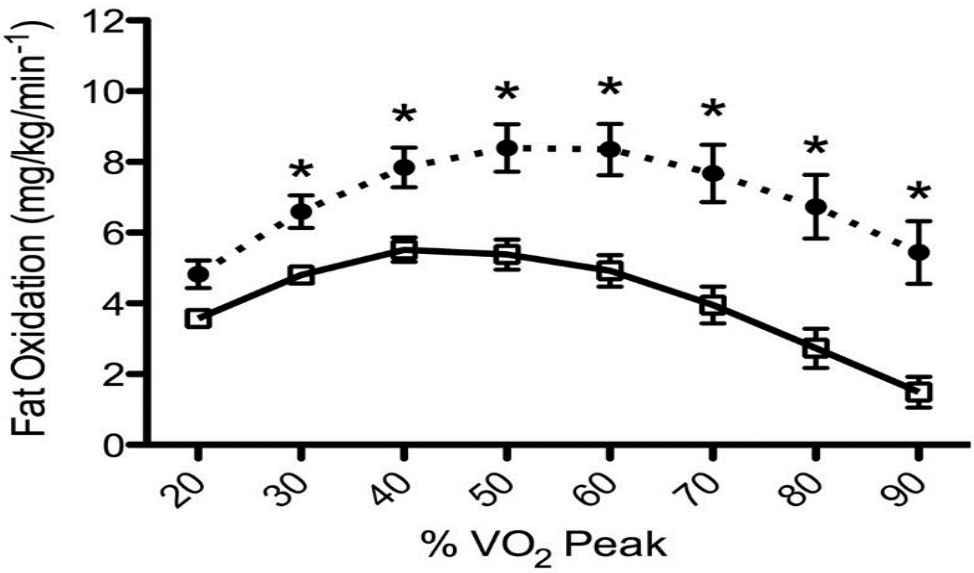

Figure 13d: Rates of fatty acid oxidation during submaximal intensity two-legged cycling: expressed in relation to the %VO_2Peak.

Sprinters (open squares) and long distance runners (shaded circles). Error bars indicate SEM; * indicates p<0.01 for group comparison.

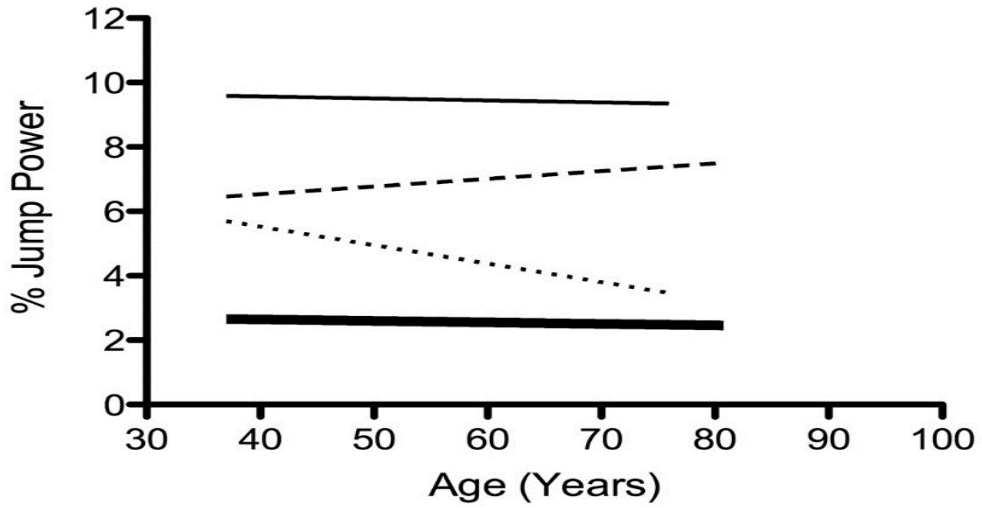

Figure 14: Power output at peak fat oxidation and peak oxygen uptake expressed relative to the peak jump power (all in W).

Peak jump power is expressed as 100% power output; power at VO_2peak in long distance runners (upper thin black line) and in sprinters (dashed line); power at peak fat oxidation in long distance runners (dotted line) and in sprinters (lower thick black line) are all presented as a proportion of peak jump power (100%). Axis has been truncated to show differences in power output between variables.

6.4 Discussion

Regular exercise is the most effective way to combat the declines in physical capability that occurs with advancing older age. However, the cross-sectional data from very athletic people aged 38-90 years suggest declines of around 10-12% per decade in peak anaerobic and aerobic power. The trajectory was almost identical for sprint and endurance athletes. These results reveal the extent of changes within the neuromuscular and cardiopulmonary systems that are not attenuated by competitive athletic training.

The participants recruited for this study were representing their country at the European Masters Athletic Championships and are considered to be amongst the most athletic people for their ages. The cohort included 5 gold medal winners. Most notably, the 37 mL/kg/min VO_2peak for the 90 year old lady is the highest that the author is are aware of for nonagenarians and is equivalent to values expected for females who are 50 years younger (Loe et al., 2013).

As expected, sprinters had much higher power output compared with endurance athletes (Michaelis et al., 2008). Normalising the peak muscle power to total body mass halved the

performance advantage, but the sprinters still maintained 22% higher power than endurance runners (*Figure 10b*). Conversely, the absolute values for VO_2peak (L/min) and rates of fatty acid oxidation were similar in sprint and endurance athletes. Thus, after normalisation to body mass the sprinters consumed 17% less oxygen during peak aerobic exercise than the endurance runners (*Figure 10d*), which is in agreement with previous studies (Kusy et al., 2012; Barnard et al., 1979; Child et al., 1984). Even greater divergence existed between sprint and endurance athletes in the peak rates of fatty acid oxidation, where the values for sprinters were 30% lower than those for the endurance athletes after normalisation to total body mass (*Figure 13b*). In the general population, rates of fatty acid oxidation increase progressively from rest to moderate intensity exercise, after which they decrease as intensity continues to increase (Venables et al., 2005). Older people have lower rates of fatty acid oxidation than younger adults (Sial et al., 1996; Toth and Tchernof, 2000), but it can increase with endurance training (Sial et al., 1998; Johnson et al., 2010; Toth et al., 1995). The peak rate of fatty acid oxidation occurred at 40-50% VO_2peak in sprinters, which is similar to younger adults in the general population (Venables et al., 2005), but rates were higher in endurance runners at all but the lowest exercise intensity and the peak occurred at 50-60% VO_2peak (*Figure 13d*).

A previous study estimated the rate of decline in peak jump power of sprinters and endurance runners as 13% and 10%, respectively, per decade (Grassi et al., 1991; Chamari et al., 1995; Michaelis et al., 2008), which is similar to the values reported previously in this study. A study of VO_2peak in endurance runners (Wiswell et al., 2001) and in the general population (Loe et al., 2013) estimated declines of around 7% per decade, which is less than the values reported in this study. Notwithstanding the fact that VO_2peak in masters athletes declined at a greater rate than those in the general population (see Michaelis et al. (2008) for anaerobic power and Loe et al. (2013) for aerobic power), the values for both types of athlete were substantially higher than averages from the general population, which is a consistent finding (Proctor and Joyner, 1997; Pollock et al., 1987; Tanaka et al., 1997; Fitzgerald et al., 1997; Pimentel et al., 2003).

Using the jump data from the masters sprinters in the study of Michaelis et al. and the VO_2peak data from Wiswell et al., 2001 (*i.e*. a scenario that takes the highest anaerobic and

aerobic, respectively, power occurring with specific training), it was estimated that the proportion of total power that can be supplied through aerobic processes is around 30% of the peak anaerobic power (Rittweger et al., 2009). This high value may be due to the derivation of jump data from master sprinters and the VO_2peak data from master endurance runners. A true estimation of the 'anaerobic reserve' can only come from measurements completed in the same individuals, as was the case in the present study. The workload (in Watts) at VO_2peak was only around 9% of the peak jump power (in Watts) in endurance runners and was significantly lower at around 7% of the peak jump power in sprinters. The peak rate of fatty acid oxidation in endurance runners was around 5% of peak muscle power, but was significantly lower in sprinters at just 3% peak muscle power. Due to the very similar rates of decline in anaerobic and aerobic power, as well as sprint and endurance runners, the anaerobic reserve greater than 90% was relatively stable with ageing. These results put into context the very small proportion of muscle power that is utilised to complete most daily activities such as walking, which are completed at intensities close to the peak rate of fatty acid oxidation.

A well-motivated person could continue to exercise at an intensity that elicits VO_2peak for only around 2 min before succumbing to fatigue. The VO_2peak is limited in healthy young adults by the supply of oxygen to the working muscles during whole body exercise (Saltin and Calbet, 2006). An indication of the extent of the central limitation can be gained from the comparison of one-leg to two-leg cycling VO_2peak (McPhee et al., 2009). Single leg cycling was less economical than conventional two-leg cycling, which might reflect a prolonged recruitment of less efficient Type 2 muscle fibres during the slower cadence and longer muscle activation. The endurance runners achieved higher relative single-leg performance compared with sprinters, indicating that the leg muscles of endurance runners had disproportionately high oxidative capacity and thus, a greater central limitation to whole-body aerobic performance. This is in fitting with observations that muscle mitochondrial enzymes and those of fatty acid metabolism have a remarkable potential to improve size and density following endurance training (Hoppeler et al., 1985; Gollnick et al., 1973), which probably also explains the very high rates of fat oxidation in the endurance athletes. It is also possible that the sprinters had larger fibre cross-sectional areas, which increases the diffusion distance for oxygen from capillary to mitochondria resulting in earlier

onset of fatigue. The ratio of one-leg to two-leg performance remained relatively constant across the ages, indicating that the limitations to VO_2peak are similar for younger and older athletes. It is therefore likely that strategies to improve cardiovascular supply of oxygen to the working muscles of the older endurance athletes, such as increasing stroke volume, will improve the VO_2peak still further.

In recent years, numerous studies showed that a few weeks of high intensity interval training or sprint interval training increased VO_2peak, muscle capillarisation and enzymes of oxidative metabolism that are synonymous with endurance training, although these studies were undertaken using untrained participants (Bacon et al., 2013). The results in the present study demonstrate the specificity of neuromuscular and cardiopulmonary function of the athletes who have been competing in the different disciplines for many years.

Limitations:

The main limitation of the present study was the cross-sectional design. It is possible that such physiological profiles are the product of heritable pre-disposition and as such are beyond the reach of most people, but the intensive exercise training programmes undoubtedly contributed to their outstanding physical capabilities. In this respect, it is interesting to note that former athletes have improved physiological profiles over the average of the general population (Teramoto and Bungum, 2010). Furthermore, it is also not possible to determine whether the divergent profiles of the endurance runners and the sprinters are due to their specific training programmes and/or to heritable factors, but it is likely that both play a role. As in the previous chapters, due to the nature of the study data collection it was not possible to control for female menstrual cycle or for diet of the participants.

Conclusion

As hypothesised, master athlete sprint runners had 22% higher peak power output but 17% lower VO_2peak and 30% lower rate of fatty acid oxidation than master athlete endurance runners. The data shows that muscle aerobic power constitutes a very small fraction of peak muscle power in both sprint and endurance trained masters athletes and show for the first time that the aerobic capacity as a fraction of total power changes little with ageing regardless of training modality. Nevertheless, peak anaerobic and aerobic power decrease

in sprint and endurance masters athletes at rates of around 10-12% per decade. These observations taken together with the improvements of VO_2max and metabolic outcomes after 12 weeks SIT, suggest that both endurance and sprint type running promote similar adaptations over the lifespan that may help to maintain muscle power more in sprinters. However when compared, endurance athletes have higher weight normalised VO_2peak and rates of fatty acid oxidation when compared to sprint athletes, suggesting that endurance type training may provide a metabolic advantage over sprint type training.

Chapter 7
General Discussion and Future Research

7.1 Main Findings and Implications

The overall aim of the work presented in this thesis was to measure the physiological effects of sprint interval training (SIT) in males and females recruited from the general population of Manchester, UK.

Objective 1 was to recruit males and females from the general population to complete 12 weeks cycling SIT and monitor changes relating to health and physiological function. The results presented in Chapters 3, 4 and 5 show the physiological responses to 12 weeks SIT and highlight possible gender differences in some responses. The main findings were:

- After 12 weeks SIT, both males and females showed significant improvements to primary outcomes of VO_2max (9%), FATmax (15.2%) and body fat reductions (1.2%).
- Females improved in VO_2max (ml/kg/min) more than males as a result of SIT (18% and 6% respectively, $p=0.009$), males reduced body fat % more than females (gender comparison: $p=0.015$).
- Untrained males had 38% (gender comparison: $p<0.001$) higher knee extensor torque than females, but when strength was normalised to muscle cross sectional area there was no difference between males and females (gender comparison: $p=0.200$).
- Males and females increased knee extensor muscle size by 4.1% in males ($p=0.033$) and 5.8% in females ($p=0.011$). There was no significant gender difference in the CSA response to training and the strength normalised to CSA did not change significantly after 12 weeks SIT ($p=0.200$).
- Knee extensor fatigue resistance increased after 12 weeks SIT ($p=0.003$), with no differences in training response between males and females (gender comparison: $p=0.153$).
- Males and females did not alter resting circulatory cytokine and adipokine concentrations after 12 weeks SIT ($p>0.05$). However, circulating levels of adiponectin and lipocalin were associated with SIT induced improvements in VO_2max and body fat changes.

Objective 2 was to recruit Masters Athlete sprint and endurance runners to complete measurements of health and physiological function. The results presented in Chapter 6 show the physiological profile of exceptionally athletic older males and females who had trained specifically for sprint or for endurance running. The main findings were:

- Sprint athletes have higher peak power outputs than endurance athletes throughout older age.
- Endurance trained athletes have higher VO_2max and fatty acid oxidation rates than sprint trained athletes throughout older age.
- Aerobic muscle power in competitive master endurance and sprint athletes constitutes a small portion of peak muscle power (~6%) and this proportion does not change during ageing..
- Declines of 10-12% per decade of both peak and aerobic muscle power outputs are seen in both endurance and sprint trained master athletes.

Gender dimorphism in the physiological responses to sprint interval training

The first aim of this thesis (pages 7-9) was to compare the physiological effects of a 12 week SIT intervention in males and females recruited from the general population. It was hypothesised that SIT would increase both VO_2 max and reduce body fat mass in males and females from the general population, supported by an increase in the rate of fat oxidation during exercise. This work progressed knowledge from the previous studies that reported an increased cardiorespiratory fitness and significant reductions in body fat mass in young males, or small samples of obese people. The new results presented in Chapter 3 showed significant improvements in VO_2 max and fat oxidation, alongside decreases to body fat mass after 12 weeks SIT in a group recruited from the general population, this equated to just 4 minutes of sprinting per week for the duration of the intervention. Although there were gender differences in the magnitude of responses in VO_2max and loss of body fat mass, the results nevertheless suggests that SIT is an effective method to raise VO_2 max, which in itself is an indicator of health (Blair et al., 1996), and fat oxidation rates during subsequent exercise and physical activity with a very minimal time commitment. This evidence will be of significant importance to health practitioners with the aim of improving public health and longevity of “health span”, especially in those who experience a lack of

time to commit to high volume aerobic training programmes demanded by endurance training programmes (Garber et al., 2011; Booth et al., 1997). Although both males and females both improved in the measured physiological outcomes, the observed gender dimorphism may impact exercise prescription by practitioners.

Interestingly, 7 participants in Chapter 3 were classified as 'very poor' or 'poor' against normative data for VO_2max (Shvartz and Reibold, 1990). After 12 weeks SIT, all of these participants were classified as 'fair' and above. Furthermore, 4 participants were above the NHS healthy range guidelines of total cholesterol/HDL ratio of >4 (National Health Service, 2015) with again all of these being considered in the healthy range after SIT (<4). This may suggest that there is a large population effect of SIT, but there is also a large individual and clinical effect to this modality of training. This has implications for exercise prescription practitioners who may wish to supplement higher, supramaximal exercise into a patient prescription in order to improve a certain variable in their diagnosis.

The gender dimorphism after SIT interventions is poorly understood, primarily due to the literature largely involving young males or athletes. If any dimorphism is present, this information is vitally important when prescribing exercise for health but also to athletic performance programmes. VO_2 max increase is a common aim of a great deal of exercise intervention studies. As a highly integrative physiological measure it provides an in depth assessment of an individual's respiratory, cardiovascular and metabolic status, being used as a typical indicator of health and quality of life (Blair et al., 1989). In order to improve VO_2 max measurements, an individual must therefore increase respiratory muscle efficiency, vascular conductance, cardiac output and muscle oxidative capacity in order to elicit any improvement. Therefore, the effect of exercise training is not only measuring an improvement in physical fitness, but a plethora of physiological systems working as one entity. Due to the often divergent response in VO_2 max to endurance training between males and females (Ogawa et al., 1992) and the similarity of this response between endurance and sprint type training (Burgomaster et al., 2008), dimorphism may exist after SIT interventions. The results of this question are presented in chapter 2, and show an increase in VO_2 max in both males and females, but interestingly suggest a tendency for females to increase VO_2 max to a higher extent than males after SIT. From the data gathered in this study it is not clear where the difference between males and females's VO_2 max response to SIT lies, with limited evidence in the literature to help explain this finding.

Nevertheless, it is an important observation of the physiological systems described above which contribute to VO_2 max and therefore suggest a divergent response between males and females in one of these physiological systems responses to SIT. In individuals at risk of, or to reduce an individual's risk of cardiovascular disease, SIT may therefore provide a useful tool for improving maximal oxygen consumption via improvements to respiratory muscle efficiency, vascular conductance, cardiac output and/or muscle oxidative capacity. However, where this difference lies between males and females to give a divergent response in VO_2max warrants further investigation in order to best prescribe SIT exercise to females and/or males specifically.

Optimal rates of fatty acid oxidation during physical activity are vital for reducing the onset of metabolic syndrome through an increase in insulin resistance via interference with the insulin signalling cascade (Montell et al., 2001). Both endurance and SIT intervention studies have yielded similar results in terms of rates of fat oxidation and suggest similar mechanisms in the form of increases in metabolic enzymes (Burgomaster et al., 2008) and skeletal muscle capillarisation (Cocks et al., 2013). Despite the differences in untrained males and females rates of fat oxidation (Venables et al., 2005), little evidence exists as to whether there is a divergent exercise training response between males and females. Males and females possess significantly different metabolic qualities, such as females maintaining higher rates of fat oxidation through prolonged exercise (Horton et al., 1998), a higher relative oxidative capacity (Kent-Braun and Ng, 2000), a slower muscle phenotype with proportionally larger Type I fibres (Staron et al., 2000) and lower mitochondrial fractional synthetic rate after training (Karakelides et al., 2010). Data presented in chapter 2 shows increases in rates of fat oxidation after SIT in both males and females. Despite the different metabolic profiles of males and females described above, rates of fat oxidation improved similarly in both males and females. This data is of importance to practitioners prescribing exercise for improvement of metabolic profile, primarily those at risk of cardiovascular disease and Type 2 Diabetes, in which an increase in fat oxidation rates and weight loss would provide huge reductions in risk factors associated with these diseases. SIT could therefore be considered as a time effective and successful training modality to improve metabolic profile of those at risk of metabolic disorder.

A large proportion of the population of both genders are actively attempting weight loss strategies with ~40% of those asked utilising increased physical activity as a tool for fat loss

(Serdula et al., 1999). High body fat mass is a key indicator of disease risk and mortality with subsequent loss of body fat mass through physical activity proving a successful method of decreasing risk and onset of disease (Christiansen et al., 2010). As well as higher rates of fat oxidation in females than males in the untrained state in the majority of the literature, paradoxically, females have been observed to possess a higher percentage of body fat mass as age and training status matched males (Bijlsma et al., 2013). This higher relative body fat mass level in females than males was observed in the data presented in chapter 3. Both males and females lost fat mass after 12 weeks SIT, this was true in both absolute terms (kg) and in relative terms (as % body mass) in both genders. However, in terms of body fat reduction in relative terms, body fat was more significantly reduced in males than females. Loss of body fat is a regularly seen response to endurance training and SIT intervention studies, however this data is the first to observe the divergent response between males and females. The mechanism for this reduced fat loss in females compared to males is unclear from this study, especially as changes in rates of fat oxidation measurements were not correlated with changes in fat mass. However, this finding is an intriguing insight to the outcomes of SIT in a general population group, proposing a low volume and highly effective fat loss methodology in a general population group.

Secondly, this thesis aimed to compare muscle size, strength and fatigue resistance in males and females after the SIT intervention, with the expectation that these characteristics would be improved after training. No increase in knee extensor strength characteristics were observed after 12 weeks SIT, however a modest increase in CSA and an improvement of skeletal muscle resistance to fatiguing exercise was seen (chapter 4). This adds evidence to the claims of this type of exercise mirroring the skeletal muscle adaptations seen after endurance exercise (Burgomaster et al., 2008; Gibala et al., 2006). The lack of increase in strength characteristics (isometric torque) after training parallels the observations a similar length endurance training protocol 10 sedentary adult males (mean age= 27±2 years, VO_2peak= 41.4±2.6 ml/kg/min) (McCarthy et al., 1995). This concludes that SIT is an ineffective method in the improvement of muscle strength in healthy individuals. However, 12 weeks SIT cycling significantly improved knee extensor resistance to fatigue equally in both males and females, which is most likely due to improvements in oxidative capacity through increases in skeletal muscle mitochondrial proteins and capillarisation of the muscle (Gibala et al., 2006; Cocks et al., 2013). This improvement in resistance to fatigue after SIT is

specifically relevant to practitioners and athletes alike who have the aim of improving exercise performance, but also to reach the aim of improving oxidative capacity for overall metabolic health.

The circulatory inflammatory profile associations with sprint interval training physiological response

This thesis also aimed to investigate the circulatory concentrations of inflammatory markers (cytokines and adipokines) as a result of 12 weeks SIT, as well as the potential for these markers to be associated with the training associated change in circulatory concentrations and physiological training adaptations, namely VO_2max and Body Fat levels.

Chapter 5 presents the possibility that cytokine, adipokine and lipoprotein concentrations are linked to body fat levels and cardiorespiratory fitness, both of which are directly linked to disease risk and all-cause mortality. Circulatory lipocalin and PAI-1 concentrations in plasma were inversely correlated to absolute VO_2 max (L/min), where only circulatory adipsin concentrations were positively correlated to body fat mass (kg). Previous work is consistent with this finding that examined the levels of plasma lipocalin and PAI-1 are significantly elevated in Type 2 diabetic patients and cardiovascular disease patients (Wang et al., 2007; Trayhurn and Wood, 2004), thereby linking cardiorespiratory fitness with these circulatory markers.

Chapter 5 shows that 12 weeks of SIT had no effect on resting circulatory inflammatory profile in healthy males and females. The adipokine response to SIT (acute and chronic) is an understudied area and thus far, healthy and athletic populations only have seen improvements in circulatory adiponectin concentrations (Shing et al., 2013; Jürimäe et al., 2005). However, other studies with healthy females (Jamurtas et al., 2013) and overweight males (Jamurtas et al., 2006) showed no increase in plasma adiponectin. This contradiction in data could be explained due to the large constant pool of adiponectin in the plasma, suggested by Jürimäe et al, therefore any changes in plasma concentration is obscured (Jürimäe et al., 2005). Notwithstanding this lack of change in circulatory inflammatory profile, significant correlations were drawn between the change in VO_2max and body fat after 12 weeks SIT with primarily circulatory adipokine concentration before training. Higher circulatory levels of resistin, lipocalin and PAI-1 at baseline were significantly correlated with a higher improvement in VO_2max (both weight normalised and absolute) after SIT. All three

of these adipokines have previously been observed as elevated in obese populations at rest (Gharibeh et al., 2010; Steppan et al., 2001). Of particular interest are the baseline levels of resistin, lipocalin and adiponectin were significantly related to SIT induced change to body fat levels, this not only clarifies previous findings linking concentrations of these adipokines to health status, but also opens an avenue of investigation in that circulatory concentrations of these adipokines are predictive of training induced physiological change. However, training response from baseline concentrations and analysis in this manner has not commonly been reported, therefore this finding requires further clarification to suggest a mechanism by which this improvement comes about.

Master athlete sprinters as a model of lifelong sprint exercise and training

The second primary objective of this thesis was to investigate the potential for lifelong sprint exercise to bring about similar preservation of physiological function as long term endurance exercise, utilising master athletes as a model.

A cross sectional study using collecting data from master athletes competing at the 18th European Veterans Athletics Championships was carried out to examine the possible differences between endurance and sprint event athletes. This provided an excellent insight into the potential for sprint athletes to be studied over lifespan, examining how SIT could potentially affect physiological outcomes during the ageing process.

Despite high training volumes and remaining competitive throughout lifespan, the athletes examined declined in aerobic and anaerobic power by approximately 10-12% per decade, suggesting that even high volumes of endurance and sprint training cannot fully attenuate decline in cardiopulmonary and neuromuscular systems. Sprint athletes did have some performance advantages over endurance athletes at old age which may be relevant to an ageing non athletic population, in that peak power was significantly higher in sprinters. In absolute terms, VO_2peak (l/min) and FATmax (g/min) were similar between sprinters and endurance athletes, however due to sprinters being significantly heavier than the endurance athletes, this similarity was curtailed, with endurance athletes consuming 17% more oxygen than sprinters during peak aerobic exercise. Furthermore, rates of fat oxidation were much higher in endurance athletes than sprinter once normalised to body mass at all but the lowest exercise intensities. Further to this finding, previous evidence suggests that endurance training can improve rates of fat oxidation during exercise in older

people (Toth et al., 1995; Johnson et al., 2010; Sial et al., 1998), suggesting endurance type protocols could be more beneficial to older persons to improve metabolic health factors. The novel data in this chapter came with the analysis of the power at VO_2peak as a percentage of peak jumping power in endurance and sprint master athletes, which showed that the reserve of anaerobic power over lifespan is seemingly preserved at approximately 90% in master athletes. This data shows that the majority of daily tasks such as walking only utilise approximately 3-5% of total muscle power output. Declines in VO_2peak were observed at 12% per decade in this athlete population, which was similar between endurance and sprint athletes, this corresponds to a number of previous studies (Pollock et al., 1997; Tanaka et al., 1997; Proctor and Joyner, 1997). One legged to two legged cycling ratio was also similar across lifespan and lower performance was observed in sprint athletes compared to endurance athletes. The latter observation suggests that older endurance athletes have an increased oxidative capacity at the working muscle, this can be ascertained due to the lack of cardiovascular limitation during one legged cycling (Davies and Sargeant, 1974; Saltin et al., 1976). The data presented in chapter 5 therefore gives an impression as to the lifelong training effect of sprint and endurance discipline training, from this it is possible to gain an insight into the long term physiological responses to sprint training.

The evidence for utilising High Intensity Interval Training (HIIT) or SIT in public health guidelines for exercise was recently the topic of a UK Physiological Society 'Crosstalk' debate, with authors presenting the arguments for and against High Intensity Interval Training in prevention and treatment of disease. Although as pointed out throughout this thesis there are a number of significant health benefits to SIT and HIIT, Holloway and Spriet (2015) illustrate there is still a relevant place for endurance type training in health promotion and maintenance. In the case of cardiac rehabilitation, there is significant amounts of evidence that continuous, moderate intensity exercise improves cardiovascular risk factors, such as reduced blood pressure in hypertensive patients (Cornelissen and Smart, 2013). SIT, whilst being effective in healthy populations as demonstrated here, may be contraindicative in patient populations, with the potential requirement for a "lead in" training period in some populations before committing to this higher intensity of workload in a rehabilitation-training programme (Gillen and Gibala, 2014). There is no human study data available as to how this method of exercise may affect a cardiovascular patients heart

or long term clinical outcomes, it is therefore unadvisable for this method of training to be utilised in a rehabilitation programme. In rodent studies, high intensity exercise in hypertensive rats has been seen to adversely remodel the left ventricle (da Costa Rebelo et al., 2012). In further investigation, high sodium fed hypertensive rats took part in either moderate intensity endurance training, or high intensity interval training 5 times per week for 4 weeks. The high intensity trained rats showed an increase in cardiac weight (~20%, $p<0.05$), a reduction in cardiac muscle CSA (~20%, $p<0.05$) and no effect on cardiac capillary to muscle fibre ratio, suggesting adverse pathological remodelling as a result of higher intensity of exercise in hypertensive models (Holloway et al., 2015). In the same study, high sodium fed hypertensive rats who took part in moderate intensity continuous training showed significantly reduced left ventricular fibrosis (~40%, $p<0.05$) and higher cardiac capillary to fibre ratio (~20%, $p<0.05$) from baseline, with no effect on cardiac weight or cross sectional area (Holloway et al., 2015). Therefore, more evidence is required in order to consider implementing SIT/HIIT in clinical populations (after a "lead in" of build-up training) so as not to promote acceleration of cardiovascular disease pathogenesis, however may still present a highly time effective manner of improving public health in healthy populations. Although SIT presents a viable tool to increase metabolic health and physical performance in non-clinical populations, in clinical patients this method of exercise may be contraindicative, with moderate intensity continuous exercise remaining the primary mode of rehabilitation.

7.2 Limitations

Each study included in this thesis discusses individual limitations to the interpretation of the data collected in turn. However, it is relevant to discuss some general limitations to the body of work as a whole.

The same cohort of recruited participants were studied in one or more of the studies presented in chapters 3, 4 and 5 of this thesis. Deviations in the number of participants, from chapter to chapter and in some cases analyte to analyte, are because of limitations in data collection such as issues with capacity of equipment (e.g. the amount of kits for ELISA) and lack of access to equipment over testing periods, with testing taking place over approximately 6 months.

As alluded to in the experimental chapters, menstrual status was not ascertained in the female participants of this study, along with menstrual cycle, this potentially may have some

impact on the results of the data, due to the well-established effects links between sex steroid hormones (primarily oestrogen) and metabolic variables such as fatty acid oxidation (Oosthuyse and Bosch, 2010). This issue is discussed at length in Chapter 2.5 as well as throughout this thesis, however taken together, this data suggests a gender dimorphism is present between males and females in response to SIT. This opens an interesting avenue for the future direction of research to establish the extent of this potential training response difference.

Furthermore, it is not possible to ascertain as to whether the participants involved in these studies are pre-disposed to certain exercise training responses which may be described as a potential limitation to the study of athletes (Tucker and Collins, 2012). This may limit somewhat the interpretation of whether SIT is viable to be applied to a population group and the effect size of the training responses. The future large scale study of SIT responses in the context of pre-disposition to training response will alleviate this issue, however currently the evidence suggests that SIT exercise improves physiological outcomes in a large number of participant group types (Gillen and Gibala, 2014; Weston et al., 2014).

Finally, the diet of participants was not controlled, with the participants being told to follow normal eating habits. Therefore the participants may have increased or reduced caloric intake, as well as increased/decreased carbohydrate, protein or fat intake. All of which may have a chronic or acute effect on physiological variables, specifically substrate utilisation during exercise (Cameron-Smith et al., 2003; Coyle et al., 2001).

7.3 Directions for future research

Gender comparisons between males and females after 12 weeks SIT deserves further investigation. This finding could implicate not only athletes wishing to improve performance, but a large proportion of the population who wish to increase physical activity in order to lose fat mass and improve health. The key metabolic characteristics that cause this divergent physiological response between males and females, which may be due to responses in key metabolic enzymes (Talanian et al., 2007), oxidative capacity (Burgomaster et al., 2005), or central adaptation to SIT (Tjonna et al., 2013) seen previously, needs further clarity. Future study should further control for variations in the female menstrual cycle, which may have an effect on physiological variables in female participants. In the present studies, the stage of menstrual cycle was not controlled due to the large numbers of

participants being recruited into the study. However, evidence suggests that the phase of the menstrual cycle has no effect on the menstrual cycle during moderate intensity exercise. Horton et al. (2002) saw no difference in lipid oxidation in 13 eumenorrhoeic females (mean age= 29±5) between the early follicular, mid follicular or midluteal phases of the menstrual cycle at rest, or during a 90 minute exercise bout at 50% VO_2max. Similarly at high intensities of exercise (40 minutes at 80% VO_2max) substrate oxidation was no different between menstrual phases in 8 eumenorrheoic females (mean age= 25±6) (De Souza et al., 1990). Similarly, the role of oral contraceptives use and its effect on substrate oxidation is unclear, with evidence pointing toward an increase in fatty acid mobilisation, but not oxidation (Casazza et al., 2004; Jacobs et al., 2005). Despite this, it would be useful in future studies to correctly control the entry point of females in terms of menstrual cycle into direct comparison studies, as well as controlling for the use of oral contraceptives. Although it is contradictory as to whether this would affect rates of fatty acid oxidation, the dimorphism between males and females may be due to a difference in steroid hormones within the females populations of the studies which may alter substrate oxidation. The use of SIT as a tool to improve the metabolic profile through increases in fatty acid oxidation rates therefore requires further investigation in terms of the effects that steroid hormone concentrations.

Future work could also involve the use of dietary interventions combined with a SIT intervention. Alteration of dietary intake has direct effects on levels of substrate utilization during exercise, with a high fat diet increasing rates of fat oxidation during exercise (Helge et al., 2001). Shen et al. (2015) observed that in rats fed a high fat diet, high intensity interval training increased CPT-I and REV-ERBα mRNA (a signalling molecule suggested to increase transcription of a number of enzymes associated with fatty acid transport and oxidation, including CPT-I (Solt et al., 2012). In humans, Helge et al. (2001) studied 13 healthy males (mean age= 27 years, VO_2max= 3.9 l/min) by endurance cycle training (~60 minutes at 65%VO_2max, 4 times per week) and feeding a high fat diet (n=7, 62% fat, 21% carbohydrate) or high carbohydrate diet (n=6, 20% fat, 65% carbohydrate) for 7 weeks. The participants fed the high fat diet demonstrated a significantly higher fatty acid uptake (~73%, $p<0.05$) than high carbohydrate fed participants as well as demonstrating a significantly higher rate of fat oxidation during exercise (~52%, $p<0.05$). However, there is little data as to how this would affect longer term health outcomes through an increase in

fatty acid oxidation related enzymes, as well as whether these positive adaptations can be maintained over longer periods (ie, >12 weeks). Similarly, it remains to be seen as to how higher fat or carbohydrate diets may have an effect on the physiological responses to SIT.

The fatigue resistance increase measured by isolated knee extension exercise warrants further study. This increase in a single leg, isolated exercise can only be due to an increase in oxidative capacity of the working muscle, as cardiovascular supply is not a limiting factor, similar to one legged cycling (Andersen and Saltin, 1985; Richardson et al., 1999). More focussed studies examining cardiac output, blood volume, haematocrit and blood flow distribution are needed to clarify this finding.

The reasons for the gender differences in training responses of body fatness and metabolism are not clear (Devries, 2015), endurance training interventions with mechanistic methods are required using muscle and adipose biopsies to add molecular mechanisms to the substantially higher fat loss in males after SIT interventions. Molecular contributions to skeletal muscle adaptation have whole body outcomes in terms of physical activity and health, implementing a focus on muscle and adipose tissues and relating this to higher physiological outcomes such as loss of body fat will increase understanding of gender differences in exercise response.

The chronic inflammatory state has received much attention in the past few years, with SIT type interventions also being utilised to study reduction in systemic inflammatory markers. However exercise intervention studies are now required to understand the most effective modality of physical activity to reduce the circulatory inflammatory proteins commonly associated with disease risk (King et al., 2003). After observing associations between baseline circulatory concentrations of inflammatory markers such as adiponectin and significant decreases in body fat after training, further data is needed to establish a link between circulatory and molecular level expression of circulatory inflammatory proteins and training adaptations to SIT or endurance exercise. Although in this study there was no significant divergent response in adipokine or cytokine circulatory concentrations between males and females after SIT, females demonstrated an 84% higher circulatory adiponectin concentration than males at baseline ($p=0.006$). This is also seen in other large cohort studies such as the Copenhagen city heart study (n=5,624 males and females age 20-94 years) (Lindberg et al., 2013) and in well-functioning elderly participants (n=3,075 males and females age 69-79 years) (Poehls et al., 2009), linking high circulatory adiponectin

concentration to reduced mortality and disease risk (Alehagen et al., 2015). This gender difference in circulatory adiponectin could have significant effects on health but also to training outcome, whether this higher concentration of adiponectin affects the training response to physiological variables in males and females remains unseen and requires further enquiry. This could have significant benefits to exercise prescription and act as future biomarkers for disease risk (Laughlin et al., 2007).

In conclusion, SIT presents a viable and effective mode of exercise training to improve and maintain the condition of healthy males and females of a variety of ages in the light of increasing incidence of cardiovascular diseases and metabolic syndrome. The evidence presented in this thesis suggests that males and females respond somewhat differently to the training stimulus experience during SIT, however positive health benefits are universal. The mechanisms of these physiological adaptations to SIT are mostly likely to be the result of adaptations commonly seen in endurance exercise. However, as presented here, further enquiry into gender differences in circulatory adipokine and sex steroid hormone concentrations and their association to training adaptation between males and females is warranted in order to fully understand the mechanism of gender dimorphism in response to SIT.

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