THE IMPACT OF REDUCED-EXERTION HIGH-INTENSITY INTERVAL TRAINING ON INSULIN SENSITIVITY AND AEROBIC CAPACITY

RICHARD SEAN METCALFE

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Department for Health

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Abstract

Despite clear recommendations on the minimal amount of physical activity for achieving health benefits and reducing risk of chronic disease, the majority of people in the Western world remain sedentary. As a ‘lack of time’ has been identified as one of the main barriers to becoming and remaining physically active, in the past decade research has focused on high-intensity interval training (HIT) as a time-efficient alternative to aerobic exercise. Although initial studies convincingly demonstrated equal or better health benefits with various HIT protocols compared to much larger volumes of aerobic exercise, these HIT protocols tend to be very strenuous and as such are unlikely to be adhered to by sedentary populations. Furthermore, most HIT protocols are not as time-efficient as sometimes claimed, with the total time per exercise session generally exceeding 20-30 minutes. This thesis aimed to characterise the effects of a novel reduced-exertion HIT (ReHIT) protocol, requiring a maximum of 2 × 20 s all-out sprint efforts in a 10 min training session, upon insulin sensitivity, aerobic capacity, glycogen utilisation and associated acute metabolic responses. The ReHIT exercise bouts were well tolerated by participants, but were associated with a substantial disturbance of physiological homeostasis including muscle glycogen degradation, lactate accumulation, excursions in plasma volume, post-exercise oxygen consumption, respiratory exchange ratio and heart rate, as well as a skeletal muscle signalling response through AMPK, and increases in skeletal muscle GLUT4 and PGC1α mRNA expression (Chapter 4 and 5). The combined training studies (n=49) provide some support for improvements in key disease biomarkers following ReHIT, with improvements in insulin sensitivity observed in men, and increased aerobic capacity observed in men and women (Chapter 7). These observations highlight a potential regulatory role for glycogen in exercise-induced adaptation. However, the mean improvements in insulin sensitivity in men were not consistent between the two training studies (Chapter 2 and 6), and there was a high level of variability observed between individuals (Chapter 6 and 7). Therefore, the impact of ReHIT on insulin sensitivity needs to be further explored in the context of a randomised controlled trial, and the mechanisms underpinning the large variability in adaptive response need to be characterised.
Dedication

This thesis is dedicated to my Mum. The level of dedication you have given to each of your three boys is an inspiration. We wouldn’t be where we are today without your enduring love, guidance and support.
Acknowledgements

This thesis represents the culmination of four and a half years of scientific endeavour. It’s been a slightly longer and bumpier journey than first anticipated, and there are a number of people to be thanked for getting me to this stage.

Firstly, I would like to offer my appreciation to my supervisor, Dr Niels Vollard, for providing me with the opportunity to undertake this PhD project, for giving up countless hours to provide me with guidance and support, and for taking the numerous blood samples which have contributed to the work in this thesis. Your calm and considered approach to complex scientific problems is definitely to thank for keeping me vaguely sane throughout this process!

I would also like to thank Dr Dylan Thompson, Dr James Betts, Dr Francoise Koumanov and Dr José Ruffino for providing me with the opportunity to collect and analyse the muscle biopsy samples for Chapter 5 of this thesis, and also for providing invaluable guidance/advice at various points along the way.

To all the fantastic friends I’ve made throughout the last four and half years (Enhad, Dan, Max, Oly, Tom, Andy, Becki, Becki, Stanley and Rhi), and to both my immediate and extended family, thank you for the ongoing support, guidance, laughter and tea and biscuit breaks you provided along the way. I can’t thank you all enough.

To all the participants who gave up their time, blood and muscle to the studies presented in this thesis. You are too numerous to all be thanked individually but far too important not to be acknowledged at all. Thank you to all of you!

Saving the biggest thank you for last: José, although we came together towards the end, we managed to keep other vaguely sane during the most challenging stages, and they were a lot more enjoyable for it. We got there in the end! Now let the real fun begin.
Publications

Chapters 2 of this thesis has contributed to the following publication:

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List of Abbreviations

ACC: Acetyl-CoA Carboxylase
ADP: Adenosine Diphosphate
AER: Aerobic Exercise
Akt/PKB: Protein Kinase B
AMP: Adenosine Monophosphate
AMPK: AMP Activated Protein Kinase
ANOVA: Analysis of Variance
aPKC: atypical Protein Kinase C
AS160: Akt substrate of 160
ATP: Adenosine Triphosphate
AUC: Area Under Curve
BMI: Body Mass Index
Ca2+: Calcium Ions
CaMPK: Calcium Activated Mitogen Protein Kinase
CGM: Continuous Glucose Monitoring
CHO: Carbohydrate
CO2: Carbon Dioxide
COX: Cytochrome Oxidase
CV: Coefficient of Variation
CVD: Cardiovascular Disease
EDTA: Ethylenediaminetetraacetic Acid
ELISA: Enzyme-Linked Immunosorbent Assay
eNOS: Endothelial Nitric Oxide Synthase
EPOC: Excess Post-Exercise Oxygen Consumption
FATmax: Maximal Fat Oxidation
GIP: Gastric inhibitory polypeptide
GLP-1: Glucagon-Like Peptide-1
GLUT-4: Glucose Transporter Protein 4
GSK-3 α/β: Glycogen Synthase Kinase α/β
HbA1c: Glycated Hemoglobin
HDL: High Density Lipoprotein
HEC: High Exercise Capacity
HIT: High-Intensity Interval Training
HOMA-IR: Homeostatic Model Assessment-Insulin Resistance
HR: Heart Rate
IMTG: Intramuscular Triglycerides
IPAQ: International Physical Activity Questionnaire
IR: Insulin Receptor
IRS-1: Insulin Receptor Substrate-1
IVGTT: Intravenous Glucose Tolerance Test
LDL: Low Density Lipoprotein
<table>
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<tr>
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<th>Definition</th>
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<tr>
<td>LEC</td>
<td>Low Exercise Capacity</td>
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<tr>
<td>MET</td>
<td>Metabolic Equivalent</td>
</tr>
<tr>
<td>METSYN</td>
<td>Metabolic Syndrome</td>
</tr>
<tr>
<td>MGU</td>
<td>Muscle Glucose Uptake</td>
</tr>
<tr>
<td>MLSS</td>
<td>Maximal Lactate Steady State</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide adenine dinucleotide (NADH is the reduced form)</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non-Esterified Fatty Acids</td>
</tr>
<tr>
<td>NHS</td>
<td>National Health Service</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen</td>
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<tr>
<td>OGTT</td>
<td>Oral Glucose Tolerance Test</td>
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<tr>
<td>p-ACC</td>
<td>Phospho Acetyl-CoA Carboxylase</td>
</tr>
<tr>
<td>p-AMPK</td>
<td>Phospho AMPK activated protein kinase</td>
</tr>
<tr>
<td>p-p38</td>
<td>Phospho p38 Mitogen Activated Protein Kinase</td>
</tr>
<tr>
<td>PAR-Q</td>
<td>Physical Activity Readiness Questionnaire</td>
</tr>
<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>PDK</td>
<td>Phosphoinositide-dependent Kinase 1</td>
</tr>
<tr>
<td>PDH</td>
<td>Pyruvate Dehydrogenase</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>Peroxisome-proliferator Activated Receptor γ Coactivator</td>
</tr>
<tr>
<td>PIP2</td>
<td>Phosphoinositide 4,5-Bisphosphate</td>
</tr>
<tr>
<td>PIP3</td>
<td>Phosphatidylinositol 3,4,5-Triphosphate</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphoinositide 3-Kinase</td>
</tr>
<tr>
<td>PYY</td>
<td>Peptide Tyrosine Tyrosine</td>
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<td>p38 MAPK</td>
<td>p38 Mitogen Activated Protein Kinase,</td>
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<td>Rac1</td>
<td>Ras-related C3 Botulinum Toxin Substrate 1</td>
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<tr>
<td>Rab-GDP</td>
<td>Rab Guanosine Diphosphate,</td>
</tr>
<tr>
<td>Rab-GTP</td>
<td>Rab Guanoside Triphosphate,</td>
</tr>
<tr>
<td>RER</td>
<td>Respiratory Exchange Ratio</td>
</tr>
<tr>
<td>ReHIT</td>
<td>Reduced-Exertion High-Intensity Interval Training</td>
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<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>RPE</td>
<td>Rating of Perceived Exertion</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
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<tr>
<td>TG</td>
<td>Triglyceride</td>
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<tr>
<td>T2D</td>
<td>Type 2 Diabetes</td>
</tr>
<tr>
<td>V̇CO₂</td>
<td>Carbon Dioxide Production</td>
</tr>
<tr>
<td>V̇E</td>
<td>Volume Expired</td>
</tr>
<tr>
<td>V̇I</td>
<td>Volume Inspired</td>
</tr>
<tr>
<td>V̇O₂</td>
<td>Oxygen Consumption</td>
</tr>
<tr>
<td>V̇O₂max</td>
<td>Maximal Oxygen Consumption</td>
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Chapter 1

General Introduction and Literature Review
Chapter 1

General Introduction and Literature Review

1.1. Introduction

Humankind is in the midst of a well-publicised epidemic of chronic disease with a consequential elevated risk of premature morbidity and mortality (Bauer et al., 2014). The latest statistics from the World Health Organisation suggest that 400 million people worldwide were obese in 2008, and this number is expected to have almost doubled by 2015. At the same time, the worldwide prevalence of type 2 diabetes (T2D) has increased from 153 million people in 1980 to 347 million people in 2008 (Danaei et al., 2011). Both T2D and obesity substantially increase the risk of cardiovascular diseases (CVD), including coronary heart disease and stroke, which remain the major cause of premature death in modern societies (Allender et al., 2008b). In addition to the associated morbidity, loss of physical function and premature mortality for the patient, the current epidemic of chronic disease places great strain upon public health care systems (Allender et al., 2008b; Zhang et al., 2009; Zhang et al., 2010).

The accumulation of excess adiposity, in particular visceral fat, is considered the key driver of the development of T2D and CVD (Lebovitz & Banerji, 2005; Pischon et al., 2008). The classic hypothesis emphasises that visceral adiposity develops initially, and then leads to the induction of local and systemic inflammation, the accumulation of ectopic fat in various tissues, and eventually severe insulin resistance, hyperglycaemia and T2D (Lebovitz & Banerji, 2005). However, although a lot of focus has been placed on the role of obesity, this is just one contributing factor, and there is a growing appreciation that more emphasis should be placed upon physical inactivity (Booth et al., 2002; Chakravarthy & Booth, 2004; Thyfault & Krogh-Madsen, 2011). Indeed, recent epidemiological evidence suggests inactivity is a larger contributor to premature death compared with obesity (Ekelund et al., 2015), and laboratory experiments have shown that physical inactivity plays an important (and possibly primary) role both in the accumulation of total/visceral fat and in the development of chronic metabolic and vascular disease (Booth et al., 2002; Chakravarthy & Booth, 2004; Thyfault & Krogh-Madsen, 2011). On the other hand, regular physical activity and/or structured exercise
training is recognised as an important strategy to prevent obesity, T2D and CVD (Pan et al., 1997; Knowler et al., 2002; Szostak & Laurant, 2011; Thompson et al., 2012).

The knowledge that inactivity contributes to, and regular exercise prevents, the development of chronic metabolic disease has led to a concerted worldwide effort to promote exercise participation in the general population. Indeed, the first position stand and guidelines for exercise participation were published in 1975 and have been updated continually since (Blair et al., 2004). In spite of this, current data suggest that the majority of individuals do not accumulate appropriate amounts of exercise required to improve and maintain metabolic health (Allender et al., 2008a). Of course the reasons for this are multifactorial. Whilst psychological factors will undoubtedly be heavily involved and part of a large scale solution, it is also clear that the current focus on a high volume of moderate intensity exercise fails to take into account key perceived barriers to exercise participation, the most common of which appears to be ‘a lack of time’ (Reichert et al., 2007; Korkiakangas et al., 2009). As such, over the last 10 years there has been a drive from exercise physiologists to develop alternative, more time-efficient exercise interventions which remain effective at improving health (Gibala et al., 2012).

The purpose of this literature review is to: i) explore the role of inactivity in the development of chronic disease, with a focus on insulin resistance, T2D and CVD, ii) explore the current evidence for the role of exercise in alleviating risk factors for T2D and CVD, and iii) to review current progress in developing time-efficient alternatives to current exercise recommendations for improving metabolic and cardiovascular health.

1.2. Evolutionary Perspectives on Physical Activity and Chronic Disease

The efficiency of human metabolic processes, and by extension long term metabolic health status, is determined by the complex interactions between our genes, our behaviours and our environment. Even at this point, it is important to take a step back and consider that the human genome was naturally selected in a context (50000-10000 BC; Late-Palaeolithic era) where our ancestor’s existence was dependent on the procurement of wild food resources through hunting or foraging (Booth et al., 2002). As such, the attainment of adequate nutrition, and indeed other activities of daily living, were inextricably linked to a high physical activity energy expenditure, and this would
likely have been characterised by a mixture of ‘endurance’, ‘strength’ and ‘peak efforts alternating with rest’ (Astrand, 1992; Eaton & Eaton, 2003). In line with the ‘survival of the fittest’ principle of evolution, it follows that individuals with a high level of physical fitness, and a high capacity for performing physical activity, would have thrived in such an environment, whilst unfit individuals with a low capacity for performing physical activity would have been unable to secure sufficient nutrition, and likely perished prior to reaching reproductive age (Booth et al., 2002; Chakravarthy & Booth, 2004).

At the same time, we must consider that for prehistoric humans the availability of food was likely inconsistent, and hence it would have been common for our ancestors to undergo cyclical periods of feast interspersed with periods of food shortage (Chakravarthy & Booth, 2004). Neel (1962) speculated that these enforced phases of famine and feast, in combination with those of physical activity and rest, would have favoured the selection of a ‘thrifty’ genotype that was exceptional both in energy utilisation and energy storage. By prioritising the replenishment of intramuscular substrates (i.e., glycogen and triglyceride) and the storage of a larger proportion of ingested calories in fat depots during periods of feast/low-activity, individuals possessing a more ‘thrifty’ genotype would have been better adapted for survival during subsequent periods of famine/high-activity (Neel, 1962; Chakravarthy & Booth, 2004). Taken together, this hypothesis would suggest that the human genome, and by extension human physiology/biochemistry, has become programmed to expect regular physical activity (Booth et al., 2002; Chakravarthy & Booth, 2004).

This genetic make-up is unlikely to have changed over the last ~40,000 years (Eaton & Konner, 1985), whilst in many cultures the past century alone has seen dramatic changes to the environment in which these genes were naturally selected. For the vast majority of individuals, physical activity is no longer obligatory for many activities of daily living and a large portion of the day is spent sedentary (Allender et al., 2008a). At the same time, access to food is no longer inconsistent; on the contrary we have easy access to a large amount of calorie rich but nutritionally poor foods (French et al., 2001). Hence, the cycling of feast/low-activity and famine/high-activity is no longer a part of our environment. As a result, metabolic genes which were naturally selected to expect a high level of physical activity are now maladapted, leading to an
excess of energy storage in a context where fuel utilization/turnover is vastly reduced (Booth et al., 2002; Chakravarthy & Booth, 2004). Over the same time period, there has undoubtedly been a substantial increase in the age-specific prevalence of many chronic diseases, in particular in obesity, T2D and CVD (Danaei et al., 2011; Wang et al., 2011). Whilst the potential genetic propensity for each of these diseases is recognised (Marenberg et al., 1994; Poulson et al., 1999; Zdravkovic et al., 2002; O’Rahilly & Farooqi, 2006), the major underlying cause for the rapid increases in prevalence must be at least partially related to the changes in physical activity and feeding patterns (Roberts & Barnard, 2005). In particular there is an emerging understanding of the fundamental role of physical inactivity in the development of each of these diseases (Booth et al., 2002; Chakravarthy & Booth, 2004; Katzmarzyk & Janssen, 2004; Thyfault & Krogh-Madsen, 2011).

The contention that physical inactivity is associated with detrimental effects upon human health has long been recognised. For example, over 2000 years ago the Greek physician Hippocrates noted: ‘That which is used develops, and that which is not used wastes away. . . If there is any deficiency in food or exercise the body will fall sick’, whilst in 1769 William Buchan wrote: ‘It seems to be catholic law that no creature, without exercise, can enjoy health. Man alone deviates from this original law, and suffers accordingly’. Over the last 60 years a substantial body of scientific literature has been accrued to support these statements. In 1953, Jerry Morris and colleagues reported on data which examined the relationship between occupational physical activity and rates of CVD. They showed that sedentary London bus drivers had twice the rate of cardiovascular mortality compared with active London bus conductors and suggested that physical activity was providing the cardioprotective effects (Morris et al., 1953). Following on from this, there are now large well-controlled epidemiological studies demonstrating that low levels of physical activity independently associate with a ∼50% increased risk of T2D, a ∼40% increased risk of coronary artery disease, a ∼60% increased risk of stroke, a ∼30% increase risk of hypertension, a ∼60% increase risk of osteoporosis, and a ∼40% increased risk of colon cancer (Katzmarzyk & Janssen, 2004). Perhaps even more convincing is the strong inverse relationship between levels of cardiorespiratory fitness, the risk of chronic disease, and levels of all-cause mortality (Blair et al., 1989; Barlow et al., 1995; Blair et al., 1995; Blair et al., 1996; Lee et al., 1998; Lee et al., 1999; Myers et al., 2002). The variance in individual levels of
cardiorespiratory fitness, usually defined as the maximal level of oxygen uptake during incremental exercise, is explained by both genetic (~50%) and environmental (~50%) factors (Bouchard et al., 1999). Even so, it noteworthy that the only environmental factor known, with any relevance, to alter VO$_2$max is physical activity (Church, 2009). Thus, the epidemiological data linking VO$_2$max to long term health status also partly indirectly implicates the key role of physical inactivity in the development of chronic disease. Taken together, there is strong epidemiological evidence that physical inactivity is an important risk factor for chronic disease.

1.3. Physical Inactivity, Insulin Resistance and Chronic Disease

The biological mechanisms linking physical inactivity to an increased risk of chronic metabolic disease are partly linked to the impact of physical inactivity upon insulin action and glucose homeostasis (Krogh-Madsen et al., 2010; Th岳ault & Krogh-Madsen, 2011; Mikus et al., 2012). This section will explore the physiological regulation of insulin and glucose metabolism in humans, and subsequently examine the effects of inactivity and exercise training upon these variables.

1.3.1. Overview of Metabolic Control in Humans

In humans, glucose and lipids are quantitatively the most important substrates for maintaining cellular energy balance, and their absorption, production, uptake, storage and utilisation are intricately controlled. This is largely achieved through the complex cross-talk between skeletal muscle, adipose tissue, the liver and the endocrine pancreas, but there is also a more broad regulatory input from the central nervous system which is influenced by signals from each of these tissues (Wasserman, 2009; Jordan et al., 2010). In response to different nutritional and physical activity stimuli, human cells, with the notable exception of the brain, are readily able to switch between glucose and fatty acid oxidation to meet their ATP demands, which is termed ‘metabolic flexibility’ (Kelley & Mandarino, 2000).

In the fasted state, when glucose availability is low, non-esterified free-fatty acids (NEFA) delivered from adipocytes are preferentially oxidised in tissues such as skeletal muscle where the uptake and utilisation of NEFA appears to be largely dependent on availability (Frayn et al., 2006). Nevertheless, plasma glucose
concentrations during times of fasting must still be maintained for use in the brain and erythrocytes which rely solely on this sugar, and this is achieved through the coordination of hepatic glycogenolysis and gluconeogenesis for subsequent hepatic glucose output (Wasserman, 2009). These actions are supported by the low insulin/glucagon ratio in the circulation. Insulin is the primary suppressant of adipose tissue lipolysis and hepatic glucose production, as well as the primary activator of glucose disposal in peripheral tissues, whilst glucagon stimulates endogenous glucose production in the liver and helps to promote NEFA release in adipocytes (Abdul-Ghani & DeFronzo, 2010). The secretion of other hormonal factors, such as adrenaline, work in concert with the low plasma insulin concentrations and high glucagon concentrations to activate lipolysis in adipose tissue and hepatic glucose output in the fasted state and during exercise (Wasserman, 2009).

In response to a mixed meal or glucose challenge there is a rise in plasma glucose and lipid availability, and the body switches from a predominantly catabolic state to a predominantly anabolic state, with a drive for nutrient uptake and storage. There is a strong suppression of adipose tissue lipolysis and hepatic glucose output whilst glucose uptake in skeletal muscle and lipid uptake in adipose tissue are stimulated. In the postprandial state, glucose becomes the primary substrate for oxidation (until blood glucose concentrations are normalised), but a proportion of the ingested glucose will also be used to replenish both liver and skeletal muscle glycogen stores. These effects are largely achieved through the release of insulin into the circulation as well as reduced glucagon secretion (Wasserman, 2009).

1.3.2. Insulin

Insulin is an endocrine peptide hormone which exerts a myriad of cellular effects essential for the maintenance of metabolic homeostasis. Synthesised and released by the pancreatic β-cells, insulin is secreted in a biphasic manner in response to increasing plasma glucose concentrations, with a rapid first phase occurring within 10 min of glucose provocation and subsequently giving way to a more enduring phase over several hours (Eliasson et al., 2008). Insulin secretion is much greater following an oral glucose load compared with the intravenous injection of glucose (DeFronzo, 2009). This effect is accounted for by the release of incretin hormones in the small intestine, in particular
the release of GLP-1 and GIP are known to potentiate insulin secretion (Defronzo, 2009). GLP-1 is also known to suppress glucagon release from the pancreatic α-cells and thus indirectly inhibits hepatic glucose output and helps to reduce glucose entry into the circulation in the fed state (Defronzo, 2009).

The secretion of insulin results in the coordination of several multi-tissue metabolic processes which work in concert to restore blood nutrient homeostasis. In skeletal muscle insulin stimulates glucose uptake, glucose oxidation and glycogen synthesis. In adipocytes, insulin also stimulates glucose uptake but more importantly suppresses lipolysis and hence NEFA release as well as promoting lipid uptake and triglyceride synthesis. In the liver, insulin is not involved in glucose uptake, but does regulate hepatic glycogen synthesis, and is a potent inhibitor of hepatic glycogenolysis and gluconeogenesis which plays a key role in blunting the postprandial rise in blood glucose concentrations. The suppression of hepatic glucose output occurs at relatively low insulin concentrations whereas actions in peripheral tissues require a much higher insulin concentration (Prager et al., 1986). Importantly, insulin also acts on endothelial cells to stimulate nitric oxide (vasodilator) and endothelin 1 (vasoconstrictor) production, alter blood flow dynamics and augment its own delivery to each of these tissues (Clark et al., 2003; Muniyappa et al., 2007; Barrett et al., 2011).

Quantitatively, skeletal muscle is widely accepted to be the major tissue responsible for insulin mediated glucose disposal. Under conditions of euglycaemia hyperinsulinemia >75% of infused glucose is taken up by the skeletal muscle bed (Baron et al., 1988). However, it should be noted that if the glucose is administered orally then a substantial proportion will be taken up by other tissues. For example, after an oral glucose bolus (1 gram/kg body weight), 29% of the ingested glucose was taken up by the liver, 26% by skeletal muscle, 23% by the brain and the other 22% was taken up by other unspecified tissues (Kelley et al., 1988). Likewise, following a high carbohydrate mixed meal (75 g carbohydrate, 37 g protein, and 17 g fat), skeletal muscle glucose uptake accounted for ~50% of the absorbed glucose, with the rest taken up by other tissues (Capaldo et al., 1999). Nevertheless, insulin-mediated glucose uptake plays a key role in regulation of whole-body metabolic control. Importantly, the beneficial effects of exercise upon insulin sensitivity are largely explained by improvements in skeletal
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muscle glucose uptake (Dela et al., 1992; Dela et al., 1995), and therefore insulin’s effects in skeletal muscle will be the main focus of this review.

1.3.3. Insulin action in skeletal muscle

In order to bind to the plasma membrane insulin receptor insulin must access the interstitial fluid compartment bathing the peripheral target cells, first through delivery to the peripheral capillary bed, and then by crossing the endothelial cell barrier. It is now recognised that insulin plays a major role in controlling its own delivery, and delivery of ingested nutrients, to the interstitial fluid surrounding the cell membrane, and there is evidence that these hemodynamic actions of insulin represent an important rate-limiting step in glucose disposal in muscle and adipose (Clark et al., 2003; Muniyappa et al., 2007; Barrett et al., 2011). The hemodynamic effects of insulin encompass three distinct mechanisms: 1) insulin has been shown to stimulate the dilation of resistance vessels to promote an increase in total blood flow to muscle and hence an increase in insulin and glucose delivery to muscle; 2) insulin results in recruitment of muscle capillaries to simultaneously promote insulin and glucose delivery and, perhaps more importantly, broaden the endothelial exchange area and encourage endothelial hormone/substrate crossing; and 3) insulin directly augments its transport through the endothelial cell lining (i.e., through the endothelial cell itself). These effects are largely regulated through the stimulation of nitric oxide production from the endothelium. The haemodynamic effects of insulin have recently been covered in several excellent reviews and the reader is referred to these for a more detailed discussion (Clark et al., 2003; Muniyappa et al., 2007; Barrett et al., 2011).

Following entry into the interstitial fluid insulin stimulates the translocation of glucose transporter protein 4 (GLUT4) to the plasma membrane, as well as the activation of key intramyocellular enzymes involved in glucose phosphorylation, glucose oxidation and glycogen synthesis, all of which work synergistically to promote the effective clearance of glucose from the blood/interstitium into skeletal muscle. In the fasted state, GLUT4 is stored in intracellular microvesicles within the cytoplasm. However, under postprandial conditions (when plasma and interstitial insulin concentrations are elevated) the GLUT4 vesicles are moved to the plasma membrane and GLUT4 protein fuses with the plasma membrane and allows facilitated transport of
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glucose into the muscle cell. Acceleration of these intracellular events is mediated through the binding of insulin to a transmembrane receptor and the subsequent initiation of an intracellular phosphorylation/dephosphorylation signalling cascade (Wojtaszewski & Richter, 2006). Insulin signalling is an extremely complex process (figure 1.1), involving both positive and negative enzyme crosstalk, and ultimately requires coordination of several propagating pathways to be fully efficient, especially in the case of GLUT4 vesicle trafficking (Wojtaszewski & Richter, 2006).

The process is initiated when insulin binds to the extracellular α-subunits of the insulin receptor which leads to a structural reconfiguration, increased activity of a tyrosine kinase within the transmembrane β-subunits, binding of ATP to the transmembrane β-subunits, and the subsequent receptor auto-phosphorylation (Saltiel & Kahn, 2001). This causes the association and complementary tyrosine residue phosphorylation of numerous insulin receptor substrates, the most important of which appears to be IRS-1 (Wojtaszewski & Richter, 2006; Abdul-Ghani & DeFronzo, 2010). The subsequent docking of the phosphorylated IRS-1 with the p85 regulatory subunit of the enzyme phosphatidylinositol 3-kinase (PI3K), leads to the activation of the p110 catalytic subunit of the same enzyme and initiates its movement to the plasma membrane (Wojtaszewski & Richter, 2006). Here PI3K mediates the conversion of phosphatidylinositol diphosphate (PIP2) to phosphatidylinositol triphosphate (PIP3) within the plasma membrane and the consequential recruitment of phosphoinositol-dependent protein kinase 1 (PDK-1) to the plasma membrane in association with Akt/PKB and atypical protein kinase C (aPKC), all of which then become activated (Wojtaszewski & Richter, 2006). In addition, PI3K is thought to activate Rac-1, independently of PIP3 production, and the activation of Rac-1 is proposed to directly affect GLUT-4 trafficking (Chiu et al., 2011). The activation of Akt/PKB leads to the propagation of two pathways important in insulin-mediated glucose disposal. Firstly, Akt/PKB is known to phosphorylate glycogen synthase kinase 3 (GSK-3 α/β) which subsequently removes the inhibitory action upon glycogen synthase and hence glycogen synthesis (Saltiel & Kahn, 2001). Secondly, Akt/PKB is thought to result in the activation of AS160 (also known as TBC1D4) (Bogan, 2012). In the fasted state, AS160 inhibits the activity of Rab proteins; however, upon insulin stimulation the activated Akt/PKB phosphorylates AS160, releases the inhibition of Rab proteins which leads to the reorganisation of the muscle cytoskeleton which promotes GLUT4 translocation.
(Bogan, 2012). Insulin also stimulates GLUT4 translocation through a signalling cascade that is independent of PI3K, since genetic ablation of PI3K does not completely prevent skeletal muscle glucose uptake (Kanzaki, 2006). The PI3K independent pathway is thought to involve a separate pathway promoted via the phosphorylated insulin receptor which involves activation of TC10 which is directly involved in GLUT4 translocation (Bogan, 2012).

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**Figure 1.1.** Insulin signalling related to glucose disposal in skeletal muscle. Solid lines represent canonical signalling events, whilst dotted lines represent the final metabolic effect. aPKC atypical protein kinase C, Akt/PKB protein kinase B, GLUT4 glucose transporter 4, IR insulin receptor, IRS-1 insulin receptor substrate-1, PDK phosphoinositide-dependent kinase 1, PI3K phosphoinositide 3-kinase, PIP2 phosphoinositide 4,5-bisphosphate, PIP3 phosphatidylinositol 3,4,5-triphosphate, Rab-GDP Rab guanosine diphosphate, Rab-GTP Rab guanosine triphosphate, AS160 Akt substrate of 160 kDa, GSK-3 α/β glycogen synthase kinase α/β

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1.3.4. **Insulin Sensitivity and Insulin Resistance**

The effectiveness of insulin action upon insulin sensitive tissues, for example in skeletal muscle, can be characterised in terms of insulin sensitivity and insulin responsiveness. Insulin sensitivity refers to the concentration of insulin required to elicit a half maximal metabolic effect, whilst insulin responsiveness describes the magnitude of the effect that is induced by the insulin concentration that is maximally effective (Holloszy, 2005). Insulin resistance describes a state whereby ‘normal’ insulin concentrations are associated with a reduced physiological action; in other words, there is a downward right shift in the insulin action dose response curve meaning there is a reduced metabolic response for any given concentration of insulin. The body’s primary initial response to tissue insulin resistance is an increase in insulin secretion which is required to overcome the tissue resistance and ensure metabolic homeostasis is maintained (figure 1.2).

![Figure 1.2](image-url)

**Figure 1.2.** The relationship between insulin sensitivity and insulin secretion. Figure taken from Roberts et al (2013). If an individual goes from being physically active (point A) to being sedentary (point B) there will be a fall in insulin sensitivity and a compensatory rise in insulin secretion to maintain normal glucose tolerance. The reverse would be true for an individual going from a sedentary to an active lifestyle. A sedentary lifestyle characterised by reduced insulin sensitivity creates a permissive environment whereby insulin sensitivity may continue to deteriorate (see text). Progression to prediabetes (impaired fasting glucose or impaired glucose tolerance) (point C) and type 2 diabetes (point D) takes many years and is characterised by a further reduction in insulin sensitivity, but also a drop in insulin secretion.
There are a number of different methods for the assessment of insulin sensitivity/insulin resistance. The gold standard measure is the hyperinsulinemic euglycemic clamp (Muniyappa et al., 2008) and this is a popular method for demonstrating changes in insulin sensitivity with exercise training. This method uses a steady state infusion of exogenous insulin to raise plasma insulin levels, and is combined with a continuous infusion of exogenous glucose which is manipulated to keep plasma glucose concentrations (measured every 5-10 minutes) constant. Under hyperinsulinemic conditions, it is assumed that endogenous glucose output from the liver is completely suppressed; thus the rate of glucose infusion required for maintaining plasma glucose concentrations at a constant levels can be considered equal to the rate of glucose disappearance into all the tissues in the body. In combination with tracers, this technique can be used to determine tissue specific insulin sensitivity. Whilst this technique is considered the reference standard, it was deemed unsuitable for the series of studies described in the present thesis for several reasons: (i) it is difficult to perform, quite invasive and requires well trained and experienced clinical staff; (ii) it is time consuming to perform for both participant and researcher; and (iii) it is expensive to perform. It could also be argued that this measure is not representative of the physiological challenge of meal feeding, which requires the effective coordination of multiple tissues, substrates and hormones.

The oral glucose tolerance test (OGTT) maps the blood glucose and insulin response to an orally ingested glucose drink and is commonly utilised in clinical settings to detect patients with impaired glucose tolerance and T2D. The OGTT is cost-effective and simple to perform: following a 10-12 hour overnight fast, blood samples are drawn before and then at various time-points for up to 180 minutes after the oral ingestion of a 75 g glucose solution and are analysed for glucose and insulin concentrations. The OGTT gives a more valid indication of the body’s response to a normal physiological challenge that is encountered on a daily basis. The OGTT response is reflective of the whole body but calculations have been developed which give an indication of liver (e.g., HOMA-IR) and peripheral specific (e.g., Cederholm) insulin sensitivity. These indexes have been shown to correlate well with the gold standard clamp measures of insulin sensitivity (Piche et al., 2007). However, the between-test reliability of the response to an OGTT can be poor (CV 8-15% for repeated tests and is therefore not ideal for
measuring small or subtle changes in insulin sensitivity (Libman et al., 2008; Jimenez-Navarro et al., 2010). However, with interventions that are specifically targeting relatively large changes in insulin sensitivity, for example exercise training, then the OGTT should be able to detect these. The measurement of glycated haemoglobin, which reflects long term regulation of blood glucose concentration over the preceding 6 weeks, has also been used in research studies.

The regulation of whole-body insulin action is complex and involves coordination of multiple tissues. All of these tissues are able to acutely (i.e. hours to days) adjust the level of insulin sensitivity at the cellular level depending largely upon intracellular nutrient availability (Schenk et al., 2008). Specifically, in response to perturbations in nutrient availability, for example a reduction in skeletal muscle fibre glycogen content after prolonged contraction, each cell is able to intrinsically modify insulin action and hence nutrient (e.g. glucose) uptake to allow return of intracellular nutrient homeostasis (Schenk et al., 2008). To add another level of complexity, these acute fluctuations in cellular insulin action will take place (and be modified) in the context of chronic extracellular signals determined by interactions between the level of obesity, exercise training status, and also genetic variations (Schenk et al., 2008). The progressive development of insulin resistance over time, which eventually manifests in appearance of metabolic abnormalities (e.g. T2D), is largely determined by changes in the chronic factors regulating insulin action (Schenk et al., 2008). However, it is important to note that an absence of the intrinsic cellular signals which act to increase insulin sensitivity may, over a prolonged period of time, influence the extent to which such chronic deterioration of whole body insulin sensitivity occurs (Schenk et al., 2008). This is particularly evident when discussing the role of physical inactivity in the development of insulin resistance and T2D.

The development of insulin resistance, in particular in skeletal muscle, is the strongest predictor of the future development of T2D (DeFronzo & Tripathy, 2009). The metabolic consequences of T2D, including microvascular and macrovascular damage can largely be explained by the characteristic high blood glucose concentrations in this disease (Gerich, 2003). However, the development of T2D takes many years and is characterised by a slow loss of glycaemic control over time, in other words small increases in fasting and postprandial glucose levels. In a meta-analysis including 95,783
individuals of varying levels of glucose tolerance, there appeared to be an exponential relationship between the risk of cardiovascular events and both fasting and postprandial glucose concentrations irrelevant of any impaired or diabetic threshold (Coutinho et al., 1999). Likewise, studies have shown strong relationships between levels of glycated haemoglobin and the risk of cardiovascular disease which is present in both healthy and diabetic ranges (Stratton et al., 2000; Khaw et al., 2001). Thus, even in healthy individuals controlling postprandial glucose excursions by maintaining or improving insulin sensitivity across the lifespan represents an important outcome for preventing cardiovascular disease (Lin et al., 2009). Prior to any deterioration in glycaemic control the reduction in insulin sensitivity leads to an elevation in insulin secretion and systemic hyperinsulinemia. Elevations in systemic postprandial insulin concentrations have also been shown to be predictive of future cardiovascular disease in healthy individuals (Pyörälä et al., 1998; Pyörälä et al., 2000).

In 1988, Reaven presented the ‘insulin resistance syndrome’ hypothesis, where he outlined potential evidence supporting the primary role of insulin resistance (i.e. hyperinsulinemia) in the development of a clustered cardiometabolic disease risk profile (Reaven, 1988). The scientific evidence for this hypothesis is strong (DeFronzo, 2010). Briefly, it appears as though chronically elevated levels of insulin in the context of an energy surplus promotes de novo lipogenesis, elevated VLDL synthesis, the accumulation of visceral fat, chronic low grade systemic inflammation, hypertension and atherogenesis (DeFronzo, 2010). Thus it appears that insulin resistance represents an important risk factor for T2D and cardiovascular disease.

1.3.5. The Role of Inactivity in the Development of Insulin Resistance

At the metabolic and molecular levels, the causes of skeletal muscle insulin resistance are numerous and complex, and include: i) impaired insulin and glucose delivery to, and across, the endothelium (Clark, 2008); ii) abnormal secretion and action of adipokines, such as leptin and adiponectin (Rabe et al., 2008); iii) impaired insulin-mediated glycogen synthesis (Petersen et al., 2007); iv) impaired mitochondrial function (Petersen et al., 2004); (v) lipid oversupply and elevated intramyocellular fatty acid species, such as diacylglycerol (Samuel et al., 2010); vi) chronic systemic inflammation
(Shoelson et al., 2006); and vii) oxidative stress (Meigs et al., 2007), all of which may be intertwined. The driver of these defects is generally considered to be the accumulation of visceral adiposity and ectopic fat, however, the role of physical inactivity cannot be underestimated, and may in fact be the primary pathological driver (Thyfault & Krogh-Madsen, 2011). Support from this hypothesis comes from numerous studies which have examined changes in insulin sensitivity, metabolic flexibility, lipid trafficking, and substrate oxidation in response to enforced periods of inactivity (Bergouignan et al., 2011; Mikus et al., 2011; Thyfault & Krogh-Madsen, 2011).

It has repeatedly been shown that a reduction in insulin sensitivity occurs in humans following a period of bed rest and can occur in as little as 3 days (Lipman et al., 1972; Hamburg et al., 2007; Alibegovic et al., 2009; Alibegovic et al., 2010). These findings have subsequently been replicated using more applicable models of reduced physical activity. For example, Krogh-Madsen and colleagues examined the effect of reducing the average daily step count from ~10,000/day to ~1500/day over a period of 14 days in healthy men who were recreationally active (Krogh-Madsen et al., 2010). They demonstrated a 17% reduction in insulin sensitivity (Krogh-Madsen et al., 2010). The change in insulin sensitivity appears to be linked specifically to a reduction in peripheral glucose disposal (i.e. muscle glucose uptake) in response to a glucose load, whilst there does not seem to be any changes in hepatic glucose output (Krogh-Madsen et al., 2010).

An enforced period of inactivity also leads to an increased accumulation of visceral fat (Olsen et al., 2008; Krogh-Madsen et al., 2010). Therefore, an important question to answer is whether the reduction in insulin sensitivity is a result of the inactivity *per se* or a reflection of positive energy balance. However, a recent study using the bed rest model indicates that inactivity causes insulin resistance even when energy balance is clamped (Bergouignan et al., 2009). Likewise, Stephens recently showed that the deleterious impact of 1 day of sitting upon insulin sensitivity was present both with and without an energy surplus across the same time period (Stephens et al., 2011). Furthermore, although Olsen and colleagues showed a 7% increase in visceral fat following two weeks of step reduction, they also observed a reduction in lean mass and a 1.2 kg drop in body mass suggesting that no overall energy surplus was present over the two weeks (Olsen et al., 2008; Krogh-Madsen et al., 2010). Lastly, the rapid increase
in fat pad mass in rodents following forced cessation of voluntary wheel running occurs even when energy balance is maintained (Laye et al., 2007). These studies strongly suggest that inactivity causes both a reduction in peripheral insulin sensitivity and an increase in visceral fat. The effects do not appear to be caused by positive energy balance; rather, an enforced period of inactivity alters postprandial fate of ingested nutrients (by altering insulin sensitivity) and alters energy storage (by increasing fat mass) (Thyfault & Krogh-Madsen, 2011).

The reduction in skeletal muscle insulin sensitivity during a period of inactivity appears to take place before any detectable increase in visceral fat mass (Knudsen et al., 2012). Thus, it has been hypothesised that the initial reduction in insulin sensitivity with inactivity may be a mechanism to alter the metabolic fate of ingested nutrients away from a fuel replete skeletal muscle cell and towards storage in adipose tissue (Thyfault & Krogh-Madsen, 2011). This contention is supported by well controlled laboratory experiments showing a re-partitioning of energy in response to changes in skeletal muscle insulin sensitivity in humans (Blanc et al., 2000; Petersen et al., 2007; Rabøl et al., 2011). Specifically, the greater insulin and glucose spikes induced by muscle insulin resistance in response to reduced activity (Krogh-Madsen et al., 2010; Mikus et al., 2012) may provide a greater opportunity to take up dietary fat into adipose tissue and for excess glucose to be diverted toward de novo lipogenesis (Blanc et al., 2000; Petersen et al., 2007; Rabøl et al., 2011). This is supported by recent studies demonstrating that exercise prevents both the deterioration in skeletal muscle insulin sensitivity and the accumulation in visceral fat mass in response to a two-week step reduction and/or profound energy surplus (Walhin et al., 2013; Krogh-Madsen et al., 2014). Overall, the re-partitioning of ingested nutrients in response to long-term inactivity may initiate a cycle of events which leads to accumulation of visceral fat, systemic hyperlipidemia, elevated systemic glycemia, excess ectopic fat storage, chronic low-grade inflammation, metabolic inflexibility and atherogenesis (Bergouignan et al., 2011; Mikus et al., 2011; Thyfault & Krogh-Madsen, 2011). This hypothesised cycle of events is depicted in figure 1.3A and 1.3B, and described in further detail in the figure legends.

In summary, recent evidence supports that physical inactivity plays an important role in the development of skeletal muscle insulin resistance. This is not to downplay the role of other metabolic factors (described above), but rather suggesting that physical
inactivity is the primary initiating factor and provides a permissive environment by which such metabolic dysfunction emerges over time (Booth et al., 2002; Chakravarthy & Booth, 2004; Bergouignan et al., 2011; Thyfault & Krogh-Madsen, 2011). When one then considers that physical inactivity also results in an accelerated loss of cardiorespiratory fitness and lean muscle tissue, two factors which are known to also play a role in the development of chronic metabolic disease, then the role of inactivity in chronic disease development becomes even more apparent (Krogh-Madsen et al., 2010). The data described above does not definitively prove that physical inactivity causes cardiometabolic disease, however the changes that have been demonstrated are moving in a pathological direction and this contention is well supported by epidemiological evidence (Katzmarzyk & Janssen, 2004).
In the context of high physical activity

Figure 1.3A. Healthy metabolic cycling in the context of high levels of physical activity and/or exercise. Figure adapted from Chakravarthy and Booth (2004). As described in text, human evolution took place in the context of cycles of physical activity/famine and inactivity/feast. During periods of feast the short periods of inactivity allowed replenishment of muscle glucose and fat stores that would then be utilised during the subsequent period of fasting and physical activity. This ‘thrifty’ genotype was shaped during evolution but likely persists today. TG triglyceride, GLUT4 glucose transporter 4, AMPK amp-activated protein kinase
In the context of low physical activity

Thrifty Storage
(High storage of excess glucose and TG in adipose tissue; little goes to skeletal muscle)

Cycle Stalled
(Excess glucose shunted to de novo lipogenesis; Excess FFA shunted towards unhealthy storage)

Unhealthy Cycle Starts

Unhealthy Cycle Stalls

Unlimited food supply without exercise induced reduction of glucose and TG in skeletal muscle, nor exercise induced increase in GLUT4 and AMPK

Type 2 Diabetes & Cardiovascular Disease

Further reductions in insulin sensitivity

Metabolic and Molecular Dysfunction

30-40 years

Unhealthy Cycle Starts

In the context of low levels physical activity and/or exercise. Figure adapted from Chakravarthy and Booth (2004). When skeletal muscle stores of glucose and fat are not turned over by regular physical activity and/or exercise, ‘thrifty’ mechanisms continually shunt excess glucose and fat into adipose tissues and ectopic fat stores. The continual inactivity and excess fat and glucose intake create a permissive environment which, over 30–40 years, will lead to the manifestation of type 2 diabetes and cardiovascular disease. TG triglyceride, GLUT4 glucose transporter 4, AMPK amp-activated protein kinase.
1.4. Exercise and Insulin Sensitivity

1.4.1. Epidemiological Evidence

There is good evidence for the positive effect of increasing physical activity in the prevention of T2D (Pedersen & Saltin, 2006). For example, numerous correlational studies where physical activity has been measured by questionnaire generally report that a higher level of physical activity, independent of other risk factors (e.g. body composition), is associated with a reduced risk of developing T2D (Helmsrich et al., 1991; Manson et al., 1991; Hu et al., 1999; Laaksonen et al., 2002). Of course, these studies do not indicate causation and it is also possible that a high level of physical activity is a sign that you are in good health, rather than the physical activity leading to good health. However, more convincing evidence comes from several well controlled clinical trials which have demonstrated that interventions involving physical activity alone (Pan et al., 1997) or in combination with dietary changes (Pan et al., 1997; Tuomilehto et al., 2001; Knowler et al., 2002) are associated with a reduced risk of developing T2D in populations with impaired glycaemic regulation and therefore at high risk of developing the disease. Pan et al. (1997) included an exercise only intervention group and observed that the risk of developing T2D was reduced by 46% with exercise over a 6 year period, a risk reduction that was comparable to their intervention which combined exercise and dietary changes. It is particularly noteworthy that the preventative effects of lifestyle changes (i.e. better diet and more physical activity) are greater than those observed with currently recommended pharmacological treatments (Knowler et al., 2002). The beneficial impact of exercise training upon the development of T2D is thought to be partly related to the maintenance or improvement of whole body insulin sensitivity.

The beneficial effects of physical activity/exercise upon insulin sensitivity are largely explained by improvements in glucose uptake into skeletal muscle (Dela et al., 1992; Dela et al., 1995). In this respect, exercise is thought to exert three distinct regulatory roles on skeletal muscle glucose uptake. Firstly, skeletal muscle contractions are known to increase glucose uptake independent of insulin (Ploug et al., 1984; Nesher et al., 1985; Zorzano et al., 1986; Wallberg-Henriksson et al., 1988; Gao et al., 1994). However, in the immediate recovery period the glucose response to an OGTT may
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actually be elevated in healthy subjects uptake due to enhanced intestinal glucose extraction in spite of the increased muscle glucose uptake (Knudsen et al., 2014). The effect of contraction on muscle glucose uptake is transient, subsiding completely ~2-3 hours after the cessation of the muscle contractions (Lauritzen et al., 2010). However, it appears to be replaced by an acute enhancement of insulin-stimulated recruitment of GLUT4 and hence postprandial glucose disposal in the exercised muscle, which can be detected for 24-48 hours post-exercise, and may be linked to the replenishment of skeletal muscle glycogen stores (Bogardus et al., 1983; Cartee et al., 1989; Gulve et al., 1990). Lastly, the cumulative effect of many repeated bouts of acute exercise (i.e., exercise training) brings about a favourable change in skeletal muscle phenotype and body composition which correlates with a more prolonged increase in insulin sensitivity that can be detected for several days after the final training bout (Dela et al., 1995). Each of these effects will be reviewed in turn.

1.4.2. Direct Effects of Exercise upon Glucose Uptake

Glucose uptake into skeletal muscle cells is increased in an intensity dependent manner during skeletal muscle contraction through signalling mechanisms that are independent of and additive to insulin (Ploug et al., 1984; Nesher et al., 1985; Zorzano et al., 1986; Wallberg-Henriksson et al., 1988; Gao et al., 1994). However, similar to insulin-stimulated mechanisms, muscle contractions stimulate glucose transport via the translocation of GLUT4 protein from cytosolic microvesicles to the plasma membrane and t-tubules, allowing glucose uptake through facilitated diffusion (Richter & Hargreaves, 2013). Whilst muscle contractions do not activate any components of the proximal insulin signalling cascade, it appears that the two pathways converge on downstream protein targets (e.g. AS160) which regulate GLUT4 vesicle translocation (Richter & Hargreaves, 2013). There is also some evidence that insulin and contractions recruit GLUT4 from distinct intracellular pools (Douen et al., 1990; Coderre et al., 1995; Lemieux et al., 2000), a finding that would potentially explain the additive impact of insulin and contraction on rates of muscle glucose transport (Richter & Hargreaves, 2013). The effects of muscle contraction upon glucose uptake appears to involve molecular signalling events linked to altered cellular energy charge, increased sarcoplasmic calcium concentrations, and the production of nitric oxide (Figure 1.5) (Richter & Hargreaves, 2013). In addition, activation of Rac-1 and subsequent
rearrangement of the actin cytoskeleton appears to be important for GLUT4 translocation and tethering at the plasma membrane (Richter & Hargreaves, 2013).

Figure 1.4. Exercise independent signalling for glucose disposal in skeletal muscle. AMP adenosine monophosphate, ATP adenosine triphosphate, AMPK amp-activate protein kinase, Ca\(^2+\) calcium ions, CaMPKII calcium activated mitogen protein kinase II, AS160 Akt substrate of 160 kD, NO nitric oxide, Rac1 Ras-related C3 botulinum toxin substrate 1, Rab-GDP Rab guanosine disphosphate, Rab-GTP Rab guanoside triphosphate, GLUT4 glucose transporter 4

1.4.3. Effects of Acute Exercise upon Insulin Sensitivity

An acute bout of exercise increases ATP turnover and results in degradation of skeletal muscle glycogen and triglyceride levels, the extent of which is determined largely by the relative exercise intensity in combination with the exercise duration (Romijn et al., 1993, 2000; van Loon et al., 2001). In the recovery from a single bout of
Chapter 1: General Introduction

exercise a number of cellular processes are accelerated in order to replete muscle substrate stores and restore metabolic homeostasis. In fact, the acceleration in cellular processes may lead to an increase in muscle glycogen stores over and above pre-exercise levels, a phenomenon known as glycogen supercompensation (Bergström & Hultman, 1966).

Numerous studies have shown that insulin action is enhanced in the hours following an acute bout of aerobic (Richter et al., 1984; Perseghin et al., 1996; Brestoff et al., 2009) and resistance (Fenicchia et al., 2004; Koopman et al., 2005; Venables et al., 2007) exercise. This effect appears to be localised to the skeletal muscles that were activated during the exercise bout (Richter et al., 1984), but can be detected using measures of whole-body insulin sensitivity as well (Venables et al., 2007; Brestoff et al., 2009). There is some evidence that this response can be detected for up to 48 hours post exercise (Perseghin et al., 1996). Importantly, this effect is observed in lean healthy men and women (Perseghin et al., 1996; Brestoff et al., 2009), overweight/obese individuals (Levinger et al., 2014; Little et al., 2014; Nelson & Horowitz, 2014), and in people with insulin resistance (Perseghin et al., 1996; Fenicchia et al., 2004; Bordenave et al., 2008; Rynders et al., 2014).

The specific mechanisms underpinning the improvements in insulin sensitivity during the recovery from a single bout of exercise are not completely understood, but an increased translocation of GLUT4 to the plasma membrane is involved (Holloszy, 2005; Maarlberg et al., 2011). However, the enhancement in glucose transport in the hours following exercise is not limited only to insulin stimulation, but can be observed with other stimuli such as hypoxia (Holloszy, 2005; Maarlberg et al., 2011). This indicates that a late step in the control of GLUT4 translocation that is common to the contraction, insulin and hypoxic pathways may be enhanced post exercise (Holloszy, 2005; Maarlberg et al., 2011). Indeed, there appears to be no enhancement of proximal insulin signalling events (e.g. PI3K, Akt/PDK) following acute exercise, but recent evidence suggests that there may be enhanced signalling via AS160 (Holloszy, 2005; Maarlberg et al., 2011). It has also been hypothesised that as the GLUT4 molecules return to intracellular storage sites following the cessation of muscle contraction, they are periodically in a ‘high-sensitivity’ area of the cell (e.g., within close proximity the cell membrane) and therefore more susceptible to stimulation with lower
insulin/contraction/hypoxia signalling stimulus (Holloszy, 2005; Maarbjerg et al., 2011).

There is evidence that the improvement in insulin sensitivity is linked to replenishment of skeletal muscle glycogen stores. In support of the effect of muscle glycogen levels, the magnitude of glycogen depletion correlated with the enhancement of insulin sensitivity measured 4 hours post-exercise (Wojtaszewski et al., 1997). Likewise, several studies in humans and in rodents suggest that carbohydrate restriction following exercise enhances, whilst carbohydrate feeding following exercise diminishes, the effect upon insulin sensitivity which tracks with skeletal muscle glycogen replenishment (Bogardus et al., 1983; Cartee et al., 1989; Gulve et al., 1990; Kawanaka et al., 2000; Holtz et al., 2008; Newsom et al., 2010). The rate of skeletal muscle glycogen synthesis is increased following a single bout of aerobic exercise (Perseghin et al., 1996; Jensen et al., 2012).

1.4.4. Effects of Exercise Training Upon Insulin Sensitivity

Björntorp and colleagues were the first to demonstrate, in a cross-sectional study, that exercise trained men had a lower plasma insulin response to a standardised glucose bolus compared with non-exercising controls, indicating improved insulin sensitivity with exercise training (Björntorp et al., 1972). Since then, cross-sectional studies unequivocally demonstrate that whole-body insulin sensitivity measured with the gold standard hyperinsulinemic euglycaemic clamp is elevated in endurance athletes compared with matched sedentary individuals (King et al., 1987; Hardin et al., 1995; Amati et al., 2009; Nelson & Horowitz, 2014a). This relationship is present regardless of age; hence, older endurance athletes have a similar insulin sensitivity level compared to matched young athletes and both athletic groups are more insulin sensitive compared to the young and older sedentary counterparts (Amati et al., 2009).

Large longitudinal trials have also shown that previously sedentary individuals can substantially improve their insulin sensitivity by undergoing a period of structured endurance training. For example, Boulé et al (2005) reported the findings from the HERITAGE family study which examined changes in insulin sensitivity following 20 weeks of progressive aerobic exercise training in 596 individuals classified as sedentary according to self-report. They demonstrated that insulin sensitivity improved on average
by 10% when measured 24 hours following the final training bout. Moreover, an increase in insulin sensitivity was evident across a range of subpopulations, including both men and women, overweight and lean individuals, and people of white and black ethnic origin (Boulé et al., 2005). Improvements in insulin sensitivity with aerobic exercise have also been demonstrated in elderly men and women (Evans et al., 2005; Davidson et al., 2009) and in populations with impaired glucose tolerance and T2D (Hughes et al., 1993). Indeed, there is some evidence that insulin sensitivity improves to a greater extent in populations who are insulin resistant (Barwell et al., 2008).

The improvements in insulin sensitivity that occur with structured endurance training may be related to chronic training adaptations or may simply be a response to the last bout of exercise that was performed. For example, in high level endurance athletes, abstinence from their training regime for 10 days markedly increased the magnitude and the duration of the plasma glucose and insulin response to an orally ingested glucose beverage (i.e. returning insulin sensitivity to levels measured in a matched group of sedentary subjects) (Heath et al., 1983). In another study, insulin sensitivity measured by hyperinsulinemic euglycaemic clamp returned to levels comparable with untrained sedentary individuals just 60 hours after training cessation, and then remained stable, and comparable to levels in sedentary subjects, during a further week of training abstinence (Burstein et al., 1985). Completing a single session of endurance training after the 10 days of exercise abstinence almost completely restored the insulin and glucose response during an OGTT to “training” levels (Heath et al., 1983). These studies suggest that the effects of aerobic training upon insulin sensitivity are largely explained by the effects of the final exercise bout. However, there are several studies which suggest that there may also be a role for training adaptations in the improvement in insulin sensitivity. For example, in the study of Perseghin et al (1996), whole body glucose uptake was improved 48 hours after a single session of moderate intensity endurance exercise but seemed to be further enhanced after 6 weeks of training in both insulin resistant offspring of T2D parents and healthy controls (~40% vs. ~25%), suggesting there was an accumulated training effect as well as a last bout effect. In addition, after a 10 week period of single-legged endurance training the improvements in insulin stimulated glucose clearance across the trained leg (measured ~16 hours post exercise) could not be mimicked by a single bout of the same exercise in the untrained leg (Dela et al., 1992, 1995), however, these improvements may (Dela et al., 1995) or
may not (Dela et al., 1992) be maintained after 6 days of detraining. Likewise, Segal et al. (1991) found no changes in insulin sensitivity following 12 weeks of aerobic training in lean, obese and diabetic men when measurements were taken 5 days after the final training bout. However, recent data from the STRRIDE randomised controlled trial indicated that some the improvements in insulin sensitivity after a 9-month period of endurance training were retained after 15 days of training cessation and that the retention was dependent on exercise dose (minutes/week) and not on the relative exercise intensity (% VO$_{2\text{max}}$) (Bajpeyi et al., 2009). It seems likely that the enduring effect in this study was the result of alterations in body mass and body fat distribution (Slentz et al., 2004; Bajpeyi et al., 2009). To summarise, an acute bout of endurance exercise can increase whole body insulin sensitivity and this may make an important contribution to improvements observed with endurance training. However, there appears to be an effect of endurance exercise training over and above the effects of the last bout although training adaptations may be lost quickly if a further bout of exercise is not performed.

Another important question pertains to whether relative exercise intensity, exercise duration or overall exercise dose (e.g., kcal/week) is most important for the adaptations in insulin sensitivity with aerobic exercise training. An early study compared the effect of 6-months of low-moderate intensity walking exercise with 6-months of moderate-vigorous intensity running on insulin action and glycaemic control (OGTT) in a group of 11 healthy older men and women with normal glucose tolerance (Seals et al., 1984). The 6-month low-moderate intensity walking intervention consisted of 3-4 weekly sessions exercising for 30 minutes at ~40% of heart rate reserve and was then immediately followed by the 6-month moderate-high intensity running intervention which consisted of 3-4 weekly sessions exercising for 30-40 minutes at ~75% of heart rate reserve. There were no changes in glucose AUC after either exercise intervention. However, whilst there were no changes in insulin AUC after the low-moderate intensity intervention, after the moderate-high intensity intervention insulin AUC was reduced by 31% relative to baseline (Seals et al., 1984). Of course, a clear caveat to the results of this study is that the low and high intensity exercise interventions were not matched for energy expenditure and hence the importance of intensity independent of overall exercise dose cannot be determined from these data.
Several studies have compared energy-matched low and high intensity exercise training programs on insulin sensitivity but the results do not give a clear consensus (Kang et al., 1996; Houmard et al., 2004; O'Donovan et al., 2005; Coker et al., 2006; DiPietro et al., 2006). These studies are summarised in table 1. In a small cohort of overweight and previously sedentary older women, 9-months of high intensity treadmill running was more effective at improving insulin stimulated muscle glucose uptake compared with 9-months of energy matched low-moderate intensity treadmill walking (DiPietro et al., 2006). Similar results have been reported in a comparable cohort of elderly participants after 3 months of low and high intensity cycling (Coker et al., 2006) and after 7 days moderate or high intensity cycling in obese men (Kang et al., 1996). However, other studies have reported contradictory findings. For example, energy matched moderate intensity cycling was just as effective as vigorous intensity cycling for improving fasting indices of insulin sensitivity in untrained middle aged males (O'Donovan et al., 2005) and increasing glucose disposal rate during conditions of euglycaemia hyperinsulinemia in older subjects (Hughes et al., 1993). Also, low-moderate intensity exercise may be just as effective as vigorous intensity exercise for lowering HbA1c in T2D patients (Hansen et al., 2009) although this finding is contested by a meta-analysis which concluded that exercise intensity predicted improvements in HbA1c more comprehensively than exercise duration (Boulé et al., 2003). Finally, to add another layer of complexity, Houmard et al. (2004) investigated the effects of 3 different 6-month aerobic training paradigms (low volume/moderate intensity, low volume/high intensity and high volume/high intensity) on insulin action in a large cohort of matched overweight, sedentary and dyslipidemic subjects. The low volume/moderate intensity and the low volume/high intensity interventions were matched on calorific expenditure (1200 kcal/week) whilst the low volume/moderate intensity and the high volume/high intensity were matched on exercise duration (~170 minutes/week). Post training insulin sensitivity testing was performed 24 hours after the final training bout so may reflect the combined effect of acute and chronic exercise. There were improvements in insulin sensitivity with all three training interventions but significantly greater effects were noted with increased exercise duration, irrelevant of the training intensity (Houmard et al., 2004). More recent data from the same laboratory suggested that the training induced improvements in insulin sensitivity after the interventions with longer sessions (~170 minutes/week) are retained for a longer period of time following training cessation compared with the intervention involving shorter sessions (Bajpeyi et
al., 2009). In agreement, a recent study demonstrated that while both exercise dose (kcal/week) and exercise intensity (kcal/min) correlated with changes in insulin sensitivity after a 16 week varied aerobic training program, the relationship was slightly stronger for exercise dose, and exercise dose was a better predictor of changes in insulin sensitivity than exercise intensity in multivariate regression analysis (Dube et al., 2011). Thus it remains unclear whether exercise intensity, exercise duration or exercise dose is the most important variable underpinning the improvements in insulin sensitivity with aerobic training.
Table 1.1. Studies comparing the effects of exercise training intensity on training induced changes in insulin sensitivity

<table>
<thead>
<tr>
<th>Reference</th>
<th>Exercise Duration</th>
<th>Measure of ISI</th>
<th>Energy Matched</th>
<th>Exercise Protocols</th>
<th>Participants</th>
<th>Δ ISI</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>DiPietro et al (2005)</td>
<td>9 Months</td>
<td>Clamp (40 mJ.m².min⁻¹)</td>
<td>Yes</td>
<td>Mod: 4 session/week on Treadmill -65% VO₂max until 300kcal expended.</td>
<td>Elderly F (n = 9) Healthy/Sedentary/Untrained BMI: 28 ± 4.5</td>
<td>MGU ↑16%</td>
<td>Pre vs. Post: N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72 hours post</td>
<td></td>
<td>High: 4 sessions/week on Treadmill -80% VO₂max until 300kcal expended.</td>
<td>Elderly F (n = 9) Healthy/Sedentary/Untrained BMI: 26.3 ± 3</td>
<td>MGU ↑21%</td>
<td>Pre vs. Post: P&lt;0.05</td>
</tr>
<tr>
<td>Houmard et al (2004)</td>
<td>6 months</td>
<td>IVGTT (SI calculated using minimal model)</td>
<td>Yes</td>
<td>Low Volume/Mod Intensity 1200 kcal/week at 40-55% VO₂max ~170 minutes/week</td>
<td>Middle Aged (n = 41; 17F, 24M) Sedentary/Untrained/Overweight BMI: 30 ± 0.5</td>
<td>SI ↑65%</td>
<td>ΔISI vs. Low/High: P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 hours post</td>
<td></td>
<td>Low Volume/High Intensity 1200 kcal/week at 65-80% VO₂max ~115 minutes/week</td>
<td>Middle Aged (n = 30; 15F, 15M) Sedentary/Untrained/Overweight BMI: 30 ± 0.5</td>
<td>SI ↑29%</td>
<td>Pre vs. Post P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>High Volume/High Intensity 2000 kcal/week at 65-80% VO₂max ~170 minutes/week</td>
<td>Middle Aged (n = 43; 20F, 23M) Sedentary/Untrained/Overweight BMI: 30 ± 0.5</td>
<td>SI ↑57%</td>
<td>Pre vs. Post P&lt;0.05</td>
</tr>
<tr>
<td>O'Donovan et al (2004)</td>
<td>6 months</td>
<td>Fasting Blood Samples</td>
<td>Yes</td>
<td>Mod: 3 session/week on Bike ~60% VO₂max until 400 kcal expended</td>
<td>Middle Aged M (n = 10) Healthy/Sedentary/Untrained BMI: 27.5 ± 6</td>
<td>SI ↑12%</td>
<td>vs. Control: P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 Hours Post</td>
<td></td>
<td>High: 3 session/week on Bike ~80% VO₂max until 400 kcal expended</td>
<td>Middle Aged M (n = 13) Healthy/Sedentary/Untrained BMI: 26.2 ± 2.6</td>
<td>SI ↑10%</td>
<td>vs. Control: P&lt;0.05 vs. High: N.S.</td>
</tr>
<tr>
<td>Coker et al (2003)</td>
<td>3 months</td>
<td>Clamp (40 mJ.m².min⁻¹)</td>
<td>Yes</td>
<td>Mod: 4-5 sessions/week on Bike 1000 kcal/week at ~50% VO₂max</td>
<td>Sedentary/Untrained/Overweight BMI: 31 ± 1</td>
<td>MGU ↓7%</td>
<td>Pre vs. Post: N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72 hours post</td>
<td></td>
<td>High: 4-5 sessions/week on Bike 1000 kcal/week at ~75% VO₂max</td>
<td>Elderly (n=7; 4F, 3M) Sedentary/Untrained/Overweight BMI: 29 ± 1</td>
<td>MGU ↓28%</td>
<td>Pre vs. Post: P&lt;0.05</td>
</tr>
<tr>
<td>Kang et al (1996)</td>
<td>7 Days</td>
<td>OGTT</td>
<td>Yes</td>
<td>Mod: 7 days on Bike 70 minutes at 50% VO₂peak</td>
<td>Obese M (n=6)</td>
<td>Ins AUC ↔ Glu AUC ↔</td>
<td>Pre vs. Post: N.S.</td>
</tr>
<tr>
<td></td>
<td>12-24 hours post</td>
<td></td>
<td></td>
<td>High: 7 days on Bike 50 minutes at 70% VO₂peak</td>
<td>Same as above (crossover design)</td>
<td>Ins AUC ↓21% Glu AUC ↔</td>
<td>Pre vs. Post: P&lt;0.05</td>
</tr>
</tbody>
</table>

M male, F female, IVGTT intravenous glucose tolerance test, MGU muscle glucose uptake, SI insulin sensitivity, N.S. not significant, OGTT oral glucose tolerance test.
1.4.5. Resistance Training and Insulin Sensitivity

Resistance trained subjects have elevated whole body insulin sensitivity compared to sedentary subjects and similar whole body insulin sensitivity compared to endurance trained athletes when expressed relative to body weight (Yki-Jarvinen & Koivisto, 1983). Likewise, the vast majority (Eriksson et al., 1998; Poehlman et al., 2000; Holten et al., 2004) but not all (Fenicchia et al., 2004) longitudinal training studies have demonstrated that insulin action can be improved in previously healthy sedentary subjects after a period of resistance training. These effects have been observed in a wide ranging number of populations, including healthy older men (Miller et al., 1994; Holten et al., 2004), lean young females (Poehlman et al., 2000), individuals with impaired glucose tolerance (Eriksson et al., 1998) and in patients with T2D (Ishii et al., 1998).

Whilst resistance trained subjects are more insulin sensitive compared to sedentary subjects when expressed in absolute terms, insulin stimulated glucose disposal is similar in resistant trained athletes and matched sedentary controls, and lower in both compared with endurance trained athletes, when expressed relative to lean body mass (Yki-Jarvinen & Koivisto, 1983; Takala et al., 1999). This suggests that although absolute insulin sensitivity is high in resistance trained athletes, this can be explained by their increased muscle mass and an increased distribution volume, rather than the muscle being intrinsically more insulin sensitive, as appears to be the case with endurance athletes. Training studies are equivocal on this topic; on the one hand, some studies have shown that improvements in insulin sensitivity with structured resistance training can occur independent of changes in muscle mass (Miller et al., 1994) whilst some studies have refuted these findings (Poehlman et al., 2000). Andersen et al. (2003) determined insulin sensitivity using the gold standard euglycaemic clamp method and thigh muscle mass using gold standard MRI after a period of heavy resistance training and again after a period of de training. They observed that glucose disposal per unit of skeletal muscle mass decreased by 33% during the transition from the trained state to a detrained state. Whilst the effects of the training programme compared to baseline were not determined, such observations suggest that resistance training improves the intrinsic sensitivity of muscle to insulin. The observation that resistance training is associated with the up regulation of key proteins involved in insulin signalling and glucose transport lends support to these data (Tabata et al., 1999; Holten et al., 2004). Regardless of whether
resistance exercise improves the intrinsic sensitivity of muscle to insulin, increasing muscle mass will improve insulin action in absolute terms by increasing distribution volume, and hence resistance exercise will be effective in improving and maintaining glucose homeostasis.

1.4.6. Exercise Training and Insulin Sensitivity: Potential Mechanisms

There are several mechanisms thought to contribute to the improvements in insulin sensitivity with exercise training. Notwithstanding the beneficial changes in body composition which may alleviate inhibitory mechanisms upon the insulin signalling cascade and increase the glucose and insulin distribution volume (Poehlman et al., 2000; Slentz et al., 2004; Schenk et al., 2008; Bajpeyi et al., 2009), there may also be improvements in the delivery of glucose and insulin to the intracellular space, and elevated levels and/or activity of proteins involved in intramyocellular glucose transport, glucose phosphorylation and glycogen synthesis (Wojtaszewski & Richter, 2006).

The classic hypothesis centres on GLUT4 protein levels which are increased following both aerobic (Houmard et al., 1991; Dela et al., 1993; Hughes et al., 1993; Langfort et al., 2003) and resistance training (Holten et al., 2004). Indeed, the overexpression of GLUT4 has been shown to increase insulin sensitivity in rats (Hansen et al., 1995), whilst muscle specific deletion of GLUT4 in mice results in muscle insulin resistance and glucose intolerance (Zisman et al., 2000). In humans, muscle GLUT4 protein content has been shown to correlate with measures of muscle specific glucose disposal (Dela et al., 1993), however, changes in GLUT4 content with training do not appear to correlate with changes in insulin sensitivity (Hughes et al., 1993), suggesting other intracellular mechanisms may also be involved.

Other changes may involve an improvement in the capacity for insulin signalling to GLUT4 translocation following exercise training. Studies generally report that the expression of proteins involved in the proximal steps of insulin signalling, for example IRS1 and PI3K, remain unchanged following exercise training (Yu et al., 2001; Holten et al., 2004; Frøsig et al., 2007). There may be in an increased phosphorylation and activity of these proteins in the insulin stimulated state following training (Houmard et al., 1999; Kirwan et al., 2000; Glynn et al., 2008) although this is not a universal finding (Tanner et al., 2002). In contrast, both the expression and activity of distal signalling
proteins, such as Akt/PKB and AS160, have been shown to increase following exercise training (Holten et al., 2004; Frøsig et al., 2007; Vind et al., 2011). These data suggest that exercise training may augment the insulin signalling cascade which would be expected to result in a more efficient coordination of GLUT4 translocation to the cell membrane and an increased capacity for glucose uptake in the exercise training state.

Lastly, maintaining a high positive gradient between the interstitial fluid and the cytosol is important for maintaining a high rate of glucose uptake into the muscle cell in the insulin stimulated state (Wasserman, 2009). There are several exercise induced adaptations which may augment this gradient. Firstly, the delivery of glucose (and insulin) may be improved by an increase in muscle capillarisation in the trained state (Lillioja et al., 1987; Cocks et al., 2013). Indirect evidence for this as a mechanism comes from the observation that muscle capillarisation correlated with insulin sensitivity in a large cohort of non-diabetic men (Lillioja et al., 1987). There is also some evidence that insulin-mediated increases in muscle blood flow in the postprandial state may be enhanced in rodents and humans following a period of exercise training (Rattigan et al., 2001; Mikus et al., 2011). Secondly, there is augmented capacity for insulin-stimulated glycogen synthesis in the both the aerobic and resistance trained state, mediated by an increase in glycogen synthase expression and activity (Perseghin et al., 1996; Holten et al., 2004; Vind et al., 2011).

1.5. Cardiorespiratory Fitness and Health

Cardiorespiratory fitness, commonly assessed as the maximal attainable rate of oxygen uptake (VO$_2$max) or time to exhaustion during an incremental exercise test to volitional exhaustion, has emerged as an important determinant of all-cause morbidity and mortality in both men and women (Blair et al., 1989; Barlow et al., 1995; Blair et al., 1995; Blair et al., 1996; Lee et al., 1998; Lee et al., 1999; Myers et al., 2002; Gulati et al., 2003). This was first shown in 1989 by Blair and colleagues using data collected from the Aerobics Centre Longitudinal Study; in a cohort of 10,224 men and 3120 women, they demonstrated that the risk of all-cause mortality was reduced by ~50% in moderately fit compared with low fit individuals (Blair et al., 1989). Since then it has been shown that a major portion of the lowered risk of all-cause mortality is due specifically to a reduction in the risk of cardiovascular mortality (Lee et al., 1999; Lee
et al., 2011). Remarkably, the predictive impact of cardiorespiratory fitness holds even in populations who already present with other recognised risk factors for cardiovascular disease, including overweight/obesity (Barlow et al., 1995; Lee et al., 1999), hypertension (Sui et al., 2007), diabetes (Church et al., 2005), and smoking (Lee & Blair, 2002). The impact of cardiorespiratory fitness on cardiovascular and all-cause mortality was recently subject to comprehensive meta-analysis (Kodama et al., 2009). Using data from 33 studies and >103,000 participants, Kodama et al. (2009) showed that, compared with individuals in the high-fitness group, individuals in the low fitness category had 70% higher all-cause and 56% higher CVD mortality rates. They also examined the dose response relationship and showed that for every 1 MET score achieved during the incremental exercise test there was a 13% reduction in CVD mortality (Kodama et al., 2009). Importantly, a more recent analysis of the Aerobics Centre Longitudinal Study has shown that improving cardiovascular fitness in individuals who had previously presented with low fitness significantly lowers the risk of all-cause and CVD mortality (Lee et al., 2011).

The specific mechanisms which link cardiorespiratory fitness to a lower risk of cardiovascular disease are not well understood. However, a recently developed murine model selectively bred for low (LEC) and high exercise capacity (HEC) has provided some potential metabolic mechanisms. For example, Wisloff et al. (2005) demonstrated that, after 11 generations, exercise capacity was 347% lower in LEC compared with HEC, and tracked with the emergence of elevated fasting glucose, fasting insulin, fasting lipids, visceral adiposity and blood pressure, as well as endothelial dysfunction.

Cardiorespiratory fitness is partly determined by genetic factors (50%) but also in large part by environmental factors (50%) (Bouchard et al., 1999). Importantly, the only environmental factor known to improve aerobic fitness is physical activity and, in particular, structured exercise training (Church, 2009). Numerous large longitudinal studies have shown that previously sedentary individuals will, on average, improve their aerobic capacity following a period of standardised aerobic training, and the average response may be increased with a greater exercise dose or relative exercise intensity (Wenger & Bell, 1986; Sisson et al., 2009; Garber et al., 2011). However, regardless of the exercise training dose or intensity the magnitude of the response is always highly variable between individuals. Improvements in aerobic capacity do not appear to be
explained by initial fitness levels, age, sex or race, so long as exercise dose/intensity is standardised amongst individuals (Skinner et al., 2000). Rather, the variance appears to be partly (~50%) the result of heritable factors (Bouchard et al., 1999). Changes in aerobic fitness are largely determined by central adaptation in the oxygen transport system and therefore oxygen delivery to the peripheral tissues during exercise (Bassett & Howley, 2000). In summary, it appears as though cardiorespiratory fitness is an important factor for cardiometabolic health and regular exercise plays an important role in maintaining/improving aerobic fitness throughout ageing.

1.6. Current Statistics on, and Barriers to Exercise Participation

This literature review has shown that physical inactivity is an important risk factor for chronic cardiometabolic disease. On the contrary, evidence has been presented which shows that the risk of both type 2 diabetes and cardiovascular disease may be reduced with regular exercise training and/or increased physical activity. However, it might be stating the obvious, but in order to accrue the health benefits of exercise, people actually have to do the exercise on a regular basis. Current statistics in the UK suggest that exercise remains an underemployed strategy, with just 40% of men and 28% of women in the UK achieving the recommended minimum amount of physical activity (150 min/week of moderate intensity exercise) when measured by self-report (Allender et al., 2008a). However, when physical activity is measured objectively by accelerometer the number falls to 6% of men and 4% of women (Allender et al., 2008a).

Some of the problem relates to the willingness of health care professionals, as well as the average individual, to primarily look towards drug therapies in situations where exercise prescription and/or other lifestyle changes are likely to be more effective (Church & Blair, 2009). Likewise, many researchers continue to look for the so called ‘exercise pill’ but such efforts are unsubstantiated and are likely to remain futile (Hawley & Holloszy, 2009). It is well accepted by exercise physiologists and physical activity practitioners alike that the focus should be to find novel ways of increasing motivation for, and long term adherence to, regular physical activity and/or exercise (Hawley & Holloszy, 2009). Therefore, the identification of the common barriers that prevent adherence and motivation to exercise is essential for alternative exercise paradigms to be developed. A recent systematic review of studies examining common barriers to
exercise identified a ‘lack of time due to work or family commitments’ as the most frequently cited, being identified in 9 out of the 13 studies which were reviewed. Logically, this would suggest that the current focus on a relatively high volume of moderate intensity physical activity on most days of the week is not ideal in terms of stimulating exercise participation. Whilst this is by no means the only reason people do not perform regular exercise, this particular barrier offers a unique opportunity to exercise physiologists as we could theoretically be able to offer a potential solution. It has recently been postulated that high-intensity interval training (HIT) may offer a time-efficient alternative to high volume aerobic exercise training.

1.7. High-Intensity Interval Training (HIT)

1.7.1. What is HIT?

High-intensity interval training (HIT) involves performing brief repeated bouts of high-intensity, submaximal, maximal or supramaximal exercise interspersed with periods of low intensity or resting recovery. The concept of HIT is not new; this type of training paradigm is frequently utilised in athletic populations to stress metabolic pathways that are relevant to performance (Gibala et al., 2012). However, more recently there has been growing interest in HIT as a potential time-efficient alternative to more traditional endurance or resistance exercise for inducing adaptations that are relevant to health (Gibala et al., 2012).

The design of a HIT session is similar to resistance exercise in that both exercise modes involve short bursts of high intensity muscular work interspersed with periods of rest. However, the nature of the HIT exercise stimulus (i.e., running or cycling), which involves high frequency and rhythmic contractions-relaxation cycles, is more like traditional cardiorespiratory exercise, albeit at a much higher intensity. Simply by its nature, cardiorespiratory exercise performed at intensities above the maximal lactate steady state (MLSS) results in the accumulation of metabolites implicated in fatigue and, as a result, these power outputs cannot be maintained for a prolonged period of time before the onset of fatigue. In order to accumulate a larger volume of exercise at these intensities, the muscle must be given periods of recovery in between each bout; in other words, the exercise must be performed in intervals. The length of time the high-intensity
bursts can be performed for is directly related to intensity - an exercise bout performed at a power output just above that which elicits MLSS may be maintained for 20-30 minutes, whilst an ‘all-out’ effort generating power outputs 2-3 fold higher than that which elicits \( \dot{V}O_{2\text{max}} \) (e.g., Wingate sprints), will result in a drop in power output after just a few seconds, and may only be maintained for up to ~30 seconds.

There is a wide scope for variation in protocols with HIT as the frequency, intensity and the duration of the high-intensity bursts, as well as the characteristics and duration of the recovery periods, may all be manipulated to change the nature of the exercise stimulus and thus potentially the adaptations associated with training (Ross & Leveritt, 2001; Gibala et al., 2012). As such, there have been a wide range of HIT protocols used throughout the literature and, generally speaking, limited standardisation of the terminology used to describe the different protocols. HIT protocols can be broadly grouped into submaximal, maximal and supramaximal, based on the intensity utilised during the high-intensity bouts relative to maximal aerobic power (Figure 1.6). However, the majority of protocols utilised to date have used either submaximal or supramaximal intensities. Based on this terminology, an example of a submaximal HIT protocol would be the 10 × 1 min at ~90% of maximal heart rate with 1 min resting recovery, whilst an example of a supramaximal HIT protocol would be the classic 4-6 × 30 s ‘all-out’ Wingate type sprints (Figure 1.6). Throughout this thesis, the term ‘HIT’ will be used to encompass all literature pertaining to high-intensity interval training. In some cases, there may be value in providing a description of the complete protocol and, where appropriate, this will be used when describing the results of specific studies.
1.7.2. Acute responses to HIT

Substrate Utilisation and Cardiovascular Responses

Substrate utilisation during the many varieties of HIT protocol has not been determined directly. Our knowledge of fuel metabolism during HIT mainly comes from studying the transition from low-moderate-high intensity exercise and from the application of single and repeated ‘all out’ sprinting protocols (e.g., Wingate sprints). This section will explore the changes in substrate metabolism that occur during the transition from submaximal to maximal to supramaximal exercise intensities.

At rest and in the fasting state, skeletal muscle primarily relies on the oxidation of plasma NEFA to meet its free energy requirements but there is also a small contribution from blood glucose (van Loon et al., 2001). The physiological importance of primarily utilising NEFA delivered from the vast adipose tissue depots in the resting post-absorptive state is the sparing of limited carbohydrate stores in the liver and in skeletal muscles. With the onset of any exercise, there is a change from primarily oxidising NEFA to utilising some mixture of NEFA, blood glucose, muscle glycogen.

Figure 1.5. Schematic of common HIT protocols vs. current recommendations. Adapted from Gibala et al (2014)
and intramuscular triglycerides. Whilst the composition of the fuel mixture being oxidised during exercise can be altered by several variables including gender, temperature, pre-exercise substrate stores, preceding diet and training status, the intensity of the exercise is a dominant influencing factor (Romijn et al., 1993, 2000; van Loon et al., 2001). Energy requirements are dictated largely by the absolute exercise intensity, whilst the composition of the fuel mix being oxidised is dependent on the relative exercise intensity (Romijn et al., 1993, 2000; van Loon et al., 2001).

As the relative exercise intensity increases, the ratio of carbohydrate to fat utilisation increases also (Romijn et al., 1993, 2000; van Loon et al., 2001). Nevertheless, in spite of this relative decrease in fat utilisation with increasing exercise intensity, the absolute energy demands of the exercise increase and so does the absolute rate of fat oxidation during low to moderate exercise intensities (Achten et al., 2002; Achten & Jeukendrup, 2003; Achten et al., 2003). In fact, the relationship between absolute fat oxidation and exercise intensity is inversely parabolic, with rates of fat oxidation reported to reach a zenith (i.e. a maximal rate, sometimes referred to as FAT_{max}) at ~55% of VO_2_{max} and then falling sharply as the exercise intensity is increased further (Achten et al., 2002; Achten & Jeukendrup, 2003; Achten et al., 2003). On the contrary, there appears to be a linear relationship between relative exercise intensity and absolute and relative utilisation of blood glucose and muscle glycogen, with muscle glycogen becoming quantitatively more important at each exercise intensity increment (van Loon et al., 2001). At intensities which elicit maximal oxygen uptake there is almost a complete inhibition of lipid contribution to ATP demands (Achten et al., 2002; Achten & Jeukendrup, 2003; Achten et al., 2003).

The metabolic pathways involved in fuel utilisation during exercise can be broadly grouped into ‘aerobic’ (i.e., oxidative phosphorylation – requiring oxygen) and ‘anaerobic’ (substrate level phosphorylation – not requiring oxygen). Oxidative phosphorylation is largely considered ‘metabolically clean’, whilst substrate level phosphorylation, either through depletion or generation of metabolites, is implicated in fatigue during high intensity exercise. Nevertheless, although exercise is often categorised as being ‘anaerobic’ or ‘aerobic’, there will always be some blending of oxidative and substrate level phosphorylation contributing to ATP resynthesis during exercise. Indeed, there is some lactate appearance into the systemic circulation from
skeletal muscle at low ‘aerobic’ exercise intensities (Richardson et al., 1998), whilst
during a supramaximal sprint (considered ‘anaerobic’) some ATP resynthesis from
oxidative phosphorylation is evident even during the initial seconds and increases in
importance as the sprint progresses (Parolin et al., 1999). The relative blend of the two
pathways is largely dependent on the relative exercise intensity, with the relative
proportional contribution of substrate level phosphorylation increasing with increasing
exercise intensity, and probably partly reflecting the hierarchical recruitment of type IIa
and IIx muscle fibres during incremental exercise (Mmphedran et al., 1965; Gordon et
al., 2004). However, at lower exercise intensities the generation of fatigue-causing
metabolites through substrate level phosphorylation is well matched with the activity of
pathways designed to remove those metabolites. Such a trend continues until the
maximal lactate steady state is reached, after which the balance between
production/removal is disrupted, and metabolites will continue to accumulate until
fatigue is initiated. So, at exercise intensities which elicit maximal oxygen uptake, fat
oxidation is almost completely inhibited, and glycolysis and carbohydrate oxidation are
maximally activated. However, there is also a heavy contribution from substrate level
phosphorylation which acts as an ATP buffer, and a significant proportion of pyruvate
will be converted to lactate in order to both maintain NAD+/NADH redox balance and
the level of flux through glycolysis. As a result, exercise at VO\textsubscript{2}max can only be
maintained for a few minutes before fatigue ensues (Bangsbo et al., 1990).

Exercise at supramaximal intensities presents a unique challenge to skeletal
muscle. As processes which increase oxygen delivery and oxygen uptake require several
minutes to be maximally active (Bangsbo et al., 1990), the relative contribution of
substrate level phosphorylation is greater in the initial seconds of a sprint. Substrate
utilisation during isolated supramaximal sprint exercise is extremely complex, involving
the rapid depletion of phosphocreatine (PCr) stores, reductions in intramyocellular ATP
and glycogen concentrations, a rapid accumulation of lactate, but also an increasing
emphasis on oxidative metabolism as the duration of the sprint increases (Gaitanos et
al., 1993; Bogdanis et al., 1996; Esbjornsson-Liljedahl et al., 1999; Parolin et al., 1999;
Esbjornsson-Liljedahl et al., 2002). The hydrolysis of phosphocreatine (PCr) provides
the majority of the energy for ATP resynthesis during the initial stages of the sprint when
peak power outputs are achieved, but as PCr stores are rapidly depleted other fuels
become more important and power output progressively falls (Parolin et al., 1999).
Glycolysis is maximally activated ~6 seconds into a sprint (maybe even sooner) and is maintained for a further 10 seconds, but is then strongly attenuated, returning to baseline levels by the end of a 30-s bout (Parolin et al., 1999). Stored muscle glycogen provides almost all of the glycolytic substrate with little or no contribution from extracellular glucose; concordantly, a single Wingate sprint has consistently been shown to reduce total skeletal muscle glycogen concentrations by ~20-30% (Esbjornsson-Liljedahl et al., 1999; Parolin et al., 1999; Esbjornsson-Liljedahl et al., 2002). As the resulting increase in pyruvate levels far exceeds its level of oxidation there is a large excursion in both muscle and blood lactate concentrations (Parolin et al., 1999); the associated reduction in muscle pH likely inhibits glycolytic enzyme activity, interferes with the calcium dependent contractile mechanism, and may contribute to a decrease in central motor drive, making ‘all-out’ sprinting extremely fatiguing (Billaut and Bishop, 2009). Pyruvate dehydrogenase (PDH) is maximally active by 15 s meaning pyruvate oxidation is more closely matched to rate of pyruvate production, correlating with a shift towards aerobic energy production and a reduction in the rate of lactate accumulation as the sprint progresses (Parolin et al., 1999).

Energy provision during repeated sprinting is substantially altered. The total work done and mean power output are reduced during subsequent bouts (Gaitanos et al., 1993; Parolin et al., 1999). There appears to be no activation of glycogen phosphorylase during repeated bouts; hence the contribution of glycogenolysis to energy provision is negligible and there is no further reduction in intramyocellular glycogen stores (Parolin et al., 1999). Phosphocreatine is regenerated rapidly following cessation of exercise and hence remains an important source of ATP synthesis in the initial seconds of repeated bouts of sprinting, as long as the recovery period is sufficient (~4 min) (Parolin et al., 1999). Oxidative metabolism provides the majority of subsequent energy requirements following the degradation of PCr stores with a very small contribution of anaerobic glycolysis (Parolin et al., 1999). The inhibition of glycogenolysis in the third bout compared with the first is presumably to prevent excessive glycogen breakdown as a plentiful supply of substrate remains from the first bout (Parolin et al., 1999). Similar increases in pyruvate dehydrogenase activity are noted in the third bout meaning the primary fate of pyruvate is oxidative metabolism and hence the rate of lactate accumulation is negligible during the third bout (Parolin et al., 1999).
The substantial energy demands placed upon skeletal muscle during sprint exercise have a dramatic knock-on effect upon the cardiovascular system. Not only is there a large aerobic component to each sprint, but the utilisation of phosphocreatine and glycogen in the initial seconds of the sprint also creates a substantial oxygen deficit. Thus, oxygen demands during the latter part of the sprint and early on in the recovery are high and, in fact, VO$_2$ approaches maximal values following each sprint effort (Hazell et al., 2014; Townsend et al., 2014). In order to aid oxygen delivery and removal of metabolic products (e.g. lactate, CO$_2$), ventilation increases dramatically and blood flow to active muscles can increase by ~100 fold during maximal dynamic exercise (Hawley et al., 2014). There is a rapid increase in heart rate which will also approach maximal levels during an ‘all-out’ sprint largely as a result of the large spike in adrenaline with sprint exercise (Boutcher, 2011). Nevertheless, there will also be localised reductions in oxygen tension within the activated skeletal muscles, particularly during the early onset of the sprint (Bhambhani, 2004). In addition, the increase in heart rate, cardiac output and peripheral hyperaemia is associated with an increase in mean arterial blood pressure and endothelial shear stress (Hawley et al., 2014).

Substrate utilisation in the hours following HIT has not been well characterised given that its measurement is hindered by the limitations of indirect calorimetry during,

**Figure 1.6.** Changes in substrate utilisation during a repeated Wingate sprint protocol. The contribution of PCr hydrolysis, glycolysis and oxidative phosphorylation to ATP re-synthesis during the first (A), and third (B) ‘all-out’ 30-s bout of maximal cycling. Figure from Parolin et al (1999).
and following, high intensity exercise which places great strain on muscle and blood buffering mechanisms (Hazell et al., 2012). However, it does appear as though HIT results in a more prolonged elevation in post-exercise oxygen consumption (EPOC) compared with moderate intensity aerobic exercise. Indeed, there are now several studies reporting that HIT results in similar elevations in 24 hour oxygen uptake compared with moderate aerobic exercise, despite large estimated differences in energy expenditure during the exercise bout itself (Hazell et al., 2012; Skelly et al., 2014). In addition, similar to submaximal aerobic exercise, studies that have measured substrate utilisation several hours following HIT suggest there is a shift towards a greater reliance on lipid substrates during recovery (Chan & Burns, 2013; Whyte et al., 2013; Williams et al., 2013).

**Hormonal Responses**

Single or repeated sprints result in alterations in the systemic hormonal milieu. Perhaps most importantly, plasma concentrations of adrenaline and noradrenaline are increased dramatically following both single and repeated Wingate sprints, an effect that may (Gratas-Delamarche et al., 1994), or may not (Vincent et al., 2004), be attenuated in women. The rise in concentrations is short-lived and returns to baseline levels very quickly following cessation of exercise (Zouhal et al., 2008; Williams et al., 2013). The physiological significance of the increase in catecholamines is likely related to the stress response during the exercise bout itself, for example, acting to increase heart rate, cardiac output and oxygen delivery to the peripheral tissues. There is also a 10-fold increase in systemic levels of growth hormone (Nevill et al., 1996) and a 15% increase in cortisol (Esbjörnsson et al., 2009) following sprint exercise.

**Molecular Responses in Skeletal Muscle**

HIT presents an acute ‘energy crisis’ in the contracting skeletal muscle cell, resulting in excursions in intracellular calcium (Ortenblad et al., 2000), rapid degradation of phosphocreatine and glycogen (Gaitanos et al., 1993; Parolin et al., 1999), alterations in the ATP/ADP/AMP ratio (Karatzafieri et al., 2001a), disruption of the cellular redox balance (Cuevas et al., 2005; Kang et al., 2009), and a significant accumulation of metabolites and electrolytes (Gaitanos et al., 1993; Parolin et al., 1999). Such intracellular changes are associated with the activation of several signalling
cascades which regulate the acute metabolic response and switch on processes which aid recovery and adaptation. HIT results in adaptive improvements in carbohydrate/fat metabolism and increased oxidative capacity (see below), and HIT also stimulates signalling pathways which are linked to mitochondrial biogenesis and transcription of genes involved in carbohydrate and lipid handling. For example, HIT has been associated with increased signalling through AMP activated protein kinase (AMPK), p38 mitogen activated protein kinase (p38 MAPK) and p53 (Gibala et al., 2009; Little et al., 2011b; Bartlett et al., 2012; Fuentes et al., 2012; Fuentes et al., 2013; Cochran et al., 2014). The current understanding of the signalling response to HIT in relation to muscle adaptation is summarised in Figure 1.8.

Firstly, both single and repeated ‘all-out’ sprint protocols are associated with increased phosphorylation of AMPK in both men and women (Gibala et al., 2009; Fuentes et al., 2012; Fuentes et al., 2013). In addition, matched work submaximal high intensity interval and continuous moderate intensity running exercise is associated with a similar increase in AMPK activation (Bartlett et al., 2012). Such a response is probably largely determined by an increased AMP/ADP: ATP ratio, but may also be regulated by the level of glycogen degradation with high intensity exercise (Moore et al., 1991; Corton et al., 1994; McBride et al., 2009; McBride & Hardie, 2009). AMPK has been linked with transcription of genes relating to mitochondrial biogenesis and carbohydrate metabolism in skeletal muscle (Holmes et al., 1999; Jørgensen et al., 2006; Winder et al., 2006; Pogozelski et al., 2009).

p38 MAPK is activated by signals of intracellular stress, including elevations in intracellular calcium (Wright et al., 2007), oxidative stress (Gomez-Cabrera et al., 2005), glycogen availability (Chan et al., 2004) and osmotic tension (Sheikh-Hamad & Gustin, 2004). The activation of p38 MAPK has been shown following repeated sprint exercise (Gibala et al., 2009; Bartlett et al., 2012) but does not appear to occur with a single sprint effort (Fuentes et al., 2012; Fuentes et al., 2013). The role of p38 in regulating mitochondrial biogenesis is emphasised by recent data which demonstrated that cellular ablation of the gamma subunit attenuated mRNA expression of PGC-1α and mitochondrial biogenesis following aerobic exercise training (Pogozelski et al., 2009).

There is also some evidence that p53, which has arisen as a key regulator of mitochondrial adaptation with exercise training (Bartlett et al., 2014; Saleem et al.,
2014), is activated with HIT. To the authors’ knowledge, only one study has examined the response of p53 to HIT (Bartlett et al., 2012). Bartlett et al (2012) demonstrated a 2.5 fold increase in p53\textsuperscript{ser15} phosphorylation 3 hours following a bout of high intensity interval running (6 × 3 min at 90% VO\textsubscript{2}max). The increase in activation of p53 was similar compared with an acute 50 min bout of continuous running at 70% of VO\textsubscript{2}max. It would be interesting to examine the response of p53 to lower volume supramaximal HIT protocols.

Signalling through AMPK, p38 MAPK and p53 is proposed to up-regulate mitochondrial adaptation by signalling through PGC-1α (peroxisome-proliferator activated receptor γ coactivator), which has been termed ‘the master regulator’ of mitochondrial biogenesis (Puigserver & Spiegelman, 2003). The proposed role of PGC-1α in mitochondrial adaptation mainly comes from rodent studies which have demonstrated that overexpression of this enzyme is associated with an elevated activity of oxidative enzymes and an improvement in exercise tolerance (Calvo et al., 2008). PGC-1α has also been shown to promote the transcription of GLUT4 mRNA and in some situations may be involved in the regulation of insulin sensitivity (Summermatter et al., 2013). In response to an acute bout of HIT, the total content of PGC-1α does not appear to increase (Little et al., 2011b; Bartlett et al., 2012). However, there is evidence of translocation of PGC-1α from the cytosol to the nucleus following HIT (Little et al., 2011b). This may be of key importance for interaction with transcription factors and regulation of gene expression. In addition, increases in the transcription of PGC-1α mRNA have repeatedly been shown following acute bouts of HIT (Gibala et al., 2009; Little et al., 2011b). Likewise, the levels of PGC-1α protein have repeatedly been shown to increase following 2-6 weeks of HIT (Burgomaster et al., 2005; Gibala et al., 2006; Little et al., 2010b; Hood et al., 2011).
Figure 1.7. Putative signalling cascades regulating adaptation following HIT. Solid lines represent a pathway which there is strong evidence for, whilst dotted lines represent proposed signalling pathways. AMP adenosine monophosphate, ATP adenosine triphosphate, AMPK amp-activate protein kinase, Ca$^{2+}$ calcium ions, GLUT4 glucose transporter 4, ROS reactive oxygen species, p38 MAPK p38 mitogen activated protein kinase, PGC-1α peroxisome-proliferator activated receptor γ coactivator, Tfacs transcription factors

1.7.3. Chronic Adaptations following HIT

Effects on Aerobic Capacity

As previously reviewed in this thesis, evidence suggests that VO$_2$max represents one of the strongest predictors of adverse health and all-cause mortality. There are now a number of studies which have investigated a variety of different HIT protocols on aerobic capacity, and these studies have recently been summarised by three meta-analyses (Sloth et al., 2013; Gist et al., 2014; Weston et al., 2014a).
The magnitude of improvement in absolute VO\textsubscript{2}max with 2-6 weeks of HIT is in the region of ~10%, and appears to be comparable to changes observed following traditional continuous aerobic training at 60-70% of VO\textsubscript{2}max (Burgomaster \textit{et al.}, 2008; Rakobowchuk \textit{et al.}, 2008; Cocks \textit{et al.}, 2013). This response does not appear to be different between men and women (Astorino \textit{et al.}, 2011) and similar effects are observed in overweight and obese individuals (Whyte \textit{et al.}, 2010; Trilk \textit{et al.}, 2011). Interestingly, several studies report that VO\textsubscript{2}max may be improved with a remarkably small volume of supramaximal HIT. For example, Ma \textit{et al.} (2013) have shown that 4 sessions/week of a HIT protocol, requiring eight 20 s cycling efforts at ~170% VO\textsubscript{2}max with 10 s recoveries, resulted in a 19% increase in VO\textsubscript{2}max in a group of young healthy men. Likewise, Hazell \textit{et al.} (2010) reported that just two weeks of supramaximal HIT, utilising 4-6 \times 10 sec ‘all-out’ sprints, resulted in a 9% improvement in VO\textsubscript{2}max. This response was similar compared with a group of participants performing 4-6 \times 30 sec ‘all-out’ sprints over the same time period (Hazell \textit{et al.}, 2010), which suggests that some mechanisms intrinsic to the intensity of the exercise bout is important for adaptations in maximal oxygen uptake.

Improvements in VO\textsubscript{2}max have also been observed in a variety of clinical populations using submaximal/maximal HIT interventions; for example, in patients with metabolic syndrome (Tjønna \textit{et al.}, 2008), cardiovascular disease (Rognmo \textit{et al.}, 2004), and, quite remarkably, in patients recovering from heart failure as well (Wisløff \textit{et al.}, 2007; Moholdt \textit{et al.}, 2011). Despite the relatively high training intensities, HIT is reported to be well tolerated in these patient groups, and importantly from a patient recuperation perspective, the majority of studies report that submaximal/maximal HIT is the superior stimulus for improving VO\textsubscript{2}max when compared to volume matched continuous moderate intensity cardiorespiratory exercise (Rognmo \textit{et al.}, 2004; Wisløff \textit{et al.}, 2007; Tjønna \textit{et al.}, 2008; Ciolac \textit{et al.}, 2010). Question marks over the safety of HIT interventions in these populations may be assuaged by recent data reporting no elevated risk of cardiovascular events during HIT compared with traditional high volume continuous exercise (Rognmo \textit{et al.}, 2012) although further studies on the safety of HIT in clinical populations are warranted.
Effects on Aerobic Performance and Oxidative Capacity

In their seminal research papers, Gibala and colleagues demonstrated that just six sessions of supramaximal HIT, requiring a maximum of 3.5 min of high-intensity exercise each session, over a period of two weeks was sufficient to double the time to exhaustion during an exercise bout at 80% of pre-training VO₂peak, and improve performance during a time-trial matched on total work, both of which rely heavily on oxidative metabolism (Burgomaster et al., 2005; Burgomaster et al., 2006). This improvement in aerobic performance were subsequently confirmed in independent laboratories (Babraj et al., 2009), and shown to be of similar magnitude to the improvements observed with traditional high-volume continuous exercise training, even though total exercise volume was ~90% lower, and total time commitment was ~75% lower, with the HIT intervention (Gibala et al., 2006).

The improvements in performance in tasks relying primarily on aerobic metabolism with HIT can likely be ascribed to local adaptations in oxidative enzymes and substrate storage within the skeletal muscle. The maximal activity of citrate synthase is increased with HIT, as is the maximal activity of cytochrome c oxidase (COX), the expression of COX II and COX IV protein subunits, and the expression of pyruvate dehydrogenase (Burgomaster et al., 2005; Burgomaster et al., 2006; Gibala et al., 2006; Burgomaster et al., 2008). In addition, the maximal activity of β-HAD, which is the rate-limiting enzyme in the oxidation of fatty acids, is up-regulated with HIT (Burgomaster et al., 2008), and so is the expression of perilipin 2 and perilipin 5 which are thought to be important in regulating lipolysis of IMTG during exercise (Shepherd et al., 2013). Lastly, resting stores of IMTG and glycogen are both increased with HIT (Burgomaster et al., 2005; Shepherd et al., 2013). Overall, such adaptations afford better metabolic control in exercising skeletal muscle, such that lipid utilisation is increased, and glycogen utilisation, phosphocreatine utilisation and lactate accumulation are reduced during matched intensity exercise (Burgomaster et al., 2006; Burgomaster et al., 2008; Perry et al., 2008), and greater voluntary power outputs can be maintained during matched work time-trials (Burgomaster et al., 2006; Burgomaster et al., 2008). Taken together, it appears as though HIT represents a time-efficient stimulus for inducing a range of adaptations classically seen following high-volume continuous aerobic exercise training.
Effects on Insulin Sensitivity

The metabolic and morphological adaptations associated with HIT appear to coincide with improvements in insulin action and glycaemic control (summarised in table 1). Whilst a couple of studies had tentatively suggested improvements in fasting insulin and glucose following submaximal HIT interventions (Tjønna et al., 2008; Trapp et al., 2008), the first comprehensive data came from Babraj et al. (2009), who reported a substantial reduction in both the duration and magnitude of the plasma glucose and insulin response to an orally ingested glucose solution (i.e., OGTT) following two weeks of supramaximal HIT, indirectly indicating improved whole-body insulin sensitivity for at least three days following the final training bout. This finding was subsequently replicated in a similar cohort of recreationally active men and women using the gold standard euglycemic hyperinsulinemic clamp technique, suggesting that the observed improvements in whole body insulin sensitivity following SIT are probably attributable to enhanced peripheral glucose handling, most likely in skeletal muscle (Richards et al., 2010). More recent work has shown that the improvements in insulin sensitivity following 6 weeks of supramaximal HIT appear to be of a similar magnitude to those observed following 6 weeks of continuous moderate intensity training, at least in young sedentary male subjects (Cocks et al., 2013).

Follow up investigations have begun to characterise the impact of different models of HIT on insulin action in populations either at an elevated risk of developing insulin resistance, such as overweight and obese men and women (Whyte et al., 2010; Gillen et al., 2013), or in populations already presenting with severe insulin resistance such as patients with T2D (Little et al., 2011a). Whyte et al (2010) demonstrated an improvement in insulin sensitivity in overweight men at 1 day, but not 3 days, following a two week supramaximal HIT intervention. A subsequent study by the same group found no change in insulin sensitivity measured 14-16 hours following a single bout of HIT in a matched cohort, suggesting that the improvements observed following the 2 week training intervention were the result of chronic adaptations (Whyte et al., 2013), albeit more transient than observed in lean individuals (Babraj et al., 2009; Richards et al., 2010; Cocks et al., 2013). In a cohort of overweight women, Gillen et al (2013) utilised a 6 week maximal HIT protocol (10 × 1 min at ~90% HRmax) and could detect no mean change in insulin sensitivity, although they did note a small reduction in glucose.
AUC during an OGTT. Little et al. (2011a) examined the effect of the same HIT protocol on glycaemic control, as measured by 24-h continuous glucose monitoring, in 8 patients presenting with T2D. Follow up measurements taken 48-h following the final training revealed a ~13% reduction in mean 24-h blood glucose concentrations, as well as a 29% reduction in the aggregate glucose AUC to the standardised breakfast, lunch and evening meals. Taken together, it appears as though HIT results in improvements in insulin sensitivity in a variety of different populations (table 1.2).

The mechanisms underpinning the improvements in insulin action with HIT remain speculative, but could be explained either by improvements in postprandial insulin and glucose delivery resulting from muscle angiogenesis and enhanced NO-mediated increases in postprandial muscle blood flow (Cocks et al., 2013), by an improved intramyocellular glucose transport capacity (Burgomaster et al., 2007; Hood et al., 2011; Little et al., 2011a; Gillen et al., 2013) and increased drive for muscle glycogen storage (Burgomaster et al., 2005; Gibala et al., 2006), or most likely some combination of these. Firstly, insulin-mediated increases in macro- and microvascular flow signalled through NO is a vital step in peripheral glucose uptake and is attenuated in people with insulin resistance (Barrett et al., 2011). Cocks et al. 2013) recently reported a 27% increase in muscle capillary density as well as a 36% increase in muscle eNOS content following HIT, which coincided with a 26% increase in insulin sensitivity. Whether or not HIT results in augmented postprandial increases in blood flow and transendothelial transport remains to be determined. From an intracellular perspective HIT results in a robust increase in intramuscular GLUT4 protein content (Burgomaster et al., 2007; Hood et al., 2011; Little et al., 2011a; Gillen et al., 2013) and elevations in muscle glycogen inferring a greater drive for glycogen synthesis (Burgomaster et al., 2005; Gibala et al., 2006) both of which might play an important regulatory role in increasing insulin sensitivity. Following on from this, it is also plausible that the reduction in abdominal fat (Gillen et al., 2013), enhanced oxidative capacity (Burgomaster et al., 2005; Gibala et al., 2006) and greater intramuscular lipid stores (Shepherd et al., 2013) following HIT result in better overall lipid handling in skeletal muscle, less accumulation of fatty-acid intermediates, and hence an augmentation of the insulin signalling cascade. Indeed, Gillen et al (2013) reported a significant relationship between the reduction in abdominal fat and the reduction in insulin area under the curve following 6-weeks of HIT in a cohort of 16 overweight...
women. However, a reduction in intramyocellular intermediate lipid species and an improved postprandial insulin signalling response remains to be shown following HIT. When considering skeletal muscle adaptations to HIT it must also be borne in the mind that the extreme exercise intensities recruit a larger muscle mass, and both type 1 and type 2 muscle fibres, which is then susceptible to adaptation.

The effect of a singlebout of HIT on insulin sensitivity has also been explored and the majority of studies in healthy lean or overweight populations report that there is no effect when measure 14-72 hours post-exercise (Brestoff et al., 2009; Richards et al., 2010; Whyte et al., 2013). However, Ortega et al. (2014) recently reported a substantial increase in insulin sensitivity (measured via intravenous glucose tolerance testing (IVGTT)) which lasted for at least 48 hours post-exercise. Likewise, Little et al (2014) recently reported a reduction in mean 24 h glucose concentrations and 24 h postprandial glucose AUC following a single bout of maximal HIT (10 × 1 min >90% HR\textsubscript{max}) in a small cohort of overweight men. The reasons for these discrepancies is unclear but may be related to the different method of assessing insulin sensitivity (IVGTT and CGM vs. OGTTs). Further studies examining the acute effect of HIT on insulin sensitivity in healthy populations are required, ideally using the gold standard clamp technique. Only one study has examined the acute effects of HIT in clinical populations. In 7 men with T2D, Gillen et al (2012) reported that a single bout of HIT (10 × 1 min >90% HR\textsubscript{max}) reduced postprandial glucose excursions and time spent in hyperglycaemia over a 24 h period measured using CGM.
# Table 1.2. Studies examining changes in insulin sensitivity following HIT

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Protocol</th>
<th>Duration (wks)</th>
<th>Si Method</th>
<th>∆Glucose</th>
<th>∆Insulin</th>
<th>∆Si</th>
</tr>
</thead>
<tbody>
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<td>Tjonna et al (2008)</td>
<td>♂♀ sed metsyn</td>
<td>4 x 4 min 90% HRmax running</td>
<td>16</td>
<td>Fasting</td>
<td>-0.3 mmol/L</td>
<td>n.s.</td>
<td>+24%*</td>
</tr>
<tr>
<td>Trapp et al (2008)</td>
<td>11♂ lean sed</td>
<td>8 s all-out/12 s recovery × 60 reps</td>
<td>15</td>
<td>Fasting</td>
<td>n.s.</td>
<td>-31% Fasting*</td>
<td>-</td>
</tr>
<tr>
<td>Babraj et al. (2009)</td>
<td>♂ lean untr</td>
<td>4-7 x 30 s all-out cycling</td>
<td>2</td>
<td>3 d post (OGTT)</td>
<td>-12% AUC</td>
<td>-37% AUC</td>
<td>+23%</td>
</tr>
<tr>
<td>Richards et al. (2010)</td>
<td>♂♀ lean untr</td>
<td>4-7 x 30 s all-out cycling</td>
<td>2</td>
<td>3 d post (Clamp)</td>
<td>-</td>
<td>-</td>
<td>+26%</td>
</tr>
<tr>
<td>Whyte et al. (2010)</td>
<td>10♂ over sed</td>
<td>4-7 x 30 s all-out cycling</td>
<td>2</td>
<td>3 d post (OGTT)</td>
<td>n.s.</td>
<td>-15% AUC</td>
<td>+23%</td>
</tr>
<tr>
<td>Nybo et al. (2010)</td>
<td>♂ untr</td>
<td>5 x 2 min &gt;95% HRmax running</td>
<td>12</td>
<td>Fasting</td>
<td>-0.5 mmol/L</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Little et al (2011a)</td>
<td>♂ sed T2D</td>
<td>10 x 1 min 90% HRmax cycling</td>
<td>2</td>
<td>CGM Mean 24 h glu</td>
<td>-1 mmol/L</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hood et al (2010)</td>
<td>4♂ 3♀ sed</td>
<td>10 x 1 min 80-95% HRmax cycling</td>
<td>2</td>
<td>Fasting</td>
<td>n.s.</td>
<td>-16% Fasting</td>
<td>+35%</td>
</tr>
<tr>
<td>Skerlyk et al (2013)</td>
<td>♂ ovre sed</td>
<td>8-12 x 10 s all out cycling</td>
<td>2</td>
<td>Fasting</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s</td>
</tr>
<tr>
<td>Gillen et al. (2013)</td>
<td>♂ ovre sed</td>
<td>10 x 1 min 90% HRmax cycling</td>
<td>6</td>
<td>3 d post (OGTT)</td>
<td>-6% AUC</td>
<td>n.s.</td>
<td>n.s</td>
</tr>
<tr>
<td>Cocks et al. (2014)</td>
<td>♂ lean untr</td>
<td>4-7 x 30 s all-out cycling</td>
<td>6</td>
<td>3 d post (OGTT)</td>
<td>-17% AUC</td>
<td>-34 AUC%</td>
<td>+48%</td>
</tr>
<tr>
<td>8♂ lean untr</td>
<td>60 min 65% VO2max cycling</td>
<td>6</td>
<td>3 d post (OGTT)</td>
<td>-12% AUC</td>
<td>-19 AUC%</td>
<td>+27%</td>
<td></td>
</tr>
</tbody>
</table>

♂ men, ♀ women, SED sedentary, METSYN metabolic syndrome patients, UNTR untrained, OVR overweight, N.S. not significant, AUC area under the plasma curve, HRmax heart rate max, OGTT oral glucose tolerance test, CGM continuous glucose monitor, GLU glucose, SI insulin sensitivity

**Effects on Blood Lipid Profile and Postprandial Lipaemia**

The impact of a period of HIT on the systemic lipid profile has not been explored to the same extent as the effect upon insulin sensitivity. Babraj et al. (2009) showed a trend for a reduction in fasting plasma NEFA following 2 weeks of supramaximal HIT in a cohort of young healthy men. However, Whyte et al (2010) could find no alterations.
in fasting NEFA, triglycerides, HDL cholesterol or total cholesterol following 2 weeks of supramaximal HIT in a group of 10 overweight males, despite an improvement in insulin sensitivity. The effect of a single bout of HIT upon postprandial lipid responses have been better characterised and, in general, appear to show a small but meaningful reduction in the blood lipid response to a high fat meal (Freese et al., 2011; Gabriel et al., 2012; Bellou et al., 2013; Gabriel et al., 2013), although this is not a universal finding (Tan et al., 2013). For example, Gabriel et al (2012) demonstrated a reduction in the triacylglycerol response to a high fat breakfast and lunch meal 18 hours following a bout of supramaximal HIT in young men. Likewise, Bellou et al (2013) reported an elevation in plasma triglyceride clearance between 14-20 hours following a single bout of supramaximal HIT in lean young men.

**Effects on Blood Pressure and Endothelial Function**

Several studies have examined changes in other indices of cardiovascular health including blood pressure and endothelial function. With respect to blood pressure, relatively few studies have examined changes following HIT interventions and, given the large variability and poor reproducibility of blood pressure measurements, are limited by small sample sizes (Rakobowchuk et al., 2008; Tjønna et al., 2008; Whyte et al., 2010; Tjønna et al., 2013). Nevertheless, Whyte et al (2010) demonstrated a small reduction in both systolic and diastolic blood pressure following 2 weeks of supramaximal HIT in a group of overweight men. Likewise, submaximal/maximal HIT interventions are associated with reductions in both systolic (~6-10 mm Hg) and diastolic (~6 mm Hg) pressure in both overweight men (Tjønna et al., 2013), and in patients with metabolic syndrome (Tjønna et al., 2008). Remarkably, Tjonna et al (2013) reported similar reductions following protocols using 1 × 4 min and 4 × 4 min intervals at ~90% maximum heart rate, highlighting the potency of a small volume of intense exercise upon cardiovascular health. However, it should be noted that Rakobowchuk et al (2008) found no reduction in a group of lean young men who had a similar baseline blood pressure following 6 weeks of supramaximal HIT. Perhaps the reductions in blood pressure following HIT are limited to individuals who are overweight and/or already presenting with elevations in blood pressure.
With regards to other vascular adaptations, Rakobowchuk et al. (2008) have shown that the distensibility and flow-mediated dilation of the popliteal artery is improved following supramaximal HIT, suggestive of improvements in endothelial structure and function. These improvements were observed following 6 weeks of training which involved 6-7 × 30 s ‘all-out’ sprints performed 3×/week in young healthy males, and were similar to improvements observed following 6 weeks of continuous moderate intensity training, despite a 90% lower training volume with HIT. More recently, Cocks et al. (2014) have shown, using a comparable HIT protocol and experimental design, that improvements in peripheral microvascular density and endothelial eNOS content are also observed. In support of this finding, several studies have observed improvements in peripheral oxygen uptake kinetics which indirectly suggests augmented microvascular perfusion (Bailey et al., 2009; McKay et al., 2009).

**Effects on Body Composition**

With respect to creating a meaningful energy deficit and hence promoting reductions in body fat content, it was previously thought to be optimal to focus on continuous steady state exercise for several hours per week. However, there is concern over the efficacy of such interventions under free-living conditions as there may be a compensatory stimulation of appetite and hence energy intake, negating any increase in total energy expenditure created with the exercise (Turner et al., 2010). At the other end of the spectrum, several studies over the last 5 years have reported positive changes in direct and indirect measures of body composition with different models of HIT (Whyte et al., 2010; Heydari et al., 2012; Gillen et al., 2013), and one study has demonstrated greater reductions in total and central abdominal fat mass when compared with prolonged moderate intensity continuous training (Trapp et al., 2008). Boutcher et al. (2011) has previously reviewed the potential mechanisms underlying these observed effects, and suggested this may be related to recently demonstrated prolonged elevations in post-exercise energy expenditure (Hazell et al., 2012; Skelly et al., 2014) or suppressions upon appetite and daily energy intake (Sim et al., 2013).

**1.7.4. Summary of HIT Research**

This literature review demonstrates that HIT is an effective exercise intervention for altering various physiological factors in a direction that would be expected to reduce
the risk of developing chronic cardiometabolic disease. Indeed, in many studies HIT has been shown to similarly effective to traditional high volume aerobic exercise interventions (Gibala et al., 2006; Rakobowchuk et al., 2008; Cocks et al., 2013; Shepherd et al., 2013). Moreover, the beneficial effects of HIT have been demonstrated in various populations including recreationally active and sedentary young to middle aged males and females, overweight and obese men and women, cohorts presenting with T2D and metabolic syndrome, and in patients recovering from cardiovascular events (Gibala et al., 2012). As such, the effectiveness of current models of HIT for improving metabolic and cardiovascular health is not in question.

However, HIT has been proposed to be a time efficient alternative to current exercise recommendations. Whilst this is technically correct given that the actual exercise time is reduced, the inclusion of recovery intervals means that the total time commitment of the main HIT protocols which have been used to date is not dramatically reduced (Figure 1.6). Moreover, many of the HIT protocols that have been utilised are associated with high levels of fatigue and can be associated with feelings of nausea. Thus they require a high level of participant motivation. Given that motivation to perform exercise is low in the majority of individuals, and ‘lack of time’ and ‘feelings of discomfort/fatigue’ are major barriers toward exercise participation, it is evident that the current design of HIT protocols is not appropriate for recommendation to the general population. Thus there is scope for research to examine whether or not it is possible to reduce the number of sprints and/or the duration of the sprints whilst still maintaining the positive effects. By reducing the sprint number and duration it should also be possible to reduce the total time commitment of the exercise bout and also the feelings of fatigue. In this context it is noteworthy that signalling pathways associated with training adaptations in oxidative capacity and carbohydrate/lipid metabolism are activated following a single 30 second ‘all-out’ sprint (Fuentes et al., 2012; Fuentes et al., 2013). Likewise, several recent HIT training studies have offered hints that shorter/easier protocols may still be effective at improving health markers so long as the intensity remains high enough (Hazell et al., 2010; Ma et al., 2013; Tjønna et al., 2013). However, this topic requires further exploration.
1.7.5. Overview of Experimental Work

The initial aim of this thesis was to design a HIT exercise intervention that was both time-efficient and not associated with high levels of fatigue and characterise its effects upon human health and metabolism. Chapter 2 outlines the theory which underpins the design of the HIT intervention, and then provides an initial characterisation of its effects upon insulin sensitivity and aerobic capacity. Having observed promising effects in Chapter 2, subsequent studies were undertaken to explore acute effects on insulin sensitivity (Chapter 3), and whole-body and skeletal muscle metabolism (Chapters 4 and 5). A further training study was then undertaken to explore the effects on key metabolic health parameters (Chapter 6).
Chapter 2

Towards the minimal amount of exercise for improving metabolic health: beneficial effects of reduced-exertion high-intensity interval training

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Chapter 2

Towards the minimal amount of exercise for improving metabolic health: beneficial effects of reduced-exertion high-intensity interval training (ReHIT)

2.1. Introduction

Several recent studies have suggested that high-intensity interval training (HIT), a training model involving a series of 30-s ‘all-out’ cycling sprints (i.e. Wingate sprints) with 4 min of rest/recovery between each bout, may provide a time-efficient strategy for inducing adaptations that are similar to traditional cardiorespiratory training (Burgomaster et al., 2005; Gibala et al., 2006; Burgomaster et al., 2007; Burgomaster et al., 2008; Rakobowchuk et al., 2008; Trilk et al., 2011; Cocks et al., 2013; Shepherd et al., 2013). Furthermore, Babraj et al. (2009) recently demonstrated the beneficial impact of HIT on insulin sensitivity, a finding that has since been confirmed by several other independent groups (Richards et al., 2010; Whyte et al., 2010; Little et al., 2011; Cocks et al., 2013). However, whilst these observations are interesting from a human physiological perspective, their translation into physical activity recommendations for the general population is uncertain for two reasons; firstly, the relatively high exertion associated with ‘classic’ HIT sessions requires strong motivation and may be perceived as too strenuous for many sedentary individuals (Hawley & Gibala, 2009); secondly, although a typical HIT session requires only 2-3 minutes of actual sprint exercise, when considered as a feasible exercise session including a warm-up, recovery intervals and cool-down, the total time commitment is more than 20 minutes, reducing the time-efficiency (Garber et al., 2011). Thus, there is scope for further research to determine whether the current HIT protocol can be modified to reduce levels of exertion and time-commitment while maintaining the associated health benefits.

Over the past decade it has become clear that skeletal muscle glycogen concentrations play a key role in regulating the skeletal muscle’s response to insulin, and it has been suggested that high levels of glycogen depletion observed during repeated 30-second Wingate sprints may play an important role in mediating the
improvements in insulin sensitivity following HIT (Babraj et al., 2009; Whyte et al., 2010). From an acute perspective, it has been demonstrated that glycogen levels modulate the effect of insulin on skeletal muscle GLUT4 content and glucose transport (Derave et al., 2000). Moreover, muscle glycogen availability influences the activity of glycogen synthase and the potency of proximal insulin signalling (Jensen et al., 2006). Consequently, several studies have demonstrated an inverse relationship between skeletal muscle glycogen content and skeletal muscle insulin sensitivity (Derave et al., 2000; Kawanaka et al., 2000; Laurent et al., 2000; Jensen et al., 2006; Litherland et al., 2007). The logical assumption here is that the glycogen depleted muscle cell up-regulates the glucose transport system to efficiently replenish glycogen stores when dietary carbohydrate becomes available, and this response appears to largely explain the improvements in insulin sensitivity that can be detected during the recovery from a single bout of endurance exercise (Bogardus et al., 1983; Cartee et al., 1989; Gulve et al., 1990). Whether or not repeatedly degrading and replenishing skeletal muscle glycogen (i.e., increasing glycogen turnover) partly underpins the more prolonged adaptive increase in insulin sensitivity that occurs with exercise training is less clear, but given that glycogen synthesis is a major fate for the glucose taken up into the muscle cell, and the major role of muscle glycogen is to support high intensity muscle contractions, it is entirely conceivable that glycogen would exert a major regulatory role in the adaptive response for muscle carbohydrate metabolism with exercise. The discovery that the β1-subunit of the AMP-activated protein kinase (AMPK) complex contains a glycogen-binding domain (Hudson et al., 2003; Polekhina et al., 2003) provides a plausible mechanism for this hypothesis: muscle glycogen depletion through exercise leads to release of glycogen-bound AMPK, the increased AMP/ATP ratio with exercise elevates AMPKα2 activity, initiates the translocation of AMPKα2 to the nucleus, and increases GLUT4 mRNA (Holmes et al., 1999; Jørgensen et al., 2006; Steinberg et al., 2006). Alternatively, it may be that release of other proteins associated with glycogen may initiate signalling mechanisms influencing insulin sensitivity (Graham et al., 2010). Regardless of the potential mechanisms however, if muscle glycogen regulates insulin sensitivity then exercise protocols aiming to reduce glycogen levels should be effective.

It has consistently been shown that a single 30-second Wingate sprint can reduce muscle glycogen stores in the vastus lateralis by 20-30% (Esbjornsson-Liljedahl et al.,
1999; Parolin et al., 1999; Esbjornsson-Liljedahl et al., 2002; Gibala et al., 2009). What is intriguing however, is that glycogenolysis is only activated during the first 15 seconds of the sprint and is then strongly attenuated during the final 15 seconds (Parolin et al., 1999). Moreover, activation of glycogenolysis is inhibited in subsequent repeated sprints (Parolin et al., 1999). This suggests that the traditional HIT protocol (4-6×30 seconds) may be unnecessarily strenuous as similar glycogen depletion may be achieved using 1-2 sprints of shorter duration (15-20 seconds). In turn, this would make the training sessions more time-efficient, less strenuous and more applicable to the largely sedentary general population. Therefore, in the current study we investigated the impact of a reduced-exertion HIT (ReHIT) intervention on insulin sensitivity in previously sedentary humans. We hypothesised that despite reducing sprint time and number, ReHIT would still be effective at improving glucose tolerance.
2.2. Methods

2.2.1. Subjects

Twenty-nine sedentary but healthy young men (n=13) and women (n=16) were recruited to take part in the study and allocated to a training group or a control group. Subjects allocated to the training group completed the full experimental protocol whilst subjects in the control group completed the pre- and post-training assessments without an exercise intervention. Baseline characteristics for each subject group are shown in Table 1. No significant differences existed between the ReHIT and control groups at baseline (Tables 3.1-3.3). All participants were classified as sedentary according to the criteria of the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003) and fit to take part in strenuous exercise according to the conditions of the physical activity readiness questionnaire (PAR-Q) (Thomas et al., 1992). Further exclusion criteria included clinically significant hypertension (>140/90 mm Hg) and a personal history of metabolic or cardiovascular disease. All subjects were fully informed of the experimental protocol and any associated risks, both verbally and in writing, before providing written informed consent to participate. In addition, the potentially confounding effect of changing dietary or physical activity patterns was fully explained to all participants and they were asked to maintain their normal lifestyle patterns throughout the study period. The experimental protocol was approved by the Heriot-Watt University School of Life Sciences Ethics Committee and was conducted in accordance with the Declaration of Helsinki.

2.2.2. Experimental Design

All participants underwent pre- and post-intervention testing for insulin sensitivity and aerobic capacity. Insulin sensitivity was assessed using an oral glucose tolerance test (OGTT) and aerobic capacity was assessed using a conventional VO$_2$max cycling test. The baseline OGTTs were performed two weeks before training commenced and post training OGTTs were conducted 3 days after the final training bout, at the same time of day as the pre-intervention OGTTs. This meant that there were exactly 8 weeks between the pre- and post-training OGTTs which ensured that female
subjects were in the same stage of their menstrual cycle. The $\dot{V}O_2\text{max}$ tests took place 1-2 days after the OGTTs.

### Table 2.1. Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Training Male (n=7)</th>
<th>Training Female (n=8)</th>
<th>Control Male (n=6)</th>
<th>Control Female (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>26±7</td>
<td>24±7</td>
<td>19±1</td>
<td>21±3</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.74±0.05</td>
<td>1.62±0.05</td>
<td>1.78±0.07</td>
<td>1.65±0.08</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.7±15.9</td>
<td>59.6±7.5</td>
<td>80.1±16.1</td>
<td>60.8±15.1</td>
</tr>
<tr>
<td>BMI (m/kg$^2$)</td>
<td>24.4±4.9</td>
<td>22.7±3.1</td>
<td>25.2±3.9</td>
<td>22.2±4</td>
</tr>
<tr>
<td>$\dot{V}O_2\text{max}$ (ml/kg/min)</td>
<td>36.3±5.8</td>
<td>32.5±4.4</td>
<td>38.0±6.7</td>
<td>32.9±6.0</td>
</tr>
</tbody>
</table>

Data is shown as mean ± SD

#### 2.2.3. Oral Glucose Tolerance Test (OGTT)

Prior to OGTTs subjects performed no moderate or vigorous intensity physical activities for three days, and refrained from drinking alcohol for one day. Furthermore, subjects completed a 3-day food diary before each OGTT which was analysed for total energy and macronutrient content using commercially available dietary analysis software (Dietplan6, Forestfield Software, UK). There were no significant differences in total energy, carbohydrate, fat or protein content over the 3 days before the pre- and post-intervention OGTTs in any of the subject groups (table 2.2).

On the day of the OGTT subjects reported to the laboratory between 7:30 and 9:30 am following an overnight fast from 10 pm the previous evening. A fasting blood sample was obtained from a forearm antecubital vein by venepuncture using the vacutainer system. 75 g of anhydrous glucose (Fisher Scientific, Loughborough, UK) dissolved in 100 ml of water was then orally administered and further blood samples were taken at 60 and 120 min after glucose ingestion. Blood samples were collected into pre-cooled plastic tubes containing EDTA, immediately placed on ice, and then centrifuged for 10 min at 1600 g to separate the plasma, which was stored at -20°C prior to the determination of plasma glucose and insulin concentrations. Plasma glucose concentration was determined by the glucose oxidase reaction using an automated...
analyser (YSI Stat 2300, Yellow Spring Instruments, Yellow Spring, OH). Plasma insulin concentration was measured using a commercially available ELISA according to the manufacturer’s instructions (Invitrogen, UK). All glucose and insulin assays were carried out in duplicate; the coefficient of variation (CV) for repeated measures of insulin and glucose was 2.6% and <1%, respectively. Area under the curve (AUC) for plasma glucose and insulin was calculated using the trapezoid model. Peripheral insulin sensitivity was determined using the Cederholm index (Cederholm & Wibell, 1990) which is calculated using the formula:

\[
75000 + \frac{(G_0 - G_{120}) \times 1.15 \times 180 \times 0.19 \times BW}{120 \times G_{\text{mean}} \times \log(I_{\text{mean}})}
\]

Where BW is body weight, \(G_0\) and \(G_{120}\) are plasma glucose concentration at 0 and 120 min (mmol·l\(^{-1}\)), and \(I_{\text{mean}}\) and \(G_{\text{mean}}\) are the mean insulin (mU·l\(^{-1}\)) and glucose (mmol·l\(^{-1}\)) concentrations during the OGTT. The Cederholm Index has previously been shown to correlate well with the gold standard insulin clamp method (Piche et al., 2007).

Table 2.2. Nutritional intake over the 3 days prior to the OGTTs

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>REHIT (n=7)</td>
<td>Control (n=6)</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Carbohydrate (g/day)</td>
<td>217±38</td>
<td>240±90</td>
</tr>
<tr>
<td>Fat (g/day)</td>
<td>60±20</td>
<td>64±30</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>82±22</td>
<td>88±33</td>
</tr>
<tr>
<td>Alcohol (g/day)</td>
<td>5±8</td>
<td>0±0</td>
</tr>
<tr>
<td>Energy (Kcal/day)</td>
<td>1818±244</td>
<td>1948±649</td>
</tr>
</tbody>
</table>

Data is shown as mean ± SD calculated from nutritional scores averaged over the 3 days

### 2.2.4. \(\dot{V}O_2\text{max Test}\)

Maximal oxygen uptake capacity (\(\dot{V}O_2\text{max}\)) was determined using a graded cycling test to volitional exhaustion on a mechanically braked cycle ergometer
Subjects cycled at 60 W for one minute after which the resistance was increased by 30 W·min\(^{-1}\) until the pedal cadence could no longer be maintained at 60 rpm. Participants respired through a rubber mouthpiece which was connected to an online gas analysis system (Sensor-Medics, Bilthoven, the Netherlands). Respiratory volume, flow and levels of expired O\(_2\) and CO\(_2\) were measured and used to determine \(\dot{V}O_2\) via indirect calorimetry (Frayn, 1983). The breath-by-breath data was inspected visually using scatterplots in Microsoft Excel and any outlying (i.e., unphysiological) data points were removed. 15-breath rolling averages for \(\dot{V}O_2\) were subsequently calculated and \(\dot{V}O_2\)max was taken as the highest 15-breath value achieved during the test. In all tests two or more of the following criteria were met: a plateau in \(\dot{V}O_2\) despite increasing intensity, RER > 1.15, heart rate within 10 beats of age-predicted maximum, and/or volitional exhaustion.

### 2.2.5. Training Protocol

Participants allocated to the training group completed three exercise sessions per week for 6 weeks, completing 18 sessions overall. All exercise sessions lasted 10 min in total, including a warm up and cool down, which meant a total training time of 30 min per week. Each training session consisted of low intensity cycling (60 Watts) and one (1\(^{\text{st}}\) session) or two (all other sessions) all-out cycling sprints. Just before each sprint, resistance was removed, subjects increased the pedal cadence to their maximal speed, a braking force equivalent to 7.5% of body weight was then applied to the ergometer, and participants sprinted against the braking force for a designated time period. The duration of the sprints increased from 10 seconds in week 1, to 15 seconds in weeks 2 and 3, and 20 seconds in the final 3 weeks. A full schematic of the training protocol is shown in Figure 3.1. Training sessions were fully supervised and verbal encouragement was given during each sprint. A rating of perceived exertion (RPE) was collected using the 15-point Borg scale (Borg, 1970) at the end of the first session, and subsequently at the end of each training week, immediately following the completion of the 10-minute training session. When giving their RPE scores, participants were asked to consider the whole 10-minute training session.
2.2.6. **Statistical Analysis**

All data are presented as mean ± SD unless otherwise stated. Data were analysed using the commercially available SPSS statistics package (PASW Statistics, version 17.0). Three-way mixed-model ANOVAs (sex [male, female] × group [ReHIT, control] × time [pre, post]) were performed to test the effects of the ReHIT intervention on body mass, VO$_2$max, glucose AUC, insulin AUC and insulin sensitivity. For variables with significant sex × time interactions, males and females were analysed separately using 2-way mixed model ANOVAs (group × time). ANOVA were performed regardless of any minor deviations from a normal distribution (Maxwell & Delaney, 2004), and with Greenhouse-Geisser corrections applied for contrasts where $\epsilon<0.75$ and the Huynh-Feldt corrections applied for less severe asphericity (Atkinson, 2002). Comparisons in RPE data between men and women were made using an independent sample t-test. Significance was accepted at P<0.05.

**Figure 2.1.** Schematic of the ReHIT training protocol. Grey boxes represent ‘all-out’ sprints against a fixed resistance of 7.5% of body mass
2.3. Results

2.3.1. Exercise adherence and characteristics

Ten out of fifteen training group participants completed all 18 training sessions (100% adherence), with a mean adherence to the ReHIT training programme of 97% for all subjects combined.

RPE data over the course of the ReHIT training program is shown in Figure 3.2. On the whole, despite the incorporation of brief but intense sprints, the training sessions were well tolerated by all of the participants. Mean RPE values peaked at the end of session 12 (week 4; 2×20 s), corresponding to ‘somewhat hard’ and between ‘somewhat hard’ and ‘hard’ in men and women respectively. None of the participants gave an RPE score higher than 15. When mean RPE values over the whole training program were calculated, female participants found the training program significantly harder than male participants (13.6±0.5 vs. 12.5±0.7, P<0.05).

The heart rate response (averaged across participants) during training sessions 4, 10 and 18 are shown in figure 2.3. The ReHIT sprints were associated with rapid excursions in heart rate, reaching in excess of 90% of maximum heart rate during each sprint effort.

![Figure 2.2. Ratings of perceived exertion during the ReHIT training sessions. Data is shown as mean ± SD.](image-url)
Figure 2.3. Heart rate responses during the ReHIT training sessions. Data shown represents the mean response for all participants during that training session. Black boxes denote timings of the all-out sprints. Dotted line represents the mean maximum heart rate.

2.3.2. $\dot{V}O_2$max and Body Mass

There were no changes in weight or BMI following the ReHIT training programme in any of the groups (Table 2.3). For $\dot{V}O_2$max there were significant main effects of sex ($P<0.001$) and time ($P<0.01$), and a significant interaction effect for group $\times$ time ($P<0.01$): following ReHIT $\dot{V}O_2$max was increased by on average 15% in men and by 12% in women with no significant sex difference in this effect (Table 2.2). These results were the same when $\dot{V}O_2$max was expressed in l·min$^{-1}$ or ml·kg$^{-1}$·min$^{-1}$. 
Chapter 2: Training Effects of ReHIT

Table 2.3. Effects of ReHIT on BMI, VO$_2$max, and glucose and insulin AUC

<table>
<thead>
<tr>
<th></th>
<th>Male REHIT (n=7)</th>
<th>Male Control (n=6)</th>
<th>Female REHIT (n=8)</th>
<th>Female Control (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>BMI (kg/m$^2$)</strong></td>
<td>24.4±4.9</td>
<td>24.0±4.6</td>
<td>25.2±3.9</td>
<td>25.3±4.0</td>
</tr>
<tr>
<td><strong>VO$_2$max (ml/kg/min)</strong></td>
<td>36.3±5.8</td>
<td>41.6±3.9*</td>
<td>38.0±6.7</td>
<td>38.0±5.6</td>
</tr>
<tr>
<td><strong>Glucose AUC (mmol/min/l)</strong></td>
<td>789±171</td>
<td>695±141</td>
<td>762±101</td>
<td>801±134</td>
</tr>
<tr>
<td><strong>Insulin AUC (mU/min/l)</strong></td>
<td>11713±4840</td>
<td>7147±3048</td>
<td>8728±4688</td>
<td>9944±1018</td>
</tr>
</tbody>
</table>

Note: Male training group VO$_2$peak data is shown for n=5 and female training VO$_2$peak is shown for n=6 due to technical problems during the pre-training tests.

Data shown are mean ± SD

*: P<0.01 for the group × time interaction effect

^: P<0.05 for the main effect of time in women

2.3.3. Glucose and Insulin Responses to the OGTT

Effects of the ReHIT intervention on glucose and insulin responses to an oral glucose load are shown in Figure 2.2, with glucose and insulin AUCs shown in Table 2.2. As we observed a significant interaction effect for sex × time for glucose AUC (P<0.05), results for male and female subjects were analysed separately using a two-way mixed-model ANOVA (group × time). No significant effects were observed for men. A significant main effect for time for women (P<0.05) indicated that post-intervention values for glucose AUC were increased, but there was no significant difference between the female ReHIT and control groups. No significant changes in insulin AUC were observed (Table 2.2).

A significant interaction effect for sex × time was also observed for insulin sensitivity (P<0.01), so male and female subjects were analysed separately. Following ReHIT, insulin sensitivity significantly improved by 28% in male subjects (P<0.05), but not in female subjects (Figure 2.3).
Figure 2.4. The impact of ReHIT on plasma glucose and insulin responses to OGTTs. Plasma Glucose (A and B), Plasma Insulin (C and D), Men (A and C), Women (B and D); Solid lines ReHIT group, Dotted lines control group; Circles pre, Triangles post. For clarity, the data is shown as mean ± SEM.

Figure 2.4. The impact of ReHIT on insulin sensitivity in men (A) and women (B). Data is presented at mean ± SEM for clarity. * denotes P<0.05 for group x time interaction effect.
2.4. Discussion

The present data demonstrate that a 6-week novel exercise intervention, consisting of very brief sessions with relatively low ratings of perceived exertion, is associated with improved insulin sensitivity in sedentary young men, and improved aerobic capacity in men and women. These beneficial effects were observed independently of significant changes in body mass and since post-training measurements were taken 3 days after the final exercise bout, are likely to represent chronic training adaptations. Importantly, the improvements were observed despite the low time commitment (totalling 30 minutes per week) and low required effort: RPE peaked at an average of 14 (‘somewhat hard’) in week 4 which is comparable with RPE scores reported with prolonged cycling at 50-75% VO$_{2\text{max}}$ (Borg, 1982). These data extend the previous literature demonstrating the beneficial effects of HIT (Burgomaster et al., 2007; Rakobowchuk et al., 2008; Babraj et al., 2009; Richards et al., 2010; Trilk et al., 2011; Whyte et al., 2010; Cocks et al., 2013; Shepherd et al., 2013), by showing that the sprint number and duration can be substantially reduced whilst still maintaining the associated positive effects.

Based on our current knowledge, when considering the health effects of exercise solely from a physiological perspective, it is fair to state that more (within reason) is better (Garber et al., 2011). In other words, in order to optimise the metabolic, cardiovascular and psychological benefits that exercise can offer, people should be encouraged to perform a large volume (at least 30 minutes per day) of both moderate and vigorous intensity cardiorespiratory exercise on most days of the week, as well as sessions focused on strength and flexibility 2-3 times per week (Garber et al., 2011). The associated health benefits with exercise are expected to increase with larger volumes of exercise and at higher intensities (Houmard et al., 2004; Swain, 2005; DiPietro et al., 2006; Church et al., 2007; Bajpeyi et al., 2009; Sisson et al., 2009). However, although such guidelines may be effective in those people who adhere to them, they remain largely ineffective at a population level (Allender et al., 2008a), partly because they fail to sufficiently consider key barriers which prevent people from performing regular exercise, such as ‘lack of time’ (Reichert et al., 2007; Korkiakangas et al., 2009). For exercise prescriptions to provide a positive effect for society there must be a balance between providing adequate health benefits and helping to generate motivation to
perform exercise by overcoming key barriers. One possible alternative strategy could be to define the minimum volume of exercise required to improve health indices with the aim of increasing exercise adherence. At the time of analysis, the training programme utilised in the current study represented the smallest volume of exercise (when considered per session) that has been shown to induce positive effects on health.

Insulin sensitivity was improved by 28% in men following ReHIT, a finding which is strengthened by the inclusion of a ‘lifestyle maintenance’ control group in which we measured no significant change over the same time period. The magnitude of improvement in insulin sensitivity with ReHIT is comparable with responses observed following 2-6 weeks of classic HIT in young sedentary or recreationally active men and women (Babraj et al., 2009; Richards et al., 2010; Cocks et al., 2013; Shepherd et al., 2013), as well as in obese men (Whyte et al., 2010). We designed our intervention based on the hypothesis that glycogen degradation would be similar to that observed with the classic HIT protocol, since glycogenolysis is attenuated during latter sprints (Parolin et al., 1999). Our observation that insulin sensitivity still improved to a similar extent supports the idea that repeated glycogen turnover might be a key determinant of improved insulin sensitivity following HIT, at least in young lean sedentary male subjects. However, as we did not determine glycogen degradation during the ReHIT training sessions this remains speculative and further studies are required to elucidate the mechanisms by which ReHIT improves insulin sensitivity.

The improvements in insulin sensitivity after ReHIT appear to be sex-specific as mean insulin sensitivity was not improved in the female subjects after the training programme. This is in contrast to a previous study which did not observe any sex differences in the improvements in insulin sensitivity in 12 recreationally active subjects after 2 weeks of classic HIT (Richards et al., 2010), but in agreement with more recent studies which could detect no change in insulin sensitivity in sedentary overweight women following a 6 week modified HIT protocol (Gillen et al., 2013; Gillen et al., 2014). No other study has investigated the effects of HIT on insulin sensitivity in women. Following a traditional aerobic exercise intervention in a large cohort, insulin sensitivity improved to a greater degree in men when compared with women but (similar to our study) the female participants had a higher baseline level of insulin sensitivity which may have impacted on the subsequent training response (Boulé et al., 2005). The
sex differences in the change in insulin sensitivity in response to our ReHIT intervention may in part be caused by the low statistical power of our study, with only 8 female subjects performing the ReHIT intervention. However, it can be speculated that differences in metabolic perturbations during the brief high-intensity cycle sprints may contribute to the observed sex difference, as women have been shown to break down up to 50% less glycogen during a single Wingate sprint (Esbjornsson-Liljedahl et al., 1999; Esbjornsson-Liljedahl et al., 2002). New data from Gillen et al who showed a lesser increase in skeletal muscle GLUT4 protein content in women compared with men following 6 weeks of HIT would fit with this hypothesis (Gillen et al., 2014). From this perspective, it would be interesting to determine whether the extent of muscle glycogen breakdown during a ReHIT training bout correlates with changes in GLUT4 protein content, insulin-stimulated canonical signalling protein content and activation, glycogen synthase activity, and insulin sensitivity following the training programme. Alternatively, it could be that our small sample included several non-responders. Previous studies have comprehensively demonstrated that following a period of exercise training part of the population will not adapt for specific parameters (non-responders), and for insulin sensitivity this has been shown to be the case for up to 40% of the population (Bouchard & Rankinen, 2001; Boulé et al., 2005; Vollaard et al., 2009). Therefore, further studies with larger sample sizes will be needed to confirm or refute our initial observations. Furthermore, the post-intervention OGTT was scheduled three days following the final exercise session, and we cannot rule out that insulin sensitivity was improved in female subjects at an earlier time-point. Finally, although we did not measure power output during the sprints, we observed that some of the female volunteers struggled with the transition from 60 W to the all-out sprints, and were unable to substantially increase their pedal frequency, and thus their power output during the sprints. This may have increased the aerobic contribution to energy supply and reduced glycogen depletion. For sedentary women substituting the 60 W cycling with unloaded pedalling may make the sprints more effective.

Maximal oxygen uptake capacity increased by 15% and 12% in men and women respectively after the ReHIT intervention, a physiologically important observation since a high aerobic capacity is associated with a lower risk of cardiometabolic disease (Wei et al., 1999; Church et al., 2005). Interestingly, since women improved their aerobic capacity but not their insulin sensitivity, it appears that there is dissociation between
changes in aerobic capacity and changes in insulin sensitivity. Our results are in agreement with previous HIT studies that have observed increases in VO$_2$ max ranging from 5 to 13% irrespective of sex or initial body mass index (Hazell et al., 2010; Astorino et al., 2011; Trilk et al., 2011), although it must be noted that improved aerobic capacity is not a universal finding with HIT (Burgomaster et al., 2005). Studies utilising reduced volume HIT protocols are inconsistent. Hazel et al utilised 4-6 × 10 s ‘all-out’ sprints over 2 weeks and demonstrated a ~10% increase in VO$_2$ max in recreationally active subjects (Hazell et al., 2010), whilst Gillen et al utilised 3 × 20 s all out sprints over 6 weeks and showed a similar increase in overweight men and women (Gillen et al., 2014). On the other hand, Skleryk et al utilised 8-12 × 10 s ‘all-out’ sprints over 2 weeks and could detect no change in aerobic capacity in 10 overweight/obese men (Skleryk et al., 2013). Each of these studies used relatively small sample sizes and as a result the impact of ReHIT on VO$_2$ max requires confirmation with a larger cohort.

Average RPE values reported on immediate completion of the ReHIT training sessions were comparable with RPE values obtained during prolonged moderate intensity cardiorespiratory exercise at 50-75% V̇O$_2$ max (Borg, 1982). However, there are limitations to the use of the RPE scale in the current study, as the RPE scale is designed for use during (or immediately following) continuous exercise at a constant intensity and may not be a valid measure of exertion during interval based exercises, especially when values are given retrospective of the most intense exercise, as was the case in this study. RPE values obtained in this manner may underestimate exertion during the sprints; indeed, other studies where RPE has been obtained following a 20-second all-out sprint have reported higher values of ~16-18 (Baker et al., 2001; Gearhart et al., 2005). However, we were interested in gaining an exertion measure to characterise our entire training intervention and our subjects were asked to consider the whole 10 min exercise session when giving their ratings. Whether the effort required to perform ReHIT sessions would deter individuals from performing this type of intervention is a question to be answered in future studies.

In conclusion, in this study we have shown that a very brief and feasible exercise intervention is associated with improvements in metabolic health and aerobic capacity. Our findings suggest that this ReHIT protocol may offer a genuinely time-efficient
alternative to HIT and conventional cardiorespiratory exercise training for improving risk factors of T2D.
Chapter 3

The impact of an acute bout of ReHIT and continuous vigorous intensity cardiorespiratory exercise on insulin sensitivity
Chapter 3

The impact of an acute bout of ReHIT and continuous vigorous intensity cardiorespiratory exercise on insulin sensitivity

3.1. Introduction

In the first study of this thesis (Chapter 2), we demonstrated that a modified HIT protocol, consisting of 10 min of low intensity cycling interspersed with two 20-s ‘all-out’ sprints (ReHIT), was effective in improving insulin sensitivity in sedentary men, and aerobic capacity in sedentary men and women. Importantly, these benefits were observed despite the very low time commitment and low ratings of perceived exertion. This makes ReHIT a more promising intervention for improving health in sedentary populations compared to other HIT protocols, and this protocol is worthy of further study.

The majority of HIT intervention studies demonstrating improvements in insulin sensitivity have performed their measurements between 48-72 hours after the final training bout and hence the changes are likely attributable to chronic training adaptations (Babraj et al., 2009; Richards et al., 2010; Cocks et al., 2013). This is supported by recent acute studies demonstrating no change in insulin sensitivity 14-16 hours (Brestoff et al., 2009; Whyte et al., 2013) and 72 hours (Richards et al., 2010) following a single bout of HIT. Our assessment of insulin sensitivity following the 6 week ReHIT intervention was also performed 3 days following the cessation of training (Chapter 2) and it seems unlikely that there would be any residual impact of the final exercise bout. However, this contention needs to be confirmed experimentally. Therefore, the aim of this study was to determine the impact of a single bout of ReHIT on insulin sensitivity measured the following day in comparison to a no-exercise control condition. We hypothesised that there would be no increase in insulin sensitivity with ReHIT. As a hypothesised positive control, we also included a condition involving an acute prolonged bout of moderate-vigorous intensity cardiorespiratory exercise, which has previously been shown to improve insulin sensitivity at a similar time point (Brestoff et al., 2009).
3.2. Methods

3.2.1. Participants

Fourteen healthy young men (n=8) and women (n=6) gave their written informed consent to take part in this study which received ethical approval from the Heriot-Watt University School of Life Sciences Ethics Committee (Table 3.1). All participants were classified as sedentary according to the conditions of the International Physical Activity Questionnaire (Craig et al., 2003) and were eligible to take part on the basis that they were fit to perform strenuous exercise according to the conditions of the Physical Activity Readiness Questionnaire (Thomas et al., 1992), free from metabolic disease, cardiovascular disease, acute viral infection and clinically relevant hypertension (i.e., >140/90 mmHg). All participants were weight stable in the preceding 3 months and were asked to maintain their normal diet and patterns of physical activity throughout the study period.

3.2.2. Baseline Testing and Familiarisation

Prior to the main experimental trials participants visited the laboratory on four separate occasions. During the initial visit peak oxygen uptake capacity (\(\dot{V}O_2\)max) was determined during a continuous incremental cycling test to their limit of tolerance on an electronically braked cycle ergometer (Lode, Excalibur Sport, the Netherlands). Resting oxygen consumption was first determined over a 5-min period and then a 25 W min\(^{-1}\) continuous ramp (starting from 0 W) was initiated with participants cycling at a self-selected cadence until the cadence could no longer be maintained above 50 rpm. Breath-by-breath analysis of respiratory volume, flow, and \(O_2\) and \(CO_2\) concentrations (SensorMedics, Bilthoven, the Netherlands) allowed determination of pulmonary gas exchange via indirect calorimetry (Frayn, 1983). A 15-breath rolling average for \(\dot{V}O_2\) was calculated and \(\dot{V}O_2\)max was taken as the highest 15-breath value achieved during the test. During each test, at least two of the following criteria were met: a plateau in \(\dot{V}O_2\) despite increasing intensity, RER > 1.15, heart rate within 10 beats of age-predicted maximum, and/or volitional exhaustion.

Following the incremental cycling test the participants performed three familiarisation sessions (two for ReHIT and one for AER). The AER familiarisation
session was mainly used to check the intensity predicted to elicit 75% \( \dot{V}O_2 \text{max} \) (calculated using linear regression from the \( \dot{V}O_2 \) responses during the incremental cycling test). Participants cycled for 15 min at the prescribed intensity and \( \dot{V}O_2 \) was measured continuously throughout (SensorMedics, Bilthoven, the Netherlands). If necessary, adjustments were made to the intensity used during the main trials. The ReHIT sessions were used to familiarise participants with the procedures and the effort required during Wingate-type sprints. Ten-second sprints were used in the first familiarisation and twenty-second sprints in the second.

### Table 3.1. Participant Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males (n=8)</th>
<th>Females (n=6)</th>
<th>Combined (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>22 ± 5</td>
<td>24 ± 5</td>
<td>23 ± 5</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.78 ± 0.08</td>
<td>1.62 ± 0.04</td>
<td>1.71 ± 0.11</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.1 ± 12</td>
<td>60.0 ± 16.8</td>
<td>67.3 ± 14.9</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>22.6 ± 4.2</td>
<td>22.9 ± 5.6</td>
<td>22.7 ± 4.7</td>
</tr>
<tr>
<td>( \dot{V}O_2 \text{max} ) (L/min)</td>
<td>2.95 ± 0.68</td>
<td>1.90 ± 0.34</td>
<td>2.50 ± 0.76</td>
</tr>
<tr>
<td>( \dot{V}O_2 \text{max} ) (ml/kg/min)</td>
<td>41.4 ± 9.1</td>
<td>32.0 ± 3.4</td>
<td>37.4 ± 8.6</td>
</tr>
<tr>
<td>Maximal Workload (W)</td>
<td>254 ± 45</td>
<td>176 ± 14</td>
<td>220 ± 53</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD

#### 3.2.3. Main Experimental Trials

Participants completed three main experimental trials (ReHIT, AER and REST) in a randomised cross-over design, with each trial taking place over a 2-day period. During each trial participants underwent an oral glucose tolerance test (OGTT) on the morning after performing either: (1) a single bout of vigorous intensity aerobic exercise (AER); (2) a single bout of reduced-exertion high intensity interval training (ReHIT); or (3) a no-exercise control condition (REST). Each trial was separated by at least 1 week and prior to each trial participants were asked to avoid any strenuous or tiring physical activities for 48 hours and avoid consuming any alcohol or caffeine for 24 hours.

On the evening prior to each OGTT, participants attended the laboratory between 4:30 pm and 7:00 pm to perform the exercise session. 30 min after completion of the exercise bout, participants were given a standardised evening meal consisting of a
microwaveable ready meal, a yogurt, a cereal bar and orange juice (Table 3.1). For each participant, the time of attendance was standardised and staggered so that the time of completion of the exercise bouts and consumption of the post-exercise evening meal was consistent between each experimental condition. Having fasted overnight (since the post-exercise meal), participants returned to the laboratory the following morning between 7:00 am and 9:30 am, rested quietly for 15 minutes, and then the OGTT was performed.

### Table 3.2. Characteristics of the standardised evening meals

<table>
<thead>
<tr>
<th>Dietary Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (Kcal)</td>
<td>773 ± 118</td>
</tr>
<tr>
<td>Total Carbohydrate (g)</td>
<td>107 ± 17</td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>48 ± 8</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>21 ± 7</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>35 ± 10</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>8 ± 3</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD

### 3.2.4. Exercise Protocols

All exercise protocols were performed on an electronically braked cycle ergometer (Lode, Excalibur Sport, the Netherlands). The aerobic exercise protocol was identical to that used by Brestoff et al. (2009) and involved 45 min at an intensity predicted to elicit ~75% of VO\(_2\)peak. Cadence was self-selected and the exercise was completed in intervals of 15 min with 2 min of resting recovery between each interval. VO\(_2\) was collected during the final 5 min of the first bout (SensorMedics, Bilthoven, the Netherlands), heart rate was measured throughout (Polar Electro, Vansbro, Sweden), and an RPE was collected during the final minute of each 15-min interval using the 15-point scale (Borg, 1970). The ReHIT condition involved 10 min of low-intensity cycling (unloaded pedalling) and two ‘all-out’ sprints. Just before each sprint, participants increased their pedal cadence to their maximal speed, a braking torque was applied to the ergometer, and participants sprinted maximally against the braking torque for 20 s. The braking torque (Nm) was calculated by multiplying the participants body mass (Kg)
with a torque factor of 0.70 Nm·Kg\(^{-1}\) for males and 0.60 Nm·Kg\(^{-1}\) for females. Heart rate and power output were measured throughout each sprint. RPE scores were collected at the end of each sprint and on completion of the 10 min protocol; for RPE scores obtained at the end of each sprint participants were asked to consider their exertion at that exact moment in time, whilst for RPE scores collected at the end of the full protocol, participants were asked to consider the whole exercise session.

Figure 3.1. Overview of the experimental design. Aerobic exercise (AER) and reduced-exertion high intensity interval training (REHIT) protocols. VO\(_{\text{peak}}\), peak oxygen uptake capacity; OGTT, Oral glucose tolerance test.
3.2.5. Oral Glucose Tolerance Test and Plasma Analysis

The OGTTs were started on average 14.5 hours (range: 13.5-16.0 hours) following completion of the exercise bouts and the timings were consistent between conditions. A baseline blood sample was obtained from a forearm antecubital vein by venepuncture using the vacutainer system. 75 g of anhydrous glucose (Fisher Scientific, Loughborough, UK) dissolved in 100 ml of water was then orally ingested and further blood samples were collected at 60 and 120 minutes after the glucose ingestion. Blood samples were collected into pre-cooled plastic tubes containing EDTA and immediately placed on crushed ice. At the end of the OGTT, samples were centrifuged for 10 minutes at 2000 g and 4°C to separate the plasma, which was stored at -20°C prior to the determination of glucose and insulin concentrations. Plasma glucose concentration was determined in duplicate using the glucose oxidase method on an automated analyser with a CV of <1% (YSI Stat 2300, Yellow Spring Instruments, Yellow Spring, OH). Plasma insulin concentrations were measured in duplicate using a commercially available ELISA according to the manufacturer’s instructions (Invitrogen, UK). The CV for repeated measures of insulin was 4%.

3.2.6. Calculations and Statistics

All statistical analysis was performed using the commercially available Statistics Package for Social Sciences (SPSS) software. To simplify analysis and interpretation of an otherwise complex data set, the OGTT responses for each condition were converted into simple summary statistics (i.e., within subject fasting, total AUC and insulin sensitivity scores). The total areas under the glucose and insulin curves were calculated using the trapezoid rule, whilst the Cederholm Index of insulin sensitivity was calculated as previously described in Chapter 2. For all OGTT derived variables, two-way repeated measures ANOVA revealed no gender × group interactions so all data was pooled, and comparisons were made using 1-factor repeated measures ANOVA with post hoc Ryan Holm Bonferroni corrected t-tests if appropriate. Significance was accepted at P<0.05 and unless otherwise stated all data are presented as mean ± standard deviation of the mean (SD).
3.3. Results

3.3.1. Exercise Characteristics

All participants successfully completed all exercise bouts. During the AER exercise session participants cycled at 116 ± 44 W; this elicited 76 ± 4 % of $\bar{V}O_2$max, and 86 ± 7 %, 90 ± 6 % and 91 ± 6 % of maximal heart rate during bouts 1, 2 and 3 respectively. Participants gave RPE scores of 14 ± 1 during bout 1, 15 ± 1 during bout 2 and 16 ± 2 during bout 3. The power output characteristics of the ReHIT exercise bout are shown in table 3.3; the ReHIT session was well tolerated by all participants and was associated with a whole-session RPE score of 12 ± 2 (i.e., between ‘light’ and ‘somewhat hard’).

<table>
<thead>
<tr>
<th>Table 3.3. Characteristics of the ReHIT exercise bout</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sprint 1</strong></td>
</tr>
<tr>
<td>Duration (s)</td>
</tr>
<tr>
<td>Torque (nM)</td>
</tr>
<tr>
<td>Peak Power (W)</td>
</tr>
<tr>
<td>Average Power (W)</td>
</tr>
<tr>
<td>Minimum Power (W)</td>
</tr>
<tr>
<td>Fatigue Index (%)</td>
</tr>
<tr>
<td>Peak Heart Rate (% max)</td>
</tr>
<tr>
<td>RPE (Borg Scale)</td>
</tr>
<tr>
<td><strong>Sprint 2</strong></td>
</tr>
<tr>
<td>Duration (s)</td>
</tr>
<tr>
<td>Torque (nM)</td>
</tr>
<tr>
<td>Peak Power (W)</td>
</tr>
<tr>
<td>Average Power (W)</td>
</tr>
<tr>
<td>Minimum Power (W)</td>
</tr>
<tr>
<td>Fatigue Index (%)</td>
</tr>
<tr>
<td>Peak Heart Rate (% max)</td>
</tr>
<tr>
<td>RPE (Borg Scale)</td>
</tr>
</tbody>
</table>

Data is presented as mean ± SD; * P≤0.05 sprint 1 vs. sprint 2

3.3.2. Glucose and Insulin Responses to the OGTTs

The plasma glucose and insulin responses to the 75 g OGTT are shown in figure 3.2, whilst the insulin sensitivity (Cederholm Index) data is presented in figure 3.3. There were no significant differences in fasting glucose concentrations, fasting insulin concentrations, total plasma glucose AUC, total plasma insulin AUC or insulin sensitivity (Cederholm Index) between any of the three conditions.
Figure 3.2. Plasma glucose (A+B) and insulin (C+D) responses to acute exercise. For clarity, the responses over time to the OGTT are presented as mean ± SEM, whilst the AUC data is presented as mean ± SD. ReHIT reduced-exertion HIT, AER aerobic exercise, REST no exercise control.

Figure 3.3. Insulin sensitivity responses to acute exercise. Data is presented as mean ± SD. ReHIT reduced-exertion HIT, AER aerobic exercise, REST no exercise control.
3.4. Discussion

In this study we investigated the effects of a single bout of ReHIT, compared with a bout of vigorous intensity endurance exercise, on insulin sensitivity measured 14-16 hours following the cessation of exercise. We could not detect any effect of either exercise bout on either fasting glucose, fasting insulin or in a dynamic index of whole-body insulin sensitivity. The lack of improvement in insulin sensitivity following a single bout of ReHIT strengthens our contention that the increase in insulin sensitivity we detected 3 days following the 6-week ReHIT intervention in male subjects (Chapter 2) could be ascribed to chronic training adaptations.

In line with our hypothesis, there was no acute impact of ReHIT on insulin sensitivity measured 14-16 hours post-exercise. This is in agreement with the majority of previous studies which have measured insulin sensitivity by OGTT at a similar time-point after more strenuous Wingate based HIT protocols in both recreationally active subjects (Brestoff et al., 2009; Richards et al., 2010) and in overweight/obese men (Whyte et al., 2013). Similarly, HIT did not appear to attenuate the systemic glucose or insulin response to a high-fat mixed meal challenge administered 14 hours post-exercise, although the overall lipemic response was reduced (Freese et al., 2011; Gabriel et al., 2012). However, a recent HIT study has demonstrated improvements in estimates of insulin sensitivity measured using an intravenous glucose tolerance test which remained for 48 hours into the recovery period (Ortega et al., 2014). Thus the acute impact of HIT remains unresolved. Nevertheless, the current data have important implications for the prescription of HIT/ReHIT as a preventative intervention in the general population. If reductions in postprandial systemic insulin and glucose concentrations are the primary targeted endpoint then single bouts will not be effective; rather HIT/ReHIT needs to be repeated regularly over several weeks in order for adaptations to be accrued. The situation may differ for specific populations: the only study in men presenting with T2D has demonstrated that an acute bout of HIT (10 x 60 s at 90% W\text{max}) performed in the morning reduced peak postprandial glucose excursions, the overall postprandial glycaemic response, and the proportion of time spent in hyperglycaemia over the following 24-h (Gillen et al., 2012). Whether this holds true for ReHIT, or in other populations such as those with prediabetes, will need to be answered in future investigations.
In disparity to our hypothesis, we could detect no increase in insulin sensitivity measured 14-16 hours following an acute bout of vigorous intensity aerobic exercise (45 minutes at ~75% VO_{peak}). This is in contrast to recent data from Brestoff et al. (2009) who, using a comparable cohort of participants, identical exercise bout and a similar post-exercise time point, demonstrated a ~25% reduction in insulin AUC during an OGTT. However, when considered as a whole the literature is somewhat inconsistent, with many studies in healthy lean individuals reporting no measurable changes at similar time-points following acute aerobic exercise of varying intensities and durations (Bogardus et al., 1983; Devlin & Horton, 1985; Baynard et al., 2005; Venables et al., 2007; Hasson et al., 2010; Short et al., 2012), whilst others have shown improvements for as long as 48 hours post-exercise (Mikines et al., 1988; Young et al., 1989; Perseghin et al., 1996; Short et al., 2013). This makes the interpretation of the literature difficult; however, the lack of change in our study may be explained by a combination of two factors. Firstly, the timing and composition of post-exercise feeding appears to have a strong influence on the response. For example, several studies show that restriction of carbohydrate intake appears to prolong any increase in insulin sensitivity post-exercise both in rodents (Cartee et al., 1989; Gulve et al., 1990; Kawanaka et al., 2000) and in healthy lean humans (Bogardus et al., 1983; Holtz et al., 2008; Newsom et al., 2010). This makes sense from an evolutionary perspective, as any acceleration in metabolism following exercise is presumably an attempt to restore intramuscular substrate stores as quickly as possible so that further exercise may be performed (Chakravarthy & Booth, 2004). Secondly, there is evidence that individuals with lower baseline levels of insulin sensitivity (e.g. overweight/obese, type 2 diabetics) tend to exhibit a more prolonged increase in post-exercise insulin sensitivity which can be detected even after several meals have been consumed (Burstein et al., 1985; Devlin & Horton, 1985; Gill et al., 2004; Nelson & Horowitz, 2014). This is perhaps reflective of the decrement in insulin action resulting in delayed restoration of intramuscular substrate stores after exercise, thereby necessitating a more prolonged increase in insulin sensitivity. In any case, given that our cohort of participants already had a relatively high baseline level of insulin sensitivity, and we fed them a meal containing ~100 g of carbohydrate approximately 30 minutes post-exercise which would be expected to stimulate rapid glycogen resynthesis (Ivy et al., 1988a; Ivy et al., 1988b), it is perhaps not all that surprising that we observed no change in insulin sensitivity following the aerobic exercise bout in the current study.
There are several limitations to the current analysis which provide opportunity for further study. Firstly, financial restrictions only permitted the inclusion of three time-points during the OGTT for our calculation of insulin sensitivity. Whilst this protocol was sensitive enough to detect the relatively large changes in insulin sensitivity observed following the ReHIT training intervention, it must be acknowledged that we may have missed more subtle changes in the current analysis. It would therefore be useful to repeat the current study using the more sensitive gold standard euglycemic clamp methodology. Secondly, we only included a 14-16 hour post-exercise time point in this study and cannot therefore rule out that ReHIT impacts on insulin sensitivity in the more immediate post-exercise period (i.e., in response to the first feeding). Lastly, in order to be able to make firm comparisons between the current acute study and the previous training intervention (chapter 3) we recruited a similar cohort of participants who, although sedentary, were young, lean and otherwise healthy, with a high level of baseline insulin sensitivity. It is therefore necessary to investigate the acute impact of ReHIT in populations with insulin resistance, particularly in light of the recent finding that other models of HIT substantially improve glycaemic control in middle aged men presenting with T2D (Gillen et al., 2012).

In summary, these data demonstrate no effect of an acute bout of ReHIT on insulin sensitivity, suggesting that the utility of HIT/ReHIT for improving insulin sensitivity may be limited to a training response.
Chapter 4

Acute metabolic and appetite responses following ReHIT and continuous moderate-intensity aerobic exercise
Chapter 4

Acute metabolic and appetite responses following ReHIT and continuous moderate-intensity aerobic exercise

4.1. Introduction

Previously in this thesis it was demonstrated that a modified version of the ‘all-out’ HIT protocols (reduced-exertion HIT; ReHIT), consisting of 10 min of low-intensity cycling interspersed with two 20-s ‘all-out’ sprints, was effective in improving insulin sensitivity in sedentary men, and aerobic capacity in sedentary men and women (Chapter 2). Importantly, these benefits were observed despite the very low time commitment (a total of 30 min per week) and relatively low ratings of perceived exertion (peaking at an average of 13, i.e. ‘somewhat hard’). As the ReHIT protocol is both highly time-efficient (total training time of 30 min per week) and manageable (mean RPE ~13 or ‘somewhat hard’) it may provide a suitable alternative or adjunct to current physical activity recommendations. However, further larger studies will be needed to confirm these initial positive observations (Metcalf et al., 2012; Gillen et al., 2014).

At the same time it also needs to be established why such a small volume of high-intensity exercise (total 40-60 s per session) appears to be so effective for promoting beneficial skeletal muscle and whole-body adaptations. Previous studies investigating more time-consuming and more strenuous HIT protocols have demonstrated a molecular response which includes a 20-30% drop in muscle glycogen concentrations, substantial lactate accumulation, an altered ATP:ADP ratio, and increases in AMPK activity, p38 MAPK activity, and a 6-fold increase in mRNA expression of PGC1α (Esbjornsson-Liljedahl et al. 1999; Gibala et al. 2009; Parolin et al. 1999). What’s more, the acute disturbance in muscle metabolism is associated with a substantial disruption of whole-body/systemic homeostasis, including a prolonged increase in post-exercise oxygen consumption (EPOC), and alterations in the appetite regulating hormones, acylated ghrelin and peptide YY, in a direction which would be expected to suppress appetite (Hazell et al. 2012; Williams et al. 2013; Deighton et al. 2013a). Such changes may explain why studies have observed beneficial alterations in body composition following several weeks of HIT (Whyte et al. 2010; Gillen et al. 2013; Heydari et al. 2012; Trapp...
et al. 2008). However, to date little is known about whether these acute responses to ‘classic’ HIT are retained with shorter, more manageable protocols such as REHIT. This represents an important area of research since it may provide information about the initial stimuli responsible for the activation of signalling pathways and chronic phenotypic adaptations. The aim of the present study was to characterise the acute metabolic and appetite responses to ReHIT compared with traditional aerobic exercise recommendations (30 min at 50% \( \dot{V}O_2_{\text{max}} \); AER). We hypothesised that ReHIT would result in a more pronounced disturbance in metabolic homeostasis and a greater suppression of appetite.
4.2. Methods

4.2.1. Subjects

Eight healthy young men (mean ± SD age: 21±2 y; height: 1.79±0.07 m; weight: 80.7±16.4 kg; BMI: 25±4 kg·m⁻²; \( \dot{V}O_2 \)max: 39±10 ml·kg⁻¹·min⁻¹) gave written informed consent to take part in this study, which received approval from the Research Ethics Approval Committee for Health (REACH) at the University of Bath. Participants were recruited on the basis that they were able to take part in strenuous exercise according to the Physical Activity Readiness Questionnaire (Thomas et al., 1992), were sedentary or moderately active according to the International Physical Activity Questionnaire (Craig et al., 2003), and were free from metabolic disease, cardiovascular disease, acute viral infection, and clinically relevant hypertension (i.e. blood pressure ≥ 140/90 mmHg and/or resting heart rate ≥ 100 bpm). All participants were weight-stable during the 6 months prior to the study and were not dieting. They were requested to maintain their normal diet and patterns of physical activity throughout the study period.

4.2.2. Baseline Testing and Familiarisation

Prior to the main experimental trials participants visited the laboratory on four separate occasions. During the initial visit participants underwent a continuous incremental cycling test to determine maximal oxygen uptake capacity (\( \dot{V}O_2 \)max). Briefly, the test started at a work rate of 60 W and increased in increments of 30 W every 3 min until volitional exhaustion. Expired air was collected into Douglas bags during the final min of the first four incremental stages and then during the final min before volitional exhaustion. Heart rate and ratings of perceived exertion (Borg, 1970) were obtained at 1-min intervals throughout the test and at the point of exhaustion. In all tests two or more of the following criteria were met: heart rate within 10 beats of age-predicted maximum, RER ≥ 1.10, RPE ≥ 19, and/or volitional exhaustion.

Following the incremental cycling test the participants performed three familiarisation sessions (two for ReHIT and one for AER), each lasting approximately 15 min. The AER familiarisation session was mainly used to check the intensity predicted to elicit 50% \( \dot{V}O_2 \)max (calculated using linear regression from the \( \dot{V}O_2 \) responses during the incremental cycling test). Participants cycled for 10 min at the
prescribed intensity and an expired air sample was collected during the final min. If necessary, adjustments were made to the intensity used during the main trials. The ReHIT sessions were used to familiarise participants with the procedures and required effort required during Wingate-type sprints. Ten-second sprints were used in the first familiarisation and twenty-second sprints in the second.

4.2.3. Main Experimental Trials

Participants completed three main experimental trials (ReHIT, AER and REST) in a randomised cross-over design. Prior to each experimental trial, participants were asked to refrain from strenuous physical activity for 48 hours and refrain from alcohol or caffeine for 24 hours. In addition, prior to the first trial a 24-hour food record was completed and participants were asked to replicate this diet prior to subsequent trials. To ensure adequate hydration participants drank ~1 pint of water upon waking on the morning of each experimental trial.

Participants reported to the laboratory between 8:30 and 9:30 am having fasted overnight from 10 pm the previous evening. Participants rested quietly for 15 min prior to collection of a 5-min baseline expired air sample, venous blood sample, and subjective feelings of appetite and physical tiredness (through visual analogue scales). These measurements were repeated at 0, 15, 30 and 90 min post-exercise or 30 min of rest. During the post-exercise period participants rested quietly, either reading, watching TV or working at a computer. Water was allowed ad libitum throughout each trial.

4.2.4. Exercise Protocols

All exercise bouts were performed on a mechanically-braked cycle ergometer (Monark 894E, Varberg, Sweden). In the AER condition, participants cycled continuously for 30 min at an intensity predicted to elicit 50% of VO2max. Heart rate, RPE and an expired air sample were collected during the final min of exercise. The ReHIT condition involved 20 min of rest followed by a 10-min sprint interval exercise protocol. This involved 10 min of low intensity cycling (60 Watts) and two ‘all-out’ sprints. Just before each sprint, participants increased the pedal cadence to their maximal speed, a braking force equivalent to 7.5% of bodyweight was then applied to the ergometer, and the participants sprinted against the braking force for 20 s. Power output
was measured continuously throughout each sprint. During the REST trial participants rested quietly for 30 min.

4.2.5. Collection and Analysis of Expired Air

Participants respired through a rubber mouthpiece connected to a two-way Hans Rudolph valve and expired air was collected into pre-evacuated Douglas bags. During each gas collection, samples of ambient (i.e. inspired) CO₂ and O₂ concentrations were measured within close proximity to the participant (Servomex miniMP 5200) rather than just assuming standard atmospheric concentrations, as has recently been recommended (Betts & Thompson, 2012). This is in order to account for small changes that could occur throughout the day in an enclosed laboratory environment.

Expired concentrations of O₂ and CO₂ were measured using paramagnetic and infrared analysers respectively (Servomex miniMP 5200). Douglas bags were then completely evacuated using a dry gas meter (Harvard Apparatus, Kent, UK) and the total volume and temperature (Model C, Edale Instruments, Cambridge, UK) were recorded for calculation of \( \dot{V}O_2 \), \( \dot{V}CO_2 \), and RER. All values were corrected to reflect standard temperature and pressures. In order to estimate EPOC, \( \dot{V}O_2 \) from the REST trial was subtracted from both of the exercise trials, excess \( \dot{V}O_2 \) from each 5-min measurement period was totalled, and then total area under the oxygen curve was calculated.

4.2.6. Subjective Appetite and Tiredness Ratings

Perceptions of hunger and prospective food consumption were assessed using validated 100 mm visual analogue scales, with standard descriptors anchored at each end (Flint et al., 2000). Perceptions of physical tiredness were assessed using 100-mm visual analogue scales ranging from “not at all physically tired” to “as physically tired as I have ever felt”.

4.2.7. Blood Collection and Analysis

Venous blood samples were collected through a cannula (BD Venflon Pro, BD, Helsingborg, Sweden). Haematocrit and haemoglobin concentrations were determined
from 1-ml aliquots of EDTA-treated whole blood at each time point. Haematocrit was determined manually using a micro-centrifuge and Hawksley reader with a CV of 2.4% (Hawksley & Sons Ltd, Sussex, UK). Haemoglobin concentration was analysed in duplicate on an automatic analyser with a CV of 1.2% (Sysmex R-3000, Sysmex Ltd, UK). Changes in plasma volume from baseline during each trial were then calculated using published equations (Dill & Costill, 1974). Hormone responses were corrected for changes in plasma volume. For plasma glucose, the uncorrected and corrected responses have been presented.

For glucose, NEFA, lactate and total PYY analysis, whole blood was dispensed into a 4-ml EDTA tube, immediately spun at 3466 g and 4°C for 10 min to separate the plasma, before being stored at -80°C until subsequent analysis. Plasma concentrations of glucose and non-esterified fatty acids (NEFA) were analysed on an automatic analyser using commercially available kits with a CV of <1% for both measures (Randox Daytona, Country Antrim, UK). Plasma concentrations of lactate were also analysed in duplicate on an automatic analyser with a CV of <1% (YSI Stat 3000, Yellow Springs Instruments, Yellow Spring, OH). Plasma total PYY concentrations were analysed in duplicate using commercially available ELISA with a CV of ~5% (Millipore, Hertfordshire, UK).

For analysis of acylated ghrelin, 2 ml of whole blood was first dispensed into a 4-ml EDTA tube containing 20 µl of p-hydroxymercuribenzoic acid (PHMB). Samples were then spun at 3466 g for 10 min at 4°C, and 500 µl of the supernatant transferred into a separate plain tube containing 50 µl of 1 M hydrochloric acid in order to preserve acylated ghrelin (Hosoda et al., 2004). Samples were then centrifuged again at 3466 g for 10 min at 4°C and the supernatant transferred into a separate tube and frozen at -80°C until subsequent analysis in duplicate using commercially available ELISA with a CV of ~6% for repeated measures (Bertin Pharma, Montigny le Bretonneux, France).

4.2.8. Statistics

All data were analysed using the commercially available Statistics Package for Social Sciences (SPSS) software. Area-under-the-curve (AUC) calculations were performed using the trapezoid rule. Two way repeated measures ANOVAs (condition [ReHIT, AER, REST] × time [Pre, 0, 15, 30, 90]) were used to analyse the response of
all measured variables over time. ANOVA were performed regardless of any minor deviations from a normal distribution (Maxwell & Delaney, 2004), and with Greenhouse-Geisser corrections applied for contrasts where $\varepsilon<0.75$ and the Huynh-Feldt corrections applied for less severe asphericity (Atkinson, 2002). Post hoc comparisons were made using paired sample t-tests corrected using the Ryan-Holm Bonferroni stepwise adjustment. Hormone AUCs were compared using 1-factor repeated measures ANOVA with post hoc paired sample t-tests. Significance was accepted at $P<0.05$ and, unless otherwise stated, all data are presented as mean ± standard deviation (SD).
4.3 Results

4.3.1. Exercise Characteristics

During the AER exercise bout participants cycled at 86 ± 5 W, which elicited 53 ± 5 % of VO₂max, a respiratory exchange ratio (RER) of 0.96 ± 0.03, and 70 ± 4% of maximum heart rate. Peak power, average power and minimum power for the ReHIT session were 9.2 ± 1.2, 7.3 ± 1.1 and 5.5 ± 1.1 W·kg⁻¹ for the first sprint, and 8.2 ± 1.4, 6.5 ± 1.1 and 4.5 ± 1.4 W·kg⁻¹ for the second sprint. Perceptions of physical tiredness increased immediately following both exercise bouts (both P<0.01 vs. REST) and tended to increase more following ReHIT compared with AER (P=0.06; Figure 5.1). Elevated physical tiredness ratings remained at 15 min post-exercise (both P<0.05 vs. REST) but there were no longer any differences between ReHIT and AER at either of this time-point. No differences between any of the groups existed at the 30 or 90 min time points.

Figure 4.1. Changes in perceptions of physical tiredness. Data is presented as mean ± SEM for clarity. * denotes P<0.05 for ReHIT vs. REST; # denotes P=0.06 for ReHIT vs. AER; † denotes P<0.05 for AER vs. REST. Black square, ReHIT; White rectangle, aerobic exercise.
4.3.2. Oxygen Uptake and Respiratory Exchange Ratio

\(V\text{O}_2\) was elevated immediately after both exercise bouts relative to REST (both \(P<0.01\)) and to a greater extent following ReHIT compared with AER (\(P<0.01\); Table 4.1). \(V\text{O}_2\) had returned to baseline levels 15 min after AER but remained elevated for at least 30 min following ReHIT (all \(P<0.05\)), resulting in a higher EPOC following ReHIT compared with AER (5.1 ± 0.9 vs. 1.0 ± 0.8 L; \(P<0.01\)).

RER was increased following both exercise bouts compared with REST (both \(P<0.01\)) but the magnitude of the increase was greater following ReHIT compared with AER (\(P<0.01\); Table 4.1). Whilst RER had returned to resting values at 15 min following AER, there was a decline in RER below baseline values which reached significance at 30 min (\(P<0.01\)) and appeared to persist for 90 min post-exercise (\(P<0.05\) vs. AER and baseline, \(P=0.18\) vs. REST).

Table 4.1. Changes in oxygen uptake and respiratory exchange ratio

<table>
<thead>
<tr>
<th>Post-Exercise Time (Min)</th>
<th>Pre</th>
<th>0-5</th>
<th>15-20</th>
<th>30-35</th>
<th>90-95</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V\text{O}_2) (L/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REST</td>
<td>0.31±0.07</td>
<td>0.29±0.05</td>
<td>0.29±0.05</td>
<td>0.28±0.05</td>
<td>0.30±0.06</td>
</tr>
<tr>
<td>AER</td>
<td>0.31±0.06</td>
<td>0.46±0.10†</td>
<td>0.31±0.05</td>
<td>0.30±0.05</td>
<td>0.30±0.05</td>
</tr>
<tr>
<td>ReHIT</td>
<td>0.30±0.04</td>
<td>0.62±0.10*#</td>
<td>0.38±0.06**</td>
<td>0.34±0.05***</td>
<td>0.31±0.04</td>
</tr>
<tr>
<td>RER ((\text{VCO}_2/\text{VO}_2))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REST</td>
<td>0.84±0.04</td>
<td>0.82±0.04</td>
<td>0.81±0.05</td>
<td>0.82±0.02</td>
<td>0.81±0.04</td>
</tr>
<tr>
<td>AER</td>
<td>0.86±0.06</td>
<td>1.04±0.06†</td>
<td>0.82±0.09</td>
<td>0.81±0.07</td>
<td>0.83±0.05</td>
</tr>
<tr>
<td>ReHIT</td>
<td>0.82±0.06</td>
<td>1.29±0.08**</td>
<td>0.76±0.05***</td>
<td>0.68±0.04**#</td>
<td>0.75±0.06#</td>
</tr>
</tbody>
</table>

Data is presented as mean ± SD. * denotes \(P<0.05\) for ReHIT vs. REST; † denotes \(P<0.05\) for AER vs. REST; # denotes \(P<0.05\) for ReHIT vs. AER.

4.3.3. Plasma Volume

Both exercise bouts resulted in a reduction in plasma volume relative to REST (both \(P<0.0001\)) but ReHIT resulted in a greater reduction compared with AER (\(P<0.01\); Figure 5.2). Whilst plasma volume had normalised at 15 min post-exercise in AER, it remained reduced in ReHIT at this time-point (\(P<0.001\) vs. REST and AER).
4.3.4. Glucose, Lactate and NEFA

Both exercise bouts resulted in an increase in plasma lactate concentration relative to REST which persisted for at least 30 min following AER (all P<0.01) and at least 90 min following ReHIT (all P<0.01; Table 4.2). The increase in plasma lactate concentration was greater following ReHIT compared with AER and concentrations remained higher at all measured time-points (all P<0.05; Table 4.2).

For plasma NEFA there was a main effect of time (P<0.01) but no main effect of condition and no condition × time interaction effect; i.e. plasma NEFA concentrations increased over time and this effect was not changed by either exercise bout (Table 4.2).

When uncorrected plasma glucose concentrations were compared, there was an increase in plasma glucose following ReHIT which persisted for at least 15 min post-exercise (all P<0.05; Figure 4.3A), whilst concentrations remained constant throughout the REST and AER trials. However, when plasma glucose concentrations were corrected for changes in plasma volume there was no change in glucose levels following ReHIT with a small reduction immediately post-exercise in AER compared with REST (P<0.05), after which concentrations remained similar between trials throughout the rest of the recovery period (Figure 4.3B).
Chapter 4: Acute Metabolic and Appetite Responses to ReHIT

Table 4.2. Changes in plasma lactate and NEFA concentrations

<table>
<thead>
<tr>
<th></th>
<th>REST</th>
<th>AER</th>
<th>ReHIT</th>
<th>REST</th>
<th>AER</th>
<th>ReHIT</th>
<th>REST</th>
<th>AER</th>
<th>ReHIT</th>
<th>REST</th>
<th>AER</th>
<th>ReHIT</th>
</tr>
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<tbody>
<tr>
<td><strong>Time (Min)</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td>1.45±0.56</td>
<td>1.88±1.11</td>
<td>1.51±0.99</td>
<td>1.46±0.25</td>
<td>0.43±0.24</td>
<td>0.46±0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>15</strong></td>
<td>1.03±0.31</td>
<td>3.54±1.81 †</td>
<td>15.37±3.53 †#</td>
<td>0.45±0.19</td>
<td>0.46±0.31</td>
<td>0.34±0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>30</strong></td>
<td>1.01±0.33</td>
<td>1.88±0.75 †</td>
<td>12.51±2.81 †#</td>
<td>0.47±0.21</td>
<td>0.60±0.40</td>
<td>0.39±0.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>90</strong></td>
<td>1.00±0.38</td>
<td>1.41±0.45 †</td>
<td>7.73±2.08 †#</td>
<td>0.54±0.23</td>
<td>0.46±0.28</td>
<td>0.36±0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data is presented as mean ± SD. * denotes P<0.05 for ReHIT vs. REST; # denotes P=0.06 for ReHIT vs. AER; † denotes P<0.05 for AER vs. REST.

Figure 4.3. Uncorrected (A) and corrected (B) plasma glucose responses Data is presented as mean ± SEM for clarity. * denotes P<0.05 for ReHIT vs. REST; # denotes P=0.06 for ReHIT vs. AER; † denotes P<0.05 for AER vs. REST. Black square, ReHIT; White rectangle, aerobic exercise.

.3.5. Acylated Ghrelin and Peptide YY

Plasma acylated ghrelin decreased immediately after both exercise bouts when compared with REST (both P<0.05). Whilst concentrations were similar in AER and REST from 15 minutes post-exercise, they continued to decline in the ReHIT trial, and were lower at the 15- and 30-min time-points compared with both REST and AER (all P<0.01; Figure 4.4A). There were no significant differences between trials at the 90-min time-point. Post-exercise total AUC for acylated ghrelin was lower in ReHIT (4612 ±
3281 pg·ml⁻¹·90 min) compared with REST (8845 ± 4690 pg·ml⁻¹·90 min; P<0.01) and AER (8904 ± 5532 pg·ml⁻¹·90 min; P<0.01).

Plasma total PYY concentrations decreased over time (P<0.05) and this effect was not altered by either exercise bout (Figure 4.4B). There were no significant differences in post-exercise total AUC for PYY between any of the groups (ReHIT: 5984 ± 1516 pg·ml⁻¹·90 min; AER: 5454 ± 1418 pg·ml⁻¹·90 min; REST: 5732 ± 2634 pg·ml⁻¹·90 min).

**Figure 4.4.** Changes in plasma acylated ghrelin (A) and PYY (B) concentrations. Data is presented as mean ± SEM for clarity. * denotes P<0.05 for ReHIT vs. REST; # denotes P=0.06 for ReHIT vs. AER; † denotes P<0.05 for AER vs. REST. Black square, ReHIT; White rectangle, aerobic exercise. Concentrations of both hormones have been corrected for changes in plasma volume.

### 4.3.6. Subjective Appetite

For perceptions of hunger there was a main effect of time (P<0.05) but no main effect of condition or condition × time interaction, i.e. perceptions of hunger increased over time and this effect was not altered by either exercise bout (Table 4.3). For PFC there was no main effect of condition, but there was a main effect of time (P<0.05) and a tendency for lower ratings following ReHIT (P=0.08 for the time × condition interaction; Table 4.3).
### Table 4.3. Changes in subjective perceptions of appetite (n=8)

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Pre</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hunger</strong></td>
<td>REST</td>
<td>36±30</td>
<td>41±31</td>
<td>45±34</td>
<td>49±30</td>
</tr>
<tr>
<td></td>
<td>AER</td>
<td>33±28</td>
<td>45±28</td>
<td>46±27</td>
<td>46±27</td>
</tr>
<tr>
<td></td>
<td>ReHIT</td>
<td>30±24</td>
<td>37±24</td>
<td>38±25</td>
<td>37±26</td>
</tr>
<tr>
<td><strong>PFC</strong></td>
<td>REST</td>
<td>43±28</td>
<td>46±30</td>
<td>50±29</td>
<td>55±29</td>
</tr>
<tr>
<td></td>
<td>AER</td>
<td>37±27</td>
<td>54±25</td>
<td>53±25</td>
<td>54±24</td>
</tr>
<tr>
<td></td>
<td>ReHIT</td>
<td>36±22</td>
<td>42±22</td>
<td>38±23</td>
<td>39±26</td>
</tr>
</tbody>
</table>

Data is presented as mean ± SD. $^*$ denotes main effect of time (P<0.05).
4.4. Discussion

We have previously demonstrated that an exercise training intervention requiring a maximum of 40 s of high-intensity exercise within a 10-min training session (ReHIT) can be effective in improving insulin sensitivity and maximal aerobic capacity over a 6-week period (Chapter 2). In the present study we examined the acute metabolic and appetite response to ReHIT. We demonstrate that circulating concentrations of acylated ghrelin are reduced by >50% following ReHIT, indicating that ReHIT may be associated with a reduction in appetite. Furthermore, our data show that a single bout of ReHIT induces a markedly greater disturbance of homeostasis compared with 30 min of cycling at an intensity of 53±2% of VO$_{2}$max. Although ratings of physical tiredness directly after cessation of exercise were moderately higher for ReHIT compared to AER, this difference had disappeared within 15 min after exercise, confirming our previous observations that ReHIT is not overly strenuous (Chapter 2). The substantial disturbance of physiological homeostasis (presumably ultimately responsible for chronic training adaptations), extreme time-efficiency, and low associated perceived exertion and fatigue suggest that ReHIT has potential as a feasible alternative, or adjunct to, current physical activity recommendations for the general population.

4.4.1. Appetite and EPOC following REHIT

The current study demonstrates that circulating levels of the appetite-stimulating hormone acylated ghrelin are significantly reduced following ReHIT, which with a total duration of 10 min makes it the lowest volume of exercise to be associated with such an effect to date. However, the decrease in acylated ghrelin was no longer significant 90 min after cessation of exercise. Similarly, EPOC was significantly greater following ReHIT compared to aerobic exercise, but for a limited duration only. These findings are of importance as several previous HIT studies have observed reductions in fat mass following training with some evidence for superior reductions compared with traditional aerobic training (Trapp et al., 2008; Whyte et al., 2010; Heydari et al., 2012; Gillen et al., 2013). Since the direct exercise energy expenditure is much lower with HIT compared with prolonged steady state aerobic exercise, the (potentially superior) reductions in fat mass following HIT have been hypothesised to be a result of either a more prolonged EPOC and/or suppression in appetite resulting in reduced energy intake.
Hazell et al. (2012) provided some initial support for a prolonged EPOC following HIT by demonstrating that 24-h total oxygen consumption was similar (~100 L above rest) following a bout of HIT (4 x 30-s Wingates) compared with 30 min of continuous aerobic exercise at ~70% \( \dot{V}O_2\text{max} \), due to a small but more prolonged elevation in metabolic rate following HIT. However, in follow up investigations which have either utilised identical ‘all-out’ HIT protocols (Chan & Burns, 2013; Williams et al., 2013) or a modified less intense HIT protocol (10 x 1 min at ~90% HR\text{peak}; (Kelly et al., 2013)), the elevation in oxygen uptake has been modest and any effect lost ~45 min into the recovery period. In the present study we show that ReHIT is associated with a 5-fold greater EPOC compared with moderate intensity aerobic exercise, but the duration and magnitude of EPOC is similar to that observed in these latter HIT studies (Chan & Burns, 2013; Kelly et al., 2013; Williams et al., 2013). On balance, the current state of evidence suggests that EPOC would only make a small contribution to any reduction in fat mass with HIT.

It has also been suggested that HIT may result in suppression of appetite during recovery and hence lead to a reduction in energy intake across a training intervention (Boutcher, 2011). From a physiological perspective, appetite is thought to be controlled by the hypothalamus, which receives information on short and long term nutrient availability via the complex interaction between several gastrointestinal organs, adipose tissue, and the circulating episodic and tonic hormonal milieu (Wynne et al., 2005; Stensel, 2010). The gut hormones acylated ghrelin (orexigenic effects) and PYY (anorexigenic effects) have emerged as important episodic regulators of hunger, feeding latency and caloric intake (for review see Wynne et al. (2005)) and may therefore play a role in mediating any changes in appetite with exercise. Following ReHIT systemic concentrations of acylated ghrelin were reduced by >50% in the immediate post exercise period, with little or no change observed following a bout of continuous cycling at a moderate intensity. This is consistent with other HIT studies (Deighton et al., 2013a; Sim et al., 2013) and fits with previous research which highlights the key effect of relative exercise intensity on modulating the response of acylated ghrelin to aerobic exercise, with suppression occurring at higher (≥70% \( \dot{V}O_2\text{max} \)) but not lower (≤50% \( \dot{V}O_2\text{max} \)) exercise intensities (Broom et al., 2007; Broom et al., 2009; Ueda et al., 2009; King et al., 2010; Wasse et al., 2013). The current study suggests that the total duration of ‘all-out’ sprint exercise is of less importance than intensity in suppressing acylated
ghrelin levels. Conversely, circulating levels of total PYY appear to be unaffected by ReHIT. The effect of other HIT protocols on total PYY is currently inconsistent with one study demonstrating an increase in the immediate post-exercise period (Deighton et al., 2013a) and another showing no detectable changes (Sim et al., 2013). It is worth pointing out however that in the study by Deighton et al. (2013a), PYY values were not corrected for plasma volume changes, and considering the large drop in plasma volume following ReHIT in the current study the increase in PYY levels may partly have been caused by hemoconcentration. A limitation of the current study is that we only measured total PYY concentrations whereas it appears that the PYY$_{3-36}$ isoform is the most potent regulator of satiety (Karra et al., 2009).

Whereas there was a strong attenuation of acylated ghrelin following ReHIT, there was no significant suppression of ratings of appetite (although it is noteworthy that ratings of prospective food consumption tended to be lower in the REHIT trial). To date, there is limited data on the effects of HIT on indices of appetite and findings are equivocal (Deighton et al., 2013a; Sim et al., 2013; Williams et al., 2013). Whilst Deighton et al. (2013a) demonstrated a clear ‘anorexic’ effect of HIT on appetite-regulating hormones as well as subjective appetite ratings immediately post exercise, this did not translate into a reduction in caloric intake during laboratory controlled ad libitum lunch and dinner buffet meals. On the other hand, in a group of overweight men, there was a less robust effect of HIT on gut hormone responses and no effect on subjective appetite ratings, yet ad libitum energy intake during a laboratory-controlled breakfast meal and over the following day (as assessed using self-report food diaries) were both substantially reduced (Sim et al., 2013). Although the physiological relevance of short-term suppression of circulating levels of acylated ghrelin with HIT remains unclear, we can confirm this effect and demonstrate that it can be achieved with a substantially smaller amount of exercise than previously reported. Future research should look to further characterise the effect of ReHIT on appetite and examine changes in body composition following a training intervention.
4.4.2. Metabolic Responses Following ReHIT

In the present study we observed a substantial disturbance of metabolic homeostasis following ReHIT. Whereas the ATP requirements of moderate intensity aerobic exercise can largely be met through oxidative pathways, predominantly via a constant supply of NEFA and glucose from the circulation (Romijn et al., 1993; Romijn et al., 2000; van Loon et al., 2001), the brief ‘all-out’ sprints in the ReHIT protocol present an acute ‘energy crisis’ to the muscle cell. The supramaximal exercise intensity leads to rapid depletion of intracellular stores of phosphocreatine, the instantaneous activation of glycogen phosphorylase, and degradation of glycogen stores by 20-30% within the first of repeated sprints (Esbjornsson-Liljedahl et al., 1999; Parolin et al., 1999; Esbjornsson-Liljedahl et al., 2002; Gibala et al., 2009). This extremely rapid mobilisation of muscle glycogen is a unique characteristic of ‘all-out’ Wingate-type sprints and likely explains a number of the differences between ReHIT and AER observed in the present study. The considerable amount of glucose-6-phosphate released in the early stages of the initial ReHIT sprint poses a challenge to the intramyocellular milieu. Although the glycolytic conversion of glucose-6-phosphate results in a rapid accumulation of pyruvate, only a small fraction of this is immediately oxidised, and most is instead converted to lactate in order to maintain flux through glycolysis (Parolin et al., 1999), as evidenced by the large spike in plasma lactate concentrations observed in our study. The strong reliance on glycolysis for ATP resynthesis will also result in a reduction in both intramyocellular and blood pH levels. Consistent with previous observations following more strenuous and time-consuming HIT protocols (Hazell et al., 2012; Chan & Burns, 2013), we observed a sharp increase in RER immediately after ReHIT which then rebounded and remained lower throughout the ReHIT recovery period compared with AER and REST. This pattern of change in RER is characteristic of the immediate bicarbonate-mediated buffering of the metabolic acidosis induced by high-intensity exercise, followed by the retention of CO₂ to replenish the depleted blood bicarbonate stores during recovery (Laforgia et al., 1997). Given that the majority of glycogenolysis, and hence metabolic acidosis, occurs during the first sprint of a repeated sprint protocol (Parolin et al., 1999) it is not surprising that a protocol involving a smaller number of shorter sprints poses a similar challenge to buffering mechanisms compared with more strenuous HIT protocols, and hence still have this effect on RER (Hazell et al., 2012; Chan & Burns, 2013). This regular overloading (and recovery) of
muscle and blood buffering mechanisms may explain the adaptations in buffering capacity observed following six weeks of HIT (Gibala et al., 2006).

Another consequence of the rapid glycogenolysis occurring during the ReHIT sprints is that the accumulation of metabolic derivatives will result in a hypertonic intramyocellular environment leading to an influx of water (Raja et al., 2006), which may explain the considerable drop in plasma volume observed following ReHIT. Potentially this subsequently places the cell in a transient state of osmotic stress (Haussinger, 1996). Intriguingly, in various cell types osmotic swelling has previously been shown to independently activate various signalling cascades, including the MAP-kinases, which are known to be involved in cellular remodelling (Haussinger, 1996; Liu & Cao, 2009). It is interesting to speculate as to whether this type of osmotic stress could be a mechanism of skeletal muscle adaptation with HIT. From a practical research perspective, a consequence of the change in plasma volume following ReHIT (which is presumably similar following other supramaximal HIT protocols), is that various plasma protein levels may have to be corrected for plasma volume changes if measured during the initial post-exercise recovery period. Similarly, it seems reasonable to suggest that hemoconcentration due to the rapid drop in plasma volume explains previously observed increases in plasma glucose concentrations with sprint exercise (Vincent et al., 2004; Esbjørnsson et al., 2009; Stokes et al., 2013), rather than an increase in glucose rate of appearance.

In conclusion, the present findings demonstrate that ReHIT suppresses circulating acylated ghrelin levels and induces a more pronounced disturbance in whole-body metabolic homeostasis when compared with continuous moderate intensity exercise. Future studies should determine whether the suppression of acylated ghrelin following acute bouts of ReHIT may reduce calorific intake across a chronic training intervention and thus reduce body fat content.
Chapter 5

The impact of ReHIT on glycogen degradation, AMPK and p38 MAPK phosphorylation, and mRNA expression of GLUT4 and PGC-1α in human skeletal muscle
Chapter 5

The impact of ReHIT on glycogen degradation, AMPK and p38 MAPK signalling, and mRNA expression of GLUT4 and PGC-1α in human skeletal muscle

5.1 Introduction

High-intensity interval training (HIT) has recently emerged as a potent exercise strategy for inducing adaptations similar to traditional cardiorespiratory training (Gibala et al., 2006; Burgomaster et al., 2008). As an example, marked increases in skeletal muscle proteins related to oxidative metabolism and glucose transport have been demonstrated following HIT (Burgomaster et al., 2005; Burgomaster et al., 2007), and such adaptations at least partially explain the parallel improvements in skeletal muscle and whole-body insulin sensitivity observed (Babraj et al., 2009; Richards et al., 2010). At the cellular level, such adaptations are thought to result from the cumulative transient increases in gene transcription that follow each acute exercise bout, with the metabolic perturbations induced by contraction activating various signalling kinases which, in turn, target downstream transcriptional coactivators and regulators (Perry et al., 2010). Two important signalling kinases implicated in the adaptive response for skeletal muscle insulin sensitivity with exercise are AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (p38 MAPK). Both have been shown to be activated following aerobic exercise (Little et al., 2010; Bartlett et al., 2012) and HIT (Gibala et al., 2009; Little et al., 2011b; Bartlett et al., 2012; Fuentes et al., 2012; Fuentes et al., 2013), and are known to target downstream transcriptional regulators of genes related to skeletal muscle glucose transport (e.g., GLUT4) and mitochondrial biogenesis (e.g., PGC 1α) (Holmes et al., 1999; Jørgensen et al., 2006; Winder et al., 2006; Pogozelski et al., 2009).

Whilst skeletal muscle glycogen has long been appreciated as an important acute regulator of skeletal muscle metabolism, accumulating evidence also supports its role as a signalling molecule potentiating exercise-induced adaptation (Philp et al., 2012). Several important signalling kinases, for instance AMPK, contain glycogen binding domains (Hudson et al., 2003; Polekhina et al., 2003), suggesting that their activity is
regulated, at least in part, by glycogen availability (Akerstrom et al., 2006; Steinberg et al., 2006; McBride et al., 2009; McBride & Hardie, 2009). It has also been speculated that rapid glycogen degradation may be indirectly linked to activation of other stress signalling kinases, for example p38, through changes in intramyocellular osmotic pressure (Philp et al., 2012). Concordantly, performing acute exercise in a low-glycogen state augments signalling through AMPK and p38 (Wojtaszewski et al., 2003; Chan et al., 2004), enhances transcription of various metabolic genes including GLUT4 (Pilegaard et al., 2002; Steinberg et al., 2006), and stimulates a superior adaptive metabolic response, for example in oxidative capacity, over the course of an exercise training intervention (Morton et al., 2009; Hulston et al., 2010). Of course, the benefits of exercising in a low glycogen state on skeletal muscle are limited mainly to athletic and highly trained individuals. However, in skeletal muscle which usually experiences very little glycogen turnover (i.e., repeated degradation and resynthesis of glycogen), such as in sedentary persons, it is plausible that even modest reductions in glycogen concentrations may be sufficient to stimulate an adaptive response. Our current working hypothesis is that glycogen turnover with exercise (e.g. HIT) underpins several key adaptive metabolic responses with training in sedentary individuals, in particular the adaptations in skeletal muscle carbohydrate metabolism which beneficially impact upon whole-body insulin sensitivity.

Glycogen use increases with increasing exercise intensity, becoming the major substrate at intensities >55% Wmax (van Loon et al., 2001). Glycogen would therefore be expected to contribute largely to energy provision during a classic HIT protocol. Indeed, several authors have cited glycogen depletion as a potential mechanism underpinning improvements in insulin sensitivity with HIT (Babraj et al., 2009; Whyte et al., 2010). However, during a repeated sprint protocol glycogenolysis only appears to be robustly activated during the first ~15 s of the first sprint (Parolin et al., 1999). Therefore, whilst a single 30-s sprint reduces glycogen stores by approximately 20-30% (Parolin et al., 1999; Gibala et al., 2009), it is likely that similar degradation may be achieved with shorter sprints (e.g., 20-s). Moreover, performing repeated sprints will not result in further glycogen degradation (Parolin et al., 1999; Gibala et al., 2009). In chapter 3 of this thesis we demonstrated improvements in insulin sensitivity following a HIT intervention requiring a maximum of 2 x 20-s ‘all-out’ sprint efforts (reduced-exertion HIT; ReHIT). Our observation that insulin sensitivity improved to a similar
extent as with more strenuous protocols (Babraj et al., 2009; Richards et al., 2010; Cocks et al., 2013; Shepherd et al., 2013) provides support for the hypothesis that repeated glycogen turnover is a key determinant of improved insulin sensitivity following HIT. However, the contention that ReHIT induces similar glycogen depletion compared to supramaximal HIT protocols involving 4-6 × 30 s ‘all-out’ sprints (~20-30% from resting) is yet to be confirmed experimentally. Thus, the primary aim of the current study was to determine glycogen utilisation in the vastus lateralis muscle during a single bout of ReHIT. In addition, we measured changes in the activation of signalling molecules thought to be influenced by glycogen availability (AMPK and p38), and alterations in gene expression related to carbohydrate metabolism (GLUT4) and mitochondrial biogenesis (PGC1α). We hypothesised that ReHIT would be associated with reductions in glycogen, increased phosphorylation of AMPK and p38 MAPK, and increased expression of GLUT4 and PGC1α.
5.2 Methods

5.2.1. Participants

Twelve healthy active men (n=6) and women (n=6) agreed to take part in this study after being advised of the full experimental procedures and potential risks. The study protocol was given ethical approval from the NHS South West Research Ethics Committee (Frenchay REC Reference: 12/SW/0258) and all subjects provided written informed consent. We were unable to obtain usable muscle biopsies in one male subject, so they were excluded from the analysis and the final sample size was n=11. Baseline characteristics are presented in table 1. Exclusion criteria for this study were: anyone aged <18 or >40, anyone with contraindications to strenuous exercise as determined by a standard PARQ, anyone with clinically relevant hypertension (≥140/90 or resting heart rate ≥100 bpm) or pre-existing disease, anyone with a known bleeding disorder or on the anticoagulant Warfarin, and anyone with a tendency to keloid scarring. The study was conducted in accordance with the *Declaration of Helsinki*.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males (n=5)</th>
<th>Females (n=6)</th>
<th>Combined (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>26 ± 7</td>
<td>26 ± 7</td>
<td>26 ± 7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.81 ± 0.06</td>
<td>1.64 ± 0.06</td>
<td>1.71 ± 0.11</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.7 ± 6.7</td>
<td>62.3 ± 9.2</td>
<td>67.9 ± 10.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9 ± 2.5</td>
<td>23.2 ± 2.7</td>
<td>23.1 ± 2.5</td>
</tr>
<tr>
<td>(\dot{V}\text{O}_2\text{max} \text{ (L/min)})</td>
<td>4.3 ± 0.8</td>
<td>2.8 ± 0.5</td>
<td>3.5 ± 1.0</td>
</tr>
<tr>
<td>(\dot{V}\text{O}_2\text{max} \text{ (ml/kg/min)})</td>
<td>57.9 ± 12.6</td>
<td>45.7 ± 5.2</td>
<td>51.2 ± 10.9</td>
</tr>
</tbody>
</table>

Data is presented as mean ± SD.

5.2.2. Baseline Testing and Familiarisation

Prior to the main experimental trial participants visited the lab on three separate occasions. During the initial visit peak oxygen uptake capacity was determined during a continuous incremental ramp test to limit of tolerance on an electronically braked cycle ergometer (30 W·min⁻¹ ramp; Lode, Excalibur Sport, the Netherlands) with \(\dot{V}\text{O}_2\) measured continuously using an online metabolic cart (ParvoMedics TrueOne 2400, Utah, USA). On separate days each participant performed two ReHIT sessions on a mechanically braked cycle ergometer (Monark 894e, Vansbro, Sweden) in order to
become familiarised with the procedures and required effort during Wingate-type sprints. Ten-second sprints were used in the first familiarisation and twenty-second sprints in the second.

5.2.3. Main Experimental Trial

At least 1 week following the final familiarisation, and having refrained from strenuous/prolonged physical activity for 2 days, and alcohol and caffeine for 1 day, participants attended the lab at 8 am following an overnight fast. After resting quietly for 15 min, two small incisions (~5 mm) were made in the vastus lateralis of one leg under local anaesthetic (1% lidocaine) for collection of muscle biopsy samples using the Bergstrom needle technique adapted for suction (Tarnopolsky et al., 2011). One of these incisions was used for collection of the baseline muscle sample and immediately closed with steristrips, whilst the second was initially covered with sterile gauze and used for collection of the sample immediately post-exercise. Further muscle biopsy samples were collected at 30 min and 180 min post-exercise through separate incisions and on the opposite leg to the first two samples. The starting leg (i.e. dominant or non-dominant) for collection of the muscle samples was counterbalanced, and the first biopsy on each leg was always taken at a more distal point to the second (~3 cm between incisions). Following each muscle biopsy procedure, the muscle sample was immediately snap-frozen and stored in liquid nitrogen until subsequent analysis. Throughout the recovery period participants remained fasted (i.e. water only) and sat quietly in the lab and read, watched TV or worked at a computer.

5.2.4. ReHIT Exercise Bout

The ReHIT exercise bout involved 10 min of low intensity cycling (60 Watts) and two ‘all-out’ sprints against a braking resistance of 7.5% body mass (Monark 894e, Vansbro, Sweden). Just before each sprint, participants increased their pedal cadence to their maximal speed, a braking torque was applied to the ergometer, and participants sprinted maximally against the braking torque for twenty seconds.
5.2.5. Skeletal Muscle Analysis

Glycogen Analysis

Approximately 20-mg of frozen wet tissue was freeze-dried, powdered, and dissected free of visible blood and connective tissue. Samples were then suspended in dH₂O (0.5-mg/100µl) and homogenised on ice; first manually using a dounce homogeniser (30-40 passes) and then by sonication (4 x 10 s bursts at 10 microns with 50 seconds in between bursts). Homogenates were then heated at 95°C for 5 min, centrifuged at 13000 rpm for 5 min, before the glycogen content of the resulting supernatant was determined in duplicate using an enzymatic assay adapted for fluorimetry according to the manufacturer’s instructions (Abcam Glycogen Assay Kit (ab65620), Cambridge, UK). Glycogen concentrations were determined as glucosyl units (mmol/kg dry weight) and background glucose concentrations were determined and subtracted from the final glycogen readings. The intra-assay coefficient of variation was ~3%.

Western Blotting

Approximately ~30 mg of frozen wet tissue was freeze dried, powdered and dissected free of visible blood and connective tissue, and added to ice cold lysis buffer (1 mg/100 µl; 20 mM Tris (pH 7.8), 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 1% Triton X-100, 10% (w/v) glycerol, 1 mM EDTA, 1 mM dithiothreitol) which was supplemented with protease (Thermo Scientific) and phosphatase inhibitor cocktail (Calbiochem Ref 539630). The sample was homogenised on ice using a dounce homogeniser (40-50 passes) and subsequently incubated for 1 hour at 4°C with continuous rotation. The sample was then centrifuged at 13000 rpm for 5 minutes and the supernatant collected. The protein content of the supernatant was determined using a bicinchoninic acid assay (BCA) (Thermo Scientific), diluted to a standard protein concentration in 1× Laemmli buffer, before being boiled at 95°C for 5 minutes. For each blot, 60 µl of sample was loaded, together with a marker protein standard, onto self-cast 7% or 10% tris-glycine SDS-polyacrylamide gels and separated by electrophoresis (~1 hour at 200 V) in Tris-glycine running buffer (0.025 M Tris-HCl, 0.2 M glycine, 0.1% SDS, pH 8.3). Gels were electroblotted by semi-dry transfer (0.8 mA/cm² for 110 min (10% gels) or 150 min (7% gels)) onto nitrocellulose membranes in SDS transfer buffer.
(48 mM Tris base pH 8.8, 39 mM glycine, 0.0375% SDS, 20% Methanol). After transfer, membranes were washed (6 x 5 minute cycle) in TBS-T (Tris-buffered saline buffer containing Tween 20; 0.09% NaCl, 1M Tris-HCl pH 7.4, 0.1% Tween,) and then incubated for 1 hour in a blocking solution consisting of 5% skimmed milk (Marvel, Premier International Foods Ltd, UK) made up with TBS-T. Having been thoroughly rewashed (6 x 5 min cycles) again in TBS-T, membranes were then incubated overnight at 4°C with primary antibodies against p-AMPK\textsuperscript{T172}, total AMPK, p-ACC\textsuperscript{S79} (acetyl-CoA carboxylase), p-p38 MAPK\textsuperscript{T180}, and total p38 MAPK (All from Cell Signalling Technologies except p-ACC\textsuperscript{S79} which is from Millipore). After incubation, membranes were washed thoroughly in TBS-T (6 x 5 min cycles) before being incubated with an appropriate dilution of anti-species IgG horseradish peroxidase-conjugated secondary antibodies made up with blocking solution (5% skimmed milk made up with TBS-T). Having been rewashed, membranes were then exposed to an enhanced chemiluminescence reagent for 2 mins and visualised using a chemiluminescent imager (EpiChemi II Darkroom, UVP, Upland, Canada). Band densities were quantified using Labworks Image Acquisition and Analysis Software Version 4.0.0.8 for Windows (UVP, Upland, Canada). All samples taken from each subject were run on the same gel. Representative blots for each protein are shown in figure 1. Blots for p-AMPK and p-p38 have been normalised to the total amount of each respective protein, and p-ACC has been normalised to total AMPK content. Post-exercise time-points are expressed as a fold-change relative to pre-exercise values.

*Quantitative real-time PCR*

Gene expression analysis was only performed on muscle samples collected at baseline and 180 min post-exercise. Total RNA was extracted from ~10 mg of frozen muscle using Trizol reagent according to the manufacturer’s instructions (Life Technologies Ltd, Paisley, UK). Briefly, samples were suspended in 1 ml of Trizol, homogenized using a mechanical glass-Teflon (or Potter-Elvenheim) homogeniser, and then mixed with chloroform to produce an aqueous RNA phase which was subsequently mixed with isopropanol to precipitate RNA. The resulting RNA pellet was then washed with 75% ethanol, dried and resuspended in nuclease free water. RNA quantity and quality was subsequently assessed using a NanoVue Spectrophotometer (Thermo Fisher Scientific, Leicester, UK). RNA samples with a 260:280 ratio (i.e., the absorbance at 260
RNA was stored at -80°C before being reverse transcribed to cDNA using the High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Life Technologies, Paisley, UK). Quantitative real-time PCR for GLUT4 (Hs00168966_m1) and PGC1α (HS01016719_m1) relative to GAPDH (Hs.pt.39a.22214836; Integrated DNA technologies) was carried out using TaqMan Fast Universal, No AmpErase UNG chemistry in combination with a StepOne Fast 7500 real-time PCR system (All Applied Biosystems, Warrington, UK). cDNA (45 ng) was amplified under the following conditions: 95°C for 20 seconds, followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds. All PCR reactions were carried out in duplicate and a non-template control (i.e., nuclease free water) was included for each housekeeping gene/gene of interest on all plates. The resulting amplification plots were used to generate C_T values for genes of interest/housekeeping gene and the ΔC_T was calculated. The comparative C_T method was used to calculated gene expression at 180 min post-exercise relative to baseline.

5.2.6. Data Handling and Statistical Analysis

Due to difficulties with sample extraction and the subsequent size and quality of sample, in some participants we were unable to obtain the full time course for all measured variables. The number of samples missing for each analysis, the reason and the approach to data handling is summarised in table 1. For analysis of glycogen, two participants had missing data immediately post-exercise (i.e., time 0). However, as values for 30 minutes post-exercise were available for these participants, and because the mean difference between 0 and 30 min post-exercise time points in the other 9 participants was negligible (~15 mmol/kg dry wt; P>0.05), it was deemed appropriate to substitute in the 30 min value at 0 min for these particular cases. For the western blotting there was more variation between participants and between time points so this approach was not appropriate; in this case missing data points have been replaced with the mean response for the group at that time point. Since comparisons for gene expression were only made between two time points, and thus any missing data point prevented that comparison being made, any participant with a missing data point was excluded from the statistical analysis.
Table 5.2. Summary of missing data and approach to data handling and statistics

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Number of Missing Samples</th>
<th>Reason</th>
<th>Approach for Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>Pre 0 30 180</td>
<td>Small size of sample</td>
<td>Substitution of value from 30 minute time point for that subject</td>
</tr>
<tr>
<td>Western Blots</td>
<td>0 2 0 1</td>
<td>Small size of sample</td>
<td>Substitution of mean value for that time point</td>
</tr>
<tr>
<td>qPCR</td>
<td>6 n/a n/a 6</td>
<td>No amplification of target genes</td>
<td>Data excluded (n=6)</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using the commercially available Statistics Package for Social Sciences (SPSS) software. Gene expression data at pre and 180 min were compared using a Wilcoxon Rank Sum Test, since non-parametric tests are more appropriate when using very small sample sizes (Siegel & Castellan, 1988). Skeletal muscle glycogen and signalling protein data were analysed using a one factor (time) repeated-measures analysis of variance (ANOVA) with Ryan Holm Bonferroni correct t-tests used to locate any differences between time points. Significance was set at P<0.05 and all data is presented as mean ± SD unless stated otherwise.
5.3. Results

All participants successfully completed the ReHIT exercise bout. Peak power, average power and minimum power for the ReHIT session were 9.4 ± 1.5, 8.1 ± 1.1 and 6.2 ± 1.1 W·kg\(^{-1}\) for the first sprint, and 8.4 ± 1.6, 7.2 ± 1.2 and 5.5 ± 0.8 W·kg\(^{-1}\) for the second sprint.

5.3.1. Glycogen Breakdown

Whole muscle glycogen concentrations were reduced on average by ~19% immediately post-exercise and remained reduced to a similar extent throughout the recovery period (Figure 5.1; P<0.05 for all post-exercise time points vs. pre).

![Figure 5.1](image-url)

**Figure 5.1.** The effect of ReHIT on skeletal muscle glycogen concentrations. Data is presented as mean ± SD. *P<0.05 vs. pre

5.3.2. AMPK, pACC and p38 MAPK signalling

Phosphorylation of AMPK did not change significantly at any of the post-exercise time points (Figure 5.2A). However, ACC phosphorylation was strongly increased both immediately and 30 min post-exercise (both P<0.05), returning to baseline levels at 180 min (Figure 5.2B). There was no change in phosphorylation of p38 MAPK at any of the post-exercise time points (Figure 5.3).
Figure 5.2. The effect of ReHIT on AMPK (A) and ACC (B) phosphorylation. Data is presented as mean ± SD. * denotes P<0.05 vs. pre. Representative blots are shown above each respective figure.

Figure 5.3. The effect of ReHIT on p38 MAPK phosphorylation. Data is presented as mean ± SD. A representative blot is shown above.
5.3.3 GLUT4 and PGC1α gene expression

Due to poor quality of extracted RNA, gene expression analysis for GLUT4 and PGC1α is presented for n=5 only. Nevertheless, the expression of PGC-1α was increased significantly 180 min following ReHIT (P<0.05) and the change was consistent across all 5 subjects (Figure 5.4B). In addition, the expression of GLUT4 showed a tendency for an increase (P=0.08), with an increase observed in 4 subjects and no change observed in 1 subject (Figure 5.4A).

Figure 5.4. The effect of ReHIT on PCG1α (B) and GLUT4 (A) gene expression. Data is presented as median with individual responses. * denotes P<0.05 vs. pre. 180 denotes the time in minutes after the ReHIT bout.
5.4. Discussion

These data show that just 40 seconds of ‘all-out’ exercise is sufficient to reduce glycogen by a fifth and increase the mRNA expression of PGC-1α in human skeletal muscle. However, it remains unclear whether the reduction in glycogen is associated with a signalling response through AMPK or an increase in GLUT4 gene expression. Specifically, whilst we were unable to detect a significant change in AMPK phosphorylation, there was a robust activation of ACC-β suggesting there may have been a level of AMPK activation in our subjects. In addition we noted a trend for an increase in GLUT4 gene expression, but this will require confirmation with a larger sample size.

5.4.1. Glycogen Depletion

In line with our hypothesis, glycogen levels in the vastus lateralis were reduced by ~20% on average immediately following ReHIT. This response fits with the data of Parolin et al who examined the response of glycogen phosphorylase during maximal cycling exercise and demonstrated that, during a repeated 30-s sprint bout, glycogen phosphorylase (and hence glycogenolysis) was predominantly activated during the first 15-s of the first sprint (Parolin et al., 1999). More importantly, these data confirm our hypothesis that glycogen degradation with ReHIT is similar to that observed following HIT protocols incorporating a greater number of more prolonged (e.g., 4 x 30-s) sprint efforts (Parolin et al., 1999; Gibala et al., 2009) and provide further indirect support for our hypothesis that regular turnover of muscle glycogen stores may be associated with improved insulin sensitivity (Chapter 3).

5.4.2. AMPK and p38 MAPK signalling responses

AMPK is an αβγ heterotrimer with a well-established role in regulating acute energy flux (Jensen et al., 2009). In addition, AMPK is thought to play a role in regulating the metabolic adaptation to exercise by signalling through several transcription factors (Holmes et al., 1999; Jørgensen et al., 2006; Winder et al., 2006; Pogozelski et al., 2009). Classically, AMPK activity was thought to be regulated primarily by the cytoplasmic AMP:ATP ratio (Moore et al., 1991; Corton et al., 1994), but recent evidence has suggested an important role of glycogen availability as well (McBride et al., 2009; McBride & Hardie, 2009). Specifically, AMPK is known to
contain a glycogen binding domain and it is thought that, in a glycogen replete state, a certain amount of AMPK is bound within glycogen granules (Philp et al., 2012). In turn, it would be expected that degrading a certain level of glycogen during exercise would release a respective amount of the bound AMPK which may then become activated if the cytosolic concentration of AMP also increased (Philp et al., 2012). The current study shows a ~20% reduction in skeletal muscle glycogen levels, whilst previous studies suggest that cytoplasmic AMP concentrations (not measured here) would also have been increased with ReHIT (Karatzaferi et al., 2001a; Karatzaferi et al., 2001b). Therefore, it was hypothesised that there would be a corresponding increase in AMPK activity following ReHIT. We measured phosphorylation of AMPK at the threonine 172 site, which is the main site regulated by muscle contraction and has previously been shown to be phosphorylated with single and repeated Wingate sprint protocols (Gibala et al., 2009; Fuentes et al., 2012; Fuentes et al., 2013). However, we could not detect a statistically significant increase in AMPK phosphorylation at any measured time point following ReHIT. On the other hand, we did detect a robust activation of ACC-β, a known downstream target of AMPK (Davies et al., 1990; Ha et al., 1994), and this suggests there may have been a level of AMPK activation which we were unable to detect. Indeed, some authors rely solely on ACC-β phosphorylation as a marker of AMPK activity following exercise (Little et al., 2011b; Cochran et al., 2014). The fact that the numerical increase in AMPK phosphorylation 30 min post-exercise was similar to that observed in previous studies following a single Wingate sprint (Gibala et al., 2009; Fuentes et al., 2012; Fuentes et al., 2013) provides some additional support for this contention. However, ACC-β may also be regulated via AMPK independent mechanisms and dissociation between AMPK and ACC-β activity exists during prolonged low intensity exercise, for example (Vavvas et al., 1997; Wojtaszewski et al., 2002; Jørgensen et al., 2004). Therefore, whether ACC-β provides a representative index of AMPK activity is not certain, meaning it is not possible to determine with any certainty whether AMPK activity was altered in the present study.

The rapid breakdown in muscle glycogen may be associated with an increase in intramyocellular osmotic tension (Low et al., 1996; Philp et al., 2012). Indeed, in Chapter 4 we demonstrated a substantial drop in plasma volume which was speculated to be related to the rapid glycogen breakdown and subsequent fluid uptake. Interestingly, increased osmotic tension has previously been shown to activate p38 MAPK in isolated
cell preparations (Sheikh-Hamad & Gustin, 2004). As such, we hypothesised that the rapid reduction in muscle glycogen (and simultaneous increase in intramyocellular osmotic tension) with ReHIT would be associated with an increase in p38 MAPK phosphorylation. In disparity to this hypothesis there was no increase in phosphorylation of p38 following ReHIT in the current study. Previous studies have shown no effect of a single Wingate sprint on p38 MAPK phosphorylation (Gibala et al., 2009c; Fuentes et al., 2012; Fuentes et al., 2013); however p38 has been shown to be activated following 4 repeated 30-second Wingates (Gibala et al., 2009; Little et al., 2011b). As glycogen depletion, and the associated increase in osmotic pressure, would likely have been similar amongst these three sprint protocols, it seems likely that p38 MAPK phosphorylation is regulated by factors unrelated to glycogen depletion with sprint exercise. For example, p38 MAPK has been shown to be regulated by increases in cytosolic calcium and activation of CAMK II and there is evidence that p38 MAPK regulates PGC-1α activity in a calcium dependent manner (Wright et al., 2007). However, current evidence for an increase in CAMK activity following HIT in humans is lacking (Gibala et al., 2009). It may be that the time points used to assess CAMK II activity in previous HIT studies, and p38 MAPK activity in the present study, were not appropriate as these effects will be transient.

Whilst in the current study we have measured AMPK and p38 MAPK phosphorylation in a whole-muscle biopsy sample, it must be recognised that changes in the cellular localisation of these signalling proteins with exercise will likely also be of key importance for the adaptive response. Certainly, the regulation of gene transcription will either require direct translocation of the protein to the nucleus or a signalling cascade which transcends the nuclear membrane. Previous studies have observed translocation of AMPK, p38 MAPK and PGC-1α subunits to the nucleus during or in the recovery from an acute exercise bout (McGee et al., 2003; Chan et al., 2004; Steinberg et al., 2006; Little et al., 2011b). Whether a similar response occurs with HIT/ReHIT is a question for future research studies.
5.4.3. GLUT4 and PGC-1α mRNA expression

Although it was only possible to obtain mRNA data for n=5 in the current study, we were still able to detect a significant increase in PGC-1α gene expression following ReHIT. This suggests that ReHIT may be associated with an adaptive increase in mitochondrial biogenesis in skeletal muscle with training. In agreement with this proposal, it has recently been shown that several markers of oxidative capacity in skeletal muscle, such as citrate synthase and COX IV, are increased following a ReHIT like exercise training programme (Gillen et al., 2014). Furthermore, increases in PGC1α gene expression is a common acute response (Gibala et al., 2009; Little et al., 2011b), and an enhanced muscle oxidative capacity is a well characterised training response (Burgomaster et al., 2005; Gibala et al., 2006; Little et al., 2010b; Hood et al., 2011), with a variety of submaximal and supramaximal HIT protocols. The current study provides promising data to suggest that this response may be achieved with a much smaller volume of exercise than previously thought, but this will require confirmation with a larger sample size.

We also observed a tendency for an increase in GLUT4 mRNA content following ReHIT, despite the limited statistical power. More specifically, there was an increase in GLUT4 gene expression in four subjects, whilst the expression remained unchanged in one subject. Whilst only tentative at present, this suggests that ReHIT may also be associated with a gene expression response linked to improved carbohydrate transport and insulin sensitivity, and this represents the smallest volume of exercise to be associated with an increase in GLUT4 gene expression to date. Moreover, this would provide a potential mechanism for the improvements in insulin sensitivity observed following 6 weeks of ReHIT (Chapter 3). These data fit with recent work from Gillen et al. (2014) who showed an increase in muscle GLUT4 protein content following a 6-week ReHIT like intervention in sedentary men and women. Numerous other studies have also shown increase in GLUT4 following both submaximal and supramaximal HIT protocols (Burgomaster et al., 2007; Little et al., 2010; Hood et al., 2011). The tendency for GLUT4 gene expression to increase with such a small volume of intense exercise, which nevertheless results in a relatively large reduction in muscle glycogen stores, would suggest that glycogen levels may play an important role in regulating GLUT4 gene expression. Indeed, it makes physiological sense that the regulation of GLUT4 gene expression (and indeed protein expression) would be coupled to muscle glycogen levels,
as this represents the main signal of carbohydrate availability in skeletal muscle. From an evolutionary perspective, such coupling would have provided a rapid adaptive increase in muscle glucose transport following a period of intense exercise, resulting in a more efficient replenishment of muscle glycogen stores when dietary carbohydrate became available (Chakravarthy & Booth, 2004).

### 5.4.4. Conclusions

To summarise, these data confirm the hypothesis that ReHIT is associated with similar levels of glycogen degradation (~20%) compared with more strenuous HIT protocols. There also appeared to be increases in the expression of GLUT4 and PGC-1α following ReHIT, but these responses will require confirmation with a larger sample size. Likewise, there was some evidence of AMPK activation following ReHIT but further studies will be required to clarify this response.
Chapter 6

The impact of six weeks of ReHIT on aerobic capacity and insulin sensitivity in sedentary men and women
Chapter 6

The impact of six weeks of ReHIT on aerobic capacity and insulin sensitivity in previously sedentary men and women

6.1. Introduction

Aerobic capacity and insulin sensitivity represent key physiological variables in the development of human metabolic disease and are therefore logical targets for preventative intervention (Blair et al., 1989; Blair et al., 1995; Blair et al., 1996; Myers et al., 2002; Balkau & Eschwege, 1999; Petersen et al., 2007; Lorenzo et al., 2010). Previously in this thesis we presented data which suggested that both of these variables could be modified with as little as two minutes of sprint exercise per week in a group of young sedentary males (Chapter 2). We noted potential sex differences in the response to this type of exercise, with female subjects showing an improvement in aerobic capacity but no change in insulin sensitivity.

Both of these findings have recently been confirmed by an independent research group and supported via the measurement of skeletal muscle GLUT4 protein content which showed a similar sex-dependent increase (Gillen et al., 2014). It is our view that the very low time commitment and relatively low ratings of perceived exertion and tiredness reported by participants makes our ReHIT protocol the most viable HIT protocol for recommendation to the general population. However, both of the studies examining the effects of ReHIT to date have been limited by small sample sizes and, considering the known variation in the human metabolic and cardiorespiratory responses to exercise training (Bouchard et al., 1999; Boulé et al., 2005; Sisson et al., 2009), a detailed reappraisal of the insulin sensitivity and aerobic capacity adaptations to ReHIT is required in a larger cohort. As such, the primary aim of this study was to re-examine the impact of 6 weeks of ReHIT on insulin sensitivity and aerobic capacity in sedentary men and women. Given that we didn’t observe any improvement in insulin sensitivity in females in Chapter 3, it is necessary to investigate whether the current ReHIT protocol can be modified to promote an adaptive response for insulin sensitivity in women. In our previous study we observed that some female subjects struggled with the transition
from 60 W to ‘all-out’ sprinting and were not always able to substantially increase their pedal cadence (and hence their power output) during the sprints. We hypothesised that this may have increased the aerobic contribution to the sprints, reduced glycogen degradation, and hence attenuated our hypothesised adaptive response related to glycogen turnover (Chapter 3). In an attempt to ameliorate this, in the current study we substituted the 60-W warm-up for unloaded pedalling and reduced the braking force applied to the bike during the sprints from 7.5% to 5% body mass. Therefore, we hypothesised that 6 weeks of ReHIT would be effective at improving insulin sensitivity and aerobic capacity in both sedentary men and women.
6.2. Methods

6.2.1. Subjects

Fifty young or middle aged men (n=27) and women (n=23) gave their written informed consent to take part in this study, which received ethical approval from the NHS South West Research Ethics Committee (Central Bristol REC Reference: 12/SW/0018). Seven participants dropped out prior to completing baseline testing, nine discontinued the intervention, and we were unable obtain post-training blood samples from one male subject who was excluded from the analysis (figure 6.1); thus, thirty-four men (n=16) and women (n=18) completed the full experimental procedures and are included in this completion-only analysis (table 6.1).

Participants were recruited based on the following inclusion criteria: aged 18-50; classified as sedentary according to the IPAQ self-report questionnaire (Craig et al., 2003); no contraindications to strenuous exercise according to the standard PAR-Q (Thomas et al., 1992); weight stable and no conscious change in diet or physical activity patterns over the preceding 6 months; no evidence of clinically significant hypertension (>140/90 mm Hg and/or resting heart rate ≥ 100 bpm); and no personal history of metabolic or cardiovascular disease. The potentially confounding impact of changes in diet and exercise patterns was fully explained to all participants and (outwith the study intervention) they were asked to maintain their normal lifestyle over the 8 week study period.

Habitual physical activity status was estimated over a representative 7 day period at baseline using synchronised accelerometry and heart rate monitoring with branched model equations (Actiheart, Cambridge Neurotechnology Ltd., Cambridge, UK). The Actiheart is a non-invasive physical activity monitor that is both reliable and valid, can accurately estimate energy expenditure across low, moderate and high intensity physical activities, and provides useful quantitative data on patterns of physical activity, allowing a comprehensive characterisation of physical activity status (Thompson et al., 2006). Participants wore the monitor continuously (day and night) and were instructed to only remove it when showering or bathing.
Table 6.1. Participant Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males (n=16)</th>
<th>Range</th>
<th>Females (n=18)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>33±9</td>
<td>21-43</td>
<td>36±9</td>
<td>18-50</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.75±0.08</td>
<td>1.59-1.87</td>
<td>1.67±0.07</td>
<td>1.54-1.84</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>76.9±7.2</td>
<td>66.6-88.9</td>
<td>66.2±9.6</td>
<td>54.3-85.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1±2.1</td>
<td>21.0-28.9</td>
<td>24.0±3.5</td>
<td>18.4-29.1</td>
</tr>
<tr>
<td>V̇O₂max (L/min)</td>
<td>3.0±0.6</td>
<td>1.7-4.0</td>
<td>2.1±0.3</td>
<td>1.6-2.7</td>
</tr>
<tr>
<td>V̇O₂max (ml/kg/min)</td>
<td>39.2±8.6</td>
<td>24.5-57.3</td>
<td>31.7±4.6</td>
<td>25.7-39.0</td>
</tr>
<tr>
<td>Maximal Workload (W)</td>
<td>221±33</td>
<td>132-270</td>
<td>167±22</td>
<td>134-214</td>
</tr>
<tr>
<td>PA Score (IPAQ)</td>
<td>258±190</td>
<td>0-692</td>
<td>328±355</td>
<td>0-1386</td>
</tr>
<tr>
<td>PAL</td>
<td>1.61±0.22</td>
<td>1.27-1.85</td>
<td>1.64±0.22</td>
<td>1.33-2.00</td>
</tr>
<tr>
<td>Sedentary (min/day)*</td>
<td>985±178</td>
<td>743-1261</td>
<td>973±178</td>
<td>606-1235</td>
</tr>
<tr>
<td>Light (min/day)*</td>
<td>341±122</td>
<td>153-521</td>
<td>335±122</td>
<td>159-609</td>
</tr>
<tr>
<td>Moderate (min/day)*</td>
<td>114±63</td>
<td>26-175</td>
<td>132±63</td>
<td>45-224</td>
</tr>
<tr>
<td>Vigorous (min/day)*</td>
<td>7±7</td>
<td>1-19</td>
<td>7±7</td>
<td>1-23</td>
</tr>
</tbody>
</table>

*Sedentary = <1.5 METs; Light = <3 METs; Moderate = ≥3 METs; Vigorous = ≥6 METs

6.2.2. Experimental Design

All participants underwent pre- and post-intervention testing for fasting lipid profile, insulin sensitivity and maximal aerobic capacity. Insulin sensitivity was assessed during an oral glucose tolerance test (OGTT) and aerobic capacity was assessed during an incremental cycling testing to limit of tolerance. The baseline OGTTs were performed two weeks before training commenced and post-training OGTTs were conducted 3 days after the final training bout, at the same time of day as the pre-intervention OGTTs. This meant that there were exactly 8 weeks between the pre- and post-training OGTTs which ensured that female subjects were in the same stage of their menstrual cycle. The V̇O₂peak tests took place 1-2 days after the OGTTs. Given that we included a ‘lifestyle maintenance’ control condition in our previous training study and observed no improvements in our primary endpoints (i.e., insulin sensitivity and maximal aerobic capacity), we did not deem it necessary (or ethical) to include a control condition in the present analysis.
6.2.3. \( \dot{V}O_2 \)peak test

Maximal oxygen uptake capacity (\( \dot{V}O_2 \)max) was determined during an incremental cycling test to the limit of tolerance. However, as more sophisticated equipment (an electronically braked ergometer and an online gas analysis system) became available to us during the course of this study, two different protocols and sets of equipment were used to determine \( \dot{V}O_2 \)max, with protocols kept identical for each subject. We were unable to perform a post-training \( \dot{V}O_2 \)max test in one female participant due to technical difficulties, so \( \dot{V}O_2 \)max data is presented for 16 men and 17 women.

13 participants (6 men and 7 women) completed the test on a mechanically braked cycle ergometer (Ergomedic 874e, Monark, Vansbro, Sweden) with expired air analysed using the Douglas bag method. The test started at 60 W and then increased in increments of 30 W every 2 min until volitional exhaustion. Participants respired
through a rubber mouthpiece connected to a two-way Hans Rudolph valve and were asked to indicate when they felt they were nearing exhaustion, after which expired air was continuously collected into pre-evacuated Douglas bags until the point of actual fatigue (i.e. cadence dropped below 60 rpm for > 5 secs). Expired concentrations of O\textsubscript{2} and CO\textsubscript{2} were measured using paramagnetic and infrared analysers respectively (Servomex miniMP 5200). Douglas bags were then completely evacuated using a dry gas meter (Harvard Apparatus, Kent, UK) and the total volume and temperature (Model C, Edale Intruments, Cambridge, UK) were recorded for calculation of \( \dot{V}O_{2\text{max}} \), \( \dot{V}CO_{2\text{max}} \), \( VE_{\text{max}} \) and \( RER_{\text{max}} \) by indirect calorimetry (Frayn, 1983). All values were corrected to reflect standard temperature and pressures, and during each gas collection, samples of ambient (i.e. inspired) CO\textsubscript{2} and O\textsubscript{2} concentrations were measured within close proximity to the participant (Servomex miniMP 5200) rather than just assuming standard atmospheric concentrations, as has recently been recommended (Betts & Thompson, 2012).

The other 20 participants (10 men and 10 women) completed the test on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) with expired air analysed using an online metabolic cart (ParvoMedics TrueOne 2400, Utah, USA). Participants cycled at 50 W for 5 minutes for determination of submaximal heart rate and substrate oxidation responses, and then a 15 W\textperiodcentered min\textsuperscript{-1} continuous ramp protocol was initiated with participants cycling at a self-selected cadence until volitional exhaustion (i.e., when cadence dropped below 50 rpm for longer than 5 seconds). Participants respired through a rubber mouthpiece connected to a two-way Hans Rudolph valve and continuous breath-by-breath analysis of respiratory volume, flow, and expired levels of O\textsubscript{2} and CO\textsubscript{2} (ParvoMedics TrueOne 2400, Utah, USA) allowed determination of pulmonary gas exchange via indirect calorimetry (Frayn, 1983). \( \dot{V}O_{2} \), \( \dot{V}CO_{2} \), VE and RER responses were first inspected manually in Microsoft Excel using scatterplots and any clear outlying (i.e., not physiological) values removed; a 15-breath rolling average was calculated and the respective maximum for each variable was taken as the highest 15-breath value achieved during the test. In all tests two or more of the following criteria were met: a plateau in \( \dot{V}O_{2} \) despite increasing intensity, RER > 1.15, heart rate within 10 beats of age-predicted maximum, and/or volitional exhaustion (Howley \textit{et al.}, 1995).
6.2.4. Oral Glucose Tolerance Test

Prior to OGTTs subjects were asked to perform no moderate or vigorous intensity physical activities for three days, refrain from drinking alcohol and caffeine for one day, and drink 1 pint of water on the morning of the test to ensure adequate hydration. On the day of the OGTT participants reported to the laboratory between 7:30 and 9:30 am following an overnight fast from 10 pm the previous evening. Having rested quietly for 15 minutes, a cannula (BD Venflon Pro, BD, Helsingborg, Sweden) was inserted into an antecubital vein and a fasting venous blood sample was drawn. Subjects then consumed a drink containing 75 g of glucose dissolved in 300 ml of water (Polycal, Nutricia, UK), and further blood samples were drawn at 30 min intervals for 2 hours. All blood samples were collected into pre-cooled plastic tubes containing EDTA, stored on ice for 30 min, and then centrifuged at 5000 rpm and 4°C for 10 min. The plasma was subsequently dispensed into 0.5 ml aliquots and stored at -80°C until analysis. Plasma glucose concentrations were determined in duplicate on an automated analyser with a CV for repeated measures of <1% (Randox RX Daytona, Co. Antrim, UK). Plasma insulin concentrations were determined in duplicate using a commercially available ELISA kit, with a CV for repeated measures of 3.2% (Mercodia, Uppsala, Sweden).

6.2.5. Training Protocol

All training sessions were carried out on a Monark cycle ergometer (Ergomedic 894e, Vansbro, Sweden). Participants completed three supervised exercise sessions per week for 6 weeks with 1-2 days recovery in between each session, completing 18 sessions overall. All exercise sessions lasted 10 min in total, including a warm up and cool down, which meant a total training time of 30 min per week. Each training session consisted of unloading pedalling and one (1st session) or two (all other sessions) all-out cycling sprints. Just before each sprint, subjects increased their pedal cadence to their maximal speed, a braking force equivalent to 5% of body weight was then applied to the ergometer, and participants sprinted against the applied braking force for a designated time period. The duration of the sprints increased from 10 seconds in week 1, to 15 seconds in weeks 2 and 3, and 20 seconds in the final 3 weeks. Strong verbal encouragement was given during each sprint. An RPE score was also collected at the
end of the 10 min training session to reflect the session as a whole (i.e., participants were asked to consider the whole training session when giving their ratings).

6.2.6. Calculations and Statistics

All variables were analysed using the commercially available Statistics Package for Social Sciences (SPSS) software. In order to simplify the statistical analysis and aid interpretation of a complex data set, the insulin and glucose responses to the OGTT pre- and post-training were converted into simple summary statistics (within subject fasting and peak concentrations, total AUCs and estimates of insulin sensitivity (Cederholm Index (Cederholm & Wibell, 1990)). The impact of ReHIT on body mass, maximal incremental cycling variables, and OGTT summary statistics was analysed by two-way mixed model ANOVA (sex [male, female] × time [pre, post]). ANOVA were performed regardless of any minor deviances from a normal distribution (Maxwell & Delaney, 2004), and with Greenhouse-Geisser corrections applied for contrasts where \( \epsilon < 0.75 \) and the Huynh-Feldt corrections applied for less severe asphericity (Atkinson, 2002). Correlations between variables were determined using a Pearson's product-moment correlation coefficient. Statistical significance was accepted at \( p < 0.05 \) and, unless otherwise stated, data is presented as mean ± SD.

![Figure 6.2. Schematic of the ReHIT training protocol. Grey boxes represent ‘all-out’ sprints against a fixed resistance of 5% of body mass](image)
6.3. Results

29 out of the 34 participants completed all 18 training sessions, i.e., 100% adherence. 2 participants missed a total of 3 non-consecutive sessions over the course of the 6 weeks (83% adherence), whilst 3 missed 1 session (94% adherence), meaning the overall adherence to the training programme was 98%. All subjects have been retained in the final analysis.

Whole-session rating of perceived exertion scores taken at the end of each training week are shown in Figure 7.2. The training sessions were well tolerated by all participants and rated consistently at ~14 on the Borg 6-20 scale (i.e., between ‘somewhat hard’ and ‘hard’). There were no differences in the ratings given by male and female participants.

![Figure 6.3](image)

**Figure 6.3.** Whole session RPE scores during ReHIT in men and women. RPE scores were taken at the end of each training week. Data is presented as mean ± SD

6.3.1. Changes in Body Mass

There was a small but significant increase in body mass (main effect for time, P<0.05) and a tendency for increased BMI (main effect for time, p=0.06) following ReHIT with no significant impact of sex upon this effect (Table 6.2).
6.3.2. \( \dot{V}O_2 \text{max} \)

An independent t-test performed on absolute change in \( \dot{V}O_2 \text{max} \) (l\( \cdot \)min\(^{-1} \)) revealed no significant difference between the two protocols used, so the data was pooled. \( \dot{V}O_2 \text{max} \) increased in both men (+9%) and women (+10%) following ReHIT (Figure 6.3, main effect for time, p<0.05), and this was associated with an increased time-to-exhaustion (main effect for time, p<0.05), an increase in maximal CO\(_2\) production (main effect for time, p<0.05), but no significant change in maximal minute ventilation, maximal RER or maximal heart rate (Table 6.2). There was no mediating effect of sex on any of the maximal exercise responses that were measured. Although the mean \( \dot{V}O_2 \text{max} \) improved in both men and women with ReHIT, there was considerable individual variability associated with this response (+240±220 ml O\(_2\); Figure 6.7). 15 subjects demonstrated a response that was within 5% of the mean change, 7 subjects showed a much greater than average response (17 to 34% increase) and 11 subjects showed either no change or a poor response (7% decrease to 4% increase). The likelihood of showing a low/average/high response for \( \dot{V}O_2 \text{max} \) did not appear to be influenced by sex (Figure 6.7), and there was no correlation between baseline \( \dot{V}O_2 \text{max} \) and the subsequent training response (\( r^2=0.08 \)).

**Figure 6.4.** Changes in maximal oxygen uptake following ReHIT. N=16 for men and n=17 for women. * denotes p<0.05 for main effect of time (i.e., pre- vs. post-training). Data is presented as mean ± SD.
6.3.3. Plasma Glucose and Insulin Responses to the OGTT

Mean fasting and peak plasma glucose concentrations were unaltered in both men and women in response to ReHIT (Table 6.2), as was the total glucose AUC during the OGTT (Figure 6.4A and 6.4B). Similarly, there were no changes in mean fasting and peak insulin concentrations (Table 6.2); however, there was a trend for reduced insulin AUC following ReHIT (P=0.076 for time main effect) with no sex × time interaction (Figure 6.4C and 6.4D). Insulin sensitivity as estimated using the Cederholm Index remained unchanged following training (Figure 6.5).

Figure 6.5. Plasma glucose and insulin responses to the OGTTs. Plasma glucose (A) and insulin (C) responses to the OGTT are presented as mean ± SEM for clarity. Total plasma glucose (B) and insulin (D) AUC in response to the OGTTs are presented as mean ± SD. N=16 for men and n=18 for women. For men, the 0, 30 and 90 minutes OGTT points are n=15; for women 30, 60 and 90 minute OGTT points are n=15, n=17 and n=15, respectively, due to difficulties during blood draws.
Similar to the VO$_2$max responses, there was considerable variability associated with the training induced change in glucose AUC (mean ± SD: -2.4±17%; range: -24 to +62%), insulin AUC (mean ± SD: -0.7 ± 28.1%; range: -53.7 to +69.6%) and insulin sensitivity (mean ± SD: +5.8±21.2%; range -48.2 to 55%), which can be seen in figure 6.7. There was a significant but weak correlation between the pre training value and the change score (%) for fasting glucose (r$^2$= 0.14, p<0.05), fasting insulin (r$^2$=0.13, p<0.05), glucose AUC (r$^2$=0.18, p<0.05), insulin AUC (r$^2$=0.14, p<0.05) and Cederholm Index (r$^2$=0.19, p<0.01).

Figure 6.6. Changes in insulin sensitivity following ReHIT. N=16 for men and n=18 for women. Data is presented as mean ± SD
Table 6.2. The impact of ReHIT on BMI, maximal gas exchange, and fasting and peak plasma glucose and insulin concentrations

<table>
<thead>
<tr>
<th></th>
<th>Males (n=16)</th>
<th>Females (n=18)</th>
<th>Combined (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>76.9±7.2</td>
<td>77.6±7.6*</td>
<td>66.2±9.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1±2.1</td>
<td>25.3±2.2†</td>
<td>23.8±3.5</td>
</tr>
<tr>
<td>Time to Exhaustion (secs)</td>
<td>887±163</td>
<td>986±133*</td>
<td>651±166</td>
</tr>
<tr>
<td>VO₂max (L/min)</td>
<td>2.95±0.56</td>
<td>3.22±0.54*</td>
<td>2.08±0.30</td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>38.6±8.3</td>
<td>41.9±8.5*</td>
<td>32.0±4.5</td>
</tr>
<tr>
<td>VCO₂max (L/min)</td>
<td>3.62±0.72</td>
<td>3.90±0.76*</td>
<td>2.51±0.31</td>
</tr>
<tr>
<td>VE max (L/min)</td>
<td>110.6±35.9</td>
<td>110.8±29.3</td>
<td>77.0±13.0</td>
</tr>
<tr>
<td>RER max</td>
<td>1.23±0.09</td>
<td>1.23±0.08</td>
<td>1.23±0.09</td>
</tr>
<tr>
<td>HR max (beats/min)</td>
<td>192±10</td>
<td>190±12</td>
<td>187±9</td>
</tr>
<tr>
<td>Fasting Glucose (mmol/l)</td>
<td>5.29±0.47</td>
<td>5.25±0.51</td>
<td>4.96±0.46</td>
</tr>
<tr>
<td>Fasting Insulin (mU/ml)</td>
<td>5.7±3.3</td>
<td>6.6±3.4</td>
<td>5.6±4.0</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.30±0.93</td>
<td>1.47±0.95</td>
<td>1.29±1.02</td>
</tr>
<tr>
<td>Peak Glucose (mmol/l)</td>
<td>9.26±1.68</td>
<td>8.91±2.17</td>
<td>7.41±1.69</td>
</tr>
<tr>
<td>Peak Insulin (mU/ml)</td>
<td>111.3±82.0</td>
<td>97.9±51.5</td>
<td>61.3±43.2</td>
</tr>
</tbody>
</table>

All data are shown as mean ± SD. * denotes p<0.05 pre- vs post-training. † denotes p=0.06 pre vs. post training. Maximal cycling and gas exchange data for females are shown for n=17 (combined n=33) due to technical difficulties during the post-training assessment.
Figure 6.7. Variability in training responses following ReHIT. Dots represent the training adaptation for individual subjects (VO₂max n=33, OGTT variables n=34) as a percentage of their individual baseline (i.e., 90% represents a 10% decrease, 110% represents a 10% increase). Green dots are female and red dots are male subjects. Note that for VO₂max and insulin sensitivity (Cederholm) an ‘improvement’ is represented by a % increase, whilst for glucose AUC and insulin AUC an ‘improvement’ is represented by a % decrease.
6.4. Discussion

The current data confirm that ReHIT improves \( \text{VO}_2\text{max} \) to a similar extent in sedentary men and women; however, in contrast to our previous observations, mean improvements in insulin sensitivity were not evident in either sex, although it is worth highlighting that there was a statistical trend for a reduction in insulin AUC post training. The current study raises some concerns over the efficacy of ReHIT as a strategy for improving insulin sensitivity, and highlights the potential problems with studies utilising small sample sizes in the studies of metabolic responses to exercise training.

6.4.1. Insulin Sensitivity Responses with ReHIT

The current study is the largest to date to examine the impact of HIT on insulin sensitivity. We could not detect any mean improvements in OGTT estimated insulin sensitivity in men or women following 6-wks of ReHIT. This is in contrast both with previous work in this thesis (Chapter 3) and recent data from Gillen et al. (2014) who showed improvements in glycaemic control in men (but not women) after a ReHIT like protocol using 24-hr CGM measurements under standardised dietary conditions. They also provided direct biochemical support for this finding by showing a similar sex-dependent increase in muscle GLUT4 protein content post-training (Gillen et al., 2014). There are a number of potential explanations for the differences between current findings and previous work which require discussion. Firstly, it is possible that altering the sprint load (7.5% to 5% body mass) diminishes the adaptive response for insulin sensitivity in men and not alter the response in females. Secondly, it cannot be discounted that ReHIT does not, in either of its current forms, results in improved insulin sensitivity in either men or women. Thirdly, as there is naturally a high level of variability associated with exercise training induced improvements in insulin sensitivity the variation observed in the current study may have masked the impact of ReHIT on insulin sensitivity. Each of these possibilities will be discussed next.

Firstly, in the current study we substituted the 60-W warm-up for unloaded pedalling and reduced the mass applied during the sprints from 7.5% to 5% of body mass. Therefore, we were examining the impact of an altered training intervention in the current study, and it is logical to question whether this has impacted on the alterations in observed training response. Indeed, whilst Gillen et al. (2014) also used an applied
resistance of 5% body mass, they included an extra 20-s sprint during their 10-min training sessions and this may have compensated for any reduction in applied resistance. Although we noted similar improvements in VO$_2$max in the current study, there was no correlation between the change in VO$_2$max and the change in insulin sensitivity, implying that the determinants of these two adaptations are distinct. However, it is important to point out that an ‘all-out’ sprint against 5% body weight still produces an average exercise intensity in the region of ~200% of peak aerobic power (W$_{max}$) and, physiologically, it is likely that this would be associated with a very similar muscular and whole-body metabolic disturbance (Chapter 4 and Chapter 5). Therefore, although it cannot be proven at this point, it seems likely that the training stimulus provided in the current study would have been of similar (and sufficient) magnitude to that which was used previously.

Alternatively, it is possible that our positive finding in Chapter 2 was the result of a type 1 statistical error, and it could simply be the case that ReHIT just does not provide any benefit to insulin sensitivity. In agreement with this possibility are a number of studies which, using a variety of HIT models, have been unable to detect any statistically significant mean change in OGTT derived measures of insulin sensitivity at similar post-training time points (Whyte et al., 2010; Gillen et al., 2013). This possibility is more difficult to refute; however, there are several pieces of information which require further discussion. Firstly, it is particularly noteworthy that the reduction in insulin AUC following training approached statistical significance. In addition, analysis of the individual responses (figure 6.7) reveals that for each OGTT-derived variable, the majority of subjects showed evidence of some ‘improvement’ post-training (62%, 58% and 62% of subjects for glucose AUC, insulin AUC and insulin sensitivity, respectively). For each variable we also observed particularly poor responses in several subjects, a phenomenon that is not limited only to the current study (Boulé et al., 2005; Bouchard et al., 2012; Kuehnbaum et al., 2014). In the context of relatively low statistical power (n=34) and large interindividual variability, such extreme low and high responders can have a large effect on the statistical outcome. Indeed, the observation that ~40% of our subjects demonstrated no measurable change (or a worsening) in insulin sensitivity with ReHIT is strikingly similar to reports with high volume aerobic exercise training (Boulé et al., 2005), the major difference being that Boulé et al trained over 600 subjects and were therefore able to detect a ~10% average increase in insulin sensitivity in their
cohort. Of course, it is possible that these observations simply represent physiological ‘noise’ associated with performing repeated OGTTs eight weeks apart. The observed weak correlations between baseline and subsequent training response for each OGTT-derived variable would indeed suggest a certain amount of ‘regression to the mean value’ in our current data set. However, without a ‘lifestyle maintenance’ control group it is difficult to estimate the error associated with our measurement, and this represents a limitation to the current analysis. At this point, we cannot draw any firm explanations or conclusions on these data. Further studies with much larger sample sizes will be required to determine the true impact of ReHIT on insulin sensitivity.

### 6.4.2. Aerobic Capacity Responses with ReHIT

Maximal aerobic capacity improved on average by ~10% in both men and women following ReHIT in the current analysis, confirming our previous observations from Chapter 3. Whilst improved aerobic capacity is now a well-established finding with HIT (Weston et al., 2014b), this finding remains important as ReHIT still represents the smallest volume of sprint exercise which has been shown to improve this parameter. The fact that VO_{2max} appears to improve so consistently following such a small volume of maximal exercise would suggest that intensity is the key variable underpinning adaptations in the oxygen transport system following exercise training in humans. This has implications for exercise prescription given that aerobic capacity is a powerful predictor of cardiovascular and metabolic disease (Blair et al., 1989; Blair et al., 1995; Blair et al., 1996; Myers et al., 2002).

Aerobic capacity is thought to be limited by central and peripheral oxygen delivery rather than peripheral oxygen utilisation (Bassett & Howley, 2000). As such, changes in aerobic capacity are thought to be explained by structural, functional and compositional changes in the central and peripheral cardiovascular system resulting in enhanced maximal cardiac output and hence oxygen delivery to the periphery (Bassett & Howley, 2000). Recent work suggests that expansion of blood volume may be of key importance, since removing the individually calculated training-induced change in blood volume via phlebotomy simultaneously abolishes any improvement in both maximal cardiac output and aerobic capacity (Bonne et al., 2014). In addition, in a selectively bred murine model, Lessard et al. (2013) demonstrated that low responders for exercise
capacity with aerobic training also exhibit an impaired angiogenic response in skeletal muscle, suggesting that increased muscle capillarisation with training might be of crucial importance. There are currently no studies which have examined the change in blood volume with HIT, however, Cocks et al. (2014) have shown that HIT and endurance exercise are equally as effective at increasing muscle microvascular density. Mechanisms underlying the increase in blood volume and capillary density are incompletely understood, but are likely related to a number of factors, including contractile activity; exercise-induced increases in blood flow, blood pressure and shear stress; localised reductions in O\textsubscript{2} tension; and signalling mechanisms related intramyocellular metabolic processes (Gustafsson et al., 1999; Egginton, 2009; Wagner, 2011). From this perspective, it is noteworthy that HIT/ReHIT is associated with an exaggerated acute cardiorespiratory response, resulting in rapid increases in heart rate (and presumably blood pressure and shear stress), prolonged elevations in oxygen uptake, and a substantial drop in plasma volume (Chapters 3 and 4). Linking these acute responses with training induced adaptations will be a fruitful area for future research.

Although aerobic capacity improved on average following ReHIT it should be noted that this improvement was still associated with substantial variation. Indeed ~10% of our subjects demonstrated no measurable response for VO\textsubscript{2}max and several others responded poorly. Equally, our sample also included several individuals who showed a particularly exaggerated improvement (~30% increase from baseline), i.e., high responders. The existence of such variation in trainability for VO\textsubscript{2}max with aerobic training has been known for many years (Bouchard et al., 1999; Sisson et al., 2009), and this issue has lately been highlighted in response to HIT as well (Astorino & Schubert, 2014). At least part of this variability is explainable by genetic factors (Bouchard et al., 1999); in fact, Timmons et al. (2010) have now validated a set of predictor genes which can establish the magnitude of change in aerobic capacity prior to the initiation of aerobic training. An important question that will need to be answered is whether the same predictor can be used to establish the variability in aerobic capacity responses to different modes of training, for example, HIT/ReHIT. This would appear likely since the predictor genes are not responsive to exercise, but rather their expression appears pre-set and related to processes such tissue development (e.g., angiogenesis), which appears a key mechanism underpinning improvement in aerobic capacity with aerobic exercise and HIT (Timmons et al., 2010; Cocks et al., 2013; Lessard et al., 2013).
6.4.3. Conclusions

In summary, this study represents the largest study to date of the effects of HIT upon cardiometabolic health. We confirm that 2 min of all out intermittent exercise per week for 6 weeks improves maximal aerobic capacity in sedentary men and women; however, the previously observed beneficial impact upon insulin sensitivity was not apparent. The variability for changes in OGTT derived variables was extremely high and warrants further investigation.
Chapter 7

General Discussion
Chapter 7

General Discussion

7.1. Overview

From the perspective of public health, it is well known that regular exercise, in general, is a beneficial behaviour. Indeed, whilst many questions remain unanswered, there is a wealth of descriptive literature demonstrating that various exercise interventions, on average, acutely and chronically modulate key cardiometabolic risk factors in a direction that would be expected to reduce the risk of developing chronic disease (Garber et al., 2011). In fact, regular exercise alone, or in combination with other lifestyle interventions (e.g., ‘healthier diet’) has been shown to reduce the risk of developing T2D by at least 50% (Pan et al., 1997; Knowler et al., 2002). When one then considers the detrimental impact of enforced periods of physical inactivity on a range of cardiometabolic risk factors (Krogh-Madsen et al., 2010; Knudsen et al., 2012), and epidemiological evidence detailing the independent relationship between sedentary behaviours and chronic disease risk (Helmrich et al., 1991; Hu et al., 1999; Laaksonen et al., 2002; Grøntved & Hu, 2011), the need to become and remain physically active becomes even more apparent. Nevertheless, the level of exercise participation in general population remains poor (Allender et al., 2008a).

As a result, the challenge now being undertaken by many exercise physiologists is not solely to establish the impact of exercise upon human health, but also to search for exercise modalities which may help to promote motivation to exercise, and exercise adherence in the general population. In particular, many researchers have focused on overcoming the so-called ‘exercise time barrier’, as this is commonly reported as the major barrier to exercise participation in the general population (Reichert et al., 2007; Korkiakangas et al., 2009). High-intensity interval training (HIT) has shown promising results from this perspective; indeed, when performed repeatedly over a period of a few weeks, HIT yields substantial gains in aerobic capacity, oxidative capacity, insulin sensitivity, and vascular function, for example (Gibala et al., 2006; Burgomaster et al., 2008; Rakobowchuk et al., 2008; Babraj et al., 2009; Richards et al., 2010; Cocks et al., 2013; Shepherd et al., 2013; Weston et al., 2014b). What’s more, there is some evidence that participants find HIT a more enjoyable mode of exercise training (Bartlett et al.,
However, the extrapolation that HIT would stimulate greater exercise adherence based on these findings currently lacks empirical evidence. Indeed, at this point in time, this is largely irrelevant as major questions remain concerning the optimal structure of a HIT intervention (in terms of mode, frequency, duration, intensity of sprints and recovery), both from the perspective of promoting healthy adaptations and in terms of suitability for the general population. Specifically, the HIT models that have been studied to date, whilst generally effective at improving physiological functioning, require very high levels of participant motivation, are very fatiguing, can be associated with feelings of nausea, and/or are still associated with a total time commitment of >20 minutes per session (Trapp et al., 2008; Little et al., 2010; Richards et al., 2010; Gillen et al., 2013). As such, it seems unlikely that these protocols would be suitable for recommendation to the general population.

The research presented in this thesis has attempted to overcome some of the concerns with HIT protocols to date. The initial aim was to design a HIT protocol that was genuinely associated with reduced total time-commitment and also lower levels of exertion, and characterise its effect on biomarkers of human health in the context of a training intervention. Having designed an intervention requiring a total of 2 × 20 sec sprints in a 10 min training session (reduced-exertion HIT; ReHIT), and shown promising improvements in aerobic capacity (men and women) and insulin sensitivity (men only) in Chapter 2, subsequent studies were undertaken to examine acute effects upon insulin sensitivity (Chapter 3), skeletal muscle and whole-body metabolic responses (Chapters 4 and 5) and potential effects upon appetite (Chapter 5) with ReHIT. Chapter 6 aimed to further establish the training effects of a modified ReHIT protocol upon insulin sensitivity and aerobic capacity in a larger cohort of sedentary men and women.

7.2. Discussion of Major Findings

7.2.1. Effects of ReHIT on Insulin Sensitivity

Insulin sensitivity represented a primary outcome measure in the current thesis based on its relevance for the development of metabolic and cardiovascular disease (Balkau & Eschwege, 1999; Petersen et al., 2007; Lorenzo et al., 2010). Indeed, the
ReHIT intervention was designed specifically to improve insulin sensitivity based on the hypothesis that glycogen degradation during exercise may underpin the adaptations in whole-body insulin sensitivity with exercise training (Chapter 2 and 6). However, the two training studies carried out in this thesis have revealed contrasting results. Neither study has revealed mean improvements in insulin sensitivity in women; on the other hand mean improvements were apparent in men in Chapter 2, but not in Chapter 6. In combination, a total of 49 sedentary individuals have been ReHIT trained for the studies in this thesis, including 23 men and 26 women. The combined average responses for glucose AUC and insulin AUC are shown below (figure 7.1).

Figure 7.1. The effect of ReHIT on glucose and insulin AUC (n=49). Data shown is training responses combined from Chapters 3 and 7 (mean ± SD). The statistics shown represent two way ANOVA (sex × time) on combined data. *P<0.01 pre vs. post in men.

Overall, the combined results from this thesis tentatively suggest that ReHIT, consisting of a maximum of 40 seconds of intense exercise per session, may improve insulin action on average in men, but not in women. This contention is supported by recently published data from Martin J Gibala’s laboratory which showed a similar sex-dependent improvement in glycaemic control (measured via CGM) 72 h following a ReHIT like intervention (Gillen et al., 2014). However, it is recognised that the coefficient of variation for repeated measures of insulin sensitivity over 6 weeks under control conditions using the OGTT are high (13% in study 1), and this makes it difficult to pick up the true signal of HIT in the present thesis. Thus, these results will require confirmation in a further much larger study, ideally using a randomised controlled
research design. In agreement with the majority of previous (Brestoff et al., 2009; Richards et al., 2010; Whyte et al., 2013) but not all (Ortega et al., 2014) previous literature, there was no impact of a single ReHIT session on insulin sensitivity 14 h post-exercise (Chapter 4), so the utility of ReHIT for improving insulin sensitivity may be limited to training adaptations. Nevertheless it is important to consider temporal issues with regards to the assessment of insulin sensitivity in the training studies in this thesis. Specifically, we used a 3 day post-training time point for our assessment and it is possible that insulin sensitivity may have been improved (either more consistently or to a greater extent) in the days prior to the assessment. Further studies will be required to appraise this caveat.

7.2.2. Effects of ReHIT on Aerobic Capacity

Aerobic capacity was consistently increased in both men and women following ReHIT, with an average improvement of ~10% (Chapters 2 and 6). Whilst changes in insulin sensitivity were the primary target with ReHIT, the consistent improvement in aerobic capacity arguably has greater clinical/preventative utility given that aerobic fitness consistently manifests as the strongest predictor of future morbidity and mortality (Blair et al., 1989; Barlow et al., 1995; Blair et al., 1995; Blair et al., 1996; Lee et al., 1998; Lee et al., 1999; Myers et al., 2002). Based upon the results of Lee et al. (2011) the average increase of ~3-4 ml/kg/min in VO$_2$max (i.e., ~1 metabolic equivalent) would be expected to reduce the risk of all-cause mortality by 15% and cardiovascular mortality by 19%, respectively.

7.2.3. Sex Differences in the Adaptive Response to HIT/ReHIT

Until this programme of research was undertaken, women were, like in many physiological fields, a relatively understudied population in response to HIT. This thesis has revealed potential gender differences in the response to supervised ReHIT training, with females showing no average improvement in insulin sensitivity (Chapter 2 and 6; Figure 7.1). This observation is now supported by several studies using varying models of HIT (Gillen et al., 2013; Gillen et al., 2014). However, it is important to point out that a number of the female participants from all three of these studies did show an improvement in insulin sensitivity ((Gillen et al., 2013; Gillen et al., 2014); Figure 7.2).
Likewise, Richards et al. (2012) showed a consistent improvement in insulin sensitivity measured using the gold standard euglycaemic clamp method following 2 weeks of classic HIT in females. Therefore, it remains unclear at this point whether the lack of average improvement in insulin sensitivity in women is a genuine finding, or a chance product of a large number of ‘non-responders’ in the context of relatively small sample sizes. Only with much larger studies that employ a greater level of control over, for example the menstrual cycle amongst other factors, will this question be able to be definitely answered (Gibala et al., 2014).

Several studies in females have reported improvements in aerobic capacity, skeletal muscle oxidative potential, and changes in vascular function following HIT (Astorino et al., 2011; Trilk et al., 2011; Astorino et al., 2013a; Astorino et al., 2013b; Gillen et al., 2013; Gillen et al., 2014). Likewise, in this thesis ReHIT was associated with a marked improvement in aerobic capacity in women (Chapters 3 and 7). Thus, in spite of the question marks over the utility for improving insulin sensitivity in women, HIT/ReHIT remains associated with metabolic and vascular changes linked to improved health outcomes. The dissociation between average changes in aerobic capacity, muscle oxidative potential and insulin sensitivity in women in this thesis suggest that the molecular determinants of these adaptations are likely also to be distinct.

In light of the suggestion that ReHIT doesn’t improve insulin sensitivity on average in women, the potential underlying mechanisms for this finding are worthy of further discussion. Firstly, it is important to further highlight that a consistent finding in this thesis is that women on average had a higher level of baseline insulin sensitivity compared with men (Chapter 2 and 6). This is consistent with previous observations (Nuutila et al., 1995; Boulé et al., 2005; Karakelides et al., 2010) and might impact upon the capacity for a subsequent training response. The modest correlation between the baseline level of insulin sensitivity and the subsequent training response noted in the female participants in this thesis may reflect this fact ($R^2 = 0.21; P<0.05; n=26$), or may indicate a level of ‘regression to the mean value’ in this data set. In any case, the correlation is only modest and therefore the variation in training responses is not completely explained by the baseline level.

In Chapter 2 it was hypothesised that differences in the amount of glycogen broken down during a HIT/ReHIT training session may explain sex differences in the
training response for insulin sensitivity. This hypothesis was based on previous studies which had shown that women break down up to 42% less glycogen in type 1 muscle fibres during a typical ‘all-out’ 30 sec sprint (Esbjornsson-Liljedahl et al., 1999; Esbjornsson-Liljedahl et al., 2002), a finding which is supported both by the smaller excursion in blood lactate (Gratas-Delamarche et al., 1994; Esbjornsson-Liljedahl et al., 2002), and a greater contribution of aerobic metabolism to ATP demand (Hill & Smith, 1993), in women during sprint exercise. The initial goal of Chapter 6 in this thesis was to examine potential sex-differences in glycogen breakdown, glycogen related signalling and GLUT4 gene expression with ReHIT. However, as it was not possible to obtain usable muscle samples in all of our participants a meaningful gender-comparison was not feasible. Nevertheless, preliminary data from 5 men and 6 women suggest similar glycogen breakdown between sexes when measured in whole muscle biopsy samples (Mean ± SD; Men 71 ± 38 vs Women 105 ± 63 mmol/kg dry weight). However, it would be informative to test whether there are sex-differences in glycogen breakdown in distinct fibre types, or even potentially in distinct subcellular stores of glycogen as different fibre types and subcellular glycogen stores are related to distinct physiological functions (Nielsen et al., 2010a; Nielsen et al., 2010b). On the other hand, recent evidence has suggested that females have lower levels of total muscle and mitochondrial protein accumulation over the course of a 6-week HIT intervention (Scalzo et al., 2014). At the same time, a recent study demonstrated a smaller increase in muscle GLUT4 protein content following 6-weeks of HIT (Gillen et al., 2014). Therefore, the diminished adaptive response in women may be unrelated to glycogen breakdown and the hypothesised transcriptional response per se; perhaps instead there is down-regulation of the pathways that link modulations in energy metabolism (and resulting gene transcription) and the protein synthetic response, which ultimately defines the change in phenotypic function (i.e., insulin sensitivity).

7.2.4. *The importance of human variation in the adaptation to exercise*

Perhaps the most important discussion point from the current thesis concerns the issue of human variation in the adaptive response to exercise training. For example, the change in insulin sensitivity with ReHIT ranged from a 100% increase to a 45% reduction (Figure 7.3A). Likewise, the change in VO2max with ReHIT ranged from a gain of 730 ml of oxygen, to a reduction of 290 ml of oxygen (Figure 7.2C). The
observed variation in response to ReHIT shows a remarkably similar pattern to that observed in the HERITAGE family study, the largest supervised aerobic exercise training study to date ([Boulé et al., 2005]; figure 7.3). Indeed, such variability in cardiovascular and metabolic adaptation to exercise training has been recognised for several years, and is in fact a hallmark feature of all major disease risk biomarkers in all large exercise training studies (Bouchard et al., 1999; Boulé et al., 2005; Sisson et al., 2009; Bouchard et al., 2012). Perhaps more importantly, in some individuals, an adverse change in some, or several, disease risk biomarkers may be observed (Bouchard et al., 2012). It should however be noted that the biological basis for an adverse metabolic response to physical activity per se is unclear and this finding needs to be confirmed in the context of a large randomised controlled trial. Indeed, the deterioration of metabolic health parameters under ‘lifestyle maintenance’ control conditions is a common finding in human research studies (Patel et al., 2011). Irrespective of this, however, the variability in exercise-induced adaptation and the existence of low responders remains an important caveat to all exercise related research.

One of the major issues is that such variation is often ignored in favour of presenting a mean ‘effect or improvement’ associated with an intervention, a method which completely overlooks the stark reality that many individuals will not experience this proposed improvement (Vollaard et al., 2009; Timmons, 2011). The ramifications of such an approach to data analysis become immediately apparent when we consider that public health recommendations on levels of exercise are largely based upon these average responses. Of course this one-size-fits-all approach is largely driven by the need to convey a simple public health promotion message (Timmons, 2011), but crucially has limited utility for any given individual starting a new exercise programme. In fact, if the adverse response hypothesis (Bouchard et al., 2012) turns out to be physiologically valid then this approach arguably contrasts one of the first ethical principles of modern medicine: ‘primum non nocere’, ‘first, do no harm’ (Smith, 2005).
Figure 7.2. Variability in insulin sensitivity (A+B) and aerobic capacity (C+D) changes with ReHIT (A+C) and aerobic exercise (B+D). A+C: combined data from Chapters 2 and 6 (i.e., individual responses to ReHIT). B+D: modified from Boulé et al. (2005) and Bouchard and Rankinen (2001), respectively; this data comes from the HERITAGE family study (i.e., individual responses to standardised aerobic exercise).

The clear importance of the variation in the metabolic adaptation to exercise training has led investigators in the logical direction of searching for methods of predicting the magnitude of adaptation to exercise prior to the start of an intervention. As both ∆VO₂max and ∆Insulin Sensitivity cannot, in any large magnitude, be explained by basic descriptive traits (e.g., age, weight, body composition etc.), and variance in single genes appear also to explain little of the variance (Gill & Malkova, 2006; Bouchard et al., 2011), this research has moved towards using more global molecular OMIC (e.g., genomics, transcriptomics, metabolomics) techniques (Timmons et al., 2010; Bouchard et al., 2011). The majority of research has focused on predicting ∆VO₂max with exercise training and has been discussed previously in this thesis (Chapter 6). The robustness of the aerobic capacity predictor likely centres around a combination of three factors: 1) at least 50% of both baseline and ∆VO₂max are
explained by genetic variance (i.e., a relatively large portion); 2) apart from physical activity, there are few (if any?) free-living factors that impact upon VO$_2$max, in other words, the changes in VO$_2$max observed following a supervised exercise intervention are likely to be solely explained by the change in physical activity behaviour; and 3) the mechanisms contributing to changes in VO$_2$max with chronic exercise training are well understood and largely limited to the cardiovascular system. In contrast, insulin sensitivity (particularly when measured at the whole-body level) requires the coordination of multiple tissues which each can be influenced by a multitude of environmental factors modified by the underlying genetic profile. In addition, whilst there appears to be a genetic influence over insulin sensitivity and the development of T2D (Poulsen et al., 1999), the heritability of changes in response to chronic exercise training have not been published. However, it is clear that variance in single genes explain little of the training response and are inconsistent across different populations (Gill & Malkova, 2006). Thus the development of predictive biomarkers for changes in insulin sensitivity with exercise training is likely to be extremely challenging. Nevertheless, a large scale clinical trial (NCT01920659; www.metapredict.eu) is currently underway with the aim of developing such biomarkers and the data presented in Chapter 6 of this thesis will be used to validate this predictor.

### 7.2.5. The role of glycogen in adaptation to exercise

The studies in this thesis have not directly assessed the role of glycogen in insulin sensitivity adaptation to exercise training. However, the hypothesis that glycogen has a role in exercise induced adaptation has been a central justification of several chapters presented (Chapters 2, 5 and 6). In this thesis there was some evidence that targeting glycogen degradation with ReHIT resulted in similar acute responses (e.g. AMPK activation, PGC-1α and GLUT4 mRNA expression) and chronic adaptation (improvements in insulin sensitivity and VO$_2$max) compared with HIT protocols utilising a greater number of sprints (Babraj et al., 2009; Gibala et al., 2009; Richards et al., 2010). However, we did not directly compare the effects of ReHIT (2 × 20 s) against the classic HIT protocol (4-7 × 30 s) so this contention is unsubstantiated at present. It may equally be that other metabolic disturbances (unrelated to glycogen degradation) that are unique to the early portion of an ‘all-out’ sprint (e.g., rise in AMP, Ca$^{2+}$, and oxidative stress) underpin such acute responses and chronic adaptations. It is clear that
glycogen can play a regulatory role in muscle adaptation under certain conditions (e.g. low glycogen training) (Morton et al., 2009; Hulston et al., 2010), but whether or not smaller amounts of glycogen turnover are associated with similar adaptive effects remains speculative.

7.3. General Discussion Points

7.3.1. Why is HIT so potent?

The observation that as little as 40 sec of intense exercise is associated with such a robust change in aerobic capacity, and potentially in insulin sensitivity, raises the question as to why this type of exercise is such a potent stimulus for adaptation. Of course the answer in its entirety is likely to be complex, but at the most rudimentary level is probably explained by three basic principles of exercise physiology: overload, supercompensation and specificity. Briefly, a single bout of exercise will cause a disruption of homeostasis (i.e., overload). So that the system is better able to deal with a similar disturbance in the future, an adaptive process occurs whereby homeostasis is returned to a higher level than before the bout of exercise (i.e., supercompensation). Importantly, the nature of the adaptations is specific to system that has been overloaded.

It is clear that, whilst brief, the short ‘all-out’ sprints which constitute a ReHIT exercise session present an extreme challenge to both muscular metabolism and the cardiovascular system (Chapters 4 and 5), for example resulting in rapid reduction in muscle glycogen, large excursions in blood lactate, overloading of muscle and blood buffering mechanisms, a drop in plasma volume, rapid increases in heart rate (and likely blood flow, blood pressure, shear stress and altered oxygen tension) and a prolonged increase in oxygen consumption (Chapters 4 and 5). For many sedentary individuals, who in some cases will have been sedentary for the majority of their life, this is likely the first time they (i.e., their muscle and cardiovascular system) will have experienced such a dramatic disturbance in homeostasis. In addition, an often overlooked principle of exercise physiology, which is important in understanding the effects of exercise intensity on metabolic parameters, relates to the pattern of fibre recruitment (Mcphedran et al., 1965; Gordon et al., 2004). According to this principle, a contracting muscle during low-moderate exercise contains both active and inactive muscle fibres; as
exercise intensity increases further muscle fibres are recruited, and only during maximal exercise are the vast majority of muscle fibres ‘active’ (Mcphedran et al., 1965; Gordon et al., 2004). As such, compared with moderate intensity exercise, HIT/ReHIT is associated with the activation of a much larger muscle mass and, in turn, a greater number of fibres will adapt. This may have particular implications in terms of insulin action and metabolic control, as a larger portion of ‘metabolically hungry’ tissue will be available to take up glucose and to impact upon whole-body metabolic parameters (Defronzo & Tripathy, 2009). Likewise, it is likely that the modest disturbances in metabolic homeostasis experienced during moderate intensity continuous aerobic exercise require more prolonged and more frequent exercise bouts in order to activate relevant signalling pathways and initiate metabolic adaptations. In contrast, the disturbance in homeostasis with HIT predominantly occurs at the start of the initial sprint and is not enhanced much further with longer or repeated sprints (Parolin et al., 1999).

In addition, certain metabolic changes, for example a drop in cytosolic ATP concentration, are unique to ‘all-out’ sprint exercise (Esbjornsson-Liljedahl et al., 1999; Esbjornsson-Liljedahl et al., 2002). The fact that the majority of the disturbance of physiological homeostasis occurs in the first portion of the first sprint may explain why the induced adaptations, for example an increase in VO\textsubscript{2}max, are similar for HIT protocols which utilise few and many, as well as short and long, sprint efforts so long as the required intensity is high enough (Hazell et al., 2010; Tjønna et al., 2013).

### 7.3.2 Insulin Sensitivity: Improvement or Maintenance in Healthy Individuals?

The studies in this thesis have largely been concerned with the impact of acute and chronic exercise on whole-body insulin action and glucose tolerance; in other words the changes in circulating blood glucose and insulin concentrations in response to standardised glucose loads. It is important to point out that the studies presented in this thesis have examined metabolically healthy individuals who, whilst sedentary, had no evidence of impaired glycaemic control. In such individuals, it could logically be argued that preventing the deterioration of insulin sensitivity over time could be just as important clinically as showing robust improvements in insulin sensitivity. In this regards, the assessment of postprandial insulin and glucose concentrations only represents one aspect of the potential effects of regular exercise. Perhaps as equally important in this population could be changes in the metabolic fate of ingested nutrients.
(Petersen et al., 2007; Rabøl et al., 2011). For example, a single bout of aerobic exercise has been shown to divert glucose away from hepatic de novo lipogenesis and towards more healthy muscle glycogen storage. Such changes were observed independently of changes in systemic insulin and glucose concentrations, and are likely the result of the exercise-induced reduction in muscle glycogen creating an increase drive for muscle glycogen resynthesis (Petersen et al., 2007; Rabøl et al., 2011). Over a prolonged period of time this may help to prevent both the development of dyslipidemia and the deterioration of whole-body insulin sensitivity (Petersen et al., 2007; Rabøl et al., 2011). It would be interesting to determine if single or repeated bouts of HIT/ReHIT are associated with a similar effect across a wide range of populations with normal and impaired glycaemic control.

7.3.3. HIT/ReHIT in the context of overweight and obesity

The studies in this thesis have focused on sedentary individuals who in many cases were not overweight or obese. This decision was taken as inactivity, independent of body fatness, is an independent risk factor for both type 2 diabetes and cardiovascular disease (Booth et al., 2002; Chakravarthy & Booth, 2004; Katzmarzyk & Janssen, 2004; Thyfault & Krogh-Madsen, 2011). In addition, low cardiorespiratory fitness, not body fatness, is the strongest predictor of morbidity and mortality (Barlow et al., 1995; Lee et al., 1998; Lee et al., 1999), and an inactive lifestyle predisposes to low levels of aerobic fitness (Krogh-Madsen et al., 2010; Knudsen et al., 2012).

Nevertheless, in the context of a culture where the prevalence of overweight and obesity are ever increasing, and exert major strains on public health care systems, the question is raised as to whether HIT/ReHIT would be beneficial in overweight men and women. In particular, one of the major motivations (both personally and clinically) of increasing physical activity in such populations is to aid in the loss of body fat. Whilst we have not assessed this directly in this series of studies, there is an accumulating number of papers showing that HIT is effective for reducing adiposity (Trapp et al., 2008; Whyte et al., 2010; Heydari et al., 2012; Gillen et al., 2013). This appears to be explained by a prolonged increase in energy expenditure (i.e., for up to 24 hours post-exercise!) and possibly also a suppression in appetite (Sim et al., 2013; Williams et al., 2013). In chapter 4, we showed modulation of key appetite regulating hormones and an
increased post-exercise energy expenditure with ReHIT. As such, studies examining long term changes in body fat content with ReHIT are warranted. In the context of cardiometabolic fitness, which is arguably more important from the perspective of disease prevention, there is no reason to believe that HIT/ReHIT would not be effective in overweight/obese individuals (Whyte et al., 2010; Gillen et al., 2013; Gillen et al., 2014). Indeed, a recent study reported improvements in aerobic capacity (men and women), oxidative capacity (men and women) and glycaemic regulation (men only) in a group of overweight individuals following HIT (Gillen et al., 2014).

7.4. Limitations

7.4.1. Limitations of training studies

The findings of the two exercise training studies in this thesis must be considered within the context of several limitations which are implicit to all long term intervention studies. Firstly, although we performed a supervised intervention in both studies meaning incomplete training adaptations can be discounted, it was an otherwise free-living study meaning that other chronic changes in lifestyle may have contributed to individual alterations in insulin sensitivity and aerobic capacity. Whilst we asked each participant not to make any conscious changes to their current diet and physical activity levels, we have no objective measures to confirm that this was adhered to.

Perhaps more important to consider is our standardisation procedure for the 3 days before each OGTT and aerobic capacity assessment. Specifically, we measured nutritional intake over the 3 days before each OGTT with food diaries; however, there are issues with regards to the validity and sensitivity of this measurement (Cook et al., 2000). In future studies, in order to better isolate the effects of the training intervention, it would be beneficial (but costly) to provide each participant with their meals over these three day periods. We also asked each participant not to perform any moderate or strenuous exercise over the three days. We confirmed this with each participant verbally, but have no objective measure as support, and crucially this may overlook other important aspects of physical activity which could acutely influence the response to an OGTT. In future studies, it would be beneficial to obtain an objective measure of physical activity prior to each OGTT.
A number of recent studies have shown that signalling and subsequent transcripitional response to an acute bout of exercise can be altered in response to changes in pre- and post-exercise nutrition. For example, carbohydrate restriction in the post-exercise period can magnify the increase in GLUT and PGC1α mRNA levels during recovery (Holloszy, 2005), whilst carbohydrate feeding prior to exercise suppresses the subsequent activation of AMPK (Guerra et al., 2010). In light of these findings, it is worth noting that in the current series of training studies we did not control the timing or composition of nutrition in the hours before and after each training session. As such, it is entirely plausible to speculate that this may be a major cause of the variability in changes in insulin sensitivity in response to ReHIT (figure 7.2; figure 6.7) and indeed in response to other exercise interventions. This represents a limitation of the current thesis but offers multiple opportunities for future research.

The timing of the post-training OGTT should also be considered as a limitation. We chose a 3 day post-exercise time point as we were specifically interested in isolating training adaptations, and previous studies have shown that the effects of a single bout of exercise on insulin sensitivity can last up to 48 hours (2 days) (Perseghin et al., 1998). However, training adaptations can be short-lived and we may have missed clinically important (or greater improvements) in insulin sensitivity in response to feedings on day 1 and day 2 after completion of the training intervention. Indeed, previous HIT studies in obese individuals have demonstrated no effect of a single bout of HIT on insulin sensitivity (Whyte et al., 2013), yet improvements after a 2 week training intervention were evident at 1 day, but not at 3 days, after the final exercise session (Whyte et al., 2010).

7.4.2. Characterisation of physical activity at baseline

Throughout this thesis we have primarily investigated the effects of ReHIT in participants who were sedentary at baseline. However, we characterised physical activity levels using a self-report questionnaire; participants were classified as sedentary if they were not achieving current guidelines for exercise participation according to the IPAQ (600 MET minutes consisting of 5 x 30 minutes/day of moderate, 3 x 20 minutes/day of vigorous, or some combination of the two). However, there are issues with using self-reported physical activity measurements. Of particular relevance is the
fact that IPAQ is not designed to pick up on other important physical activity dimensions such as total physical activity energy expenditure. It is possible to be classified as sedentary according to public health guidelines for exercise participation but still have high daily physical activity energy expenditure (Thompson et al., 2014). Likewise, the IPAQ is not designed to pick up very short sporadic bouts of very high intensity physical activity, yet this thesis has demonstrated this to have very important acute and chronic impacts on metabolism. It is possible that differences in baseline physical activity (not picked up by our IPAQ measure) could have impacted upon the response to the additional exercise stimulus applied in the training studies (Thompson et al., 2014). Indeed, this may be another contributing source to the large variability in adaptive response for some variables (e.g. insulin sensitivity) to exercise training (Thompson et al., 2014).

7.4.3. Limitations of the fasting OGTT

The main outcome measure in this thesis was insulin sensitivity as measured during a fasting OGTT. The test-retest reliability of the OGTT has previously been highlighted as a concern; with relatively high coefficients of variation for repeated measures of glucose AUC (10%), insulin AUC (21%) and insulin sensitivity (14%) in 6 week lifestyle maintenance control subjects (Chapter 1). This will clearly impact on our ability to pick up the true signal of the ReHIT intervention in the current series of studies. In this context, the lack of a control group in the second training study represents a major limitation.

Over and above the variability of the OGTT, it is also important to note that the OGTTs were performed in a fasted state. After an overnight fast, liver glycogen levels will be depleted and, as the liver has first refusal of any glucose taken up from the gut, the liver will be a major determinant of the glucose and insulin response to an oral glucose load on the fasted state. As such, the impact of changes in skeletal muscle insulin sensitivity (which are most likely to be altered with exercise (and ReHIT)) will be underestimated using a fasting based OGTT. This can be neatly demonstrated with a comparison of recent studies examining the acute effects of HIT on insulin sensitivity. Several studies, including Chapter 3 of this thesis, utilising a fasting OGTT have unequivocally shown that there are no acute alterations in insulin sensitivity with HIT
(Brestoff et al., 2009; Richards et al., 2010 Whyte et al., 2013). However, a more recent study using an intravenous glucose tolerance test (IVGTT), which measures the response to a glucose load injected directly into the blood (and therefore misses the liver as a first passing), demonstrated large increases in insulin sensitivity which lasted up to 48 hours post-exercise (Ortega et al., 2014). Presumably this is because the effect of changes in skeletal muscle insulin sensitivity is being measured more directly with the IVGTT. Therefore, it would be worthwhile investigating the impact of ReHIT on insulin sensitivity (acute and training effects) using the IVGTT or the gold standard insulin clamp method.

7.4.4. Limitations of the acute metabolic and molecular studies (chapter 4 and 5)

In chapters 4 and 5 we examined the acute metabolic and molecular response to a single bout ReHIT. There are several limitations to consider in the interpretation of the main findings of these studies. Firstly we found that EPOC was more pronounced with ReHIT compared with a bout of aerobic exercise; however we did not take into account oxygen consumption during the exercise bout itself and total oxygen uptake would perhaps have been a more interesting comparison. Secondly we found alterations in acylated ghrelin following ReHIT in a direction which would be expected to suppress appetite (subjective appetite was not altered); however we did not include a measure of ad libitum energy intake to support these data. As such the relevance of the changes in acylated ghrelin with ReHIT on the final outcome remains unclear. The conclusions on both of these measures are limited to the 1.5 hour post-exercise measurement period.

In chapter 5 we measured glycogen concentrations, AMPK and p38 MAPK activity and GLUT and PGC1a gene expression in response to a single bout of ReHIT. We assessed these changes in whole muscle homogenate. However, for glycogen this neglects possible regional subcellular differences in utilisation which may be important in determining downstream signalling and transcriptional responses. Likewise, this method fails to take into account potential changes in the localisation and regional activation of AMPK and p38 MAPK in response to exercise/ReHIT which are likely similarly important for initiating transcriptional changes with exercise. Moreover, although we measure increases in the transcription of GLUT4 and PGC1a in response to ReHIT, we did not include a protein measurement at a later time point, so have no
way of knowing whether this led to a change in respective protein levels. Lastly, due to the invasiveness of the muscle biopsy technique, we could only include 3 post-exercise time points. As the activation in AMPK and p38 MAPK can be temporal it is possible we may have missed changes in activation at different time points during the recovery.

**7.5. Recommendations for Future Research**

The data presented in this thesis raise several potential areas for future research, including further characterisation of the training effects of ReHIT in healthy and clinical populations, further examination of the acute impact of ReHIT on insulin sensitivity at different post-exercise time points, further examination of the role of glycogen in acute exercise signalling responses and training adaptations, as well as wider issues relating long term adherence and clinical benefit in a free-living setting. In this section we will explore some study designs which could address these issues; where appropriate proposed experimental designs will be provided.

**7.6.1. Further characterisation of the training effects of ReHIT**

As demonstrated in figure 7.1, this thesis has provided some evidence to suggest that 6 weeks of ReHIT may improve insulin sensitivity in sedentary men, but overall this contention remains unsubstantiated. As such, it is logical to suggest that future research should aim to further examine the effects of ReHIT on insulin sensitivity (and other biomarkers), not only in sedentary individuals but also in populations with impaired glucose tolerance (i.e., at high risk) and type 2 diabetics. The design for any future study attempting to answer these questions must take the form of a randomised controlled trial with a larger number of subjects, and should measure insulin sensitivity using the gold standard hyperinsulinemic euglycaemic clamp. A missing aspect of the training studies in the current thesis was the inclusion of tissue specific measures of insulin signalling and GLUT4 protein levels to support changes in the whole-body outcomes; future research should aim to include these.

**7.6.2. Further characterisation of the acute effects of ReHIT on insulin sensitivity**

In chapter 3 of this thesis we showed that neither a single bout of ReHIT or vigorous aerobic exercise provided any benefit to insulin sensitivity when measure 14-
16 hours post-exercise. Participants were provided with a standardised meal (relatively high in carbohydrate content) immediately post-exercise, so in effect we were measuring insulin sensitivity in response to the second feeding. As such, a logical follow up question to ask is: does ReHIT or aerobic exercise improve insulin sensitivity in response to the first post-exercise feeding? The proposed research design to answer this question is shown in figure 8.3. Once again it would be optimal to substitute the OGTT measurement utilised in Chapter 3 for the gold standard hyperinsulinemic euglycaemic clamp. This represents a relatively straight forward research design in its simplest form, but again could be combined with tissue specific measures of gene expression or insulin signalling to support whole body measures. The application of tracer technologies during the OGTT/mixed meal/hyperinsulinemic clamp to track the fate of ingested nutrients would provide some extremely useful mechanistic data.

![Figure 7.3](image.png)

**Figure 7.3.** A potential randomised cross-over research design to examine if a single bout of ReHIT or aerobic exercise improves insulin sensitivity in response to the first feeding.

### 7.6.3. The Role of Glycogen in Regulating Adaptations with Exercise Training

The hypothesis that glycogen has a role in exercise induced adaptation has been a central theme throughout several chapters in this thesis (Chapters 2, 4, 5 and 6). We demonstrated that ReHIT reduced muscle glycogen levels by ~20% and this may largely explain several of the downstream signalling/transcriptional responses (Chapter 5) and the changes in whole body metabolic homeostasis (Chapter 4). The fact that the combined training studies of this thesis show some improvement in mean insulin
sensitivity in response to ReHIT (Chapter 7) supports the hypothesis that glycogen turnover regulates adaptations in carbohydrate metabolism. However, this is by no means conclusive and further studies are required to examine this relationship.

It is also plausible to hypothesise that glycogen degradation may indirectly be linked to the consistent improvements in aerobic capacity with ReHIT (Chapter 2 and 6). Briefly, the rapid breakdown of glycogen is likely to create a hypertonic intracellular environment and draw water from the cardiovascular system into the intracellular space (Philp et al., 2014). This may therefore explain the substantial drop in plasma volume measured in the immediate recovery (Chapter 4). Interestingly, increases in blood volume appear to play an important role in the improvements in aerobic capacity with exercise training (Bonne et al., 2014) and it is plausible that a reduction in plasma volume may evoke signalling mechanisms underpinning this change with ReHIT.

Linking acute disturbances in skeletal muscle and whole-body metabolisms with longer term adaptations to training is an important area of research, yet few studies examine these two effects in the same group of subjects and within the context of the same intervention period. With the aim of examining the role of glycogen depletion in exercise induced adaptation, it would be interesting to correlate acute glycogen utilisation, plasma volume changes and associated signalling and transcriptional responses in muscle, with long term changes in insulin sensitivity and aerobic capacity. Similar approaches have been used to examine whether acute changes in muscle protein synthesis with resistance exercise can predict the magnitude of hypertrophy with resistance training (Mitchell et al., 2014). A schematic of a potential research design to examine this question is shown in figure 8.4.

Briefly, each participant would complete a 6-week exercise training intervention with assessments of insulin sensitivity (clamp) and aerobic capacity measured pre- and post-training. During the first and last training session the acute responses (substrate utilisation, plasma volume changes, signalling response and gene expression) would be measured. More complex measures such as changes in specific subcellular glycogen deposits (by transmission electron microscopy) and localisation of signalling proteins in muscle (by separating the nuclear, mitochondrial and cytosolic subfractions) could be built into these acute testing days. The pre exercise biopsy during the acute sessions would also allow the muscle specific adaptations to the exercise training intervention to
be characterised. The acute changes in glycogen and associate signalling/transcription responses would then be correlated with chronic changes in insulin sensitivity, whilst the acute changes in plasma volume would be correlated with chronic changes in aerobic capacity.

There are of course practical limitations to such a study design; it is relatively resource/time heavy, would require significant financial support, and the inclusion of multiple testing days and extensive blood and tissue sampling would place significant burden on the participants. However, the development of new techniques for obtaining muscle biopsies (e.g. the biopsy gun rather than the Bergstrom needle) is making it easier to administer and less invasive, whilst still allowing sufficient quantity and quality of muscle to be obtained for analysis. Likewise, the number of muscle biopsies could be reduced to 6 by removing the 24 hour sample. Overall, the potential quality of the data, and indeed the breadth of research questions able to be answered with such a study design, means it would be well worth the associated time and resources.

![Figure 7.4](image.png)

**Figure 7.4.** Schematic of a possible study design to examine the interactions between acute responses and chronic adaptations. Insulin sensitivity and aerobic capacity would be assessed before and after the 6-week exercise intervention. During the first (1) and last (18) training sessions, the acute responses (substrate utilisation, signalling, gene expression, protein expression etc.) to the exercise bout would be assessed.
7.7. Conclusions

The work in this thesis has attempted to characterise the effect of a reduced-exertion HIT (ReHIT) protocol on insulin sensitivity and aerobic capacity. Despite the exaggerated disturbance of skeletal muscle and whole-body metabolic homeostasis (Chapters 4 and 5), data collected on levels of exertion during a ReHIT bout suggest that the exercise was well tolerated by participants (Chapters 2, 4 and 6). There was some evidence for improvements in key disease biomarkers following ReHIT, with improvements in insulin sensitivity observed in men (see section 7.2.1 of general discussion) and improvements in VO$_2$max observed in both men and women (Chapter 2 and 6). These findings provide support for the hypothesis that glycogen plays an important regulatory role on the adaptive responses to exercise training (Chapter 2). However, the improvements in insulin sensitivity in men were not consistent between the two training studies conducted (Chapter 2 and 6) and there was a high level of variability observed between individuals (see section 7.2.3 of general discussion). As such, the impact of ReHIT on insulin sensitivity needs to be further characterised in the context of a randomised controlled trial. Likewise, studies examining the mechanisms underpinning the variability in the adaptive response to exercise/ReHIT are warranted.
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