Non-pharmacological interventions for the treatment and prevention of cardio-metabolic disease

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Non-pharmacological interventions for the treatment and prevention of cardio-metabolic disease

by

Benjamin Michael Kelly

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of Loughborough University

October 2014

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Abstract

In recent years there has been a surge in interest concerning high intensity intermittent exercise training (HIT) due to its ability to confer rapid notable cardio-metabolic health benefits. Specifically, HIT has been shown to improve insulin sensitivity and glycaemic control as well as other cardiovascular health factors after just 2 weeks of training (typically 6 training sessions). This thesis investigated the potential therapeutic role of HIT training within obese cohorts specifically addressing metabolic health, inclusive of inflammatory profiles and glycaemic control.

Data demonstrated that HIT training significantly improved maximal aerobic capacity (Chapter 6) over a medium (6 week) training term, however was not successful in doing so after shorter-term (2 weeks) training (chapter 5). HIT training induced significant up-regulation in M2 macrophage gene expression in adipose tissue after 6 weeks of training (chapter 6) however did not induce significant improvements in plasma inflammatory profile (chapters 5 and 6). Glucose control and body composition were unchanged throughout regardless of training programme length. HIT training failed to induce any changes in fat loss, with data to suggest that excess post-exercise oxygen consumption (EPOC) contributed minimally as a fat loss mechanism (Chapter 4).

In conclusion, the findings in this thesis demonstrate that sub-maximal low volume HIT training may have positive effects on localised inflammation and aerobic capacity, however further work is required in order to ascertain the true therapeutic potential of HIT on glucose control and body composition.

Keywords: Obesity; inflammation; high intensity intermittent training; adipose tissue.
Acknowledgements

I would like to thank Prof Myra Nimmo for her advice and guidance throughout the completion of this thesis. I would also like to pay thanks to my internal PhD moderator Dr David Stensel who has provided me with much valued advice and encouragement in times of need. My gratitude is extended to Professor Jamie Timmons for providing me with new challenges and experiences that have gone a long way to re-instilling my passion for this subject.

The support, friendship and technical skills of Dr James King have been invaluable throughout the completion of my work and I thank him for his dedication to my professional development. Equally the continued guidance and friendship of Dr Joao Viana is something I truly treasure.

Particular thanks must go to current and past PhD students for making this process bearable. Particular thanks to Conor Taylor, Kevin Deighton, Soteris Xenophontos, Sion Parry, Andy Shaw, Adam Fry, Liam Heaney, Maurice Dungey, Julie Hunt and Tom Paulson.

There are no words to summarise the support given to me from a very special person in my life, my fiancée Faye Webb. Her boundless patience and endless love over the past 8 years has been simply incredible. Her caring demeanour, positivity and incredible humour continue to offer an escape from the challenges of academia. I will forever be in her debt. Additionally I must pay huge thanks to the entire Webb family,
Kim, Gary, Jack, Kirsten and Archie. A beautiful family with the kindest of hearts whom I will forever cherish.

Critically I would like to thank the entire (immediate and extended) Kelly family. I have been blessed with incredible role models and thank Susie, Juliette and Michael for directly influencing my career aspirations and for consecrating my love for all things FAC 51.

Lastly and quite simply I dedicate this work in its entirety to you, Mum and Dad. Without your endless love, support, encouragement and belief in my abilities this journey would not have been possible. My development as an academic and critically a human being is merely a reflection of your tireless devotion and selflessness. You are my true inspirations and to you both I owe everything, Thank you.
Publications

Publications that have arisen from this thesis:

**Journal Articles:**


**Scientific meeting proceedings:**


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## Abbreviations

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<tr>
<td>%</td>
<td>percent</td>
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<tr>
<td>°C</td>
<td>degrees centigrade</td>
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<tr>
<td>µL</td>
<td>microliter</td>
</tr>
<tr>
<td>ACSM</td>
<td>American College of Sports Medicine</td>
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<tr>
<td>AIT</td>
<td>aerobic interval training</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<td>ATP</td>
<td>adenosine tri-phosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
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<td>b.min⁻¹</td>
<td>beats per minute</td>
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<td>BMC</td>
<td>bone mineral content</td>
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<td>BMI</td>
<td>body mass index</td>
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<td>blood pressure</td>
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<td>BSA</td>
<td>Bovine serum albumin</td>
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<td>CD</td>
<td>cluster of differentiation</td>
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<td>cDNA</td>
<td>complementary DNA</td>
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<td>CHO</td>
<td>carbohydrate</td>
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<td>CI</td>
<td>confidence interval</td>
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<td>centimeter</td>
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<td>control</td>
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<td>COX</td>
<td>cytochrome oxidase</td>
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<td>CPT-1</td>
<td>carnitine palmitoyltransferase</td>
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<td>CRP</td>
<td>c-reactive protein</td>
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<tr>
<td>CS</td>
<td>citrate synthase</td>
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<td>CT</td>
<td>cycle threshold</td>
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<td>coefficient of variation</td>
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<td>cardiovascular</td>
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<td>DAG</td>
<td>diacylglycerol</td>
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<td>diastolic blood pressure</td>
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<td>DEFRA</td>
<td>Department of Farming and Rural Affairs</td>
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<td>DEXA</td>
<td>dual energy X-ray absorptiometry</td>
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<td>deoxyribose nucleic acid</td>
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<td>ECG</td>
<td>electrocardiogram</td>
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<td>ethylenediaminetetra acetic acid</td>
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<td>energy expenditure</td>
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<td>ELISA</td>
<td>enzyme linked immunosorbant assay</td>
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<td>excess post exercise oxygen consumption</td>
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<td>ER</td>
<td>endoplasmic reticulum</td>
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<td>EU</td>
<td>European Union</td>
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<td>FCS</td>
<td>fetal calf serum</td>
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<td>free fatty acid</td>
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<td>fasting plasma glucose</td>
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<td>FPI</td>
<td>fasting plasma insulin</td>
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<td>G</td>
<td>mean plasma glucose during OGTT</td>
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<td>GA</td>
<td>gauge</td>
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<td>GLUT4</td>
<td>glucose transporter type 4</td>
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<td>gp</td>
<td>glycoprotein</td>
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<td>HADH</td>
<td>hydroxyacyl-CoA-dehydrogenase</td>
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<td>HbA1c</td>
<td>glycated haemoglobin</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>HIF</td>
<td>hypoxic inducible factor</td>
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<td>HIIE</td>
<td>high intensity intermittent exercise</td>
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<td>high intensity interval training</td>
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<td>HMOX</td>
<td>heme-oxygenase</td>
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<td>HMW</td>
<td>high molecular weight</td>
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<td>HR&lt;sub&gt;max&lt;/sub&gt;</td>
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<td>heart rate reserve</td>
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<td>horse-radish peroxidase</td>
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<tr>
<td>/</td>
<td>mean plasma insulin</td>
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<td>ICAM-1</td>
<td>intercellular adhesion molecule</td>
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<td>IgG</td>
<td>immunoglobulin G</td>
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<td>IKK&lt;sub&gt;α&lt;/sub&gt;</td>
<td>inhibitor of nuclear factor kappa-B kinase subunit alpha</td>
</tr>
<tr>
<td>IKK&lt;sub&gt;β&lt;/sub&gt;</td>
<td>inhibitor of nuclear factor kappa-B kinase subunit beta</td>
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<td>IKK&lt;sub&gt;γ&lt;/sub&gt;</td>
<td>inhibitor of nuclear factor kappa-B kinase subunit gamma</td>
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<td>IL</td>
<td>interleukin</td>
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<td>insulin receptor substrate 1</td>
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<td>ISI-HOMA</td>
<td>insulin sensitivity index-homeostatic model assessment</td>
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<td>ITGAX</td>
<td>gene encoding for CD11c</td>
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<td>IU</td>
<td>international unit</td>
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<td>IV</td>
<td>intra-venous</td>
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<td>c-Jun N-terminal kinases</td>
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<td>kg</td>
<td>kilogram</td>
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<tr>
<td>kJ</td>
<td>kilojoule</td>
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km.h  kilometres per hour
L      litre
LDH    lactate dehydrogenase
LMW    low molecular weight
m      meter
M      molar
MAPK   mitogen activated protein kinase
MCP    monocyte chemo-attractant protein
MCT    moderate continuous exercise
min    minute
MIP-1α macrophage inflammatory protein
ml     millilitre
mmHg   millimetres of mercury
mmol   millimolar
MRC-1  mannose receptor 1
mRNA   messenger ribo-nucleic acid
NADPH  nicotinamide adenine dinucleotide phosphate
NF-κB  nuclear factor kappa-light-chain-enhancer of activated B cells
nm     nano-meter
NSAID  non-steroidal anti-inflammatory drug
OGTT   oral glucose tolerance test
OPD    o-phenylenediamine
PAI-1  plasminogen activator inhibitor
PCr    phosphocreatine
PDH    pyruvate dehydrogenase
PFK    phosphofructokinase
pg     picogram
PGC-1α  peroxisome proliferator-activated receptor gamma co-activator 1-alpha
pVO₂max  power at VO₂max
QPCR  quantitative polymerase chain reaction
RE-HIT  reduced exertion high intensity intermittent training
RER  respiratory exchange ratio
Reps  repetitions
RMR  resting metabolic rate
RNA  ribonucleic acid
ROS  reactive oxygen species
RP  rapid phase
RPE  rating of perceived exertion
RPM  revolutions per minute
RT  reverse transcription
s  second
sIL  soluble interleukin
SAT  subcutaneous adipose tissue
SBP  systolic blood pressure
SD  standard deviation
SIT  sprint interval training
SP  slow phase
SPSS  statistical package for the social sciences
T2DM  type 2 diabetes mellitus
TAG  triacylglycerol
TBS  tris buffered saline
TBST  tris buffered saline-tween
t-lim  maximum time at peak velocity
TLR  toll like receptor
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<th>Term</th>
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<tr>
<td>TNF-α</td>
<td>tumor necrosis factor alpha</td>
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<tr>
<td>$v\Delta 50$</td>
<td>intensity between velocity at maximal lactate steady state and $v\dot{V}O_{2\text{max}}$</td>
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<tr>
<td>VAT</td>
<td>visceral adipose tissue</td>
<td></td>
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<tr>
<td>VCO₂</td>
<td>volume of carbon dioxide per unit of time</td>
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<td>$vLT$</td>
<td>velocity at the lactate threshold</td>
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<td>$VO_{2\text{max}}$</td>
<td>maximal oxygen uptake</td>
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<td>$VO_{2\text{peak}}$</td>
<td>peak oxygen uptake</td>
<td></td>
</tr>
<tr>
<td>$v\dot{VO}_{2\text{max}}$</td>
<td>velocity at $\dot{VO}_{2\text{max}}$</td>
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<tr>
<td>W</td>
<td>watts</td>
<td></td>
</tr>
<tr>
<td>W/V</td>
<td>weight to volume</td>
<td></td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<tr>
<td>$W_{\text{max}}$</td>
<td>maximal wattage</td>
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<tr>
<td>$\beta$-HAD</td>
<td>beta-hydroxy acyly-CoA dehydrogenase</td>
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CHAPTER 1

INTRODUCTION
1.1 Interval training – A historical perspective

The subsequent studies within this thesis aimed to profile to the effectiveness of HIT type training for improving determinants of metabolic health in pre-clinical populations. Within the first experimental chapter (4) this thesis demonstrated that HIT type training did not induce prolonged elevation of energy expenditure across a 12 h time course and as such was unlikely to contribute significantly to potential changes in body fat across the course of a short-term HIT training programme such as that described previously (Leggate et al. 2012). Chapter 5 aimed to profile the effectiveness of a recently popularised (Little et al. 2011) short term low-volume HIT training protocol for improving glycaemic control, inflammatory profile and aerobic capacity within an obese cohort. Additionally this chapter utilised a sub-group in order to assess the potential effects of reduced training frequency on the aforementioned clinical outcomes. Chapter 5 failed to demonstrate any improvements in any measurement parameter in either experimental group. The results gave rise to the conjecture that the training protocols implemented within the chapter were not effective tools for reducing markers associated with cardio-metabolic health risk. The final experimental chapter within this thesis aimed to extend findings by previous authors (Larsen et al. 2014) by profiling the effectiveness of a novel HIT type intervention on markers associated with metabolic disease. Along with glycaemic control, systemic inflammation and aerobic capacity, this chapter focussed on adipose tissue, assessing tissue specific inflammation at both the RNA and protein levels. Results demonstrated that 6 weeks of HIT training significantly improved aerobic capacity, with RNA markers of M2 anti-inflammatory macrophages being significantly up-regulated. No further changes in glycaemic control or inflammation were demonstrated. These data would suggest that a protocol with greater volume and/or intensity may be required in to elicit positive
changes in glucose control and inflammation with cohorts similar to those used within this thesis.

Interval training was first described within academic literature by Reindell and Roskamm (1959), and later popularised after the Second World War by Emil Zatopek, winner of Olympic gold in the 5000 m, 10000 m and marathon during the Helsinki games in 1952. Zatopek was reported to have repeated up to 100 x 400 m repetitions per day run at a pace close to that of ‘hard-work’, interspersed with 200 m of recovery. Furthermore Roger Bannister, the first sub-4-minute miler, employed interval training in varying forms 5 days per week almost all year round. Reportedly, Banister’s coach preferred the athlete to focus on the quality of the work performed as opposed to quantity; significantly reducing the amount of continuous miles run by Bannister.

During the 1960’s research into intermittent exercise grew with the work of Per Olof Åstrand and colleagues describing the fundamental physiological responses to intermittent exercise. Even at this early stage Åstrand hinted at the potential broader benefits of interval exercise stating that “This is of interest not only for the training of sportsmen, but also for the rehabilitation of patients…” This early data (Åstrand et al. 1960a, 1960b; Christensen, Hedman and Saltin 1960) demonstrated that interval training at a velocity between critical velocity (the vertical asymptote of the velocity-time relationship) and velocity at \( \dot{V}O_{2\text{max}} \) (\( v\dot{V}O_{2\text{max}} \) - defined as the velocity associated with \( \dot{V}O_{2\text{max}} \) determined by an incremental work test on a treadmill) could elicit \( \dot{V}O_{2\text{max}} \) despite complete rest between 2-3 min hard repetitions. Åstrand considered this one of the best forms of interval training to improve \( \dot{V}O_{2\text{max}} \) since all cardiorespiratory parameters were stressed to their maximum. Åstrand (1960a) also demonstrated that the mechanical efficiency during intermittent work was not different to that of
continuous exercise. This work was extended by the same group to profile shorter exercise:rest intervals. Christensen and colleagues (1960) were the first to propose very short (10-15 s) interval running at an intensity equivalent to 100 % \( \dot{V}O_2\max \), interspersed with 10-15 s of recovery. This study profiled the metabolic response to such short intervals and demonstrated that a runner, 24 year old Bengt Saltin, with a \( \dot{V}O_2\max \) of 67 ml.min.kg\(^{-1}\) reached 99 % \( \dot{V}O_2\max \) in the final intervals of the 30 min intermittent exercise session composed of 10-15 s sprints. Sometime later, Karlsson and Saltin (1971) published data describing the metabolic response within skeletal muscle during high intensity interval work. This group took muscle biopsies following each of 5 repetitions of 1 min of exercise at approximately 120 % of the power required to reach \( \dot{V}O_2\max \) (p\( \dot{V}O_2\max \)), followed by 5 min of rest. Data showed that creatine phosphate was progressively depleted after each repetition with muscle lactate reaching 25 mmol.L.kg\(^{-1}\) (wet muscle). This series of early work can be credited with being the first to extend understanding of the metabolic consequences of the intermittent exercise paradigm.

During the seventies, \( \dot{V}O_2\max \), and sub-maximal increments, began to be systematically measured regularly, with major advancements in training physiology coming about through the work of Alois Mader and others who aided in the determination of the blood lactate threshold at 4 mmol.L\(^{-1}\) using 5 min stages of constant velocity (Mader et al. 1976). The eighties saw the emergence of running talents such as Sebastian Coe who performed interval training along with circuit training for strength and power gains. During this decade coaches would use specific velocities to calibrate interval training without taking into account physiological markers. It is now well established that the minimal velocity that elicits \( \dot{V}O_2\max \) in a steady state condition is below \( \dot{V}O_2\max \) and is actually set at between the velocity at
the maximal lactate steady state and \( \text{vVO}_2\text{max} \), this being defined as \( v\Delta 50 \) (Billat et al. 2000).

The work of Veronique Billat and colleagues during the nineties and early 2000’s began to further explain that improvements in \( \text{VO}_2\text{max} \) correlated strongly with time spent exercising at \( \text{VO}_2\text{max} \). One of the best examples of this work compared time spent at \( \text{VO}_2\text{max} \) during both high intensity interval and continuous type exercise (Billat et al. 2000). The group hypothesised that continuous running at \( v\Delta 50 \) allowed participants to remain closer or at \( \text{VO}_2\text{max} \) for a longer period than intermittent running at \( v\text{VO}_2\text{max} \), whilst eliciting a similar accumulation of blood lactate. The time spent at \( \text{VO}_2\text{max} \) within an intermittent trial was 8 min (83 % of total time run at \( v\text{VO}_2\text{max} \)) and 2 min 42 s for the continuous exercise trial (approximately 50 % of time spent at \( v\Delta 50 \)).

When comparing blood lactate concentrations, end values averaged 7.4 ± 1.8 and 8.0 ± 1.2 mmol.L\(^{-1}\) for intermittent and continuous exercise respectively, with the values not being significantly different from one another. This work clearly demonstrated that time spent at \( \text{VO}_2\text{max} \) was critical for the improvement of aerobic capacity.

An expansive volume of literature exists relating to interval training and aerobic capacity with the work of Billat representing a small fraction relevant specifically to applied performance physiology. Interestingly this work has a wider relevance to health physiology, given the strong association between \( \text{VO}_2\text{max} \) and the prediction of all-cause morbidity and mortality (Carnethon et al. 2003; Chase et al. 2009; Kodama et al. 2009; Lee et al. 2010). Interval training with a focus on improving \( \text{VO}_2\text{max} \) may in part act as an achievable intervention for improving health outcomes in pre-clinical populations.
Those who are overweight and obese represent one such pre-clinical population and represent a developing issue globally, with a rapid increase in prevalence resulting in a congruent rise in metabolic diseases such as insulin resistance and type 2 diabetes mellitus (T2DM) (Danaei et al. 2011). It is clear that lifestyle interventions that are both economical and accessible are urgently required in order to prevent and treat the growing epidemic.

The core defect underlying the development of type 2 diabetes mellitus (T2DM) is skeletal muscle insulin resistance (DeFronzo and Tipathy. 2009). Mechanisms and primary contributing factors of insulin resistance are both vast and complex. Evidence suggests that physical inactivity may be the principal initiating factor (Thyfault and Krogh-Madsen. 2011). Inactivity leads to reduced energy expenditure, which when combined with increased energy intake promotes adipose tissue expansion and with it the development of obesity and a state of chronic inflammation. Inflammation has been independently implicated in the development of insulin resistance and T2DM (Wellen & Hotamisligil. 2005) and is characterised by abnormal cytokine production, increased production of acute phase reactants as well as activation of a network of inflammatory signalling pathways (Hotamisligil 2006). Insulin stimulates tyrosine phosphorylation of insulin receptor signalling (IRS) proteins, which is a crucial event in mediating insulin action and is a significant signalling defect of systemic insulin resistance. Inflammatory mediators promote insulin resistance through inhibitory serine phosphorylation of IRS-1. IRS-1 serine phosphorylation disrupts insulin-receptor signalling through several distinct mechanisms, ultimately blocking insulin action (Hotamisligil et al. 1996; Zick. 2005).
Regular exercise improves insulin sensitivity and is effective in preventing T2DM (Goodyear and Kahn. 1998). Traditionally, health oriented physical activity guidelines have centred on individuals undertaking moderate intensity, continuous forms of exercise on most days of the week (Bull et al. 2010). Given that the most commonly cited reason for not participating in physical activity is lack of time, and the recent evidence that individuals prefer an intermittent exercise protocol in comparison to continuous exercise (Bartlett et al. 2012), it is now timely to consider novel forms of exercise that may be more readily adopted.

Recent work suggested that sprint interval training (SIT) training involving a series of 30 s all out cycle sprints (i.e. Wingate sprints) with 4 min recovery between bouts provided an efficient strategy for inducing adaptations comparable to those seen following traditional endurance based training (Burgomaster et al. 2005; Gibala et al. 2006; Burgomaster et al. 2007; Burgomaster et al. 2008; Rakobowchuck et al. 2008; Trilk et al. 2010). Furthermore several authors have demonstrated that sprint interval SIT and HIT may have favourable effects on metabolic control after as few as 6 sessions. (Burgomaster et al 2006, 2008; Gibala et al. 2006; Wisløff et al. 2007; Tjonna et al. 2008; Babraj et al. 2009; Haram et al. 2009; Nybo et al. 2010; Richards et al. 2010; Whyte et al. 2010; Gaesser and Angadi. 2011; Hood et al. 2011; Little et al. 2011; Shepherd et al. 2013). Additionally data demonstrate that reduced-exertion sprint based exercise, the most recent variation to high intensity exercise consisting of just 20 s sprints, was successful in improving metabolic health and aerobic capacity (Metcalf et al. 2011).

HIT and SIT training are in part characterised through activation of large muscle mass and associated with high glycogen breakdown/turnover (Burgomaster et al. 2006).
Consequently a greater proportion of muscle fibres require replenishment of their carbohydrate stores. It is this high level of glycogen depletion following HIT/SIT that may play a pivotal role in the improvement of insulin sensitivity in cohorts at risk of metabolic disease. Muscle glycogen availability is inversely related to muscle cell membrane GLUT4 content during insulin stimulation (Derave et al. 2000), glycogen synthase activity (Jensen et al. 2006), expression of GLUT4 mRNA (Stenberg et al. 2006) and therefore insulin sensitivity (Derave et al. 2000; Jensen et al. 2006; Kawanaka et al. 2000; Laurant et al. 2000; Litherland et al. 2007; Richter et al. 2001). It may be suggested therefore that exercise modalities such as SIT and HIT, which reduce muscle glycogen levels, are likely to be effective in reducing metabolic disease profiles.

It is the aim of this thesis to explore further the paradigm of interval training and how best to apply this form of exercise within an obese population. Specifically this thesis aims to examine the effects of different high intensity intermittent exercise protocols on improving markers of metabolic disease including glucose tolerance, insulin sensitivity and inflammation within an obese population.
CHAPTER 2

LITERATURE REVIEW
2.1 The development of the high intensity interval training (HIT) paradigm

Regular endurance training is an effective training strategy to improve health (Fletcher et al. 1996). Guidelines recommend 150 min of moderate continuous exercise per week in order to maintain a state of good health (Department of Health 2011). This for many is an achievable prospect yet for others this time commitment is too high rendering typical continuous moderate exercise unattainable. In recent years there has been a growth in interest surrounding alternative strategies to gain health benefits including aerobic interval training (AIT) sprint interval training (SIT), low-volume high intensity interval training (HIT) and reduced exertion HIT training (RE-HIT) which may represent novel strategies to induce adaptations normally associated with endurance training.

2.1.1 Aerobic interval training

A form of interval training, known as aerobic interval training (AIT) is an exercise variant deemed suitable for de-trained and clinical populations. The work of Talanian and colleagues (2007) formed a manageable exercise protocol that looked at the broader application of interval training. Healthy women completed a total of thirteen days of training. Training consisted of 10 x 4 min intervals at 90 % \(\dot{V}O_{2\text{peak}}\) separated by 2 min recovery. This group (Talanian et al. 2007) demonstrated a 13 % increase in \(\dot{V}O_{2\text{peak}}\) after just 7 sessions of AIT. During a 60 min continuous cycle test, whole body fat oxidation was significantly higher at 30 min, 45 min and 60 min after training, showing a 36 % increase compared to baseline values. Muscle analysis provided data consistent with others (Rodas et al. 2000; Burgomaster et al. 2005, 2006; Gibala et al. 2006), showing a 20 % increase in maximal citrate synthase (CS) activity after training. Furthermore, activity of \(\beta\)-HAD (the rate limiting enzyme of \(\beta\)-Oxidation) increased by
32 % after training. Although resting muscle glycogen content was unaffected by training the net muscle glycogen utilisation was decreased by 12 % after 60 min of cycling, with net phosphocreatine (PCr) degradation significantly decreased by 40 % after HIT training. Talanian et al (2007) were the first to demonstrate that interval training at an intensity equivalent to 90 % VO\textsubscript{2peak}, an intermediate between classical sub-maximal and sprint training paradigms, was able to increase both VO\textsubscript{2peak} as well as markers of oxidative and glycolytic potential. This was based on increases in β-HAD (32%) and CS (20%). Despite presenting novel findings they failed to include a moderate intensity control group and as such do not definitively define AIT exercise as being of greater benefit compared to matched continuous moderate training in improving aerobic capacity and muscle metabolism.

Wisløff et al (2007) addressed the use of AIT (referred to as HIT in publication) training within a patient population. Specifically patients with post infarction heart failure (ejection fraction < 40 %) were assigned to a HIT group, a moderate continuous training (MCT) group or a non-exercising control group. The HIT training was carried out 3 x per week and consisted of 4 x 4 min uphill walking intervals at 90 % - 95 % peak heart rate (HR\textsubscript{peak}) separated by 3 min recovery. After twelve weeks of exercise training VO\textsubscript{2peak} increased by 46 % and 14 % for AIT and continuous groups respectively. AIT also improved work economy to a greater extent than continuous training, evidenced by a 15 % reduction in oxygen cost, reduced heart rate (HR) and blood lactate at any given submaximal walking speed. Protein levels of peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) increased by 47 % in the HIT group and correlated highly with improvements in VO\textsubscript{2max} (r = 0.71). This study (Wisløff et al. 2007) demonstrated that AIT was feasible in those with clinical pathologies. As with the testing of any clinical population, the measurement of aerobic
capacity can often provide falsely low values prior to training and then more accurate elevated values after an exercise intervention. All but 2 individuals within the Wisløff study met the criteria for true maximal oxygen uptake as defined by a levelling off of oxygen uptake despite increases in workload and an RER above 1.05. As such the data can be deemed robust. The study is limited by low numbers, however as a proof of concept design this work acted as a vehicle for further investigation into the clinical application of AIT.

Tjønna and colleagues (2008) extended previous work by the same group using an identical exercise regimen, this time in a cohort diagnosed with metabolic syndrome. After sixteen weeks, AIT reduced factors of the metabolic syndrome more than a continuous moderate exercise group. At the end of the AIT training period 46 % of individuals were no longer diagnosed with the metabolic syndrome, compared to the 37 % following continuous exercise. Insulin receptor (IR) phosphorylation was increased to a greater extent following AIT compared with any other intervention. This is particularly pertinent given that the measurement of IR phosphorylation is a reflection of peripheral insulin sensitivity in obese and T2DM patients. This study (Tjønna et al. 2008) along with further work from the same group in a rat model (Haram et al. 2009) provided clear justification for further investigation into the application of AIT based training across a spectra of chronic disease states.

With relevance to the current thesis, Leggate and colleagues (2012) replicated a previous AIT protocol (Talanian et al. 2007) within an overweight and obese cohort. After just 2 weeks (6 sessions) of training data demonstrated a significant reduction in inflammatory profile specifically in plasma and adipose tissue. These data (Leggate et al. 2012) were the first to establish that sub-maximal interval training could potentiate
health improvement via improved inflammatory profile and aerobic capacity. Clearly from the data provided by this group (Leggate et al. 2012) further work is warranted in order to ascertain if exercise volume can be further reduced whilst still instigating health improvements within the pre-clinical population and is reflected in this thesis.

2.1.2 Sprint interval training

The most utilised high intensity protocol within the literature consists of ‘all-out’ repeated 30 s ‘SIT’ sprints. Sprints are repeated 4-6 times with each bout separated by ~ 4 min of recovery. Typically a braking force of 0.075 kg per kg of body mass is applied during the 30 s cycle sprint, with this force reduced or removed entirely during recovery.

MacDougall and colleagues (1998) examined aerobic and anaerobic components of fitness in healthy males, focusing on glycolytic and oxidative enzyme activity following 7 weeks (3 x per week) of SIT exercise training. After training, both peak power output and total work over 30 s significantly increased. V̇O₂max increased from 3.73 ± 0.13 to 4.01 ± 0.08 L.min⁻¹. Activity increases in hexokinase, glycogen phosphorylase, phosphofructokinase, lactate dehydrogenase, CS, succinate dehydrogenate, malate dehydrogenase and 3-hydroxyacyl-CoA-dehydrogenase were indicative of increased oxidative and glycolytic potential.

Rodas et al (2000) utilised a more manageable protocol based on greater recovery periods. This group presented similar findings to that of MacDougall and colleagues (1998). Two weeks of training consisted of mixed sessions of 15 s all out reps with 45 s rest periods and 30 s SIT reps with 12 min rest periods. The initial training session
consisted of 2 bouts of 15 s and 7 bouts of 30 s. Bouts were increased by 1 every 2
training sessions. Data (Rodas et al. 2000) demonstrated that after training, muscle
PCr was significantly increased by 31 % and muscle glycogen by 32 %. Following 2
weeks of training lactate accumulation was significantly lower at the end of a 30 s ‘all
out’ performance test when compared against pre-training values. Performance gains
were significantly increased following training with maximum power output increasing
by 10 % and \( \dot{V}O_2 \text{max} \) by 11 %. Despite demonstrating beneficial oxidative and glycolytic
responses this study lacked statistical power given the small sample size \((n = 5)\) and
as such data should be viewed with caution especially with respect to performance
gains. Further, despite the high intensity repetitions being of short duration the overall
training volume was high. This time commitment may not pose an attractive prospect
for many given that ‘lack of time’ is cited as being one of the biggest barriers to exercise
(Leslie et al. 1999; Stutts et al. 2002; Trost et al. 2002) and would suggest a need for
similar exercise at a much reduced volume.

In the mid 2000’s Gibala and colleagues published a body of work looking towards a
significantly reduced volume of exercise to elicit similar metabolic responses to that of
regular continuous moderate exercise. This work profiled the metabolic responses to
low volume SIT. The first paper in a series of publications, now regarded as being
central to our understanding of SIT came from Burgomaster et al (2005). Burgomaster
examined the effects of 6 sessions of SIT over 2 weeks on muscle oxidative potential,
\( \dot{V}O_2 \text{peak} \) and cycling endurance time to fatigue in recreationally active participants.
Participants were assigned to either an exercising or non-exercising group. Results
demonstrated a 100 % increase in cycle endurance time to fatigue compared with
baseline in the exercising group. SIT training did not improve \( \dot{V}O_2 \text{peak} \) nor were there
any changes in power output between the first and last training sessions. Maximal
activity of CS increased by 38 % after training with resting muscle glycogen increasing by 26 % after training which is comparable to earlier work (MacDougall et al. 1998; Rodas et al. 2000) despite a significantly reduced overall training volume. The increase in muscle oxidative potential was comparable to that reported following 6-7 days of traditional endurance exercise training (Chesley et al. 1996; Spina et al. 1996).

Building on the finding of improved oxidative capacity following low volume SIT exercise, Burgomaster et al (2006) revealed time taken to complete a 250 kJ time trial decreased by 9.6 % following SIT training which was further reflected by a 5.4 % increase in average power output. In agreement with their previous work (Burgomaster et al. 2005) VO\textsubscript{2max} did not increase after training, differing from the results obtained by MacDougall et al (1998) and Rodas et al (2000) after using SIT training, suggesting a yet to be elucidated optimal intensity / duration intercept which must be achieved in order to improve aerobic capacity. Burgomaster provided the first evidence that SIT improved exercise performance during a time trial scenario and further validated previous findings (Burgomaster et al. 2005) that skeletal muscle oxidative capacity may be enhanced by a brief 2 week training period equivalent to only 16 min of high intensity work.

Gibala et al. (2006) later established that following SIT training), the time required to complete a 750 kJ time trial significantly decreased by 10.1 % versus a decrease of 7.5 % following endurance training (consisting of 90 min - 120 min of continuous cycling 3 x per week). Mean power output during a time trial increased to a greater degree following SIT (22 W) compared to endurance training (13 W). The maximal activity of mitochondrial oxidative enzymes (cytochrome oxidase and its specific subunits II and IV) increased after SIT training despite no differences between groups
for muscle oxidative capacity. This work (Gibala et al. 2006) was the first to directly demonstrate that 6 sessions of low volume SIT over 2 weeks induced comparable improvements to traditional high volume endurance training in muscle oxidative capacity and exercise capacity despite a 90% reduced overall training volume however changes in $\dot{V}O_{2\text{max}}$ were not reported making it impossible to determine changes in aerobic capacity. By proposing SIT training as an efficient strategy for inducing performance benefits Gibala and colleagues provided a catalyst for the recent intensification of research into the area of high intensity exercise.

Extending the SIT training model beyond the classic 2 week period through to 6 weeks Burgomaster et al (2008) compared SIT training with exercise recommended by public health guidance (American College of Sports Medicine 1998) making the study one of the first to focus on the potential for SIT training to re-inform exercise prescription and policy. The SIT training utilised was identical to that used previously (Burgomaster et al. 2005, 2006; Gibala et al. 2006). Endurance exercise consisted of 40 min – 60 min of continuous cycling at 65% $\dot{V}O_{2\text{peak}}$ (American College of Sports Medicine 1998). Following training, $\dot{V}O_{2\text{peak}}$ significantly increased with no differences between groups despite a 90% lower training volume in the SIT group. Mean power output increased only following SIT training by 7%. After training there were no differences between groups in oxygen uptake during 60 min constant load cycling, RER values during constant load cycling, total fat or carbohydrate oxidation with all parameters improving after 6 weeks of training. Here Burgomaster et al. (2008) demonstrated that SIT was indeed a time efficient strategy to induce changes in selective markers of whole body and skeletal muscle metabolism with positive gains being comparable to those of high volume moderate intensity exercise. This work demonstrated that the potential health
benefits gained via government prescribed exercise may also be achieved with significantly lower training volumes through SIT.

Early SIT literature failed to clarify the exercises wider application specifically its deployment within clinical populations and associations with health improvements. Later work from Babraj et al (2009) demonstrated 6 sessions of SIT over a 2 week period reduced area under the plasma glucose, insulin and free fatty acid (FFA) concentration curves (AUC) by 12 %, 37 % and 26 % respectively in a group of young sedentary males following an OGTT test, further supporting the notion that SIT may be used as a novel strategy to reduce metabolic risk factors.

Whyte et al (2010) described reductions in waist circumference of 1.4 cm as well as a significant reduction of 15 % in 2 h insulin AUC following 2 weeks of SIT in an obese cohort. Significant improvements in \( \text{VO}_{2\text{max}} \) were demonstrated following 2 weeks of training. The authors attributed significant improvements to a relatively low level of baseline fitness within the cohort. This may be unlikely given that baseline aerobic capacity neither positively nor negatively associates with gains in exercise training induced maximal aerobic power (Hautala et al. 2006; Timmons 2011). This is still a contentious issue given recent work demonstrating a fairly weak yet significant Pearson correlation of -0.4 between basal \( \text{VO}_{2\text{max}} \) and % improvement in aerobic capacity following 2 weeks of SIT (Astorino, Schubert 2014). Changes in \( \text{VO}_{2\text{max}} \) reported by Whyte and colleagues (2010) are confounded by a lack of control group and are further attributed to a possible “learning effect”. This may have been reduced had at least 2 baseline tests been conducted. Equally, in line with current understanding, it is most probable that peripheral adaptations may be almost exclusively responsible for improved exercise capacity commonly witnessed after
short SIT interventions (Gibala & McGee 2008). Similar adaptations were
demonstrated by Richards et al (2010) whom following the identical training
programme (Burgomaster et al. 2005) demonstrated via hyper-insulinaemic
euglycaemic clamp, significant increases in insulin sensitivity despite no significant
changes in basal glucose and insulin concentrations. Additionally, reduced glucose
and insulin AUC as well as improved insulin sensitivity as assessed via OGTT, has
been shown after 6 weeks of SIT training (Shepherd et al. 2013), with improvements
being similar to high volume continuous moderate cycling. The data described above
(Babraj et al. 2009; Richards et al. 2010; Whyte et al. 2010; Shepherd et al. 2013)
demonstrated the therapeutic potential for high intensity exercise to improve the
wellbeing of individuals at risk of developing metabolic disease.

For more than a decade, the regulation of energy provision during repeated bouts of
maximal isokinetic cycling such as SIT has been examined (Jones et al. 1985; McCartney et al. 1986, Putman et al. 1995, Spriet et al. 1989, Trump et al. 1996).
During sprint exercise, power outputs are elicited in excess of 2 to 3 times that at which
\( \dot{V}O_{2\text{max}} \) is achieved. To meet the maximal demand for ATP, the majority of energy for
short maximal exercise is derived via substrate phosphorylation from PCr hydrolysis
and glycolysis (Jones et al. 1985, Karlsson 1971, Margaria, Edwards and Dill. 1933).
However, studies using intermittent exercise consisting of 2 to 4 bouts of 30 s of
maximal cycling separated by 4 min of rest have demonstrated that there is a
progressive shift to oxidative metabolism in later bouts (Bogdanis et al. 1996; Putman
et al. 1995, Spriet et al. 1989, Trump et al. 1996). It is not surprising therefore, that
aerobic capacity has been shown to significantly increase following low volume HIT
training as explored within the current thesis (Chapter 6)
In addition, significant lactate accumulation occurs in the first bout compared with the third and fourth bouts, where lactate accumulation is negligible (McCartney et al. 1986, Putman et al. 1995, Spriet et al. 1989). Reduced flux through the glycolytic pathway and a greater degree of activation of pyruvate dehydrogenase before a third bout is accompanied by an increase in oxygen uptake, which contributes to reduced accumulation of lactate and increased oxidation of pyruvate (Putman et al. 1995). It is likely therefore that exercise constructs consisting of 5-10 bouts as demonstrated within this thesis will be defined by similar metabolic pathways. Associated with a reduction in lactate accumulation is an increase in oxidative phosphorylation in a third bout of SIT compared with the first. With this in mind, a rationale develops for the improvement of aerobic and oxidative capacity following 4-6 sprints in as little as 2 weeks as will be investigated further within this work.

During maximal sprint exercise, glycogen is the primary substrate, with extracellular glucose providing ~2–3 % of the total (Katz et al. 1986; Putman et al. 1995). Therefore, glycogen phosphorylase catalyses the rate-limiting step in glycogenolysis and sets the upper limit for the rates of glycolysis. This mechanism is particularly pertinent to the obese / pre-diabetic cohort. Depleting glycogen stores via HIT type training may allow for an increased ‘sink’ or ‘capacity’ for exogenous glucose intake. With this in mind this may begin to rationalise the use of the HIT training modality within those at risk of hyperglycaemia.

2.1.3 High intensity interval training

Of critical relevance to this thesis is HIT training. This training modality is composed of ~1 - 3 min high intensity intervals separated by matched or reduced recovery times.
The workload is often set at 80 % - 100 % $\text{VO}_{2\text{max}}$ and performed intermittently over 10-30 min with a high number of repetitions completed. Previously many HIT models have been restricted to young and relatively healthy participants; with much less known about the application of HIT based training within a sedentary or overweight population, who may be at a greater risk of developing disease (Trost et al. 2002). In the first of a series of studies, Hood et al (2011) explored the effectiveness of a novel HIT intervention within a sedentary but otherwise healthy population. An exercise model consisting of 10 x 1 min bouts at ~ 95 % of heart rate reserve (HRR) with 1 min recovery was completed 3 times per week for 2 weeks. Data revealed comparable adaptations in mitochondrial enzymes to those achieved following 6 weeks of SIT based training (Burgomaster et al. 2007) and high volume endurance exercise (Pilegaard et al. 2003). Fasting insulin showed a marked decrease of 16 % after training with insulin sensitivity calculated by homeostasis model assessment (HOMA) improving by 35 %. In spite of a low sample size, this study showed for the first time that with a low total exercise volume and training time commitment, a novel HIT intervention was successful in improving glycaemic control within a sedentary population.

Given the striking results obtained by Hood and colleagues (2011), Little and colleagues (2011) utilised the identical training protocol to extend its application within a group of T2DM patients. Before and after the training period continuous glucose monitoring was employed over a 24 h period to assess average blood glucose concentrations and post-prandial hyperglycaemic excursions under a standardised diet. Training induced a marked 13 % reduction in average blood glucose concentration over 24 h, further reflected in reduced 24 h blood glucose AUC. Equally, the sum of 3 h postprandial AUC was significantly lower after training. Although
reducing fasting hyperglycaemia is a significant aspect of T2D treatment, evidence suggests that lowering postprandial hyperglycaemia is just as important for achieving adequate glycated haemoglobin (HbA1c) levels (Woerle et al. 2007).

HbA1c is a form of haemoglobin that is measured primarily to identify average blood glucose concentrations over an extended period of time. When circulating levels of blood glucose are elevated glucose molecules attach to haemoglobin in red blood cells. The amount of glucose that binds to the haemoglobin is proportional to the length of time an individual spends in hyperglycaemia. Once haemoglobin is glycated it remains in this form and therefore a build-up within the red blood cell reflects the average level of glucose the cell has been exposed to over its life-course. HbA1c is reflective of an individual’s blood glucose levels during a previous 1–3 month period.

Compared with fasting glucose, HbA1c has several advantages as a diagnostic test; it has higher repeatability (Selvin et al. 2007), can be assessed in the non-fasted state, is the preferred test for monitoring glucose control (American Diabetes Association. 2009). Elevated HbA1c levels are a risk factor for macrovascular disease (Selvin et al. 2010).

As demonstrated previously following high intensity training (Gibala et al. 2006; Little et al. 2011), maximal activity of mitochondrial enzymes was elevated following training with skeletal muscle mitochondrial protein content also showing significant elevations. Total GLUT4 protein was increased by ~369% which was greater than that demonstrated by Hood et al (2011) within a sedentary population. Functional performance was increased with the maximal workload achieved during a ramp cycling test improved by 10% following training, with HR and rate of perceived exertion (RPE)
reducing significantly as a consequence of training. These pilot data demonstrated that low volume HIT may rapidly reduce hyperglycaemia and increase skeletal muscle oxidative capacity in patients with T2DM. Notably these improvements were achieved following a total time commitment of 75 min per week, which was half of the 150 min moderate weekly exercise previously advised (Bull et al. 2010). The work described here (Little et al. 2011) did not include a moderate intensity control group by which to compare the benefits of HIT based training over continuous.

Research manipulating HIT training is ever increasing and it is probable that this modality of training is the most achievable for the sedentary and clinical populations when performed at sub-maximal intensities and when recovery time is adequate.

2.1.4 Reduced exertion high intensity intermittent training

The mechanisms underpinning improvements in metabolic control following high intensity based training are yet to be fully elucidated. The work of Babraj et al (2009) and others (Whyte et al. 2010) suggested that high levels of glycogen depletion observed during the classical 30 s SIT protocol may play an important role in mediating improvements in insulin sensitivity following high intensity training by providing a ‘sink’ for glucose disposal. This theory is bolstered by the work of Horowitz et al (2005) who demonstrated that exercise induced energy deficit attenuated a reduction in carbohydrate oxidation 24 h after exercise. If indeed muscle glycogen depletion did regulate insulin sensitivity then exercise protocols that reduce muscle glycogen concentration should be effective in improving glycaemic control. The 30 s SIT sprint protocol such as that utilised by Burgomaster and colleagues (2005) has been shown to reduce muscle glycogen stores by up to 30 % (Esbjornsson-Liljedahl et al. 1999;
Parolin et al. 1999; Esbjornsson-Liljedahl et al. 2002). According to the work of Parolin et al. (1999) glycogenolysis is only activated during the first 15 s of the first sprint and is then attenuated in the final 15 s. The activation of glycogenolysis is seemingly further inhibited in repeated sprints. This suggests that the classic SIT model may be unnecessarily demanding for participants as similar glycogen depletion may be achieved in shorter sprint formats, making the exercise more applicable to sedentary and clinical cohorts. The work of Metcalfe et al. (2011) worked towards identifying a minimal amount of exercise required for improving parameters of metabolic health. Healthy but sedentary participants underwent a 6 week RE-HIT training consisting of 1 (1st session) or 2 (all other sessions) all-out cycling sprints with sprint bouts separated by low intensity cycling at 60 W. The duration of sprints increased from 10 s in week 1, to 15 s in weeks 2 and 3 and 20 s in the final 3 weeks with no sessions lasting longer than 10 min including warm up and cool down. RE-HIT training increased \( \dot{V}O_2 \) peak by ~ 15 %. Of critical importance however was a significant 28 % improvement in insulin sensitivity in participants following RE-HIT training with this magnitude of change being comparable with responses to 2 weeks of classic SIT training in both active (Babraj et al. 2009; Richards et al. 2010) and obese (Whyte et al. 2010) individuals. The work of Metcalfe et al. (2011) demonstrated for the first time that the volume of the classic 30 s SIT protocol could be substantially reduced whilst still improving metabolic health and aerobic capacity. Further replication of this protocol within a diabetic population is required in order to ascertain if the increases in potential health benefits are as marked. Although not directly defined within the study, the authors concede the potential for 'non-responders' to lie within the data set, suggest it would be logical for RE-HIT work to be repeated with a larger sample size. This would allow comparisons to be made to other high intensity protocol used within the same population (Little et al. 2011).
The application of high intensity training within clinical populations is still a relatively new paradigm. Evidence clearly suggests that for insulin resistant and T2DM populations low volume high intensity work may prove as beneficial as high volume continuous moderate exercise from both a health and time saving perspective. There is a great deal of work still to be done in order to elucidate the mechanisms through which metabolic health is improved following HIT exercise as well as working towards a true ‘minimum’ in terms of the total exercise stimulus necessary to improve fundamental health markers and co-morbidities of disease. For a summary of notable high intensity interventions please refer to reference table (A) within appendix 1.

2.2 Glucose regulation – An overview

The ultimate purpose of glucose homeostasis is to provide sugar to the brain (Unger, 1991; Cryer, Gerich. 1985). Neuronal tissues lack the ability to synthesise ketone bodies and oxidise their own amino acids or fatty acids without undergoing adverse structural and functional changes (Auer, 1986). Therefore provision of free glucose for neuronal oxidation is essential for survival. Hypoglycaemia leads to altered states of consciousness and can lead to death. Secondly the maintenance of euglycaemia is important for the integrity of proteins throughout the body. Prolonged hyperglycaemia leads to excessive glycosylation of proteins, changing their structure and function, which eventually effects every organ system in the body.

Glucose can be produced from multiple sources i.e. from fats, proteins via gluconeogenesis; and from the muscle and liver via glycogenolysis. Most of the bodies energy reserve is stored as fat (DeFronzo, Ferrannini, 1995). Glucose is also stored as glycogen. Breakdown of glycogen stores from the liver results in a ready supply of
glucose for the brain and other tissues with the need for free glucose and is the primary energy source in rested conditions. Muscle glycogen serves as a local source of glucose as muscle lacks glucose-6-phosphatase and cannot release free glucose into the circulation. Proteins serve as a source of substrates for gluconeogenesis (Gerich, Campbell, 1988). Proteins when necessary are broken down to glucose via gluconeogenesis by the liver and kidney (Owen et al. 1969). The body protects its protein supply given that excessive protein breakdown can lead to cell death. Protein breakdown is often witnessed during prolonged fasting.

Under standard nutritional conditions the liver contains 70 g of glycogen which acts as an efficient storage form of glucose. During fasting, glycogen phosphorylase initiates glycogen breakdown by cleaving a single glucose-1-phosphate which is converted then to glucose-6-phosphate by a de-branching enzyme, Glucose-6-phosphatase then converts glucose-6-phosphate to free glucose. During fasting, glycogen is depleted at a rate of 9% an hour. When fasting exceeds 8 hours, gluconeogenesis progressively replaces glycogenolysis to preserve glycogen stores.

As hepatic glycogen decreases blood glucose levels fall as does insulin secretion. A decrease in plasma insulin leads to an increase in glucagon secretion, mobilisation of gluconeogenic precursors and free fatty acids, and increased hepatic gluconeogenesis. Gluconeogenesis requires a total of 12 ATPs; 2 in the form of guanosine diphosphate (GDP), four in the form of ATP, and six in the form of NADH. The first of these reactions includes conversion of pyruvate to oxaloacetate to phosphoenolpyruvate. The second of these reactions is the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate, which represents the rate-limiting reaction for gluconeogenesis. The final reaction is the conversion of glucose-6-phosphate to free
glucose, which is necessary for glucose to enter the plasma and is unique to the hepatocyte. Following a 10-hour fast, gluconeogenesis accounts for 70% of total hepatic glucose production.

Gluconeogenesis is regulated by the pancreatic hormonal milieu, intracellular substrate levels, fatty acid levels, counter-regulatory hormones, and the central nervous system. Hepatic glucose production is highly responsive to insulin. Half-maximal suppression of glucose production occurs at insulin levels of 25 mU.ml\(^{-1}\), whereas half-maximal stimulation of glucose utilisation occurs at insulin levels of 50 mU.ml\(^{-1}\) (Rizza et al. 1981). A rise in insulin secretion of 5 to 10 mU.ml\(^{-1}\) results in a marked decline in hepatic glucose output (Cahill, 1970). Insulin decreases gluconeogenesis by decreasing intracellular cyclic adenosine monophosphate (cAMP) levels, which inhibits phosphorylation of enzymes. Insulin also inhibits hepatic glucose production indirectly by restraining lipolysis and proteolysis systemically, which reduces delivery of glucose precursors to the liver (DeFronzo, Bonadonna, Ferrannini, 1992; DeFronzo, 1988).

Glucagon has a potent effect on hepatic glucose production by stimulating both glycogenolysis and gluconeogenesis, and it increases glucose output within minutes. Glucagon’s effects on gluconeogenesis are persistent, whereas its effects on glycogenolysis are short-lived (Gerich, Campbell, 1988). Glucagon stimulates gluconeogenesis by increasing intracellular cAMP levels. Increased hepatic glucose production, in turn, results in suppression of glucagon secretion (Gerich, Charles, Grodsky, 1976).
Counter-regulatory hormones such as cortisol, growth hormone, thyroid hormones, catecholamines, and angiotensin II prevent hypoglycaemia by stimulating hepatic glucose production. Epinephrine promotes glycogenolysis, whereas cortisol promotes gluconeogenesis (Gann, Foster, 1999). The actions of norepinephrine and angiotensin II are mediated by the intracellular signals of phosphatidylinositol and calcium to promote glycogenolysis (Coffee, 1998; Gann, Foster, 1999). Cortisol and epinephrine also limit pyruvate use, allowing it to be converted to free glucose. The central nervous system modulates hepatic glucose production via parasympathetic fibres to the liver (DeFronzo, Ferrannini, 1995; Shimazu, 1987). Hypoglycaemia stimulates hepatic glucose production via sensors in the hypothalamic region of the brain, which activate oxaloacetate in the tricarboxylic acid cycle and directly contribute hepatic glycogenolysis via sympathetic fibers to the liver (DeFronzo, Ferrannini, 1987; DeFronzo, Ferrannini, Hendler, 1983).

Humans require a constant source of energy, most of which is derived from oxidation of glucose. First, glucose must enter the cell via glucose transporters. Five glucose transports have been identified (GLUT 1 through to GLUT 5), of which GLUT 4 has the strongest association to the current thesis. GLUT 4 is an insulin-dependent transporter for muscle and adipose tissue. In the basal state most GLUT 4 transporters are not located on the membrane but in intracellular vesicles. Insulin causes a translocation of GLUT 4 from intracellular vesicles to the cell membrane. Insulin also augments GLUT 4 activity; therefore muscle and adipose tissue glucose clearance rates increase 10-fold with increases in plasma insulin levels (DeFrozo, Bonadonna, Ferrannini, 1992; Birnbaum, 1992; Klip et al, 1994; Stephens, Pilch, 1995; DeFronzo et al. 1985).
Having entered the cell, glucose is oxidized into energy via the Embden-Myerhof glycolytic pathway. Glycolysis occurs in the cytosol of all cells and consists of 10 steps that convert 1 mole of glucose into 2 moles of ATP, 2 moles of NADH, and 2 moles of pyruvate. ATP is utilised for cellular energy, whereas NADH and pyruvate provide substrates for other energy-producing reactions. Glycolysis represents the major source of energy (ATP) in cells that lack mitochondria (e.g., erythrocytes) and for cells functioning under anaerobic conditions (e.g., contracting muscle).

The rate-limiting step of glycolysis is the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate. This step is considered irreversible. The other two irreversible steps are the conversion of glucose to glucose-6-phosphate by hexokinase/glucokinase and the conversion of phosphoenolpyruvate to pyruvate by pyruvate kinase. These three reactions are significant because they are the major obstacles that must be overcome in the formation of glucose during gluconeogenic reactions.

When there is an adequate supply of oxygen to tissues, the overall reaction for the glycolytic pathway ends in pyruvate, which is then converted to acetyl CoA, which enters the tricarboxylic acid (TCA) cycle. Approximately 90 % of the potential energy from glucose oxidation is available in the form of pyruvate. The TCA cycle nets an additional 36 moles of ATP for every mole of glucose. Under anaerobic conditions the cells rely on glycolysis for ATP, and pyruvate is converted to lactate. Conversion of pyruvate to lactate regenerates cofactors needed for glycolysis which is particularly pertinent to the current thesis and the source of energy provision required for HIT type training.
2.3 Obesity, adipose tissue and inflammation

Adipose tissue is accepted as being a complex endocrine organ (Trayhurn and Beattie 2001). It is composed of several cell types with approximately 50 % of adipose tissue being composed of adipocytes with the remainder made up of blood cells, endothelial cells, adipose tissue precursors and macrophages (Compher and Badellino 2008). Excess adiposity leads to a marked secretion of adipokines including the inflammatory cytokines and several growth factors. As the largest endocrine organ in the human body (Trayhurn 2005) adipose tissue is able to produce and secrete over 75 inflammatory proteins (Wood et al. 2009) with augmented production of interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α) and intercellular adhesion molecule -1 (ICAM-1) being noted in the obese population, in comparison to lean individuals (Hotamisligil et al. 1995; Kern et al. 1995; Bosanska et al. 2010). Increased adiposity, with concomitant rises in inflammatory cytokine production contributes to a chronic state of inflammation which promotes metabolic disorders (Maachi et al. 2004) including insulin resistance, the metabolic syndrome and T2DM (Shoelson et al. 2006) (Figure 2.1).
Figure 2.1 - Obesity induced changes in adipokine secretion and the development of insulin resistance. Expansion of adipose tissue leads to increased macrophage infiltration and inflammation with enhanced production of pro-inflammatory cytokines (Adapted from Galic et al. 2010).
2.4 Mechanisms of increased inflammation in adipose tissue

Adipocytes are both the source and the target of inflammatory signals (Weisberg et al. 2003; Canzio et al. 2005). Macrophages are mononuclear phagocytes known to infiltrate adipose tissue, residing primarily in the stromal vascular fraction (Weisberg et al. 2003; Xu et al. 2003). Once resident within adipose tissue, macrophages release pro-inflammatory cytokines such as TNF-α, IL-6 and plasminogen activation inhibitor (PAI-1) (Gordon 1998). Weisberg and colleagues (2003) demonstrated that macrophage accumulation in adipose tissue is directly proportional to the measures of adiposity in humans and is significantly higher in the obese compared to lean. The same group (Weisberg et al. 2003) estimated that the percentage of macrophages in adipose tissue ranged from under 10 % in lean humans, to approximately 40 % in obese humans.

Adipocyte size as opposed to overall obesity may act as a trigger for macrophage infiltration. New, smaller subcutaneous adipocytes act as a sink or buffer, absorbing FFA and triacylglycerol (TAG) in the postprandial period. Once these cells have reached an upper threshold, in which they are no longer able to accommodate storage, then preferential storage within visceral adipocytes is engaged (Skurk et al. 2007). As adipocytes grow larger they become dysfunctional, with abdominal adipose mass positively correlated with macrophage infiltration (Curat et al. 2004; Tchoukalova et al. 2004). This is confirmed by data showing that adipocyte hypertrophy in the rat model is coincident with macrophage recruitment and activation as well as inflammation within adipose tissue (Cinti et al. 2005).
Adipocyte size is an important determinant in the release of cytokines, with IL-6, TNF-α, monocyte chemo-attractant protein (MCP-1), and interleukin 1 (IL-1) found in the highest concentrations in the largest adipocytes (Skurk et al. 2007). This is likely due to a greater mean surface area and cell volume. It has been proposed previously that hypoxia may occur in areas within adipose tissue in obesity as a result of adipocyte hypertrophy (Wood et al. 2009). Adipocyte hypertrophy compromises oxygen supply from the vasculature, and thereby initiates the inflammatory response through the recruitment of the transcription factor hypoxic inducible factor 1 (HIF-1) (Trayhurn and Wood 2004). Hypoxia also induces inflammatory responses in macrophages and inhibits differentiation of pre-adipocytes (Lewis et al. 1999; Oda et al. 2006; Ye et al. 2007). Collectively there is strong evidence to suggest that cellular hypoxia as a result of adipose tissue expansion may be a significant contributor to adipose tissue inflammation.

Huang et al (2001) demonstrated that MCP-1 is an essential signal for macrophage activation and recruitment, suggesting this chemokine may be the most likely candidate for the stimulation of macrophage recruitment. Additionally Xu et al (2003) showed that increased expression of MCP-1 in adipose tissue preceded insulin resistance in obese mice, strengthening the suggestion of a causative role of this chemokine in macrophage infiltration.

As well as adipose tissue volume and adipocyte size, cytokine levels may be influenced by fatty acid composition within the diet as demonstrated by Garcia-Escobar and colleagues (2010), who showed that rat adipocytes release greater concentrations of IL-6 following the consumption of a diet high in fatty acids as compared against mice fed on saturated or polyunsaturated fatty acid diets. This
further demonstrates that inflammatory regulation is likely to be moderated by more than one single factor with one such factor being related to nutrition.

2.5 Subcutaneous and visceral adipose tissue

Increasing visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) are independently regarded as risk factors for insulin resistance and T2DM. Around 80 % of all body fat is located in subcutaneous areas (Arner 1997). In contrast VAT makes up only 10 % - 20 % of total fat in men and 5 % - 8 % in women (Wajchenberg 2000).

When the storage capacity of SAT is exceeded, or its ability to generate new adipocytes is impaired because of genetic predisposition or stress for example, fat begins to accumulate in areas outside of the subcutaneous region (Ibrahim 2010). It is storage within the visceral region that is thought to pose the greatest risk of metabolic disease (Gastaldelli et al. 2002).

VAT and SAT differ with respect to their endocrine function, lipolytic activity, response to insulin and other hormones (Ibrahim 2010). Because of its anatomical position, visceral fat venous blood is drained via the portal vein directly to the liver as opposed to venous drainage associated with SAT. Drainage differences may contribute to the strong association between VAT and insulin resistance (Bergman et al. 2006) given that portal drainage allows direct hepatic access to FFA and adipokines secreted by visceral adipocytes. This direct release into the portal vein may contribute to insulin resistance downstream (Figure 2.2).
Figure 2.2 - Energy surplus results in accumulation of TAG in adipocytes at subcutaneous adipose tissue, which acts as a metabolic sink. When capacity of subcutaneous fat is exceeded or if it is impaired, fat will accumulate in areas outside the subcutaneous compartment (Adapted from Ibrahim et al. 2010)

The inflammatory profile of VAT and SAT differ substantially. The dominant source of inflammatory protein release is the visceral adipose depot (Fried et al. 1998; Bruun et al. 2005; Bosanska et al. 2010), although it has been previously shown (Gletsu et al. 2006) that IL-6 was more abundant in the SAT rather than VAT. Additionally adiponectin abundance has been shown to be greater within SAT (Fain et al. 2004; Lihn et al. 2004). Despite reported higher concentrations of inflammatory proteins in VAT, when contextualised SAT may provide a more substantial contribution toward an overall state of chronic low-grade inflammation in comparison to VAT because of the volume of tissue.

2.6 Chronic low grade inflammation, insulin resistance and T2DM

Hotamisligil and colleagues (1993) and others (Feinstein et al. 1993) first showed that the pro-inflammatory cytokine TNF-α induced insulin resistance. Later additional
inflammatory mediators were described as being products of adipose tissue (Zhang et al. 1994; Shimomura et al. 1996; Fried et al. 1998; Steppan et al. 2001; Fukuhara et al. 2005). Whilst the likes of leptin and adiponectin are true ‘adipokines’ being produced exclusively by adipocytes, others such as TNF-α, IL-6, MCP-1 are highly expressed in other cells and activated macrophages. The relative inflammatory contribution of the aforementioned cell types to inflammatory output is still uncertain.

Primarily insulin resistance is mediated by 2 pathways, JNK and IKKβ / NF-κB with each being operationally diverse. JNK has been shown to promote insulin resistance through phosphorylation of serine residues in IRS-1, inhibiting the normal tyrosine kinase cascade through counter-regulatory serine/theonine phosphorylation (Zick et al. 2005). In contrast IKKβ liberates NF-κB for translocation into the cell nucleus where it promotes the expression of numerous target genes whose products induce insulin resistance (Figure 2.3).

In addition to inflammatory cytokines other stressors such as reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress have been shown to activate JNK and NF-κB. Markers of oxidative stress increase in line with increases in adiposity which is consistent with a role for ROS in the development of obesity induced insulin resistance. A potential mechanism for this action has been described and involves the activation of NADPH oxidase by lipid accumulation within the adipocyte with a complimentary rise in ROS production (Ruperez, Gil and Aguilera 2014).
Figure 2.3 - Potential cellular mechanisms for activating inflammatory signalling. Obesity activates IKKβ / NF-κB and JNK pathways in adipocytes, hepatocytes and associated macrophages. Intracellular stresses such as ROS and ER have also been demonstrated as stimuli able to activate these pathways (Adapted from Shoelson, Lee and Goldfine 2006).
2.7 Exercise training and chronic low-grade inflammation

A large body of evidence stretching beyond the scope of this current review exists as to the effects of exercise training on inflammatory regulation (Gleeson et al. 2011) in health and disease (Adamopoulos et al. 2002; Zoppini et al. 2006; Oberbach et al. 2008; Gray et al. 2009; Balducci et al. 2010; Leggate et al. 2012). The true mechanisms contributing to an exercise induced anti-inflammatory environment remain un-clear. Mechanisms such as those relating to increased release of cortisol and adrenaline; reduced expression of toll like receptors (TLRs) on macrophages; inhibition of adipose tissue infiltration by macrophages are amongst many pathways all of which are likely to be involved in reducing inflammation (Gleeson et al. 2011). An important consideration is how specific exercise modalities and intensities contribute to the promotion of these anti-inflammatory mechanisms. High volume continuous moderate exercise for example may act via adipose tissue specific pathways whereas higher intensity shorter duration exercise is likely to mediate catecholamine modulated pathways. The contribution of these independent exercise modalities to inflammation related health status within the ‘at-risk’ population remains un-defined.

Most studies investigating the interaction between exercise and inflammatory status show commonality in use of low to moderate intensity exercise, often less than 60 % $\dot{V}O_{2\text{max}}$ with only recently work (Leggate et al. 2012) beginning to look at the potential benefit of exercise at higher intensities and differing exercise paradigms. For example, work in the healthy population (Gray et al. 2009) has demonstrated that a 12 week walking intervention was unsuccessful in inducing changes in plasma c-reactive protein (CRP), IL-6, sIL-6R, TNF-α or its receptors, in previously inactive participants (< 30 min of moderate exercise 5 x per week). In support, Keller et al (2005a, 2005b)
ran a 10 week training study in 7 untrained but otherwise healthy males. Despite training for 1 h per day, 5 x per week at 75 % maximal power output no significant reductions in inflammation occurred. Clearly sample size must be considered when reviewing this work and may explain the non-significant changes. Utilising a larger sample size Ho and colleagues (2013) subjected 64 overweight and obese individuals to 12 wks of aerobic exercise consisting of 30 min of treadmill running at 60 % HRR, 5 x per week. Data reveal that after training there were significant reductions in TNF-α but no change in IL-6. Correspondingly, Oberbach et al. (2006) found no change in IL6, CRP, IL-10 or adiponectin following 4 weeks of moderate intensity exercise in a healthy cohort. Of note however was a reduction in inflammatory profile within those with prior metabolic abnormalities.

Exercise of a higher intensity was used by Gray and colleagues (2009), who conducted training in sedentary, overweight but otherwise healthy males. After 12 and 24 weeks of progressive moderate intensity training (~ 70 % \( \dot{V}O_{2\text{max}} \)) IL-6 was significantly reduced. Through utilisation of a non-exercise de-training period the authors (Gray et al. 2009) reported that IL6 increased, returning close to baseline levels; bringing into question the strength of the initial exercise effect. Furthermore these data give rise to speculation that exercise intensity may be the critical factor in inducing positive changes within the inflammatory profile. Leggate et al (2012) demonstrated within an overweight and obese group, that in just 2 weeks plasma sIL-6R and MCP-1 as well as IL-6 in adipose tissue decreased following HIT based training. Training consisted of 10 x 4 min of cycling at 85 % \( \dot{V}O_{2\text{max}} \) performed 3 x per week. Balducci and colleagues (2010) demonstrated that 12 months of training, twice per week at 70 %- 80 % \( \dot{V}O_{2\text{max}} \) reduced inflammation in T2DM patients and in those with metabolic syndrome. Levels of IL-6, TNF-α and CRP were all reduced with levels
of IL-10 increased. Increased IL-10 in association with increased levels of adiponectin after training promotes the current theory that adiponectin induces production of IL-10 in macrophages and monocytes (Wolf et al. 2004). Given the disparity between results in this (Balducci et al. 2010) work and that of previous studies suggests that higher exercise intensities are critical in order to reduce TNF-α especially when body mass is unchanged as found by Balducci et al. (2010). Associations with adiponectin need further attention given the work to suggest the independent inflammatory roles of multiple adiponectin isoforms (Lara-Castro et al. 2006).

These data and those mentioned above give rise to the conclusion that exercise intensity is a key component in the reduction of inflammation. Evidence suggests that exercise training may be a suitable treatment / prevention strategy for the treatment of inflammation in the circulation and in adipose tissue. There is emerging evidence to suggest that intensity of exercise may be critical in inducing an anti-inflammatory response with the possibility of an inflammation-exercise intensity dose response relationship although more work is required to confirm or deny this.

Work suggests that resistance training may be an alternative exercise stimulus capable of stimulating a beneficial effect on some inflammatory mediators. For example 1 year of moderate intensity resistance training, at least twice per week in overweight women was shown to induce reductions in CRP and increase adiponectin despite no changes detected in IL-6, ICAM-1 and irrespective of there being no changes body fat or fat mass (Olson et al. 2007). The comparison of resistance and aerobic training was made by Donges et al (2010). Ten weeks of resistance training but not aerobic training reduced levels of circulating CRP in an overweight cohort. Despite this change, no changes were detected in IL-6 for either group. With support
of the above evidence it may be suggested that resistance training offers some potential benefits for reduction of some pro-inflammation proteins notably CRP, yet may not induce improvements in others such as IL-6, at least within a healthy population. Resistance exercise may act as an effective supplement to a mixed modality exercise programme.

Further studies have investigated the effects of exercise training in diseased populations including those with metabolic diseases such as T2DM and the metabolic syndrome; that are partly defined by having elevated levels of chronic low-grade inflammation. Twelve weeks of exercise has been shown to be effective in reducing IL-6, sIL-6R, and TNF-α in chronic heart failure patients (Adamopoulos et al. 2002) with patients having exercised for 30 min, 5 x per week at 60 % - 80 % of maximal heart rate. These findings were not completely replicated in a T2DM population who undertook 4 weeks of moderate intensity exercise 4 days per week (Oberbach et al. 2006). T2DM patients and those with impaired glucose tolerance failed to change IL-6 or IL-10 after training however did show improvements in CRP and increases in adiponectin. Moderate intensity exercise induced reduction of inflammatory proteins such as intercellular adhesion molecule 1 (ICAM-1) which has been shown on a number of occasions in ‘at risk’ populations, specifically the obese, those with impaired glucose tolerance and those with T2DM. Short and long term exercise interventions have reduced ICAM-1 concentrations in T2DM patients specifically with interventions ranging between 3 / 4 weeks - 6 months with frequencies ranging from 1–7 days per week (Roberts et al. 2006a, 2006b; Zoppini et al. 2006; Tonjes et al. 2007). Interestingly the same exercise interventions were proven unsuccessful in healthy participants (Zoppini et al. 2006). Exercise interventions incorporating both aerobic and resistance exercise were effective in reducing MCP-1 concentrations in male
T2DM patients (Troseid et al. 2004). Interestingly, in the majority of the aforementioned investigations changes in MCP-1 or ICAM-1 were associated with significant reductions in weight loss. This suggests that adiposity may be the critical factor moderating inflammatory profile subsequently rendering it impossible to determine the true independent effect of exercise. These data further suggest that exercise training may be an effective method of treating inflammation with clinical and sub-clinical populations.

From evidence reported here this gives rise to the suggestion that diseased populations may benefit from accessing monitored and controlled high intensity training which may give rise to more marked changes in inflammatory profile over a shorter period of time, however may equally benefit from longer duration exercise modalities which target adipose specific inflammatory pathways.

As alluded to, the anti-inflammatory effect of exercise is likely mediated via a collective of pathways some of which are specific to adipose tissue. With this in mind weight loss or more specifically reductions in adipose tissue plays an important role in the reduction of inflammation. Reported weight loss in previous work has been variable and primarily attributed to poor dietary control. Interventions that have controlled dietary restriction well, alongside exercise for weight loss have demonstrated positive improvements. Bruun and colleagues (2006) demonstrated a ~18 kg reduction in body mass with accompanying reductions in circulatory IL-6, CRP and MCP-1 following 15 weeks of both moderate intensity exercise and a hypo-calorific diet in a morbidly obese population.
Supporting the work of Bruun et al (2006) using a similar protocol, Christiansen et al (2010a) found that IL-6 and MCP-1 were significantly reduced with adiponectin significantly improving. This 3 month combined diet and exercise intervention induced ~ 12 kg weight loss with an exercise only group demonstrating a much smaller ~ 3.5 kg weight loss. This less prominent weight change was accompanied by no change in inflammatory proteins or adiponectin. These data give rise to the suggestion that targeting inflammatory pathways specifically associated with adipose tissue, using exercise and caloric restriction, poses as an effective strategy for the reduction of inflammation. It would seem however that weight reduction in the region of ≥ 3-5 kg may be required for meaningful inflammation related health benefits.

Clearly exercise training is able to improve inflammation in both healthy and diseased populations. Interventions utilising exercise ≤ 60 % \( \text{VO}_{2\text{max}} \) may not be as effective in reducing inflammation as exercises set at intensities ≥ 70 % \( \text{VO}_{2\text{max}} \). Additionally, reduction in inflammation is partly driven by changes in adiposity; hence exercise interventions with accompanying dietary restriction may be the most successful method of improving inflammatory profiles. New data investigating HIT exercise proves promising, providing a new exercise paradigm that may induce rapid reductions in inflammatory profile.

2.8 High intensity interval training and body composition

Aerobic exercise programmes, typically of low to moderate intensity of 30 min - 40 min over 3 or 4 days of the week have been shown to increase cardiorespiratory fitness (Ross et al. 2000) and preserve fat free mass (Evans et al. 1999). These training programmes have been the most frequently adopted by the general population to
improve health, however the resultant overall fat loss has been shown to be minimal (Boutcher and Dunn 2009; Wu et al. 2009). Furthermore given that the estimated optimal dose of aerobic exercise required to significantly reduce visceral fat is 3780 kcal expended per week (Ohkawara et al. 2007) it is likely that the overweight and obese populations may find difficulty in adhering to such high weekly workloads.

HIT may offer a novel alternative to aid in the reduction of body fat. In a recent study Heydari et al. (2012) subjected 14 men and 13 women to either a 20 week programme of endurance training (4 - 5 sessions per week, 30 – 45 min in duration at an intensity equating to 60 % - 85 % of maximal HRR) or a 15 week high intensity programme (10 s - 30 s of high intensity bouts at 60 % – 70 % of maximum work output in 10 s, separated by recovery dependant on heart rate recovery). Body composition was assessed by using the sum of 6 skin folds, with an average taken from 3 valid measurements. Results demonstrated striking differences in total EE between the 2 training paradigms. Despite no change in body weight pre and post training in either endurance (60.6 ± 13.4 kg and 60.1 ± 12.1 kg respectively) or HIT group (63.9 ± 11.0 kg and 63.8 ± 11.5 kg respectively), the HIT group had a tendency for greater reductions (14 %) in the sum of 6 skinfolds. The endurance training group reduced sum of 6 skinfolds by 5.7 %. However when looking at the baseline sum of 6 skinfold measurements, it would appear that the HIT group had 17 % higher values compared to the endurance trained group. This difference is larger than the difference found pre and post exercise for skinfolds in the interval training group (14.8 %). Evidence suggests that given a higher degree of body fatness, the greater the potential to show reductions following training, as described in the correlation presented by Trapp et al (2008). Had groups been more accurately matched, then the difference between the groups would not be as marked.
Trapp et al (2008) compared fat loss between HIT and continuous based exercise as well as including a non-exercising control group. Body composition was assessed via DEXA scans which were taken before and after the training programme. The HIT training consisted of 8 s ‘all out’ bouts of cycling followed by 12 s rest. These reps continued for a maximum of 60 efforts, with the authors stating exercise time to be between 5 min and 20 min depending on ability. The continuous steady state exercise group performed 20 min - 40 min of cycling at an intensity equivalent to 60 % $\dot{V}O_{2\text{peak}}$. A control group consisted of non-exercising participants who maintained dietary and physical activity diaries as evident within the exercising groups and simply carried out pre and post assessments. Data revealed a significant degree of fat loss achieved in the HIT group (2.5 ± 0.83 kg) compared to increased fat mass shown in the continuous moderate exercise group (0.44 ± 0.88 kg) and control group (0.33 ± 0.47 kg). Furthermore, HIT exercise induced a significant reduction in central abdominal fat (-0.15 ± 0.08 kg). Both continuous and control groups showed increases of 0.1 ± 0.08 kg and 0.3 ± 0.04 kg respectively. The authors attribute the stronger response from HIT to be enhanced lipid utilisation however the precise mechanisms have yet to be elucidated. The methods employed by the authors to estimate EE lacked precision and therefore it cannot be reliably concluded that energy balance was unaffected in the HIT group. Energy expenditure was estimated by converting the workload for each session into oxygen consumption in L.min$^{-1}$. A more accurate methodology may well include continuous on-line measurement during exercise. This would provide breath-by-breath data allowing for estimation models to be applied to accurate $O_2$ and $CO_2$ data. It may also be feasible that changes in fat mass may have been influenced by unreported changes in diet. Additionally the same may be true when rationalising the increase in fat mass in both continuous exercise and control groups. The use of various ethnicities within the study may also be reason to view this data with caution.
It is recognised for example that the South Asian population have a higher percentage of body fat at any BMI compared to a Caucasian population (Lear et al. 2007), and thus a greater potential for fat loss. There may also be evidence to suggest that the metabolism of fat differs between ethnicities (Kuller 2004) and as such using a mixed ethnicity cohort may not be the most accurate method of assessing changes in body fat.

Possible mechanisms underlying fat loss following HIT based exercise involve increased exercise and post-exercise fat oxidation with potentially decreased post-exercise appetite. Gaitanos et al. (1993) suggest that towards the end of a HIT session consisting of multiple repetitions, an inhibition of anaerobic glycogenolysis occurs. Moreover it is proposed that ATP resynthesis is mainly derived from PCr degradation and intramuscular TAG stores. The increase in blood glycerol has been shown to accompany HIT in trained (Trapp, Chrisholm and Boutcher 2007) and un-trained (Talanian et al. 2007) cohorts, thus supporting the theory that progressively through training, HIT exercise may result in increased fatty acid transport. An increased capacity for fat oxidation has been described previously (Burgomaster et al. 2005) following as little as 6 session of HIT.

In summary HIT has been shown to be beneficial for fat loss in a limited number of studies, however the various limitations within these studies warrant further investigations that impose more robust measurement strategies, looking exclusively at manageable forms of HIT as the training stimulus and in direct comparison with continuous exercise modalities across a diverse range of experimental cohorts.
2.9 HIT and excess post exercise oxygen consumption

One possible mechanism previously proposed (Boucher et al. 2011) for fat loss relates to elevations in post-exercise metabolic rate. Excess post exercise oxygen consumption (EPOC) has been proposed as a possible mechanism for fat loss following exercise (Boutcher 2011). It is particularly pertinent to address the effect of HIT upon EPOC which will be explored in detail in chapter 3.

The term EPOC was developed by Gaesser and Brooks (1984) and describes elevations in metabolic rate above that of basal levels, over an extended period of time following exercise. EPOC can be divided into 2 distinct components, firstly the rapid component, which describes the sum of components that decays within approximately 1 h; and a slow component with a half-life in the order of several hours (Bahr 1992). Processes that are active beyond the first hour after exercise must be responsible for the slow EPOC component (Borsheim and Bahr 2003).

Brockman et al (1993) investigated HIT in five well-trained females. Females completed both 10 min of running at 80 % $\dot{V}O_{2\text{max}}$ as well as a HIT protocol, which incorporated 7 x 2 min of sprinting at an intensity of 90 % $\dot{V}O_{2\text{max}}$, with 2 min of active recovery between bouts (~ 5 km.h$^{-1}$), noting an increased EPOC of 3.31 L. The same group also completed a 2 h walk at 25 % $\dot{V}O_{2\text{max}}$ which produced only a small EPOC of 1.52 L suggesting that HIT based training may be more successful than continuous exercise in elevating mechanisms associated with increased metabolic rate after training.
A key study comparing continuous versus interval exercise came from the work of LaForgia et al (1997). Data indicated that the intermittent bout resulted in an EPOC of 15.0 L (measured over 9 h after exercise) which was 2.5 times higher than the EPOC achieved following the energy matched continuous exercise bout (6.9 L). The duration of EPOC also varied significantly, with the EPOC attributed to intermittent exercise lasting 9 h before returning to baseline values. This was in contrast to an EPOC lasting only 2 h following the continuous protocol. Interestingly, when comparing these data to that reported within the Gore and Withers (1990a, 1990b) it is clear that their reported EPOC following 80 min of continuous exercise at 70 % \( \dot{V}O_{2\text{max}} \) (14.6 L) is similar to that achieved following intermittent exercise in LaForgia (1997), despite the continuous exercise involving twice the amount of total work done. Furthermore relating to the LaForgia study, EPOC measurements were not taken during the recovery periods during the intermittent protocol. Despite not measuring this parameter the authors did address this limitation and estimated that the total EPOC from these rest periods would probably amount to an additional oxygen consumption of 2-3 L.

In conclusion these data further demonstrate the importance of exercise intensity for significant elevations in post-exercise metabolic rate. Of equal importance is the suggestion that greater elevations in EPOC are demonstrated following the intermittent exercise modality over classical continuous forms of exercise, regardless of intensity.

Further work matching total sub-maximal work done in both continuous and intermittent exercise has been conducted. McGarvey, Jones, Peterson (2005) aimed to identify if \( O_2 \) consumption was different over a 2 h post-exercise period following
continuous and HIT based exercise. The findings from this study indicated a higher total O$_2$ consumption and RER during exercise in the intermittent protocol compared to the continuous protocol. Although precise values were not reported, the authors quote that EPOC duration was on average 4 min longer for the intermittent exercise condition compared to continuous. Despite significant differences being found during exercise, there was no significant difference between the magnitudes of EPOC following intermittent or continuous exercise. This work is limited given that baseline resting metabolic rate (RMR) values were collected 20 min prior to exercise. This would potentially provide elevated V̇ O$_2$ levels due to the anticipatory response of the subject. Furthermore, through the work of MacDonald et al (1998) it is appreciated that in the supine position V̇ O$_2$ kinetics are slowed to a rate lower than that of a participant sat in an upright position.

Continuing with the work of McGarvey et al (2005), the physiological responses to their 2 exercise modalities varied, with RER values being lower in intermittent exercise compared to continuous, for nearly the whole 2 h recovery period. Although the initial dip in RER within the 30 min - 40 min of recovery (for both modalities) may be attributed to CO$_2$ retention and lactate buffering, the continued low RER into the second hour for the intermittent exercise would seem to be reflective of a greater reliance of fat metabolism. This is in agreement with the work of Yoshioka et al (2001) who investigated how HIT based exercise acutely altered EE and lipid utilisation. Participants were assigned to complete rest, continuous moderate or HIT based exercise. The group failed to give the full details of the protocols employed however utilised intensities of 35 % and 77 % VO$_{2\text{max}}$ for the moderate and intermittent exercise respectively. They further note that fat oxidation tended to be greater following the high intensity condition compared to moderate.
Warren et al (2009) made the point that EE would have to remain significantly elevated for several hours post exercise in order to have a beneficial effect on fat loss. Knab and colleagues (2011) investigated the effects of ~45 min of continuous cycling on 24 h EE (during exercise and at rest) using a whole room calorimeter system. Specifically this group used a protocol consisting of 2 min at 50 %, 2 min at 75 % and 41 min at 50 % and a further 2 min at 50 % of ‘maximum work load’ (57 % maximum work load = 70 % $\dot{V}O_{2\text{max}}$). The exercise protocol itself resulted in a net EE of 519 ± 60.9 kcal. Of greater importance EE remained significantly elevated for 14.2 h following exercise, corresponding to an additional 190 ± 71.4 kcal. These data suggest that HIT exercise may have a significant effect upon post-exercise metabolism.

Recently Larsen and colleagues (2013) investigated the impact of metabolic syndrome on EPOC response to high intensity exercise. Seven metabolic syndrome patients completed 1 x 4 min cycling at 85 % - 95 % $HR_{\text{max}}$, 4 x 4 min cycling at the same intensity, separated by 3 min recovery periods vs. 47 min continuous cycling at 70 % $HR_{\text{max}}$ all separated by at least 48 h. Data demonstrated that EPOC length did not differ significantly between HIT and continuous exercise trials. These data should be viewed with caution given the short baseline RMR measurement period. Additionally oxygen samples were collected as 1 min averages which may mask some variability within the $\dot{V}O_2$ kinetics, subsequently effecting AUC calculations. These data were replicated by Williams et al (2013) who demonstrated no significant differences in EPOC following 4 x 30 s all out SIT sprints, 60 min continuous moderate cycling at 60 % $\dot{V}O_{2\text{peak}}$ or a resting control as measured over a 3 h period.

In summary, exercise has been shown to increase metabolic rate above that of baseline. There is to date no clear evidence that HIT exercise is more effective than
traditional endurance exercise in elevating EPOC over an extended period of time. There is a clear need for more commonly utilised HIT protocols to be profiled with regard their contribution to EPOC. It is the aim of the next chapter to explore further the effects of HIT on EPOC and to ascertain a detailed profile of HITs benefit for fat loss. It is the aim of the next chapter to explore further the effects of HIT on EPOC and to ascertain a detailed profile of HITs potential benefit for fat loss.

**Aims of thesis:**

- To profile the EPOC associated with HIT and AIT training and to draw conclusions upon this mechanisms contribution to fat loss.

- To establish the minimum HIT training volume required in order to improve markers of cardio-metabolic health.

- To determine if short and medium term HIT training can improve glucose control in an obese population

- To determine if short and medium term HIT training can reduce the inflammatory profile in overweight and obese individuals.
CHAPTER 3

GENERAL MATERIALS AND METHODS
3.1 Participants and ethical approval

All studies presented in this thesis were approved by the Loughborough University Ethical Advisory Committee and were conducted in accordance with the Declaration of Helsinki (2008). The volunteers gave informed written and verbal consent after being advised of all possible risks and discomforts associated with the procedures used in the study designs.

All participants were asked to complete a health-screening questionnaire at the time of consent to ascertain suitability for the study. Any participants reporting haematological or inflammatory disorders, or were taking any non-steroidal anti-inflammatory drugs (NSAIDs) were excluded from taking part.

All participants with a BMI > 30 kg.m$^2$ were subjected to a physical exam including resting ECG and full cardiopulmonary stress test to ensure a normal cardiovascular response to incremental exercise. Any participants presenting with arrhythmia at rest or during the exercise test were excluded from the research and referred for further clinical investigation at cardiology outpatients (Leicester Glenfield Hospital).

3.2 Anthropometric measurements

Participant’s height and body mass were measured prior to all experimental tests. Height and body mass were determined using a balanced beam scale with attached stadiometer, with participants wearing shorts, t-shirt and no footwear. Waist and hip circumferences were measured with a measuring tape. Waist circumference was measured half way between the iliac crest and the lowest rib. Hip circumference was
measured at the widest part of the hips. These measurements were used to calculate mean waist-hip ratio.

Total body composition was measured by dual-energy X-ray absorptiometry (DEXA) using a Lunar Prodigy (GE corporation, Fairfield, Connecticut, USA). The output segmented 3 compartments of fat mass, bone mineral content and fat free soft tissue, the last 2 of which constitute fat-free mass and percentage body fat. DEXA has been validated as a measure of body fat in obese and normal weight individuals (Paradisi et al. 1999; Van, Mayclin 1999; Bertin et al. 2000).

3.3 High intensity intermittent training

A HIT protocol utilised in chapters 4 and 5 consisted of 10 x 1 min intervals at a load equivalent to ~ 90 % HRpeak separated by 1 min recovery performed on a cycle ergometer (Little et al. 2011). Participants completed a familiarisation trial for the HIT protocol before all main trials. During this visit participants completed 5 x 1 min intervals followed by 1 min recovery. This session allowed the external work to be calculated for the HIT trial.

3.4 Glucose tolerance testing

Participants attended the laboratory having fasted for at least 12 h overnight. Plasma insulin and glucose were determined from venous blood samples collected into EDTA monovettes from a 21 GA cannula inserted into an antecubital vein. Blood samples were collected before and 30 min, 60 min, 90 min and 120 min after ingestion of 82.5 g dextrose monohydrate dissolved in 200 ml of water. This solution was immediately followed by 100 ml of water. Blood samples were immediately centrifuged at 1600 x g
(10 min at 4°C) and the plasma aliquoted into labelled Eppendorf tubes and stored at -80°C until the analysis of glucose and insulin. Insulin sensitivity was determined by the insulin sensitivity index (ISI) calculated using the oral glucose tolerance test (OGTT) glucose and insulin values from the formula proposed by Matsuda and DeFronzo (1999). The AUC for plasma insulin and glucose were calculated from before to 120 min after ingestion of the dextrose drink using the trapezoidal method.

3.5 Blood pressure

In chapters 5 and 6 arterial blood pressure was measured using a digital automatic blood pressure monitor (Omron M7. Omron Healthcare UK Ltd, Milton Keynes, UK). Participants remained in a seated position for 5 min before the 1st measurement. A cuff was placed around the upper dominant arm, which rested on a firm surface at the level of the heart during all measurements. Blood pressure was measured 3 times with results reported as an average of the 2nd and 3rd readings.

3.6 Blood sampling and handling

Repeated blood samples were collected from a 21 GA BD Venflon cannula (Becton Dickinson Infusion Therapy, Helsingborg, Sweden) via an antecubital vein. The cannula was kept patent via regular flushing with 0.9% (w/v) saline solution. The first 2 ml of blood extracted from the cannula via a syringe was discarded. Blood samples were collected into BD vacutainers (BD, Plymouth, UK) containing either 1.8 mg EDTA per ml of blood (glucose and inflammatory hormones) or 17 IU lithium heparin per ml of blood (insulin). Blood samples were gently inverted 8 times and then placed on an SRT6 Stuart roller mixer (Bibby Scientific Ltd, Stone, UK) to ensure mixing. Whole blood glucose was analysed immediately via a glucose oxidase reaction, using an
automated analyser (YSI Stat 2300, Yellow Spring Instruments, Yellow Spring OH, USA). Remaining samples were centrifuged at 1600 x g for 10 min at 4 °C (Heraeus Labofuge 400 R, Kendro Laboratory Products, Langenselbold, Germany). The resulting plasma was aliquoted into Eppendorf tubes and stored at -80 °C for subsequent analysis. Participants were in a seated position throughout all sampling periods with the arm continuously air warmed in order to collect arterialised venous samples.

### 3.7 Dietary Controls

Prior to entry to the lab, participants were asked to record their dietary intake over a 24 h period as accurately as possible. Participants were familiarised with electronic weighing scales and were advised on how to record food weights and times of consumption. Prior to all subsequent visits, participants were asked to replicate this diet as accurately as possible, so to ensuring consistency across trial periods. Additionally participants were asked to restrain from alcoholic or caffeinated drinks in the 24 h prior to trial but were free to consume water ad libitum, this information also being recorded.

Within chapter 6, participants were advised not to change their diets during a 6 wk training period. In order to monitor this, participants completed 4 day diet diaries on 2 separate occasions (separated by at least 1 week) prior to trial commencement. As above, participants were fully familiarised with electronic food scales and advised on how to accurately complete the 4 day diary. This provided an average baseline for dietary intake. Participants completed the 4 day diet diary again during week 3 of
training in order to ensure maintenance of the pre-trial food intake. For detail please review methodology within the chapter.

3.8 Enzyme linked immunosorbent assays

Insulin, adiponectin and MCP-1 were quantified using commercial sandwich enzyme linked immunosorbent assays (ELISAs). Both TNF-α and IL-10 were measured via high sensitivity assays (R & D systems, Minneapolis, MN, USA).

Plasma IL-6, sIL-6R, and CRP were analysed via ‘in-house’ ELISAs. All materials and chemical reagents were obtained from Sigma-Aldrich Ltd (Poole, UK) unless otherwise specified. All incubation periods were at room temperature and during each incubation stage the plate was placed on a Stuart Mini Orbital Shaker (Bibby Scientifc Ltd, Stone, UK) at 60 revs.min⁻¹ unless otherwise stated. Wash steps for ELISAs were carried out manually using a multi-channel pipette (Sartorius, NY, USA). The absorbance of wells was read using a Varioskan Flash Mutimode Reader (Thermo Scientific, Vantaa, Finland). Protein concentration of samples was determined in relation to a 4-parameter logistic standard curve. The equation of the curve is given below:

\[ X = \log \text{EC50} - \log_{10}\left(\frac{(\text{Top} - y)}{(y - \text{Bottom})}\right)^{\frac{1}{\text{Hillslope}}}. \]

Where \( X \) is the sample concentration; \( y \) is the absorbance; \( \text{EC50} \) is the middle of the curve. All samples were analysed in duplicate and were repeated if the coefficient of variation (CV) between duplicates was more than 10 %.

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3.9 Interleukin 6 assay

An ELISA for the detection of plasma IL-6 was optimised using a human IL-6 antibody set (OptEIA, BD Biosciences, Oxford, UK) containing a primary and secondary antibody and recombinant human IL-6 (rhIL-6) for standards. Immulon 4HBX Flat 96-well microtiter plates (Nunc, Thermo Scientific, Roskilde, Denmark) were coated with 100 µl anti-human IL-6 monoclonal capture antibody diluted 1:250 in a 0.1 M sodium carbonate buffer. The next day the plates were washed with Tris Buffered Saline (TBS), pH 7.5 with 0.05 % Tween 20 (TBS-T) 3 times, and then blocked with 5 % bovine albumin serum (BSA; Probumin, Millipore, Illinois, USA) in TBS. The plates were incubated for 1 h at room temperature. Afterwards, plates were washed and 100 µl of samples and standards were added to the wells in duplicate. Plasma samples were diluted 1:5 in TBS with 10 % foetal calf serum (FCS). The rhIL-6 was serially diluted in TBS with 10 % FCS from 20 to 0.156 pg.ml⁻¹. TBS with 10 % FCS served as the zero standard. After 2 h plates were washed 6 times and the 100 µl of biotinylated anti-human IL-6 monoclonal antibody detection antibody diluted 1:250 in TBS-T with 1 % BSA was added per well. Plates were further incubated for 1h before being washed 7 times. The enzyme streptavidin alkaline phosphatase (AKP) was diluted 1:2000 in TBS with 1 % BSA and 100 µl added per well. Plates were then incubated for 45 min. Washes were repeated as in the previous step before the addition of an ELISA amplification system (Invitrogen, Paisley, UK). In the first step 50 µl of substrate solution was added to all wells and plates incubated on the bench top before adding 50 µl of the amplification solution. The reaction was stopped within 15 min of the amplification solution being added by the addition of 10 % hydrochloric acid (stop solution) and the absorbance read at 490 nm with a correction wavelength of 690 nm. This assay measured total IL-6 content.
3.10 Soluble interleukin 6 receptor assay

Plasma sIL-6R concentration was determined by ELISA via a method adapted from Gray et al. (2008). Antibody pairs M5 (capture) and M182 (detection) were used to detect sIL-6R. Immulon 4HBX Flat 96-well microtiter plates were coated with 100 µl Purified Mouse Anti-Human CD126 capture antibody (Clone M5, BD Biosciences, San Diego, USA), diluted in 0.1 M sodium carbonate buffer to give a concentration of 2 µg·ml⁻¹ and were stored at 4 °C overnight. The next day plates were washed 3 times with phosphate buffered saline (PBS) with 0.05 % Tween 20 (PBS-T) and wells subsequently blocked with PBS with 10 % FCS (assay diluent). Plates were incubated for 1 h before being washed again and adding 100 µl standards or samples to wells. Samples were diluted 1:200 in assay diluent. The rhIL-6R (R&D systems, Minneapolis, MN, USA) was serially diluted in assay diluent to give standards ranging from 500 to 0.5 pg·ml⁻¹, was added to each well. Plates were incubated for a further hour before washing 5 times. The enzyme Streptavidin Horse Radish Peroxidase (HRP) (BD Biosciences) was diluted 1:4000 in assay diluent and 100 µl added per well. Meanwhile a substrate solution was prepared. After 45 min the plate was washed 7 times and 100 µl of the working substrate solution added for 30 min before the addition of 50 µl of stop solution. The final absorbance was read at 450 nm with a correction wavelength of 570 nm.

3.11 C-reactive protein assay

CRP was quantified from an ELISA method adapted from (Pawluczyk et al. 2011). Immulon 4HBX Flat 96-well microtiter plates were coated with 100 µl of primary anti-CRP rabbit polyclonal antibody (Abcam, Cambridge, UK), diluted to a working concentration of 3.5 µg·ml⁻¹ in 0.05 M sodium carbonate, pH 9.6. The plates were
incubated at 4 °C overnight. The following morning the plates were washed 4 times with PBS-T and the wells blocked with 100 µl PBS with 1 % BSA (Probumin, Millipore, Illinois, USA). The plates were then incubated for 1h at room temperature and then washed as before. Afterwards, 50 µl of plasma samples (diluted 1:100 in PBS) or standards were added to the wells in duplicate. Human CRP (NIBSC, Potters Bar, UK) was used as a calibrant for this ELISA method. A CRP stock of 50 µg·ml⁻¹ was serially diluted in PBS to give a standard curve ranging from 1000 to 1 ng·ml⁻¹. The plate was incubated at 4°C overnight. On the 3rd day the plates were washed as before and 50 µl of a secondary mouse monoclonal antibody to CRP (Abcam, Cambridge, UK) diluted to a working concentration of 2.7 µg·ml⁻¹ in PBS was added to each well. Plates were then incubated for 2 h and the wash step was repeated. For the next step, polyclonal rabbit anti-mouse IgG conjugated to HRP (Dakeo, Ely, UK) was diluted 1:1000 in PBS and 50 µl of the diluted solution was added to the wells and plates were incubated for 1 h. A soluble substrate solution for the detection of peroxidase activity was prepared using SIGMAFAST OPD tablets shortly before the next wash step. One o-Phenylenediamine dihydrochloride (OPD) tablet and one urea hydrogen peroxide tablet were dissolved in 20 ml deionised water to give a final concentration of 0.4 mg·ml⁻¹ OPD, 0.4 mg·ml⁻¹ urea hydrogen peroxide, and 0.05 M phosphate-citrate, pH 5.0. The plates were washed a further 4 times and 50 µl of the substrate solution was immediately added to all wells and the colour was left to develop for 5 min before stopping the reaction with 100 µl of 10 % hydrochloric acid. The absorbance of the wells was read at 490 nm with a correction wavelength at 650 nm.
3.12 Insulin sensitivity index

Insulin sensitivity was estimated using the Matsuda index of insulin sensitivity (Matsuda, DeFronzo 1999) which is validated as being an accurate measure, correlating highly ($r = 0.73$) with the rate of whole body glucose disposal during the euglycaemic insulin clamp. Insulin sensitivity was calculated using the following equation:

$$\text{Insulin Sensitivity} = \frac{10,000}{\sqrt{(\text{FPG} + \text{FPI}) \times (G + I)}}$$

Where $\text{FPG}$ is the fasting plasma glucose; $\text{FPI}$ is the fasting plasma insulin; $G$ is the mean plasma glucose during the OGTT and $I$ is the mean plasma insulin.

3.13 Statistical analysis

All statistical analysis was performed using SPSS 21.0 software (Statistical Package for the Social Sciences Inc., Chicago, Illinois, USA). Data is presented as mean and standard deviation ($\pm$ SD) unless specified otherwise. All data was checked for normality using the Shapiro-Wilk test before the main analysis. If data was not normally distributed the data was transformed and checked for normality again. The transformed, normally distributed data was then used for any subsequent statistical analysis to comply with the assumptions of parametric models of analysis.

Data was analysed using a within group, repeated measures analysis of variance (ANOVA) model where there were more than 2 comparisons. Post-hoc pair-wise comparisons were performed where appropriate according to the Bonferroni adjustment method. Paired sample t-tests were used to analyse within group differences when there were only 2 comparisons. Delta ($\Delta$) changes between
independent groups were analysed via independent samples t-tests. Statistical significance was accepted at $p \leq 0.05$. 
CHAPTER 4

THE IMPACT OF HIGH INTENSITY INTERMITTENT EXERCISE ON RESTING METABOLIC RATE IN HEALTHY MALES

¹This chapter was accepted for publication (2013)

4.1 Abstract

High intensity intermittent training (HIT) may favourably alter body composition, potentially mechanistically driven by post exercise oxygen consumption (EPOC). This chapter assessed the impact of 2 common aerobic interval training (AIT) and HIT protocols on energy expenditure (EE) and 11 h EPOC. Oxygen consumption (L·min⁻¹), respiratory exchange ratio (RER) and EE were measured in 9 healthy, lean males (Age:18.5-29.9 years; BMI: 20.3 - 25.0 kg·m⁻²) over 12 h under three conditions: control (CON), HIT1 (10 x 1min high intensity cycling bouts followed by 1min rest) and HIT2 (10 x 4min high intensity cycling bouts followed by 2 min rest). Total exercise period EE during HIT1 (1151 ± 205 kJ) (mean ± SD) was significantly lower than HIT2 (2788 ± 322 kJ; \(p < 0.001\)). EE within the 60 min after exercise was significantly higher after HIT1 (388 ± 44 kJ; \(p = 0.02\)) and HIT2 (389 ± 39 kJ; \(p = 0.01\)) compared with CON (329 ± 39 kJ), with no difference between exercise conditions. RER during this period was significantly lower in HIT1 (0.78 ± 0.06; \(p = 0.011\)) and HIT2 (0.76 ± 0.04; \(p = 0.004\)) compared with CON (0.87 ± 0.06). During the ‘slow phase’ of EPOC (1.25 h – 9.75 h) there were no significant differences in EE or RER between trials. Single sessions of HIT induce a transient increase in resting metabolic rate however the subtleness of this effect suggests that altered metabolic rate may not contribute significantly to previously documented changes in body composition with HIT.
4.2 Introduction

Despite the common acceptance that regular physical activity reduces the risk of many chronic conditions such as obesity, type 2 diabetes and cardiovascular disease, participation in physical activity remains low (WHO 2010). Specifically, within the UK approximately two-thirds of men and women do not perform sufficient activity to benefit their health (WHO 2010). Traditionally, health oriented physical activity guidelines have centred on individuals undertaking moderate intensity aerobic, continuous forms of exercise on most days of the week (Department of Health 2011). Given that the most commonly cited reason for not participating in physical activity is lack of time, and the recent evidence that individuals preferred an intermittent exercise protocol in comparison to continuous exercise (Bartlett et al. 2011), it is now timely to consider other forms of exercise which can potentially be more readily adopted.

In recent years there has been a surge in interest concerning HIT due to its ability to confer notable cardio-metabolic health benefits rapidly, with great time efficiency (Gibala & Little 2010). Specifically, HIT has been shown to improve insulin sensitivity and glycaemic control (Burgomaster et al 2006, 2008; Gibala et al. 2006; Wisloff et al. 2007; Tjonna et al. 2008; Babraj et al. 2009; Haram et al. 2009; Nybo et al. 2010; Richards et al. 2010; Whyte et al. 2010; Gaesser and Angadi 2011; Hood et al. 2011; Little et al. 2011; Shepherd et al. 2013) as well as other cardiovascular health factors (Fletcher et al. 1996) in as little as 2 weeks of training (typically six training sessions).

A body of research has also emerged documenting beneficial changes in body composition and/or mass in response to HIT (Boutcher 2011) with studies reporting favourable changes within short time frames. Accordingly, Whyte et al (2010)
subjected overweight and obese males to six training sessions over two weeks with each session consisting of four to six 30 s cycle sprints, interspersed with 4.5 min of recovery. Along with positive changes in several metabolic health parameters, this group noted a significant 2.4 cm reduction in waist circumference. Leggate and colleagues (2012) also observed a significant reduction in waist circumference (1.4 cm) in a group of overweight and obese males following the completion of two weeks of aerobic interval training (AIT). In this study each training session consisted of 10 x 4 min bouts of cycling at ~ 85 % $\dot{V}O_{2peak}$, separated by 2 min of recovery. Additionally, research has hinted that interval training may be superior to continuous moderate-intensity exercise training for the purpose of adiposity reduction (Macpherson et al. 2011). In this study 6 weeks of treadmill based SIT induced a two-fold greater reduction in total adiposity (12.4 % vs. 5.8 %) compared with a much greater volume of continuous moderate-intensity running. Given the relatively short duration of these interventions and accumulated exercise volume, the energy expended during exercise was unlikely to have been sufficient to account for the reported changes in waist circumference/adiposity. However, definitive information regarding this aspect of HIT/AIT is lacking and consequently the total impact of low volume interval training on energy balance is unknown.

Recently, Knab et al (2011) reported that metabolic rate is augmented for 14 h after a single bout of moderate-high intensity aerobic exercise, equating to an additional 795 kJ EE on top of that expended during exertion. The authors of this previous paper suggest that this elevated oxygen consumption may significantly contribute to fat loss over the course of exercise training programmes. Although research has been conducted to profile excess post exercise oxygen consumption (EPOC) after continuous forms of exercise (Borsheim and Bahr 2003; LaForgia et al. 2006), less
attention has been given to high intensity forms of exercise (McGarvey et al. 2005; Malatesta et al. 2008). Specifically, how high intensity intermittent exercise (HIIE – in this chapter representing one single bout of AIT or HIT) impacts upon EE and substrate utilisation, both during and after exercise, is not well understood. Given recent reports demonstrating notable changes in body composition and/or mass with HIT (Boutcher 2011) it is necessary to understand how HIT influences these parameters. Recent reports have provided preliminary information on this issue (Williams 2013; Chan & Burns 2013; Hazell et al. 2012) however in these studies the focus has been on SIT based exercise. This type of training may not be feasible for many who are inactive or who have chronic conditions e.g. type 2 diabetes or cardiovascular disease. More manageable forms of HIT have emerged which are more suitable for such individuals; however there is currently no information regarding the impact of these protocols on EE and substrate utilisation, during or after exercise.

The present investigation sought to accurately characterise the EE during exercise and EPOC (11 h post-exercise) of two commonly used HIT protocols which have been developed to be implemented with untrained individuals and/or clinical groups. The first protocol (HIT1) examined a HIT intervention which has been demonstrated as being suitable for individuals with type 2 diabetes, conferring rapid and marked gluco-regulatory benefits (Little et al. 2011). The second protocol (HIT2) examined was that previously implemented by Leggate et al (2012), where a significant decrease in the waist circumference of overweight/obese men was observed after just six AIT training sessions. The present investigation aimed to characterise the EE elicited by an acute bout of HIT type training in terms of both the immediate and delayed effect. In doing so, determining the potential contribution of HIT induced EE to previously documented reductions in body fat and/or waist circumference.
4.3 Materials and methods

4.3.1 Participant characteristics

Nine untrained and moderately active men (defined as doing no more than $2 \times \leq 30$ min of moderate intensity exercise sessions per week with no involvement in any structured training for $\geq 2$ years) volunteered to take part in the current study. The study inclusion criteria demanded that participants had a BMI 18.5-29.9 kg m$^{-2}$, and had been weight stable for at least three months prior to the study. Participants were free of cardiovascular, metabolic and haematological disease and were not taking any medications or supplements. The participants were fully informed of the aims, risks and discomforts associated with the investigation before providing written informed consent. This study conformed to local guidelines, the declaration of Helsinki, and was approved by the local ethics committee.

4.3.2 Preliminary measurements

Before main trials resting metabolic rate (RMR) was measured on two separate occasions in order to obtain reliable baseline results (Compher et al. 2006; Roffey et al. 2006). For these tests participants reported to the laboratory in the morning (~07:30) having not eaten since the previous evening. Resting metabolic rate assessments followed the guidance of Compher et al (2006). In brief, participants were asked: not to exercise during the preceding 24 h; not to consume caffeine, alcohol or drugs during the preceding 24 h; to remain fasted for at least 10 h prior to laboratory entry; to record what they had eaten in the preceding 24 h in order to replicate this feeding pattern prior to the second RMR measurement; to eliminate uncontrolled activity by travelling to the laboratory by motorised transport, with any essential walking being kept to $< 50$ m. The laboratory temperature was maintained at 23.5 -
24.5 °C. To minimise movement participants were assisted when necessary by wheelchair.

All resting metabolic measurements were performed using an open circuit, ventilated hood indirect calorimetry system (GEM Nutrition Ltd., Cheshire, England). This technique has been validated in the measurement of RMR in both adults and children (Weissman et al. 1990; Tissot et al. 1995). After a 30 min seated resting period, participants were instructed to lie in a comfortable supine position. The clear hood canopy was placed over the head area and plastic sheeting attached to the hood was placed around the body to form a seal between the air inside and outside the hood. Participants were instructed not to talk or fidget and listened to the radio throughout the measurement to reduce boredom and to prevent sleeping. $\dot{V}O_2$ (L·min$^{-1}$), $\dot{V}CO_2$ (L·min$^{-1}$), RER and EE (kJ) were determined at 30 s intervals (as pre-determined by software) over a 30 min period. For analysis, the first 10 min of data were discarded to account for any initial short-term respiratory artefact. Minute averages for $\dot{V}O_2$ along with RER caloric equivalents were used to estimate EE. The mean percentage difference between the two baseline measurements was 1.6 %.

Before main trials participants also performed a $\dot{V}O_2$peak test to volitional exhaustion using an electromagnetically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). $\dot{V}O_2$ was measured continuously throughout the test using an online breath-by-breath gas analysis system (Cortex Metalyzer, CPX International Inc, Berlin, Germany). For this test participants first warmed up for 3 min against a resistance of 50 W. After this, the workload was increased to a starting work rate which was subsequently increased by 30 W at intervals of 3 min. The initial work load for the test was individualised for each participant, being selected so that the test would last for
approximately 12 min (4 stages). The test was terminated when participants could no longer maintain pedal cadence at 50 RPM. Data points were collected as 5 s averages with \( \dot{V}O_{2\text{peak}} \) identified over the highest 30 s period during the test. HR was measured during the test using a telemetric heart rate monitor (Polar RS100, Polar Electro, UK Ltd., Warwick, UK).

4.3.3 Experimental protocol

All participants completed two HIIE main trials and one control trial with each study visit being separated by at least four days. Figure 4.1 provides a schematic overview of the main trial protocols. Prior to each main trial participants abstained from structured physical activity and standardised their dietary intake within the prior 24 h. On the morning of trials participants arrived at the laboratory via motorised transport to eliminate extraneous activity. An 11 h EPOC assessment was made across each main trial and for clarity and comparability across trials the beginning of this measurement point was defined as time zero (0 h). Thus, exercise protocols ended at the same time (prior to the start of EPOC assessment) which meant that it was necessary for the HIIE start times to differ. Consequently, the beginning of the control trial and HIT2 is defined at -67 min which reflects the 60 min exercise period (in HIT2) before EPOC measurement, 2 min cool down, and an additional 5 min resting period necessary to allow \( CO_2 \) levels to return to values which fall within acceptable limits for transfer to the ventilated hood (essential for the exercise trials). Accordingly, the beginning of HIT1 is defined as -27 min which reflects the duration of the 20 min exercise protocol as well as the aforementioned 7 min recovery period after exercise (Figure 4.1).
Figure 4.1 - Schematic representation of testing schedule

For the control trial participants reported to the laboratory in a fasted state at ~ 07:30. Participants then relaxed in a comfortable seated position for 30 min prior to the start of the trial (~ 67 min). At this point breath collection via online gas analysis (Cortex Metalyzer, CPX International Inc, Berlin, Germany) began whilst participants remained in a seated position. Gas collection occurred continuously for 67 min. This period prior to post-exercise assessment is defined as the ‘exercise period’ (EP). Immediately after the EP, the rapid phase (RP) of EPOC was measured continuously over 60 min using the ventilated hood (0 h – 1 h). During this time, measurements were taken continuously with participants resting in a supine position. Immediately after the RP, participants were seated next to the ventilated hood where breakfast was provided (1 h – 1.15 h). Participants consumed breakfast within 15 min, after which point ventilated hood measurement recommenced. Subsequently, expired gas analysis was performed continuously for 15 of every 30 min over the next 9.75 h. This period was identified as the EPOC slow phase (SP). Standardised meals were also consumed by participants at 4 h and 8 h with each meal being consumed within 15 min.

Identical procedures were completed in the exercise trials except a bout of HIIE was completed at the beginning of trials. During exercise, gas analysis data was collected
continuously with $\dot{V}O_2$, $\dot{V}CO_2$, RER and HR averaged every 15 s. Within each protocol the 2 min cool down period was composed of cycling against no resistance. The exercise protocol within HIT1 was identical to that described by Little et al (2011) (See general methods). The exercise protocol within HIT2 was identical to that described previously by Leggate et al (2012), consisting of 10 x 4 min intervals at an RPM of 60 - 80, each separated by 2 min of light pedalling against a set resistance of 50 W. The workload was manipulated during the trial to ensure that the average corresponding $\dot{V}O_2$ during high intensity bouts equated to ~ 85 % $\dot{V}O_2peak$.

4.3.4 Meals for experimental trials

During main trials participants were provided with 3 standardised meals which were identical across trials. All meals were consumed within 15 min. The total amount of energy provided for participants at each meal was individualised to meet daily energy requirements for an inactive day. This value was calculated by multiplying each individuals average RMR (from preliminary assessments) by 1.375 (Harris et al. 1918). Breakfast was provided immediately after the EP (at 1 h) and consisted of toasted white bread and jam (30 % of daily energy requirements). Lunch and dinner were provided at 4 h and 8 h with both meals consisting of a tuna mayonnaise sandwich, salted crisps, chocolate muffin and green apple (each meal 35 % of daily energy requirements). The macronutrient composition of the food provided across each trial was typical of a UK diet and consisted of 38 % fat, 14 % protein and 48 % carbohydrate (Department for Education, Food and Rural Affairs 2010). Mean energy intake equated 12, 385 kJ.
4.3.5 Statistical analysis

The statistical analysis for this study was performed using SPSS (Ver.19). All data are reported as Mean ± SD. For both the EP and RP periods, \( \dot{\text{VO}}_2 \), RER and EE minute averages were determined for each participant, whilst for the SP period data were collected as 30 min averages for each trial hour. The latter values were subsequently multiplied by 2 to provide an estimate of hourly \( \dot{\text{VO}}_2 \), RER and EE. Data were also analysed for the RP and SP periods combined to give a total EPOC. Each of these trial segments were analysed separately using repeated measures ANOVA. Where appropriate, data was analysed using AUC which was calculated using the trapezoid method (Matthews et al. 1990). No differences were found between mean and AUC data and therefore AUC data is not reported. Where significant differences were found a Bonferroni post-hoc test was used to identify where significant differences lay. For all analyses alpha was set at 5 %.

4.4 Results

4.4.1 Participant and exercise characteristics

The physical characteristics of the study participants are summarised in Table 4.1. The average workload undertaken by study participants to elicit 90 % of peak HR during exercise within HIT1 was 204 ± 47 W. Similarly, the average workload completed to elicit 85 % of \( \dot{\text{VO}}_2 \text{peak} \) during HIT2 was 211 ± 46 W.
Table 4.1 Participant characteristics. Data are presented as mean and (standard deviation).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>21 (2)</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181.2 (7.5)</td>
<td>172.1</td>
<td>192</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.9 (7.5)</td>
<td>61.6</td>
<td>88.2</td>
</tr>
<tr>
<td>Body Mass Index (kg.m(^{-2}))</td>
<td>22.5 (1.8)</td>
<td>20.3</td>
<td>25.0</td>
</tr>
<tr>
<td>(\dot{V}O_2\text{Peak}) (ml.kg.min(^{-1}))</td>
<td>44 (6)</td>
<td>33</td>
<td>58</td>
</tr>
<tr>
<td>RMR (kJ.day(^{-1}))</td>
<td>8629 (636)</td>
<td>7478</td>
<td>9655</td>
</tr>
</tbody>
</table>

4.4.2 Energy expenditure

When analysing EE for the entire trial period (combining EP, RP and SP phases) revealed significant differences were observed between experimental conditions \((p < 0.001)\). The total energy expended within HIT2 \((7280 \pm 703\, \text{kJ})\) was significantly higher than that expended in both HIT1 \((5761 \pm 598\, \text{kJ}; p < 0.001)\) and CON \((4874 \pm 456\, \text{kJ}; p < 0.001)\). Energy expenditure in HIT1 was also significantly higher than CON \((p < 0.001)\). Figure 4.2a illustrates the EE during the EP independently. Within this period one-way ANOVA revealed a significant difference between trials \((p < 0.001)\). Specifically, EE during the EP in HIT2 \((2788 \pm 322\, \text{kJ})\) was significantly greater than HIT1 \((1151 \pm 205\, \text{kJ}; p < 0.001)\). Logically, the EE in both exercise conditions during the EP was higher than CON \((368 \pm 46\, \text{kJ}; p < 0.001)\).
Figure 4.2 Energy Expenditure (Mean ± SD) during the a) Exercise Period, b) EPOC Rapid Phase, c) EPOC Slow Phase, d) EPOC Rapid and Slow Phases Combined across all trials.

Figure 4.2 b) shows the EE during the EPOC RP across main trials. One-way ANOVA revealed a significant difference between trials for the EPOC RP ($p < 0.001$). Specifically, the EE for this period after HIT1 (388 ± 44 kJ) was significantly higher than that expended over the same period during CON (329 ± 39 kJ; $p = 0.02$). Similarly, EE during the RP in HIT2 (389 ± 39 kJ) was also higher than CON ($p = 0.01$). During this period there was no difference in EE between exercise conditions. Figure 4.2 c) illustrates the EE during the EPOC SP across main trials. Within this period one-way ANOVA found no differences between experimental trials. Energy expenditure during
the SP was 4107 ± 387, 4227 ± 426, and 4268 ± 389 kJ for CON, HIT1 and HIT2, respectively. When the total EPOC period was analysed together (RP & SP combined) significant differences between experimental trials were evident with total EE of 4437 ± 419, 4615 ± 457 and 4658 ± 421 kJ for CON, HIT1 and HIT2 (Figure 4.2 d).

4.4.3 Respiratory exchange ratio

There was a significant difference in the RER during the RP after exercise ($p < 0.001$). In the RP RER was significantly lower in HIT1 (0.78 ± 0.06; $p = 0.011$) and HIT2 (0.76 ± 0.04; $p = 0.004$) compared with that over the same period during CON (0.87 ± 0.06). There were no significant differences between HIT1 and HIT2 with respect to RER in this period. Within the SP there were no significant differences in RER between any experimental trials. Furthermore, this was also the case when extending the analysis to include the RP period component i.e. RP & SP combined.

4.5 Discussion

The primary aim of this study was to profile the energy expended during, and for up to 11 hours after 2 routinely used HIIE protocols. This study demonstrated that both HIIE interventions substantially raise EE during exertion and additionally increase EE and reduce RER for up to one hour after. Following this point however, no differences in EE or RER are apparent. These findings demonstrated that the impact of these protocols on EE and RER after exercise is transient, with effects on EE particularly being of relatively small magnitude. These data suggest that HIIE induced changes in post-exercise metabolic rate do not contribute significantly to previously documented reductions in body fat and/or waist circumference which have been observed after HIT interventions.
In the present study both HIT1 and HIT2 substantially raised EE during exertion. The EE elicited by the protocols implemented in this study were substantially higher than that induced by classical SIT training. Specifically, two recent studies have identified similar EE profiles during sessions of SIT with values of 595 kJ (Deighton et al. 2012) and 735 kJ (Hazell et al. 2012). Comparatively, both protocols utilised in the present investigation elicited substantially higher EE values compared with these previous studies, approximating two-fold higher with HIT1 and 4-4.5 fold higher with HIT2. These findings are of practical importance with the indication that these forms of training may convey dual benefit by enhancing metabolic health parameters whilst also exerting a noteworthy influence on energy balance. Such information begins to allow us to make judgements regarding the most appropriate forms of HIT suitable for various populations with differing intervention goals.

Perhaps the most notable finding from the present investigation was that both HIT protocols increased EE within the hour immediately after exercise (EPOC rapid phase), although this response was transient and of small magnitude. Specifically, the net increase in EE during this period was approximately 60 kJ in both HIT interventions, equating to just 2 (HIT2) and 5 (HIT1) percent of the energy expended during the actual exercise component. The similarity in response between protocols is noteworthy given the marked difference in the duration and EE induced in HIT1 and HIT2. This is likely due to the similarity of exercise intensity between protocols, as it is known that exercise intensity is the most important determinant of the EPOC magnitude, explaining five times more of the variance than exercise duration or total work completed (Gore & Withers 1990).
The EPOC magnitude and duration in the present investigation was small and brief, and consequently unlikely to contribute significantly over time to changes in body composition and/or mass. This finding is consistent with other studies which have examined the effects of HIT on EPOC and reported small changes in post-exercise EE (Chan & Burns 2013; Williams et al. 2013; LaForgia et al. 1997) but at odds with a recent report which described a large increase in post-exercise EE after an acute bout of continuous moderate intensity cycling (Knab et al. 2011). This latter investigation is noteworthy given the gold standard methodology employed. Specifically, post-exercise metabolic rate was measured after 45 min of moderate intensity cycling (~73 % $\dot{V}O_{2\text{max}}$) in a respiratory chamber over an extended duration and the researchers reported a 795 kJ net increase in EE over 14.2 h. At first glance, when specifically compared with HIT2 in the present study, it is unclear why such different outcomes are apparent given that the exercise intensity in HIT2 was significantly greater and associated EE comparable. Closer scrutiny of the study protocol however reveals one key difference between study methodologies. Notably, in the study conducted by Knab et al (2011) participants were fed back the energy which they expended during exercise (2171 kJ) so that they consumed a much greater amount of food on the exercise trial than their control trial. This extra energy would have significantly augmented resting metabolism (thermic effect of feeding) above that on the control trial and therefore the EPOC reported reflects the true increase with exercise plus the increase promoted by a heightened food intake. Conversely, in the present investigation participants consumed identical meals across trials and therefore the independent effect of exercise was isolated.

In the current study EE was higher only during the RP of EPOC i.e. up to one hour after the end of exercise. This outcome is consistent with other studies which have
examined the impact of HIT on post-exercise metabolic rate (Chan & Burns 2013; Williams et al. 2013). Specifically, Chan & Burns (2013) observed an increase in oxygen consumption up to 30 min after SIT. Moreover, with a similar protocol, Williams et al (2013) observed an increase in post-exercise oxygen consumption that lasted for up to 45 min. These findings, and those from HIT1 in the present investigation, are consistent with the suggestion that brief intense exercise impacts up on the EPOC early response, but that more significant and prolonged exercise is necessary to influence more prolonged changes in metabolic rate (LaForgia et al. 2006). The brevity of response, particularly with regards HIT2 in the present study, is not entirely clear given the longer protocol duration and relatively large associated EE. It is possible that the intermittent nature of the protocol attenuated the associated stress and physiological burden however other studies utilising intermittent protocols have documented significantly longer EPOC durations (Bahr et al. 1992; LaForgia et al. 1997). Both previous investigations involved supra-maximal exercise, suggesting that the higher exercise intensity is significant contributing factor. Furthermore, the post-exercise EE assessment protocols were very different compared to the current investigation with significantly lower sampling volume and hence greater interpolation of data.

In the present study both exercise protocols had a significant impact on the RER during exercise and within the EPOC RP. Specifically, during exercise the RER increased in each protocol representing an increasing reliance on carbohydrate oxidation proportionate with the high exercise intensity. Subsequently, within the immediate hour after exercise, RER decreased dramatically, indicating an enhancement of fat oxidation within the immediate hour after exercise. This response was transient however in the current study as participants consumed a breakfast meal one hour after
exercise and the resultant switch in metabolic fuel preference masked any more prolonged enhancement of fat oxidation which may otherwise have occurred. Specifically, other investigations have shown that lower RER values may persist for several hours after interval type exercise (McGarvey et al. 2005; Malatesta et al. 2008). The mechanisms responsible for this change in metabolism possibly include an elevated catecholamine response to HIT, which may stimulate mitochondrial respiration, lipolysis and fat oxidation (Mulla et al. 2000). An increase in CO₂ retention as a means of replenishing the bicarbonate used to buffer lactic acid (Bahr et al. 1987, 1991a, 1991b, 1992; Short and Sedlock 1997; LaForgia et al. 2006) may also be important. Nonetheless, given the relatively small absolute increase in EE the importance of this change in fuel preference is debatable.

This investigation has strengths and limitations which should be recognised. Firstly, the strict control of diet and physical activity, prior to and during the investigation, is a key strength of the study. Moreover, the detail of post-exercise assessment in terms of the percentage of actual sampling time, rather than data interpolation, is also notable (51 % of the total 11 h EPOC assessment). Limitations of this study include the lack of generalisability afforded by the use of a homogenous sample of young, healthy males. There would be merit in future studies to examine the impact of HIIE on EPOC in individuals who are obese whereby changes in post-exercise metabolic rate may make a greater relative contribution to the overall energy deficit imposed by exercise. Sex based differences in metabolism and energy homeostasis also demand that investigations are conducted with females.

Based on the results obtained from the current study it may be suggest that the EE attributed to EPOC is minimal when compared against a resting control trial and that
this is likely not to be the reason for the previously documented fat loss associated with HIT training programmes (Boutcher 2011). From the evidence presented here it would suggest that HIT based exercise does not stimulate an elevation in metabolic rate that would be effective in contributing to fat loss over brief interventions. There are likely further un-elucidated mechanisms contributing to reductions in body fat and/or waist circumference following HIT based exercise.
CHAPTER 5

AN EVALUATION OF LOW AND REDUCED VOLUME HIGH INTENSITY INTERMITTENT TRAINING (HIT) FOR HEALTH IMPROVEMENTS IN THE OBESE

2 This chapter has been submitted for publication and is currently under review.

2 Kelly BM, King JA, Jackson AP, Xenophontos S, Nimmo MA (2014) Two weeks of low-volume high intensity training has no impact on key determinants of metabolic health in pre-clinical populations. Appl Physiol Nutr Metab. Submitted.
5.1 Abstract

HIT type training has been described as a time-efficient strategy for inducing favourable cardiorespiratory and metabolic health adaptations. To date there has been little published evidence as to the efficacy of this training modality for health improvements within pre-clinical populations such as the obese and insulin impaired. Implementing a recently popularised low volume HIT protocol this chapter investigate the effects of 6 sessions ($n = 10$) or 4 session ($n = 8$) of HIT performed over 2 weeks on aerobic capacity, body composition, glycaemic control and systemic inflammation in an in-active overweight/obese male cohort (BMI 31.2 ± 3.6; $\text{VO}_{2\text{max}} 30.3 ± 4.4 \text{ ml.kg}^{-1}.\text{min}^{-1}$). All training sessions consisted of 10 x 1 min intervals at 90% $\text{HR}_{\text{peak}}$ separated by 1 min of active recovery with 50W resistance. After 2 weeks of training no changes in body mass occurred in either groups 1 ($\Delta = -0.1 \text{ kg}; p = 0.798$) or 2 ($\Delta = -0.2 \text{ kg}; p = 0.524$). In conjunction with no changes in lean body mass (both groups $p > 0.05$) BMI remained un-altered in groups 1 ($\Delta = 0.0 \text{ kg.m}^2; p = 0.716$) and 3 ($\Delta = 0.0 \text{ kg.m}^2; p = 0.537$). Group 1 demonstrated a non-significant reduction in systolic blood pressure ($\Delta = -8.1 \text{ mmHg}; p = 0.446$), with group 2 showing a non-significant increase ($\Delta = 4.0 \text{ mmHg}; p = 0.348$). After the interventions no changes were detected from baseline in aerobic capacity, glycaemic control or systemic inflammatory profile in either group ($p > 0.05$). It was concluded that within this pre-clinical population, the HIT regimens utilised within this chapter were not able to induce any changes in the selected health parameters within a 2 week time-frame.
5.2 Introduction

In the obese, the core defect underlying the development of T2DM is skeletal muscle insulin resistance (DeFronzo and Tipathy 2009). Mechanisms and primary contributing factors of insulin resistance are complex although evidence suggests that physical inactivity may be a principal initiating factor (Thyfault, Krogh-Madsen 2011). Inactivity leads to reduced EE, and when combined with increased energy intake, promotes adipose tissue expansion and with it the development of obesity and a state of chronic inflammation (McArdle et al. 2013). Inflammation has been independently implicated in the development of insulin resistance and T2DM (Wellen and Hotamisligil 2005) and is characterised by abnormal cytokine production, increased production of acute phase reactants as well as activation of a network of inflammatory signalling pathways (Hotamisligil 2006). Regular exercise favourably influences many aspects of health and is particularly noted for its capacity to improve insulin sensitivity and to help prevent or manage T2DM (Goodyear and Kahn 1998; Sigal et al. 2006). Exercise prescription guidelines have been developed to help individuals realise the health enhancing effects of exercise which traditionally have focused on moderate-intensity, continuous forms of exercise (Garber et al. 2011). Unfortunately, adherence to these guidelines is poor (British Heart Foundation 2012), therefore many individuals do not perform sufficient exercise to benefit their health. Given recent evidence revealing that healthy individuals (Bartlett et al. 2011) and patient populations (Tjønna et al. 2008) may prefer intermittent over continuous exercise, and the noteworthy benefits reported with some novel forms of intermittent training (Gibala et al. 2012), it is timely to consider new forms of exercise training that may be more readily adopted and sustained.
Recent work suggests that SIT and HIT provide time-efficient strategies for inducing metabolic and cardiorespiratory adaptations comparable to those seen following traditional high volume endurance training (Burgomaster et al. 2005, 2007, 2008; Gibala et al. 2006; Rakobowchuk et al. 2008; Trilk et al. 2011). Notably, several groups have demonstrated that SIT may have favourable effects on metabolic control after as few as six training sessions (Babraj et al. 2009; Burgomaster et al. 2006, 2008; Gibala et al. 2006; Richards et al. 2010; Shepherd et al. 2013; Whyte et al. 2010) and with a several-fold lower time commitment. In these previous interventions it is important to note that SIT training was employed as opposed to HIT training. SIT training requires a specialised ergometer and it is likely that this challenging regimen may not be accomplishable for all populations. In recognition of this, a revised low-volume HIT protocol has been devised and has shown promise as a regimen that can be completed by clinical (Little et al. 2011) and pre-clinical groups (Hood et al. 2011). Specifically, using this adapted protocol consisting of 10 x 1 min intervals at ~ 90 % HR$_{\text{max}}$, a striking reduction in 24 h glycaemia was reported after two weeks (six sessions) in patients with T2DM (Little et al. 2011).

In the present study it was specifically sought to advance the work of Hood et al (2011) and Little et al (2011) by characterising the impact of low-volume HIT on additional key determinants of metabolic health (systemic inflammation, glycaemic control, body composition, blood pressure, aerobic capacity) in a pre-clinical population (inactive, overweight or obese men). Furthermore, given previous information indicating that changes in aerobic capacity can be achieved with 2 sessions per week of high intensity exercise (within two weeks) (Wenger and Bell 1986), we also examined whether this reduced training frequency was sufficient to confer any favourable health adaptations. The importance of this latter line of enquiry lies within the identification of the minimum
amount of exercise capable of providing health benefits, which has implications for the sustainability of interventions.

5.3 Materials and Methods

5.3.1 Participant Characteristics

Participant characteristics are provided in Table 5.1. All participants had a BMI $\geq 27$ kg·m$^{-2}$, were inactive (no structured exercise), but were otherwise healthy. Participants were excluded if they were smokers, had impaired fasting glucose or had a BMI $\geq 40$ kg·m$^{-2}$. Participants were included in the study after they were informed both verbally and in writing of all possible discomforts and risks associated with the study. All study procedures were approved by the local ethics advisory committee and all participants gave their written informed consent before commencing the study.

5.3.2 Study assessments (Pre and 72 h Post-Training)

Arterial blood pressure was measured using a digital automatic blood pressure monitor (Omron M7, Omron Healthcare Ltd, Milton Keynes, UK) as described in general methods. Body mass and height were measured with participants wearing shorts. Body composition parameters including height, weight, waist and hip circumferences and DEXA were carried out as described in general methods.

Peak oxygen uptake ($\dot{V}O_{2peak}$) was determined using a continuous incremental exercise test on an electromagnetically-braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands) performed to volitional exhaustion. Expired air was measured continuously using an online breath-by-breath gas analysis system (Cortex
Metalyzer, CPX International Inc, Berlin, Germany). This differed from the collection methods in the previous chapter due to new testing equipment and the collection of individual breath data as opposed to 5 s averages. Participants warmed up for 3 min against a resistance of 50 W, after which point the workload increased by 30 W every 3 min from an individualised starting load. The test was terminated when the participant could no longer maintain 50 pedal revolutions per min (RPM). \( \text{VO}_{2\text{peak}} \) was identified over the highest 30 s period during the test. Heart rate (HR) was measured throughout the test using a telemetric HR monitor, which was wirelessly paired with the breath-by-breath analysis system (Polar RS100, Polar Electro, UK Ltd, Warwick, UK). Glycaemic control and insulin sensitivity were assessed via OGTT (See General Methods for detail).

5.3.3 High intensity interval training

The HIT protocol utilised in this study was based on that devised by Little and colleagues (2011) (see general methods for detail). Group 1 \((n = 8)\) completed six sessions of HIT exercise over 2 weeks whereas group 2 \((n = 10)\) completed four sessions over the same period. Group one trained on Monday, Wednesday and Friday. Group 2 trained on a Monday and Friday.

5.3.4 Dietary and physical activity controls

Before the intervention participants were explicitly told not to change their dietary habits or undertake any structured exercise during the course of the study. Prior to the pre-training assessments participants recorded their diets and subsequently replicated this before the post-training assessments.
5.3.5 Biochemical Analysis

Biochemical analysis of inflammatory mediators was carried out as described in general methods. All samples were analysed in duplicate and were repeated if the CV between duplicates was more than 10 %. The intra-assay CVs for the inflammatory proteins were as follows: adiponectin 3.5 %, IL-10 8.7 %, TNFα 7.8 %, CRP 5.3 %, IL-6 4.8 %, sIL6-R 3.9 % and MCP-1 6.4 %.

5.3.6 Statistical analysis

Statistical analysis was carried as described in general methods. Specifically insulin sensitivity was determined by the ISI calculated using the OGTT glucose and insulin values from the formula proposed by Matsuda and DeFronzo (1999). Pre to post differences in basal plasma glucose, insulin, ISI anthropometry and \( \dot{V}O_2 \)peak were assessed using paired sample \( t \)-tests. Additionally, group differences were assessed via comparison of delta change using independent \( t \)-test. To be included in the analysis participants must have completed all of the necessary training sessions.

5.4 Results

5.4.1 Body composition, blood pressure and peak oxygen uptake

There were no differences in any characteristic between group 1 and 2 at baseline (Table 5.1). Following six sessions of HIT over 2 weeks (group 1) there were no changes in blood pressure, aerobic capacity, body mass, BMI, waist or hip circumference (Table 5.1). Moreover, DEXA indicated no differences in regional fat (%), total tissue, total lean tissue, total fat tissue or BMC (all \( p > 0.05 \)) (Figure 5.1 a).
This lack of change was replicated in group 2 who completed 4 sessions of HIT over two weeks (Figure 5.1 b).
Table 5.1 Body composition, blood pressure and peak oxygen uptake for groups 1 and 2 pre and post 2 weeks of HIT.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th></th>
<th>p</th>
<th>Group 2</th>
<th></th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Training</td>
<td>Post-Training</td>
<td>Sig. &lt; 0.05</td>
<td>Pre-Training</td>
<td>Post-Training</td>
<td>Sig. &lt; 0.05</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>94.5 (13.9)</td>
<td>94.4 (14.3)</td>
<td>0.798</td>
<td>104.8 (17.6)</td>
<td>104.6 (17.0)</td>
<td>0.524</td>
</tr>
<tr>
<td>BMI (kg.m²)</td>
<td>29.7 (3.4)</td>
<td>29.7 (3.5)</td>
<td>0.716</td>
<td>32.3 (3.3)</td>
<td>32.3 (3.2)</td>
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</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>110.4 (9.7)</td>
<td>95.3 (4.4)</td>
<td>0.164</td>
<td>109.6 (10.4)</td>
<td>108.6 (10.4)</td>
<td>0.316</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>107.6 (6.9)</td>
<td>104.0 (2.6)</td>
<td>0.235</td>
<td>113.9 (7.3)</td>
<td>112.7 (9.4)</td>
<td>0.499</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>0.9 (0.03)</td>
<td>0.9 (0.03)</td>
<td>0.709</td>
<td>1.0 (0.05)</td>
<td>1.0 (0.03)</td>
<td>0.879</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>131.1 (16.8)</td>
<td>123.0 (11.5)</td>
<td>0.446</td>
<td>120.6 (8.9)</td>
<td>124.0 (5.9)</td>
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</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>84.7 (10.9)</td>
<td>76.0 (9.7)</td>
<td>0.185</td>
<td>76.8 (7.1)</td>
<td>75.8 (5.4)</td>
<td>0.829</td>
</tr>
<tr>
<td>VO₂peak (l.min⁻¹)</td>
<td>3.1 (0.6)</td>
<td>3.7 (0.8)</td>
<td>0.138</td>
<td>3.2 (0.4)</td>
<td>3.2 (0.4)</td>
<td>0.846</td>
</tr>
<tr>
<td>VO₂peak (ml.kg⁻¹.min⁻¹)</td>
<td>35.6 (5.4)</td>
<td>38.5 (5.4)</td>
<td>0.135</td>
<td>30.3 (4.2)</td>
<td>30.5 (4.2)</td>
<td>0.960</td>
</tr>
</tbody>
</table>
Figure 5.1 Body composition changes as measured by DEXA represented as % change. 
a) represents individuals having completed 4 HIT sessions over 2 weeks with b) representing those individuals having completed 6 HIT sessions over 2 weeks.

5.4.2 Systemic inflammation and insulin sensitivity

Table 5.2 shows the circulating concentrations of candidate inflammatory proteins before and after training. There were no differences seen within or between groups in any parameter (all \( p > 0.05 \)). There were also no differences found for fasting or 2 h (OGTT) insulin or glucose AUC (\( p > 0.05 \)) in either group. Consequently, ISI also
remained unaltered. The glucose and insulin responses to the 2 h OGTT before and after training are shown in Figure 5.2.

Figure 5.2 Two hour OGTT response to 75 g glucose ingestion with data representing group 1 glucose (a) and insulin (b) responses and group 2 glucose (c) and insulin (d) responses. Data are presented as mean and standard deviation. Solid lines represent pre-intervention with dashed lines representing responses 72h post training.
Table 5.2 - Inflammatory proteins in the circulation in groups 1 and 2 following 2 weeks of HIT training. Data presented as range and (median).

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Typical Ranges</td>
<td>Pre-Training</td>
<td>Post-Training</td>
<td>Pre-Training</td>
</tr>
<tr>
<td></td>
<td>Range (Median)</td>
<td>Range (Median)</td>
<td>Median ∆Change</td>
<td>Sig. &lt; 0.05</td>
</tr>
<tr>
<td>Adiponectin (µg.ml⁻¹)</td>
<td>2.0-20.0</td>
<td>1.1-14.6 (2.3)</td>
<td>0.8-10.9 (1.7)</td>
<td>-0.3</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>IL-10 (pg.ml⁻¹)</td>
<td>1.1-4.5</td>
<td>0.5-0.7 (0.5)</td>
<td>0.5-0.7 (0.5)</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>TNF-α (pg.ml⁻¹)</td>
<td>1.3-2.3</td>
<td>1.4-2.6 (2.3)</td>
<td>1.1-3.2 (2.2)</td>
<td>-0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (µg.ml⁻¹)</td>
<td>1.4-5.8</td>
<td>0.1-5.7 (1.5)</td>
<td>0.1-3.8 (1.5)</td>
<td>-0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (pg.ml⁻¹)</td>
<td>1.0-6.0</td>
<td>1.6-112.4 (3.5)</td>
<td>1.3-87.0 (3.7)</td>
<td>-9.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sIL-6R (ng.ml⁻¹)</td>
<td>20.0-45.0</td>
<td>21.3-36.3 (24.8)</td>
<td>19.8-32.3 (27.9)</td>
<td>1.2</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1 (pg.ml⁻¹)</td>
<td>100.0-200.0</td>
<td>51.4-411.1 (157.3)</td>
<td>55.8-181.0 (148.2)</td>
<td>-38.7</td>
</tr>
<tr>
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</tbody>
</table>
5.5 Discussion

This investigation examined the impact of low-volume HIT on key metabolic health parameters (systemic inflammation, glycaemic regulation, body composition, aerobic capacity) using a recently popularised protocol that has shown promise as a therapeutic intervention in clinical and pre-clinical groups (Hood et al. 2011; Little et al. 2011). Moreover, it was sought to determine whether any benefits would be obtainable with a reduced training frequency of two times per week. The novel findings in this study were that this specific regimen (training two or three times each week) was unable to induce changes within these selected health parameters after two weeks in a population at risk of developing chronic metabolic disease. These findings suggest that this protocol is not potent enough to stimulate certain metabolic health adaptations within this population and time-frame.

Exercise of varying intensities improves wellbeing and can combat some of the basal increase in inflammation often associated with obesity, T2DM and cardiovascular disease (Adamopoulos et al. 2002; Balducci et al. 2010; Leggate et al. 2012; Thompson et al. 2010; Zoppini et al. 2006). At present, there is a scarcity of data regarding the impact of HIT on inflammation. With regards to exercise *per se*, the consensus of evidence suggests that high intensity exercise is needed to reduce the inflammatory profile (Gleeson et al. 2011; Nimmo et al. 2013). This thesis was supported by a large intervention in T2DM patients where robust improvements in the circulating inflammatory profile were constrained to those who performed combined high intensity aerobic and resistance exercise training (Balducci et al. 2010). The findings in the present investigation are at odds with those previously reported whereby two weeks of HIT improved the inflammatory profile in subcutaneous adipose
tissue and the circulation (Leggate et al. 2012). In this previous study however, the HIT regimen differed; despite eliciting a similar intensity, required a four-fold greater exercise volume than the protocol implemented in the present investigation. Within a 2 week time-frame, it therefore appears that a greater volume of HIT is required to impact upon systemic inflammatory status at this intensity.

Inflammation has been independently implicated in the development of insulin resistance and T2DM (Wellen and Hotamisligil 2005). Inflammatory mediators promote insulin resistance through inhibitory serine phosphorylation of IRS-1 which disrupts insulin-receptor signalling through several distinct mechanisms; ultimately blocking insulin action (Zhang et al. 2010). The novel aspect of the present investigation was the characterisation of this modified low-volume HIT protocol in a population with a less extreme phenotype who are at risk, rather than already exhibiting chronic metabolic disease. This study also sought to provide new information regarding exercise frequency; specifically whether benefits in glycaemic control could be achieved with two rather than three bouts of HIT each week.

In the present investigation there were no measurable changes in fasting glucose or insulin following two weeks of HIT, with either two or three training sessions performed per week. Given that fasting levels of these parameters chiefly reflect the hepatic contribution to glycaemic regulation, these data indicate that HIT did not affect hepatic glycaemic control. This outcome is supported by the results from two previous studies that have shown no alteration in fasting levels of glucose and insulin after two weeks of SIT (Babraj et al. 2009; Richards et al. 2010). Conversely, implementing the same low-volume HIT protocol as per the present investigation, Hood and colleagues (2011)
observed a 16 % decrease in fasting levels of insulin. The reason for the difference between the present results and those reported by Hood et al (2011) is not clear given the high similarity in protocols and participants studied. Additional confirmatory work with a larger participant cohort is therefore required.

The present study also found no changes in the OGTT glucose and insulin responses following the intervention. It therefore also appears that in this population, this specific low-volume HIT regimen does not alter glucose profile after 4 or 6 training sessions. In similar cohorts, beneficial changes in glucose profile have been reported previously after two weeks with the classical SIT protocol when assessments have been made with the euglycaemic-hyperinsulinemic clamp (Richards et al. 2010) or OGTT (Babraj et al. 2009). The difference in outcome between protocols is possibly related to the greater breakdown of muscle glycogen and increase in GLUT4 transport proteins in response to SIT. Despite the lack of effect observed in the present study, previous research suggests that this low-volume HIT protocol may provide robust improvements in postprandial glycaemic regulation within T2DM patients who exhibit a more deleterious glycaemic profile (Little et al. 2011). Notably, these improvements were detected using continuous glucose monitors which may detect changes not identified by OGTT (Karstoft et al. 2013).

The present investigation is the first to examine the impact of low-volume HIT on aerobic capacity. After 2 weeks, neither 6 (group 1) or 4 (group 2) sessions of HIT altered peak oxygen uptake in this pre-clinical group who exhibited low baseline levels of aerobic fitness. In several previous investigations implementing SIT, changes in aerobic capacity have not directly been examined. Instead, improvements after 2
weeks of training have been inferred by reductions in time-trial performance tests and changes in skeletal muscle markers of oxidative capacity (Burgomaster et al. 2006; 2007; Gibala et al. 2006). Additionally, high-volume HIT protocols have been shown to markedly improve aerobic capacity after 2 weeks in healthy women (13 %) (Talanian et al. 2007), and overweight/obese men (12 %) (Leggate et al. 2012). The differences in findings between studies relate directly to the protocols implemented and probably relate to the time spent exercising at $\dot{V}O_2\text{max}$ (Billat et al. 2000). Un-reported data from Chapter 4 shows that within healthy untrained males, only a fraction of time is spent close to peak oxygen uptake intensity. This contrasts with SIT and high-volume HIT where large percentages of the exercise intervals are completed at or above this required intensity (Leggate et al. 2012).

The current study is the first to incorporate both standard anthropometric measures and DEXA analysis before and after a two week HIT intervention. No changes were detected for group 1 or 2 in total body mass, waist or hip circumferences or waist-to-hip ratio. Furthermore, no changes were detected in tissue fat percentage, regional fat percentage, fat mass, lean mass or BMC. These outcomes demonstrate that across this duration, this HIT protocol did not exert any influence on any body composition parameter. In contrast to these findings, improvements in body composition have been reported in response to SIT (Whyte et al. 2010) and high-volume HIT (Leggate et al. 2012) interventions after 2 weeks with reductions in waist circumference being recorded (up to 2.4 cm). Given the relatively low associated total energy deficits imposed by these regimens (Hazell et al. 2012; Leggate et al. 2012; Kelly et al. 2013) it is hard to explain these outcomes. The inherent variability associated with this measurement may have been influential.
A growing body of data indicates that longer interval training based interventions (3-6 months) may provoke changes in body composition (Gillen et al. 2013; Nybo et al. 2010; Schjerve et al. 2008; Tjønna et al. 2008; Warburton et al. 2005). Specifically, within a sample of overweight women, Gillen et al (2013) showed that 6 weeks of low-volume HIT reduced adiposity (total body, abdominal and leg) and increased lean mass (leg and gynoid regions). Collectively these data suggest that 2 weeks of low-volume HIT is not sufficient to stimulate changes in body composition, however favourable changes to fat and lean mass may occur if the regimen is extended to 6 weeks or more.

In conclusion, research has shown that HIT can favourably affect many aspects of health with some beneficial changes being achievable within as little as two weeks (Babraj et al; 2009; Hood et al. 2011; Little et al. 2011; Richards et al. 2010; Talanian et al. 2007; Whyte et al. 2010). The current chapter sought to provide new information concerning a recently popularised low-volume HIT protocol that has been deemed more suitable for clinical and pre-clinical populations. Despite previous findings showing reduced fasting insulin in sedentary overweight individuals, and attenuated 24 h glycaemia in patients with T2DM, this study failed to observe any changes in systemic inflammation, glycaemic control, body composition or aerobic capacity in inactive, overweight or obese men. A lack of change was also witnessed in a second arm of participants who reduced the training frequency from 3 to 2 bouts per week. This study has therefore shown that this particular low-volume HIT protocol was unable to exert any beneficial impact on selected health indicators within this time-frame and pre-clinical population.
CHAPTER 6

GLYCAEMIC CONTROL AND INFLAMMATION IN RESPONSE TO 6 WEEKS OF HIGH INTENSITY INTERVAL TRAINING IN THE OBESE

3 The study in the present chapter was part of a large international collaborative project (The Metapredict Trial, EU FP7) and was a sub-study specifically focussed on inflammation and adipose tissue biology with data representing ~ 10 % of the total project cohort. The author contributed significantly to all elements of study design and independently led this sub-study.

3 WWW.METAPREDICT.EU
6.1 Abstract

HIT training may act as a novel approach in the treatment of obesity induced cardio-metabolic co-morbidities. The current study assessed the impact of 6 weeks of HIT training within an obese cohort. Specifically this study focused on the potential therapeutic benefits of HIT for improving inflammatory status, glucose control, aerobic capacity and body composition. Eighteen participants (10 men and 8 women) aged 18 - 50 years all with a BMI > 27 kg·m$^{-2}$ successfully completed the study. Fasting, rested blood samples were collected before and 48 h after the final training session with analysis of inflammatory mediators (Adiponectin, MCP-1, IL-10, TNF-α, IL-6, sIL-6R, and CRP) carried out by ELISA. Pre and post adipose tissue biopsies were collected for analysis of local inflammatory gene expression (CD46, CD68, HIF1-α, HMOX-1, IL-6, IL-6R, IL-10, ITGAX, MRC-1 and TNF-α) using real time QPCR. Body composition was measured (DEXA) with glycaemic control and aerobic capacity also determined. Results demonstrated significant improvements in maximal aerobic capacity after training ($p \leq 0.05$) as well as significant up-regulation of genes encoding for the M2 macrophage phenotype ($p \leq 0.05$). No further physiologically significant changes were demonstrated. This study demonstrated that HIT based training induced significant improvements in maximal aerobic capacity with an obese cohort however was not able to improve glucose control or systemic inflammation. This study provides some evidence that HIT training may produce a localised anti-inflammatory environment within adipose tissue however more work is required in order to fully elucidate if this response.
6.2 Introduction

The previous chapter suggested that a sub-maximal HIT training intervention (Little et al. 2011) was unsuccessful in improving markers of aerobic capacity, metabolic control, body composition or inflammatory profile when performed over a 2 week period. This gave rise to the conjecture that if extended over a longer training period in order to increase total accumulated training volume, it may have been possible for a modified model (Little et al. 2011) of HIT to improve the previously measured physiological factors whilst still being ‘manageable’ within an obese cohort.

This chapter focused particularly on the gene expression of inflammatory mediators and other factors influencing metabolic regulation. Although expression of mRNA only explains ~ 40 % of protein abundance, the abundance of mRNA is often an excellent proxy for the presence of a protein, specifically, whether or not that protein is detectable within target cells (Ramakrishnan et al. 2009; Vogel and Marcotte 2012).

HIT type training has been shown to act as a potent stimulus for the up regulation of mRNA in as little as 2 wks. Using an AIT protocol described previously (Leggate et al. 2012), Perry and colleagues (2010) demonstrated that PGC-1α mRNA was increased >10-fold 4 h after the 1st training session and returned to baseline within 24 h. This ‘saw-tooth’ pattern continued until the 7th training session, with smaller increases after each bout. In contrast, PGC-1α protein was increased 24 h after the 1st bout (23 %) and plateaued at +30 – 40% between the 3rd and 7th training session. It was demonstrated that the training-induced increases in transcriptional and mitochondrial proteins resulted from the cumulative effects of transient bursts in their mRNAs. From
this work it was summarised that the transcriptional capacity of human muscle is extremely sensitive and can be activated by just one training bout.

In continuation from the above, it may also be possible to use mRNA concentrations as an estimate of potential proteomic changes over time with longer term HIT exercise because of their earlier response. In addition, given the importance of adipose tissue as an endocrine organ and its relationship with metabolic homeostasis, tissue specific mRNA was assessed in order to address localised changes in mediators of metabolic regulation and inflammation as well as direct changes in the circulation. Inflammatory proteins were measured in the circulation in order to ascertain the direct systemic effects of HIT exercise on chronic inflammation.

The exercise period was extended to 6 weeks. This duration was derived from the work of Burgomaster et al (2008) who demonstrated significant improvement in $\dot{V}O_{2\text{max}}$ following 6 weeks of SIT training although the SIT training group indicated significant improvements in aerobic capacity between 0 and 3 weeks with no further improvements beyond week 3. This data suggests that the critical maximum HIT training programme length probably lies around 3 weeks. The intensity in this study was > 100 % $V_{O2\text{max}}$. At a lower intensity and in an obese group six weeks of training has shown health benefits (Larsen et al. 2014). The training was based around the use of 1 min intervals devised by previous groups and the previous chapter (Little et al. 2011). The modification is around the recovery (increased from 60 s to 90 s) and in the training intensity of the active component which was identified at the first training session in week 1. It was determined as the maximal wattage that the subjects could sustain for 60 s whilst maintaining an RPM of 100, which on average corresponded to
128 % of the maximal wattage achieved during an incremental cycling test. Participants performed a maximum of 3 reps in week 1, increasing to 5 reps in weeks 2 which was maintained then throughout training. After the first 2 weeks, the training load was increased by 10 %. Along with significant increases in lean mass and \( \dot{V}O_{2\text{peak}} \) the authors describe a significant decrease in HbA1c levels. This 6 week protocol (Larsen et al. 2014) was sufficient in improving markers of metabolic stability and adds further justification for employing a 6 week intervention when incorporating sub-maximal HIT training.

The aim of this chapter was to extend the training programme to 6 weeks and to modify the protocol to one more closely linked to Larsen et al. (2014). It was expected that positive improvements in glucose control, inflammation, body composition and \( \dot{V}O_{2\text{peak}} \) would occur as a consequence of 6 weeks HIT training at both the mRNA and protein levels.

6.3 Materials and methods

6.3.1 Participant characteristics

All participants had a BMI > 27 kg·m\(^{-2}\), reported not taking part in any form of exercise more than 2 times per week and were otherwise healthy. Participants were excluded if they were smokers or had a BMI \( \geq 40 \) kg·m\(^{-2}\). Participants were included in the study after they were informed both verbally and in writing of all possible discomforts and risks associated with the study. All study procedures were submitted to and approved by the local ethics advisory committee and all participants gave their written informed consent before commencing the study.
6.3.2 Body composition

Approximately 8 days after initial baseline monitoring, participants entered the laboratory at 7 am following a 10 h overnight fast. Firstly body composition was analysed using DEXA (Lunar Prodigy, GE medical systems, Madison, WI, USA) (See general methods for detail). Activity EE was monitored throughout the study using the Acti-heart accelerometer (Mini Mitter, Sunriver, OR, USA). The measurement of physical activity prior to training, over a 7 day period allowed for estimation of typical daily total energy expenditures. The Acti-heart has been shown to provide accurate estimates of energy expenditures in low-to-moderate activities (Thompson et al. 2006), as well as treadmill walking and running in adults (Brage et al. 2005) with currently no data specific to its use within the obese cohort.

The Acti-heart was attached to participants chests using ECG electrodes (3M Red Dot 2271, London, Ontario, Canada), positioned at the level of the third intercostal space. Once a suitable position was confirmed by signal test, the Acti-heart was set to record data every 30 s over a 7 day period.

Energy intake was assessed via a standard 4-day diet diary, incorporating 3 weekdays and 1 weekend day in order to estimate average daily energy intake (Thompson and Byers 1994). Participants were fully instructed on food weighing with digital scales (Salter, Kent, UK) and brand recording. Subsequent data was analysed for energy content via 'micro-diet' software (Downlee systems, High Peak, UK).
6.3.3 Adipose tissue sampling

Following assessment of body composition participants moved to a sterile lab for adipose tissue biopsy. Participants lay in a semi-supine position and povidone-iodine was used to clean the inferior border of the costal margin to the superior iliac spine. 10 ml of 1 % (w/v) lidocaine was administered under sterile conditions to the area before the adipose tissue was extracted ~10 cm - 15 cm laterally from the umbilicus, using a percutaneous needle biopsy technique (Christiansen et al. 2010a) with a 14 GA needle and 20 ml syringe. A vacuum was applied to the syringe resulting in the collection of adipose tissue. The excised adipose tissue was immediately washed with 0.9 % (w/v) saline solution to limit blood levels within the biopsy sample before it was aliquoted into Eppendorf tubes using sterile forceps. The tissue was snap frozen in liquid nitrogen before being transferred to - 80 °C freezer until analysis.

6.3.4 Oral glucose tolerance test

Participants were prepared for an oral glucose tolerance test which was completed over a 2 h period (see general methods for detail). Immediately prior to OGTT testing and following 10 min of seated rest, blood pressure was measured (See general methods for detail).

6.3.5 Test of peak aerobic capacity

Twenty-four hours after body composition, biopsy and OGTT tests, participants returned to the laboratory to complete a $\dot{V}O_2$peak test. Participants had $\dot{V}O_2$peak determined using a continuous incremental exercise test on an electromagnetically-braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands), performed to
volitional exhaustion. This test differs from that described in previous chapters. Expired air was measured continuously using an online breath-by-breath gas analysis system (Cortex Metalyzer, CPX International Inc., Berlin, Germany). Participants warmed up for 5 min against a resistance of 50 W, after which point the workload was increased linearly by 16 W per minute until the participant could no longer maintain 50 RPM. This protocol differs from the step protocols utilised in previous chapters. $\dot{V}O_{2\text{peak}}$ was identified over the highest value achieved over 15 breaths, taken from a rolling average. HR was measured throughout the test using a telemetric heart rate monitor, which was wirelessly paired with the breath-by-breath analysis system (Polar RS100, Polar Electro, UK Ltd, Warwick, England).

### 6.3.6 Repeat baseline tests

On completion of the $\dot{V}O_{2\text{peak}}$ test participants were once again fitted with an Acti-heart monitor and provided with 4-day diet diary apparatus. Participants maintained both over a 7 day period which allowed for assessment of diet and physical activity stability when compared against the initial monitoring period measurement. On completion of 7-day diet and activity monitoring participants returned to the laboratory at 8 am having fasted for 10 h overnight in order to complete validation OGTT and $\dot{V}O_{2\text{peak}}$ tests. This allowed for the evaluation of basal glucose variability and allowed determination of any learning prejudice relating to the test of maximal aerobic capacity. Baseline $\dot{V}O_{2\text{peak}}$ was taken as the highest value achieved from the 2 baseline measures.
Figure 6.1 Schematic representation of the experimental protocol
6.3.7 Post-training measurements

At the beginning of week 3 participants were once again fitted with an Acti-heart monitor in order to estimate changes in habitual daily EE attributable to intervention administration. Post-intervention procedures were carried out 72 h after the final training session to abolish any effects of acute exercise (Hawley and Lessard 2008) and were identical to those performed during the pre-training data acquisition period, with Acti-heart readings collected continuously for 1 week after training.

6.3.8 High intensity interval training

Participants underwent 6 weeks of 100 % supervised training 3 x per week, with approximately 1–2 rest days between training days, similar to that described previously (Hoier et al. 2013; Larson et al. 2014). Each training session lasted for ~ 15 min and consisted of a 2 min warm-up (50 W) followed by 5 x 60 s exercise bouts with 90 s cycling at a low intensity as recovery (50 W). The training load was found at the first training session in week 1. It was determined as the maximal load that the subjects could sustain for 60 s, which corresponded to 100 % - 130 % of $W_{max}$ achieved during the incremental cycle test. After the first week of training, repetitions increased from 3 to 5 and after the first 2 weeks the training load was increased by 10 %. Participants completed a total of 18 HIT training sessions. If participants were unable to complete a minimum of 16 sessions they were excluded from the study. If after 6 weeks, only 16 sessions were achieved, 2 additional sessions were completed immediately beyond the 6 -week end point.
Table 6.1 Participant characteristics, with data presented as mean and (standard deviation). Abbreviations: BMI=body mass index, BP=blood pressure.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Training</th>
<th>Post-Training</th>
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<tbody>
<tr>
<td><strong>Mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>96.2 (17.7)</td>
<td>96.5 (18.2)</td>
<td>0.630</td>
</tr>
<tr>
<td><strong>BMI (kg.m$^2$)</strong></td>
<td>31.8 (3.7)</td>
<td>31.9 (3.8)</td>
<td>0.658</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>124.1 (7.4)</td>
<td>121.6 (9.4)</td>
<td>0.173</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>79.1 (6.1)</td>
<td>80.4 (6.9)</td>
<td>0.460</td>
</tr>
<tr>
<td>$\dot{V}O_2$peak (L.min$^{-1}$)</td>
<td>2.4 (0.7)</td>
<td>2.8 (0.8)</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>$\dot{V}O_2$peak (ml.kg$^{-1}$.min$^{-1}$)</td>
<td>25.2 (4.8)</td>
<td>28.5 (4.8)</td>
<td>&lt; 0.01*</td>
</tr>
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</table>
6.3.9 ELISAs and biochemical analysis

Adiponectin, MCP-1, IL-10 and TNF-α were quantified using commercial sandwich enzyme linked immunosorbent assays (ELISAs) of which TNF-α and IL-10 were high sensitivity ELISAs (R & D systems, Minneapolis, MN, USA). Plasma IL-6, sIL-6R, and CRP were analysed via ‘in-house’ ELISAs (See general methods for detail). All samples were analysed in duplicate and were repeated if the CV between duplicates was more than 10 %. The intra-assay CVs for the inflammatory proteins are reported in Table 6.3.

6.3.10 RNA extraction and cDNA reaction

Adipose tissue was lysed for 4 min in 1 ml of TRIzol reagent (GIBCO-BRL, Life Technologies, Roskilde, Denmark) using an automated tissue lyser system (Tissuelyser II, Qiagen, Hilden, Germany). Following tissue lysis, metal lysis beads were removed and the Eppendorf tubes incubated for 10 min at room temperature. At this stage 200 µl of chloroform was added to each sample to partition proteins into the organic phase and nucleic acids into the aqueous phase. All samples were vortexed for ~ 20 s before 20 min incubation at room temperature and then spun at 14000 x g for 20 min at 4 °C. Following the spin phase, 500 µl of clear aqueous phase was transferred to a new 1.5 ml Eppendorf tube followed by 500 µl isopropanol. This was done in order to aggregate RNA into pellet. After thorough mixing this was incubated at room temperature for 30 min and then spun at 14000 x g for 20 min at 4 °C to remove alcohol soluble salts. Isopropanol was next removed from each tube leaving only the pellet. At this point 1 ml of 70 % ice cold ethanol was added to each tube and spun at 14000 x g for 20 min at 4 °C. Ethanol was removed and the pellet allowed to
air-dry for 30 min. Once dry the pellet was left to dissolve for 10 min in 30 μl RNase free water with occasional agitation.

RNA concentration was assessed by adding 1 μl of sample to a Nanodrop spectrophotometer (NanoDrop 2000, Thermo Scientific, Vantaa, Finland). Additionally 260/280 absorbance ratios were reviewed in order to assess any potential contamination (Table 6.2). Any samples not achieving ≥ 1.80 ratios were repeated.

Table 6.2 - Average RNA concentrations across all trials and associated 260/280 absorbance ratios.

<table>
<thead>
<tr>
<th>Trial</th>
<th>RNA Concentration (ng.μl⁻¹)</th>
<th>260/280 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>153.49</td>
<td>1.86</td>
</tr>
<tr>
<td>Post</td>
<td>163.24</td>
<td>1.90</td>
</tr>
</tbody>
</table>

After extraction, 1 ng of RNA from each sample was translated into cDNA using a high capacity RNA to cDNA kit (Life Technologies, Carlsbad CA, USA). All reactions took place in 0.5 ml Eppendorf tubes with 1 x RT+ and 1 x RT- reactions performed on all samples in accordance with manufacturer’s instructions. On completion of reaction preparation all samples were subjected to thermal cycling which consisted of 1 cycle of 60 min at 37 °C, 5 min at 95 °C followed by cooling to 4°C.
6.3.11 Real-time QPCR

Real-time QPCR was performed on a real-time PCR system (ViiA 7, Life Technologies, Carlsbad CA, USA) using Taqman gene expression assays, purchased from Applied Biosystems. The following probe sets were used; For cytokines: IL-6 (Hs00985639_m1); IL6r (Hs01075666_m1), IL-10 (Hs00961622_m1), TNF-α (Hs01113624_g1), MCP-1 (Hs00611256_m1), HMOX-1 (Hs01110250_m1); For macrophage markers: CD68 (Total macrophages, Hs_Hs02836816_g1), MRC-1 (M2 macrophages, Hs00267207_m1), ITGAX (M1 macrophages, Hs00174217_m1); For marker of hypoxia HIF-1α (Hs00153153_m1); and for the house keeping gene, β2-microglobulin (B2M) (Hs00984230_m1).

Cycle thresholds (CT) values of the B2M housekeeping gene were not significantly correlated with changes in physiological parameters and remained stable across measurements making it a strong housekeeping gene for analysis of adipose tissue in this cohort.

6.3.12 Statistical analysis

Statistical analyses were carried out using SPSS version 21 (SPSS Inc, an IBM company) as described in general methods. Specifically, pre to post differences in blood pressure, basal plasma glucose and insulin, glucose and insulin AUC, anthropometry, inflammatory proteins and \( \dot{V}O_2 \text{peak} \) data were assessed using paired sample \( t \)-tests. Statistically, paired \( t \)-tests did not reveal a difference between 2 baseline OGTT measures and as such the first of the 2 measurements was accepted as baseline. Pre to post changes in gene expression were assessed via paired
samples t-test using \( \Delta CT \) values as described elsewhere (Schmittgen and Livak 2008).

The previous chapter highlighted a high degree of variability within OGTT and inflammatory parameters. The current chapter aims to look in more detail at individual responses in selected parameters. These were analysed and ranked according to percentage change. Confidence intervals (95\%) were computed for all of the variables in order to provide narrower estimates of true magnitude of change. Following previous procedures (Scharhag-Rosenberger et al. 2012; Astorino and Schubert 2014), participants were categorised as ‘responders’ if magnitude of percentage change in individual response exceeded 1 CV from baseline after accounting for measurement error. Non-responders were categorised as individuals improving less than 1 CV above baseline.

6.4 Results

6.4.1 Inflammatory proteins in the circulation

Circulatory inflammatory protein data are presented in table 6.3. No changes were measured for IL-10, TNF-\( \alpha \), CRP, IL-6, sIL-6R or MCP-1. Singularly, HMW adiponectin significantly decreased following 6 weeks of HIT training. The coefficient of variation for each protein assay is also presented in table 6.3 and reflects variation between duplicate samples on a single assay plate. Table 6.4 highlights percentage responder types for each proteomic marker with accompanying confidence intervals and ranges.
Table 6.3  Inflammatory proteins in plasma pre and post 6 weeks of HIT training. Data are presented as range and (median) with delta (Δ) change describing the mean post minus pre adaptation. % CV between duplicate samples is provided with all analyses.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Pre-Training Range (Median)</th>
<th>Post-Training Range (Median)</th>
<th>Δ Change</th>
<th>Sig*. ≤ 0.05</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>adiponectin (µg.ml⁻¹)</td>
<td>0.3-12.8 (2.4)</td>
<td>0.2-7.6 (1.7)</td>
<td>-0.96</td>
<td>0.01*</td>
<td>8.5</td>
</tr>
<tr>
<td>IL-10 (pg.ml⁻¹)</td>
<td>0-1.3 (0.1)</td>
<td>0-0.6 (0.2)</td>
<td>-0.06</td>
<td>0.710</td>
<td>5.3</td>
</tr>
<tr>
<td>TNF-α (pg.ml⁻¹)</td>
<td>1.1-5.9 (2.7)</td>
<td>1.5-5.8 (3.1)</td>
<td>0.1</td>
<td>0.458</td>
<td>8.5</td>
</tr>
<tr>
<td>CRP (µg.ml⁻¹)</td>
<td>0-6.3 (1.5)</td>
<td>0-11.3 (1.9)</td>
<td>0.7</td>
<td>0.981</td>
<td>3.9</td>
</tr>
<tr>
<td>IL-6 (pg.ml⁻¹)</td>
<td>1.5-6.9 (2.2)</td>
<td>1.5-12.2 (2.1)</td>
<td>0.45</td>
<td>0.674</td>
<td>8.6</td>
</tr>
<tr>
<td>sIL-6R (ng.ml⁻¹)</td>
<td>0.8-2.4 (1.6)</td>
<td>0.7-2.7 (1.4)</td>
<td>-0.06</td>
<td>0.667</td>
<td>6.7</td>
</tr>
<tr>
<td>MCP-1 (pg.ml⁻¹)</td>
<td>100.2-607 (347.4)</td>
<td>193.1-654.6 (307.1)</td>
<td>-14.7</td>
<td>0.842</td>
<td>8.4</td>
</tr>
</tbody>
</table>
Table 6.4  Percentage representation of participants categorised as responders or non-responders for plasma inflammatory proteomic changes following 6 weeks of HIT. Responders were defined as those achieving a favourable ≥ 1CV magnitude of change from the mean, with non-responders showing an adverse magnitude of change ≥ 1CV from the mean.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Responders (%)</th>
<th>Non-Responders (%)</th>
<th>Mean Change (%)</th>
<th>95 % CI (%)</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>33</td>
<td>47</td>
<td>10.55</td>
<td>-10.40 - 31.49</td>
<td>-37.22 - 109.82</td>
</tr>
<tr>
<td>sIL-6R</td>
<td>47</td>
<td>27</td>
<td>-1.17</td>
<td>-16.88 - 14.54</td>
<td>-43.74 - 90.35</td>
</tr>
<tr>
<td>CRP</td>
<td>47</td>
<td>53</td>
<td>82.96</td>
<td>-48.36 - 214.29</td>
<td>-156.76 - 800</td>
</tr>
<tr>
<td>adiponectin</td>
<td>6</td>
<td>73</td>
<td>-17.38</td>
<td>-33.91 - -0.85</td>
<td>-50.15 - 85.91</td>
</tr>
<tr>
<td>TNF-α</td>
<td>33</td>
<td>33</td>
<td>9.97</td>
<td>-7.04 - 26.97</td>
<td>-27.31 - -2.37</td>
</tr>
<tr>
<td>IL-10</td>
<td>47</td>
<td>47</td>
<td>55.54</td>
<td>-23.22 - 134.30</td>
<td>-103.12 - 451.03</td>
</tr>
<tr>
<td>MCP1</td>
<td>60</td>
<td>33</td>
<td>9.2</td>
<td>-19.62 - 38.03</td>
<td>-55.07 - 104.70</td>
</tr>
</tbody>
</table>
6.4.2 Adipose tissue gene expression

M2 macrophage expression as determined by MRC-1 expression was significantly up regulated following 6 weeks of HIT training ($p = 0.001$). No significant changes in gene expression were detected for HIF-1α ($p = 0.135$), TNF-α ($p = 0.866$), CD68 (Total macrophage marker; $p = 0.756$), ITGAX (M1 macrophage marker; $p = 0.781$), IL-10 ($p = 0.971$), IL-6 ($p = 0.634$), IL6-R ($p = 0.838$), HMOX-1 ($p = 0.694$) or CD46 (Encoding for MCP-1; $p = 0.138$) (Figure 6.2). Individual responder types are highlighted in Table 6.5.
Figure 6.2 Graphical representations of fold changes defined by $2^{-\Delta\Delta Ct}$ of all genes following 6 weeks of HIT training. The $2^{-\Delta\Delta Ct}$ was derived from the equation: [Sample A (CT gene of interest – CT internal control)] – [Sample B (CT gene of interest – CT internal control)]. Significant changes from baseline (*) were demonstrated for MRC-1.
Table 6.5  Percentage representation of participants categorised as responders or non-responders for gene expression changes following 6 weeks of HIT.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Responders (%)</th>
<th>Non-Responders (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD46</td>
<td>27</td>
<td>40</td>
</tr>
<tr>
<td>CD68</td>
<td>47</td>
<td>27</td>
</tr>
<tr>
<td>HIF1-α</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>HMOX-1</td>
<td>20</td>
<td>33</td>
</tr>
<tr>
<td>IL-6</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>IL-6R</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>IL-10</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>ITGAX</td>
<td>47</td>
<td>27</td>
</tr>
<tr>
<td>MRC-1</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>TNF-α</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>
6.4.3 Insulin sensitivity

After the 6 week HIT intervention there were no significant changes in fasting glucose ($p = 0.093$), fasting insulin ($p = 0.401$) or the Matsuda insulin sensitivity index ($p = 0.852$), nor were there any differences found for the area under the glucose ($p = 0.736$) or insulin ($p = 0.719$) concentration curves in response to a 2 h OGTT. The glucose and insulin responses to the 2 h OGTT before and after training are shown in figure 4.3.3. Basal insulin showed a mean cohort increase of 14.3 % ($12.77 \pm 9.42$ mU.l$^{-1}$ to $14.60 \pm 12.44$ mU.l$^{-1}$). A total of 11 % participants were responders for basal insulin improvement (95 % CI = -4.04 % - 36.32 %, Range = -58.06 % - 112.21%). Basal glucose responses ($5.13 \pm 4.64$ to $5.59 \pm 4.93$) were less marked demonstrating a group mean increase of 9 % (95 % CI = 1.34 % - 13.36 %, Range = -14.92 % - 29.49 %). For this parameter 17 % of participants were responders to HIT exercise and 50 % non-responders.
With respect to insulin AUC a mean cohort change of 2.86 % was shown following HIT (95 % CI = -17.14 % - 22.87 %, Range = -59.29 % - 112.53 %). Data demonstrated 11 % of participants as responders to training in this parameter with 22 % of participants defined as non-responders. A mean change of 2.90 % was shown in glucose AUC (95 % CI = -4.48 % - 10.29 %, Range = -19.07 % - 27.72 %) with 28 % responders and no non-responders.
6.4.4 Body composition, blood pressure and peak oxygen uptake

After 6 weeks of HIT training there were no changes in body mass ($p = 0.658$) or BMI ($p = 0.630$). DEXA data (Figure 6.4) showed no changes in tissue or regional fat (%), total tissue (g), total lean tissue (g), total fat tissue (g) or bone mineral content (BMC) (g) within this cohort ($p > 0.05$).

![Figure 6.4](image)

**Figure 6.4** Changes in tissue and regional % fat, total tissue (g), total lean tissue (g), total fat tissue (g) and bone mineral content (BMC)(g) as measured by DEXA. Data are presented as percentage change (%) and standard deviation.

No significant changes in systolic (SBP) ($p = 0.173$) or diastolic (DBP) ($p = 0.460$) blood pressure were observed following 6 weeks of HIT training. Based on individual response criteria 14 % of participants were non-responders to HIT for changes in DBP, with no participants being classified as responders. Moreover 14 % of individuals were classified as responders for changes in SBP with no participants falling into the non-responder criteria. The mean magnitude of change for SBP was -2.01 % (95 % CI = -4.64 % – 0.62 %, Range = -11.97 % – 5.98 %). Additionally the
mean % change for DBP was 1.91 % (95 % CI = -2.40 % - 6.22 %, Range = -11.39 % – 18.31 %).

\( \dot{V}O_{2peak} \) significantly improved following training in absolute and relative terms \( (P = < 0.01) \). Mean % change in relative \( \dot{V}O_{2peak} \) equated to 14.1 % (95 % CI = 9.1 % - 19.1 %, Range = -5.0 % – 36.4 %). Using relative values, 83 % of participants were classified as responders with 17 % classified as non-responders (Figure 6.5).

![Graph showing changes in VO2peak](image)

**Figure 6.5** Changes in a) relative and b) absolute \( \dot{V}O_{2peak} \) following 6 weeks of HIT training. Data are expressed as mean and standard deviation.
Data revealed that self-reported dietary intake significantly decreased following 6 weeks of HIT training ($p = 0.01$) (Figure 6.6). Additionally total energy expenditure (omitting the formal exercise period) of the full cohort, as estimated by the Actiheart device, did not change as a consequence of the exercise trial, before, during or after the intervention ($p > 0.05$) (Figure 6.7).

![Figure 6.6](image)

**Figure 6.6** Changes in self-reported dietary intake. Data represent a comparison between pre intervention and week 6 of training. Data are presented as mean energy intake in kilojoules (kJ) and standard deviation.
Figure 6.7  Changes in estimated total energy expenditure as measured by Actiheart. Data represent a comparison between 2 x pre-exercise measurements, a mid-training measurement and a 1 week post-intervention measurement. Data are presented as mean energy expenditure per day in kilojoules (kJ.d-1) and standard deviation.
6.5 Discussion

The primary aim of this study was to profile the effectiveness of a low volume HIT protocol (Larsen et al. 2014), for improving aerobic capacity, glucose control, and inflammatory profile in an obese “at risk” cohort with particular focus on adipose tissue inflammatory gene expression. This study has demonstrated that 6 weeks of HIT training was successful in improving peak aerobic capacity and successfully up-regulated expression of genes related to anti-inflammatory responses in adipose tissue. Exercise however did not improve glucose control or markers of inflammation.

The present study is the first to demonstrate significant up-regulation in the M2 macrophage marker MRC-1, following low volume HIT training. Although there is complexity around the macrophage classification and the associated inflammatory response (Martinez and Gordon 2014), classically the M1 macrophage phenotype is induced by inflammatory factors such as TNF-α and produces pro-inflammatory cytokines, with the M2 phenotype induced by factors such as IL-4 and IL-10 to produce anti-inflammatory cytokines (Sulahian et al. 2000; Gratchev et al. 2001; Martinez, Helming and Gordon 2009; Dalmas, Clement and Guerre-Millo 2011). Increases in M2 macrophage phenotypes have been shown in morbidly obese patients who lost 22 kg in 3 months, following Roux-en-Y gastric bypass (Cancello et al. 2005). Also obese mice fed high fat diets in combination with a 16 week endurance training programme demonstrated significant up-regulation of genes associated with M2 macrophages (Kawanishi et al. 2010). In the current study, data suggests no change in body fat, which might suggest that exercise alone can cause the gene expression change.
This study did not find any changes in inflammatory mediators, therefore although not measured here, the possible pathway for the up-regulation of the M2 macrophage phenotype may in part be explained by glucocorticoid hormones, which will have been released following the high intensity exercise (Deuster et al. 1998). Following secretion from the adrenal glands, glucocorticoids are metabolised by cellular enzymes in macrophages. Active glucocorticoids are lipophilic and diffuse through the membrane to bind to glucocorticoid receptor alpha (GCR-α) leading to nuclear translocation of the complex. The GCR-α binds to DNA directly to promote M2 gene expression and repress M1 gene transcription (Varga et al. 2008) either directly or indirectly through transcription factors such as NF-κB (Martinez and Gordon 2014).

Despite significant changes in macrophage polarisation, the current study saw no other changes in inflammatory markers other than plasma adiponectin, which significantly decreased. Data is variable as to the response of adiponectin to exercise training. There is clear evidence to suggest that adiponectin levels are inversely correlated with BMI (Arita et al. 1999; Weyer et al. 2001; Bruun et al. 2003; Kern et al. 2003; Ryan et al. 2003; Vilarrasa et al. 2005; Bluher et al. 2006), however a review by Simpson and Singh (2008) demonstrated that only 3 from 8 randomised control trials involving exercise training resulted in increased levels of plasma adiponectin and data suggests that in order to achieve increases in adiponectin, dietary restriction with a 10 % weight loss is required (Madsen et al. 2008). What should be made clear is that these studies fail to significantly isolate adiponectin isoforms and as such conclusions are based on the measurement of total adiponectin.
There are some studies which have indicated increases in total adiponectin with additional evidence to suggest that 1 year of high intensity exercise causes an increase in adiponectin independent of weight loss (Balducci et al. 2010). However in agreement with the current findings, Christiansen and colleagues (2010b) showed a small non-significant decrease in plasma adiponectin following 3 months of exercise training, yet in diet and diet plus exercise groups levels were increased. Leggate and colleagues (2012) also demonstrated a significant reduction in HMW adiponectin following 2 weeks of HIT training in an obese cohort. Intriguingly the current study data suggests a significant reduction in calorie intake of ~ 30 %, but this did not change body composition. Taking into account average habitual energy expenditure data (Figure 6.7) and the estimation of energy expended during a typical training session (≤ 1000 kJ) as well as no change in body mass data (Table 6.1) it may suggest that the participants under-reported within their diet diaries and as such the energy deficit may not have been as marked as that reported.

There is evidence to suggest that it is the ratio of HMW adiponectin to total adiponectin which shows the strongest correlation with insulin sensitivity in humans and not necessarily total adiponectin or individual isoforms (Pajvani et al. 2004; Lara-Castro et al. 2006). Neither low molecular weight (LMW) nor total adiponectin were measured in the current study but it is tempting to speculate that all adiponectin isoforms were decreased following exercise, with decreases in HMW adiponectin showing the least decrement and thus increasing the ratio in favour of the HMW isoform. If this were the case and given data to suggest a role for HMW adiponectin in the modulation of human monocytes and macrophages
toward an anti-inflammatory phenotype (Wolf et al. 2004) may explain why significant decreases in HMW adiponectin were found in the current study independent of changes in body mass and with maintenance of positive macrophage responses. Future work should aim to measure high and low molecular weights of adiponectin as well as total levels in order to gain a more complete conclusion based on isoform ratios. This is particularly important given that the findings in the current study are not isolated (Christiansen et al. 2010b; Leggate et al. 2012).

Data concerning systemic and adipose tissue inflammation following HIT training is limited with a majority of previous exercise models incorporating higher volume continuous-moderate exercise. Leggate et al (2012) provide the only work to date evaluating systemic and adipose inflammation after HIT training. This protocol although referred to as the umbrella term HIT falls into the category of AIT using the previously reported protocol (Talanian et al. 2007). Leggate et al (2012) demonstrated a significant reduction in plasma sIL-6R, MCP-1 and adiponectin. Within adipose tissue IL-6 was reduced with no changes detected in ICAM-1 with TNF-α, MCP-1 and IL-10 undetectable within the adipose tissue. These data (Leggate et al. 2012) show some similarity with those in the current study with regards lack of change in TNF-α and MCP-1 in circulation.

Justification for the differing responses between the findings of Leggate and colleagues (2012) and those in the current study with regards to plasma and adipose inflammatory markers is most likely attributed to the volume of exercise incorporated within the programme. Leggate and colleagues (2012) utilised ~ 240
min of high intensity efforts over 2 weeks compared to the 90 min over 6 weeks in the current study.

Polak et al. (2006) demonstrated similar results to those of the current study using an obese cohort. This group (Polak et al. 2006) showed no change in plasma adiponectin, TNF-α or IL-6 following 12 weeks of aerobic exercise. Despite a high weekly volume of 225 min, based on the aforementioned theory the exercise intensity (50 % $\dot{V}O_{2\text{max}}$) used by Polak et al (2006) may not have been high enough to induce beneficial changes. Similar findings were demonstrated by Leick et al (2007) who saw no changes in adipose tissue mRNA for adiponectin, leptin, IL-6 or TNF-α following 8 weeks of higher intensity exercise (70 % $\dot{V}O_{2\text{max}}$) within an obese cohort and detected no changes in circulatory or adipose tissue IL-18. This leads to the proposal that 90 min of high intensity effort across 6 weeks is insufficient in inducing positive changes in inflammatory profile, with a more likely weekly volume $\geq$ 120 min being required with a minimum intensity of 85 % $\dot{V}O_{2\text{max}}$

There is still a need however to ascertain the true volume – intensity intercept required to induce positive changes in inflammatory profile with HIT exercise. This chapter makes clear that a total of 90 min of high intensity effort utilised over 6 weeks is not sufficient in improving most markers of inflammation.

Inflammation in both plasma and adipose tissue has been linked with insulin resistance and T2DM. (Dandona, Aljada and Bandyopadhyay 2004; Schoelson, Lee and Goldfine 2006). Previous work suggests that HIT may result in significant
improvements in insulin resistance however results are varied and could relate to the large variety of training protocols being characterised under the one umbrella of HIT. Significant improvements in glucose control have been demonstrated in as little as 2 weeks following SIT based exercise in healthy (Babraj et al. 2009; Richards et al. 2010) and obese (Whyte et al. 2010) populations. Similar improvements have been demonstrated in the same time period following sub-maximal HIT in physically inactive (Hood et al. 2011) and T2DM (Little et al. 2011) cohorts and with a similar RE-HIT protocol over a 6 week period Metcalfe et al (2011) have shown an improvement in insulin sensitivity of 28 %. Recently Shepherd and colleagues demonstrated that 6 weeks of SIT exercise reduced glucose AUC by 17 % and insulin AUC by 33 % with an overall increase in insulin sensitivity of 56 % in a physically inactive cohort. When comparing the cohort in the current chapter to those in the aforementioned works assessing glucose control it becomes clear that baseline $\dot{V}O_{2peak}$ values are approximately 36 % lower compared to those previously reported (Kessler et al. 2012). This gives rise to the suggestion that a minimal aerobic capacity must first be achieved before improvements in glucose control occur. In agreement with the data in the current chapter and in line with this theory, Gillen and colleagues (2013) demonstrated that 6 weeks of HIT training identical to that described previously (Little et al. 2011), was not sufficient in improving insulin sensitivity in overweight women, who all demonstrated an average basal $\dot{V}O_{2peak}$ of 28 ml.kg$^{-1}$.min$^{-1}$. It may be suggested that a $\dot{V}O_{2peak}$ in the range of 30-35 ml.kg$^{-1}$.min$^{-1}$ must first be acquired in order for insulin sensitivity to improve, which may be an indirect reflection of changes in mitochondrial function (Weibel, Taylor and Huppeter 1991). From an individual response perspective data from the HERITAGE family study (Boule et
al. 2005), currently the largest ($n=600$) investigation of glucose homeostasis following exercise (20 weeks, 3 x week cycling 55 % - 76 % $\dot{V}O_{2\text{max}}$) demonstrated that 42 % of their cohort demonstrated no change or impairment in insulin sensitivity following exercise which is in comparison with 65 % in the current cohort, with HERITAGE (Boule et al. 2005) demonstrating that 54 % of individuals showed impairment in glucose AUC, in comparison to the 65 % demonstrated in the current cohort. This suggests that in the current sample, albeit large for a tissue biopsy study, was a sample weighted towards non-responders and those who were impaired. The reason for these non or adverse responses remain unclear however they are likely to be driven by pre-disposed genetic factors which are currently the subject of intensive investigation within the field. Although not directly comparable, these data demonstrate that negative responses in glucose homeostasis are not rare occurrences following exercise and that in many studies these responses may be masked by highly positive responders who dominate group means. These data further highlight the importance of reporting individual responses within data sets to gain a true reflection of exercise responses.

When looking in more detail at the current cohort and in contrast to aforementioned works (Babraj et al. 2009; Richards et al. 2010; Whyte et al. 2010) basal insulin levels were deemed elevated (Belfiore, Ianello and Volpicelli 1998). Mean basal insulin levels in the current cohort rose by 14 %, which may initially suggest that the exercise intervention may have negatively impacted upon insulin sensitivity. This response is difficult to justify given the volume of previously reported positive outcomes associated with this exercise modality, however as mentioned above may relate to individual responses driving the group mean and
hence why individual responses have been reported in this chapter. From those determined as non-responders to basal insulin change, 1 participant showed worsened basal insulin profile showing an adverse 112 % change which may partly explain the overall poor response within the cohort. Yet when this individual is removed from the cohort the median % decrement is only improved by 1.28 % (19.93 % decrement vs. 18.65 %) highlighting that this one particularly poor response is more marked than others, however is certainly not an isolated event.

One speculative theory for the worsened glucose control arises from impaired mitochondrial uptake and oxidation of fatty acids (Morino et al. 2006; Ruderman et al. 1999). A theory suggests that muscle insulin resistance arises from impaired mitochondrial uptake and oxidation of fatty acids (Ruderman et al. 2006; Morino et al. 2006), which has been specifically associated with obesity and T2DM (Kelley et al. 2002; Hojlund et al. 2008). Specifically this model suggests that long-chain acyl CoAs (LC-CoAs) derived from circulating lipids or intra-muscular TAG are diverted away from carnitine palmitoyltransferase 1 (CPT 1) and are instead preferentially partitioned towards the synthesis of signalling intermediates such as diacylglycerol (DAG) and ceramide. The accumulation of these and other lipid molecules is thought to engage stress activated serine kinases that interfere with insulin transduction (Yu et al. 2002; Holland et al. 2007). In the context of the current study it may be theorised that if lipolysis is high immediately after HIT, this may provide the catalyst for FFAs to overload the obese dysfunctional mitochondria. The subsequent incomplete fatty acid oxidation may contribute to downstream skeletal muscle insulin resistance defined in the current study by elevations in basal insulin. These findings are not isolated and are replicated in a
sub-population within the STRRIDE studies (Slentz et al. 2004) which in part investigated exercise intensities and durations within >100 obese (un-published data, Kraus et al. 2014). More work is required in order to ascertain the true contribution of this speculative mechanism.

Compelling evidence has shown that $\dot{V}O_{2\text{max}}$ is a strong and independent predictor of all cause and cardiovascular disease mortality (Carnethon et al. 2003; Chase et al. 2009; Kodama et al. 2009; Lee et al. 2010). Due to a perceived nervousness within physicians to provide therapies that improve $\dot{V}O_{2\text{max}}$, the importance of aerobic capacity is often overlooked from a clinical perspective compared with other risk factors such as obesity and insulin resistance. The current study demonstrated a 13 % improvement in $\dot{V}O_{2\text{peak}}$ following 6 weeks of HIT training. Although not demonstrate in this thesis, previous work has demonstrated significant improvements in $\dot{V}O_{2\text{max}}$ following as little as 2 weeks of HIT training (Rodas et al. 2000; Talanian et al. 2007; Whyte et al. 2010; Leggate et al. 2012). Talanian and colleagues (2007) demonstrated that following 7 HIT sessions $\dot{V}O_{2\text{peak}}$ was increased in healthy women. This followed a HIT protocol consisting of 10 repetitions of 4 min at 85 % $\dot{V}O_{2\text{peak}}$ separated by 2 min recovery. Later Whyte et al (2010) demonstrated significant improvements in $\dot{V}O_{2\text{max}}$ following 2 weeks of SIT within an obese population. With closer comparison to the current study, longer duration training studies (6 – 8 weeks) utilising HIT and SIT have also shown similar positive improvements in aerobic capacity (Burgomaster et al. 2008; Shepherd et al. 2013; Larsen et al. 2014). Shepherd and colleagues (2013) subjected healthy young participants to 6 weeks of classic SIT training 3 x per week. Results demonstrated a significant 7 % increase in
\( \dot{V}O_{2\text{max}} \) following training, with similar improvements demonstrated following 6 weeks of sub-maximal HIT training (Larsen et al. 2014). These data would suggest that the protocol in the current study was more successful in improving peak aerobic capacity compared to an arguably more demanding and intense SIT protocol.

The relative contributions of aerobic capacity and fat loss to overall morbidity and mortality are still unclear (Lavie et al. 2014). This is in part highlighted by the obesity paradox proposed by Stephen Blair, demonstrating that lean low fitness individuals were at 5.6-fold higher risk of death than obese men with strong aerobic fitness. It is likely that aerobic capacity may provide the strongest contribution to health however changes in body adiposity must not be disregarded given its importance as an endocrine organ (Trayhurn and Beattie 2001).

Low volume high intensity interval training has previously been described as a time-effective strategy for improvement in body fat in overweight and obese individuals (Tremblay et al. 1994; Mourier et al. 1997; Warburton et al. 2005; Schjerve et al. 2008; Tjonna et al. 2008; Trapp et al. 2008; Tjonna et al. 2009; Wallman et al. 2009; Whyte et al. 2010; MacPherson et al. 2011; Heydari et al. 2012). However data from the current study demonstrates that no changes in body composition occurred as a consequence of HIT training.

Previous significant body composition changes associated with short duration (2 weeks) SIT and AIT exercise (Whyte et al. 2010; Leggate et al. 2012) have been described by waist circumference measures which can be associated with high
levels of measurement error (Ulijazek and Kerr 1999) and could explain the discrepancy with the findings in the current study. Despite Leggate and colleagues (2012) utilising 40 min of high intensity effort, a low total training volume (2 weeks) makes it unlikely to have contributed significantly to fat loss. From the analysis presented throughout this thesis, the suggestion arises that higher accumulated training volumes (> 6 weeks) may be required as opposed to long duration (> 40 min) HIT exercise sessions over a short-term (2 week) period.

Shepherd and colleagues (2013) indicated that following 6 weeks of SIT training participants failed to decrease fat mass and with a similar protocol to the current study, Keating and colleagues (2014) showed that after 12 weeks of training consisting of up to 6 x 1 min bouts at 120 % \( \dot{V}O_{2\text{peak}} \) with 2 min recovery periods, 3 x per week, there were no improvements in total body mass, lean mass or any measures of fat mass. In Keating’s study a continuous moderate training arm over the same duration and frequency but consisting of 30 min – 45 min at 50 % - 65 % \( \dot{V}O_{2\text{peak}} \) demonstrated significant improvements in the aforementioned parameters both above baseline levels and in comparison to changes associated to HIT suggesting that energy expenditure associated with increased training volume may be critical in order to promote changes in body fat.

Having demonstrated high accumulated training volume, Heydari et al. (2012) demonstrated significant reductions in body fat within an obese cohort after 12 weeks of HIT. The training consisted of 8 s bouts at 90 % HR\( _{\text{peak}} \) with 12 s recovery periods. The stark differences between these data (Heydari et al. 2012) and those presented in the current study are again possibly explained by the
training volume and length. Heydari and colleagues (2012) took measurements of body composition at 3 week intervals throughout the study. The authors report that at weeks 3 and 6 no changes in body composition were reported, whereas at week 9 changes in body composition became significant. It is likely that total exercise energy expenditure in their study would have been relatively low, an assumption made against the protocol utilised in this current study and that profiled previously (Kelly et al. 2013). Despite being low the volume of high intensity work was approximately 33 % higher in the Heydari et al. (2012) protocol compared with Keating et al. (2014). Using an extended training period Keating and colleagues (2014) failed to show any changes in body fat following 12 weeks of exercise, utilising a highly similar protocol to that of the current study. Current data and previous literature suggesting that 8 – 24 week HIT studies reduce body fat (Tremblay et al. 1994; Mourier et al. 1997; Bodouou et al. 2003; Trapp et al. 2008; Dunn et al. 2009; Tjønna et al. 2009; Heydari et al. 2012) gives rise to the suggestion that had the current study been extended beyond 6 weeks to a 15 week duration, then total energy expenditure may have accumulatively provided stimulus for significant body composition changes.

From the data in the current study and that described previously it is suggested that changes in body composition are explained by differences in accumulated training volume. No clear statements currently can be made as to whether significant changes are dominated by alterations in visceral as opposed to subcutaneous compartments following HIT training given the lack of well controlled data. More work utilising more sensitivity measures such as MRI are required to explore this paradigm.
In summary this chapter has demonstrated that 6 weeks of HIT based training in an obese cohort, equating to ~ 90 min of high intensity exercise was successful in mediating anti-inflammatory effects within adipose tissue. Additionally, significant improvements in maximal aerobic capacity were observed. These findings suggest that this specific HIT intervention may be successful in improving markers of cardio-metabolic health with changes likely to be more profound if extended beyond a 6 week period. Future work should aim to use this model within a larger cohort and modify volume and intensity, generating a dose response output and thus elucidating the optimal intensity – duration intercept. Equally, this HIT protocol should be compared against an energy matched continuous protocol in order to ascertain the true contribution of the intermittent, high-intensity modality to improve cardio-metabolic health.
CHAPTER 7

GENERAL DISCUSSION
7.1 General discussion

Glucose control following acute exercise and exercise training is highly variable in nature. Data from the current thesis (chapter 6) described 2 clear populations or phenotypes, with individuals showing strong improvements in insulin sensitivity or indeed an adverse or non-response. Using a simple statistical model it has been possible to show here that group mean changes could be driven by a large ratio of adverse responses. The HERITAGE family study (Boule et al. 2005), demonstrated that 42% of participants showed no change or impairment in insulin sensitivity following exercise training. It is reasonable to suggest that cohorts presented in the current thesis were hugely varied and those with a non-responsive phenotype dominated the group mean.

With this in mind it is not simple to describe HIT as being a positive or negative health intervention based purely on the group statistical output. Contrasting to the data in this thesis significant improvements in glucose control have been demonstrated in as little as 2 weeks following SIT based exercise in healthy (Babraj et al. 2009; Richards et al. 2010) and obese (Whyte et al. 2010) populations. Similar improvements have been demonstrated in the same time period following HIT in sedentary (Hood et al. 2011) and T2DM (Little et al. 2011) cohorts with improvements also evident after longer-term interventions (Metcalfe et al. 2011; Moreira et al. 2008; Nybo et al. 2010; Shepherd et al. 2013; Tjonna et al. 2008, 2009). It is proposed here that in those with ‘responsive’ phenotypes, HIT training proves to be a useful methodology for improving insulin sensitivity however in the ‘adverse’ phenotypes such as those described here and elsewhere (Boule et al. 2005), this exercise may actually be detrimental to metabolic health. Moreover the majority of participants throughout this thesis
were within normal healthy insulin and glucose ranges prior to training. It is likely that individuals with clinically elevated metabolic parameters may stand to improve most (if of the responsive phenotype). Future work should aim to utilise the protocols described here within a cohort of individuals presenting with impaired glucose control.

In continuation from the above and owing to a strong relationship, it is clear that improved glucose control may be a reflection of a reduced inflammatory profile (Dandona, Aljada and Bandyopadhyay. 2004; Schoelson, Lee and Goldfine. 2006). This thesis has demonstrated that HIT training may directly impact upon the inflammatory status of adipose tissue (chapter 6). It has been demonstrated here for the first time that a modified low volume HIT protocol lasting < 15 min (Larsen et al. 2014) up-regulated the expression of M2 phenotype macrophages in adipose tissue. Findings imply an increased potential for the body to produce a localised anti-inflammatory environment independent of significant changes in any other inflammatory mediators (plasma or adipose) or indeed body fat.

Classically, the inflammatory response is partly described by the production and release of inflammatory mediators via macrophages. Macrophages although probably of mixed phenotypes do show 2 distinct lineages, which are pro-inflammatory (M1) and anti-inflammatory (M2). The M2 macrophage phenotype has been shown to produce and release anti-inflammatory mediators such as IL-4, IL-10 and IL-13 (Martinez and Gordon 2014). Macrophages demonstrate high levels of plasticity and can switch between phenotypes when exposed to certain stimuli.
Little human data exists as to the role of exercise in the phenotype switching of macrophages with some evidence describing this response following surgically induced weight loss (Cancello et al. 2005) and continuous exercise in the rat (Kawanishi et al. 2010). Given the lack of changes in any other inflammatory mediator a possible pathway for the up-regulation of the M2 macrophage phenotype may be explained by glucocorticoid hormones, which will have been released following high intensity exercise (Beaudry, Riddle 2012; Deuster et al. 1998). Glucocorticoids are released following exercise at intensities ≥ 80 % \( \dot{V}O_{2\text{max}} \) (Beaudry, Riddle 2012; Deuster et al 1998; Slentz et al. 2009). Once bound to the glucocorticoid receptor, the glucocorticoids promote M2 macrophage gene expression via direct binding to DNA.

If true, then increased exposure to elevated levels of glucocorticoids as well as individual mitochondrial function may partly explicate the detrimental glycaemic responses in non or adverse responders. The proposed intermittent glucocorticoid exposure via HIT training (Van raalte et al. 2011) may have provided a catalyst for FFAs to overload the obese potentially dysfunctional mitochondria in obese individuals (Ruderman et al. 2006; Morino et al. 2006). The subsequent incomplete fatty acid oxidation may contribute to downstream skeletal muscle insulin resistance (Morino et al. 2006; Ruderman et al. 2006; Slentz et al. 2009).

Other than macrophage changes in adipose tissue, this thesis demonstrated no changes in any other inflammatory mediators following short (2 weeks) or medium term (6 weeks) training. Exclusively, Leggate et al (2012) provided evidence for reduced inflammation after HIT training. Using a previously reported protocol
(Talanian et al. 2007) this group (Leggate et al. 2012) demonstrated a significant reduction in plasma sIL-6R, MCP-1 and in agreement with the current findings saw no change in TNF-α. Given that training intensities (~ 85 % VO₂max) in this thesis are comparable to that used previously (Leggate et al. 2012) differences between findings may be attributed to total training volume. The 60 min and 90 min of high intensity effort utilised in chapters 5 and 6 respectively were insufficient in inducing positive changes in inflammatory profiles. Data suggest that when restricted to short training periods (2 weeks) it is likely that an AIT protocol is required in order to induce significant whole-body inflammatory changes as described by Leggate and colleagues (2012). If a longer training period is afforded then HIT training protocols may be sufficient to reduce inflammatory profile as long as ~ 120 min of high intensity effort is achieved per week. Based on this theory had the training periods in chapters 5 and 6 been extended to ~ 8 weeks then speculatively, changes in inflammatory profile may have been detected probably due to higher accumulative net energy expenditures.

The above discussions give rise to some key considerations. Firstly, this highlights the importance of identifying individual response phenotypes prior to exercise prescription to ensure that frequency, intensity and duration is suitably tailored. Secondly it is essential that large cohort data is contextualised and interrogated as individual responses, as group means may not describe a truly meaningful physiological response. Further invasive studies should be employed to investigate the potential adverse response of some individuals following HIT training.
The obese cohorts within this thesis demonstrated a comparatively low basal \( \dot{V}O_2_{max} \) when compared with previous work (Kessler et al. 2012). Since skeletal muscle \( \dot{V}O_2_{max} \) is a function of interaction between oxygen supply and mitochondrial oxidative capacity (Hepple et al. 2002) it may suggest that those with the lowest \( \dot{V}O_2_{max} \) may have the worst mitochondrial function. Equally given the lack of change in glucose control following HIT it may give rise to the suggestion that in order for glucose control to improve, \( \dot{V}O_2_{max} \) (and thus mitochondrial oxidative capacity) must first reach a minimal threshold to cope with the increased metabolic demand associated with HIT training. Based on the data presented here and within previous investigations it is suggested that a basal \( \dot{V}O_2_{max} \) in the range of 30-35 ml.kg.min\(^{-1}\) may indirectly reflect the sufficient mitochondrial capacity required to cope with increased metabolic demand associated with HIT training.

Future work utilising HIT type protocols should aim to address markers of mitochondrial capacity before, during and after training. With concomitant frequent \( \dot{V}O_2_{max} \) measurements the relationship between aerobic capacity and mitochondrial capacity may be more extensively explored. This may provide data on the usefulness of basal \( \dot{V}O_2_{max} \) as a predictor of mitochondrial capacity.

\( \dot{V}O_2_{max} \) may act as a strong surrogate measure for other metabolic processes adding to the already established value of this measurement parameter in the prediction of all-cause morbidity and mortality (Carnethon et al. 2003; Chase et al. 2009; Kodama et al. 2009; Lee et al. 2010). Overwhelming evidence demonstrates that SIT, HIT and AIT based exercise is capable of inducing improvements in aerobic capacity (Burgomaster et al. 2008; Larsen et al. 2014;
Leggate et al. 2012; Macpherson et al. 2011; Moholdt et al. 2009; Nybo et al. 2010; Rodas et al. 2000; Rognmo et al. 2004; Schjerve et al. 2008; Shepherd et al. 2013; Talanian et al. 2007; Tjonna et al. 2008, 2009; Wallman et al. 2009; Warburton et al. 2005; Whyte et al. 2010; Wisloff et al. 2007). Despite aforementioned findings, chapter 5 failed to detect any changes in $\dot{V}O_{2\text{max}}$. Original data relating to the protocol used in Chapter 5 (Hood et al. 2011; Little et al. 2011) failed to report changes in aerobic capacity making it impossible to make direct comparisons. Data within chapter 6 are in agreement with original data (Larsen et al. 2014) having demonstrated a significant improvement in aerobic capacity after 6 weeks of training. These data give rise to an important observation in that total exercise volume (i.e. accumulated minutes spent in high intensity bouts) may be a critical factor for improving aerobic capacity when utilising HIT based training set at sub-maximal $\dot{V}O_2$ intensities (80 % - 90 % $\dot{V}O_{2\text{max}}$). Total accumulated time spent at high intensities within chapter 5 equated to 60 min whereas accumulated time at high intensities within chapter 6 equated to 90 min. Despite a 50 % lower weekly training volume in chapter 6 compared with chapter 5, the larger total accumulated training volume in chapter 6 provided stimulus for a stronger improvement in aerobic capacity. Speculatively, had the protocol used within chapter 5 (Little et al. 2011) been extended from 2 to 6 weeks it is possible that this protocol would have demonstrated the largest improvement in aerobic capacity. This suggests that in order to independently increase aerobic capacity, a sub-maximal low volume HIT protocol must ensure that participants spend > 60 min accumulated time at high intensities.
Data specifically describing changes in fat mass following low volume HIT, via methods such as DEXA or MRI are sparse, with data to suggest no changes in body fat following a HIT protocol identical to that in chapter 6 (Larsen et al. 2014). To date only in-direct measures of adipose changes have been taken following short-term (2 week) interventions (Leggate et al. 2012; Whyte et al. 2010). Although waist circumference has been correlated highly with visceral fat, the measurement error involved does not allow for these data to be deemed truly reliable. Even with the limited data available evidence suggests that short to medium term (2 - 6 weeks) HIT type interventions result in negligible body mass changes with only longer term higher volume protocols reporting meaningful changes after training (Bodou et al. 2003; Dunn et al. 2009; Heydari et al. 2012; Mourier et al. 1997; Tjonna et al. 2009; Trapp et al. 2008; Tremblay et al. 1994). It may be fair to suggest that HIT type training in association with increased energy restriction may give rise to more marked responses. This point highlights the unreliability of self-report diet data. Chapter 6 demonstrated a significant reduction in estimated self-reported energy intake however no changes in fat mass occurred suggesting under-reporting of caloric intake. Studies providing long-term dietary support and monitoring are required to definitively answer this question.

7.2 Conclusions

In conclusion, this thesis has demonstrated that HIT training within an obese cohort can significantly augment maximal aerobic capacity however from the data presented here it would suggest that accumulated time at high intensities may be critical for improving aerobic capacity in a time-efficient manner. It is suggested
that ~ 60 min - 90 min over a 6 week period is required when training at ~ 85 % VO\textsubscript{2max}.

For the first time it has been shown that the expression of anti-inflammatory macrophage phenotypes are up regulated in subcutaneous adipose tissue following HIT training. The true mechanisms for this up regulation are unclear however may relate to glucocorticoid release, with this hypothesis warranting further investigation. No other changes in inflammatory mediators were detected leading to the conclusion that training volume must be increased ≥ 120 min per week if a training load of ~ 85 % VO\textsubscript{2max} is utilised over a 6 week period. Future work should profile how training volume can be reduced in line with increasing intensity.

Glucose responses to HIT training are highly varied and as such further work is warranted to repeat experiments similar to those described in this thesis utilising much larger cohort numbers. Given the highly diverse training responses recommendations cannot yet be made for improving this parameter although it may be proposed that response phenotypes do benefit from HIT exercise.

Finally, the evidence presented throughout this thesis suggests that HIT training, within the methodological parameters described here, is not a beneficial strategy for inducing fat loss over short to medium terms (2-6 weeks). In order for fat loss to occur, HIT protocols must be extended in nature giving rise to the supposition that total or net energy expenditure is the critical determinant of fat loss and not high acute exercise energy expenditure.
It is the opinion of this author that HIT may provide a supplementary training stimulus, which should be built in to a more varied and extended training programme for obese individuals. This thesis highlights foremost the complexities of exercise prescription within pre-clinical cohorts and gives some insight into the future of exercise prescription. Data here provide a positive contribution to the evolving field of personalised exercise medicine.
References


maximal oxygen uptake for a longer time than intense but submaximal runs. *Eur J Appl Physiol* 81: 188-196


morbidly obese subjects after surgery-induced weight loss. Diabetes 54: 2277-2286


113. Hazell TJ, Olver TD, Hamilton CD, Lemon P WR (2012) Two min of sprint interval exercise elicits 24-hr oxygen consumption similar to that of 30 min of continuous endurance exercise. *Int J Sport Nutr Exerc Metab* 22: 276-83


obesity, insulin resistance, and tumor necrosis factor-α expression. *Diabetes* 52: 1779-1785


phosphorylase and PDH during maximal intermittent exercise. *Am J Physiol* 277: e890-e900


exercise for increasing aerobic capacity in patients with coronary artery

and related comorbid conditions after diet-induced weight loss in men.
Annals of Internal Medicine 133: 92-103

(1994) Sex differences in lean and adipose tissue distribution by magnetic
resonance imaging: anthropometric relationships. *Am J Clin Nutr* 59: 1277-
1285

Giralt M, Ritov VB, Menshikova EV, Kelley DE, Hidalgo J, Pedersen BK,
Potential role in the systemic response to exercise and prevention of the
metabolic syndrome. *Diabetes* 55: S48-S54

monophosphate-activated protein kinase and malonyl coenzyme A. *Obesity*
14: S25-S33

222. Ruderman NB, Saha AK, Vavvas D, and Witters LA (1999) Malonyl-
CoA, fuel sensing, and insulin resistance. *Am J Physiol* 276: E1-E18


244. Stehling M, Wallace PK, Morganelli PM, and Guyre PM (2000) Human monocytes express CD163, which is upregulated by IL-10 and identical to p155. *Cytokine* 12: 1312-1321


APPENDIX 1
Table A – A Summary of trials utilising HIT type training interventions

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Sample Size</th>
<th>Intensity/Duration</th>
<th>Glucose Regulation</th>
<th>Anthropometric Measures</th>
<th>VO₂max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tremblay et al 262</td>
<td>1994</td>
<td>Young healthy men and women</td>
<td>Cycle ergometer 4 x wk for 15 wk</td>
<td>27</td>
<td>10 - 15 x 15 - 30 s sprints at 70% max work output in 10 s with 60-90 s recovery</td>
<td>N/A</td>
<td>No change in body mass; Sum of 6 skinfolds: (-14%)</td>
<td>25.6%</td>
</tr>
<tr>
<td>McDougall et al 155</td>
<td>1998</td>
<td>Young healthy men</td>
<td>Cycle ergometer 3 x wk fo 7 wk</td>
<td>12</td>
<td>4-10 x 30 s 'all out' sprints with 4 min recovery</td>
<td>N/A</td>
<td>N/A</td>
<td>6.8%</td>
</tr>
<tr>
<td>Rodas et al 213</td>
<td>2000</td>
<td>Young healthy men</td>
<td>Cycle ergometer daily for 2 wk</td>
<td>5</td>
<td>7 x 15 s 'all out' with 45 s recovery and 7 x 'all out' with 12 min recovery</td>
<td>N/A</td>
<td>N/A</td>
<td>11.3%</td>
</tr>
<tr>
<td>Rognmo et al 215</td>
<td>2004</td>
<td>Overweight adults with CAD</td>
<td>Treadmill 3 x wk for 10 wk</td>
<td>17</td>
<td>4 x 4 min at 80-90% VO₂peak with 3 min recovery</td>
<td>N/A</td>
<td>No change in body mass</td>
<td>17.9%</td>
</tr>
<tr>
<td>Burgomaster et al 46</td>
<td>2005</td>
<td>Healthy individuals</td>
<td>Cycle ergometer 3 x wk for 2 wk</td>
<td>16</td>
<td>4-7 x 30 s 'all out' sprints with 4 min recovery</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Warburton et al 277</td>
<td>2005</td>
<td>Overweight men with CAD treated with angioplasty or bypass</td>
<td>Treadmill, stair climber and cycle ergometer 2 x wk for 16wk and continuous exercise at 65% HRres for 3 wk</td>
<td>14</td>
<td>8 x 2 min at 90% HRres/VO₂peak with 2 min recovery and continuous exercise 30 min</td>
<td>N/A</td>
<td>Body mass: (-3.4%)</td>
<td>31.8%</td>
</tr>
</tbody>
</table>

Abbreviations: BMI (body mass index); WC (waist circumference); WHR (waist:hip ratio); %BF (percentage of body fat); LBM (Lean body mass); AUC (area under the curve); wk (week); s (seconds); min (minutes) - The symbol (+) denotes increases and (-) decreases in any given parameter. VO₂max is reported as % improvement, with (0%) denoting no change reported within text without supporting figures.
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Sample Size</th>
<th>Intensity/Duration</th>
<th>Glucose Regulation</th>
<th>Anthropometric Measures</th>
<th>VO2max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burgomaster et al 44</td>
<td>2006</td>
<td>Young healthy men</td>
<td>Cycle ergometer 3 x wk for 2 wk</td>
<td>16</td>
<td>4-7 x 30 s 'all out' sprints with 4 min recovery</td>
<td>N/A</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>Talanian et al 244</td>
<td>2007</td>
<td>Healthy recreationally active women</td>
<td>Cycle ergometer 3 or 4 x wk for 2 wk</td>
<td>8</td>
<td>10 x 4 min at 90% VO2peak with 2 min recovery</td>
<td>N/A</td>
<td>N/A</td>
<td>13%</td>
</tr>
<tr>
<td>Wisloff et al 288</td>
<td>2007</td>
<td>Normal BMI older adults with post-infarction heart failure</td>
<td>Treadmill 2 x wk and home walking 1 x wk for 12 wk</td>
<td>27</td>
<td>4 x 4 min at 90-95% Hrpeak with 3 min recovery</td>
<td>No change in fasting glucose</td>
<td>No change in BMI</td>
<td>46%</td>
</tr>
<tr>
<td>Tjonna et al 253</td>
<td>2008</td>
<td>Overweight, middle aged adults with metabolic syndrome</td>
<td>3 x wk for 16 wk</td>
<td>28</td>
<td>4 x 4 min at 95% HRmax with 3 min recovery</td>
<td>Fasting glucose: -4.3%; Insulin sensitivity: +15%; No change in fasting glucose</td>
<td>Body weight: -3%; BMI: -2.3%; WC: -4.7%; No change in WHR</td>
<td>35%</td>
</tr>
<tr>
<td>Burgomaster et al 44</td>
<td>2008</td>
<td>Normal BMI, young adults</td>
<td>Cycle ergometer 3 x wk fo 6 wk</td>
<td>20</td>
<td>4-7 x 30 s 'all out' sprints with 4.5 min recovery</td>
<td>N/A</td>
<td>No change in body weight</td>
<td>7.3%</td>
</tr>
<tr>
<td>Rakobowchuck et al 206</td>
<td>2008</td>
<td>Young healthy men and women</td>
<td>Cycle ergometer 3 x wk fo 6 wk</td>
<td>20</td>
<td>4-7 x 30 s 'all out' sprints with 4 min recovery</td>
<td>N/A</td>
<td>N/A</td>
<td>7.3%</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Participant Characteristics</td>
<td>Study Design</td>
<td>Sample Size</td>
<td>Intensity/Duration</td>
<td>Glucose Regulation</td>
<td>Anthropometric Measures VO2max</td>
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<tr>
<td>Schjerve et al 224</td>
<td>2008</td>
<td>Obese middle aged adults</td>
<td>Treadmill 3 x wk (2 supervised and 1 home based) for 12 wk</td>
<td>27</td>
<td>4 x 4 min at 85-95% HRmax with 3 min recovery</td>
<td>No change in fasting glucose, insulin, C-peptide or HbA1c</td>
<td>Body mass: (-2%); %BF: (-2.2%); No change in WHR 33%</td>
<td></td>
</tr>
<tr>
<td>Moreira et al 183</td>
<td>2008</td>
<td>Overweight middle aged adults</td>
<td>Cycle ergometer 3 x wk for 12 wk</td>
<td>23</td>
<td>20 x 2 min at 20% above anaerobic threshold with 1 min recovery</td>
<td>Fasting glucose: (-16%)</td>
<td>BMI: (-1.4%); WC: (-0.8%); WHR: (-2.5%); BF%: (-0.6%) N/A</td>
<td></td>
</tr>
<tr>
<td>Trapp et al 259</td>
<td>2008</td>
<td>Inactive but healthy females</td>
<td>Cycle ergometer 3 x wk for 15 wk</td>
<td>45</td>
<td>20 min total - 8s 'all-out' against 0.5kg with 12 s recovery</td>
<td>Fasting insulin: (-31%); Insulin sensitivity (+33%)</td>
<td>Body mass: (-3.2%); Fat mass: (-11.3%); %BF: (-7.7%) 28.6%</td>
<td></td>
</tr>
<tr>
<td>Haram et al 108</td>
<td>2009</td>
<td>Male rats 3 months of age bread for metabolic syndrome</td>
<td>Treadmill 5 x wk for 8 wk</td>
<td>15</td>
<td>1h per day - 4 min at 85-90% VO2max with 3 min recovery at 70% VO2max</td>
<td>Fasting glucose: (-14.5%); 2h post glucose load:(-19.7%)</td>
<td>Body mass: (-6.3%); Retroperitoneal fat: (-64.2%) 45</td>
<td></td>
</tr>
<tr>
<td>Babraj et al 11</td>
<td>2009</td>
<td>Young men with normal BMI</td>
<td>Cycle ergometer 6 x wk for 2 wk</td>
<td>16</td>
<td>4-7 x 30 sec 'all out' sprints with 4 min recovery</td>
<td>Glucose AUC: (-12%); Insulin AUC: (-37%); Insulin sensitivity: (+23%); No change in fasting parameters</td>
<td>N/A N/A</td>
<td></td>
</tr>
<tr>
<td>Moholdt et al 182</td>
<td>2009</td>
<td>Overweight older adults, Post coronary artery bypass graft</td>
<td>Treadmill 5 x wk for 4 wk</td>
<td>59</td>
<td>4 x 4 min at 90% HRmax with 3 min recovery</td>
<td>No change in fasting glucose</td>
<td>No change in body mass 12.2%</td>
<td></td>
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<tr>
<td>Study</td>
<td>Year</td>
<td>Participant Characteristics</td>
<td>Study Design</td>
<td>Sample Size</td>
<td>Intensity/Duration</td>
<td>Glucose Regulation</td>
<td>Anthropometric Measures</td>
<td>VO2max</td>
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</tr>
<tr>
<td>Wallman et al 277</td>
<td>2009</td>
<td>Obese middle aged adults</td>
<td>Cycle ergometer 4 x wk for 8 wk and 1 h diet education seminar prior to training</td>
<td>14</td>
<td>10 x 1 min at 90% VO2peak with 2 min recovery at 30% VO2peak</td>
<td>N/A</td>
<td>No change in body mass; Upper body fat mass : (-8%)</td>
<td>24%</td>
</tr>
<tr>
<td>Munk et al 187</td>
<td>2009</td>
<td>Overweight middle aged adults with coronary artery stents</td>
<td>Cycle ergometer or running 3 x wk for 6 mnths</td>
<td>40</td>
<td>4 x 4 min at 80-90% HRmax with 3 min recovery</td>
<td>N/A</td>
<td>BMI: (-2.2%)</td>
<td>16.8%</td>
</tr>
<tr>
<td>Whyte et al 288</td>
<td>2010</td>
<td>Young overweight/obese men</td>
<td>Cycle ergometer 3 x wk for 2 wk</td>
<td>10</td>
<td>4-7 x 30 sec 'all out' sprints with 4 min recovery</td>
<td>24 h post-exercise fasting insulin: (25%); Insulin AUC: (-15%); Insulin sensitivity: (+23%); All changes lost at 72h</td>
<td>No change in body mass; WC: (-1.1%)</td>
<td>9.5%</td>
</tr>
<tr>
<td>Richards et al 211</td>
<td>2010</td>
<td>Young overweight adults</td>
<td>Cycle ergometer 3 x wk for 2 wk</td>
<td>31</td>
<td>4-7 x 30 sec 'all out' sprints with 4 min recovery</td>
<td>Insulin sensitivity: (+27%); No change in fasting glucose or insulin</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Nybo et al 190</td>
<td>2010</td>
<td>Young men, ~24.3% BF</td>
<td>Treadmill 3 x wk for 12 wk</td>
<td>28</td>
<td>5 x 2 min at 95% HRmax - recovery intervals not specified</td>
<td>Fasting glucose: (-9%); 2h post-load glucose: (-16.4%)</td>
<td>No change in body mass of %BF</td>
<td>14.2%</td>
</tr>
<tr>
<td>Hood et al 120</td>
<td>2011</td>
<td>Overweight middle aged adults</td>
<td>Cycle ergometer 3 x wk for 2 wk</td>
<td>7</td>
<td>10 x 60 s at 80-95% HR with 60 s recovery</td>
<td>Insulin sensitivity: (+35%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Participant Characteristics</td>
<td>Study Design</td>
<td>Sample Size</td>
<td>Intensity/Duration</td>
<td>Glucose Regulation</td>
<td>Anthropometric Measures</td>
<td>VO2max</td>
</tr>
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<tr>
<td>Little et al. 162</td>
<td>2011</td>
<td>Obese older type 2 diabetics</td>
<td>Cycle ergometer 3 x wk for 2 wk</td>
<td>8</td>
<td>10 x 60 s at 90% HRmax with 60 s recovery</td>
<td>Continuous 24 h blood glucose: (-13.2%); AUC 24 h glucose: (-13.5%); 3 h post-prandial glucose AUC: (-29.6%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Metcalfe et al. 181</td>
<td>2011</td>
<td>Sedentary young men and women</td>
<td>Cycle ergometer 3 x wk for 6 wk</td>
<td>29</td>
<td>2 x 20s 'all-out' with ~3 min recovery at 60 W</td>
<td>Insulin sensitivity: (+28%)</td>
<td>N/A</td>
<td>13.5%</td>
</tr>
<tr>
<td>Trilk et al. 265</td>
<td>2011</td>
<td>Overweight/obese women</td>
<td>Cycle ergometer 3 x wk for 4 wk</td>
<td>28</td>
<td>4-7 x 30 sec 'all out' sprints with 4 min recovery</td>
<td>N/A</td>
<td>No change in BMI; No change in %BF</td>
<td>12%</td>
</tr>
<tr>
<td>Macpherson et al. 168</td>
<td>2011</td>
<td>Bordeline overweight young adults</td>
<td>Treadmill 3 x wk for 6 wk</td>
<td>20</td>
<td>4-7 x 30 sec 'all out' sprints with 4 min recovery</td>
<td>N/A</td>
<td>Fat mass: (-12.4%); Lean mass: (+1%)</td>
<td>11.5%</td>
</tr>
<tr>
<td>Leggate et al. 157</td>
<td>2012</td>
<td>Overweight/obese men</td>
<td>Cycle ergometer 3 x wk for 2 wk</td>
<td>12</td>
<td>10 x 4 min at 90% HRmax with 2 min recovery</td>
<td>No change in fasting insulin or glucose; No change in insulin or glucose AUC; No change in insulin sensitivity</td>
<td>WC: (-1.4%)</td>
<td>8.3%</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Participant Characteristics</td>
<td>Study Design</td>
<td>Sample Size</td>
<td>Intensity/Duration</td>
<td>Glucose Regulation</td>
<td>Anthropometric Measures</td>
<td>VO2max</td>
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<tr>
<td>Heydari et al. 115</td>
<td>2012</td>
<td>Overweight inactive men</td>
<td>Cycle ergometer 3 x wk for 12 wk</td>
<td>46</td>
<td>20 min total - 8 s at 90% HRmax with 12 s rest</td>
<td>N/A</td>
<td>Body mass: (-1.7%); BMI: (-1.8%); WC: (-3.8%); %FM: (-6.7%); LBM: (+2.2%)</td>
<td>15.2%</td>
</tr>
<tr>
<td>Shepherd et al. 229</td>
<td>2013</td>
<td>Sedentary young men</td>
<td>Cycle ergometer 3 x wk for 6 wk</td>
<td>16</td>
<td>4-7 x 30 sec 'all out' sprints with 4 min recovery</td>
<td>Glucose AUC: (-17%); Insulin AUC: (-35%); Insulin sensitivity: (+49%)</td>
<td>No change in body mass mass; LBM: (+2.1%)</td>
<td>7.6%</td>
</tr>
<tr>
<td>Gillen et al. 98</td>
<td>2013</td>
<td>Overweight/obese women</td>
<td>Cycle ergometer 3 x wk for 6 wk</td>
<td>16</td>
<td>10 x 60 s at 90% HRmax with 60 s recovery</td>
<td>Glucose AUC: (-5.5%)</td>
<td>%BF: (-1.7%); Increased LBM: (1.3%)</td>
<td>17.6</td>
</tr>
<tr>
<td>Astorino and Schubert</td>
<td>2014</td>
<td>Active men and women and sedentary women</td>
<td>Cycle ergometer 1 x day for 6 days OR 3 x wk for 12 wk</td>
<td>40</td>
<td>4-7 x 30 sec 'all out' sprints, 4 min recovery or 10 x 60 s at 80-95% HRres with 60 s recovery</td>
<td>N/A</td>
<td>N/A</td>
<td>SIT: (6.3%); HIT: (25%)</td>
</tr>
<tr>
<td>Larson et al. 153</td>
<td>2014</td>
<td>Overweight but otherwise healthy adults</td>
<td>Cycle ergometer 3 x wk for 6 wk</td>
<td>10</td>
<td>5 x 60 s at Wmax with 90 s recovery at 25W</td>
<td>No change in fasting glucose; HbA1c: (-3%)</td>
<td>No change in body mass; LBM: (+35.9%)</td>
<td>6.80%</td>
</tr>
</tbody>
</table>