The metabolic responses of high intensity intermittent exercise in healthy untrained adults

Submitted by

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16th of August 2013

A dissertation submitted in total fulfilment of the requirements of the degree of Doctor of Philosophy

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SUMMARY

Sustaining a healthy body weight relies on maintaining energy balance. Energy balance describes the relative energy intake (dietary) to the energy expenditure (cellular energy and physical activity). In order to decrease adiposity, energy balance needs to be shifted negative, heightening energy expenditure so that it outweighs energy intake. The most malleable and cost effective method of energy balance manipulation is increasing physical activity. Diet and food intake can also be manipulated to reduce energy intake, however this can become quite costly and time consuming, particularly for those who are time poor and uneducated in healthy and low calorie foods.

High intensity interval training (HIIT) has been shown to increase fat loss more effectively than continuous moderate intensity training (Tremblay *et al.,* 1994; Trapp *et al.,* 2008; Macpherson *et al*., 2011; Gremeaux *et al.,* 2012), which is optimal for fat oxidation rates and was once considered the best exercise type employed to lose body fat. A recent study attributed this enigma to the rapid burst of intense exercise with the slight restorative effect of interspersed work and passive rest. Although the aforementioned studies were training studies, this study used single bouts of workload matched HIIE and moderate intensity, continuous exercise (CON) and found similar levels of fat utilisation between the two trials as well as significantly greater plasma and urine purine nucleotides post HIIE compared to CON, potentially resulting in greater energy loss.

This thesis aims to measure metabolic profiles for a range of HIIE models to understand the mechanism and determine which may be best at inducing an energy deficit that can lead to elevating energy expenditure, hence more rapid reductions in fat mass observed with HIIT.

Five studies were completed, assessing eight exercise models. Healthy untrained males were employed in four of the studies and healthy untrained females in the fifth. Respiratory gases, blood samples, urine samples and muscle samples were collected prior to exercise at rest, during exercise and post exercise in the following recovery period.

Study 1 investigated the metabolic profiles of two metabolically fatiguing exercise types (repeat sprint ability (RSA) and cycling capacity test (CCT)) in healthy untrained males. The same metabolic results were found despite less work performed in one of the exercise bouts, half of the energy cost of actual exercise; 22kJ for RSA compared to 52.3kJ for CCT. This study shows that when exercise is maximal effort exercise is performed; there is no difference in purine base excretion, and potential subsequent energy loss.

Study 2 employed healthy untrained males who completed three workload matched HIIE models, and showed no significant differences in metabolic profiles measured between the three trials. Hence, increasing exercise intensity and reducing exercise time and consequent increased duration of rest period, had no effect on indicators of substrate metabolism in plasma, urine or muscle. However, study 2 also contained a small pilot study where one participant's pre exercise and post exercise urine sample was analysed for urinary lactate concentrations. Results from this individual showed greater concentrations of lactate post exercise compared to pre-exercise, with a trend for progressively higher levels as exercise intensity was increased.

This lead to study 3 where four exercise types were analysed for urinary lactate pre and post exercise. Two of the models were workload matched (HIIE and CON) whilst the other two were metabolically stressing protocols employed in study 1 (RSA and CCT). It was discovered that the greater the exercise intensity, the greater the urinary lactate excretion.

This has significant metabolic considerations' for energy balance as this energy loss has never before been considered a part of the energy balance equation.

Healthy untrained males were recruited to be a part of study 4 which blended aspects of two exercise models already employed in this thesis (HIIE and RSA) and maximal effort all out HIIE was investigated. Two 10 min protocols were utilised, employing maximal effort cycling for 8 s followed by 12s rest (8:12) compared to 24 s of maximal effort cycling followed by 36 s rest (24:36). This study showed that the 24:36 HIIE protocol induced significantly greater, but previously unaccounted for energy loss via heightened urinary lactate and purine excretion compared to the 8:12 trial. Furthermore, plasma markers indicate that the 24:36 protocol may have had a direct influence on fat metabolism thus utilising greater amounts of fat to fuel the HIIE bout. Hence this may be an optimal exercise protocol for males looking to maintain a healthy body weight or aiming at reducing adiposity when repeated as such in HIIT. This study also employed healthy females performing the same HIIE models to investigate if the 24:36 protocol is the best option for females and if metabolic benefits can be demonstrated across the genders. Similar metabolic profiles were observed between the two HIIE trials as well as no discernible differences between males and females, except for that males induced significantly greater urinary lactate and purine excretion after the 24:36 HIIE trial compared to the 8:12 trial.

This thesis provided novel information regarding energy loss associated with high intensity exercise, in particular HIIE, via excretion of lactate, a metabolite that may contribute to energy balance but has never before been accounted for the energy balance equation following intense exercise. Furthermore this thesis investigated the optimal HIIE model for untrained males at inducing energy loss and fat utilisation, finding that of all HIIE

protocols tested, maximal cycling for 24 s followed by 36 s rest is the superior mode at influencing measures of metabolic loss. Lastly, insight into which HIIE may be best for females was considered, with additional research required.

The results of this thesis may have significant implications for the healthy and disease populations. Energy loss exacerbates energy expenditure of exercise, inducing a negative energy balance. These small shifts in the energy balance equation (energy in = energy expenditure) will build up over time with HIIT and significantly alter adiposity. Henceforth the optimal HIIE protocol from this thesis may be incorporated into HIIT regimes aimed at body weight maintenance for those in a time poor society, or those wishing to reduce adiposity.

Moreover, results have implications for those suffering from cardiometabolic disease where increased body fat is a prevalent risk factor. Therefore it is imperative at obtaining the best exercise protocol aimed at reducing adiposity so that it can incorporated into the prescribed exercise regime to reduce fat mass rapidly.

DECLARATION

I, Tracey Gerber, declare that the PhD thesis entitled:

The metabolic responses of high intensity intermittent exercise in healthy untrained adults

is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".

Full name: Tracey Gerber

Signature:

Date:

ACKNOWLEDGEMENTS

Completion of this PhD and thesis would not have been possible if not for the support and guidance of many people.

Firstly I would like to acknowledge and thank my supervisors, Associate Professor Alan Hayes and Dr Christos Stathis. To Alan, thank you for taking me on as an honours student and helping me receive the Vice Chancellors Scholarship to complete my PhD and the Vice Chancellor's University medal. Thank you also for your advice and input in the final thesis, I never would have completed it on time without your help. To Chris, thank you for all your assistance on every early morning trial day (there were a lot of them) and helping me in the laboratory, especially setting up the HPLC. I also wish to thank you for all of the opportunities you gave me in involving me with other areas of research and other research projects. I am eternally grateful and appreciative for all the help and encouragement you both have given me throughout the last four years.

To Bradley Gatt and Jessica Meilak, thank you for being the best laboratory managers in all of Victoria University (past and present). The guidance and help you both have given me (personally and professionally) over the last five years is invaluable and I will never forget it. You made the 4am wake ups for 6am trial days all the easier. I am grateful that as an 'outsider' you accepted me into your lab and made me feel like part of the PB team. To the PB PhD guys, thank you for accepting me as one of your own, but above all else, thank you for the crazy nights of PhD stress relief. They were definitely welcomed.

To my office girls, Nancy Capitanio, Jessica Danaher and Cara Timpani, thank you for being the amazing people that you are and making my PhD time enjoyable and very

entertaining. You girls kept me sane and brightened up every day and I thank you all for it. Sorry to Erik Hanson for all the shenanigans he had to put up with, but I'm sure he secretly loved it.

To the rest of the gals of level four of the western centre for health research and education, thank you for all the morning tea cake breaks and laughs. Please keep the traditions going once I'm gone.

To my best friend Sara Somerville, thank you for all the reassurance and support. You are more of a sister than a friend and I am grateful for all the time, advice and laughs we have shared. I'm sure there will be many more to come.

To my family, thank you for the patience and support throughout the last four years. In particular, to my mother Beverley and dad Wolf, thank you for all the love and encouragement you have given me, not just for the last five years but for the last 30 years. Thank you for all the sacrifices you made to give me everything I ever wanted and needed. To my dad, although you are not here to see me submit this thesis, I hope that you are proud that I've reached this goal I set out to complete four years ago. I wouldn't have been able to do it without all your help, especially the 2am food drop offs when I was working late in the office.

Lastly, this thesis would not have been possible without my participants. Thank you for volunteering your time to be a part of these studies. I cannot thank you enough!

LIST OF COMMUNICATIONS AND AWARDS

Presentations

Danaher J, **Gerber T**, Wellard R.M., Bishop D, Stathis CG., 'The effect of β-Alanine on buffering capacity and exercise performance during and following high intensity exercise in healthy males'. Invited Poster Presentation at European College of Sports Science (ECSS) 2012, Bruges Belgium

Gerber T, Hayes A, Stathis C.G., 'Potential for accelerated fat loss: The influence of exercise intensity on the metabolic response following workload matched high intensity intermittent exercise'. Poster Presentation at Exercise and Sports Science Australia (ESSA) 2012, Gold Coast, QLD, AUS

Danaher J, **Gerber T**, Wellard R.M., Bishop D, Stathis CG., 'The effect of β-Alanine on buffering capacity and exercise performance during and following high intensity exercise in healthy males'. Poster Presentation at Exercise and Sports Science Australia (ESSA) 2012, Gold Coast, QLD, AUS

Gerber T, Hayes A, Stathis C.G., The metabolic responses of work load matched high intensity intermittent exercise'. Poster Presentation at American College of Sports Medicine (ACSM) Annual Meeting 2012, San Francisco, CA, USA

Hanson E.D**.**, **Gerber T**, Anderson M, Bishop D.J, Stathis, C.G., 'Effects of Heat Acclimation, With or Without Simulated Hypoxic Living, on Exercise Performance'. Poster presentation at the ACSM Annual Meeting 2012, San Francisco, CA, USA

Gerber T, Hayes A, Stathis C.G., 'The metabolic responses to high intensity intermittent exercise'. Oral Presentation Victoria University Postgraduate Research Conference 2012, Melbourne, Vic, AUS

Gerber T, Hayes A, Stathis C.G., 'The effect of caffeine ingestion on performance and substrate metabolism during high-intensity intermittent exercise in healthy adults'. Poster Presentation at Exercise and Sports Science Australia (ESSA) 2010 held in Gold Coast, QLD, AUS 2010

Gerber T, Hayes A, Stathis C.G., 'Caffeine ingestion and high intensity intermittent exercise increases post exercise fat mobilisation and glycogenolysis in healthy individuals'. Poster Presentation at Australian Physiological Society (AuPS) 2010, Adelaide, SA, AUS

Gerber T, Hayes A, Stathis C.G., 'The effect of caffeine ingestion on performance and substrate metabolism during high-intensity intermittent exercise in healthy adults'. Poster Presentation at Victoria University postgraduate research conference 2010, Melbourne, Vic, AUS

Awards

University Medal of Excellence (honours year) *2010*

Most outstanding student (honours) in School of Biomedical Science *2010*

Vice chancellors Scholarship to undertake postdoctoral studies (PhD) *2009*

Most outstanding final year student, Bachelor of Science (Biomedical Science) *2006*

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ABBREVIATIONS

8:12	8s maximal effort cycling, followed by 12s rest
24:36	24s maximal effort cycling, followed by 36s rest
°C	degrees Celsius
µl	Microliters
µM	Micromole
150%HIIE	20s cycling at 150% VO_{2peak}, followed by 40s rest
200%HIIE	15s cycling at 200% VO_{2peak}, followed by 45s rest
300%HIIE	10s cycling at 300% VO_{2peak}, followed by 50s rest
ACC	Acetyl CoA Carboxylase
ACOD	Acyl- CoA Oxidase
ACS	Acyl-CoA Synthetase
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	5' adenosine monophosphate-activated protein kinase
ATP	Adenosine triphosphate
Bpm	Beats per minute
Ca^{2+}	Calcium
CCT	Cycling capacity test
CHO	Carbohydrate
Cm	Centimetre
CO_2	Carbon dioxide
CoA	Co enzyme A

CON	Continuous exercise
CONT	Continuous exercise training
CP	Creatine phosphate
CPT1	Carnitine palmitoyltransferase one
CPT11	Carnitine palmitoyltransferase two
Cr	Creatine
DEXA	Dual-energy X-ray absorptiometry
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunoassay
EMCL	Extramyocellular
EPOC	Excess post exercise oxygen consumption
ETC	Electron transport chain
F-6-P	Fructose - 6 – phosphate
FABPpm	Fatty acid binding protein
FAD	Flavin adenine dinucleotide
FADH	Flavin adenine dinucleotide dehydrogenase
FAT/CD36	Fatty acid translocase
Fatmax	Fat oxidation maximal exercise zone
FATP1	Fat protein 1
FFA	Free fatty acid
G-6-P	Glucose - 6- phosphate

G-6-P-DH	Glucose - 6- phosphate dehydrogenase
H^+	Hydrogen
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
Hex	Hexokinase
HI	High intensity
HIIE	High intensity intermittent exercise
HIIT	High intensity intermittent training
HPLC	High performance liquid chromatography
HSL	Hormone sensitive lipase
Hx	Hypoxanthine
IMCL	Intramyocellular
IMP	Inosine monophosphate
IMTG	Intramuscular triglycerides
In	Inosine
KCl	Potassium chloride
Kg	Kilograms
$KHCO_3^-$	Potassium bicarbonate
L	Litres
LCFA	Long chain fatty acid
LDH	Lactate dehydrogenase
MCFA	Medium chain fatty acid
MEHA	3-methyl-N-ethyl-B-hydroxyethyl-aniline

Mg	Milligram
MgCl	Magnesium chloride
Min	Minute
MJ	Megajoule
$ml.kg.min^{-1}$	Millilitres per kilogram per minute
mRNA	Messenger RNA
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide dehydrogenase
NA_OH	Sodium hydroxide
NEFA	Non esterified fatty acid
nm	Nanometres
NRF1	Nuclear respiratory factor one
NRF2	Nuclear respiratory factor two
O_2	Oxygen
PCA	Perchloric acid
PCr	Phosphocreatine
PDC	Pyruvate dehydrogenase complex
PDH	Pyruvate dehydrogenase
PDHa	Pyruvate dehydrogenase activity
PDHk	Pyruvate dehydrogenase total
PEP	Phosphoenolpyruvic acid
PFK	Phosphofructokinase
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator

	1 alpha
pH	Power of hydrogen
Phos a	Phosphorylase a
Phos b	Phosphorylase b
Pi	Phosphate
PI3-K	Phosphoinisitide 3-kinase
PK	Pyruvate kinase
PPi	Pyrophosphoric acid
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
RPM	Revolutions per minute
RSA	Repeat sprint ability
S	Seconds
SD	Standard deviation
SEM	Standard error of the mean
TCA	Tricarboxylic cycle
TFA	Trifluoracetic acid
TMB	3,3'5,5' tetramethylbenzidine
Ua	Uric acid
UV	Ultra violet
v/v	Volume per volume
VCO_2	Carbon dioxide production
VO_2	Oxygen consumption

VO_{2max}	Maximal oxygen consumption
VO_{2peak}	eak oxygen consumption
W	Watts
Xan	Xanthine
Yrs	Years

Chapter one: Perspectives

This thesis provides insight into metabolic and substrate utilisation profiles for a range of high intensity intermittent exercise (HIIE) models performed by healthy untrained males. It investigates the optimal HIIE protocol that may potentially enhance fat metabolism and induce an energy deficit. The ideal method found for the healthy, untrained male gender is also compared to healthy, untrained females to see if beneficial metabolic changes observed in males are transferrable across the genders.

1.1 Thesis scope

In this obesogenic environment, maintaining physical health and body weight is a primary concern for many individuals. Diet pills, weight loss shakes and fad diets are commonly promoted and employed to maintain healthy weight. However the most economical and flexible method of controlling body fat or adiposity is altering exercise and the amount of physical activity.

Skeletal muscle comprises approximately 40% of an individual's body weight (normal healthy range) and is the major site of energy production and expenditure (Gropper *et al.,* 2009). Hence it is advantageous to manipulate exercise protocols which have a significant impact on enhancing skeletal muscle metabolism.

Constant, moderate intensity exercise elicits optimal fat oxidation rates (Romijn *et al.,* 1993), exercising (walking/jogging) at 60-65% of VO_{2max}, and known as the 'fat_{max}' zone (Achten *et al.,* 2002). Recently it has been suggested that high intensity intermittent exercise (HIIE) leads to reductions in adiposity at a faster rate than submaximal continuous exercise (CON) (Tremblay *et al.,* 1994; Trapp *et al.,* 2008; Macpherson et al., 2011; Gremeaux et al., 2012). This seems counter-intuitive as HIIE is short, rapid bouts of high

intensity exercise, followed by a rest period, with this combination repeated for the duration of exercise protocol. In training studies comparing fat loss between HIIE and CON, HIIE has been shown to utilise fat sources despite high intensity exercise being a glycolytic process, utilising glycogen, resulting in lactate accumulation. This has been attributed to the work to rest nature of HIIE. Using single bouts of workload matched HIIE and CON, similar plasma FFA and glycerol concentrations were observed, indirect plasma markers reflective of fat oxidation. There are limitations of using plasma FFA and glycerol to describe fat oxidation, as plasma FFA can be re-esterified back into adipose tissue. This study also showed that HIIE induces greater ATP turnover and degradation compared to CON as measured by plasma purine accumulation and enhanced urinary purine loss (Borg *et al*, 2008), elucidating that HIIE creates metabolic disturbances that elevate subsequent energy loss with the requirement for *de novo* synthesis of ATP post exercise (Newsholme and Leech, 1983).

Much of the research pertaining to HIIE focuses on muscle adaptations associated with training (HIIT), specifically the mitochondrial enzyme protein changes, in particular the up regulation of oxidative enzyme activity and improvement in respiratory health and metabolic processes such insulin sensitivity. Without producing any succinct metabolic reasons, this collection of studies have depicted HIIT as beneficial for many population groups (Wisloff *et al.,* 2007; Tjonna *et al.,* 2009; Little *et al.,* 2011). However, without knowing metabolic mechanisms behind this exercise type, whether these beneficial changes can be further promoted or optimised remains unknown. Obvious metabolic disorders that may benefit from this exercise model are obesity and diabetes, due to proven decreases in fat mass (Tremblay *et al.,* 1994; Trapp *et al.,* 2008; Macpherson *et al.,* 2011; Gremeaux *et*

al., 2012) and improvements in insulin sensitivity with HIIT (Little *et al.,* 2011; Gillen *et al.,* 2012). In addition to metabolic illness, healthy individuals looking to maintain healthy weight or those whom have lifestyles that are too 'time poor' to partake in longer, less intense physical exercise, may also benefit from an exercise type that will yield physical changes in a timely manner, encouraging the continuation of exercise (Gibala 2007; Gibala and Little 2010). Therefore the main focus of this thesis explores possible metabolic explanations behind greater fat loss after HIIT compared to CON training, as well as optimising HIIE to further exacerbate these changes.

1.2 General aim of the thesis

The primary aim of this thesis was to determine metabolic profiles (urine, blood and muscle) of various HIIE models and to investigate the nature of HIIE (duration and intensity) that optimise the metabolic processes that elevate energy deficit for potential increase energy expenditure and influence energy balance and subsequent fat utilisation.

1.3 Significance

High intensity intermittent training (HIIT) accelerates decreases in adiposity and improves insulin sensitivity, resulting in HIIE becoming the preferred exercise model to induce favourable changes in those afflicted with weight challenges and diabetes.

The metabolic profile of HIIE, including respiratory, plasma, urinary and muscular markers, is yet to be comprehensively determined, therefore the significance of this project was to produce blood and urinary profiles of important metabolites that may help elucidate and understand the augmented fat loss associated with HIIE. The metabolic mechanisms

behind this exercise type must first be elucidated in the healthy, untrained population, thus this thesis primarily focuses on employing untrained males, and with one study also including females for comparisons across the sexes.

Results from this thesis may be applied to disease population cohorts. Numerous diseases already benefit from employing HIIT, but the improvements may be quicker, favourable and encouraging if the optimal and easiest model of HIIE is found for each specific disease state. HIIE has already been shown to improve insulin sensitivity, but there may be another protocol that can further enhance these improvements whilst decreasing fat mass at an accelerated rate, decreasing the progression of obesity and diabetes, ultimately preventing damage to the endocrine and metabolic systems. HIIE is also known to improve cardiorespiratory health in individuals suffering cardiovascular disease. It also has been shown to decrease blood lipid profile as well as decreasing blood pressure, which are two risk factors for coronary heart disease. Other risk factors associated with cardiovascular disease are obesity, type II diabetes and poor attitude to exercise, hence if each of these factors can be addressed in a single exercise model, individuals may be more likely to persevere with the exercise to obtain benefits.

Chapter two: Review of literature

This review of literature will encompass skeletal muscle metabolism through all exercise intensities, a focus being the substrate utilisation during high intensity exercise. This review will also detail the evidence regarding high intensity intermittent exercise and its action of decreasing adiposity at an accelerated rate compared to continuous exercise.

2.1 Energy balance

Physical activity and healthy diet can induce a difference in energy balance as much as 7 mega joules (MJ) per day compared to sedentary/mixed diet individual (Dionne *et al.,* 1997). An individual will accumulate fat mass when there is a positive energy balance shown by the following calculation:

energy IN_{take} > energy OUT_{put} = energy stored

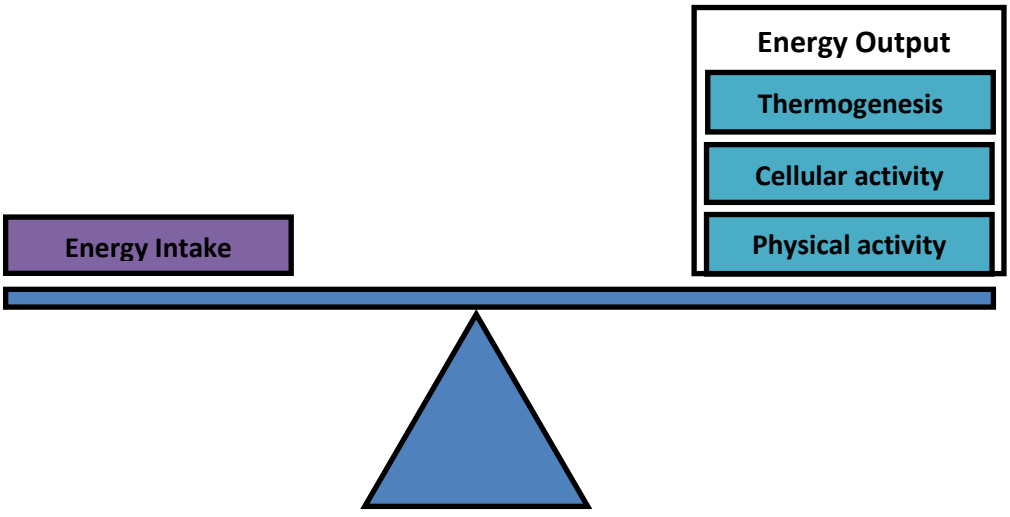

Figure 2.0: Energy balance; calories in refers to energy intake and calories out energy expenditure (Adapted from Seeley *et al.,* 2006)

Optimising or maximising physical activity to induce energy loss and fat oxidation may result in a greater loss than 7MJ per day. A 5% increase per day in energy intake can result in positive energy balance that can lead to a gain of 5 kilogram (kg) of fat mass over a one year period (Jequier, 2002). Hence while exercise clearly increases energy expenditure; it would be beneficial to manipulate substrate utilisation even further during exercise to maximise energy expenditure, if the focus was weight control and for decreased adiposity.

2.2 Substrate utilisation

Exercising skeletal muscle requires a mixture of substrates for utilisation for cellular respiration to produce adenosine triphosphate (ATP). Glucose and lipid are the primary substrate fuel sources of skeletal muscle ATP production. Up-regulating metabolism, such as with exercise, will result in the consumption of substrate and elevated energy expenditure (if diet is controlled) (LaForgia *et al.,* 2006).

2.2.1 Substrate sources

The majority of carbohydrates are digested to glucose and stored in liver and skeletal muscle as glycogen, as well circulating in the plasma (plasma glucose) for ATP production. Lactate, a metabolic by-product of high intensity exercise can also be taken up by the liver and recycled via the Cori Cycle to form glycogen (Long and White 1938; Katz and Tayek, 1998).

Sources of fat from our diet are stored in adipose tissue and in skeletal muscle as intramuscular triacylglycerol (IMTG) (Hawley *et al.,* 1998). Lipids undergo lipolysis (breakdown) to form one molecule of glycerol and three free fatty acids (FFA); the free glycerol is released into the blood its accumulation is a reflection of the rate of that lipolysis.

There are four types of FFA: small chain FFA, medium chain FFA (MCFA), long chain FFA (LCFA) and very long chain FFA. LCFA and MCFA have specific transport protein carriers which uptake fatty acids into muscle celss (Hawley *et al.,* 1998; Hulver *et al.,* 2003). However for the purpose of this thesis, all FFA's will collectively be grouped as FFA.

2.2.1.1 Transport of FFA into skeletal muscle

Movement of FFA across the sarcolemma begins with disassociation of FFA from albumin and movement to the outer layer of the sarcolemma (Holloway *et al.,* 2008). Three identified classes of proteins exist that take FFA across the sarcolemma; fatty acid binding protein (FABPpm), fatty acid translocase protein (FAT/CD36) and FATP1-6, as per figure 2.1 (Jeukendrup, 2002; Holloway *et al.,* 2006; Holloway *et al.,* 2007; Holloway *et al.,* 2008).

Muscle contraction increases the translocation of FAT/CD36 from the endosomal membrane to the sarcolemma and FFA is taken into the muscle (Holloway *et al.,* 2006; Bonen *et al.,* 2008; Holloway *et al.,* 2008). FABPpm also plays a role in uptake and is higher in slow twitch muscle fibres (Jeukendrup, 2002). FABPpm and FAT/CD36 are expressed in skeletal muscle according to rate of FFA oxidation therefore AMPK, insulin and phosphoinositide 3-kinase (PI3-K) (involved in the insulin signalling cascade), and the energy status of the muscle cell effect fat oxidation (Holloway *et al.,* 2008), more research is needed.

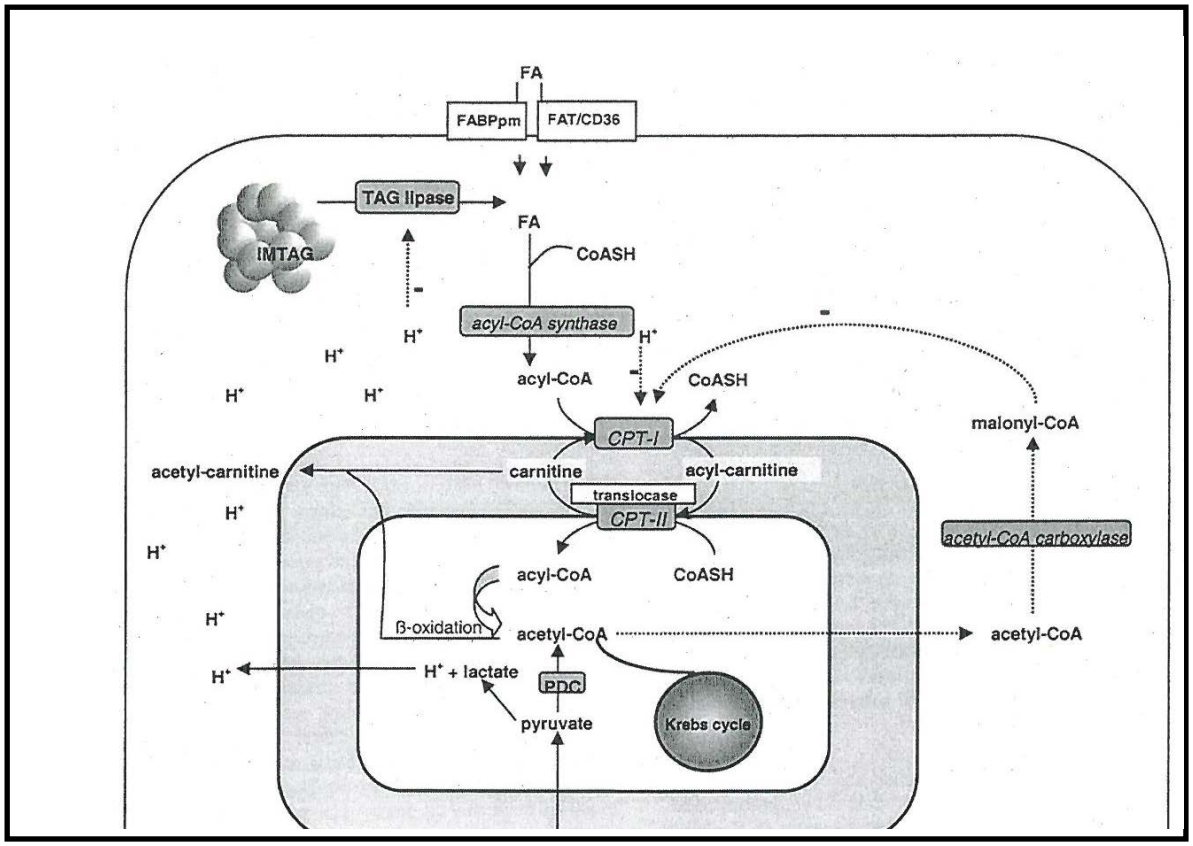

Figure 2.1: Entry of FFA into the skeletal muscle across the sarcolemma and entry into the mitochondria where acetyl CoA utilisation occurs (Adapted from Achten and Jeukendrup, 2004)

2.2.1.2 Pathway of FFA into the mitochondria for oxidation

The path of FFA (long chain FFA) entry into the mitochondria is facilitated by carnitine palmitoyltransferase one (CPT1), located on the outside of the outer mitochondrial membrane (Jeukendrup, 2002; Holloway *et al.,* 2006) (see figure 2.1). FFA binding with carnitine is the initial step in FFA movement into the mitochondria; as carnitine binds to acyl-CoA, free CoA is released and the acyl-carnitine complex is transported to the inner mitochondrial membrane by carnitine palmitoyltransferase (CPTII). Any carnitine that diffuses back across the mitochondrial membrane is now available for transport of newly arrived FFA across the membranes (Jeukendrup, 2002).

FFA, IMTG and the CHO sources muscle glycogen and plasma glucose provide energy to the body via three different energy systems during exercise. The systems are the ATP-Creatine Phosphate (CP) system (which does not utilise glycogen, FFA and glucose), anaerobic glycolysis and the aerobic system (Gastin, 2001; Gropper *et al.,* 2009), each dominant during different types of exercise.

2.3 Energy systems

When there is a low oxygen demand such as at rest, the body is fuelled aerobically with a mixture of fat and CHO as the fuel sources funding energy production. During the first few seconds of maximal effort exercise, energy requirements are met by existing ATP stores, although depleted quickly (~10 s). The diminished ATP stores are replenished rapidly by the transfer of phosphate from CP to form ATP, CP stores in muscle are approximately 4-5 times greater than that of ATP, thus able to provide energy for approximately 10-25 seconds (s) of high intensity exercise (Gastin, 2001; Gropper *et al.,* 2009). However after a single 30 s bout of maximal exercise, glycolysis plays a significant role in ATP production with approximately 25-30% of ATP synthesised coming from phosphocreatine (PCr) breakdown, 60-65% from glycolysis and a very small contribution from the aerobic system (Bogdanis *et al*., 1996; Cheetham *et al.,* 1986; McCartney *et al.,* 1986; Nevill *et al.,* 1989; Medbo and Tabata 1989; Bogdanis *et al.,* 1995). While the aerobic contribution occurs in the latter stages, the studies in this thesis utilised short duration exercise bouts < 24 s, thus primarily aerobic and as such is a highlight of this thesis.

The anaerobic pathway/energy system produces ATP in the cytosol of the muscle by the incomplete breakdown of glucose into lactate. Muscle glycogen is the primary source, with plasma glucose contributing only a small amount 5-10% (Holloszy *et al.,* 1998). At high

exercise intensities, an inadequate supply and/or the rate of utilisation of O_2 prevents aerobic phosphorylation meeting the ATP demands of exercise, thus anaerobic glycolysis to supplies ATP, with lactate as the main by product (Gastin 2001; Gropper *et al.,* 2009). When the oxygen supply is sufficient, substrate will be metabolised and energy produced aerobically. The aerobic system consists of β-oxidation, the Kreb's Cycle and the Electron Transport Chain and relies on the supply of oxygen for the complete utilisation of substrate (CHO and FFA) in the mitochondria (see figure 2.2).

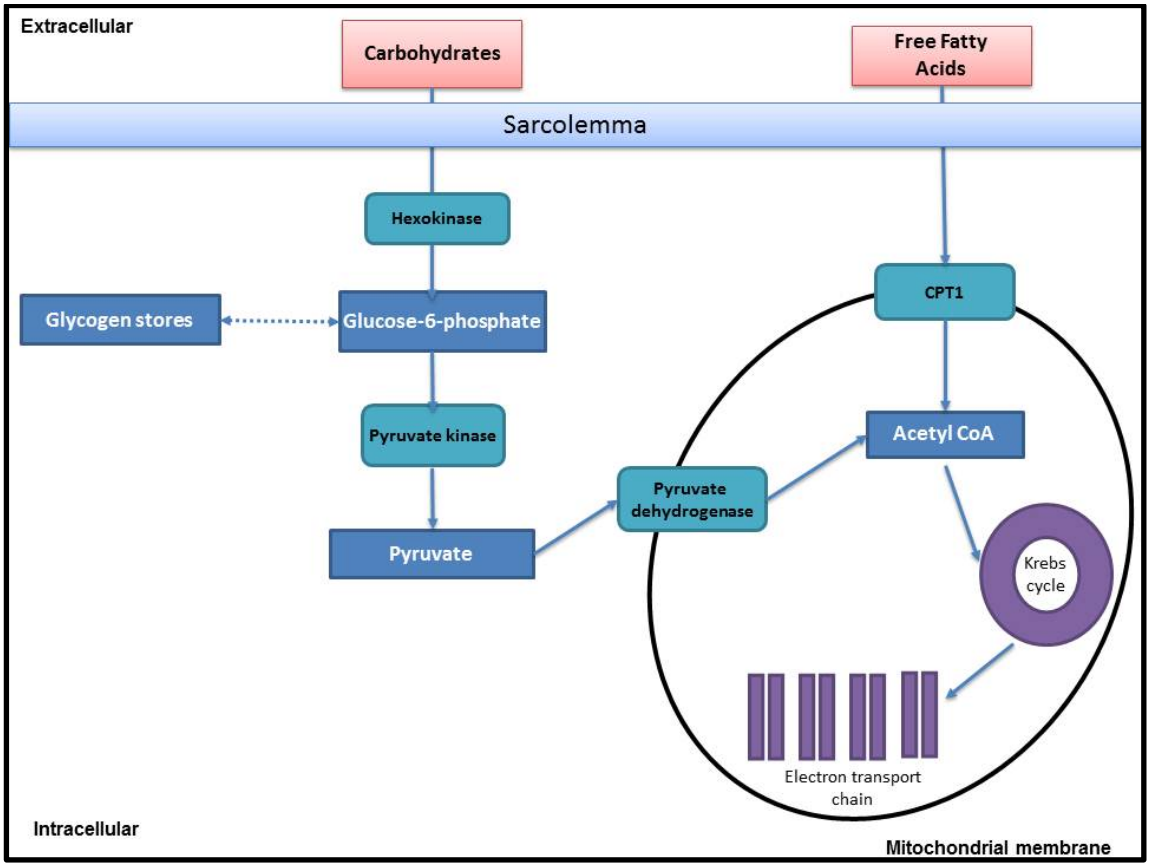

Figure 2.2: Highly simplified diagram Aerobic metabolism, showing substrate pathways for glycogen, CHO and FFA; Carnitine Palmitoyltransferase 1 = CPT1 (modified from Gropper, 2009)

2.3.1 The mitochondria: Powerhouse of the skeletal muscle

Mitochondria are contained in abundance in skeletal muscle and are the main site of energy production. Following endurance exercise, the size and mass of mitochondria can increase, potentially leading to increases in fat and CHO oxidation (Little *et al.,* 2010) due to increases in Peroxisome Proliferator-activated Receptor Gamma Co-activator (PGC1α), a regulator of mitochondrial biogenesis (Wu *et al.,* 1999; Little *et al.,* 2010).

Mitochondria have two membranes, an outer and inner, the inner mitochondrial membrane containing the cristae (see figure 2.2) (Nisoli *et al.,* 2004) and the enzymes of the Electron Transport Chain (see figure 2.3). The mitochondria ultimately utilises CHO and fat to produce ATP via the Krebs Cyle and the Electron Transport Chain.

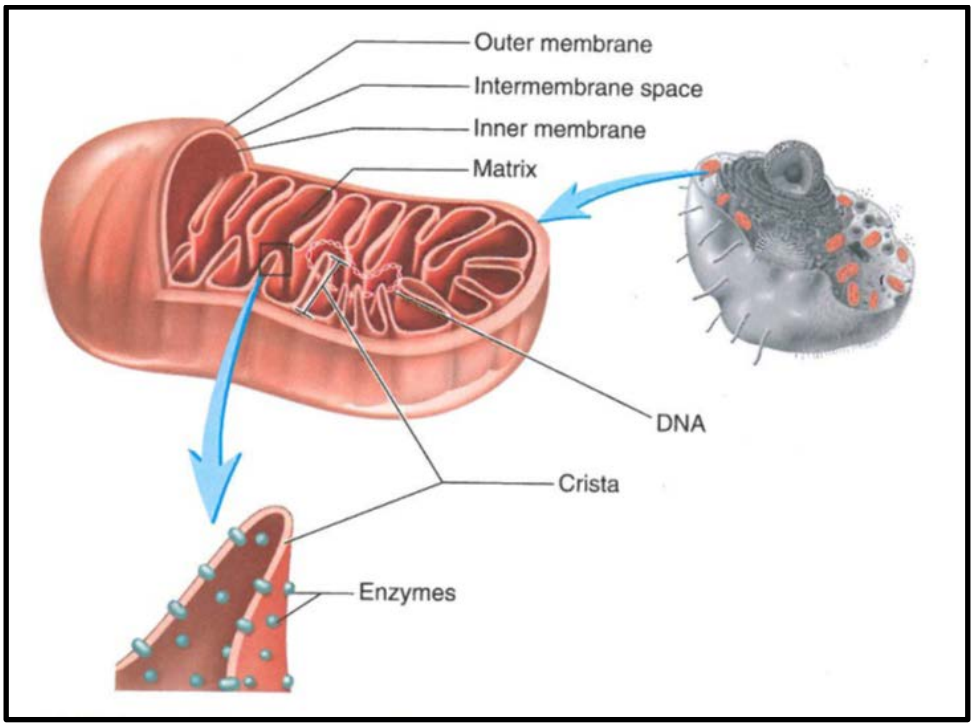

Figure 2.3: Microscopic and simplified diagram of mitochondria demonstrating the membranes and intermembrane space and matrix (Adapted from Seeley *et al.,* 2006).

The Krebs Cycle (Tricarboxylic Acid Cycle or Citric Acid Cycle), is located in the matrix of the mitochondria and is a series of biochemical reactions where substrates are

catabolised to oxidise acetate. Acetate is converted into acetyl CoA, and is expended to produce ATP. This is the starting point of the Krebs Cycle, with the transfer of a two-carbon acetyl group from acetyl CoA to the four carbon acceptor oxaloacetate to form a six carbon compound citrate (see figure 2.4). One complete cycle reduces NAD to NADH and FAD to FADH and produces carbon dioxide (CO_2). The NADH/FADH is shuttled into oxidative phosphorylation (electron transport chain) with the result being ATP.

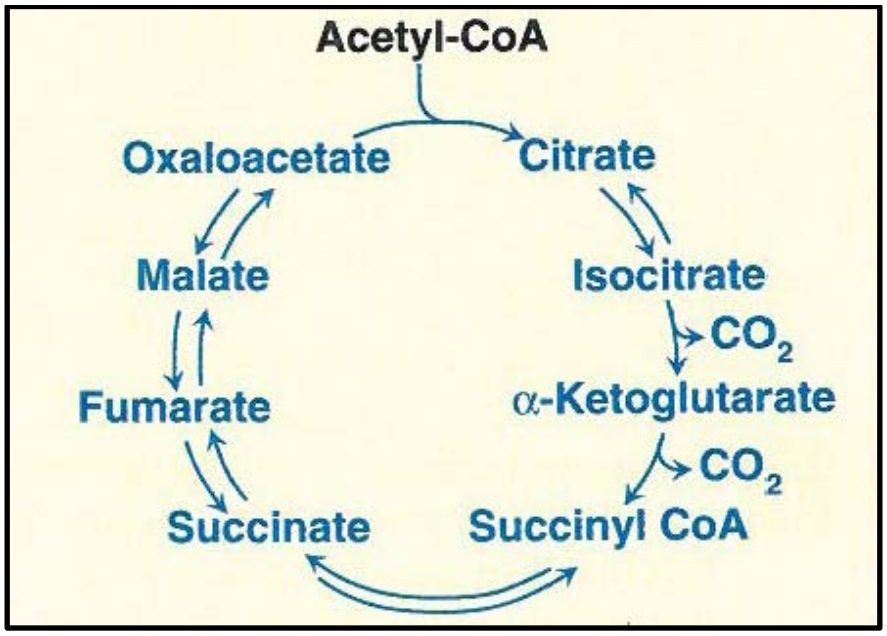

Figure 2.4: Schematic diagram of the enzymes involved in ATP production in the Krebs Cycle (taken from Champe *et al.,* 2005)

The electron transport chain is located on the mitochondrial inner membrane and is comprised of a series of protein complexes that act as electron donors and acceptors. The ETC consists of four complexes, complex I-IV and ATP synthase which translocates hydrogen protons (H^+) to produce ATP (Nisoli *et al.,* 2004), see figure 2.5 below.

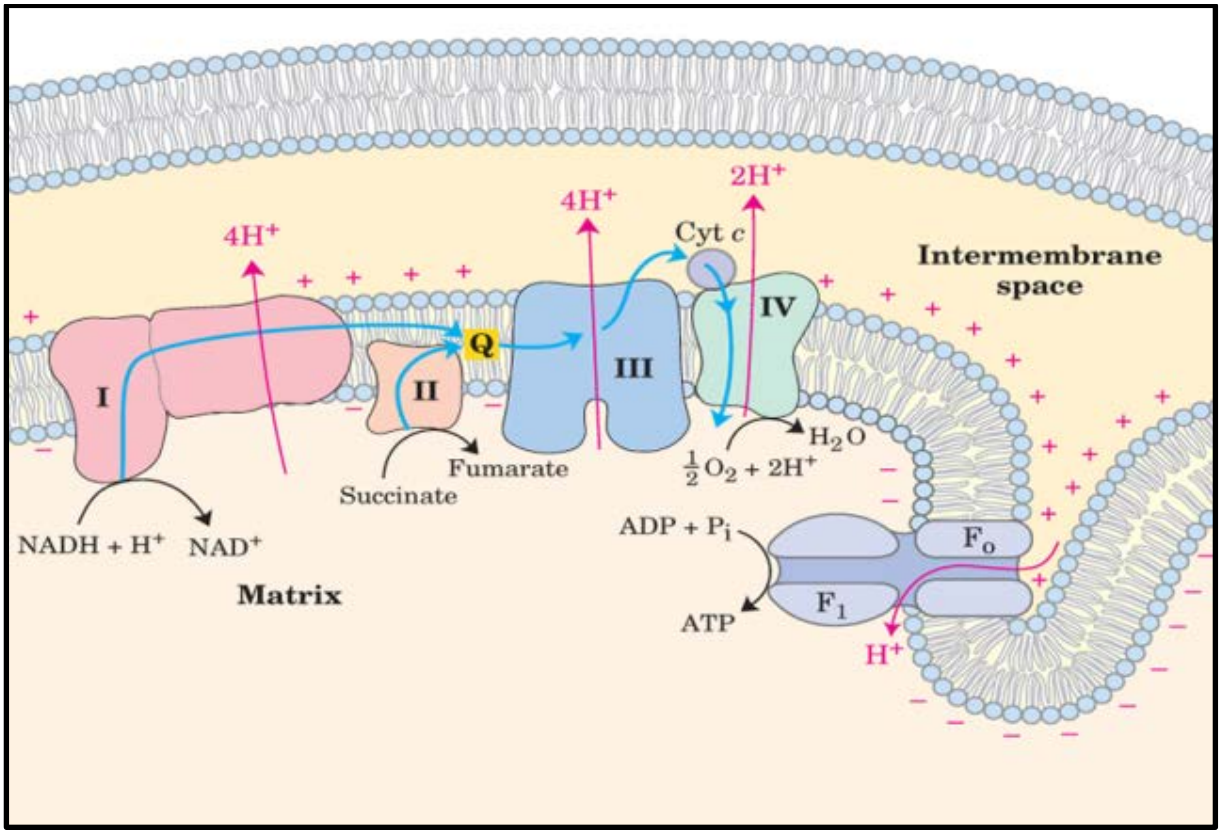

Figure 2.5: Movement of H^{+} via complexes I-IV and ATP synthase of the Electron Transport Chain to produce ATP (taken from Seeley *et al.,* 2006)

Every molecule of NADH/FADH taken up from the Krebs Cycle is a donor and passes electrons to a more electronegative acceptor such as oxygen. This transfers H^{+} across the inner-membrane of the mitochondria into the membrane space and this movement of electrons and protons creates an electrochemical proton gradient. This process continues down the chain until all electrons are passed into oxygen, oxygen being the most electronegative and last electron acceptor in the Electron Transport Chain. The movement of electrons releases energy which is used to induce a gradient across the mitochondrial membrane by actively pumping protons via three pumps (complex I, III and IV), into the inner membrane space and are used to make ATP via ATP synthase. The complete process is

called oxidative phosphorylation as adenosine diphosphate phosphate (ADP) is phosphorylated to ATP by utilising the energy provided by hydrogen oxidation.

2.4 Classifying exercise intensity and substrate utilisation

As exercise intensity increases, there are shifts in the energy system supplying ATP, consequently altering the contribution of fat and CHO to energy production. Exercise intensity is classified in regards to percentage of VO_{2max} and refers to maximal oxygen consumption, subsequently the relative exercise intensity expressed as percentage oxygen consumption (Holloszy *et al.,* 1998).

During exercise at <25% VO_{2max} (low intensity) energy supply comes primarily from lipolysis of adipose tissue with FFA. As exercise intensity increases from 25% VO_{2max} to 85% VO_{2max} (moderate exercise intensity), sources of fat include FFA and IMTG, however the proportion of fat contribution to ATP production declines and CHO oxidation increases. During high exercise intensities, >85% VO_{2max}, the contribution of fat sources decreases and there is increased CHO oxidation (Dudley *et al.,* 1982), with plasma glucose contributing only 10-15% to energy supply and glycogen the major supplier to ATP production (Holloszy *et al.,* 1998).

2.5 Substrate utilisation during low exercise intensities <25% VO_{2max}

FFA is the primary substrate utilised in the mitochondria at rest and during low exercise intensities to produce ATP (Frayn, 2006). There is an abundance of mitochondria contained in skeletal muscle, the rate of fat oxidation is regulated by FFA availability, transport across the sarcolemma and mitochondrial membrane and rate of reesterification (Romijn *et al.,* 1993; Jensen, 2003). At rest, adipose tissue is in a constant state of lipolysis

(breakdown of adipose tissue), and reesterification (the taking up of FFA) and this balance is influenced by the metabolic status of the muscle cell. Approximately 70% of reesterification of FFA occurs at rest (Borsheim and Bahr 2003), this percentage dropping to roughly 25% with the rate of lipolysis tripling during low intensity exercise, significantly increasing FFA availability for oxidation (Wolfe *et al.,* 1990; van Hall *et al.,* 2002). There is no change in contribution of fat oxidation to total energy expenditure from resting to low intensity exercise, this remaining the same until exercise intensity reaches the point of maximal fat oxidation atmoderate exercise intensities, approximately 65% VO_{2max} (van Loon *et al.,* 2001).

During low to moderate intensity exercise, lipolysis increases three-fold due to an increase in β-adrenergic stimulation via increased epinephrine concentrations. In addition, blood flow is increased and re-esterification rates halved, promoting FFA delivery to the exercising skeletal muscles (Jeukendrup 2002). A study employing graded epinephrine infusion observed progressively increased lipolysis (rate of appearance of glycerol) and release of FFA into the bloodstream during low intensity exercise.

2.6 Substrate utilisation during moderate exercise intensities 25-85% VO_{2max}

During moderate exercise, fat and CHO are utilised similarly, however in the early stage of exercise, there is a substrate shift and CHO becomes the predominant fuel source as it is used at a faster rate than FFA. However as exercise progresses, there is an additional metabolic shift from the depleted CHO source to the more abundant fat stores (Watt *et al.,* 2004). This requires an organised relationship between the liver, adipose tissue, pancreas and skeletal muscle to supply energy during prolonged submaximal exercise (Mourtzakis *et al.,* 2006). The liver stores glycogen and takes up lactate whereas the pancreas releases

hormones (insulin and glucagon) involved in glucose regulation. Nonetheless at the beginning of prolonged submaximal exercise, blood glucose and muscle glycogen are the primary fuel sources, muscle glycogen being more essential than blood glucose, until muscle and liver glycogen stores become depleted (Holloszy *et al.,* 1998).

The breakdown of glycogen stored within the muscle occurs in the cytosol and is controlled by phosphorylase. Glucose and glycogen are converted to Glucose-6-Phosphate (G-6-P) via a series of enzymatic reactions and ultimately pyruvate kinase produces pyruvate. Via pyruvate dehydrogenase (PDH), pyruvate is decarboxylated to generate acetyl CoA in the mitochondria and generate ATP.

2.6.1 The pathway of pyruvate into the mitochondria

PDH is found in the mitochondrial matrix and aids in shuttling pyruvate into the matrix where it is converted to acetyl CoA by the pyruvate dehydrogenase complex (PDC).At exercise intensities greater than 85% VO_{2max}, the mitochondria's' role in ATP production is reduced and the majority of pyruvate is converted to lactate.

2.7 Substrate utilisation during high Intensity exercise >85% VO_{2max}

High intensity exercise utilises the ATP-CP system and anaerobic glycolysis to produce ATP, meeting the high energy cost of this exercise type (Randle 1969; Brouns and van der Vusse, 1998; Gastin 2001). Glycogen is the primary fuel source, contributing to 2/3 of energy production, with the other 1/3 from fat sources (Brooks and Mercier, 1994; Coyle *et al.,* 1997). During a 30 s single bout of maximal exercise, 25-30% of ATP synthesised comes from phosphocreatine (PCr) breakdown while the remaining 65-70% of ATP production comes from glycogen entering anaerobic glycolysis (Bogdanis *et al.,* 1996;

Brouns and van der Vusse, 1998; Dawson *et al.,* 1998). PCr replenishment is an aerobic process and takes approximately 3 mins for complete recovery from cessation of exercise (Edwards *et al.,* 1973; McCartney *et al.,* 1986; Chasiotis *et al.,* 1987; Bogdanis *et al.,* 1995; Bogdanis *et al.,* 1996; Dawson *et al.,* 1997; Wadley and Rossignol, 1998; Tomlin *et al.,* 2001; Tomlin *et al.,* 2002; Dorado *et al.,* 2004; Dupont *et al.,* 2004; Thevenent *et al.,* 2007; Forbes *et al.,* 2008). Interestingly one study observed that after 6 mins of recovery, there was only 85% PCr replenishment complete. This may be due to PCr content falling even lower in fast twitch muscle fibres due to poor capillary density, fewer mitochondria number and increased H^+ ion concentration (Bogdanis *et al.,* 1995), limiting the oxidative processes required for replenishment. Nonetheless, once ATP synthesis from PCr has diminished, glycogen is the dominant fuel source used for ATP generation. As muscle glycogen content declines (Broberg and Sahlin 1989; Bogdanis *et al.,* 1995; Bogdanis *et al.,* 1996; Bogdanis *et al.,* 1998; Balsom *et al.,* 1999), muscle pyruvate and lactate concentrations increase (Broberg and Sahlin 1989; Medbo and Tabata 1989; Bogdanis *et al.,* 1995; Bogdanis *et al.,* 1996; Bogdanis *et al.,* 1998; Balsom *et al.,* 1999) as metabolic by-products of anaerobic glycolysis (see figure 2.7).

2.7.1 High intensity exercise stimulates anaerobic glycolysis

At high exercise intensities, oxygen cannot be extracted and utilised fast enough hence ATP is produced anaerobically. In the cytosol, glycogen is degraded to glucose 1 phospahte (G-1-P) which is then converted to G-6-P (see figure 2.6). Phosphofructokinase (PFK), the rate limiting enzyme of glycolysis converts downstream G-6-P to F1-6-P and ultimately the enzyme pyruvate kinase will make pyruvate. Pyruvate can be utilised aerobically discussed in section 2.6.1 or anaerobically where it is broken down further to

lactate which subsequently effluxes the muscle (see figure 2.6). The rapid hydrolysis of ATP and PCr during high intensity exercise results in the build-up of free phosphate (Pi) and AMP, increasing the activity of glycolysis. Henceforth an increased glycolytic rate elevates pyruvate production, wielding an allosteric effect on PDHa (Parolin *et al.,* 1999).

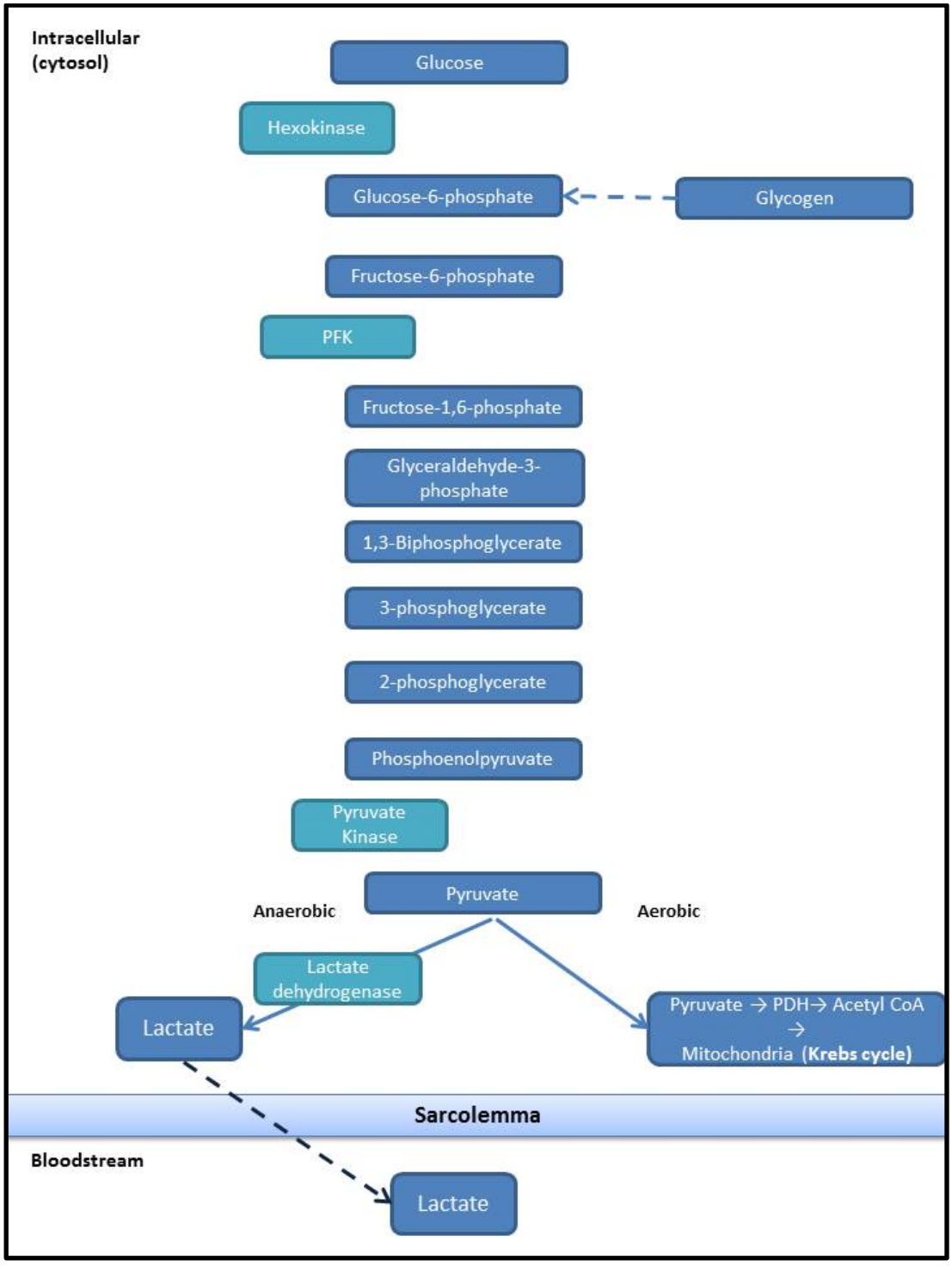

Figure 2.6: Simplified pathway of glycolysis, displaying the aerobic and anaerobic fate of pyruvate (modified from Gropper, 2009)**. Anaerobically, pyruvate is converted to lactate and aerobically it shuttled to the mitochondria.**

When glycolytic production of pyruvate overcomes the oxidation rate via PDH, accumulated pyruvate is converted to lactate via lactate dehydrogenase (LDH) (see figure 2.6). Lactate effluxes the muscle with the concentration of lactate in the blood the balance of production rate and removal (Olson 1963; Alpert, 1965; Schroder et al., 1969; Hubbard, 1973). Once in the blood stream, lactate can be taken up by exercising or non-exercising skeletal muscle, the myocardium, kidney or the liver (Hubbard 1973) where it is converted to pyruvate for gluconeogenesis (produce glycogen) and subsequent storage, this process called the Cori Cycle (Long and White 1938; Schroder et al., 1969; Katz and Tayek, 1998; Katz and Tayek, 1999). Blood lactate can also be filtered at the kidney and excreted in the urine, this will be discussed in detail in section 2.9.2.5 and chapter 6.

2.7.2 High intensity exercise decreases fat oxidation

As exercise intensity gradually increases from 25%, 65% to 85% VO_{2max}, the contribution of CHO to energy production increases (Romijn *et al.,* 1993). At intensities >85% VO2max, CHO utilisation increases (Jeukendrup, 2003; Achten and Jeukendrup, 2004), causing lactate production (Krebs *et al.,* 1964; Gastin, 2001). Contrastingly, fat oxidation decreases as exercise intensity increases, becoming depressed when exercise intensity reaches approximately 80-85% $VO_{2max,}$ as seen in figure 2.7 (Romijn *et al.,* 1993; Thompson, 1998).

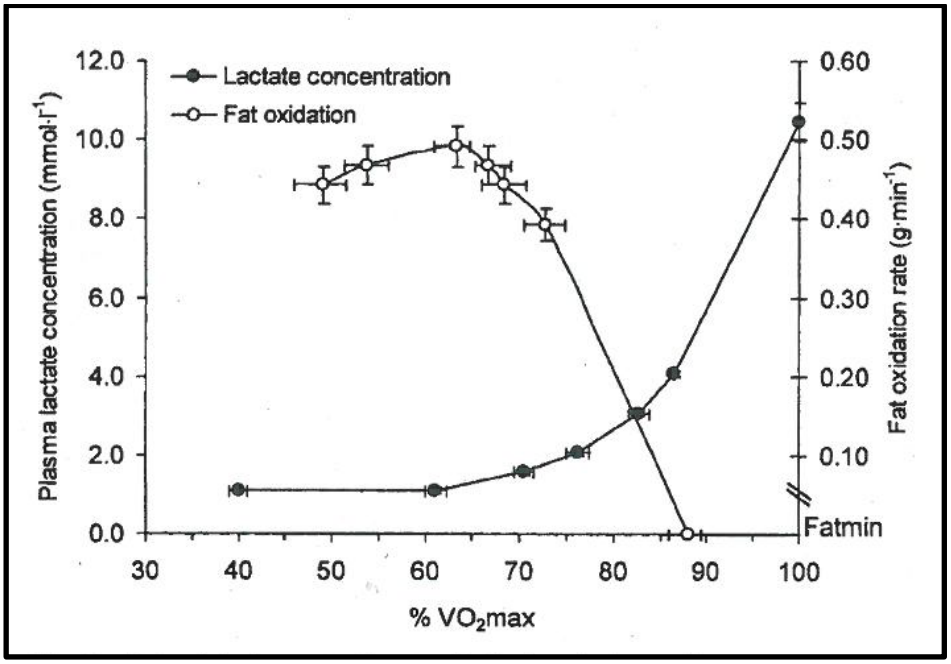

Figure 2.7: The relationship between fat oxidation and lactate concentration as exercise intensity increases (Taken from Achten and Jeukendrup, 2004a). **As lactate production increases, fat oxidation decreases.**

Numerous systemic and local factors may cause depressed fat oxidation during high intensity exercise. Vasoconstriction to adipose tissue with the majority of blood flow redirected to exercising skeletal muscles for increased substrate supply and waste removal may result in re uptake and reesterification of FFA in adipose tissue, decreasing delivery uptake and oxidation in muscle mitochondria (Helge *et al.,* 2007). However, the plasma FFA availability may not play a role in limited fat oxidation, as muscle blood flow is at its highest during high intensity exercise (van Loon *et al.,* 2001). Indeed at exercise intensities greater than 85% VO_{2max}, no difference in thigh plasma FFA oxidation has been observed, thereby the use of other fat sources at this exercise intensity such as IMTG, may be decreased, explaining decreased fat oxidation at higher exercise intensities (Helge *et al.,* 2007).

The metabolic by-products of high intensity exercise may also lead to declining fat utilisation. When glycolytic flux exceeds the utilisation of pyruvate, considerable muscle

lactate concentrations are produced. Acetylcarnitine is also produced, reducing the available free carnitine which is needed for FFA uptake into the mitochondria. Therefore fatty acid oxidation rates may be limited to the amount of free carnitine availability (van Loon 2001; Jeukendrup, 2002), providing an avenue to the reduced action of CPT1 and thus inadequate entry across the mitochondria membrane. Furthermore, increased lactate production with the subsequent H^+ ion release decreases muscle pH (Stisen *et al.,* 2006), causing intramuscular acidity. An acidic skeletal muscle environment inhibits the activity of CPT1 by 40% when pH drops from 7.0 to 6.8 (Stisen *et al.,* 2006; Sahlin and Harris 2008), limiting FFA transport into the mitochondria for oxidation (Sidossis *et al.,* 1997; Jeukendrup, 2002; Helge *et al.,* 2007). Lastly, an additional outcome of anaerobic glycolysis is the potential elevation of acetyl CoA, derived from pyruvate. High concentrations of acetyl CoA may lead to increased production of malonyl coenzyme A in the cytosol (Elayan and Winder 1991; Jeukendrup 2002; Sahlin and Harris 2008), an intermediate of fatty acid synthesis which can inhibit the action of CPT1 (Odland *et al*., 1998; Roepstorff *et al.,* 2005). This occurs under acidic conditions; hence CPT1 activity is optimal during exercise modes that do not alter muscle pH (Odland *et al.,* 1998). For this reason the optimal exercise conditions for promoting fat oxidation should be when there is sufficient FFA delivery to the exercising skeletal muscle at an exercise intensity that produces little lactate, thus not altering muscle pH.

2.8 The fat burning exercise zone

It's been established that as the intensity of exercise increases, there is a shift in substrate mobilisation and utilisation (Romijn *et al.,* 1993; Achten *et al.,* 2002). Theoretically, plasma FFA and intramuscular triglycerides (IMTG) are the primary substrate

sources while exercising at a low intensity (25-30% VO_{2max}), while a moderate exercise intensity (60-75% VO_{2max}) utilises both fats and CHO sources (Romijn *et al.,* 1993; van Loon *et al.,* 2001). Thus, it is commonly thought that prolonged, continuous exercise at low to submaximal intensities results in higher aerobic capacity for fat oxidation (Talanian *et al.,* 2007).

A number of studies have demonstrated that maximum fat oxidation occurs between exercise intensities of 33-65% VO_{2max} (Jones *et al.,* 1980; Romijn *et al.,* 1993; Sidossis *et al.,* 1997; Freidlander *et al.,* 1998; Howlett 1998; Thompson 1998; Romijn *et al.,* 2000; Stisen *et al.,* 2006; van Loon 2001 Venables and Jeukendrup, 2005). The significant variation (33-65% VO_{2max}) may be due to sex, training status, tested protocol and diet (Achten and Jeukendrup, 2004a). For instance, when employing hand bike cycling, 55% VO_{2max} is when fat oxidation is at its peak, whilst its 75% VO_{2max} for bike cycling (Knechtle *et al.,* 2004). The intensity that elicits maximum fat oxidation also increased from 54%-65% VO_{2max} in trained participants (Nordby *et al.,* 2006). However, a study from 2002 using a graded exercise test demonstrated the fat_{max} (maximal fat oxidation) zone and that 64 ± 4% VO_{2max} is the precise exercise intensity for maximal oxidation of fat (Achten *et al.,* 2002).

2.8.1 Establishing Fat_{max} zone

Achten et al. (2002) used a graded exercise test to exhaustion to measure fat oxidation over a range of exercise intensities to exhaustion. Maximal fat oxidation occurred at 64 ± 4% VO_{2max}, correlating to 74 ± 3% of maximum heart rate (Achten *et al.,* 2002). Utilising a similar protocol but with a trained cohort, fat_{max} was determined to be slightly lower than the untrained cohort, 62.5 ± 9.8% VO_{2max}, 73% maximum heart rate (Acheten and Jeukendrup 2003). The 'fat_{max} zone', hence the optimal exercise intensity zone to

maximise fat oxidation was also made clear, 55 ± 3% VO_{2max} to 72 ± 4% $VO_{2max.}$ Exercising at an intensity above this and fat oxidation begins to decline (Achten *et al.,* 2002). This supported prior results from Romijn et al. (1993) who investigated substrate utilisation at three different intensities, low, moderate and high intensities (25% 65% and 85% of VO_{2max}, respectively). Participants cycled for 120 mins at intensities of 25% and 65% VO_{2max} and 30 mins at 85% VO_{2max}, finding that highest fat oxidation occurred at 65% VO_{2max}, (see figure 2.8).

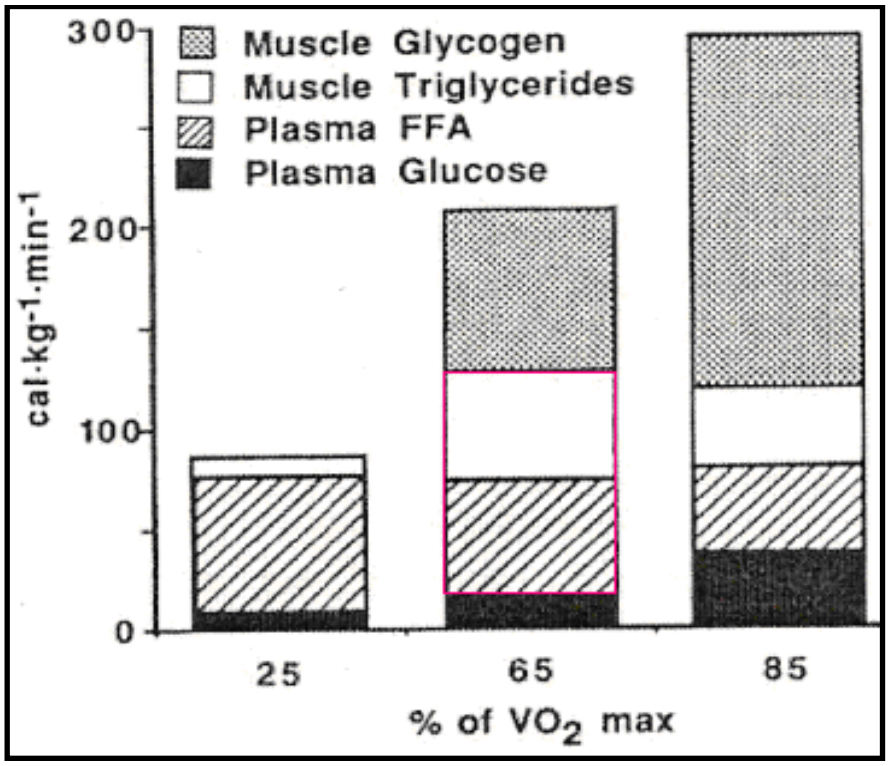

Figure 2.8: Substrate (glycogen, IMTG, plasmas FFA and plasma glucose) utilisation at 25%, 65% and 85% of VO_{2max} when exercising continuously for 120 mins (Taken from Romijn *et al.,* 1993).

2.8.2 Fat oxidation is maximal whilst exercising at 65% VO_{2max}

The percentage of CHO oxidation increases as exercise intensity increases, and total energy expenditure from lipid contribution decreases (Achten and Jeukendrup 2003; Achten

and Jeukendrup 2004), demonstrated in figure 2.8. Whilst exercising at 85% VO_{2max}, lipolysis is similar to that of 65% VO_{2max} however oxidation of fatty acids is decreased (see figure 2.8) as they may be trapped in adipose tissue due to decreased blood flow (Romijn *et al.,* 1993) or oxidation may be inhibited as detailed in section 2.7.2. Fatty acid oxidation is regulated by lipolysis, FFA delivery to muscle, transport across muscle and mitochondrial membranes and fatty (see section 2.2.1.1) and IMTG hydrolysis (Achten and Jeukendrup, 2004), therefore these pathways and mechanisms may be at optimal activity and not inhibited during exercise at 65% VO_{2max}. Figure 2.8 shows the contribution of substrate to exercise at three different exercise intensities and whilst exercising at 65% VO_{2max}, a significant portion of energy is produced from IMTG, with similar levels of plasma FFA contributing equally (Romijn *et al.,* 1993). Significant oxidation of fatty acids derived from IMTG was also observed in trained individuals cycling at 60% VO_{2max} (Watt *et al.,* 2002) and cycling between between 50-70% VO_{2max} using magnetic resonance spectroscopy whilst cycling (White *et al.,* 2003).

Despite this significant evidence, individuals participating in high intensity physical activity(<80% VO_{2max}) have less subcutaneous fat compared to those who participate in low to moderate intensity physical activity (Tremblay *et al.,* 1990; Hunter *et al.,* 1998; Benson *et al.,* 2008). More than 20yrs ago, 2623 (1366 women and 1257 men) participated in the 1981 Canada fitness survey and were measured for energy expenditure of leisure time activities, estimated maximal oxygen uptake (VO_{2max}), subcutaneous fat and anthropometric characteristics (Tremblay *et al.,* 1990). Although using indirect methods, this study reported VO_{2max} was greater in those undertaking moderate to high intensity physical activity when compared to those not completing this level of physical activity. Participants that were characterised to be a part of the high intensity activity group had a reduced waist to hip

ratio, most likely due to low waist circumferences. Moreover, women in the moderate to high intensity group and men in the high intensity activity group had lower amounts of subcutaneous fat. This may be unexpected given lower intensities of activity are associated with fat oxidation. Further when the energy cost of the physical activity was removed and not recognised as part of energy expenditure, a significant difference in subcutaneous fat was determined between participants undertaking vigorous activity and those not. Thus this shows that the effect of exercise intensity on body fat is not simply due to the energy cost of the physical activity, but to additional effects on other components of energy balance (Tremblay *et al.,* 1990).

Additionally repeated bouts of high intensity exercise or high intensity intermittent training (HIIT) has been shown to be better than submaximal continuous exercise training (CONT) at inducing fat loss (Tremblay *et al.,* 1994; Trapp *et al.,* 2008; Macpherson *et al.,* 2011; Gremeaux *et al.,* 2012). The scope of this thesis surrounds the metabolic shifts induced by HIIE which may induce energy loss, potentially contributing to heightening energy expenditure and reducing adiposity.

2.9 High intensity intermittent exercise and high intensity intermittent training

High intensity intermittent exercise (HIIE) is repeated short high intensity exercise bouts (>80%VO_{2max}) lasting between 6-120 s followed by a rest period consisting of between 12s-4mins; the total duration of exercise can last from 2.5min to 60min (Bogdanis *et al.,* 1995; Tremblay *et al.,* 1994; Talanian *et al.,* 2007; Trapp *et al.,* 2007; Burgomaster *et al.,* 2008; Perry *et al.,* 2008; Gibala *et al.,* 2009; Little et al., 2010; Little *et al.,* 2011; Macpherson *et al.,* 2011; Bartlett *et al.,* 2012; Gillen *et al.,* 2012; Gremeaux *et al.,* 2012). The

manipulation of exercise intensity and duration modifies substrate utilisation and the subsequent metabolic pathway (Holloszy and Coyle 1984).

HIIE and HIIT induce favourable changes in health, metabolic and performance markers (Price and Halabi, 2005; McKay *et al.,* 2009) at a quicker rate compared to CONT. HIIE and HIIT are also considered more enjoyable to perform (Jakicic *et al.,* 1995; Bartlett *et al.,* 2012) and is time efficient (Gibala, 2007; Boutcher, 2010; Gibala and Little, 2010; Metcalfe *et al.*, 2011). However, the main and commonly accepted changes observed by numerous studies are a significant increase in aerobic fitness (Tremblay *et al.,* 1994; Mourier *et al.,* 1997; Macdougall *et al.,* 1998; Ross and Leveritt 2001; Laursen *et al.,* 2002; Barnett *et al.,* 2004; Warburton *et al.,* 2005; Helgerud *et al.,* 2007; Talanian *et al.,* 2007; Bishop *et al.,* 2008; Burgomaster *et al.,* 2008; Dunn, 2009; Perry *et al.,* 2008; Tjonna *et al.,* 2008; Trapp *et al.,* 2008; Tjonna *et al.,* 2009; Whyte *et al.,* 2010; Astorino *et al.,* 2012; Gibala *et al.,* 2012), which may be attributed to an increase in mitochondrial content and oxidative enzyme activity (Harmer *et al.,* 2000; Rodas *et al.,* 2000; Ross and Leveritt 2001; Laursen *et al.,* 2002; Slordahl *et al.,* 2005; Burgomaster *et al.,* 2007; Burgomaster *et al.,* 2008; Gibala *et al.,* 2009) or perhaps to improved cardiovascular health, with increased stroke volume and better cardiac contractility (Helgerud *et al.,* 2007). Along with improving cardiorespiratory health, marked improvements in insulin sensitivity in healthy individuals as well as Type II Diabetics, have been observed post HIIT (Mourier *et al.,* 1997; Boudou *et al.,* 2003; Tjonna *et al*., 2008; Trapp *et al.,* 2008; Dunn, 2009; Tjonna *et al.,* 2009; Whyte *et al.,* 2010; Little *et al.,* 2011; Gillen *et al.,* 2012). Improvements in insulin resistance, enhancing glucose uptake, has been observed up to three days post HIIT consisting of 30 s all out cycling followed by 2-4 mins rest, with the authors attributing this result to skeletal muscle adaptations (Babraj *et al.,* 2009). Moreover a recent study employing participants with type II diabetes

demonstrated decreased postprandial hyperglycaemia after a single session of HIIE consisting of ten 60 s cycling bouts at 90% of the maximum workload achieved, separated by 60s rest (Gillen *at al.,* 2012).

In addition to increased aerobic capcity, there are significant decreases in adiposity after HIIT compared to CONT (Tremblay *et al.,* 1994; Trapp *et al.,* 2008; Macpherson *et al.,* 2011; Gremeaux *et al.,* 2012). This is a noteworthy change as increased adiposity is a risk factor for a range of cardiometabolic disorders such as obesity, type II diabetes and metabolic syndrome. These studies highlight the importance of using HIIT to improve health; however the precise metabolic mechanisms behind this result are not comprehensively understood. From a health and quality of life perspective, reducing adiposity is the most important HIIT induced metabolic alteration. Numerous articles show improvements in body morphology over two to twenty-four weeks of various HIIT protocols, and in various participant cohorts', from healthy untrained males and females to those with cardiovascular disease. Decreases in body mass (Tremblay *et al.,* 1993; Mourier *et al.,* 1997; Boudou *et al.,* 2003; Warburton *et al.,* 2005; Helgerud *et al.,* 2007; Perry *et al.,* 2008; Tjonna *et al.,* 2008; Trapp *et al.,* 2008; Dunn 2009; Tjonna *et al.,* 2009; Whyte *et al.,* 2010), subcutaneous fat (Tremblay *et al.,* 1993; Mourier *et al.,* 1997; Boudou *et al.,* 2003; Trapp *et al.,* 2008; Dunn, 2009 Tjonna *et al.,* 2009), abdominal trunk fat (Tremblay *et al.,* 1993; Mourier *et al.,* 1997; Boudou *et al.,* 2003; Trapp *et al.,* 2008; Dunn, 2009; Tjonna *et al.,* 2009;) and waist circumference (Mourier *et al.,* 1997; Tjonna *et al.,* 2008; Dunn 2009; Tjonna *et al.,* 2009; Whyte *et al.,* 2010) have all been observed following HIIT.

Clearly this area of research warrants further exploration as HIIT has been shown to cause greater losses in fat mass compared to CONT (Tremblay *et al.,* 1994; Trapp *et al.,*

2008), despite the CONT similar to that of the aforementioned mentioned 'fat_{max}' (Achten et al., 2001), described in section 2.8.

2.9.1 HIIT is better than CONT at rapidly reducing adiposity

To demonstrate the beneficial effect of HIIT on fat loss, HIIT was compared to other training regimes, namely CONT. Shaw et al. (2006) did a systematic review of continuous aerobic training studies and demonstrated no effect on fat loss. This result, coupled with evidence from two studies comparing HIIT and CONT show the favourable effect HIIT has on reducing adiposity. Notable articles by Tremblay et al. (1994) and Trapp et al. (2008) both employed HIIT and CONT, both observing a greater fat loss after HIIT compared to CONT.

Tremblay et al. (1994) used a training program consisting of twenty weeks of CONT compared to 15 weeks of HIIT. Diet was not restricted and training sessions were unmatched for physical work between the two regimes; energy cost was greater in the CONT compared to the HIIT (120.4MJ compared to 57.9MJ, respectively). Using an indirect method of measurement (skin-fold), the authors showed a pronounced decrease in the sum of all six skin-fold measurements after the HIIE training program. Prior to exercise CON was 79.2 ±35.1 and after 74.7 ±34.2, compared to 94.2 ± 37.7 to 80.3 ± 36.0 for the HIIE program, with the change being significant when corrected for energy expenditure as shown below in figure 2.9.

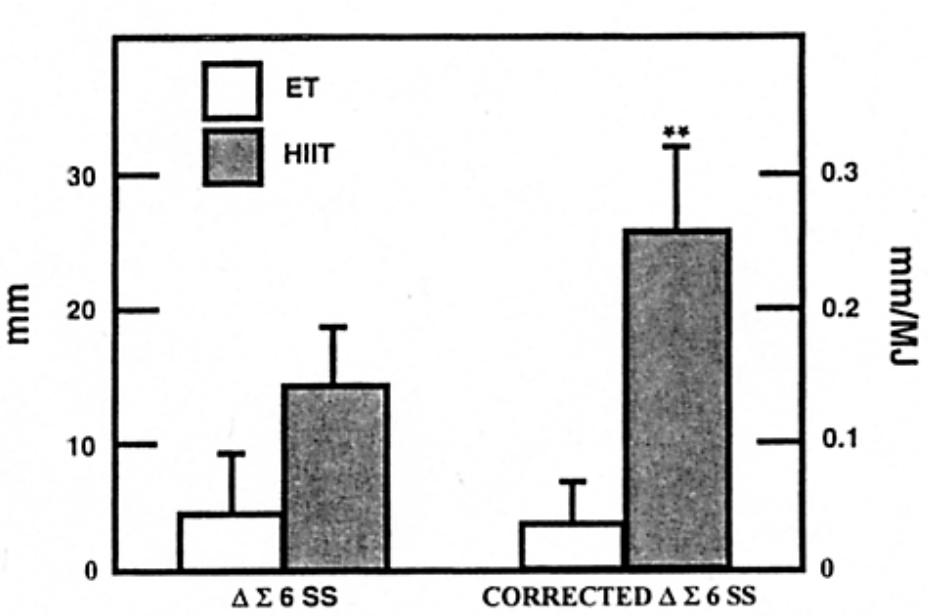

Figure 2.9: The change in skin fold measurements after HIIT and CONT (ET) (Tremblay *et al.,* 1994).

Trapp et al. 2008 compared HIIT and CONT but added an additional test "control" group, where participants maintained their normal exercise patterns for the duration of the experiment (Trapp *et al.,* 2008). In this study, fat loss was assessed over a fifteen week period, again participants instructed to maintain normal diet. Using a more sensitive method of Dual-energy X-ray Absorptiometry (DEXA), there was a significant decrease in central abdominal fat and fat mass after HIIT, as opposed to CONT and control, where no beneficial changes were observed (see figure 2.10). Central abdominal adiposity was significantly decreased after HIIT whereas no changes were observed in the CONT group. There were also no significant changes in lean body mass in the CONT group, further potentially displaying that HIIE is better at accelerating fat loss whilst maintaining muscle mass.

Although there were significant differences in fat mass loss between HIIT and CONT (SSE; figure 2.10), there was no significant difference in the energy cost of exercise,

measured in MJ between the three different programs. It is clear from this study that HIIT induces greater decreases in adiposity than CONT, which is not attributed to the energy cost of physical activity but due to additional metabolic factors creating energy loss.

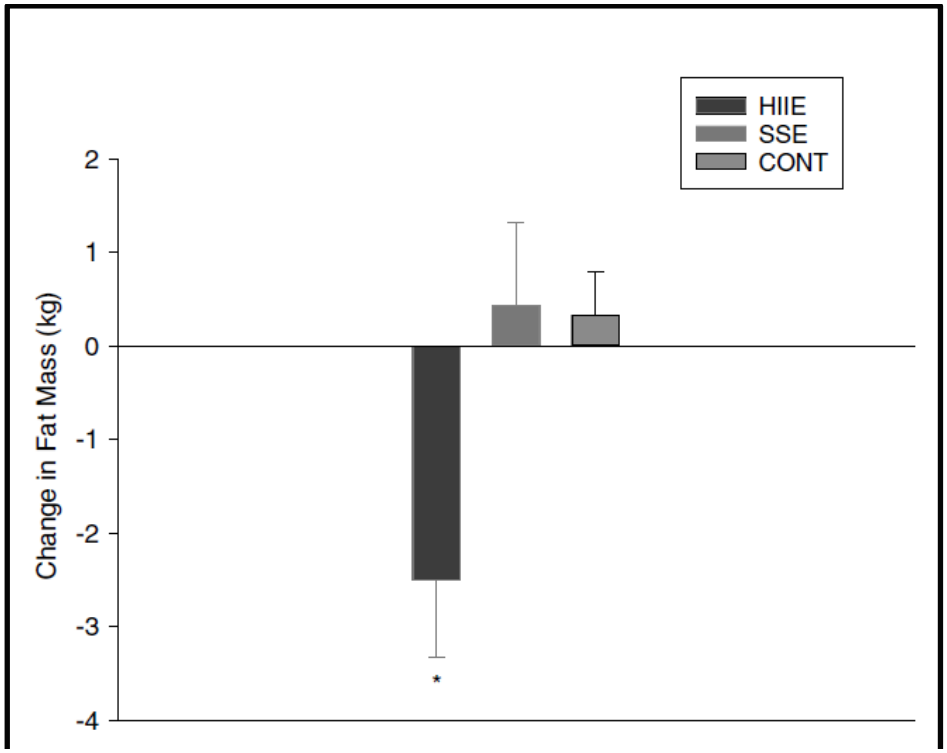

Figure 2.10: The effect of HIIE, CONT (SSE) and maintaining exercise patterns (CONT) on change in fat mass, measured via DEXA (Taken from Trapp *et al.,* 2008).

It has been suggested that the accelerated fat loss associated with HIIE may be due to the oscillations in work and rest periods, preventing skeletal muscle metabolism from adapting to the stereotypical high intensity exercise with glucose being the predominant substrate utilised. Moreover, the two abovementioned studies proposed that the work and rest model of HIIE induced substrate partitioning, where glycogen is utilised during the high intensity work periods, and fat sources contribute to energy production during the passive rest periods, fuelling oxidative metabolism. Trapp et al. (2007) proposed that shifts in fat metabolism during high intensity exercise model are evident as plasma glycerol and

epinephrine concentrations were increased post exercise, plasma glycerol reflective of lipolysis as stated earlier (section 2.2.2); epinephrine known to stimulate lipolysis. Nevertheless, these studies are limited; the experimental designs are not matched for workload, hence different number of kJ expended. Furthermore the use indirect methods of fat loss and absence of plasma, muscle and urine metabolic data do not provide a comprehensive metabolic view of substrate utilisation of HIIE compared to CON.

Interestingly, in a workload matched study where HIIT and CONT were matched for average intensity, energy cost of exercise and exercise duration and distance, there were no differences in skeletal muscle 5' AMP-activated protein kinase (AMPK) which assists in substrate metabolism and in PGC1α mRNA three hours post exercise between the two different types of exercise (Bartlett *et al.,* 2012). Therefore, similar levels of skeletal muscle adaptation to HIIT does not explain the decreases in adiposity that appear earlier in HIIT compared to CONT. Thus there must be other reasons for the fat loss observed. To accurately compare fat loss from CON and HIIE, workload, exercise intensity, (duration of exercise) and frequency of exercise need to be controlled and matched in acute bouts of exercise.

2.9.2 The metabolic differences between HIIE and CON

A recent unpublished study (submitted) from our laboratory employed HIIE and CON, successfully matching total workload, the HIIE protocol 30mins of 20 s cycling at 150% VO_{2max} with 40 s rest between bouts, whereas the CON model was 30 mins of continuous cycling at 50% VO_{2max} (Borg *et al.,* 2008). It was suggested that this study provided additional evidence of increased reliance on fat metabolism with HIIE. Plasma glycerol and FFA concentrations were not different between CON and HIIE trials, hence similar levels of

fat may have been utilised. There was a significant increase in plasma lactate during HIIE compared to CON, reflective of the greater glycolytic contribution to HIIE (Borg *et al.,* 2008). This was attributed to the work rest nature of HIIE and together these results indirectly reflect substrate partitioning, first proposed by Tremblay et al. (1994). In addition, this study showed differences in plasma and urinary purine concentrations following exercise and the recovery period may play a major role in increasing energy expenditure (Borg *et al.,* 2008) which will be discussed in section 2.9.2.4.

2.9.2.1 Substrate utilisation during high intensity intermittent exercise

There are numerous studies investigating HIIE that provide valuable insight into the substrate utilisation and metabolism of this type of exercise protocol. HIIE involves all three energy systems with the initial maximal effort high intensity bout using the ATP-CP system to meet energy demands, with glycolysis contributing to energy supply thereafter. In the following recovery period there may be replenishment of PCr, the degree of replenishment dependant on the length of the rest period, as mentioned in section 2.7 complete replenishment may take approximately 3-6 mins (Edwards *et al.,* 1973; McCartney *et al.,* 1986; Chasiotis *et al.,* 1987; Bogdanis *et al.,* 1995; Bogdanis *et al.,* 1996; Dawson *et al.,* 1997; Tomlin *et al.,* 2001; Tomlin *et al.,* 2002; Dorado *et al.,* 2004; Dupont *et al.,* 2004; Thevenent *et al.,* 2007; Forbes *et al.,* 2008). Therefore the rest duration prior to the next exercise bout will reflect the degree of glycolytic contribution, for example, in the Trapp et al. (2008) HIIT programs, the rest periods were 12 s and 36 s which are too short for complete resynthesis. Altering the duration of rest between the high intensity exercise bouts to investigate if glycolytic contribution and oxidative metabolism are altered will be explored in this thesis, specifically study 2 in chapter 5.

Depending on the duration and intensity, there may also a significant decrease in muscle glycogen stores during the first sprint of HIIE. In a previous study exploring successive bouts of 30s maximal all out cycling, there were no further decreases in glycogen or muscle lactate in the subsequent sprints, suggesting that glycogenolysis is inhibited. As mentioned earlier, PFK is the rate limiting enzyme for glycolysis and may be inhibited due to elevated cytosolic citrate concentrations (Essen and Kaijser, 1978), or perhaps it is more likely that phosphorylase b and hexokinase are inhibited due to increases in G-6-P (McCartney *et al.,* 1986; Gaitanos *et al.,* 1993). With glycogenolysis potentially inhibited, potential fuel sources for the exercising skeletal muscle may be free glucose and hexose phosphate from the muscle; however these are very unlikely to provide such high energy demands of the repeated sprint bouts. Therefore it is more likely that the primary fuel source may be IMTG.

As stated earlier, plasma glycerol, reflective of lipolysis has been shown to increase during and post HIIE (Essen *et al.,* 1977; McCartney *et al.,* 1987; Coggan *et al.,* 2007; Goto *et al.,* 2007; Trapp *et al.,* 2007; Borg *et al.,* 2008). In the previously mentioned workload matched study, plasma FFA and plasma glycerol were similar between HIIE and CON (Borg *et al.,* 2008) eluding that there was no difference in fat metabolism between the two exercise protocols. This is interesting because the CON protocol consisted of 30 mins of cycling at 50% VO_{2max}, which is close to that of the fat_{max} zone (Achten *et al.,* 2004). No change in plasma FFA concentration when plasma glycerol concentrations are increased suggests that plasma FFA may also be oxidised during this exercise type (McCartney *et al.,* 1986). Furthermore a study utilising supra-maximal HIIT (120% VO_{2peak}) observed increased plasma glycerol post HIIE training and no difference between FFA in CON and HIIT. This indicates that lipolysis and oxidation are at same level (Christmass *et al.,* 1999a). Furthermore, there was no difference observed in a HIIE and CON haematocrit, so metabolic responses may be

due to the exercise nature (Christmass *et al.,* 1999) and not blood flow or blood volume; therefore it can be proposed that high levels of fat utilisation may also occur during HIIE. The reliance on fat metabolism associated with HIIE will be explored in this thesis during various HIIE models. These models will also be manipulated to see if this reliance on fat sources can be enhanced further. Although not explored in this thesis, the fat loss associated with HIIE may also be partly due to increased concentration of catecholamine's post exercise (Trapp *et al.,* 2007), thus requires discussion.

2.9.2.2 Catecholamine release stimulate fat oxidation

Catecholamines, norepinephrine and specifically epinephrine have been shown to stimulate lipolysis and are released during and after HIIE consisting of numerous different intensities (Gratas-Delamarche *et al.,* 1994; Borsheim *at al.,* 1998; Christmass et al., 1999; Vincent *et al.,* 2004; Trapp *et al.,* 2007; Bracken *et al.,* 2009). This may be pertinent as catecholamine's drive lipolysis and prompt FFA release from subcutaneous tissue and intramuscular fat (Issekutz, 1978). With more β adrenergic receptors found in abdominal stores compared to subcutaneous (Rebuffe-Scrive *et al.,* 1989), this may be a reason HIIE associated with greater fat loss in the abdominal area (Tremblay *et al.,* 1993; Mourier *et al.,* 1997; Boudou *et al*., 2003; Tjonna *et al.,* 2008; Trapp *et al.,* 2008; Dunn, 2009; Tjonna *et al.,* 2009; Whyte *et al.,* 2010). The half-life of catecholamine's is 2-3 mins in the blood, thus the short rest periods of HIIE are not long enough for complete clearance (Bracken *et al.,* 2008), potentially prompting constant lipolytic drive throughout HIIE compared to CON. However, elevated fat loss observed with HIIT is unlikely to be due to fat utilisation during exercise alone.

Potentially increasing fat contribution to energy supply during HIIE is not strong enough stimulus to induce such changes in adiposity observed in Tremblay *et al.* (1994) and Trapp *et al*. (2008). Therefore it is likely that changes occurring post exercise may also play a significant role in the fat loss associated with HIIT. Indeed high intensity exercise is associated with greater metabolic changes than submaximal exercise and Borg *et al*. (2008) suggested the recovery period following HIIE may play a large role in inducing an energy deficit due to recovery processes associated with excess post exercise oxygen consumption (EPOC) and including purine nucleotide metabolism.

2.9.2.3 The effect of elevated excess post exercise oxygen consumption (EPOC) post high intensity exercise

EPOC occurs when VO_2 remains above rest levels (Gaesser and Brooks 1984; Bell *et al.,* 1997; Borsheim and Bahr 2003; LaForgia *et al.,* 2006; Matsuo *et al.,* 2012) and is indicative of increased energy utilisation to return the body to homeostasis (LaForgia *et al.,* 2006). Substrates are utilised during this time and as EPOC is an aerobic process, fat is the principal fuel source (Tremblay *et al.,* 1990; Treuth 1996; Hunter *et al.,* 1998; Balsom 1999; Al mulla *et al.,* 2000; Yoshioka *et al.,* 2001; Hall *et al.,* 2002; Kuo *et al.,* 2005; Benson *et al.,* 2007; Helge *et al.,* 2007; Malatesta *et al.,* 2009).

There is a defined short phase and a long phase of EPOC, encompassing the different metabolic mechanisms which are replenished, removed or returned to their pre-exercise values. During the short phase there is replenishment of myoglobin with oxygen in blood and muscle, resynthesis of ATP and CP, removal of lactate and increased body temperature, circulation and ventilation (Bell *et al.,* 1997). The prolonged phase, which can last for hours

(Iwayama and Tokuyama, 2012), is a little less understood and mechanisms more complex. There is glycogen resynthesis, protein breakdown and resynthesis (Borsheim and Bahr, 2003), increased sympathetic activity (Gladden *et al.,* 1982; Gaesser and Brooks, 1984; Hermansen *et al.,* 1984; Maehlum *et al.,* 1986; Bahr *et al.,* 1990; Sagnol *et al.,* 1989; Campos et al., 2012) regulating triglyceride/fatty acid cycling (Yoshioka *et al.,* 2001), this last process accounting for a significant portion of EPOC. The extent of EPOC is greatly attributed to the exercise intensity and duration (Iwayama and Tokyuama 2012).

2.9.2.3.1 The effect of exercise intensity and duration on EPOC

The magnitude and length of EPOC is significantly elevated as exercise duration is prolonged (Knuttgen, 1970; Bahr *et al.,* 1987; Chad and Wenger 1988; Elliot *et al.,* 1988; Sedlock *et al.,* 1989; Gore and Withers 1990; Bahr *et al.,* 1992; Sedlock, 1992; Neary *et al.,* 1993; Quinn *et al.,* 1994; Sedlock *et al.,* 1994; Imamura *et al.,* 2004), see figure 2.11.Exercising at 70% VO_{2max} for 20, 40 and 80 mins showed progressively increasing EPOC as duration increased, from 11.1L, 14.7L and 31.9L, respectively (Bahr 1992).

Increasing exercise intensity also elevates EPOC exponentially (Knuttgen, 1962; Knuttgen, 1970; Haigberg *et al.,* 1980; Sedlock *et al.,* 1989; Bahr and Sejersted, 1991; Sedlock 1991; Elliot *et al.,* 1992; Brockman *et al.,* 1993; Dawson *et al.,* 1996; LaForgia *et al.,* 1997; Phelian *et al.,* 1997), see figure 2.11. A comprehensive study comparing submaximal and high intensities, exercising over 30, 50 and 70% VO_{2max} and for 20, 50 and 80 mins showed the combination to produce the greatest EPOC was exercising at 70% VO_{2max} for 80 mins. EPOC was 7 hrs in length equating to approximately 297 kJ (Gore and Withers, 1990). It should be noted that one study produced results that contradict this trend, with the lower exercise intensity 50% compared to 70% VO_{2max}, inducing greater EPOC. Reasons behind this

conflicting result remain unclear and no method of analysis was discussed in the paper (Chad and Quigley 1991). An additional study observed no EPOC 35 mins post exercise after an exercise duration 20 mins at 80% VO_{2max} (Hagberg *et al.,* 1980). Given that intensity plays a key part in EPOC, it is logical to assume that there would be even further elevations in EPOC after supra-maximal intensity exercise.

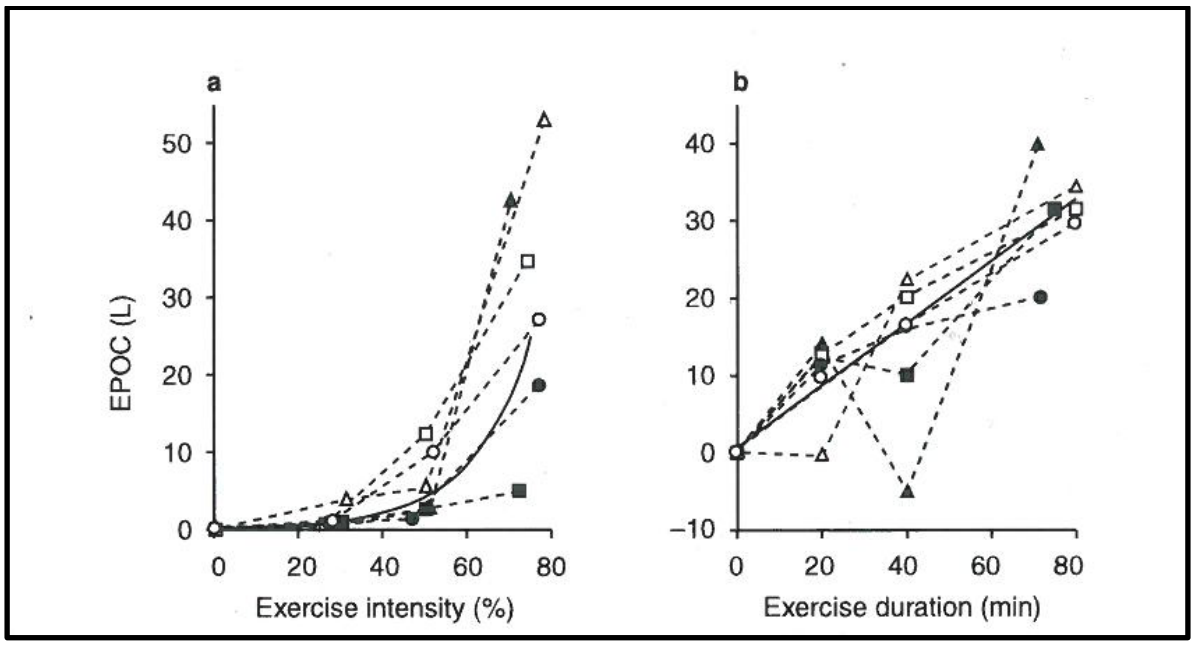

Figure 2.11: The effect of exercise intensity and exercise duration on EPOC (Taken from Borsheim and Bahr, 2003)

2.9.2.3.2 The effect of supra-maximal exercise intensity (>100% VO_{2max}) on EPOC

EPOC is significantly elevated after supra-maximal intensity exercise compared to high intensity exercise (Bahr *et al.,* 1992; Nummela and Rusko 1995), exercise intensities 105% VO_{2max} and 70% VO_{2max} respectively. There was a two-fold increase in EPOC with magnitude of supramaximal intensity exercise 15L compared to 6.9L after high intensity exercise (Bahr *et al.,* 1992).

In treadmill sprinting and cycling, HIIE bouts significantly increased the magnitude of EPOC compared to CON exercise bouts (Devlin and Horton, 1985; Maehlum *et al.,* 1986; Kaminsky *et al.,* 1990; Brockman *et al.,* 1993; Almuzaini *et al.,* 1998; Scott; 1999; Tomlin and Wenger 2001; Lyons *et al.,* 2005) although not all magnitudes and durations are significant enough to produce substantial changes to weight (LaForgia *et al.,* 2006).

2.9.2.3.3 Limitations to EPOC

Measurements of EPOC can be limited by the practicality and accuracy of gas collection and measurement in experimental exercise trials. False VO_2 measurements can be obtained in the rest period prior to exercise due to increased sympathetic nervous stimulation caused by exercise anticipation (Borsheim and Bahr, 2003). Furthermore, the collection mask or mouth piece can be uncomfortable for participants. A canopy or hood may be a better method however the paramount method of collection is placing participants in a calorimetry chamber where they are not hooked up to any apparatus and permits hours of un-impinged measurement (Iwayama and Tokuyama 2012).

EPOC associated with high intensities only induce approximately 14-15% of the net total oxygen cost of the exercise (LaForgia *et al.,* 2006). Daily accumulations of such small imbalances can contribute to inducing negative energy balance over time, but only contributes minimally (Scott, 2006). Over a twelve month period exercising at three days a week, the accumulated difference in energy balance may shift negative and may equate to a net loss of 1.5 kg of adipose tissue per year (LaForgia *et al.,* 2006). Whereas, after 15 weeks of HIIT, Trapp *et al.* (2008), showed approximately a loss of 3 kg of fat mass (Trapp *et al.,* 2008). Since EPOC is probably only a minor contribution to overall increase energy expenditure post HIIE, another metabolic mechanism that is measured post exercise and

may contribute to elevating energy expenditure is the ATP loss associated with purine base loss.

2.9.2.4 Increasing energy loss via purine nucleotide excretion post high intensity exercise

During high intensity exercise there is a high turnover of ATP in skeletal muscle. An elevated ATP turnover often results in the failure of ATP resynthesis rates matching hydrolysis (Bangsbo *et al.,* 1992; Stathis *et al.,* 1994), decreasing ATP content with consequential increases in ADP and AMP (Borberg and Sahlin, 1989). AMP is rapidly deaminated to form inosine monophosphate (IMP) which accumulates in the muscle (see figure 2.13), accumulation can be up to two-fold higher in type II fast muscle fibres compared to type I slow muscle fibres after muscle contraction (Tullson *et al.,* 1995; Karatzaferi *et al.,* 2001). Adenylosuccinate synthetase and adenylosuccinare lyase are two enzymes involved in IMP reamination, adenylosuccinate synthetase the rate limiting enzyme in the reaminating process, and may be inhibited by low concentrations of PCr and low muscle pH, (Zhao *et al.,* 2000) both induced by high intensity exercise. A portion of IMP is further degraded to hypoxanthine (Hx) (Dobson *et al.,* 1971; Harkness, 1988; Stathis *et al.,* 1994), and while significant amounts of Hx are rescued by the purine salvage pathway to resynthesise ATP (see figure 2.12), Hx can pass the sarcolemma and enter the blood stream. A small portion of Hx is taken up by the liver where it is further converted to uric acid via xanthine oxidase, and expelled predominantly via the kidneys in the urine (see figure 2.12) (Harkness, 1988; Tullson *et al.,* 1991; Tullson *et al.,* 1995; Hellsten *et al.,* 1999; Zhao *et al.,* 2000; Saiki *et al.,* 2001; Kaya *et al.,* 2006; Stathis *et al.,* 2006; Dudzinska *et al.,* 2010; Zielinski *et al.,* 2011). Hx does not flux back into the muscle from the plasma thus expulsion of Hx

and/or uric acid can be viewed as 'lost' energy that would need to be replaced post exercise (Hellsten *et al.,* 1999) to maintain energy balance.

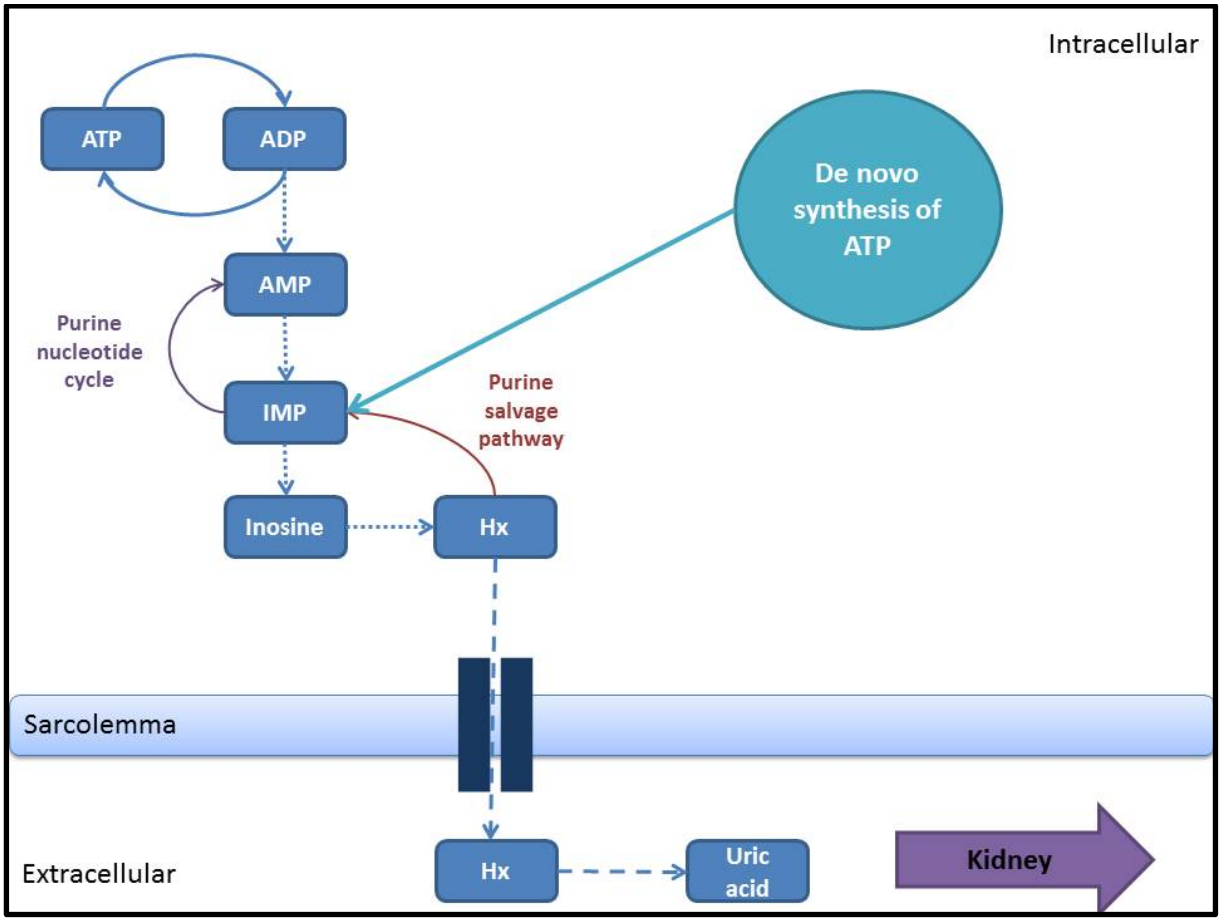

Figure 2.12: ATP and purine nucleotide metabolism during high intensity exercise. ATP degradation and de novo ATP synthesis can be observed. (adapted from Stathis *et al.*, 1994).

Plasma Hx levels peak in the blood stream around 10-20 mins post high intensity exercise and remain elevated for upwards of 45-60 mins (Bangsbo *et al.,* 1992; Saiki *et al*., 2001). The subsequent *de novo* or brand new synthesis of ATP is metabolically expensive, utilising ribose to form ATP (Hellsten *et al.,* 1999), increasing energy deficit. Therefore following high intensity exercise, the elevated restorative cost of *de novo* purine synthesis potentially elevates energy expenditure, contributing to inducing negative energy balance.

The production of Hx is very small relative to the IMP (Stathis *et al.,* 1994) thus maintaining IMP via sustaining high intensity exercise is the key factor to deamination of IMP to Hx (Tullson *et al.,* 1995) and consequent energy deficit. Significantly greater content of Hx and the downstream metabolic by-products found in the urine will be elevated even further with extended bouts of high intensity exercise. Therefore the majority of research pertaining to the effect of exercise on purine metabolism centres on repeated sprint or HIIE, as the rest periods separating the explosive sprints allows high intensity exercises to be sustained for a prolonged period.

During HIIE purine nucleotide base excretion is increased, potentially due to the increased exposure to enzymes in this pathway. After four 30 s all out, maximal sprints, separated by 4 mins rest, there were significant decreases in total adenine nucleotides (TAN), which is ATP+ADP+AMP. Concomitant with these decreases are subsequent increases in IMP, inosine and Hx, indicative of increased purine loss with HIIE (Hellsten *et al.,* 1998). Increases in plasma and urinary concentrations of Hx and uric acid occurs when the number of sprints are increased, showing that longer HIIE promotes a greater mismatch in ATP hydrolysis to resynthesis (Stathis *et al.,* 1999; Hellsten *et al.,* 1999; Saiki *et al.,* 2001; Borg *et al.,* 2008; Pechilvanis *et al.,* 2010). As the number of sprints increased from 1 to 4 to 8, significant increases in plasma inosine, Hx and uric acid were increased, remaining elevated for 60mins post exercise in the 4 and 8 sprints compared to 1 sprint (see figure 2.13). After 120 mins post exercise, plasma Hx was significantly elevated after the 8^{th} sprint compared to the 1^{st} and 4^{th}. Furthermore after 2 hrs of recovery, urinary Hx and whole body purine loss increased as the number of sprints increased (Bangsbo *et al.,* 1992; Hellsten *et al.,* 1998; Stathis *et al.,* 1999; Saiki *et al.,* 2001; Pechilvanis *et al.,* 2010). Urinary Hx and uric acid were also significantly elevated post HIIE compared to CON (Saikia *et al.,* 2001; Borg *et al.,* 2008).

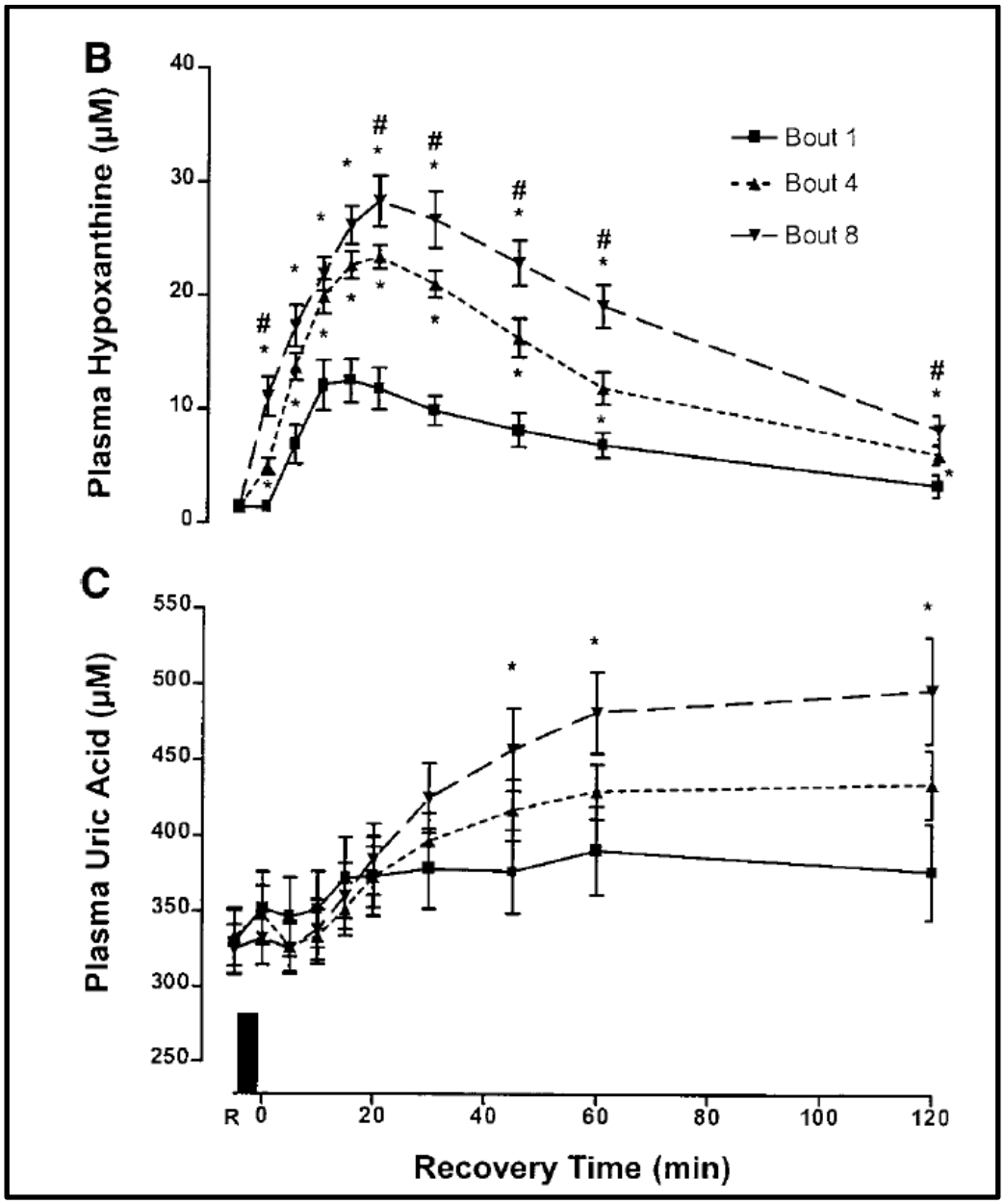

Figure 2.13: The effect of repeated sprints (1, 4 and 8) on plasma Hx and plasma uric acid concentrations during and post exercise (Taken from Stathis *et al.,* 1999)

Therefore there is substantial evidence demonstrating increased purine base loss with HIIE compared to CON (Borg *et al.,* 2008). As mentioned earlier, the occurrence of these metabolites in the plasma and urine post exercise can be extrapolated to ATP loss

from the active muscle, inducing an energy deficit which needs to be restored post exercise. This energy deficit is additional to the energy cost of the actual work performed in the exercise bout. Therefore it is clear that ATP degradation (purine bases) and the consequential purine loss, requiring subsequent metabolic restoration contributes to an energy deficit post exercise. Measuring purine base loss after different modalities of HIIE to give an indication of energy loss hence elevated energy expenditure will be one of the main focuses of this thesis. Given that purine loss is energy that needs to be replaced; perhaps there are other molecules that contribute to energy deficit in the same way or to an even greater extent, with lactate a potential contributor.

2.9.2.5 Lactate is found in the urine after exercise

Lactate produced during high intensity exercise effluxes the muscle and accumulates in the plasma, as mentioned in section 2.7.1. While lactate can be taken up by the liver as part of the Cori cycle, lactate can also be filtered at the kidney and excreted or lost in urine. Urine lactate concentration rises when blood lactate is significantly elevated, suggesting a blood lactate threshold, similar to that of glucose. Lactate is indeed similar to glucose and can be reabsorbed in the proximal convoluted tubule (Miller and Miller, 1949), the amount of lactate filtered by the glomerulus and hence reabsorbed reduced by restricted renal blood flow which accompanies high intensity exercise. Therefore urine collected immediately post exercise may only give a rough estimate of total lactate loss (Miller and Miller 1949). Urine collection must be collected once renal blood flow has returned, hence the collection period in this thesis is over the course of 60-90 mins (see section 3.5.5).

Numerous studies show post exercise lactate excretion, first reported more than 80 years ago in (Liljestrand and Wilson 1925; Johnson and Edwards 1937; Miller and Miller

1949; Pechilvanis *et al.,* 2010). After continuous treadmill running to exhaustion at 8.6 grade, there were significant concentrations of lactate and pyruvate excreted in urine, compared to rest, however pyruvate excretion was miniscule compared to lactate (Johnson and Edwards, 1937). Lactate lost from the body when excreted is energy loss, as a result of high intensity exercise and may contribute to energy deficit. This energy loss is not a part of the energy cost of the actual physical activity performed (kJ) nor is it considered in EPOC. Furthermore as with purine base excretion as there is no metabolic consequence outside replenishment via dietary means, lactate excretion has never been accounted for in energy balance. It may contribute significantly to whole energy balance, elevating energy expenditure after high intensity exercise.

The amount of urinary lactate excretion may be affected by intermittent exercise. Increased lactate excretion post intermittent exercise as measured by innovative metabolomics technology was reported (Pechlivanis *et al.,* 2010). Metabolites post repeated sprint exercise, sprints separated by either 10 s or 60 s were analysed and with shorter duration rest time, (reflective of increased plasma lactate and more glycolytic contribution), there was a significant greater urinary lactate post exercise (Pechlivanis *et al.,* 2010). Lactate lost in the urine correlates simply to elevated energy loss. This factor of exercise has been neglected from an energy balance standpoint and will be a primary focus of study 3 (chapter 6) of this thesis. Lactate lost will be quantified following different high intensity exercise protocols and estimated as an additional potential metabolic avenue for the fat loss and energy expenditure associated with HIIT compared to CONT. Lactate loss via urinary excretion may influence energy expenditure more significantly than purine nucleotide metabolism due to the difference in concentration of excretion, $\mu mol.hr^{-1}$ for purines

compared to the 1000 times greater mmol.hr^{-1} for lactate excretion. Therefore there are significantly more lactate mols lost, hence a noteworthy metabolic research avenue. Henceforth there is solid evidence that HIIE induces similar levels of fat utilisation and increases energy loss post exercise. Therefore over the 15-20 weeks of HIIT, these small daily metabolic imbalances in energy balance may result shifting energy balance negative causing significant reductions in adiposity, which was observed by Tremblay *et al.* (1994 and Trapp *et al.* (2008).

The majority of metabolic studies pertaining to understanding mechanisms of fat loss employ male participants due to hormonal differences, as study 1, 2, 3 and 4 of this thesis do. However, in order to see if HIIE causes similar metabolic responses in females, study 4 also employs this gender. Utilising females in acute metabolic studies is problematic as the ever fluctuating hormones affect lipolysis; hence testing days need to be in a specific phase of the menstrual cycle, as stated in section 3.4 of the methods chapter. There are also many differences in metabolism between the genders, highlighted in the following section.

2.10 Differences between the male and female genders in skeletal muscle metabolism

Exercise metabolism between the genders varies significantly, specifically fibre type distribution, substrate utilisation and post exercise recovery mechanisms. A study employing three 30s all out sprints with 20 mins rest separating the sprints, showed an increased rate of recovery in females compared to males (Esbjornsson-Liljedahl *et al.,* 2002). This was demonstrated by a decrease in high energy phosphates and accumulation of metabolic by products of ATP degradation in women compared to males (Esbjornsson-

Liljedahl *et al.,* 2002). Post exercise plasma concentrations of inosine, Hx, xanthine and uric acid increased, but the magnitude of accumulation was significantly lower in females compared to males. Furthermore, there were smaller exercise induced reductions of ATP, AMP, inosine and hypoxanthine in women compared to men in type II muscle fibres. The lower concentrations of inosine in females compared to male are reflective of faster reamination of IMP to ATP during the recovery periods between sprints and this may be due females containing smaller muscle mass and cross sectional area of muscle fibres; shorter diffusion distances may facilitate an easier recovery of IMP to ATP (Esbjornsson-Liljedahl *et al.,* 2002). The implication of this study may be that females exhibit a reduced potential for purine loss via urinary excretion. Therefore excretion after two HIIE models will be investigated to see if a certain protocol will induce changes more suited to energy loss with female results are compared to the male counterparts.

Differences in lipid metabolism between males and females is contentious with a number of studies stating females derive a greater proportion of energy from lipid than men (Tarnopolsky *et al.,* 1990; Phillips *et al.,* 1993; Friedlander *et al.,* 1998; Horton *et al.,* 1998; Friedlander *et al.*, 1999; Romijn *et al.,* 2000; Blaak, 2001; Carter *et al.,* 2001; Kanaley *et al.,* 2001; Henderson *et al.,* 2007; Kang *et al.,* 2007) whilst others contradict this and have demonstrated that females do not induce greater lipolysis (Burguera *et al.,* 2000; Mittendorfer *et al.,* 2002; Steffenson *et al.,* 2002; Roepstorff *et al*., 2002; Riddell *et al.,* 2003). These discrepancies are most likely due to the differences in testing procedures such as analytical techniques as well as uncontrolled trial days where hormone levels are not accounted for. Oestrogen promotes lipolysis and elevates FFA availability as well as decreasing gluconeogenesis, sparing muscle and liver glycogen stores (Braun and Horton,

2001; D'eon *et al.,* 2002). Increased fat oxidation may transpire due to the oestrogenic effects on CPT1 which is up-regulated by oestrogen, increasing FFA uptake into the mitochondria for oxidation (D'eon *et al.,* 2002) and citrate synthase, an enzyme involved in Krebs Cycle (Roepstorff *et al.,* 2005). Hence providing physiological evidence that fat mobilisation and oxidation is greater in women (Henderson *et al.,* 2007), yet conflicting evidence pertaining to IMTG utilisation remains (Mittendorfer *et al.,* 2002; Roepstorff *et al.,* 2006).

Males on the other hand may derive usable fat from other sources such as IMTG (Mittendorfer *et al.,* 2002) and have a greater total use of glycogen compared to females (Tarnopolsky *et al*. 1990; Esbjornsson-Liljedahl *et al.,* 2002) and greater lactate production (Esbjornsson-Liljedahl *et al.,* 2002) which may be due to skeletal muscle fibre type distribution. Females contain a higher percentage of type fibres 1 muscle fibres, hence more mitochondria thus opportunity for fat oxidation. This thesis will observe any differences between female and males in regards to the reliance on fat which has been shown with HIIE. A greater proportion of type I muscle fibres, implies females have lower amounts of type II glycolytic fibres hence lower glycolytic capacity compared to males. This is reflected in performance during repeat sprints when females show lower power outputs and fatigue at a faster rate. Interestingly the recovery between sprints is similar to that of males, accompanying faster resynthesis of ATP, and no differences between males and females in resting ATP and CP content (Esbjornsson-Liljedahl *et al.,* 2002; Billaut *et al.,* 2003).

The effects of hormonal elicit considerable differences between the genders, for instance catecholamine's which stimulate lipolysis. Conflicting evidence exists pertaining to adrenaline and noradrenaline as increased adrenaline has been observed in men compared

to women (Esbjornsson-Liljedahl *et al*., 2002; Roepstorff *et al*., 2006; Moro *et al.,* 2007) while similar catecholamine responses in men and women were shown in another study (Mittendorfer *et al.,* 2002).

The increased fat oxidation observed in females may be due to an increased sensitivity to β-adrenergic stimulation in adipose tissue resulting in an increased FFA availability, possibly exacerbated as females contain greater adipose tissue depots. As mentioned earlier, exercise induced glycogen depletion is also smaller in women than men in type I muscle fibres which may also be partially due to β-adrenergic induced cAMP activation. Type I muscle fibres may have a greater adrenergic response as there is a greater abundance of receptors, three-fold difference between type II and type I muscle fibres (Esbjornsson-Liljedahl *et al.,* 2002).

Irrespective of a greater fat oxidation capacity, women are not as successful at attaining decreases in adiposity in response to exercise training intervention (Ballot and Kersey 1991; Donnelly and Smith 2005, Henderson *et al.,* 2007). It is possible that this may be due to post exercise differences in metabolism between the genders (Henderson *et al.,* 2007). As mentioned earlier, females ability to reaminate IMP to ATP more effectively than males will lead to a lower requirement of *de novo* synthesis of ATP (see section 2.3.5), hence energy expenditure will be significantly lower in females compared to males (Blaak, 2001; Roepstorff *et al.,* 2006). This, along with ascertaining differences between substrate utilisation is a focus of this thesis.

2.11 Aims and hypothesis

The principal aim of this thesis was to determine metabolic profiles (urine, blood and muscle) of several varied HIIE models to determine which modality of exercise induces metabolic changes that cause energy loss (additional to the energy cost of exercise) and potentially elevate fat utilisation.

2.11.1 Aims and hypotheses of Study 1

Study 1 (chapter 4) aimed to investigate two maximal effort exercise protocols, a short duration HIIE and a supramaximal CON exercise where total exercise time is significantly different (30s to 130s respectively), and monitor plasma and urinary profiles to better understand the metabolic differences between the two modes of exercise.

The specific aims of study 1 were:

1. To provide metabolic profiles of "all out" short duration HIIE and CON (RSA and CCT, respectively). Repeat sprint ability (RSA) exercise is five 6 s sprints separated by 24 s rest while cycling capacity test (CCT) is cycling until volitional exhaustion.
2. Assess whether RSA and CCT elicit similar metabolic shifts that may contribute beneficially to energy loss.
3. To compared the metabolic responses of actual cycling time of the RSA and CCT protocols, 30 s compared to ~130 s

The primary hypothesis of this study is that both metabolically exhaustive exercise bouts will not utilise fat during exercise, but it will be enhanced post exercise, fuelling post exercise oxidative recovery processed.

A secondary hypothesis is that the RSA exercise will induce significantly greater purine loss, with greater plasma accumulation and urinary excretion of hypoxanthine and uric acid.

2.11.2 Aims and hypotheses of Study 2

The aim of study 2 (chapter 5) was to measure and compare metabolic profiles of three workload matched HIIE models, adjusted for exercise intensity and duration to maintain consistent exercise workload over the duration of the HIIE protocol.

The specific aims of study 2 were to:

1. Workload match three models of HIIE, consisting of 30 mins total duration:
    a. 20 s cycling at 150% VO_{2peak}, followed by 40 s rest (150%)
    b. 15 s cycling at 200% VO_{2peak}, followed by 45 s rest (200%)
    c. 10 s cycling at 300% VO_{2peak}, followed by 50 s rest (300%)
2. Provide metabolic profiles of three workload matched HIIE models
3. Determine if the increase in intensity but with decreased cycling time, thus same work performed, induces changes in plasma and urinary markers of glycolytic and purine nucleotide metabolism
4. Assess if fat utilisation is altered in response to different duration rest periods
5. Assess urinary lactate excretion post HIIE (as part of a pilot study)

It is hypothesised that as intensity increases to compensate for the reduced time of the work phase, glycolytic metabolic markers and purine metabolism will be increased. Furthermore, as the rest period is also altered, this will translate to different levels of fat utilisation, marked by increases in plasma glycerol and consistent concentrations of plasma FFA.

2.11.3 Aim and hypotheses of Study 3

The aim of study 3 (chapter 6) was to measure the magnitude of urinary lactate excretion of four different exercise intensity models. The amount of lactate excretion is correlated to energy loss leading to energy deficit.

The specific aims of study 4 were to:

1. Analyse the plasma and urinary lactate concentrations of four different exercise models, two metabolically fatiguing and two workload matched:
    a) RSA exercise
    b) CCT exercise bout
    c) HIIE consisting of 20 s cycling at 150% VO_{2peak} followed by 40 s rest for 30 mins total duration
    d) CON consisting of 30 mins of continuous cycling at 50% VO_{2peak}

2. To determine urinary lactate excretion is increased relative to exercise intensity

It is hypothesised that urinary lactate excretion will correlate with exercise intensity and total work performed; therefore concentration will be the highest in the CCT protocol and the CON the lowest.

2.11.4 Aim and hypotheses of Study 4

Study 4 (chapter 7) aimed to utilise short bouts of "all out" HIIE to ascertain the potential for maximising fat metabolism by inducing extreme metabolic disturbances in males and females. Results from earlier chapters of this thesis were employed to potentially identify an optimal HIIE model that may cause all metabolic changes required to cause energy loss and utilise fat.

The specific aims of study 4 were to:

1. Provide metabolic profiles of two maximal "all out" intensity HIIE
    a. 8 s of all out cycling followed by 12 s rest for 10 mins total duration **(8:12)**
    b. 24 s of all out cycling followed by 36 s rest for 10 mins total duration **(24:36)**
2. To maximise glycolysis and purine metabolism by using maximal effort HIIE protocols whilst potentially utilising fat during the rest periods.
3. Determine if the metabolic responses similar between males and females.

It is hypothesised that the recovery periods separating each high intensity sprint bout will enable fat utilisation, contributing to energy supply. In addition to this, markers of purine base and lactate metabolism will be increased significantly greater in 24:36 HIIE trial compared to the 8:12 HIIE trial.

Chapter three: Experimental Methods and Procedure

The following chapter covers all experimental methodologies used in the exercise performance and biochemistry laboratories.

3.1 Participants

Participants were recruited from the student population at Victoria University. Healthy, untrained male participants aged 18-35 years (yrs) volunteered to take part in four studies. The fifth study employed healthy, untrained females, aged 18-35 yrs.

After receiving a full explanation of each procedure and exercise protocol, all participants provided written and verbal informed consent and completed medical history forms prior to the commencement of participation. The experimental design and all procedures undertaken by participants were approved by the Victoria University Human Research Ethics Committee and performed in accordance with the ethical standards set out in the 1964 Declaration of Helsinki.

3.1.1 Exclusion Criteria for male and female participants

- Too physically fit (a peak oxygen consumption (VO_{2peak}) > 50ml/kg/min)
- Smoker
- Afflicted with a metabolic illness such as Diabetes
- Taking medication that affects metabolism
- Muscular or skeletal injury

3.1.2 Subject Characteristics

The following table contains subject characteristics for the four studies comprising this thesis.

Table 3.0: Participant characteristics of the four studies comprised in this thesis

	Number of participants	Age (years)	Weight (kg)	Height (cm)
Study 1: The effect of short duration high intensity intermittent exercise and continuous exercise on metabolic profiles in healthy untrained males	N = 11	25 ± 5.16	83± 9.56	180 ± 5.8
Study 2: The influence of exercise intensity and rest periods on the metabolic responses following workload matched high intensity intermittent exercise	N = 9 (N = 7 muscle biopsy)	22.7± 4.1	82 ± 6.2	178 ± 4.9
Study 3: Urinary lactate excretion increases as exercise intensity is increased in healthy untrained males	N = 6	28 ± 4.9	85.7± 11.4	180 ± 4.8
Study 4: The metabolic impact of all out high intensity intermittent exercise in healthy adults				
Males	N = 8	29 ± 3.8	77 ± 10	177 ± 7.7
Females	N= 7	27 ± 5	65 ± 8.4	165 ± 4.5

Values expressed as mean ± standard deviation (SD)

After signing the appropriate consent forms and completing a medical questionnaire, participants presented to the exercise performance laboratory at either Footscray Park or St Albans Victoria University campus for preliminary testing.

3.2 Preliminary testing

A digital stadiometer was used to measure height (Proscale Accurate Technology Inc, North Carolina, USA) and digital scales used to measured weight (Riteweigh, Melbourne Australia).

3.2.1 Peak oxygen consumption test (VO_{2peak})

Participants performed a peak oxygen consumption test ($VO_{2\ peak}$) on an Excalibur Lode Cycle Ergometer (Lode, Amsterdam, Netherlands). Males and females completed the same VO_{2peak} protocol but with altered wattage. A standard test protocol was pre-programmed into the Lode software (Lode Ergometry Manager, Amsterdam Netherlands), consisting of 3 min x 3 sub-maximal workloads of 50, 100 and 150 watts (W) for males and 25, 50, 75 W for females. Each subsequent workload increased every minute by 25 W until volitional exhaustion. Participants were encouraged to keep a pedal frequency of 80-90 revolutions per minute (RPM).

Participants were fitted with a Hans Rudolph mask (Hans Rudolph Inc, Kansas, USA) that ensures one way gas exchange and respiratory gas measurements were collected for the duration of the VO_{2peak} test. Respiratory gas measurements were taken using open circuit spirometry indirect calorimetry using a metabolic cart (Turbofit version 5.09 software, Vacumed, California USA). The Hans Rudolph mask was attached to a ventilometer (Pneumoscan s30, California USA) where expired air was directed into a gas chamber and analysed for O_2 and CO_2 (Ametek, Pittsburg, USA). The gas analysers were calibrated prior to each test using commercially prepared gas mixtures (BOC Gases, Australia). Room

conditions (temperature, humidity and pressure) were taken into account using a Kestrel 4000 Pocket Weather Tracker (Nielsen Kellerman, ACT, Australia) and volume was calibrated using a 3L syringe.

3.2.2 Heart rate monitoring

Heart rate was monitored continuously throughout the test by a Polar Electro heart rate monitor (Polar, Finland) with beats per minute (bpm) recorded every 30 s as a safety precaution, but also to give the participant a comprehensive view of their fitness.

3.3 Familiarisation session

The second visit to the exercise performance laboratory was a familiarisation session to habituate the participant to exercise protocol. Participants 'practiced' the exercise protocol for approximately 5 mins (Chapter 5, 6, 7 and 8) and completed the entire protocol in chapter 4 as duration is approximately 2.5 mins. Furthermore, comprehensive and detailed descriptions of all trial day procedures were given to psychologically ease the participant, and so they were aware of all invasive processes. This last aspect of the familiarisation session was particularly important in chapter 5 where muscle biopsies were collected. The importance of maintaining the same dietary and exercise regimes for 48hrs prior to any further laboratory visits was also stressed.

3.4 Experimental Design

At least three to four days after the familiarisation session and at least one week after the VO_{2peak} test, trial days were completed. For males, each trial of the study was

performed at least one week apart, whereas trial days for females were completed a month apart due to hormone fluctuation pertaining to menstruation.

Female participants not on the oral contraceptive pill were tested in the follicular phase of the menstrual cycle; if on an oral contraceptive pill, participants presented for testing between day five and day fourteen of the medication, which correlates to the follicular phase of the menstrual cycle. As can be seen in figure 3.0, reproductive hormones are more consistent during the follicular phase than the luteal phase, minimising hormonal influence.

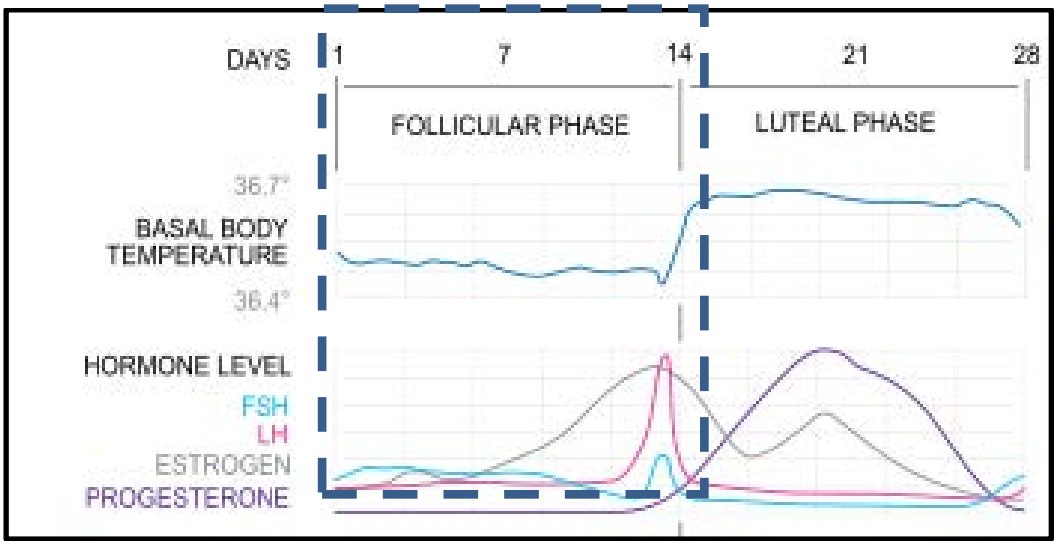

Figure 3.0: Hormonal fluctuations during the follicular and luteal phase of the menstrual cycle in human females (Adapted from Seeley *et al.,* 2006)**.**

The trial days consisted of a rest, exercise and recovery period with data collected throughout all three phases of the trial.

3.4.1 Exercise Protocols

Eight different exercise protocols were employed in this thesis, and two types of exercise bike. Seven of the eight protocols were performed on an Excalibur Lode cycle

ergometer (Lode, Amsterdam, Netherlands) (see figure 3.2), with Lode Manager Software (Lode, Amsterdam, Netherlands) utilised to program the exercise protocols. For the eighth protocol, a Wattbike (British Cycling, Nottingham) was used (see figure 3.1), and the level of resistance on this bike remained on the same sprint level for all trials (Air Brake 7.0).

Figure 3.1: Diagram of the Wattbike employed in chapter 4 and 6 (British Cycling, Nottingham).

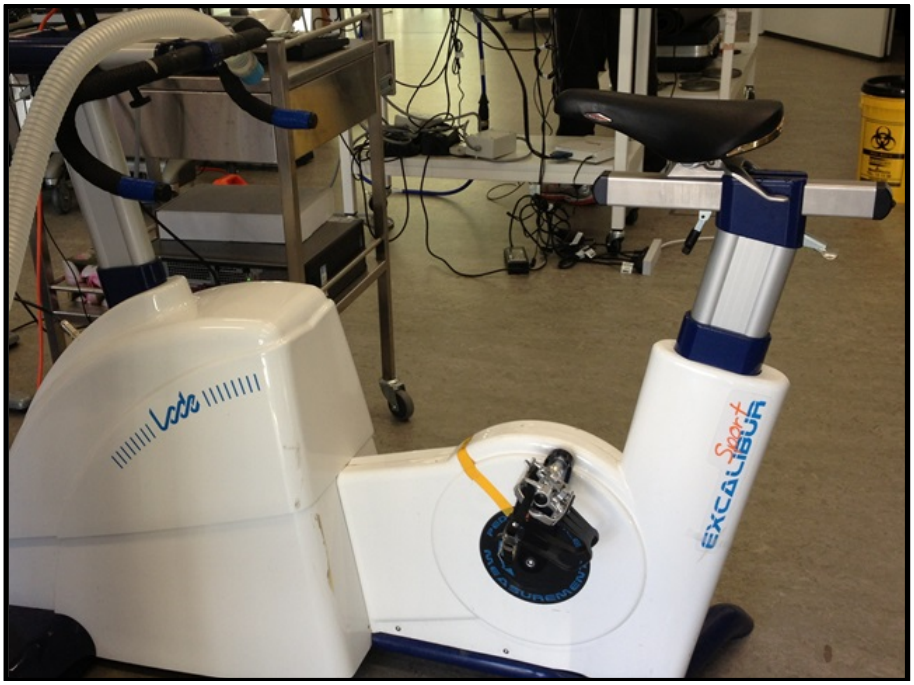

Figure 3.2: Diagram of a Lode bike employed in Chapters 5, 6 and 7 (Lode, Amsterdam Netherlands).

3.4.1.1 Exercise protocols employed in chapter 4

Chapter 4 utilised two metabolically stressing exercise protocols

1. **RSA:** 5 x 6 s all out sprints separated by 24 s (Wattbike)
2. **CCT:** Cycling until fatigue at 110% of power output achieved at VO_{2peak} (Excalibur Lode Bike)

3.4.1.2 Exercise protocols employed in chapter 5

Three HIIE models were utilised in chapter 5, the exercise protocols were matched for workload and exercise duration, exercise intensity is increased as cycling time is decreased as shown in figure 3.3. All cycling tests used the Excalibur Lode bike with HIIE protocols pre-programmed using Lode Ergometry Manager (Amsterdam, Netherlands).

1. **150% HIIE**: Cycling for 20 s at 150% VO_{2peak}, followed by 40 s rest for 30 mins
2. **200% HIIE**: Cycling for 15 s at 200% VO_{2peak}, followed by 45 s rest for 30 mins
3. **300% HIIE:** Cycling for 10 s at 300% VO_{2peak}, followed by 50 s rest for 30 mins

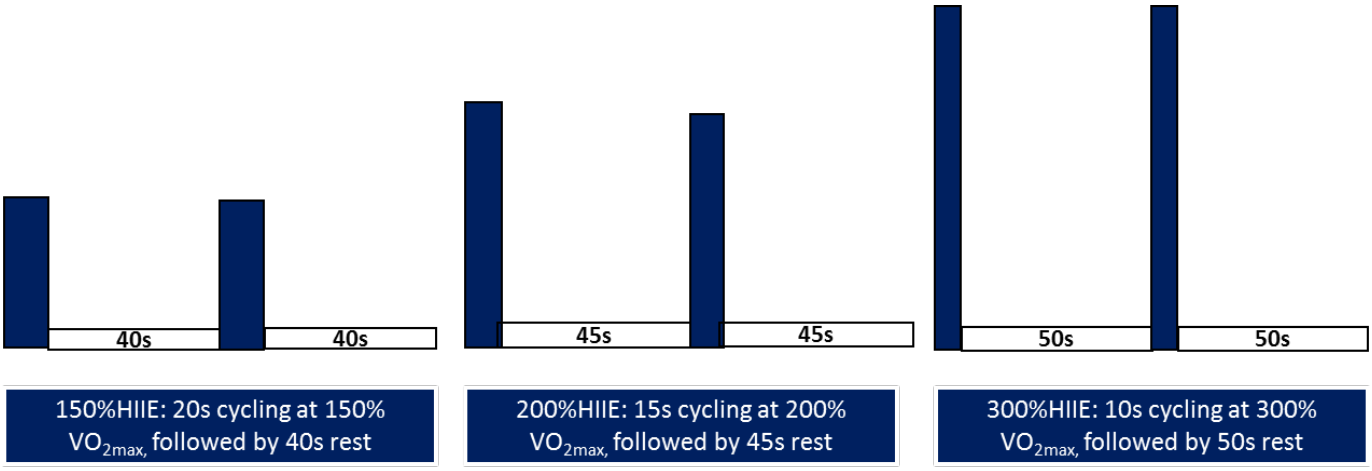

Figure 3.3 Schematic of the HIIE protocol, 150% HIIE, 200% HIIE and 300% HIIE work and rest ratio, used in chapter 5.

3.4.1.3 Exercise protocols employed in chapter 6

Four exercise protocols were employed in chapter 6:

1. Repeated Sprint Ability test **(RSA):** 5 x 6 s all out sprints separated by 24 s (Wattbike)
2. Cycling Capacity Test **(CCT):** Cycling until fatigue at 110% of power output achieved at VO_{2peak} (Excalibur Lode Bike)
3. High intensity intermittent exercise **(HIIE):** 30 mins of cycling for 20 s at 150% VO_{2peak} followed by 40 s rest (Excalibur Lode Bike)
4. Continuous exercise **(CON):** 30 mins of continuous cycling at 50% VO_{2peak} (Excalibur Lode Bike)

The HIIE and CON are workload matched, the HIIE three times the exercise intensity with actual exercise time 1/3 lower.

3.4.1.4 Exercise protocols employed in chapter 7

Chapter 7 studies used an Excalibur Lode bike and the workload applied was set against each participant's body weight on the exercise testing trial day such as with a Wingate protocol.

1. **8:12** maximal, all out cycling for 8 s, followed by 12 s rest for 10 mins
2. **24:36** maximal, all out cycling for 24 s, followed by 36 s rest for 10 mins

3.5 Measurements and Analysis

Respiratory gas exchange, heart rate monitoring, blood collection and urine collected were completed in every study as well as muscle collection in chapter 5.

3.5.1 Respiratory gas sampling

Respiratory gas exchange and calibration of equipment as per $VO_{2peak,}$ section 3.2.1. In all studies, respiratory exchange gas samples were collected for 10mins during rest at the beginning of the trial days. During the HIIE protocols, gas measurements were collected for the duration of exercise in only chapter 5.

During the recovery period, respiratory gas exchange measures were taken immediately post exercise for approximately 10-20 mins and in the last 10 mins of either the 60 or 90 mins of recovery.

Respiratory gases collected during chapter 5, chapter 7 were completed with the Turbofit system, using Turbofit version 5.09 software, (Vacumed, California USA). A Moxus system (AEI Technologies, Pennsylvania, USA) was utilised for chapter 4 and chapter 6.

Software calculated gas measurements every 15 s for the Turbofit system and every 30 s in the Moxus system. Oxygen consumption (VO_2) and respiratory exchange ratio (RER) were utilised with raw data averaged over a 5min period. Due to the supra-maximal exercise intensities and subsequent lactate and H^+ production and the subsequent influence on carbon dioxide production (VCO_2), respiratory gas exchange measures are used only as a comparative measure.

3.5.2 Heart rate monitoring

As per the VO_{2peak} test, a polar heart rate monitor was used during every trial day. Heart rate was continuously monitored as a safety precaution and was collected at rest, throughout exercise and when blood was collected in the recovery period.

During continuous exercise (CON and CCT), heart rate was collected every 30 s. During all HIIE protocols, heart rate was collected at the end of the exercise bout, during the rest period and at the end of the rest just prior to the commencement of the next subsequent exercise period.

3.5.3 Rating of Perceived Exertion (RPE)

RPE was observed at the mid-point of exercise and during the last minute of exercise using the Borg scale RPE as demonstrated in figure 3.4, in chapters 5 and 7. RPE determines the level of physical exertion of the exercise bout, thus enabling the level of physicality of different exercise protocols. Participants were asked to point to a number, corresponding to the level of physical exertion they were experiencing.

6	No exertion
7	
8	
9	
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	
20	Maximal exertion

Figure 3.4: The Borg scale rating of perceived exertion (Adapted from Borg, 1982).

3.5.4 Blood sampling and collection

Upon arrival to the exercise physiology laboratory, participants had a 20 gauge cannula (Optiva, United Kingdom) inserted in the antecubital vein with a stopcock attached for repetitive blood sampling. The cannula was kept patent with periodical flushing with isotonic saline solution (0.9% NaCl), (AstraZeneca, USA).

An aliquot of whole blood was dispensed into a lithium heparin vacutainer while a further aliquot was dispensed into an ethylenediaminetetraacetic acid Vacutainer (0.117 mL of 15% K3 EDTA (17.55mg), BD Vacutainers). Blood was dispensed into eppendorf tubes and immediately spun in a centrifuge for 2 min at 12,000RPM (Centrifuge 5415 C, Eppendorf, Hamburg, Germany) and plasma frozen in liquid nitrogen for later analysis. Plasma FFA, glycerol and insulin were analysed from the EDTA treated sample and plasma lactate, glucose, Hx, xanthine, inosine and uric acid from the lithium heparin treated sample.

3.5.4.1 Study time points

The following table contains the time points where blood samples were collected for each study. Every study collected a rest blood sample, prior to commencement of exercise with the participant resting quietly in a supine position.

Table 3.1: Blood collection time points

	Exercise	Recovery
Study 1	R00 (end of exercise)	R5, R10, R15, R30, R60
Study 2	E30 (end of exercise)	R5, R10, R15, R30, R60, R75, R90
Study 3	R00 (end of exercise)	R5, R10, R15, R30, R60, R75, R90

Study 4	E5, E10 (E10= end of exercise)	R15, R30, R60, R75, R90

3.5.4.2 Plasma analysis

3.5.4.2.1 Plasma free fatty acids (FFA)

Plasma FFA concentration were analysed in duplicate using a Wako non esterified fatty acids (NEFA) kit (NEFA ,Wako, Japan), that was adapted for a ninety six well, clear bottom plate.

Principle

NEFA are converted to Acyl-CoA, adenosine monophosphate (AMP) and pyrophosphoric acid (PPi) due to the action of Acyl-CoA synthetase (ACS). Acyl-CoA oxidase (ACOD) causes Acyl-CoA to oxidise, yielding 2, 3 transenoyl-CoA and hydrogen peroxide. In the presence of peroxide, hydrogen peroxidase gives a blue-purple pigment by quantitative oxidation condensation with 3-methyl-N-ethyl-N-(B-hydroxyethyl)-aniline (MEHA) and 4-aminoantipyrine. The colour change is diluted and spectrophotometrically quantitated according to a standard curve.

Five µl of sample was pipetted into the corresponding wells. The standards and blank were aliquotted as per table 3.2 below.

Table 3.2: NEFA standard concentrations

Sample	1.0 mEq/L NEFA standard (µL)	DD H_2O (µL)
Blank		5
25% standard	1.25	3.75
50% standard	2.5	2.5
75% standard	5	
100% standard	7.5	
125% standard	10	
150% standard	12.5	
200% standard	15	

* DD H_20 = Deionised double distilled water

Ninety µl of reagent one (Acyl-CoA synthetase, Coenzyme A, Adenosine-5' triphosphate disodium salt, 4-Aminoantipyrine, Ascorbate oxidase and phosphate buffer, pH 7.0) was pipetted into every well and was mixed for 30 s on the plate shaker on low speed. The plate was incubated for 10mins at 37°C (no longer than 15 mins as optimal enzymatic activity occurs by 10 mins of incubation). One hundred and eighty µl of reagent two (Acyl-CoA oxidase, Peroxidase, 3-methyl-N-ethyl-N-(B-hydroxyethyl)-aniline) was pipetted into every well, again mixed for 30 s on the plate shaker and incubated for 10 mins at 37°C. The plate was allowed to sit for 5 mins at room temperature and NEFA concentration was obtained spectrophometrically at 550nm using an X-mark microplate reader (Bio-Rad Laboratories, Australia). A standard curve was produced utilising the NEFA standards and the unknown sample concentrations determined.

3.5.4.2.2 Plasma glycerol

Plasma glycerol was analysed in duplicate spectrophotometrically via a commercially available kit "Serum Triglyceride Determination Kit" (Sigma Aldrich Laboratories, Sydney, Australia), adapted to be used on a ninety six well plate.

This method enzymatically produces a quinoneimine dye that shows an absorbance which is directly proportional to glycerol concentration in the sample.

Principle

$$\text{Glycerol + ATP} \xrightarrow{\textit{Glycerol kinase}} \text{G-1-P + ADP}$$

$$\text{G-1-P + } O_2 \xrightarrow{\textit{Glycerol phosphate oxidase}} \text{DAP + } H_2O_2$$

$$H_2O_2 + \text{4-AAP + ESPA} \xrightarrow{\textit{Peroxidase}} \text{Quinoneimine dye + } H_20$$

To construct a standard curve to determine unknown sample concentration, a 5mM stock of glycerol was constructed. The below table, table 3.3, demonstrates how each glycerol standard was made.

Table 3.3: Glycerol standard concentrations

Concentration (µM)	5mM glycerol stock (µl)	DD H_2O (µl)
0	0	1000
50	10	990
100	20	980
200	40	960
500	100	900

Twenty five µl of sample, standard and blank were pipetted into the corresponding well of the clear bottom plate. As fast as possible without producing bubbles, 225µl of glycerol reagent (0.75 mM ATP, 3.75 mM Magnesium salt, 0.188 mM 4-Aminoantipyrine,

2.11 mM *N*-Ethyl-*N*-(3-sulfopropyl) *m*-anisidine, sodium salt, 1,250 units/L Glycerol Kinase (microbial), 2,500 units/L Glycerol Phosphate Oxidase, 2,500 units/L Peroxidase (horseradish), buffered to 7.0, with 0.05% sodium azide (added as preservative to prevent degradation) was pipetted into every well. The plate was incubated at 37°C for 5 mins before being read in the X-mark plate spectrophotometer at 540nm. A standard curve was produced to determine unknown plasma glycerol concentrations.

1.5.4.2.3 Plasma glucose and lactate

Plasma lactate and glucose were measured in duplicate using a Yellow Springs Analyser (YSI 2300 STAT; Yellow Springs Instruments (YSI), Ohio, USA). The method is based on glucose and L-lactate oxidase methods for glucose and lactate, respectively.

Principle

L-Lactate

$$\text{L-Lactate} + O_2 \xrightarrow{\textit{L-Lactate oxidase}} \text{Pyruvate} + H_2O_2$$

$$H_2O_2 \xrightarrow{\textit{Platinum anode}} 2H + O_2 + 2e$$

Principle

Glucose

$$\text{Glucose} + O_2 \xrightarrow{\textit{Glucose oxidase}} \text{Glucomic Acid} + H_2O_2$$

$$H_2O_2 \xrightarrow{\textit{Platinum anode}} 2\,H + O_2 + 2e$$

Twenty five µL of sample was injected into the sample chamber of YSI analyser. Lactate and glucose diffused into a membrane that is placed over an electrochemical probe containing L-Lactate oxidase and glucose oxidase. L-lactate was oxidised to pyruvate and hydrogen peroxide whereas glucose was oxidised to gluconic acid and hydrogen peroxide. The hydrogen peroxide was detected amperometrically at the surface of the platinum electrode. The current flow at the electrode is directly proportional to the hydrogen peroxide concentration, thus also to the L-lactate and glucose.

3.5.4.2.4 Plasma insulin

Plasma insulin was measured in duplicate using a spectrophotometric insulin enzyme linked immunoassay (ELISA) kit (Dako, Denmark).

Principle

This ELISA kit is based on two monoclonal antibodies. When the sample and the antibody labelled enzyme are incubated in the wells of the ninety six well plate that is coated in anti-insulin antibody, a complex forms (see figure 3.5). Washing of the plate removes any unbound antibody and the bound conjugate is detected using a substrate reaction with 3,3'5,5'tetramethylbenzidine (TMB). An acid (stop solution) produces a colourimetric endpoint and is read spectrophotometrically at 450nm.

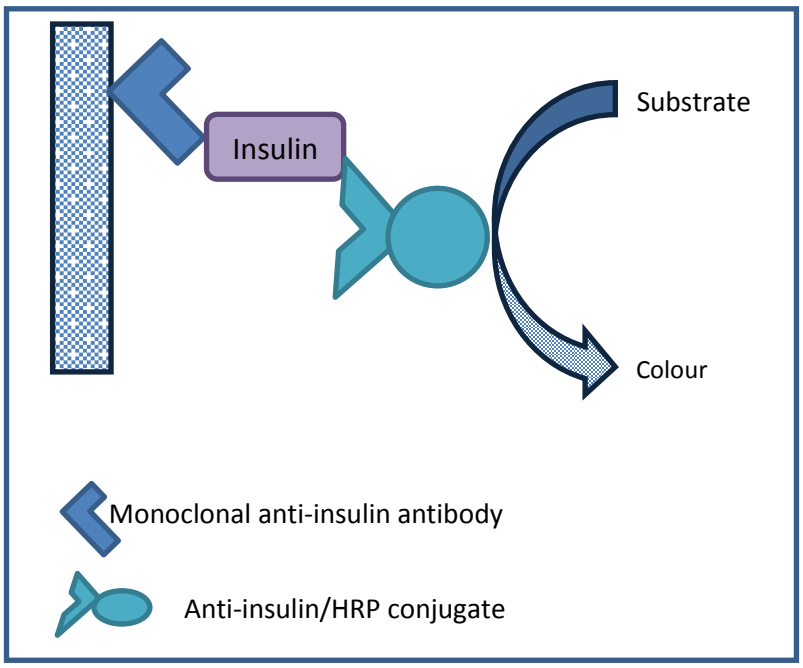

Figure 3.5: Schematic diagram of Insulin ELISA principle (adapted from Dako, Denmark).

Twenty five µl of standard or sample was pipetted into the appropriate well. The concentrations of the insulin standards are depicted in the below diagram (figure 3.6).

Calibrator	1	2	3	4	5
µIU/mL	0	5	15	75	180
pmol/L	0	30	90	450	1080

Figure 3.6: Plasma insulin standard concentrations used in the 96 well plate, provided by DAKO (Dako, Denmark).

One hundred µl of conjugate was then pipetted into each well and the plate incubated with shaking at room temperature for 60 mins to ensure binding. After 60 mins, the plate was washed three times manually, with wash buffer. 290µl of wash buffer was pipetted into each well and removed before the next wash with contents tapped dry on

absorbent paper towel. One hundred µl of substrate (TMB) was then aliquotted to each well and again incubated whilst shaking for 10 mins before the acidic stop solution was added (100µL). The plate was read immediately at 450nm in the X-mark plate reader. A standard curve was completed (four parameter sigmoid curve) and unknown plasma insulin concentrations determined.

3.5.4.2.5 Plasma purines (hypoxanthine (Hx), xanthine, inosine and uric acid)

Using high performance liquid chromatography (HPLC), plasma Hx, inosine, xanthine and uric acid, and standards, were measured in singlicate. Standards were measured to construct a standard curve from which unknown sample peak height and retention time could be correlated. Samples were extracted before run through the HPLC.

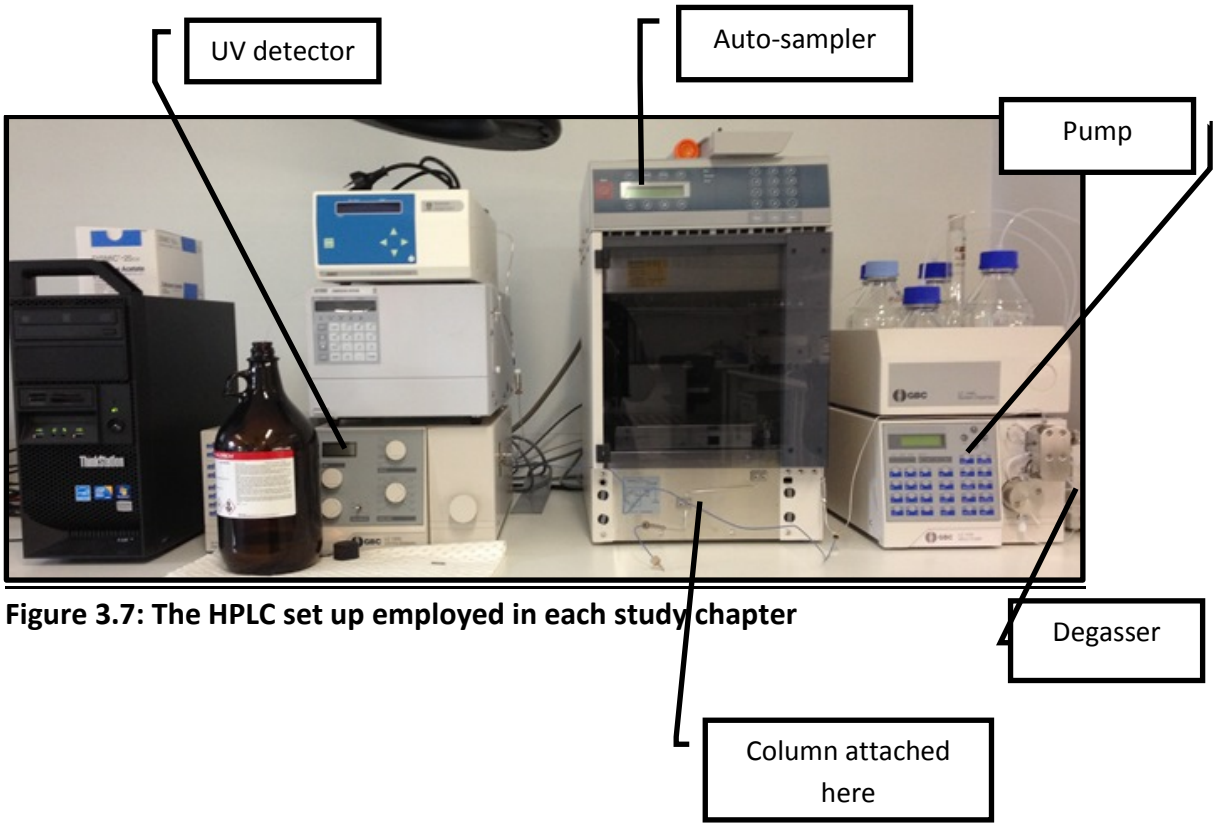

Figure 3.7: The HPLC set up employed in each study chapter

Principle

HPLC is used to separate compounds pertaining to their size. HPLC uses a pump to pass solvent that is pressurised and the sample through a column that is filled with sorbent, causing separation of components in the sample (see figure 3.7). The different components in the sample are separated at different times due to the different degree of interaction with the sorbent.

One hundred µl of sample was added to 50µl of 1.5M Perchloric acid (PCA) in an eppendorf tube and vortexed. To neutralise, 37µl of potassium bicarbonate ($KHCO_3$) was added to each eppendorf, vortexed and centrifuged at zero degrees for 15,000RPM, for 5 mins. The samples were left to sit in the fridge (2-8°C) for 5 mins before another centrifugation cycle and the supernatant pipetted and stored in HPLC polypropylene tapered vials for small injection volumes (Waters, MA, USA) until analysed.

Four standards containing different concentrations of hypoxanthine (Hx), inosine, xanthine and uric acid were constructed. The table below (table 3.4) contains the contents of each standard.

Table 3.4: Hx, inosine, xanthine and uric acid concentrations of each standard used to create a standard curve used to determine purine concentrations in each sample.

Standard	Final [Hx] (µM)	Final [inosine and xanthine] (µM)	Final [uric acid] (µM)
1	1	1	600
2	5	2.5	400
3	20	10	200
4	40	20	100

The analytical column used was a Luna 5u C^{18} Column 100A, 250mm x 4.6 mm I.D. 5 micron with a C^{18}, 3.0 mm ID guard column (Phenomenex, Lane Cove Australia). The guard column changed every 100-150 samples. The mobile phase consisted of 0.1% Trifluoroacetic acid (TFA) pH2.2 (Sigma Aldrich Laboratories, Sydney, Australia) and methanol gradient (Sigma Aldrich Laboratories, Sydney, Australia). The mobile phase gradient was as follows 99:1 0.1%TFA: Methanol (v/v) at 0 mins – held for 3 min; 70:30 0.1%TFA: Methanol (v/v) at 10 mins; 5:90 0.1%TFA: Methanol (v/v) at 11 mins and held for 2 mins; 99:1 0.1%TFA: Methanol (v/v) at 14 mins; a total of 25 min trace time. The mobile phase was continuously degassed using an ERC34152 degasser (GBC Scientific, Victoria Australia) at a flow rate of 1.0ml/min.

Prior to commencement of injection, the pump was equilibrated and each solvent de-gassed/purged for 5 mins. This was performed before the initiation of every run.

Fifteen µl of deproteinised sample was injected using and LC1650 auto-sampler (GBC Scientific, Victoria Australia) with data collected at an optimal UV wavelength absorption 250nm. Each standard was arranged in the auto-sampler to be analysed first with samples following. After every 10 samples, a standard was placed to ensure detection remained consistent. Data acquisition and analysis of retention time and peak heights were performed using EZchrom SI software (GBC Scientific, Victoria Australia) (Method adapted from Farthing *et al.,* 2007).

Using the four standards, a standard curve was constructed for each of the four purines and unknown plasma concentrations of Hx, inosine, xanthine and uric acid were calculated.

3.5.5 Urine sampling and analysis

Participants collected total urine volume at rest as well as upon completion of the recovery period (90 mins post exercise). In chapter 4 (study 3), collection was after 60 mins of recovery. Participants were instructed to consume water *ad libitum* throughout the exercise trials to ensure adequate urinary output. Volume of urinary output was measured, with an aliquot frozen for analysis of urinary purines (Hx, inosine, xanthine and uric acid). In chapters 6, 7 and 8, urine was analysed immediately for urinary lactate.

Analysis of concentration of each metabolite is per plasma analysis detailed in section 3.5.4.2.5 for HPLC analysis of urinary purine bases and 3.5.4.2.3 for urinary analysis of lactate.

Whole volume and duration of time was used to calculate excretion in $\mu mol.hr^{-1}$ for purine nucleotide bases and $mmol.hr^{-1}$ for urinary lactate.

3.5.6 Muscle sampling and collection

Muscle samples were collected in via muscle biopsy. A small amount of muscle (~120-180 mg) was extracted from the vastus lateralis muscle under local anaesthesia (20mg/2ml, Xylocaine, AstraZeneca, USA) via needle biopsy (Bergstrom, 1975). The sample was immediately snap frozen in liquid nitrogen at -172°C and stored at -80°C until cut for freeze drying.

3.5.6.1 Freeze-drying

Muscle samples were cut in liquid nitrogen to ensure they remained frozen at all times. 20-30mg (wet weight) of muscle was obtained. The normal dry to wet muscle weight ratio is approximately one to four. Twelve samples per freeze dry cycle were placed in the freeze dryer (Edwards Modulo, High Vacuum, and Britain England) for a minimum of 48 hrs.

Upon completion, samples were transferred to a desiccator with fresh, dried desiccant and were allowed to reach room temperature. The main benefit of freeze drying is that the sample is stable at room temperature.

Muscle samples were weighed to determine dry weight, thus able to calculate the dry to wet weight ratio. It should be 23% ± 2%. All muscle samples were within this range. Approximately one hour after being removed from the freeze dryer, samples are crushed and metabolites extracted.

3.5.6.2 Muscle crushing and extraction

In accordance with method of Harris *et al.* (1974), each muscle sample was weighed to get the 'dry weight'. Samples were crushed and powdered in a crucible and any connective tissue was removed. Approximately 2mg of powdered muscle was placed into an eppendorf tube for extraction of metabolites lactate, ATP-CP and creatine. An additional 1mg of powdered muscle was dispensed into another eppendorf tube for extraction of glycogen.

3.5.6.2.1 Metabolite extraction (Lactate, Creatine, ATP-CP)

This procedure was performed completely on ice and the centrifuge pre-cooled. Two hundred and fifty µl of ice cold 0.5M PCA/1mM EDTA was added to the eppendorf tube containing the 2mg powdered muscle sample. The PCA is a strong oxidizing agent, extracting all of the acid-soluble agent and acid-soluble metabolites whilst simultaneously denaturing proteins causing them to precipitate out. Each sample is 'tapped' and then vortexed in rotation for 10 mins, remaining on ice between vortex cycles. The tubes are tapped to ensure all fibres are removed from the wall of the eppendorf tube. The samples were then spun at 28,000RPM for 2 mins at the pre-cooled temperature of 0°C. Upon completion, the

eppendorf tubes were placed back in ice. 200µL of the supernatant was removed without disturbing the pellet, and placed into a second eppendorf tube. Fifty µL of $KHCO_3^-$ was added to each sample, vortexed again, and left on ice to sit for 5 mins. $KHCO_3^-$ neutralises the acid, forming another precipitate. Samples again were centrifuged at the same settings and the supernatant pipetted into the corresponding labelled cryule and stored at -80°C until analysed for ATP-CP, creatine and lactate.

3.5.6.2.2 Glycogen extraction

Prior to beginning the glycogen extraction procedure, the incubator was pre-heated to 100°C.

Two hundred and fifty µl of hydrochloric acid (HCl) was added to an eppendorf tube of each 1mg of powdered muscle sample to breakdown membrane structures and release the substrate, with the weight recorded. Samples were incubated at 100°C for 2 hrs and agitated on the hour. Upon completion, weight was recorded and each sample neutralised with 750µl of 0.667M sodium hydroxide (NaOH). Weight was recorded again and samples stored at -80°C for analysis of glycogen content.

3.5.6.3 Metabolite analysis

Methodologies for ATP-CP, creatine, lactate and glycogen were adapted from Lowry and Passonneau, (1972) and further altered for analysis using a fluroskan plate reader (Thermofisher, USA) and white bottom ninety six well plate as fluorescence is detected from the top of each well on the plate.

3.5.6.3.1 ATP-CP

Analysis of ATP and CP is a three step enzymatic process. Each sample, blank and standard was analysed in duplicate and change in fluorescence was determined using the Fluroskan.

Principle

$$\text{P-Creatine + ADP} \xrightarrow{\textit{Creatine kinase}} \text{Creatine + ATP}$$

$$\text{ATP + Glucose} \xrightarrow{\textit{Hexokinase}} \text{ADP + G-6-P}$$

$$\text{G-6-P + NADP} \xrightarrow{\textit{Glucose dehydrogenase}} \text{6-P-Gluconolactone + NADPH}$$

Ten µl of sample, blank, ATP standards, CP standards and NADH standards was pipetted in the corresponding well of the ninety six well plate. Prior to beginning plating, a NADH standard curve was constructed via recording absorbance of the NADH standards on UV-visible spectrophotometer (Shimadzu) at 340nm. Changes in fluorescence of ATP and CP standards are checked against the NADH standard curve. The reagent consisting of all items in table 3.5 was added to each well.

Table 3.5: Reagent cocktail to determine ATP-CP concentration

Reagent	Stock concentration	Final concentration	To make 100ml of cocktail reagent
Tris buffer (pH 8.1)	1M	50mM	5ml
Magnesium chloride (MgCl)	1M	1mM	100µl

Dithiothreitol (DTT)	0.5M	0.5mM	100µl
Glucose	100mM	100uM	100µl
Nicotinamide adenine dinucleotide phosphate (NADP)	0.05M	50uM	100µl
Glucose 6 phosphate dehydrogenase (G-6-P-DH)			10µl

In the presence of this combination, G-6-P dehydrogenase, gives an indication of NADPH, read on the fluroskan. Hexokinase is then added to each well initiating the 2nd reaction, producing G-6-P, needed for the third reaction to occur. The plate was incubated for 30 mins at room temperature before being read a second time. Reading one subtracted from reading two gives the change in NADPH, hence the ATP concentration in the sample. The last enzyme added is Creatine kinase and ADP. The plate was then incubated for 60 mins at room temperature before the third reading. The NADPH produced in this step is attributed to CP present in sample.

3.5.6.3.2 Creatine

Obtaining muscle creatine content is a three step enzymatic process. Sample, standards and blank was analysed in duplicate and read on the Fluroskan.

Principle

$$\text{Creatine + ATP} \xrightarrow{\textit{Creatine Kinase}} \text{ADP+P-Creatine}$$

$$\text{ADP+P-Pyruvate} \xrightarrow{\textit{Pyruvate Kinase}} \text{ADP+ Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\textit{Lactate dehydrogenase}} \text{Lactate} + \text{NAD}$$

Prior to plating sample, standards and blanks, a small volume of 15mM NADH standard was recorded on UV-visible spectrophotometer at 340nM. The change in fluorescence of CP standards was then checked against the 15mM NADH standard.

Thirty µl of sample, CP standards and blank were pipetted into the corresponding well of the ninety six well plate. The reagent cocktail contents are listed in table 3.6.

Table 3.6: Reagent cocktail to determine Creatine concentration

Reagent	Stock concentration	Final concentration	To make 100ml of reagent cocktail
Imadazole (pH 7.4)	1M	50mM	5ml
MgCl	1M	5mN	500µl
Potassium chloride (KCl)	1M	30mM	3000µl
Phosphoenolpyruvic acid (PEP)	10mM	0.1mM	960µl
ATP	Solid	0.2mM	12mg
LDH	5mg/ml	1ug/ml	20µl
PK	10mg/ml	5ug/ml	50µl

The plate was incubated for 15 mins at room temperature before reading on the Fluroskan. This reading shows any residual NADH that may be present. An enzyme consisting of creatine kinase and 0.05% bovine serum albumin, the catalyst for the second reaction was added to each well of the plate and incubated for 60 mins at room temperature. The plate is read for a 2nd time and reading three 15 mins later. Pyruvate is produced which is catalyst for reaction three.

3.5.6.3.3 Lactate

Obtaining muscle lactate concentration is a two- step enzymatic process.

Principle

$$\text{Lactate} + NAD^{+} \xrightarrow{\textit{Lactate Dehydrogenase}} \text{Pyruvate} + NADH + H^{+}$$

$$\text{Pyruvate} + \text{Hydrazine} \longrightarrow \text{Pyruvate hydrazone}$$

Prior to plating the reagent, a NADH standard curve recorded on UV-visible spectrophotometer at 340nm. Changes in fluorescence of Lactate standards are checked again the NADH standard curve.

The reagent cocktail (see table 3.7) was pipetted into the corresponding wells of the ninety six well plate and incubated for 30mins before being read on the Fluroskan. LDH initiates the reaction hence incubation and reading prior to the addition of any sample or standard.

Table 3.7: Reagent cocktail to determine Lactate concentration

Reagent	Stock concentration	Final concentration	To make 100ml of reagent cocktail
Hydrazine	1M	100mM	10ml
Glycine	1M	100mM	10ml
NAD	0.1M	0.5mM	400µl
LDH	25mg/ml		200µl

In duplicate 5µl of sample, blank and lactate standards were pipetted into the corresponding well. The plate was incubated for an additional 60 min at room temperature before reading a second time.

3.5.6.3.4 Glycogen

Obtaining muscle glycogen concentration is a twostep enzymatic process.

Principle

$$\text{Glucose + ATP} \xrightarrow{\textit{Hexokinase}} \text{ADP + Glucose 6-P}$$

$$\text{Glucose 6-P + NADP} \xrightarrow{\textit{Glucose-6-P Dehydrogenase}} \text{6-P-Gluconolactone + NADPH}$$

Prior to plating samples, blank and glycogen standards, a NADH standard curve recorded on UV-visible spectrophotometer at 340nm. Changes in fluorescence of glucose standards are checked again the NADH standard curve.

5ul of blank, glucose standards and sample in duplicate was added to corresponding well of the ninety six well plate. Reagent cocktail was added to every well of the plate before being read for the first time on the Fluroskan. The reagent components are listed below in table 3.8.

Table 3.8: Reagent cocktail to determine Glycogen concentration

Reagent	Stock concentration	Final concentration	To make 100ml of reagent cocktail
Tris buffer (pH 8.1)	1M	50mM	5ml
MgCl	1M	1mM	100µl
DTT	0.5M	0.5mM	100µl
ATP (pH. 7.0)	0.3M	0.3mM	100µl
NADP	0.05M	50uM	100µl
G-6-P-DH			10µl

Hexokinase is added to each well, resulting in second reaction, producing G-6-P. The plate is incubated for 60 mins at room temperature and read a final time.

3.6 Statistical Analysis

All results are expressed as means ± standard error of the mean (SEM), except fro subject characteristic data which is expressed as mean ± standard deviation. All data are checked for distribution and variance. All results plasma, muscle and urinary, RPE, power, heart rate and respiratory gases were analysed using Graph-pad Prism software version 6.02 (Graphpad software, USA) and two-way repeated measures ANOVA with time as the within group factor and exercise protocol as the between group factor used to detect significance. Where an interaction between trials was detected, post hoc tests were completed.

Tukeys and Bonferroni multiple comparison tests were employed to find statistical significance from exercise and recovery time points to resting values in muscle (chapter 5) and plasma results for each study of this thesis. Sidaks multiple comparison tests was utilised to detect differences between exercise bouts at specific time points and was utilised in each study for the plasma results and in chapter 5 when analysing muscle metabolites.

A matched, paired t-test was used to determine differences in total work (kJ) investigated in chapter 4, 6 and 7. Paired T tests were also performed on urinary results from each study, comparing post exercise to basal values and determining if the two recovery values were significantly different. Statistical significance was accepted at ($p < 0.05$).

Chapter four: The result of short duration high intensity intermittent exercise and continuous

exercise on metabolic profiles in healthy untrained males

4.1 Introduction

Researchers have observed that individuals who engage in regular high intensity exercise appeared to have more lean muscle mass and less fat mass compared to those performing prolonged moderate exercise activities (Tremblay *et al.,* 1990), which has been thought optimal exercise for fat oxidation (Romijn *et al.,* 1993). A review by Shaw *et al.* (2006) concluded that CONT does not reduce adiposity, whereas HIIT is a more effective exercise strategy for fat loss (Tremblay *et al.,* 1994; Trapp *et al.,* 2008; Gremeaux et al., 2011; Macpherson et al., 2012) and results in reduced adiposity even when there is lower energy cost estimates of the HIIT (Tremblay *et al.,* 1994; Trapp *et al.,* 2008). To further demonstrate this anomaly, a recent, unpublished study (submitted) from our laboratory profiling acute workload-matched submaximal continuous exercise (CON) and HIIE exercise bouts, showed that plasma and respiratory markers of fat oxidation were not different between HIIE and CON, attributed to the work-rest mode of HIIE (Borg *et al.,* 2008). This study also demonstrated significantly increased urinary purine (Hx and uric acid) loss after the HIIE compared to the CON bout. Increased urinary purine loss is equated to lost energy, with energy expenditure elevated due to metabolically costly *de novo* synthesis of ATP, compared to the purine salvage pathway (Newsholme and Leech 1983). As mentioned in section 2.9.2.4, plasma and urinary Hx concentrations and hence ATP degradation, is increased when number of sprints increases (Stathis *et al.,* 1999), due to elevated deamination of IMP to Hx (Stathis *et al.,* 1994; Tullson *et al.,* 1995). Therefore it is logical that a repeated sprint protocol such as HIIE would induce significantly greater purine loss

compared to the CON bout. Accordingly this results in greater energy loss and a greater ATP requirement to replenish stores post HIIE compared to CON, potentially elevating energy expenditure.

4.1.1 Aims and hypothesis

This study aimed to investigate and compare the metabolic differences of two metabolically stressing exercise protocols (RSA and CCT) of the same total duration, but actual exercise time is different 30s for RSA (five 6 s all out sprints followed by 24 s rest) compared to 130-140 s of CCT. Utilising plasma and urinary profiles of substrate and purine base metabolism, this study examined the energy loss and substrate utilisation of each exercise protocol to determine which may be better at increasing total energy loss and potentially reducing adiposity if utilised in a training program.

It is hypothesised that purine nucleotide concentrations will be greater in the plasma and urine in the RSA exercise trial compared to the CCT, due to repeated sprint nature of the exercise mode compared to one continuous bout. In addition, lipid sources may be used during exercise in the RSA exercise trial due to work and rest nature of the exercise mode compared to the CCT trial (Tremblay *et al.,* 1994).

4.2 Methods

4.2.1 Participant characteristics

Eleven healthy, untrained adult males aged between 18-35 years (25 ± 5.16 yrs; 83.16 ± 9.56 kg; 180.27 ± 5.86 cm; 49.24 ± 9.7 ml.kg.min^{-1}) volunteered to take part in this study. The experimental design and all procedures undertaken by participants were approved by the Victoria University Human Research Ethics Committee and performed in accordance with the ethical standards set out in the 1964 Declaration of Helsinki. Participants provided written and verbal informed consent and completed medical history forms prior to the commencement of participation.

4.2.2 Preliminary Testing

Before trial days, all participants underwent a $VO_{2\ peak}$ test on a Lode Cycle Ergometer, as described in section 3.2.1.

4.2.2.1 Respiratory gas exchange

Participants were fitted to a Hans Rudolph mask and expired air was directed through a ventilometer (Moxus; AEI Technologies, Pennsylvania, USA). For additional details referring to respiratory gas exchange collection, see section 3.2.1.

4.2.3 Familiarisation session

Participants underwent a familiarisation session as described in section 3.3.

4.2.4 Trial day procedure

Twenty-four hours prior to the trial day, participants were instructed to abstain from caffeine, physical activity and alcohol and to consume a healthy diet. They were asked to maintain a 24 hr food diary in order to replicate meals for the following trial days to prevent variation.

4.2.4.1 Experimental Design

On two separate days, participants completed a high intensity exercise bout before resting for 60 mins. The exercise models are designed to elicit metabolic fatigue and were Repeat Sprint Ability **(RSA**) in which participants performed five 6 s maximal all out sprints separated by 24 s and a Cycling Capacity Test **(CCT)** at 110% of power output achieved at VO_{2peak} (315 ± 69 W), typically lasting approximately 130-140 s.

On the day of an experimental trial, participants arrived at the laboratory in the morning in a post-absorptive state after an overnight fast of approximately 10-12 hrs. The start time for each trial for a given participant remained consistent to avoid influence of circadian variance. Participants were instructed to consume water *ad libitum.*

4.2.4.2 Respiratory gas collection and analysis

Respiratory gas exchange was measured and analysed as per section 3.2.1. Measurements were obtained at rest; 10 min post exercise and for the last 10 min of the 60 min recovery period.

4.2.4.3 Blood collection and analysis

Method of blood collection is described in section 3.5.4. All samples were analysed for plasma concentrations of FFA, glycerol, lactate, glucose, insulin, inosine, xanthine, Hx and uric acid, described in section 3.5.4.2. Blood samples were collected at the following time points: rest, immediately post exercise (R00) and during recovery at 5 (R05), 10 (R10), 15 (R15), 30 (R30) and 60 min (R60) post exercise.

4.2.4.4 Urine collection and analysis

Urine was collected and measured with methods set out in section 3.5.5. The complete volume of urine was measured, with an aliquot frozen at -20°C for analysis of inosine, Hx, xanthine and uric acid.

4.2.5 Statistical Analysis

Subject characteristic results are expressed as mean ± standard deviation (SD). All other results are expressed as means ± standard error of the mean (SEM). All results, plasma, urine, heart rate and respiratory gases were analysed using Graph-pad Prism software version 6.02, detailed in section 3.6. The plasma, heart rate and respiratory gas (VO_2 and RER) results were analysed using a two-way repeated measures ANOVA with time as the within group factor and exercise protocol as the between group factor used to detect significance for the plasma results. Where an interaction between trials was detected, post hoc tests were completed, Bonferroni, Tukeys and Sidak as mentioned in section 3.6. A matched, paired t-test was used to determine differences in total work (kJ) as well as on urinary results comparing post exercise to basal values and determining if the two recovery values were significantly different. Statistical significance was accepted at ($p < 0.05$).

4.3 Results

There were no significant differences between RSA and CCT trials at any time point of any plasma or urinary marker of metabolism.

4.3.1 Physiological markers

4.3.1.1 Total work (kJ)

Total work (kJ) was significantly different between RSA and CCT exercise, with CCT significantly greater than RSA (p=0.0004).

Figure 4.0: Total work in kilojoule (kJ) performed in RSA and CCT exercise bouts
Data are means ± SEM; N = 11
p<0.001 significantly different between trials

4.3.1.2 Oxygen consumption (VO_2)

There was a significant increase in VO_2 in both RSA and CCT trials, increasing with exercise and remaining above basal levels until 10 mins post exercise ($p<0.05$). There were no differences detected between the RSA and CCT trials.

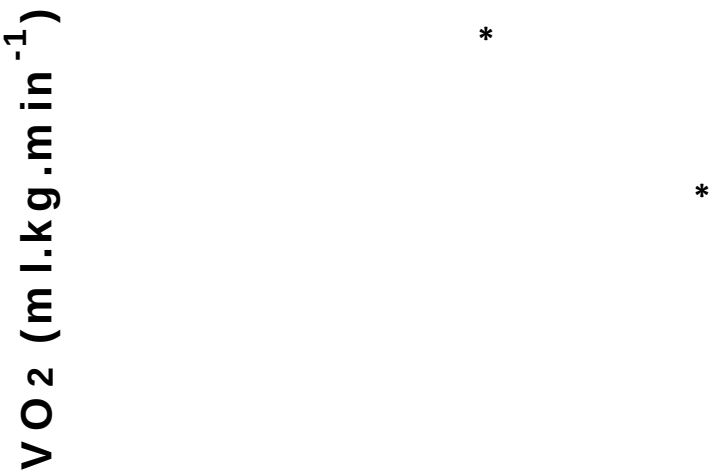

Figure 4.1: Oxygen consumption (VO2) at rest, during exercise and recovery of RSA and CCT exercise
Data are means ± SEM; N = 11
* $p<0.05$ significantly different from rest

4.3.1.3 Respiratory exchange ratio (RER)

There was a significant interaction in RER (p=0.0248). In both high intensity trials, RER rose above rest during exercise and was significantly higher in the RSA trial compared to the CCT at the end of exercise at R00 (p<0.05). RER remained elevated 10 mins into recovery (p<0.05) and then fell below rest at R55 and R60 of the recovery period in both RSA and CCT trials (p<0.05).

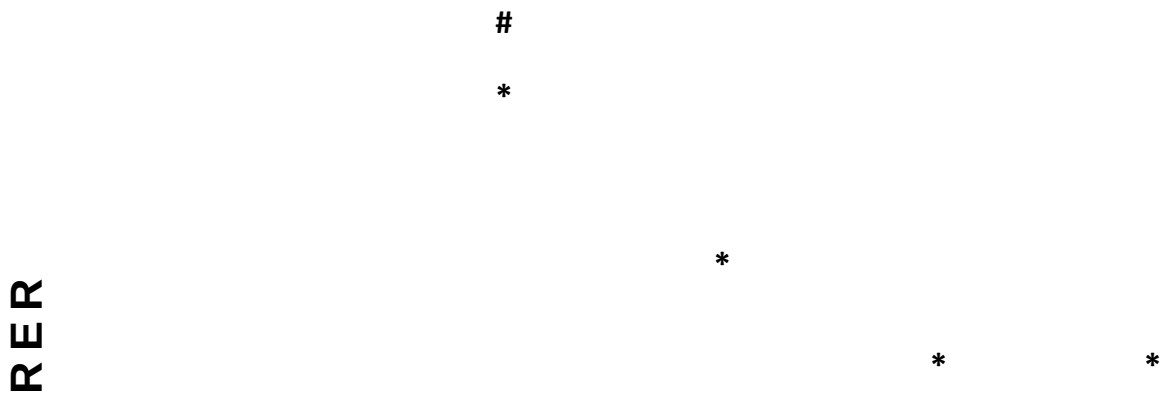

Figure 4.2: Respiratory exchange ratio (RER) at rest and during recovery of RSA and CCT exercise
Data are means ± SEM; N = 11
* p<0.05 significantly different from rest
# p<0.05 significantly different between trials

4.3.1.4 Heart rate

There was a significant increase over time of HIIE, with heart rate elevated at the end of exercise ($p<0.001$), yet no differences between RSA and CCT trials. Heart rate in the RSA trial returned to rest by 60mins post exercise, whereas heart rate in the CCT trial was still elevated above rest 60mins post exercise ($p<0.05$).

Figure 4.3: Heart rate during rest and recovery of RSA and CCT exercise
Data are means ± SEM; N = 11
** $p<0.001$ significantly different from rest
* $p<0.05$ significantly different from rest (black asterix denotes both trials are significant from rest, coloured refers to respective trial)

4.3.2 Substrate metabolism

4.3.2.1 Plasma FFA

Plasma FFA concentrations were significantly decreased from rest at the end of exercise (R00) in both trials ($p<0.05$). Plasma FFA returned to rest levels 5 min post exercise and was significantly lower from rest 30 and 60mins post exercise in the CCT trial ($p<0.05$). There were no significant differences between the two exercise bouts.

Figure 4.4: Plasma free fatty acid (FFA) profiles of RSA and CCT trials
Data are means ± SEM; N = 11
* $p<0.05$ significantly different from rest (black asterix denotes both trials are significant from rest, coloured refers to respective trial)

4.3.2.2 Plasma glycerol

Plasma glycerol concentrations increased from rest during exercise. Concentrations remained elevated until 30 min post exercise ($p<0.05$) before declining to rest by 60 mins post exercise. There were no differences in glycerol concentration between RSA and CCT trials at any time point.

Figure 4.5: Plasma glycerol profiles of RSA and CCT trials
Data are means ± SEM; N = 11
* $p<0.05$ significantly different from rest

4.3.2.3 Plasma lactate

There was an interaction in plasma lactate concentration between trials (p=0.0025) and an effect of time (p<0.0001). Concentrations increased from rest during exercise and stayed above basal levels for 30 mins post exercise (p<0.05) before returning to rest by 60 mins (p<0.05). Despite an interaction, no differences (p=0.06) between the RSA and CCT trials were detected, although a strong trend for lactate to be higher in the RSA trial compared to the CCT at R00 existed.

Figure 4.6: Plasma lactate profiles of RSA and CCT trials
Data are means ± SEM; N = 11
* p<0.05 significantly different from rest

4.3.2.4 Plasma glucose

Plasma glucose concentrations increased during the recovery and remained elevated until 15 mins of recovery (R15) ($p<0.05$). There were no differences between the RSA and CCT trials.

Figure 4.7: Plasma glucose profiles of RSA and CCT trials
Data are means ± SEM; N = 11
* $p<0.05$ significantly different from rest

4.3.2.5 Plasma insulin

Plasma insulin concentrations increased from rest in both RSA and CCT trials during exercise and remained elevated until R30 ($p<0.05$). There were no differences detected between the trials.

Figure 4.8: Plasma insulin profile of RSA and CCT trials
Data are means ± SEM; N = 11
* $p<0.05$ significantly different from rest

4.3.3 Purine Metabolism

4.3.3.1 Plasma inosine

There were no differences detected from rest or between RSA and CCT trials in plasma inosine.

Figure 4.9: Plasma inosine profile of RSA and CCT trials
Data are means ± SEM; N = 11

4.3.3.2 Plasma xanthine

There were no differences detected from rest or between RSA and CCT trials in plasma xanthine.

Figure 4.10: Plasma xanthine profile of RSA and CCT trials
Data are means ± SEM; N = 11

4.3.3.3 Plasma hypoxanthine (Hx)

Plasma Hx concentrations increased over time, with values peaking at 15 min post exercise, the only time point significantly elevated from basal levels ($p<0.05$).

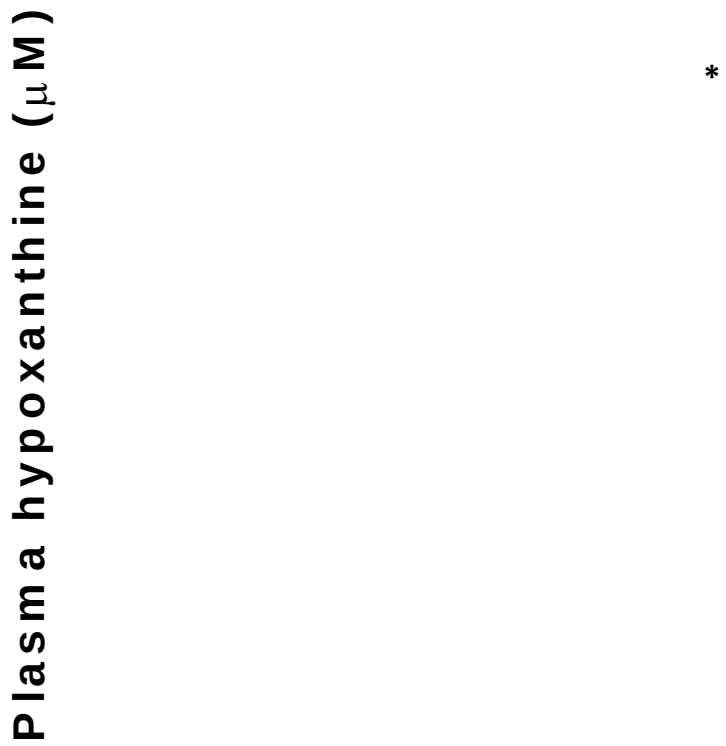

Figure 4.11: Plasma hypoxanthine profile of RSA and CCT trials
Data are means ± SEM; N = 11
* $p<0.05$ significantly different from rest

4.3.3.4 Plasma uric acid

Plasma uric acid progressively increased from rest, reaching significantly elevated levels at 30 and 60 mins post exercise completion ($p<0.05$). However, there were no differences between exercise trials.

Figure 4.12: Plasma uric acid profile of RSA and CCT trials
Data are means ± SEM; N = 11
* $p<0.05$ significantly different from rest (black asterix denotes both trials are significant from rest, coloured refers to respective trial)

4.3.3.5 Urinary purines

There was no difference in urinary inosine, xanthine or uric acid from rest or between RSA and CCT trials. Urinary Hx significantly increased post exercise compared to rest in both trials ($p<0.05$), with no differences detected between exercise bouts.

Table 4.0 Urinary purine (In, Xan, Hx and uric acid) response at rest and post RSA and CCT exercise

	Rest (umol.hr^{-1})	60 mins post exercise (umol.hr^{-1})
Inosine		
RSA	6.5	8.3
CCT	8.0	9.1
Xan		
RSA	6.17	4.9
CCT	7.5	4.0
Hx		
RSA	17.07	137.8*
CCT	7.8	94*
Uric acid		
RSA	309.2	283.9
CCT	334.1	181.89

* Denotes significance from basal concentrations

4.4 Discussion

This study demonstrated that there were no significant differences in metabolic profiles of substrates or purine base metabolism between RSA and CCT trials. Interestingly the total work of exercise, RSA = 22kJ and CCT = 52.3kJ (figure 4.0; $p<0.05$) and time of actual exercise (30 s compared to approximately 130 s respectively) were considerably lower in the RSA trial compared with the CCT trial. Hence, this suggests that similar metabolic benefit can be gained from doing approximately ½ of the physical work in less than half the time. Thus if RSA exercise is repeated such as in a training program, the energy loss via increases purine base excretion may accrue. Hence small daily imbalances in energy deficit may accumulate and energy expenditure may increase from doing ½ of the work in the RSA exercise compared to CCT exercise.

4.4.1 RSA and CCT exercise induce similar metabolic responses during and post exercise

There were no significant differences in plasma and urinary substrate profiles or purine metabolism between RSA and CCT trials. Interestingly the work performed to achieve this was significantly lower in the RSA trial compared to the CCT trial (22kJ and 52.3 kJ respectively) and roughly 30 s of cycling time compared to 130 s. For the first 10 s of maximal effort exercise, the ATP-CP energy system provides the exercising skeletal muscles with energy, and when PCr stores are diminished, glycolysis becomes the principal energy system with glycogen as the primary source (Gastin, 2001; Gropper *et al.,* 2009) (see section 2.7). Both RSA and CCT induced significant increases from rest in plasma glucose concentration, though not different between exercise trials (see figure 4.7). There was a concomitant increase in plasma lactate accumulation during exercise confirming glycogen as

the main energy source (see figure 4.6). Therefore, similar glycolytic responses were elicited when total cycling duration and total work performed were unmatched (see figure 4.0; $p<0.05$), therefore lactate production, efflux and accumulation may depend on exercise intensity more than exercise duration. As well as no differences observed in glycolytic markers of metabolism, there were no differences between the RSA and CCT trials in purine nucleotide metabolism.

Although no variation between trials, in both RSA and CCT trials, plasma and urinary Hx were significantly elevated from basal. The high energy demand of high intensity exercise results in ATP degradation characterised by increased plasma and urinary purine bases such as Hx and uric acid. It is assumed that purine bases measured above basal plasma and urinary excretion concentrations originate from the muscle as a result of elevated metabolic stress induced by exercise. This equates to energy loss and possible elevated energy expenditure (Broberg and Sahlin, 1989; Bangsbo *et al.,* 1992; Stathis *et al.,* 1994) that is estimated from the difference in energy required to salvage the purines that synthesise ATP *de novo,* validated in section 2.9.2.4. Plasma Hx rose significantly from rest and remained elevated until 15 mins post exercise (see figure 4.11; $p<0.05$) in both trials with no significant differences detected. Plasma Hx is transient and is converted to plasma xanthine and uric acid, as demonstrated by uric acid increasing above rest from 30 to 60 min after exercise in both trials (see figure 4.12; $p<0.05$). Urinary Hx excretion was also significantly increased from rest post exercise (see figure 4.15; $p<0.05$), again no differences detected between RSA and CCT trials. As there appeared to be similar purine loss across the two metabolically fatiguing exercise bouts, hence similar levels of energy required to *de novo* synthesise degraded ATP (Newsholme and Leech, 1983), there may be similar levels of

energy deficit observed with both trials, which is not affiliated with the energy cost of the actual exercise performed. This result was not expected as deamination of IMP to Hx is increased after repeated sprints compared to CON (Borg *et al.,* 2008) and for this reason it was hypothesised that RSA would induce significantly greater plasma and urinary Hx and uric acid concentrations compared to the continuous CCT bout. Therefore the modality of the exercise (continuous or intermittent) is not pertinent to inflicting metabolic differences when exercise intensity is maximal. Therefore no matter of the model or total exercise time, metabolically fatiguing exercise results in similar metabolic outcomes. Doing half the physical work over the same period of time results in similar metabolic profiles and levels of energy loss, hence elevates expenditure to the same relative level.

4.4.2 Fat utilisation during and post RSA and CCT exercise

It is unlikely that fat metabolism contributed significantly to energy supply during the CCT or RSA exercise trials or during the rest periods between sprints of the RSA trial. During exercise, there was an increase in plasma glycerol from rest ($p<0.05$) though no differences were detected between RSA and CCT trials (see figure 4.5; $p<0.05$). Elevated plasma glycerol is reflective of lipolysis; release of FFA for utilisation (McCartney *et al.,* 1987; Coggan *et al.,* 2007; Trapp *et al.,* 2007). However plasma FFA concentration significantly decreased from rest in both RSA and CCT trials (see figure 4.4; $p<0.05$). Collectively these results suggest that fat was not oxidised during either RSA or CCT exercise or during the RSA rest periods separating the five 6 s sprints. This is not consistent with fat utilisation associated with HIIE as per section 2.9.2.1 which due to the work and rest nature, the RSA exercise may have been expected to follow. It has been proposed that accelerated decreases in adiposity associated with HIIE are due to fat utilisation during this mode of exercise with heightened

fat oxidation attributed to the work and rest nature of HIIE, particularly the rest periods between high intensity sprints (Tremblay *et al.,* 1994). Perhaps the 24 s rest periods in the RSA exercise were too short a time for fat to be broken down and for FFA to be taken up by the mitochondria, and a longer duration of rest is needed for this to occur. It could be speculated that as plasma FFA concentration decreased below rest while plasma glycerol simultaneously increased, that FFA were utilised by the exercising skeletal muscle. Little research exists regarding lipid metabolism during all out, maximal effort exercise, however a study that employed isotopic palmitate tracers compared fat metabolism over three different exercise intensities; low (25% VO_{2max}), moderate (65% VO_{2max}) and high (85% VO_{2max}) and found significantly elevated plasma glycerol from rest during exercise, yet depressed plasma FFA concentration, proposing fat oxidation is lowest during higher intensity exercise (Romijin *et al.,* 1993). Furthermore, previous research suggests that elevated plasma glycerol concentrations are indicative of IMTG contribution to energy supplies of repeated wingate exercise (Greer *et al.,* 1998) which is 30 s of maximal all out cycling, separated by a 4 min rest period. For that reason, while FFA may not have contributed to energy production during the RSA and CCT trials, IMTG may have been utilised as energy source.

FFA utilisation may have also declined during high intensity exercise due to significant muscle lactate production and subsequent increased H^+ ion release, as per section 2.7.2. Upon exercise cessation, plasma lactate was significantly elevated from rest in both trials, peaking at approximately 14-15mmol/L at the end of the RSA and CCT bouts ($p<0.05$; see figure 4.6). FFA uptake into the mitochondria may be decreased due to CPT1 inhibition caused by a shift in pH, induced by lactate production, a validated metabolic

response section 2.7.2. Although this study did not measure blood pH, a study employing males and similar RSA exercise demonstrated that blood lactate peaking at 9mmol/L caused muscle pH of 6.89 (Bishop *et al.,* 2004); whereas in another study after exhaustive exercise muscle pH was 6.69 (Juel *et al.,* 2003). If 9-10mmol/L equates to a muscle pH of 6.69-6.89, it may be assumed that 15mmol/L will correlate to a much lower muscle pH, hence inhibition of CPT1 may be the reason limiting fat utilisation in this study. These results and supportive evidence suggest that fat utilisation may have been depressed during exercise and not utilised.

Although lipid was not utilised during short duration, maximal effort all out exercise, a number of earlier studies suggests fat sources may be oxidised in the recovery periods once the high intensity sprint has ceased (Tremblay *et al.,* 1990; Treuth *et al.,* 1996; Hunter *et al.,* 1998; Balsom 1999; Al mulla *et al.,* 2000; Yoshioka *et al.,* 2001; van Hall *et al.,* 2002; Benson *et al.,* 2007; Helge *et al.,* 2007) and in the EPOC period (Lyons, 2006). EPOC uses oxidative processes, characterised by elevated VO_2 above rest, to replenish and restore exercise-induced metabolic alterations, the primary substrate utilised being fat. Figure 4.1 shows a significant increase from rest in VO_2 at the end of exercise ($p<0.05$), remaining elevated until 10 min post exercise ($p<0.05$), yet no difference exists between RSA and CCT bouts. EPOC can last for several hours post vigorous exercise, established in section 2.9.2.3.4 and this duration was not a feasible measure for the current study. Additionally, due to short duration of gas exchange collection at rest in the current study, approximately 10 mins, the VO_2 collected at rest may be falsely high, due to anticipation induced prior to exercise (Borsheim and Bahr, 2003). As such EPOC may have extended further into the recovery period than is illustrated in figure 4.1.

Nonetheless, after CCT and RSA exercise bouts, lipolysis may have increased as there was a progressive elevation in plasma glycerol concentrations until 30 mins of recovery. Upon termination of exercise, plasma FFA concentrations returned to rest, remaining consistent till the end of the 60 min recovery phase. During rest, a mixture of fat and CHO are oxidised with FFA dominant, and it is likely that fat oxidation increased post exercise as RER results were closer to 0.7 (see figure 4.2), reflective of lipid as the predominant utilised substrate. In all out exercise, RER is not entirely reflective of fat and CHO metabolism due to the influence of elevated H^+ production (Bergman and Brooks, 1999) and the buffering by bicarbonate producing CO_2 influencing the RER equation as per section 2.3.3.4 (Bergman and Brooks, 1999). Nonetheless, RER supports plasma evidence of increased fat oxidation in the recovery period post exercise. Thus although not used to any great extent during high intensity exercise fat may be utilised post exercise, potentially contributing to decreasing adiposity associated with vigorous exercise.

4.4.3 Conclusion

RSA and CCT were considerably different in total work performed and cycling time (30 s compared to ~120 s respectively), however purine base and substrate metabolism were not, indicating that similar metabolic perturbations can be achieved with half of the total work in a quarter of the time.

The exercise induced metabolic changes compared to rest such as increased purine loss equating to energy loss and possibly increased fat utilisation post exercise hence may influence energy balance, potentially decrease adiposity if the exercise were repeated continually.

Chapter five: The influence of exercise intensity and rest periods on the metabolic responses following

workload matched high intensity intermittent exercise

5.1 Introduction

Exercising continuously at a submaximal intensity (~65% VO_{2max}) is considered the 'fat_{max}' protocol (section 2.8.2) (Romijn *et al.,* 1993; Achten *et al.,* 2002), this exercise model has frequently been employed in training programs aimed at decreasing adiposity by optimising fat oxidation rates. Interestingly, high intensity and supra-maximal intensity exercise also decreases adiposity (Tremblay *et al.,* 1990; Hunter *et al.,* 1998; Benson *et al.,* 2007). This however is not due to the direct energy cost of the actual exercise which is low due to the short duration, but is most likely caused by exercise induced metabolic disturbances, requiring energy utilisation post exercise to restore homeostasis. The accrual of small elevations in energy expenditure after each exercise session of a training regime may shift energy balance negative, resulting in decreases in fat mass in the long term.

Chapter 4 of this thesis highlights the effect of metabolically fatiguing RSA and CCT exercise on purine nucleotide loss (section 4.4.1) and glycolysis. Plasma and urinary Hx, a metabolite of ATP degradation was elevated post CCT and RSA exercise, equating to energy deficit with subsequent greater *de novo* synthesis rate of ATP required, further enhancing energy expenditure as established in section 2.9.2.4.

In addition, the previous chapter indicates that fat substrate may not have contributed to energy supply during high intensity exercise, but may have been elevated post exercise contributing only minimally to decreasing adiposity. Henceforth, an exercise

model that encompasses fat utilisation during exercise and creates all metabolic shifts that are restored post exercise and may attribute to elevating energy deficit may be favorable at increasing energy loss and possible elevations in energy expenditure.

HIIE is thought to be the leading exercise model employed to reduce adiposity and elevate energy expenditure (Tremblay *et al.,* 1994; Trapp *et al.,* 2008; Borg *et al.,* 2008). It may integrate oxidative metabolism and glycolysis (Tremblay *et al.,* 1994) whilst inducing metabolic disturbances carried into the recovery period, elevating energy loss via increasing overall energy expenditure. However, metabolic implications and substrate utilisation that may explain accelerated reductions in adiposity associated with HIIT remain unclear. A study from our laboratory compared acute bouts of workload matched HIIE and CON and attributed the decreased adiposity associated with HIIT to the nature of HIIE; short high intense exercise bouts followed by passive rest phases. The short, high intensity exercise bouts impact ATP degradation thus net purine loss from the muscle, leading to the requirement for new ATP synthesis, enhancing energy expenditure (Borg *et al.,* 2008) (see section 2.9.2.4). Therefore, the work to rest nature of HIIE may stimulate fat-carbohydrate substrate partitioning (Tremblay *et al.,* 1994) with fat substrate utilised during the rest period. Utilising greater fat substrate during and post high intensity exercise as well as elevating energy expenditure, may elicit significant reductions in adiposity over time.

As mentioned in section 2.9.2.4, purine nucleotide metabolism and appearance of plasma and urinary metabolites are affected by number of sprints, with greater sprint number preventing reamination of Hx to IMP, hence the greater Hx concentrations observed (Tullson *et al.,* 1995). Therefore while sprint number influences purine nucleotide

metabolism (Stathis *et al.,* 1999), the rest period between the high intensity sprints may influence substrate utilisation.

5.1.1 Aims and hypothesis

Therefore the aims of this study were three-fold; 1) to provide clarity pertaining to substrate utilisation during and post single bouts of HIIE, 2) employ manipulated HIIE models where exercise intensity and duration are altered, but with the same amount of physical work performed, to see if metabolic perturbations can be exacerbated, and 3) investigate urinary lactate excretion after workload matched HIIE in one participant as part of a pilot study to determine whether significant lactate loss occurs and such would also be a mechanism by which energy is lost.

It is hypothesised that with increasing exercise intensity, muscle, plasma and urinary markers of glycolysis and purine metabolism will be exacerbated, irrespective of rest intervals.

Secondly, the utilisation of fat for energy supply may be increased during exercise due to the rest phases of the HIIE model, with elevated plasma markers of fat mobilisation further amplified in accordance to prolonged rest.

5.2 Methods

5.2.1 Participant characteristics

Eight healthy, untrained males aged between 18-35yrs volunteered to take part in this study (22.7 ± 4.1 years, 178 ± 4.9 cm, 82 ± 6.2kg; 50 ± 5.6 ml.kg.min^{-1}) (n= 7 300% HIIE, n=6 muscle collection. Muscle metabolite data is different due to participants not consenting to muscle biopsy procedures). Participants provided written and verbal informed consent and completed medical history forms prior to the commencement. The experimental design and all procedures undertaken by participants were approved by the Victoria University Human Research Ethics Committee performed in accordance with the ethical standards set out in the 1964 Declaration of Helsinki.

5.2.2 Preliminary Testing

A $VO_{2\ peak}$ on a Lode Cycle Ergometer was performed using a standard test protocol described in section 3.2.1.

5.2.2.1 Respiratory gas exchange

Respiratory gas measurements were taken using a metabolic cart and the Turbofit software (Vacumed, California USA) as per section 3.5.1.

5.2.3 Familiarisation session

Prior to the exercise trial day, participants underwent a familiarisation session, detailed in section 3.3.

5.2.4 Trial procedure

To prevent substrate variation between trials, participants kept a 24 hr food diary in order to replicate meals for the following trial days. Furthermore participants were instructed to abstain from alcohol, physical activity and caffeine for 24 hr prior to testing.

5.2.4.1 Experimental design

In a randomised order and separated by at least one week, participants completed the 3 exercise protocols 1) **300%:** 10 s cycling at 300% VO_{2peak}: 50 s rest (357 ± 64 W); 2) **200%:** 15 s cycling at 200% VO_{2peak}: 45 s rest (476 ± 86 W); 3) **150%:** 20 s cycling at 150% VO_{2peak}: 40 s rest (714 ± 129 W) as seen below in figure 5.0. Exercise bouts were 30 mins in duration, and matched for workload by altering exercise intensity and cycling duration. To account for increases in intensity, cycling time was decreased, prolonging the rest phase. HIIE was preceded by a rest period and followed by 90 mins of recovery.

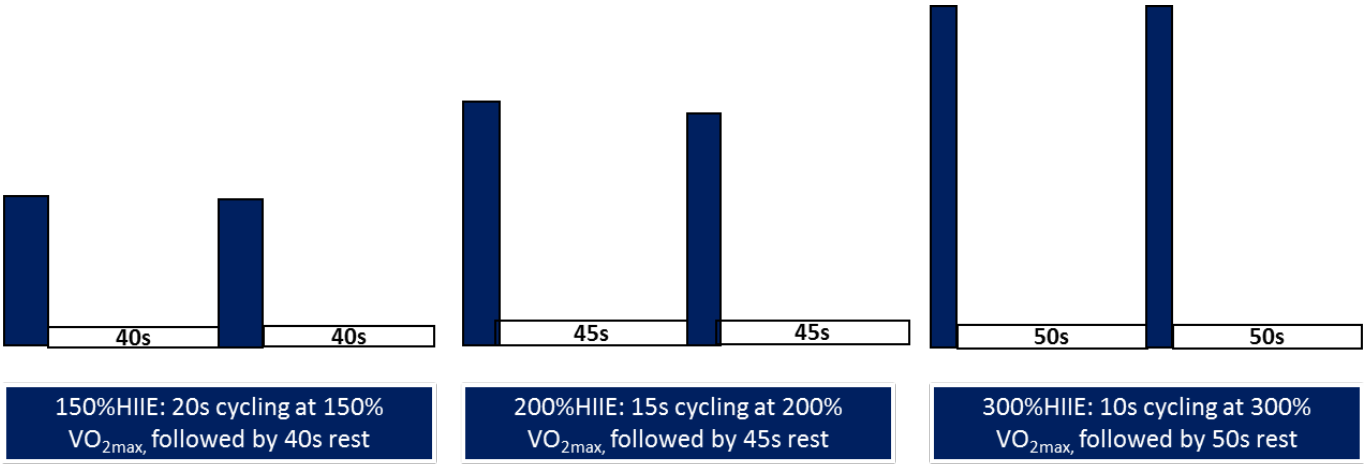

Figure 5.0: Diagrammatic HIIE protocols employed in this chapter.

Participants arrived at the laboratory in the morning in a post-absorptive state after an overnight fast (10-12 hrs) and were instructed to consume water *ad libitum*. The start

time for each trial remained consistent to avoid influence of circadian variance. While resting in a supine position, participants gave rest respiratory gas exchange measures then were prepared for blood and muscle collection.

5.2.4.2 Respiratory gas collection and analysis

Respiratory gases were collected as per section 6.2.2.1. Respiratory gases were also collected at rest, for the 30 min duration of HIIE and periodically during the 90 min recovery period.

5.2.4.3 Blood Collection and Analysis

Blood was collected as per collection methods detailed in section 3.5.4. Samples were collected at rest, at the end of exercise and recurrently during the recovery period at 5 (R5), 10 (R10), 15 (R15), 30 (R30), 45 (R45), 60 (R60), 75 (R75) and 90 mins (R90) post exercise. Plasma FFA, glycerol, lactate, glucose, insulin, inosine, xanthine, Hx and uric acid were analysed as per section 3.5.4.2.

5.2.4.4 Muscle Collection and Analysis

Muscle samples were collected via muscle biopsy at rest, in the rest period immediately after the first bout of HIIE (10-20 s), upon completion of 30 mins of HIIE and after 90 mins of supine recovery. Using a needle biopsy, a small amount of muscle was extracted from the vastus lateralis muscle under local anaesthesia (Bergstrom, 1975). The sample was immediately snap frozen and stored at -80 degrees until freeze dried for analysis of muscle metabolites glycogen,

ATP-CP, creatine and lactate, detailed comprehensively in section 3.5.6 muscle sampling and collection as well as 3.5.6.3 muscle metabolite analysis.

5.2.4.5 Urine collection and analysis

Urine was collected as per methods set out in section 3.5.5. Inosine, xanthine, Hx and uric acid were analysed using HPLC, shown in section 3.5.5. One participant's urine was analysed immediately for urinary lactate was analysed using the automated YSI analyser as per section 3.5.5.

5.2.4.6 Rating of perceived exertion

Borg scale rating of perceived exertion was shown and determined at the 15^{th} and 30^{th} bouts of each HIIE protocol, as per section 3.5.3.

5.2.5 Statistical Analysis

As per section 3.6, subject characteristic data is expressed at mean ± standard deviation (SD) whereas all other results are expressed as means ± standard error of the mean (SEM).

All results that are plasma, muscle and urinary, RPE, heart rate and respiratory gases (VO_2 and RER) were analysed using Graph-pad Prism software version 6.02 (Graphpad software, USA). A two-way repeated measures ANOVA, with time as the within group factor and exercise protocol as the between group factor used to detect significance in plasma, muscle and respiratory results. Where an interaction between trials was detected, post hoc tests were completed as detailed in section 3.6. Matched, paired t-tests were used to determine differences in RPE as well as all urinary results. Post exercise urinary results were

compared to each other and to rest concentrations. Statistical significance was accepted at ($p < 0.05$).

5.3 Results

The rate of perceived exertion (RPE) was significantly different with the 150% HIIE perceived to be less demanding physically than the 300% HIIE trial and elevated post exercise urinary Hx concentrations in the 300% compared to the 150% HIIE trial. Thus the results section will describe the effects of exercise.

5.3.1 Physiological Markers

5.3.1.1 Oxygen consumption (VO_2)

There was an interaction in VO_2, p = 0.0324. VO_2 levels increased from rest during exercise in all three trials (p<0.0001) and remained above rest until 15 mins post exercise (p<0.05).

Figure 5.1: VO_2 of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7
* p<0.05 significantly different from rest

5.3.1.2 Respiratory exchange ratio (RER)

RER increased from approximately 0.9 at rest to 1.0 in the initial stages of exercise ($p<0.05$) and although steadily falling throughout the exercise period, RER remained elevated until E30. After an initial compensatory elevation post exercise (R5; $p<0.05$), RER returned to 0.8 by 15 mins post exercise, staying stable for the rest of the recovery period.

Figure 5.2: Respiratory exchange ratio (RER) of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7
* $p<0.05$ significantly different from rest

5.3.1.3 Heart rate

Heart rate was increased significantly from rest ($p<0.001$) during exercise and decreased during recovery, returning to rest by the end of the 90 min recovery period.

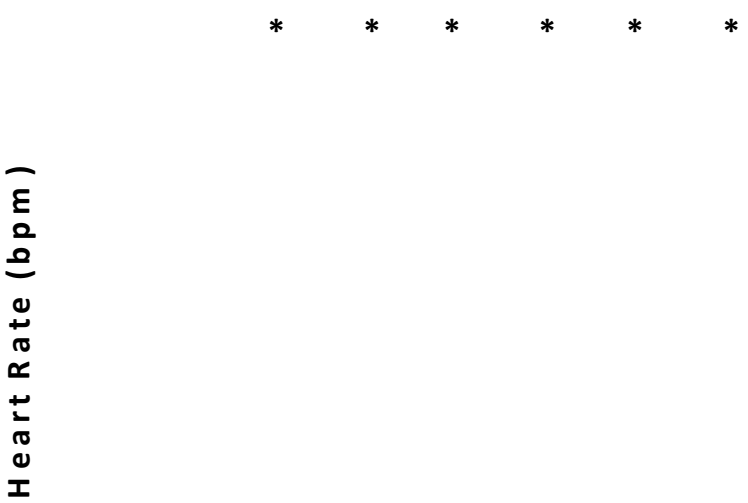

Figure 5.3: Heart rate (bpm) of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7
* $p<0.001$ significantly different from rest

5.3.1.4 Rating of perceived exertion (RPE)

After the 15th bout, the 150% HIIE and 200% HIIE protocols were perceived to be easier to complete than the 300% HIIE bout. While at the end of exercise (after 30 bouts), only the 150%HIIE was perceived to be easier than the 300%HIIE bout.

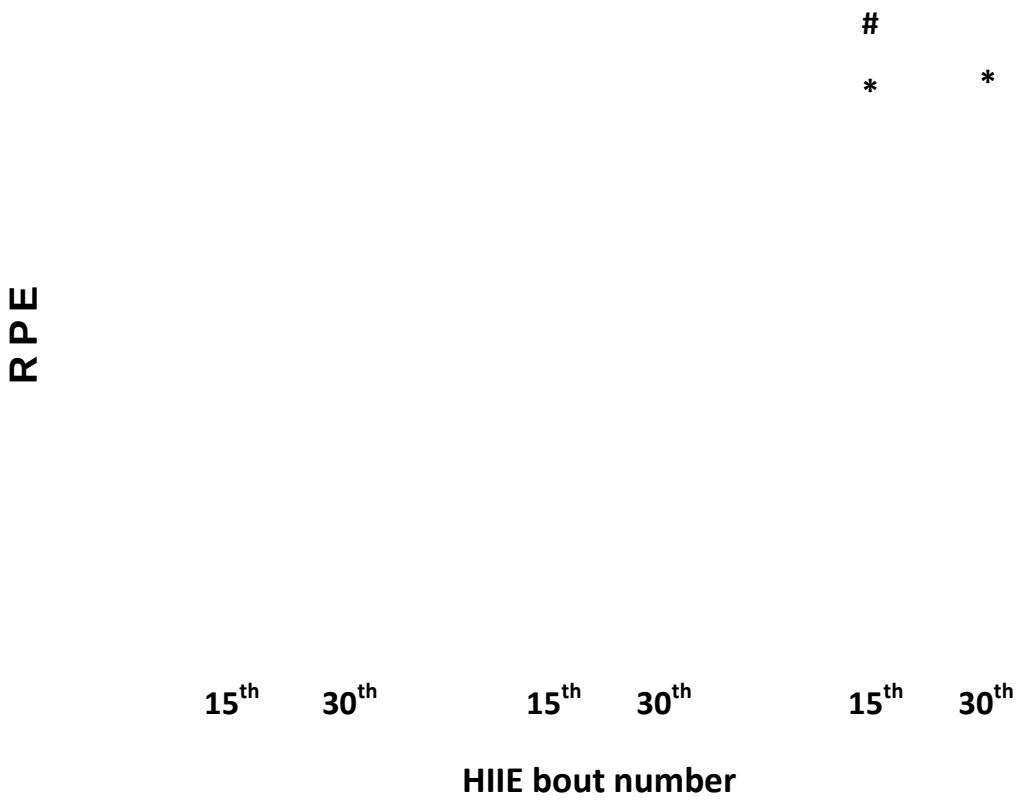

Figure 5.4: Rating of Perceived exertion of workload matched HIIE at the 15th and 30th exercise bout
Data presented as mean ± SEM; N = 8; 300% N = 7
* p<0.05 perceived to be more physically demanding than 150%
# p<0.05 perceived to be more physically demanding 200%

5.3.2 Substrate metabolism

5.3.2.1 Plasma free fatty acids (FFA)

Plasma FFA remained consistent throughout all 3 HIIE trials, however rose above basal levels at 75 and 90 min post exercise ($p<0.05$).

Figure 5.54: Plasma FFA profiles of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7
* $p<0.05$ significantly different from rest (black asterix denotes all three trials are significant from rest, coloured refers to respective trial)

5.3.2.2 Plasma glycerol

Plasma glycerol increased during exercise and remained elevated at 10 min post exercise ($p<0.05$) before decreasing to rest concentrations.

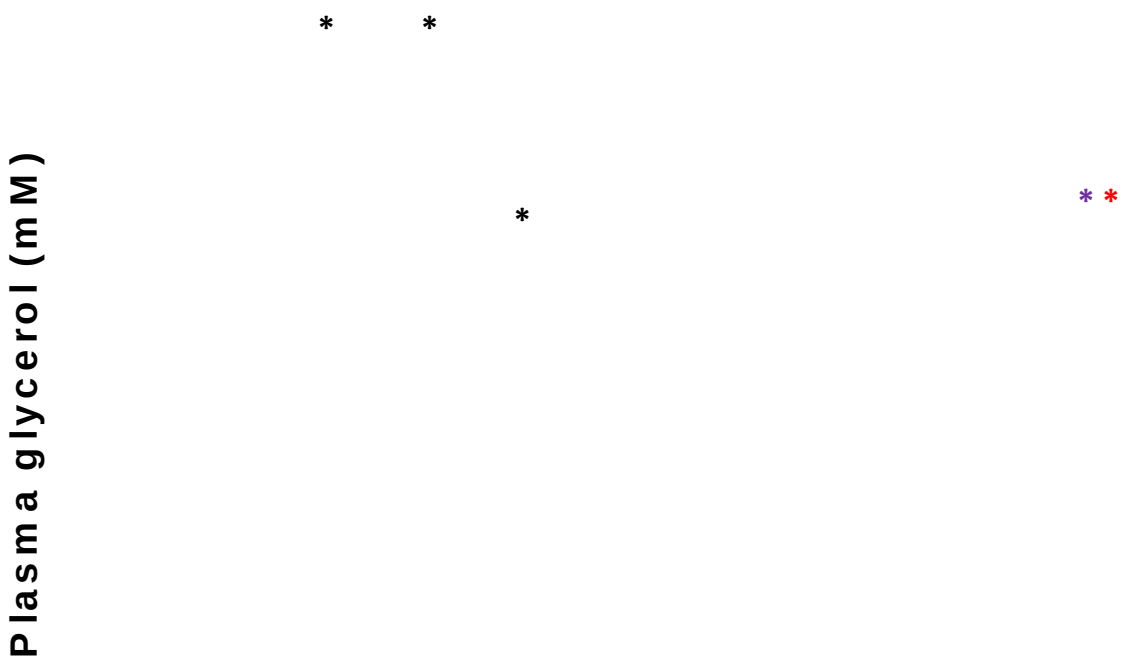

Figure 5.6: Plasma glycerol profiles of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7
* $p<0.05$ significantly different from rest (black asterix denotes all three trials are significant from rest, coloured refers to respective trial)

5.3.2.3 Plasma lactate

There was an interaction in plasma lactate profiles ($p=0.0173$) and an effect on time ($p<0.0001$). Concentrations were increased from rest at every time point ($p<0.05$) in all three trials, remaining elevated well into the recovery period. Despite the interaction, there were no differences detected between the HIIE trials.

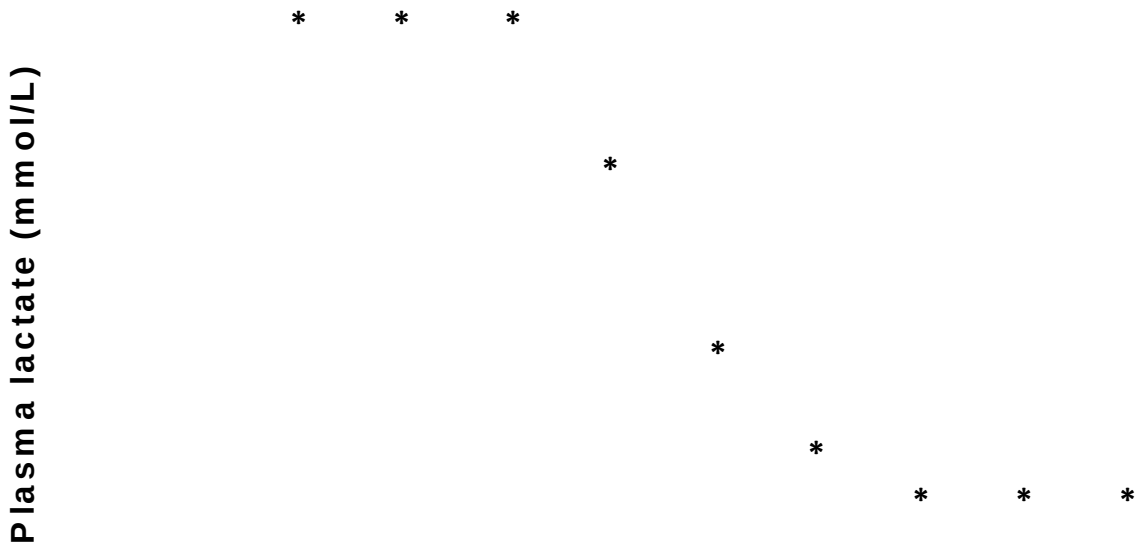

Figure 5.7a: Plasma lactate profiles of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7
* $p<0.05$ significantly different from rest

5.3.2.3.1 Plasma lactate area under the curve

There were no significant differences in area under the curve of the plasma lactate profiles between the three HIIE trials. There was however a strong trend for the 300% HIIE trial to be higher than the 150% HIIE trial (p=0.057). Due to the observed variability, more participants may prove this result significant.

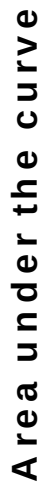

Figure 5.7b: Area under the curve of plasma lactate profiles
Data are means ± SEM; N = 8; 300% N = 7

5.3.2.4 Plasma glucose

There was a significant interaction in plasma glucose concentrations (p=0.0121). Concentration increased from rest in all three HIIE trials (p<0.05), remaining elevated into the recovery period for 10 mins before returning to rest.

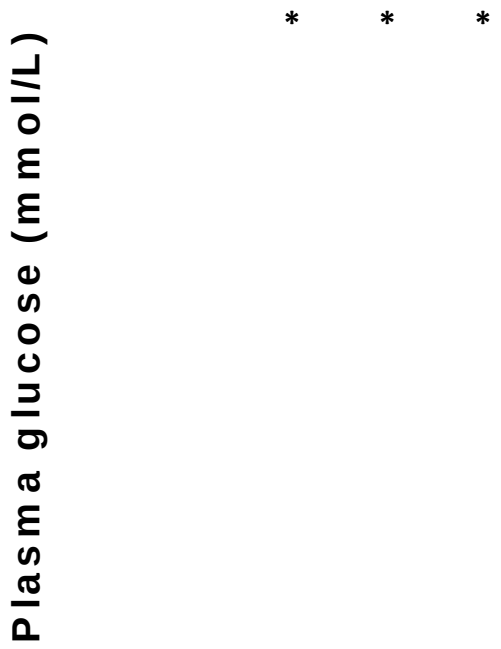

Figure 5.8: Plasma glucose profiles of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7
* p<0.05 significantly different from rest

5.3.2.5 Plasma insulin

Plasma insulin increased from rest at the end of exercise (E30), remaining elevated for an additional 15 mins (R15) ($p<0.05$) before decreasing to rest.

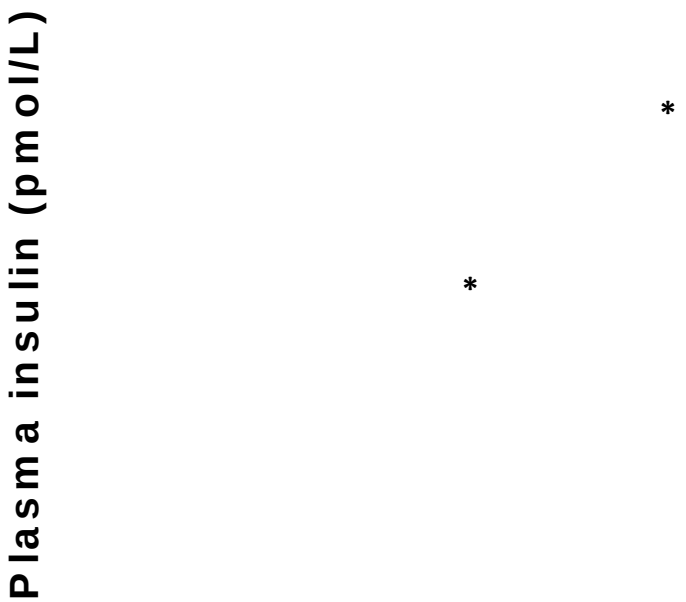

Figure 5.9: Plasma insulin profiles of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7
* $p<0.05$ significantly different from rest

5.3.2.6 Muscle glycogen

Glycogen concentrations decreased from rest after the 1st HIIE bout and even further lower at the end of 30 mins exercise ($p<0.05$). Glycogen concentrations recovered by the end of the 90 min recovery period. There were no significant differences detected between the three HIIE trials.

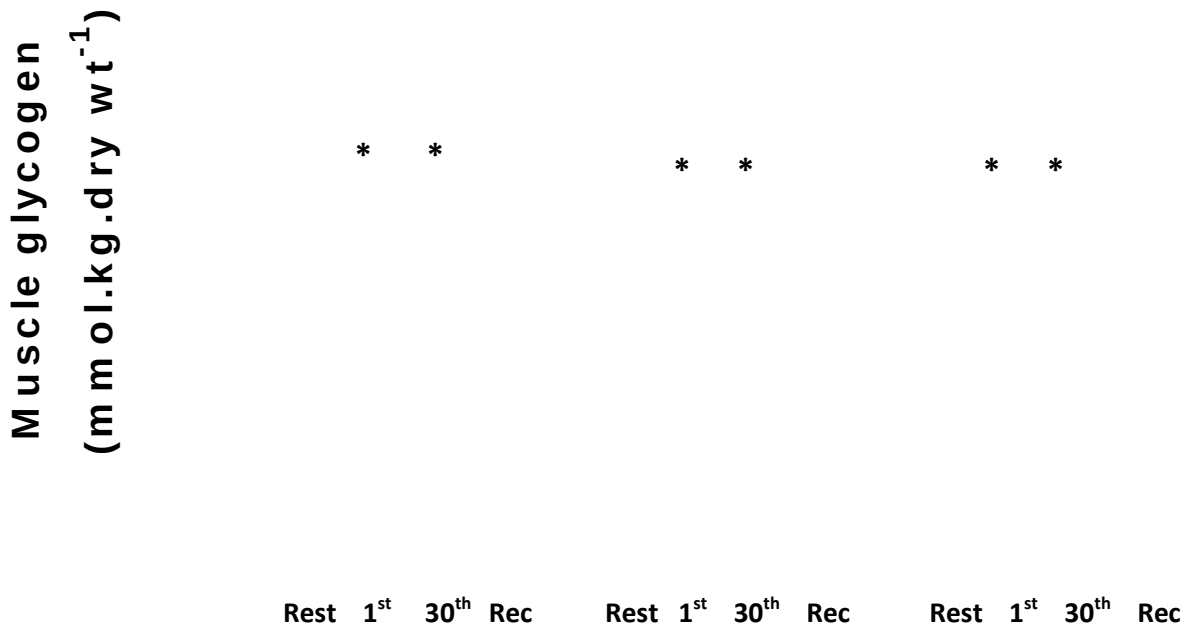

Figure 5.10: Muscle glycogen content of workload matched HIIE at rest, after the 1st bout of HIIE, upon completion of exercise and 90 mins post exercise
Data are means ± SEM; N = 6; 300% N = 5
* $p<0.05$ significantly different from rest

5.3.2.7 Muscle lactate

Muscle lactate in all three HIIE trials was significantly elevated after the first exercise bout and at the end of exercise ($p<0.05$). Muscle lactate concentrations declined post exercise, returning to rest by 90 mins post exercise.

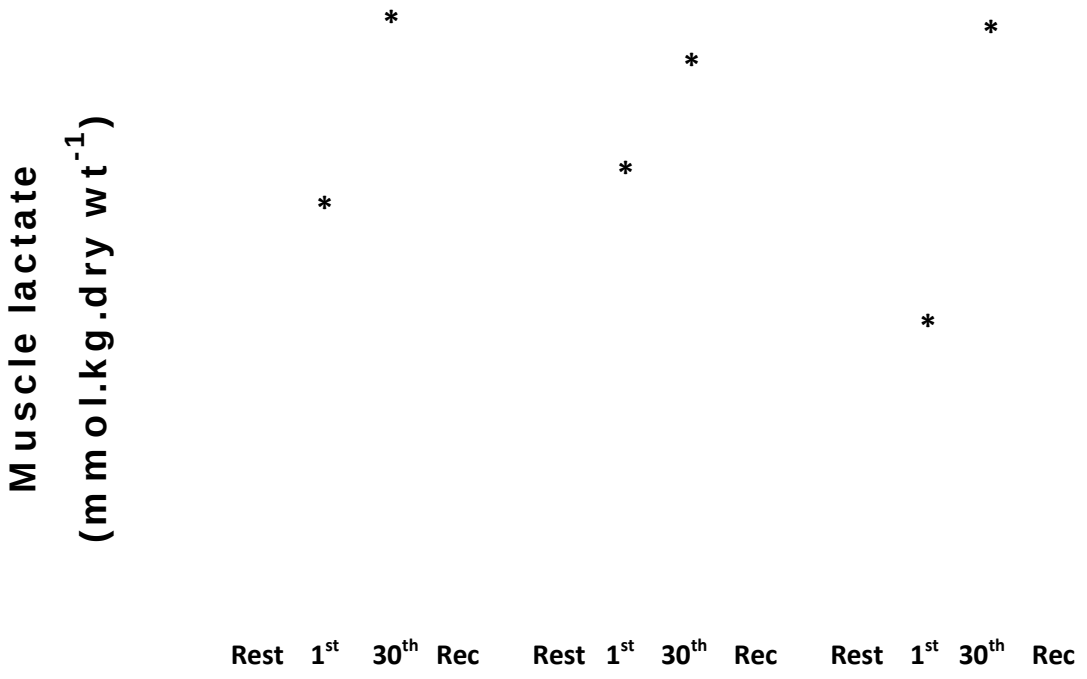

Figure 5.11: Muscle lactate content of workload matched HIIE at rest, after the 1st bout of HIIE, upon completion of exercise and 90 mins post exercise
Data are means ± SEM; N = 6; 300% N = 5
* $p<0.05$ significantly different from rest

5.3.2.8 Muscle ATP

There was no significant change in ATP content observed from rest or between the three HIIE trials.

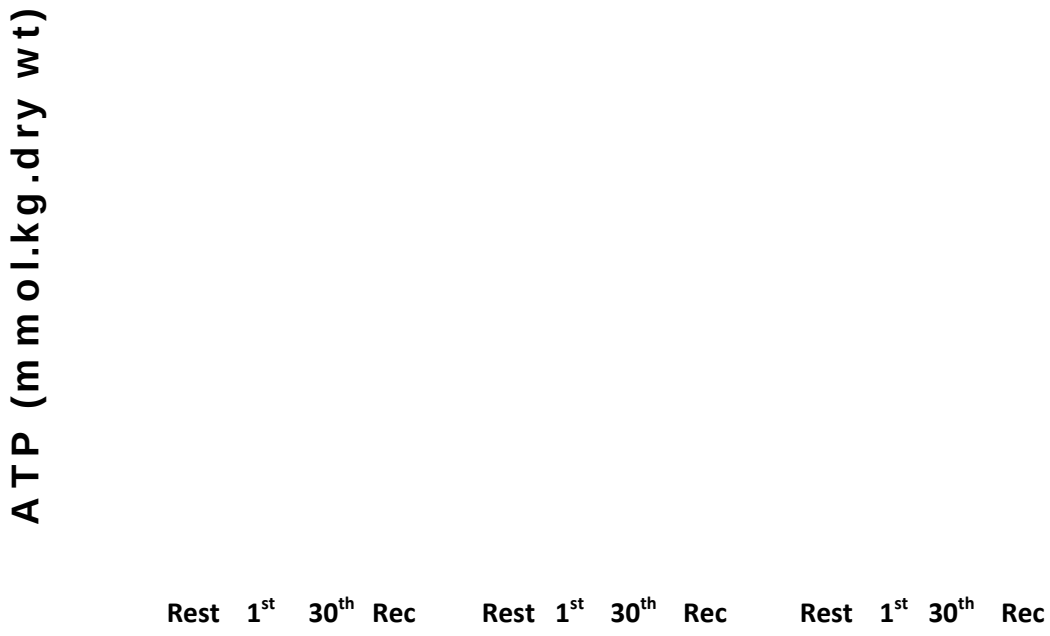

Figure 5.12: Muscle ATP content of workload matched HIIE at rest, after the 1st bout of HIIE, upon completion of exercise and 90 mins post exercise
Data are means ± SEM; N = 6; 300% N = 5

5.3.2.9 Muscle Creatine Phosphate

Muscle creatine concentrations decreased after the 1st exercise bout, decreasing further by the end of exercise. Concentrations had returned to rest by 90 mins post exercise. There were no differences observed between the HIIE trials.

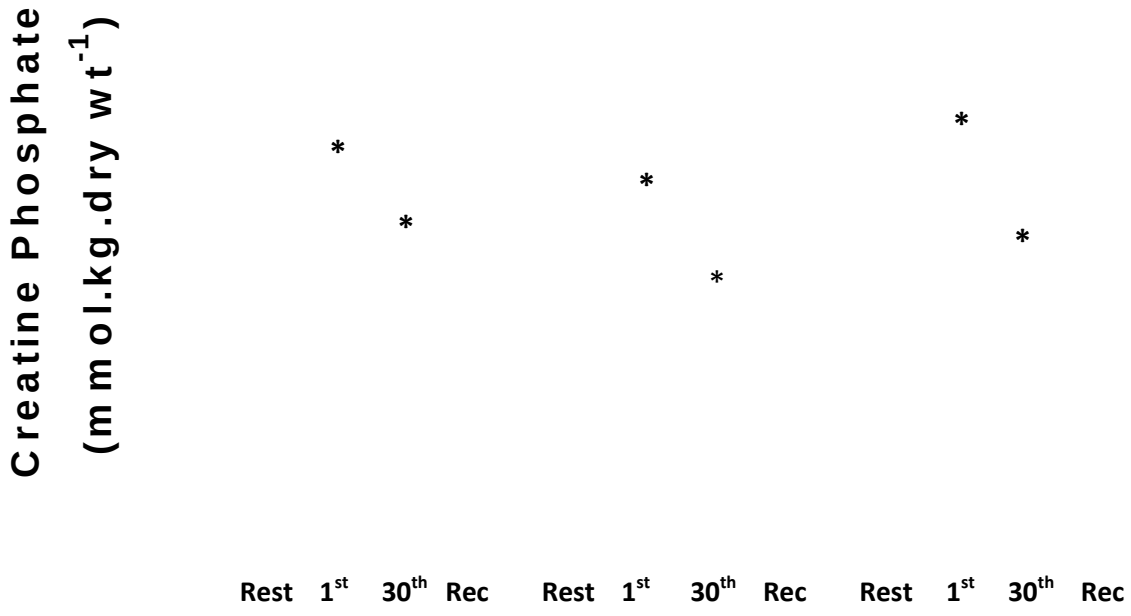

Figure 5.13: Muscle creatine phosphate content of workload matched HIIE at rest, after the 1st bout of HIIE, upon completion of exercise and 90 mins post exercise
Data are means ± SEM; N = 6; 300% N = 5
* p<0.05 significantly different from rest

5.3.2.10 Total Muscle Creatine

There were no significant differences from rest or between HIIE trials observed in total muscle creatine content. There was a trend for concentration to be decreased post HIIE, however not reaching significance.

Figure 5.14: Muscle creatine content of workload matched HIIE at rest, after the 1st bout of HIIE, upon completion of exercise and 90 mins post exercise
Data are means ± SEM; N = 6; 300% N = 5

5.3.2.11 Urinary lactate

Urinary lactate of one participant was analysed to investigate whether urinary lactate excretion is elevated post exercise. There is a trend for lactate excretion to progressively increase as exercise intensity increased.

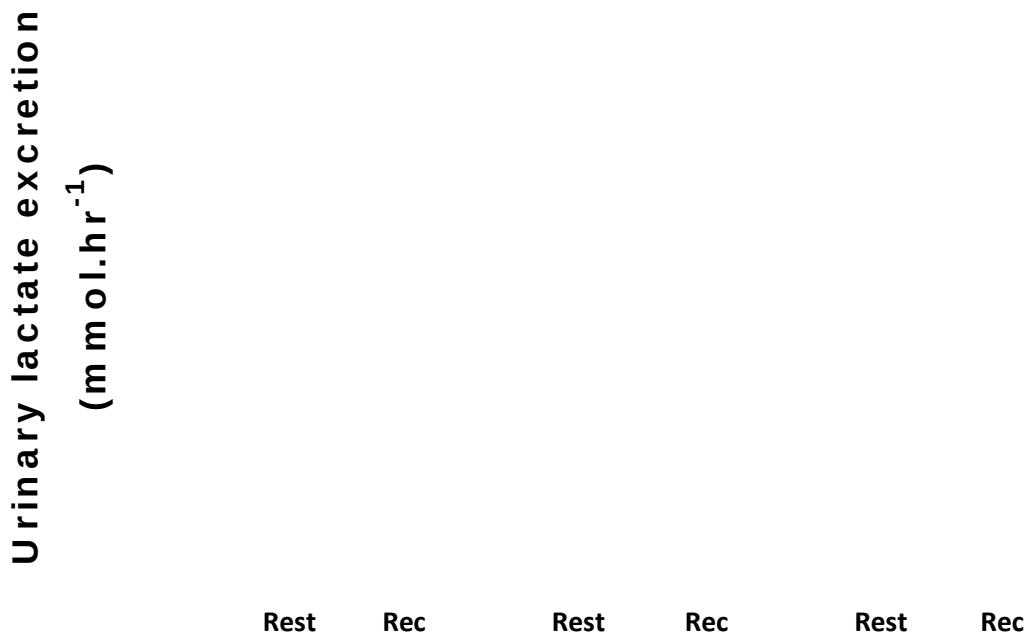

Figure 5.15: Urinary lactate excretion of workload matched HIIE, collected at rest and 90 mins post exercise
Data presented as mean ± SEM; N = 1 (pilot study data)

5.3.3 Purine Metabolism

5.3.3.1 Plasma inosine

There were no differences from rest concentrations in plasma inosine.

Figure 5.16: Plasma inosine profiles of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7

5.3.3.2 Plasma xanthine

Like plasma inosine, there were no differences in plasma xanthine concentrations from rest.

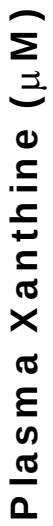

Figure 5.17: Plasma xanthine profiles of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7

5.3.3.3 Plasma Hx

Plasma Hx concentration was significantly elevated from rest post exercise at R10 ($p<0.05$).

No other differences were detected.

Figure 5.18: Plasma hypoxanthine profiles of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7
* $p<0.05$ significantly different from rest

5.3.3.4 Plasma uric acid

There was no change in plasma uric acid concentrations from rest.

Figure 5.19: Plasma uric acid profiles of workload matched HIIE
Data are means ± SEM; N = 8; 300% N = 7

5.3.3.5 Urinary purines

There was no difference in urinary excretion of inosine compared to rest excretion levels. Urinary xanthine was elevated above rest in the 300% trial but not in the 150% and 200% trial. Urinary Hx increased from rest in all three HIIE trials, (300% $p=0.0001$ and 150% and 200% $p<0.05$). Post exercise, Hx excretion was significantly elevated in the 300% HIIE trial compared to the 150% HIIE trial ($p<0.05$). Urinary uric acid concentrations were significantly elevated post exercise compared to rest in all three HIIE trials ($p<0.05$).

Table 5.0 The effect of 150%, 200% and 300% HIIE on urinary purines (In, Xan, Hx and uric acid) post exercise

	Rest (umol.hr^{-1})	90 mins post exercise (umol.hr^{-1})
Inosine		
150% HIIE	3.7	5.0
200% HIIE	4.8	4.4
300% HIIE	3.1	5.29
Xan		
150% HIIE	5.1	6.6
200% HIIE	5.9	7.6
300% HIIE	5.2	10.9*
Hx		
150% HIIE	1.9	7.6*
200% HIIE	1.9	15.8*
300% HIIE	2.0	26.3*#
Uric acid		
150% HIIE	82.0	201.2*
200% HIIE	88.7	167.1*
300% HIIE	78.8	190.3*

* Denotes significance from rest
# Denotes significance from 150% HIIE

5.4 Discussion

This study highlighted that manipulating supra-maximal exercise intensity and duration did not effect of purine loss and substrate metabolism when the same amount of physical work was performed. There were no differences between HIIE bouts in any purine base marker except for significantly elevated urinary Hx after the 300% HIIE trial compared to the 150% HIIE trial (see table 5.0; $p<0.05$). The altered duration of the rest period separating the high intensity exercise bouts did not influence plasma markers of fat mobilisation for potential oxidation. In addition, the participants rated the 300% HIIE protocol more physically demanding than the two other HIIE protocols.

5.4.1 Altering exercise intensity and duration to match workload does not affect metabolic profiles of HIIE

High intensity exercise is perceived to be more physically strenuous that low intensity exercise and metabolically there are many differences in energy metabolism demonstrated by numerous plasma, muscle and urinary variables. However, this study showed that when increasing exercise intensity, and extending the rest period so that the same amount of physical work is completed, the metabolic profiles are similar.

Physiological parameters were not different between the trials, no difference in heart rate and VO_2 between the three workload matched HIIE trials were observed, indicative of similar levels of physiological stress across the three HIIE trials. As a result, any variations, including increases from rest concentrations are due to the nature of the exercise model; short high intense cycling bouts (10-20 s) followed by a rest period (40-50 s).

5.4.1.1 Exercise induced muscle metabolic perturbations

During the exercise or work phase of HIIE, diminished ATP is replenished at a rapid rate by the transfer of phosphate from CP to ATP. After the first exercise bout there was a significant decrease in muscle CP concentrations from rest, progressively decreasing over the course of the HIIE bouts ($p<0.05$; figure 5.13). Potential partial oxidative restoration of CP may have occurred during the rest periods between each sprint, however as complete replenishment requires approximately 3-6 minutes, 40-50s of rest did not allow for this, hence complete replenishment occurring upon cessation of the 90 min recovery period (see figure 5.13). After a single 30 s bout of maximal exercise 65-70% of ATP production comes from glycogenolysis. Muscle glycogen was significantly decreased after the first bout and upon HIIE completion, levels were significantly lower from rest ($p<0.05$; see figure 5.10). This result along with significant increases in muscle lactate from rest ($p<0.05$: see figure 5.11), indicate the glycolytic contribution to HIIE, specifically during the intense cycling phases.

Muscle and plasma lactate increased significantly from rest during all 150%, 200% and 300% HIIE trials (figures 5.11 and 5.7 respectively; $p<0.05$), and remained elevated for the entire exercise duration. Exercise intensity influences plasma lactate concentration, with progressive increases as intensity is amplified. Nevertheless, when HIIE is workload matched, muscle lactate production doesn't increase any higher with increased exercise intensities (see figure 5.11). There were no differences between any of the three HIIE trials at any time point in muscle or plasma lactate concentrations, indicating a similar extent and efflux from the muscle. However there was a strong trend for an increased area under the curve of the plasma lactate profiles in the 300% HIIE trial compared to the 150% HIIE trial ($p=0.057$; see figure 5.7b). Furthermore, urinary lactate excretion concentrations in one

participant appear to be different post exercise compared to rest and different between the three HIIE bouts (see figure 5.15). It should be noted that only one participant's urine was immediately analysed for lactate concentration as part of a pilot study with results shown in figure 5.15. The remaining participant samples were analysed but were influenced by storage and results subjective due to the presence of LDH in the urine (Heintz *et al.,* 1991). Thus it would be ideal to analyse urine samples immediately or treat them with perchloric acid (PCA) upon collection to prevent degradation during storage. Nonetheless, these results suggest that as exercise intensity is increased, despite matched for workload, urinary lactate, the end point of glycolysis, is elevated. Due to the ability of lactate to efflux the muscle and be excreted in the urine, a question remains as the greater possibility of a greater production with a more intensive bout. This needs to be validated and became one of the main aims of chapter 6. Regardless, lactate in the urine is a form of energy loss which needs to be replaced. This energy deficit has never been accounted for in energy balance, thus may play a role in the energy balance equation and potentially elevating energy expenditure, perhaps even larger than purine loss, given the larger-fold difference with lactate (see section 2.9.2.5).

Additonal metabolites lost in the urine, indicative of ATP loss are Hx and uric acid. During repeated sprint exercise, ATP hydrolysis does not match ATP resynthesis, resulting in degradation and ultimately build up of IMP (Broberg and Sahlin, 1989; Bangsbo *et al.,* 1992; Stathis *et al.,* 1994). The key to elevating this imbalance is the consecutive sprints with limited recovery time, preventing reamination of Hx to IMP via the purine salvage pathway and exacerbating Hx accumulation in the plasma (Tullson *et al.,* 1995). After an exercise bout, elevated purine levels (Hx and uric acid) in the plasma and urine (above basal), equates to increased energy loss and potentially heightened energy expenditure, as there is

greater energy requirement to synthesise *de novo* ATP (Newsholme and Leech, 1983). Plasma Hx was significantly elevated from rest 10 mins post exercise in all three HIIE trials (see figure 5.18; $p<0.05$). This pattern followed into the urine with post exercise urinary Hx and uric acid significantly greater compared to rest concentrations (see figure 5.22 and 5.23 respectively; $p<0.05$). These results suggest that there may have been significant ATP degradation during all three HIIE trials, requiring energy to *de novo* replenish ATP, creating an energy deficit which may contribute elevating energy expenditure. Interestingly there were no significant decreases in ATP concentration observed (see figure 5.12). It may be that the work and rest nature of HIIE allows for a great proportion of Hx salvage to be resynthesised to ATP. However, the small amounts, although not significant statistically, are still significantly metabolically as Hx was elevated from rest and was excreted in significant amounts in the urine. Interestingly, and not in accordance with plasma results, urinary Hx excretion post exercise was significantly higher in the 300% HIIE trial compared to the 150% HIIE trial and this may equate to a greater amount of energy required to restore degraded ATP in the 300% HIIE compared to the 150% HIIE trial. This result highlights the importance that exercise intensity has on inducing metabolic perturbations resulting in potential energy loss. This is supported by a training study comparing CON and intermittent exercise, but of a lower intensity (CON equating to 30 mins at 60-75% max heart rate and intermittent consisting of two 15 min bouts at 50-65% max heart rate) and found that the exercise protocol that was higher in exercise intensity (CON protocol) produced greater weight loss (Donnelly *et al.,* 2000). Therefore even though the mode of exercise was continuous exercise, the greater exercise intensity induced greater metabolic changes pertaining to fat mass loss. Maintaining high intensity repeated sprints that promote purine and lactate loss as seen in section 2.9.2 allowing for elevated accumulation of these metabolic by products

and potential energy loss, are due to the rest period. Therefore the key aspects of exercising to lose weight may be high intensity exercise that can be sustained for a prolonged duration of time, thus incorporating a rest break. Futhermore the rest period may be elevate fat metabolism during glycolytic exercise.

5.4.1.2 The value of incorporating a rest period into HIIE

High intensity exercise is synonymous with glycogenolysis and significant lactate accumulation in the muscle and blood stream, resulting in depressed oxidation of fat (Romijn *et al.,* 1993) (see section 2.7.1). Yet a study from our laboratory proposes a role for fat metabolism during HIIE, likely due to the work - rest nature of the model (Borg *et al.*, 2008), reflecting the substrate partitioning theory first put forth by Tremblay *et al.* (1994) established in section 2.9.1. The current study aimed to determine if increased exercise intensity, hence decreased cycling duration and a subsequent prolonged rest period, affected plasma markers of fat utilisation. The workload matched protocol rest periods were 40 s to 45 s to 50 s, for the 150%, 200% and 300% HIIE bouts respectively, however no differences in plasma glycerol and plasma FFA were detected between the three HIIE trials (see figure 5.6 and 5.5 respectively). Plasma glycerol, reflective of lipolysis, rose significantly from rest during HIIE and remained elevated in the recovery periods post exercise (see figure 5.6; $p<0.05$), while plasma FFA remained at similar levels to that of rest (see figure 5.5). Together these results suggest elevated lipolysis during exercise with the released FFA potentially utilised as a fuel source during the rest periods, as demonstrated in 2.9.2. Alternatively, it is possible that IMTG were oxidised and the glycerol efflux the muscle, accumulating in the plasma and the FFA were reesterified into adipose tissue as per section 2.5. The longest difference in rest interval was 10 s (40-50 s rest durations), this change may

not have been long enough to elicit such changes in fat metabolism between the three HIIE trials.

Although no differences were observed in muscle, plasma or urinary metabolites after single bouts of the exercise, beneficial adaptations may be acquired after chronic HIIE training. Following HIIT regimes, gradual increases in mitochondria content, mitochondrial enzymes and transport proteins regulating fat metabolism have been observed (Talanian *et al.,* 2007; Perry *et al.,* 2008; Gibala *et al.,* 2009; Little *et al.,* 2011). Various models of HIIT have shown increased mitochondrial proteins and enzymes pertaining to oxidative metabolism, in particular PCG1α responsible for mitochondrial biogenesis (Little *et al.,* 2011; Gibala *et al.,* 2012), FAT/CD36, FABPpm and FATP responsible for FFA transport into the skeletal muscle (Talanian *et al.,* 2007; Perry *et al.,* 2008) and CPT1 which takes FFA into the mitochondria (Talanian *et al.,* 2010). This is an area that warrants further investigation with western blotting and implementing the 150%, 200% and 300% HIIE protocols into HIIT regimes to determine if the slight differences in rest time accrue to favourable metabolic changes within the skeletal muscle (see section 9.2.3). As well as the aforemention PGC1α, AMPK is an upstream regulator of PGC1α is increased after just one HIIT session, as is mRNA of PGC1α (Burgomaster *et al.,* 2008; Gibala *et al.,* 2009), therefore it may be pertinent to complete the above mentioned western blots on the current muscle samples. AMPK and hence PGC1α are activated by a number of factors such as decreases in ATP concentration and ratio changes of ATP:ADP/AMP. In the current study, ATP content (see figure 5.12), plasma and urinary purine nucleotides (downstream by-products of mis-matched ATP hydrolysis/resynthesis), were not significantly different between the three HIIE trials, except for urinary Hx, which was greater post 300% HIIE compared to 150% HIIE. Thus as few

significant changes were observed, however this may not translate in differences in the activation of AMPK and PGC1α.

5.4.2 Post exercise energy deficit

EPOC is the immediate time post exercise when VO_2 remains above resting levels and is reflective of elevated energy utilisation to return exercise induced metabolic disturbances to rest, maintaining homeostasis (LaForgia et al., 2006). VO_2 remained elevated above rest until 15 mins post exercise, before returning to rest ($p<0.05$; see figure5.1).

Exercise duration and intensity are the two leading factors influencing EPOC, leading to greater VO_2 after HIIE compared to continuous exercise at a submaximal intensity (Lyons *et al.,* 2006; Kaminsky *et al.,* 1990, Phelian *et al.,* 1997). Interestingly there was no difference between the three HIIE trials, 150%, 200% or 300% HIIE, in VO_2 post exercise (see figure 5.1). The effect of exercise intensity on EPOC may be diminished when intensity is accounted for by duration or may be reflective of the similarity of metabolic changes occurring in all three bouts, requiring similar energy consumption for restoration initially.

Similarly in workload-matched HIIE and CON bouts, there were no differences observed in EPOC (Borg *et al.,* 2008). Others have employed workload matched HIIE and found differences in EPOC (Kaminskey *et al.,* 1993) attributing the increases to exercise intensity (Kaminskey *et al.,* 1993), these results not consistent with those produced via our laboratory.

As mentioned chapter 4 EPOC has been shown to last for several hours post exercise (Bahr and Sejersted, 1991). True EPOC may not be apparent in this study. Due to exercise anticipation and anticipation of muscle biopsies VO_2 which may have been recorded falsely

high (Borsheim and Bahr 2003). An additional explanation alluding to decreased EPOC may be the equipment utilised is not sensitive enough to detect such changes, demonstrated in section 2.9.2.3.4. As mentioned in chapter 4, to obtain resting respiratory gas measure, our laboratory uses a Hans Rudolph mask. There are other methodologies available to obtain more precise respiratory measures at rest such as a canopy system (Moxus, AEI technologies) or a calorimetry chamber is which the participant rests. Nonetheless VO_2 and RER are used in this thesis as comparative measures due to the supra-maximal intensities of the exercise bouts. Regardless, plasma glycerol concentrations continued to increased post HIIE, whilst plasma FFA remained consistent with rest, consistent with RER of 0.8 (see figure 5.4) reflective of fat utilisation. Therefore post HIIE, plasma FFA may have been oxidised as concentrations stayed stable (see figure 5.5) rest levels and physical rest.

5.4.3 Conclusion

This study demonstrates that exercise intensity does not affect substrate metabolism when HIIE is matched for physical work. There were no consistent differences in metabolic profiles between the 150%, 200% and 300% HIIE trials, therefore the protocol perceived to the easiest provides the same metabolic changes leading to energy deficit. Therefore the 150% HIIE where participants cycled for 20 s at 150% VO_{2pea}k followed by 40 s rest is seemingly the easiest.

Furthermore, it is likely that EPOC is only a small contributor to fat use associated with exercise, whereas the big differences occur when purines are excreted, hence energy loss that requires *de novo* replacement. It is also speculated that lactate excretion may be a metabolic consequence of HIIE and along with the excretion of purine bases, the loss may contribute to whole body energy deficit.

Chapter six: Urinary lactate excretion increases as exercise intensity is increased in healthy untrained males

6.1 Introduction

There are dramatic metabolic perturbations that occur during short intense exercise and the subsequent recovery period, that may help to elucidate potential mechanisms that may explain the observation of greater reductions in adiposity with potentially lower caloric output, associated with HIIT (Tremblay *et al.,* 1994; Trapp *et al.,* 2008).

Chapter 4 of this thesis demonstrated that plasma markers reflective of substrate metabolism and purine nucleotide base loss are similar despite significant differences in the energy cost of exercise between the two metabolically exhausting exercise protocols, RSA and CCT. Furthermore, the previous chapter of this thesis showed that exercise intensity does not play a role in substrate metabolism and purine loss when workload is matched between HIIE trials. Furthermore, results from the previous chapter show increased purine loss yet no change in ATP concentration. Hence the metabolic cost of replacing the lost ATP (calculated from urinary purines) is likely to provide only a minor contribution to energy balance and inducing negative energy balance required to produce considerable morphological changes observed with chronic HIIT compared to CONT (Tremblay *et al.,* 1994; Trapp *et al,.* 2008). This indicates a further metabolic mechanism may be contributing to shifting energy balance negative.

A pilot study from chapter 5 examining urinary lactate excretion in one participant, exhibited increased urinary lactate concentrations after HIIE compared to rest, with a trend for elevations in excretion as exercise intensity increased. Therefore exercise intensity may influence urinary lactate excretion.

Lactate accumulates in the blood at magnitudes of 1000-fold greater than Hx and blood concentration (µmol.hr-1 to mmol.hr^{-1}) progressively increases with increasing exercise intensity (Billat, 1996).

Blood lactate concentration is determined by the balance of lactate production and removal (Hubbard, 1973). As discussed in section 2.9.2.5, skeletal muscles are the primary tissue responsible for lactate production which is expelled into the bloodstream through lactate transporters. Circulating lactate can be taken up by the liver (Kayne and Alpert 1964; Rowell *et al.,* 1966) where the Cori Cycle results in conversion of lactate to pyruvate hence glycogen storage (Hubbard, 1973). Other tissues can also take up lactate, (see section 2.9.2.5). However, blood lactate concentrations that occur post high intensity exercise, results in lactate filtered by the kidneys and excreted in the urine (Liljestrand and Wilson, 1925). Numerous studies have demonstrated this result (refer to section 2.9.2.5) (Liljestrand and Wright-Wilson, 1925; Johnson and Edwards, 1937; Miller and Miller 1948; Pechlivanis *et al.,* 2010), yet interestingly, from an energy balance standpoint, no study has considered lost lactate from the body as energy deficit which must be replaced.

Lactate is a three carbon molecule derivative of glucose breakdown, and contains approximately half the potential energy of a glucose molecule, with metabolic energy sufficient to re-phosphorylate 16 ATP molecules via oxidative phosphorylation in the Krebs Cycle (Gropper *et al.,* 2009). Thus in the same way that purine excretion can be considered a

loss of ATP that needs to be resynthesised, urinary lactate excretion is a direct loss of energy from the body that must be replaced. Replacement by dietary sources is independent of O_2 consumption, thus does not influence EPOC. As urinary lactate excretion escalates with more vigorous exercise bouts (Pechlivanis *et al.,* 2010), increasing exercise intensity may provide an additional means for energy loss following intense exercise protocols which has been previously unaccounted for in the energy balance equation.

6.1.1 Aims and hypothesis

The aim of this study was to measure urinary lactate excretion following four different exercise intensity models; two 30 min work-load matched exercise bouts at high and low intensity and two, short, fatiguing exercise bouts designed to maximise glycolysis and lactate production. The primary hypothesis is that higher peak plasma lactate post-exercise will result in a greater magnitude of urinary lactate excretion, indicative of increased energy loss that must be replaced. This may provide an avenue for substrate loss and thus alterations in energy balance associated with the exercise bout.

It is hypothesised that intense intermittent exercise will result in significantly higher plasma lactate concentration that will result in greater urinary lactate excretion compared to submaximal continuous exercise. Furthermore, the two short fatiguing exercise bouts will cause an elevated concentration in the lactate compared to intermittent exercise, due to the higher intensity of exercise.

6.2 Methods

6.2.1 Participants

Six healthy untrained males aged between 18-35yrs (28 ± 4.9yrs; 85.7 ± 11.4 kg; 180 ± 4.8 cm; 49.35 ±6.26 ml.kg.min^{-1}) volunteered to take part in this study which was approved by the Victoria University Human Research Ethics Committee and performed in accordance with the ethical standards set out in the 1964 Declaration of Helsinki. Before presenting for preliminary testing, each participant completed a medical questionnaire and signed consent forms.

6.2.2 Preliminary Testing

Participants' underwent a $VO_{2\ peak}$ test as per section 3.2.1.

6.2.2.1 Respiratory gas exchange

Respiratory gas measurements were collected as detailed in 3.2.1.

6.2.3 Trial procedure

In the 24 hrs prior to testing, participants were requested to abstain from exercise, alcohol and caffeine and to complete a 24 hr food recall to replicate diet for each subsequent testing session to avoid resting substrate variation.

6.2.3.1 Experimental design

In a cross-over design, participants completed four different exercise protocols in random order, each testing day at least one week apart. Two workload matched protocols

(HIIE and CON) on a cycle ergometer and a further two maximal protocols, (RSA and CCT) on a Wattbike and Lode cycle ergometer, respectively.

The **CON** protocol is 30 min of continuous cycling at a workload calculated at 50% of VO_{2peak} (125 ± 21W) while the workload matched **HIIE** consists of 30min of 20 s cycling at 3 time 50% VO_{2peak} followed by 40 s passive rest (385 ± 678 W)**.** As mentioned in chapter 4, the **RSA** trial encompasses five 6 s maximal sprints separated by 24 s rest and the **CCT,** cycling at a workload calculated as 110% of power output achieved at VO_{2peak} till volitional exhaustion (352 ± 56 W).

Participants presented to the laboratory in the morning to avoid circadian variance and after an overnight fast (10-12 hrs).

6.2.4 Measurements

6.2.4.1 Blood Sampling and analysis

For the HIIE and CON trials blood was collected at rest, and at the end of exercise and in the recovery periods at 5 (R5), 10 (10), 15 (R15), 30 (R30), 45 (R45), 60 (R60), 75 (R75) and 90 mins (R90). For the RSA and CCT trials, blood was collected at rest, upon exercise completion (R00) and in the recovery period at 5 (R5), 10 (R10), 15 (R15), 30 (R30), 45 (R45) and 60 mins (R60). 10ml of blood was collected at each time point as per section 3.5.4.

Plasma was analysed for plasma glucose and lactate using a Yellow Springs analyser as per section 3.5.4.2 plasma analysis.

6.2.4.2 Urine collection and analysis

Urine was collected and analysed for urinary glucose and lactate as described in section 3.5.5. Complete urine volume was collected for 60mins post CCT and RSA exercise

and 90mins after the HIIE and CON exercise and concentrations are corrected for time and volume. The 30 min time difference between post exercise collections, may not affect results as concentrations are corrected for volume and time. Furthermore, the lower collection time period may result in an underestimation of concentration.

6.2.5 Statistical Analysis

Subject characteristics are presented as mean ± SD while all other results are expressed as means ± SEM. All results were analysed using Graph-pad Prism software version 6.02 as detailed in section 3.6. Plasma and urinary results were analysed using two way repeated measures ANOVA, with time as the within group factor and exercise protocol as the between group factor used to detect significance. Section 3.6 details the post hoc tests employed when an interaction between trials was detected. A matched, paired t-test was used to determine differences in total work (kJ) and significance was accepted at $P<0.05$ for all results.

6.3 Results

6.3.1 Total work of exercise

The energy expended during HIIE and CON trials both used similar levels of energy, 226 and 226 kJ of energy respectively, whereas CCT and RSA used significantly lower levels of energy 47 and 22.10 kJ respectively. CCT was significantly higher than RSA ($p<0.01$).

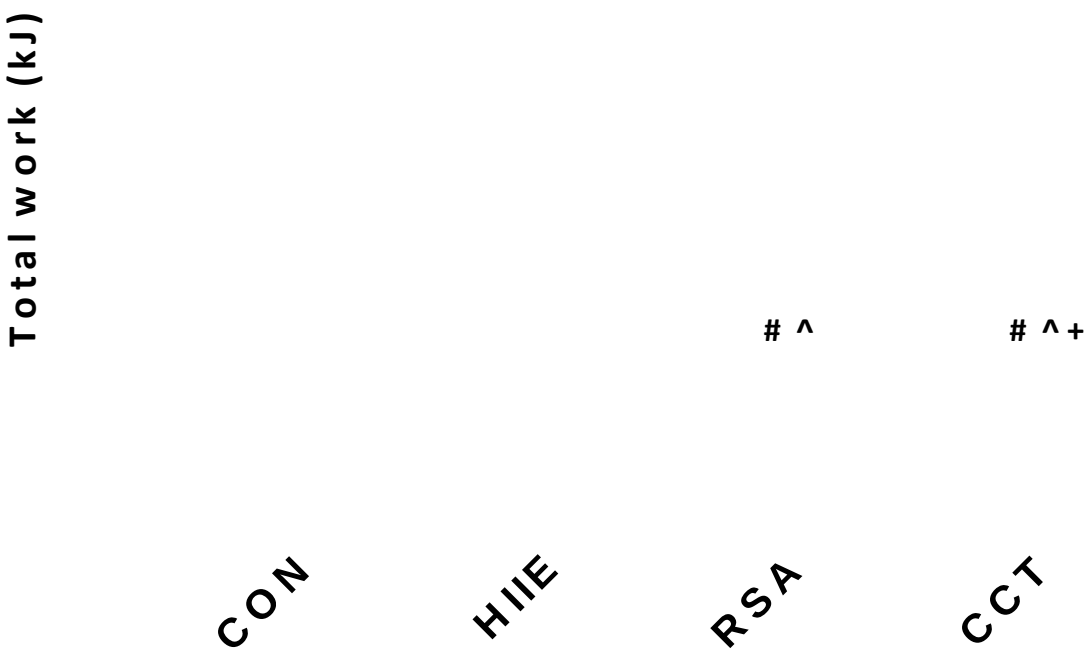

Figure 6.0: Total work in kilojoule (kJ) performed in CON, HIIE, RSA and CCT exercise bouts
Data are means ± SEM; N = 6
# p<0.01 from CON
^ p<0.01 from HIIE
+ p<0.01 from RSA

6.3.2 Plasma lactate and glucose

There was a significant interaction and effect of time on plasma lactate concentration ($p<0.05$). There was no difference in basal concentrations of the four exercise bouts and no difference in the CON bout throughout the exercise and recovery period. The HIIE, RSA and CCT trials displayed significantly elevated plasma lactate from rest upon exercise cessation (R00), at R5, R10 and R15 mins post exercise ($p<0.05$), with the RSA and CCT also remaining elevated at R30 mins ($p<0.05$). Plasma lactate in the RSA, CCT and HIIE trials were significantly greater ($p<0.05$) compared to the CON trial at R00, R05, R10 and R15 ($p<0.05$). In addition, the RSA and CCT plasma lactate were significantly higher than the HIIE trial at R05, R10 and RC15 ($p<0.05$). There were no differences in lactate concentrations between the RSA and CCT trials.

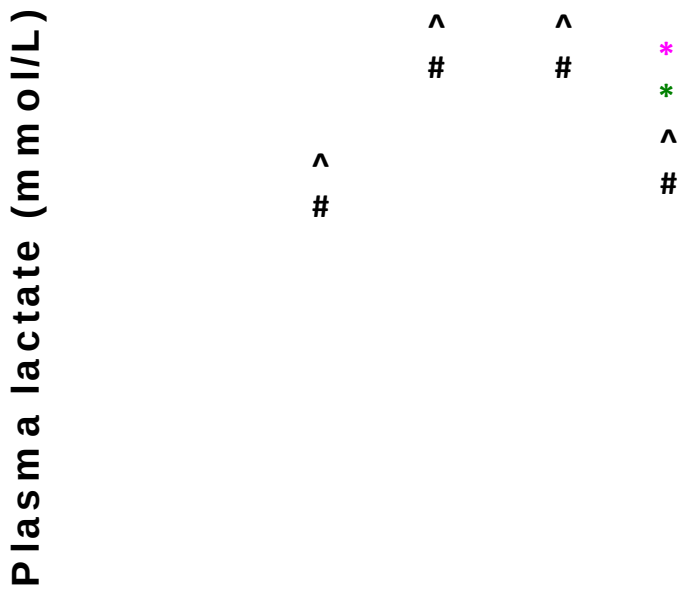

Figure 6.1: Plasma lactate concentration during and after CON, HIIE, RSA and CCT exercise
Data are means ± SEM: N = 6
* p<0.05 from rest levels (coloured asterix denotes significant from rest in that respective trial)
# p<0.05 HIIE, RSA and CCT significantly different from CON
^ p<0.05 RSA and CCT significantly different from HIIE

There was a significant interaction ($p<0.05$) and significant time effect on time ($p<0.001$) on plasma glucose concentration. There was a significant increase from rest in the RSA and CCT trials post exercise at R05, R10 and R15 ($p<0.05$). No other significance was detected.

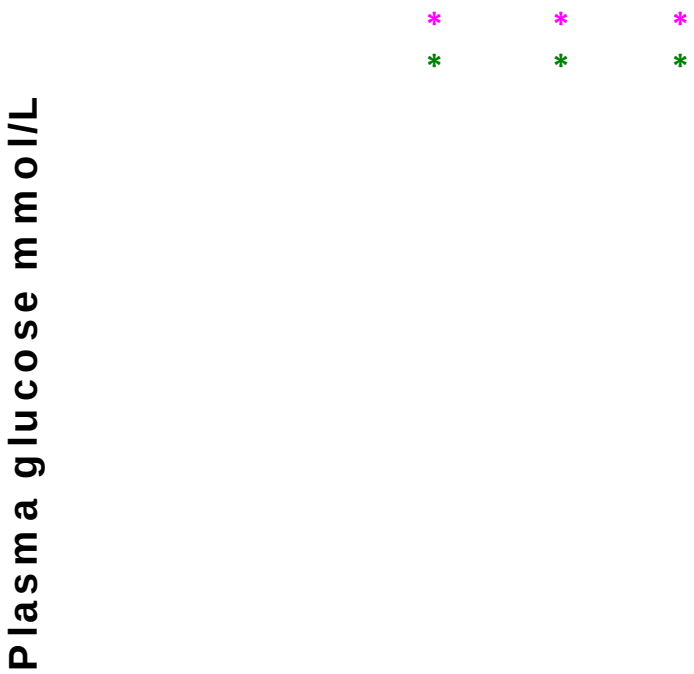

Figure 6.2: Plasma glucose concentrations during and after CON, HIIE, RSA and CCT exercise

Data are means ± SEM: N = 6

* $p<0.05$ from rest levels (coloured asterix denotes significant from rest in that respective trial)

6.3.3 Urinary lactate and glucose

Resting concentrations of urinary lactate were the same in all four trials. HIIE, RSA and CCT urinary lactate was significantly elevated from rest compared to post exercise ($p<0.05$). Post exercise urinary lactate excretion was significantly higher after the RSA and CCT trial compared to the HIIE ($p<0.01$), almost a 3-fold increase in excretion compared to HIIE; however no differences detected between RSA and CCT trials.

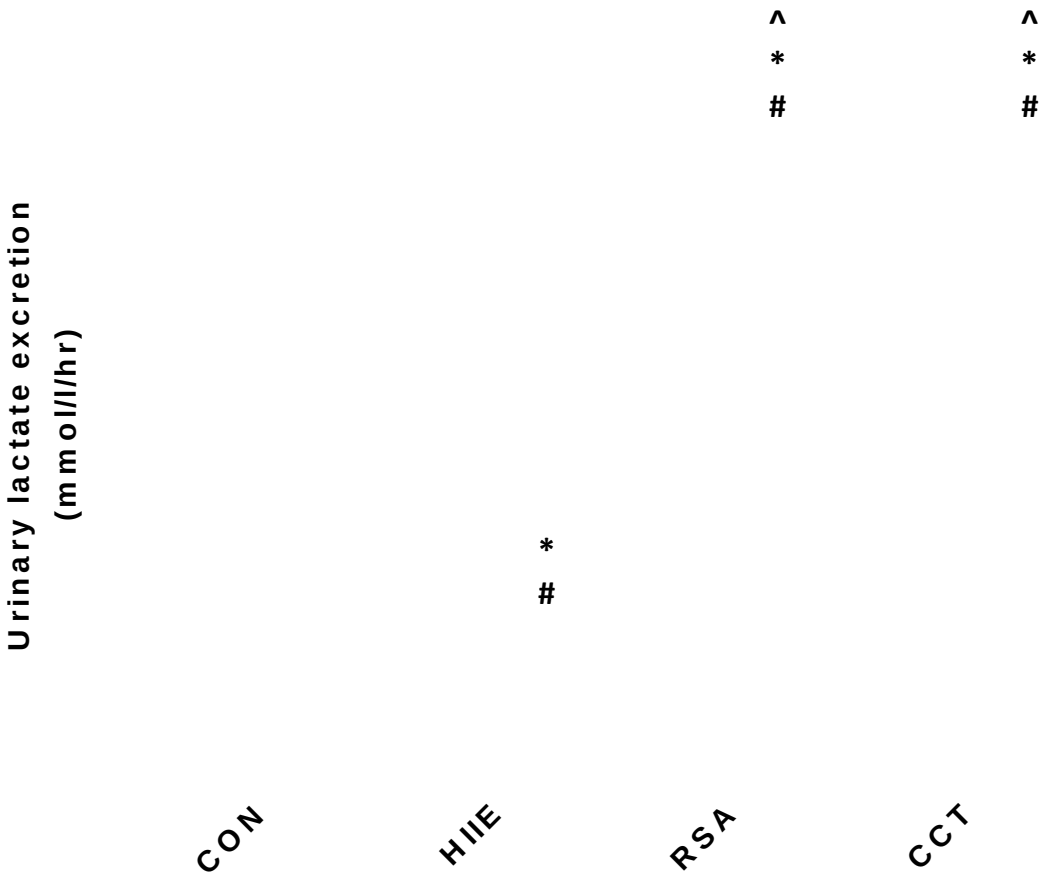

Figure 6.3: Urinary lactate excretion pre and post CON, HIIE, RSA and CCT
Data are means ± SEM; N = 6; open column = rest; full colour = post exercise
*p<0.05 significantly different from rest levels
# p<0.05 HIIE, RSA and CCT significantly different from CON
^ p<0.05 RSA and CCT significantly different from HIIE

There was a significant increase in urinary glucose excretion post exercise compared to rest in the CON trial only ($p<0.05$). No other significance was detected.

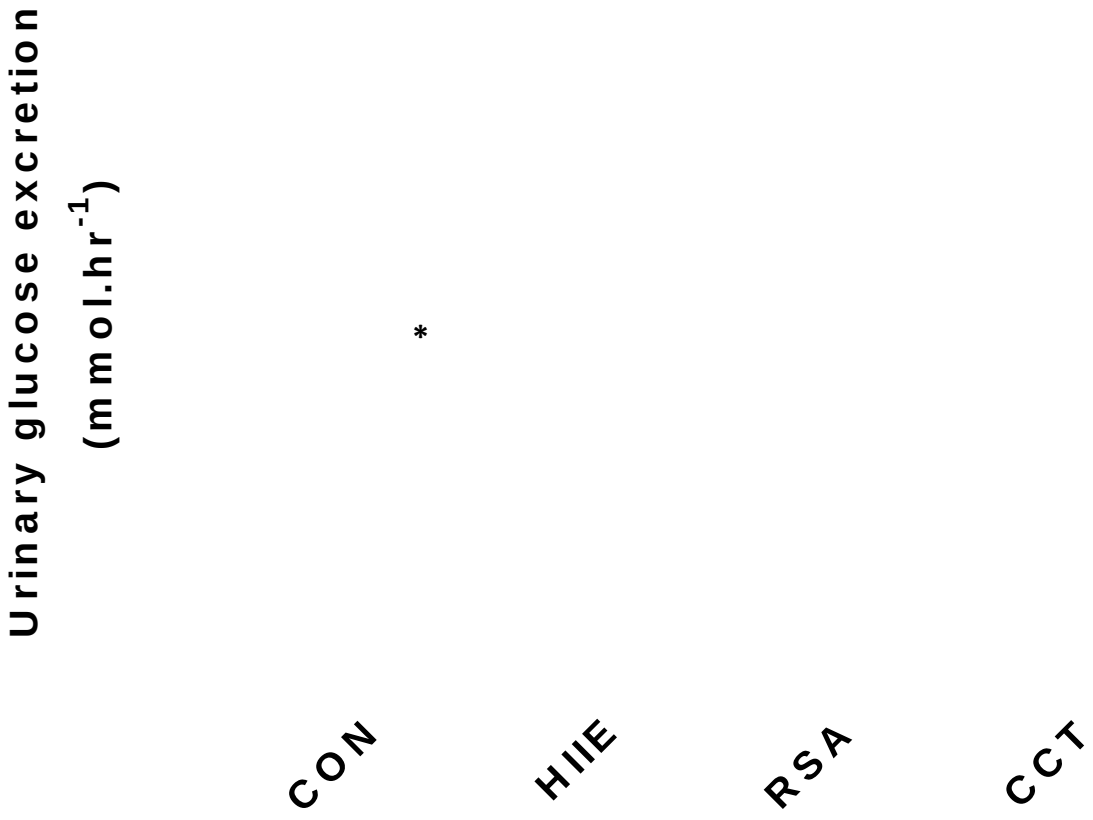

Figure 6.4: Urinary glucose excretion pre and post CON, HIIE, RSA and CCT exercise
Data are means ± SEM; N = 6; open column = rest; full colour = post exercise
* p<0.05 from rest levels

6.4 Discussion

This study demonstrated an elevated urinary lactate excretion as exercise intensity increases, with results clearly showing that lactate is significantly excreted in the urine and that excretion is increased as exercise intensity is increased. Four different exercise protocols were compared and the influence of intensity on urinary lactate excretion as a possible mechanism for energy loss from the body, which must be replaced. This unaccounted for energy loss is not considered in the energy balance equation, hence may provide another metabolic mechanism elevating energy expenditure possibly contributing to the accelerated fat loss associated with HIIT compared to CONT (Tremblay *et al.,* 1994; Trapp *et al.,* 2008).

6.4.1 Urinary lactate excretion increases as exercise intensity increases

Plasma lactate concentrations were elevated from rest in the HIIE, RSA and CCT trials with the more intense protocols producing higher plasma lactates profiles irrespective of the total workload performed (see figure 6.1). HIIE resulted in higher plasma lactate levels compared with CON ($p<0.05$) further elevated with RSA and CCT protocols compared with CON ($p<0.0001$). The work performed in the CCT was higher than the RSA ($p<0.01$) however, they amounted to 20% and 10%, respectively, of the work performed in the HIIE and CON trials, which were workload matched (see figure 6.0). This highlights the importance of the mode of exercise, in particular, the influence of exercise intensity on plasma lactate concentrations. A similar pattern was observed with urinary lactate excretion profiles which were significantly greater after HIIE compared to CON ($p<0.05$) and further elevated after

RSA and CCT ($p<0.01$, see figure 6.3). Importantly, these observations in CCT and RSA were made against a substantially reduced total work output. This suggests that exercise duration is less important than intensity for lactate production and subsequent efflux from the muscle. This is further supported by the two very high intensity, fatiguing exercise protocols (RSA and CCT), with a high glycolytic contribution of energy supply, further exacerbating plasma lactate accumulation and the urinary lactate excretion rate (see figures 6.1 and 6.3 respectively), despite performing substantially lower total work (kJ) compared with the HIIE and CON protocols (see figure 6.0) and over a much shorter duration.

Presence of lactate in the urine following physical exertion has been recognised for decades (Liljestrand and Wright-Wilson, 1925; Johnson and Edwards, 1937; Miller and Miller 1948; Pechilvanis *et al.,* 2010). Unlike plasma lactate and on occasion muscle lactate, which has been used for monitoring and understanding metabolism of physical performance and training applications (Billat, 1996), little consideration has been given to the metabolic outcome of post exercise urinary lactate excretion. During vigorous exercise, an increased rate of glycogenolysis results in elevations in muscle pyruvate and lactate concentrations (refer to section 2.7.1). Muscle lactate production is influenced by exercise intensity and duration with concentrations of lactate accumulating in the bloodstream increasing when energy demand exceeds the metabolic oxidative capacity of the muscle, requiring a greater glycolytic contribution to sustain the exercise (Broberg and Sahlin 1989; Medbo and Tabata 1993; Bogdanis *et al.,* 1995; Bogdanis *et al.,* 1996; Bogdanis *et al.,* 1998; Balsom *et al.,* 1999).

The excretion of lactate in the urine is influenced by the magnitude of the post exercise plasma lactate concentration and subsequent renal lactate handling. Pyruvate is

also excreted in the urine, however excretion of pyruvate is relatively insignificant compared to lactate (Hubbard, 1937), and was not measured in this study. A closer look at urinary excretion patterns of lactate relative to plasma accumulation after exercise indicates that lactate excretion follows a similar pattern to that of glucose excretion (Miller and Miller, 1949). At low plasma concentrations, reuptake of lactate from the filtrate in the renal tubules occurs to a threshold (~6 mmol/L) before excess lactate molecules not taken up in the tubules are subsequently excreted in the Miller and Miller 1949). The urinary excretion patterns observed in this study support this trend and a greater magnitude of urinary lactate excretion is excreted with the more intense bouts (Pechilivanis *et al.,* 2010).

Urinary lactate excretion results from this current study provides additional weight to studies where elevated urinary excretion of purine bases (Hx and uric acid) were significantly elevated after greater number of repeated sprints (Stathis *et al.,* 1994; Hellsten et al., 1999; Stathis *et al.,* 1999; Saiki *et al.,* 2001; Stathis *et al.,* 2006; Pechlivanis *et al.,* 2010) and in HIIE when compared to CON (Borg *et al.,* 2008). Purine base excretion was twice the magnitude after an acute bout of 30mins HIIE than a workload matched CON exercise (Borg *et al.,* 2008), thus more energy utilised to restore *de novo* ATP, a metabolically costly mechanism (Newsholme and Leech, 1983; Hellsten *et al.,* 1998) demonstrated in section 2.9.2.4.

Interestingly, the degree of urinary lactate excretion and the relative molecular energy loss is greater by a factor of 3200 compared with urinary purine base excretion. This is taken from the assumed *de novo* replacement cost of 5 ATP for every Hx molecule that originated from the muscle (Newsholme and Leech, 1983), compared to 16 molecules of

ATP that can be generated from a lactate molecule and the 1000 fold difference in relative excretion rates between purines and lactate.

Thus the metabolic impact of excreting lactate compared to purine bases is considerably greater in respect to energy expenditure. Losing lactate in the urine is a potential avenue for lost energy which is not accounted for in whole body energy balance equation, and may need to be considered more closely in estimations with exercise that requires significant glycolytic or adenine nucleotide replenishment or restoration, respectively. Nonetheless, together and over periods of time, these imbalances in energy balance accumulate and can explain the greater reduction in adiposity and expended energy associated with HIIE training compared with CON training (Tremblay *et al.,* 1994; Trapp *et al.,* 2008).

6.4.2 Conclusion

These results substantiate the hypothesis that urinary lactate excretion is influenced by the extent of plasma lactate accumulation and exercise intensity. The results also demonstrate that urinary lactate excretion is a major metabolic mechanism influencing energy balance during intense exercise due to exercise induced energy deficit.

Lactate lost from the body post metabolically stressing exercise results is subsequent energy loss which must be replaced. Thus these results offer an additional metabolic avenue for enhancing energy expenditure and reducing adiposity.

Chapter seven: The metabolic impact of all out high intensity intermittent exercise in healthy untrained adults

7.1 Introduction

HIIT is a very effective exercise protocol at reducing adiposity and elevating energy expenditure compared to other exercise models (Tremblay *et al.,* 1994; Trapp *et al.,* 2008; Macpherson et al., 2011; Gremeaux et al., 2012). This exercise type has additional health benefits such as improved insulin sensitivity (Little *et al.,* 2011; Gillen *et al.,* 2012) and improved cardiovascular health (Wisloff *et al.,* 2007; Helgerund *et al.,* 2010; Guirand *et al.,* 2011; Meyer *et al.,* 2012). From a fat loss perspective, it is essential to determine the best HIIE model that maximises fat utilisation and enhances energy expenditure.

Many HIIT models have been employed by various research groups; comparing CON to HIIE and show significant fat mass loss, particularly abdominal fat loss (Trapp *et al.,* 2008) after HIIT compared to CONT (Tremblay *et al.,* 1994; Trapp *et al.,* 2008), demonstrated in section 2.9.1. It is beneficial to maximise the metabolic benefits of HIIT by employing an optimal HIIE protocol which exacerbates each metabolic consideration of reducing adiposity and shifting energy balance negative. Chapter 4 of this thesis demonstrated that out all maximal effort exercise elevated purine loss, but resulted potential depressed fat utilisation. Whereas chapter 5 aimed at elevating purine loss and elevating fat utilisation by manipulating HIIE protocols and established heightened purine loss and possible reliance on fat and glycogen during exercise. However as exercise intensity increased and HIIE models

manipulated, these metabolic changes were not improved. Chapter 6 of this thesis employed exercise protocols from chapter 4 and 5 and validated another potentially more influential metabolic avenue for energy deficit that is unaccounted for in energy balance, urinary lactate excretion. Therefore it is favourable to combine "all out" maximal intensity exercise with the work and rest HIIE model to construct an exercise model that encompasses all beneficial aspects of the modalities utilised in chapter 4, 5 and 6 of this thesis will be tested in males and females.

Males and females exhibit diverse metabolic responses during rest and exercise conditions, owing to differences in male and female steroid hormone concentrations and fluctuations (D'eon *et al.,* 2012). Hormones influence substrate metabolism, specifically in females, oestrogen and progesterone exhibit antagonistic effects on lipolysis as stated in section 2.10. Moreover females typically show a greater reliance on fat as an energy source compared to males. Hence using exercise protocols aimed at decreasing adiposity, females may lose greater relative amounts of fat mass compared to males. Therefore this study will investigate substrate metabolism in males and females in maximal effort HIIE.

7.1.1 Aims and hypothesis

This study will employ 'all out cycling' maximum effort HIIE protocol, to determine whether an 'all out' HIIE model will induce all favourable metabolic changes needed to maximise energy deficit observed in the earlier chapters of this thesis. HIIE protocols from another research group will be employed but altered slightly, increasing the exercise intensity to 'all out' maximum effort and decreasing exercise duration (Trapp *et al.,* 2007). Commissioning females, Trapp *et al*. (2007) employed two 20 mins of HIIE, the two

protocols; 8 s cycling, separated by 12 s rest **(8:12)** and 24 s cycling followed by 36 s rest **(24:36).** Exercise intensity was set at the power output achieved at 70% VO_{2peak}. The current study will also use the 8:12 and 24:36 model, but increase intensity to wingate levels (resistance set against body weight) and decrease the total HIIE duration to 10mins.

The aim was to investigate the metabolic response and changes in substrate utilisation during these two different all out HIIE models in order to see if either protocol produces favourable shifts in metabolism aimed at producing metabolic markers reflective of decreasing adiposity and enhancing energy expenditure.

It is hypothesised that purine nucleotide loss and lactate excretion will be significantly greater in the 24:36 model as opposed to the 8:12 bout of the male group, reflective of greater ATP degradation and glycolysis thus opportunity for energy loss. In addition to this, as the rest period is 36 s compared to 12 s, possible greater fat utilisation will be observed in the 24:36 HIIE trial. In the female cohort, it ishypothesised that the metabolic patterns will be similar to the males, with the 24:36 HIIE trial inducing significantly greater changes in plasma and urinary markers of metabolism compared to the 8:12 HIIE trial. Although similar patterns in plasma and urine may be found, it is hypothesised that there will be a greater change in plasma glycerol in females compared to males, an indirect reflection of greater possibility of fat utilisation. In addition, plasma and urinary metabolites indicative of purine metabolism will be in lower in relative concentrations in females compared to males as females are better at reamination of Hx.

7.2 Methods

7.2.1 Participant characteristics

Eight healthy, untrained males aged between 18-35 years (29 ± 3.8yrs, 177 ± 7.7cm, 77 ± 10kg; 48 ± 5.8 ml.kg.min^{-1}) and seven healthy, untrained females aged between 18-35 years volunteered to take part in this study (27 ± 5 yrs.; 165 ± 4.5 cm, 65 ± 8.4 kg; 37±5.5 ml.kg.min^{-1}) volunteered to take part in this study The experimental design and all procedures undertaken by participants were approved by the Victoria University Human Research Ethics Committee performed in accordance with the ethical standards set out in the 1964 Declaration of Helsinki. Participants provided written and verbal informed consent and completed medical history forms prior to the commencement.

7.2.2 Preliminary Testing

At least one week prior to trial days, all participants underwent a $VO_{2\ peak}$ test comprehensive detailed located in section 3.2 regarding preliminary testing. The W was set different for males and females, the female protocol consisting of 3 min x 3 sub-maximal workloads of 25, 50 and 75 watts (W), each subsequent workload increased every minute by 25 W until volitional exhaustion as detailed in section 3.2.1.

7.2.2.1 Respiratory gas exchange

Respiratory gas measurements are described in section 3.5.1.

7.2.3 Familiarisation session

Participants underwent a familiarisation session prior to the testing days as set out in section 3.3.

Female participants underwent a familiarisation session prior to the testing days. As trial days were separated by approximately one month (see section 3.4 experimental design), two familiarisation sessions were given to each participant to ensure complete replication of the trial day.

7.2.4 Trial procedure

For 24 hrs prior to the trial testing day, participants were instructed to abstain from alcohol, physical activity and caffeine as well as maintaining a 24 hrs food diary in order to replicate meals for the following trial days to prevent substrate variation.

7.2.4.1 Experimental design

In a randomised order and separated by at least one week for males and one for female participants, completed the two exercise protocols; 8 s all out cycling followed by 12 s rest **(8:12)** and 24 s all out cycling followed by 36 s rest **(24:36).**

Exercise bouts were 10 mins duration preceded by a rest period and followed by 90mins of recovery.

Participants arrived at the laboratory in the morning in a post-absorptive state after an overnight fast of approximately 10-12 hrs and were instructed to consume water *ad libitum.* The start time for each trial for a given participant remained consistent to avoid influence of circadian variance. While resting in a supine position, participants gave rest respiratory gas measures and were prepared for blood collection.

7.2.4.2 Respiratory gas collection and analysis

Respiratory gases were collected at rest and for the first and last 10 mins of the 90 min recovery period. Collection measures are detailed in section 3.5.

7.2.4.3 Blood Collection and Analysis

Blood was collected at rest, at 5 mins of exercise (E5), at the end of exercise (E10) and recurrently during the recovery period at 5 (R5), 10 (R10), 15 (R15), 30 (R30), 45 (R45), 60 (R60), 75 (R75), 90 mins (R90). Blood sampling methods are described in section 3.5.4 with analytical methods of plasma FFA, glycerol, glucose, lactate, insulin, inosine, xanthine, Hx and uric acid found in section 3.5.4.2.

7.2.4.4 Urine collection and analysis

Urine was collected as per details in section 3.5.5. Urine samples were measured for inosine, Hx, xanthine, uric acid and lactate as per plasma analysis section 3.5.5.

7.2.4.5 Rating of perceived exertion

Borg scale rating of perceived exertion was shown at the 5^{th} and 10^{th} mins of exercise as per instruction described in 3.5.3.

7.2.5 Statistical Analysis

Detailed in section 3.6, all results is expressed as means ± SEM except for data pertaining to subject characteristics is expressed as means ± SD. All results was analysed using Graph-pad Prism software version 6.02 and statistical significance was accepted at $p < 0.05$. Two-way repeated measures ANOVA with time as the within group factor and

exercise protocol as the between group factor was completed to detect significance in all plasma, heart rate power, and respiratory gas results. Where an interaction between trials was detected, post hoc tests were completed as mentioned previously. A matched, paired t-test was performed on urinary results comparing post exercise to basal values and determining if the two recovery values were significantly different.

7.3 Results

7.3.1 Peak power

In both male and female groups, peak power decreased over the 10min HIIE bout with peak powers in minute 6 significantly lower than minute 1 ($p<0.05$) in the male group and lower than minute 1 at minute 3 for both HIIE trials in the female group. No differences were detected between 8:12 and 24:36 HIIE trials.

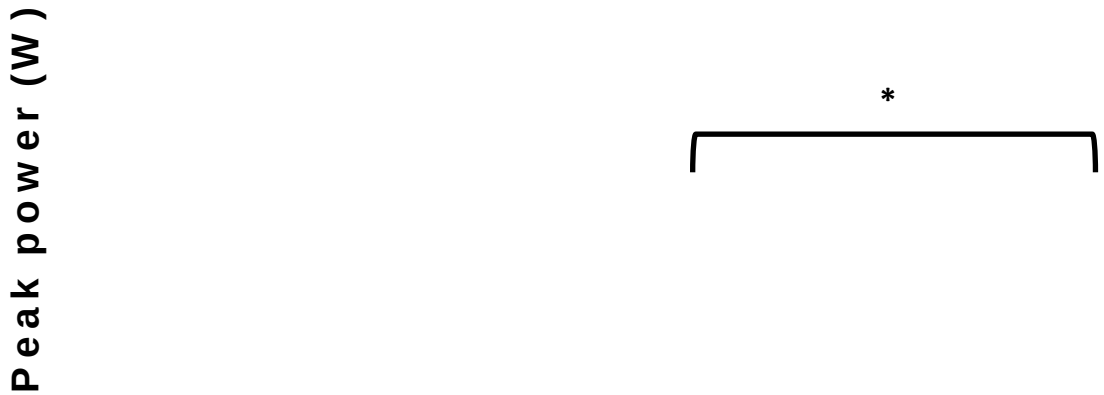

Figure 7.0: Peak power (W) of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8
* $p<0.05$ significantly different from rest
The power outputs from the 3 sprints from the 8:12 protocol were averaged in order for comparisons to the 24:36 protocol.

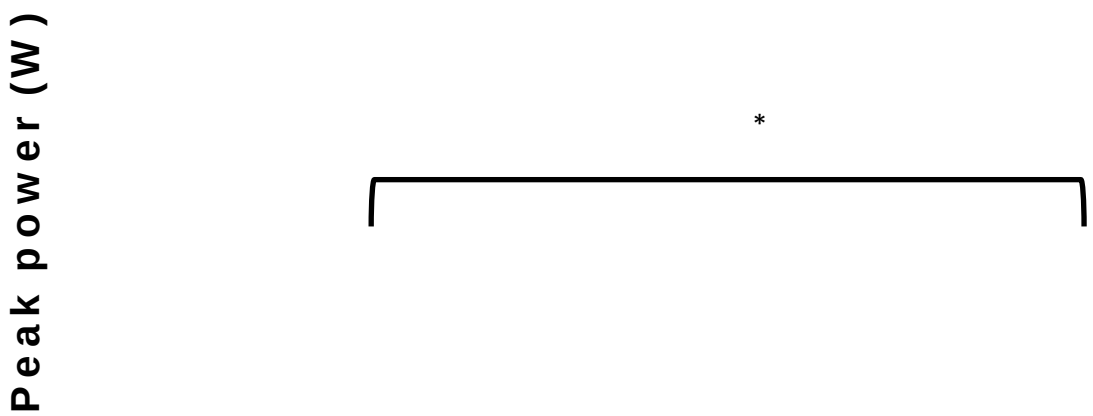

Figure 7.1: Peak power (W) of maximal all HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7
* p<0.05 significantly different from rest
The power outputs from the 3 sprints from the 8:12 protocol were averaged in order for comparisons to the 24:36 protocol.

7.3.2 Mean power

Mean power output progressively decreased over time and no differences were detected between the two HIIE trials in both male and female participant groups. In the male group, the 8:12 HIIE trial was significantly lower than minute 1 by minute 3 and by minute 2 in the 24:36 trial.

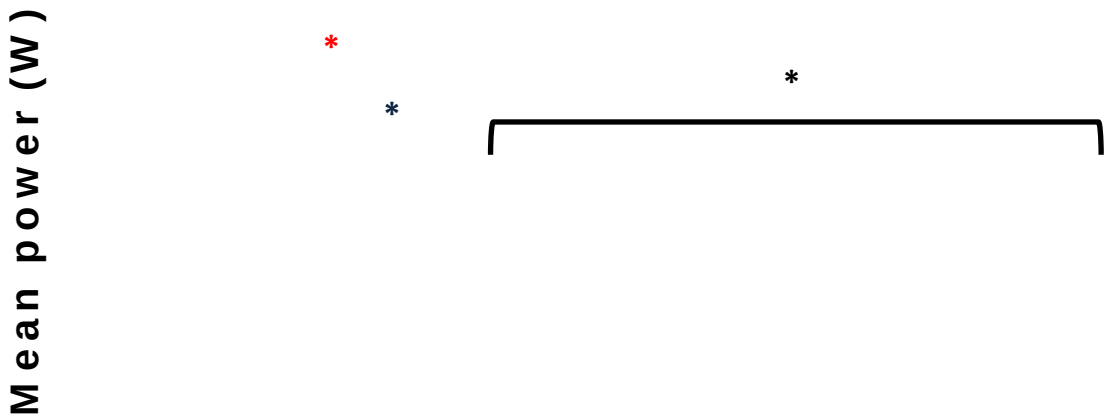

Figure 7.2: Mean power (W) of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8
* p<0.05 different from rest significance (black asterix refers to both trials, coloured pertains to respective trial)
The power outputs from the 3 sprints from the 8:12 protocol were averaged in order for comparisons to the 24:36 protocol.

In the female group, mean power decreased over time and was significantly different from minute 1 at 2 for the 8:12 trial and minute 3 for the 24:36 trial.

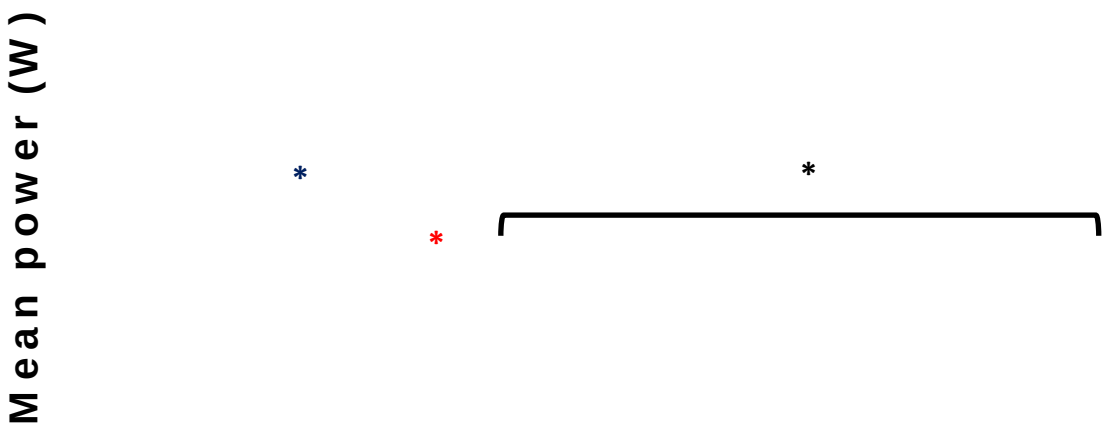

Figure 7.3: Mean power (W) of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.

Data presented as mean ± SEM; N = 7

* p<0.05) significantly different from minute 1 (black asterix refers to both trials, coloured pertains to respective trial)

The power outputs from the 3 sprints from the 8:12 protocol were averaged in order for comparisons to the 24:36 protocol.

7.3.3 Oxygen consumption (VO_2)

In the male group, VO_2 rose in both HIIE trials from rest during exercise before progressively decreasing post exercise, but remaining about basal levels until 20 min post exercise. There was no difference between the 8:12 and 24:36 HIIE protocols.

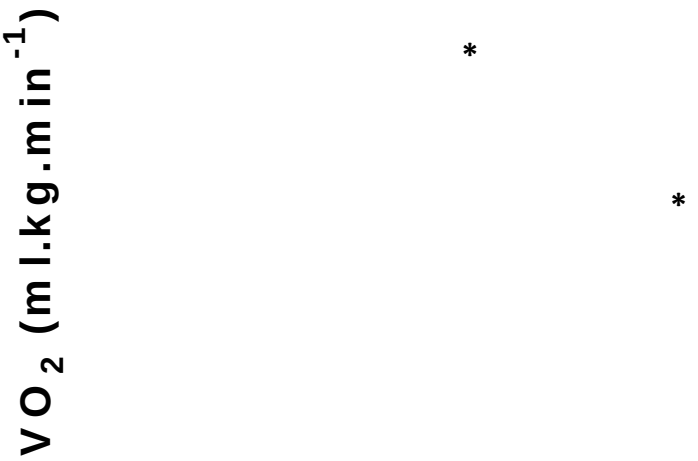

Figure 7.4: VO_2 of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8
* p<0.05 different from rest

VO_2 of the female cohort, increased in both trials from rest during exercise ($p<0.05$), before returning to resting levels by R15. At 5 mins post exercise the 8:12 trial has significantly elevated VO_2 compared to the 24:36 ($p<0.05$).

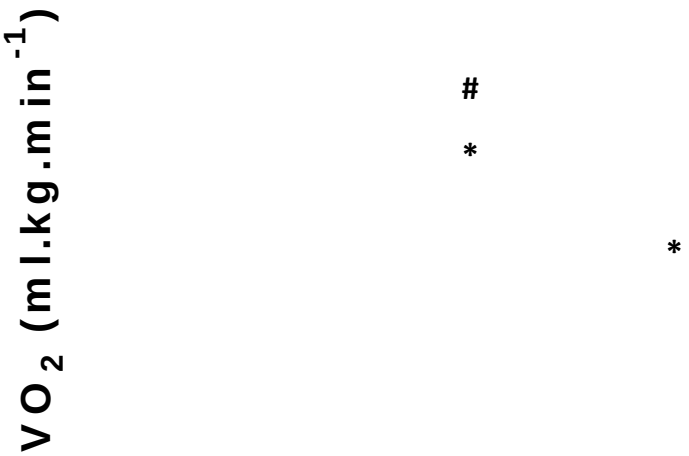

Figure 7.5: VO_2 of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7
* $p<0.05$ different from rest (black asterix refers to both trials, coloured pertains to respective trial)
# $p<0.05$ different between 8:12 and 24:36 HIIE bouts

7.3.4 Respiratory exchange ratio (RER)

In both male and female groups, RER increased above rest during exercise in both 8:12 and 24:36 HIIE trials and there were no difference between 8:12 and 24:36 at any time point.

In the male group, RER returned to rest levels by R20, then declined below resting values at 85 to 90 mins ($p<0.05$) post exercise.

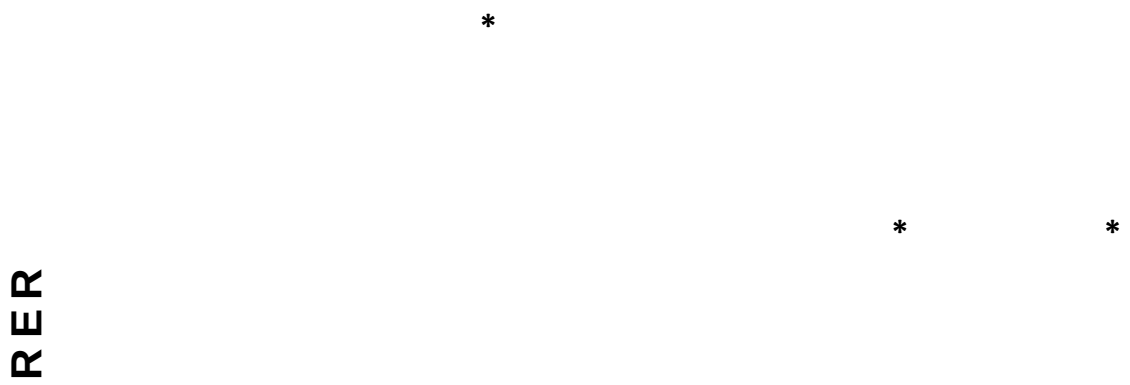

Figure 7.6: Respiratory exchange ratio (RER) of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8
* $p<0.05$ different from rest

RER results of the female group was significantly elevated from rest during exercise and decreased below resting levels in the end stages of the recovery period at R85 and 90 ($p<0.05$).

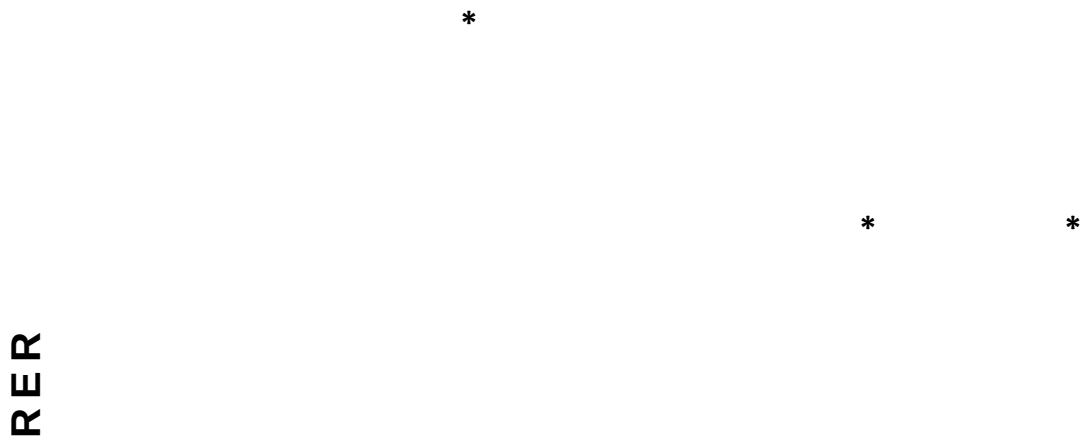

Figure 7.7: Respiratory exchange ratio (RER) of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7
* $p<0.05$ different from rest

7.3.5 Heart rate

During exercise, heart rate of both male and female cohorts increased significantly from rest in both HIIE trials, before declining once exercise had ceased. Heart rate in both groups remained elevated until 45 mins post exercise ($p<0.05$) and no differences between 8:12 and 24:36 HIIE trials were detected. In the male group there was a significant interaction ($p=0.0258$) but not present in the female group.

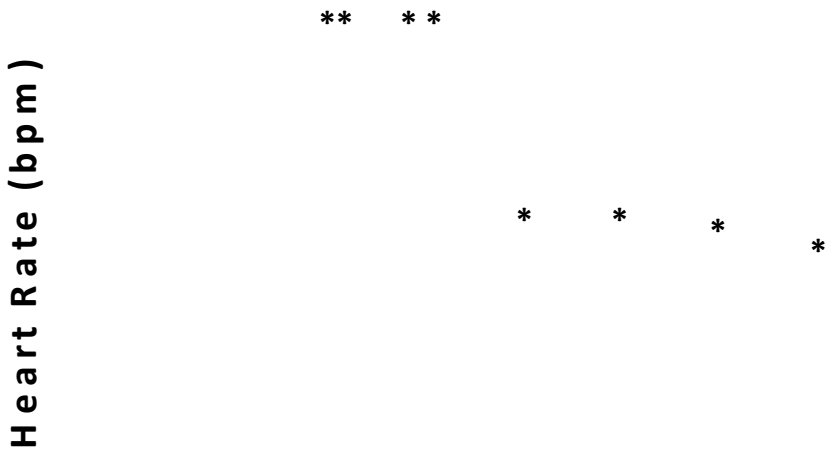

Figure 7.8: Heart rate (bpm) of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8
** $p<0.001$ significantly different from rest
* $p<0.05$ different from rest

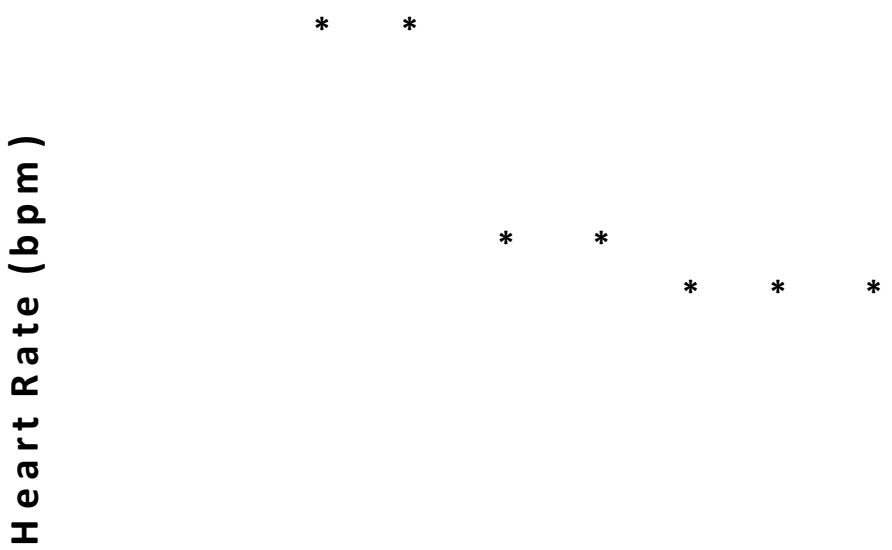

Figure 7.9: Heart rate of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.

Data presented as mean ± SEM; N = 7

** p<0.001 significantly different from rest

* p<0.05 different from rest

7.3.6 Rating of perceived exertion (RPE)

At the end of exercise (E10), both 8:12 and 24:36 HIIE trials were considered to be harder compared to the 5th bout by both gender groups. There were no differences between the two trials.

Figure 7.10: Rating of perceived exertion (RPE) of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8
* p<0.05 different from E5

Figure 7.11: Rating of perceived exertion (RPE) of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7
* p<0.05 different from E5

7.3.7 Substrate Metabolism

7.3.7.1 Plasma FFA

There was no difference in plasma FFA concentration between exercise protocols or from rest in the male participant group.

Figure 7.12: Plasma FFA profiles of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8

Plasma FFA concentrations in the female group and in both trials decreased significantly from rest at the end of exercise (E10) in both trials ($p<0.05$), with the 24:36 trial lower than the 8:12 ($p<0.05$). Concentrations had returned to resting levels by 5mins post exercise. During the recovery period, plasma FFA concentrations remained consistent except for the 24:36 trial at R45 where concentrations decreased below resting ($p<0.05$). At 75 and 90 mins post exercise, concentration in the 8:12 trial appear to be greater than the 24:36 trial, however results are not statistically significantly different.

Figure 7.13: Plasma FFA profiles of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7
* $p<0.05$ different from rest (black asterix refers to both trials, coloured pertains to respective trial)
# $p<0.05$ different between 8:12 and 24:36 HIIE bouts

7.3.7.2 Plasma glycerol

Plasma glycerol increased in both trials from rest ($p<0.05$) in both male and female groups. In the male group, plasma glycerol concentrations remained above rest until 30 mins post exercise in the 8:12 HIIE protocol, and until R45 in the 24:36 protocol. Furthermore, plasma glycerol in the 24:36 HIIE bout was significantly higher than the 8:12 protocol 15 mins post exercise ($p<0.05$).

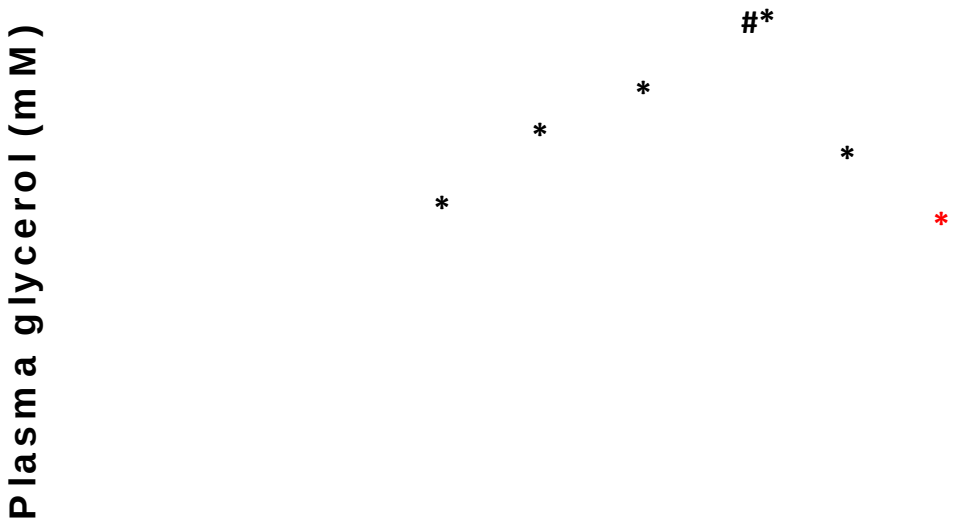

Figure 7.14: Plasma glycerol profiles of maximal all HIIE, 8:12 compared to 24:36 trials in the male group. Data presented as mean ± SEM; N = 8
* $p<0.05$ different from rest (black asterix refers to both trials, coloured pertains to respective trial)
# $p<0.05$ different between 8:12 and 24:36 HIIE bouts

There was no difference between trials in plasma glycerol concentrations in the female group. There was a significant increase from rest at R5 to R15 ($p<0.05$). At R30 plasma glycerol concentration remained above rest in the 24:36 trial ($p<0.05$) only.

Figure 7.15: Plasma glycerol profiles of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7
* $p<0.05$ different from rest (black asterix refers to both trials, coloured pertains to respective trial)

7.3.7.3 Plasma lactate

Plasma lactate increased significantly from rest in both gender groups, peaking at R5 ($p<0.05$) in the male group, before progressively decreasing during the recovery period and returning to basal at R60 for the 8:12 HIIE trial and R75 for the 24:36 HIIE trial. Additionally, in the male group, the 24:36 HIIE trial produced significantly higher concentrations compared to the 8:12 trial during exercise and until 75 min post exercise.

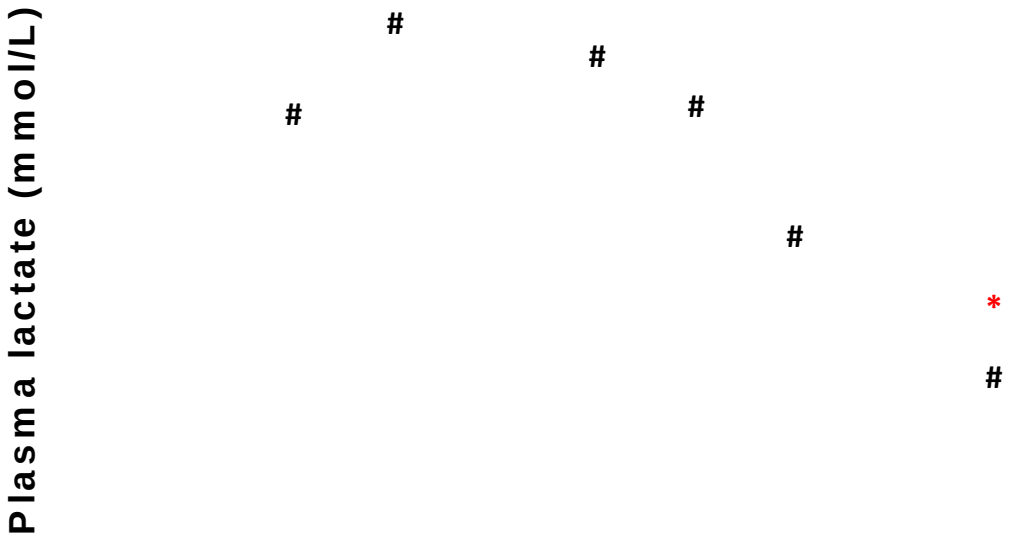

Figure 7.16: Plasma lactate profiles of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8
* $p<0.05$ different from rest (black asterix refers to both trials, coloured pertains to respective trial)
# $p<0.05$ different between 8:12 and 24:36 HIIE bouts

In the female group, plasma lactate remained elevated from rest until R60 in the 8:12 trial and R75 in the 24:36 trial respectively. The only difference between trials was at 15 mins post exercise, where the 24:36 trial had significantly greater plasma lactate concentrations than the 8:12 trial ($p<0.05$).

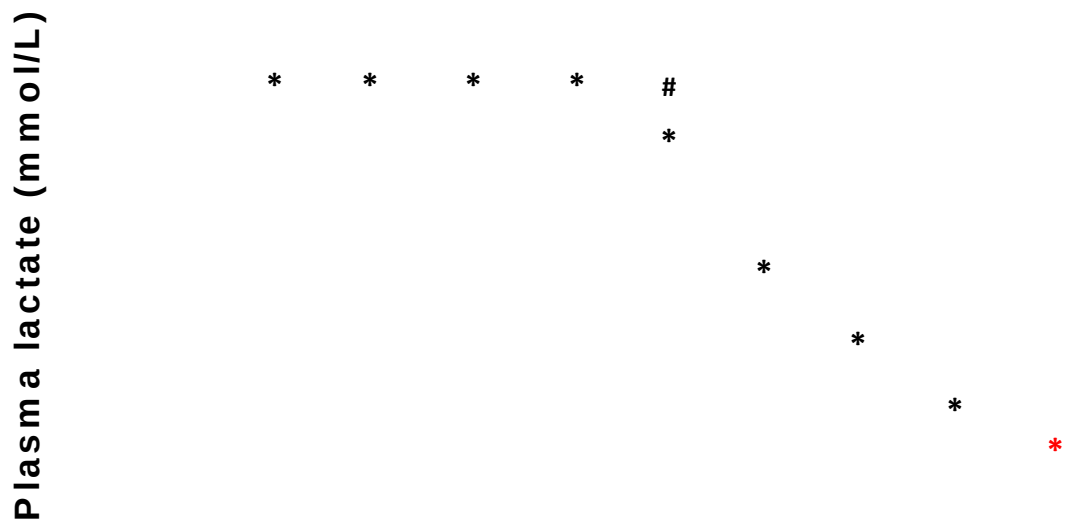

Figure 7.17: Plasma lactate profiles of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7
* $p<0.05$ different from rest (black asterix refers to both trials, coloured pertains to respective trial)
# $p<0.05$ different between 8:12 and 24:36 HIIE bouts

7.3.7.4 Plasma glucose

There were no significant differences in plasma glucose between 8:12 and 24:36 HIIE trials for both male and female genders. There was however a significant rise from rest in both HIIE trials at the end of exercise (E10) ($p<0.05$). Plasma glucose concentration peaked at R5 before declining to rest levels at R15 for the 8:12 HIIE trial and R30 for the 24:36 bout.

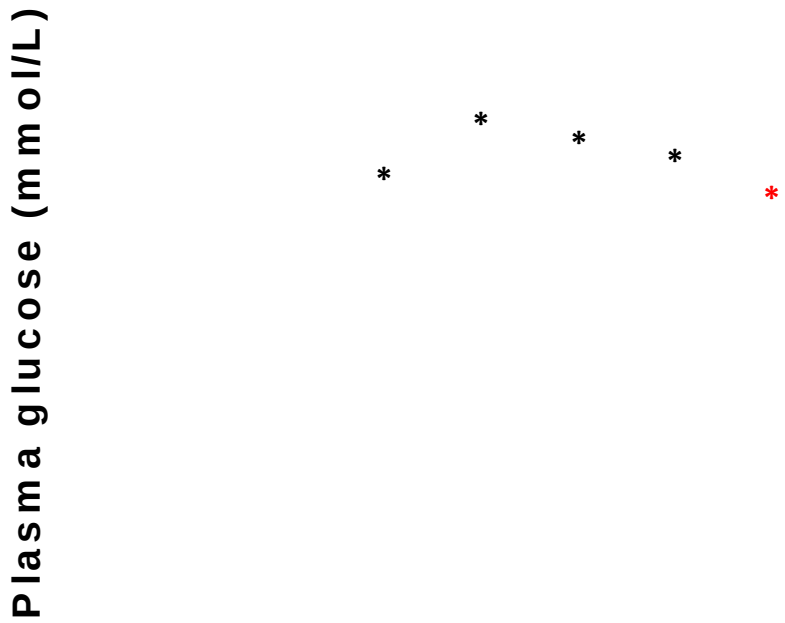

Figure 7.18: Plasma glucose profiles of maximal all HIIE, 8:12 compared to 24:36 trials in the male group
Data presented as mean ± SEM; N = 8
* $p<0.05$ different from rest (black asterix refers to both trials, coloured pertains to respective trial)

The rise of plasma glucose concentrations in the female group was delayed compared to the males and were increased significantly from rest at R5 to R15 ($p<0.05$).

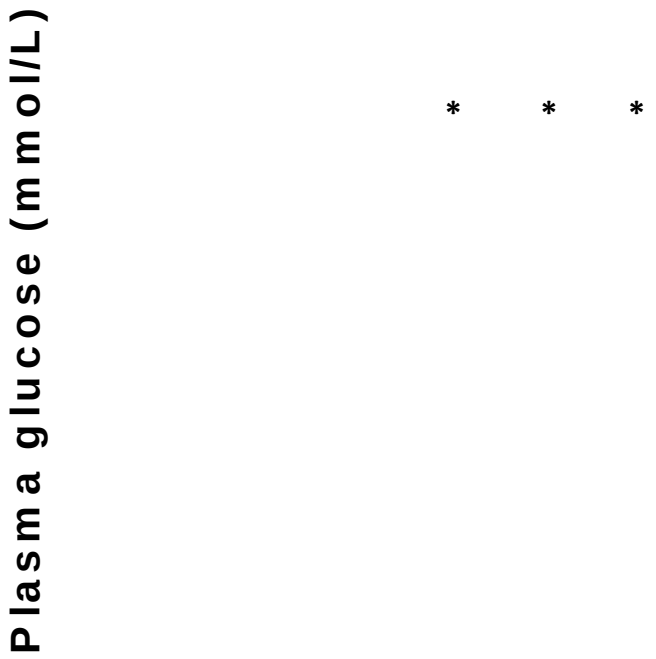

Figure 7.19: Plasma glucose profiles of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7
* $p<0.05$ different from rest

7.3.7.5 Plasma insulin

Plasma insulin was significantly elevated from rest at 15 min post exercise ($p<0.05$) in both HIIE trials and in the two gender groups. Concentrations returned to rest concentrations by 60 mins in both exercise trials and both gender groups, with no differences between bouts.

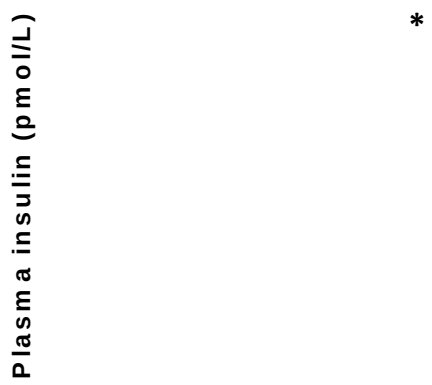

Figure 7.20: Plasma insulin profiles of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8
* $p<0.05$ different from rest

Figure 7.21: Plasma insulin profiles of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7
* $p<0.05$ different from rest

7.3.7.6 Urinary lactate

Urinary lactate was significantly greater post exercise compared to rest ($p<0.05$) after the two HIIE trials and in the male and female groups.

In the male cohort, post recovery excretion rates significantly higher in the 24:36 HIIE trial compared to the 8:12 HIIE trial ($p<0.01$).

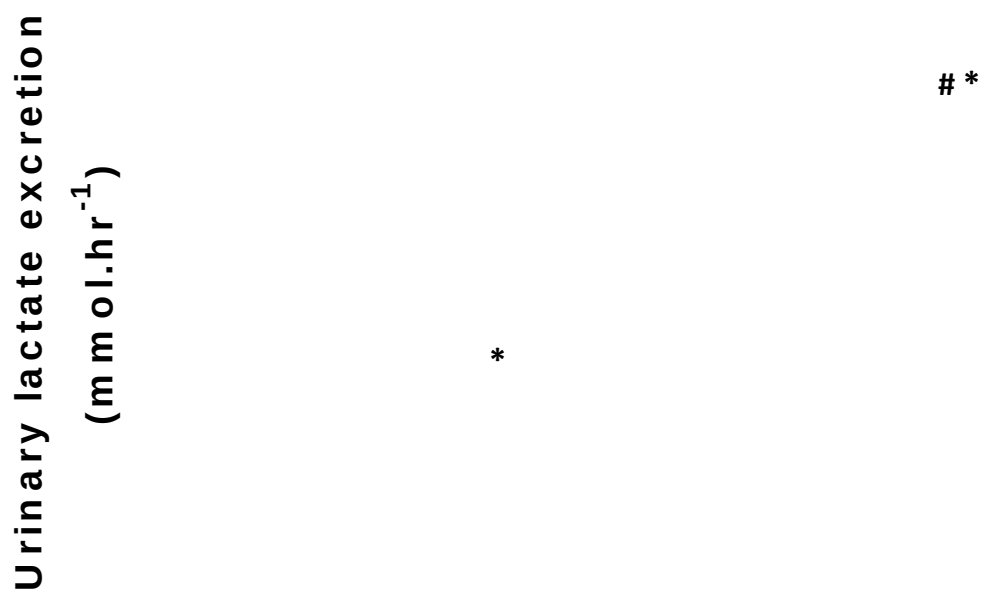

Figure 7.22: Urinary lactate excretion of the male group, measured at rest and 90 mins post maximal all HIIE, 8:12 compared to 24:36 trials.
Data presented as mean ± SEM; N = 8; open columns = rest; full colour = 90mins post ex
* $p<0.05$ different from rest
# $p<0.05$ different between 8:12 and 24:36 HIIE bouts

No differences were observed between the 8:12 and 24:36 HIIE trials in the female group.

Figure 7.23: Urinary lactate excretion of the female group, measured at rest and 90 mins post maximal all out HIIE, 8:12 compared to 24:36 trials.
Data presented as mean ± SEM; N = 7; open columns = rest; full colour = 90 mins post exercise
* p<0.05 different from rest

7.3.8 Purine Nucleotide Metabolism

7.3.8.1 Plasma inosine

There were no differences from rest or between 8:12 and 24:36 HIIE trials in plasma inosine in both the male and female groups.

Figure 7.24: Plasma inosine profiles of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8

Figure 7.25: Plasma inosine profiles of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7

7.3.8.2 Plasma xanthine

There were no differences from rest or between 8:12 and 24:36 HIIE trials in plasma xanthine concentrations in both the male and female groups.

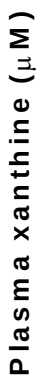

Figure7.26: Plasma xanthine profiles of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8

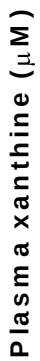

Figure 7.27: Plasma xanthine profiles of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7

7.3.8.3 Plasma Hx

In the male participant group, plasma Hx was significantly increased from rest at the end of exercise (E10) in the 24:36 trial, but concentrations did not rise above basal in the 8:12 trial until R10 ($p<0.05$). Concentrations remained elevated above rest until R30 and R60 in both the 8:12 and 24:36 trials respectively. At 10 mins post exercise, the 24:36 HIIE trial Hx concentration was significantly higher than the 8:12 HIIE trial ($p<0.05$).

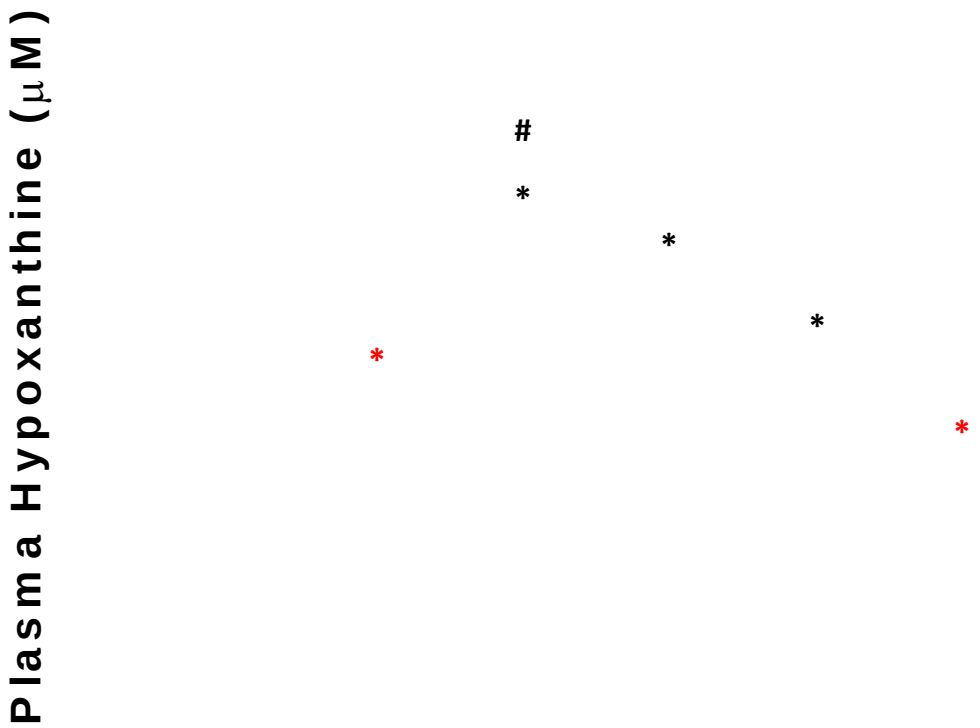

Figure 7.28: Plasma hypoxanthine profiles of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8
* $p<0.05$ different from rest (black asterix refers to both trials, coloured pertains to respective trial)
# $p<0.05$ different between 8:12 and 24:36 HIIE bouts

Concentrations of plasma Hx in the female group in the 8:12 HIIE trial increased from rest at the end of exercise (E10) and remained above rest until R30 ($p<0.05$), whereas the only time point elevated from rest in the 24:36 trial was R15 ($p<0.05$). However there were no differences detected between the two HIIE trials.

Figure 7.29: Plasma hypoxanthine profiles of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7
* $p<0.05$ different from rest

7.3.8.4 Plasma uric acid

Plasma uric acid was elevated from rest at 30 mins post exercise (R30) in the male group and remained elevated until 90 mins post exercise. There were no differences detected between the two HIIE trials in the male group.

Figure 7.30: Plasma uric acid profiles of maximal all HIIE, 8:12 compared to 24:36 trials in the male group.
Data presented as mean ± SEM; N = 8
* p<0.05 different from rest
# p<0.05 different between 8:12 and 24:36 HIIE bouts

In the female group, plasma uric acid concentrations increased progressively in both 8:12 and 24:36 trials and reached significantly greater concentration than rest at R30, remaining so for the duration of the 90 min recovery period ($p<0.05$). Concentrations were significantly higher at R60 and R90 in the 24:36 trial compared to the 8:12 trial ($p<0.05$).

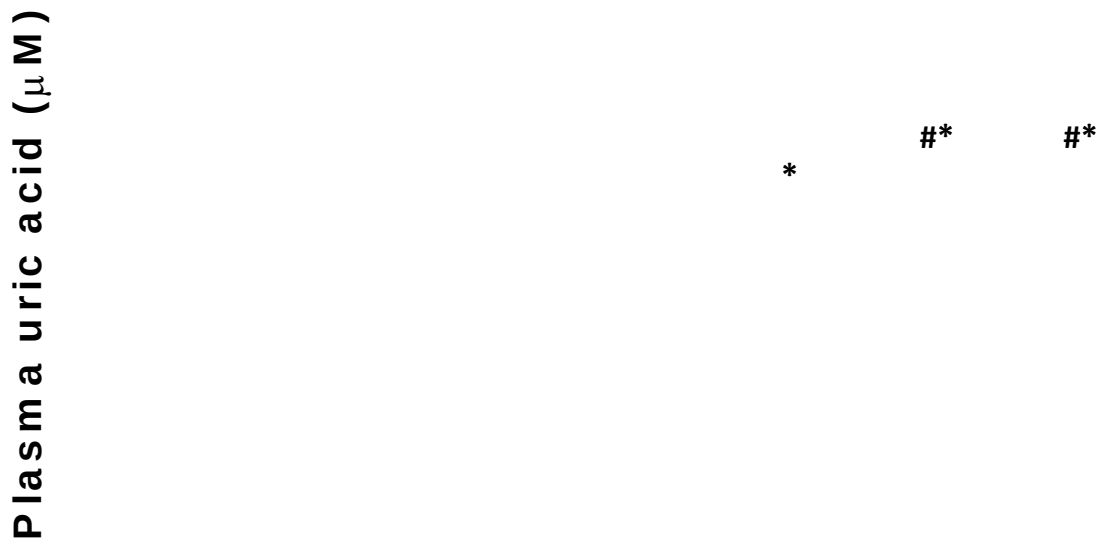

Figure 7.31: Plasma uric acid profiles of maximal all out HIIE, 8:12 compared to 24:36 trials in the female group.
Data presented as mean ± SEM; N = 7
* p<0.05 different from rest
# p<0.05 different between 8:12 and 24:36 HIIE bouts

7.3.8.5 Urinary purines

There were no differences detected in urinary inosine or xanthine excretion from rest or between trials for both genders. Hx was significantly elevated from rest after 90 mins of recovery in both 8:12 and 24:36 trials ($p<0.05$) for both the male and female groups. There was a significant interaction in the male group ($p= 0.0131$) and concentrations in the male group 24:36 bout was significantly greater than the 8:12 trial ($p<0.01$). There were no differences between the two HIIE trials in the female group. For the male participants, urinary uric acid was different from rest in the 24:36 (<0.05) trial but not the 8:12 trial ($p=0.07$). Post recovery, the 24:36 bout was also significantly higher compared to the 8:12 post recovery sample ($p<0.05$). There were no differences observed in urinary uric acid from rest or between 8:12 and 24:36 HIIE trials for the female group.

Table 7.0 The effect of 8:12 HIIE and 24:36 HIIE trials on urinary purines (In, Xan, Hx and uric acid)

	Rest (umol.hr^{-1})	90 mins post exercise (umol.hr^{-1})
Inosine		
Males 8:12	8.6	11.4
Males 24:36	8.1	10.6
Females 8:12	1.5	7.4
Females 24:36	2.9	9.3
Xan		
Males 8:12	7.6	8.2
Males 24:36	8.4	24.3
Females 8:12	7.5	4.6
Females 24:36	4.2	6.1
Hx		
Males 8:12	6.0	88.9*
Males 24:36	8.2	119.3$^{*\#}$
Females 8:12	1.8	37.8*
Females 24:36	4.7	67.1*
Uric acid		
Males 8:12	177.4	299.1
Males 24:36	143.0	433.3$^{*\#}$

Females 8:12	112.0	148.5
Females 24:36	169.0	218.5

* denotes significance from rest

# denotes significance between trials

7.4 Discussion

Although no observed differences between 8:12 and 24:36 trials in RPE (see figure 7.10) and power in the male group, there were significant differences in purine base excretion and lactate loss. Whereas, the patterns observed in the female group were similar, displaying no difference between the two HIIE trials. Additionally, there was the possibility of elevated lipolysis in the male participant group, reflected by significantly increased plasma glycerol in the 24:36 trial compared to the 8:12 however the female lipid profiles did not follow this pattern, demonstrating the diverse metabolic responses to HIIE by different genders.

7.4.1 There is greater lactate excretion after the 24:36 trial compared to 8:12 trial in the male participant group

HIIE uses glycogen as a principal fuel source to fund the high energy demand of the exercise bouts. Lactate, a by-product of glucose degradation, is expelled from the muscle where it accumulates in the blood stream (Hubbard, 1973), reflective of increased glycogenolysis. In the male group, plasma lactate concentrations increased significantly from rest in both 8:12 and 24:36 HIIE trials during exercise, remaining elevated well into the recovery period ($p<0.05$, see figure 7.16). Concentrations were significantly greater in the 24:36 trial compared to 8:12 trial during exercise and recovery ($p<0.05$), indicating a greater glycogenolysis in 24:36 HIIE compared to the 8:12 HIIE. As lactate is removed from circulation via uptake into various tissues as discussed in section 2.9.2.5, plasma lactate progressively decreases during recovery (see figure 7.16). As well as taken up by numerous tissues, lactate is filtered via kidneys and excreted in the urine (Liljestrand and Wilson 1925; Jervell 1928; Johnson and Edwards 1937; Miller and Miller 1949; Pechilvanis *et al.,* 2010).

Chapter 6 of this thesis confirms significantly greater urinary lactate excretion after exercise compared to rest, with concentrations further exacerbated after all-out maximal effort high intensity exercise compared to a HIIE model where cycling was 20 s set at 150% $VO_{2peak,}$ followed by 40 s rest, specifically section 6.4.1. In the current chapter, urinary lactate excretion post 90 mins recovery was significantly elevated compared to rest in both all-out HIIE trials ($p<0.05$, see figure 7.22), with excretion significantly higher in the 24:36 HIIE trial compared to 8:12 HIIE ($p< 0.05$). Thus the 24:36 trial induced a significantly greater loss of this metabolite which may be construed as energy loss, potentially contributing to energy deficit increasing energy expenditure.

7.4.2 Purine nucleotide metabolism in the male cohort

Additional high intensity exercise metabolites that are found in the urine, plasma Hx and uric acid (see section 2.9.2.4), were significantly elevated post exercise from rest in both HIIE trials in the male group. Plasma Hx and uric acid concentrations were significantly greater after the 24:36 HIIE trial compared to the 8:12 HIIE trial ($p<0.05$; see figures 7.28 and 7.30 respectively), this pattern following into the urinary results. Urinary excretion of Hx and uric acid were significantly elevated from rest, with the 24:36 trial producing even greater excretion of these two metabolites than the 8:12 trial ($p<.0.05$; see figures 7.36 and 7.38). Therefore, coupled with the lactate results, a great energy loss is observed with the 24:36 HIIE trial compared to 8:12 HIIE trial, thus indicating that the 24:36 HIIE protocol may be better at increasing energy deficit than the 8:12 protocol.

Interestingly, as established in chapter 2, section 2.9.2.4 purine nucleotide metabolism is aggravated with greater numbers of successive sprints (Stathis *et al.,* 1999; Hellsten *et al.,* 1998; Borg *et al.,* 2008; Saiki *et al*., 2011; Pechilvanis *et al.,* 2010), with

greater deamination and limited utilisation of the purine salvage pathway (Tullson *et al.,* 1995). However this study conflicts with this result as the 8:12 HIIE "all out" trial consisted of 30 8 s maximal effort sprints, whilst the 24:36 "all out" HIIE protocol used 10 maximal effort 24 s sprints, yet there was greater plasma accumulations of Hx and uric acid and elevated excretion of these two metabolites in the 24:36 trial compared to the 8:12 trial. Consequently this study shows that continuous duration of maximal effort work may be more important that sprint number at inducing purine loss. Whilst possibly exacerbating energy loss, the interspersed rest periods may lead fat utilisation, further potentiating the effect of HIIE on reductions in adiposity.

7.4.3 Fat utilisation during and post HIIE in healthy untrained males

In the male group plasma glycerol was significantly elevated from rest during exercise in both HIIE trials ($p<0.05$), remaining greater than rest into the recovery period, indicative of increased lipolysis (see figure 7.14). Plasma FFA was not different at any time point from rest or between trials. These results are consistent with other studies that have investigated fat metabolism during HIIE, discussed in section 2.9.2.1. With greater lipolysis there may be more FFA available in the plasma for uptake across the sarcolemma hence greater FFA uptake into the muscle and greater potential for oxidation in the mitochondria. It should be noted that FFA may be available for use, it does not necessarily mean that they will be oxidised. As mentioned previously in section 2.5.1, FFA may be re-esterified back into adipose tissue for storage. Lipolysis, indicated by elevated plasma glycerol concentrations peaked at 15 mins post exercise, thus greater fat mobilisation after exercise ceased. It is well-documented that sources of fat are metabolised post exercise to return exercise

induced metabolic shifts to rest levels, maintaining equilibrium within the body, section 2.9.2.3.

Oxidation of lipid once exercise has ceased is essential to fuel metabolic restorative processes. This goes hand in hand with elevated VO_2 post exercise, known as excess post exercise oxygen consumption (EPOC). VO_2 remains elevated for a time post exercise, and is indicative of elevated energy expenditure (Lyons *et al.,* 2006). Figure 7.4 shows VO_2 was not different between the two HIIE trials and remained elevated from rest until 20mins post exercise. As mentioned in section 2.9.2.3.4, chapter 4 and 5 of this thesis, this may not represent true EPOC which can last for hours post high intensity exercise (Borsheim and Bahr 2003). Nonetheless, during recovery RER, decreased post exercise to below resting value of 0.8 to approximately 0.7 in both HIIE trials, which is significantly lower than rest ($p<0.05$; see figure 7.6). Although only employed as a comparative measure in high intensity exercise (section 2.3.3.4), an RER of 0.7 correlates to a greater proportion of fat utilised to fuel cellular metabolism. This is supported by plasma results; Plasma glycerol was elevated in both HIIE trials post exercise, yet greater in the 24:36 trial ($p<0.05$), peaking at 15mins post exercise whilst FFA remained similar to rest. The female group displayed similar metabolic patterns, with the responses tempered compared to the males.

7.4.4 Metabolic responses to 8:12 and 24:36 HIIE in females

In the female group, plasma lactate was increased significantly from rest ($p<0.05$; see figure 7.17) indicative of increased glycogenolysis, with glycolytic contribution similar between the two HIIE trials. Characteristic of high intensity exercise, lactate accumulates in the plasma with the end product of lactate metabolism urinary excretion (established in

section 2.7.1). Plasma lactate remained elevated into the 90 min recovery period (see figure 7.17) and urinary lactate was significantly elevated post exercise in both trials compared to rest (see figure 7.23; $p<0.05$). Therefore both 24:36 and 8:12 HIIE trials appeared to cause the same relative energy loss via lactate excretion after HIIE and would thus induce similar energy deficit.

As mentioned previously, purine loss via urinary excretion also contributes to increasing energy deficit. Plasma Hx was significantly elevated from rest during the recovery period in both trials (see figure 7.29; $p<0.05$) while plasma uric acid concentrations also significantly elevated from rest, ($p<0.05$; see figure 7.31) with concentrations even further exacerbated in the 24:36 HIIE trial ($p<0.05$). No differences were detected from rest or between trials were detected in urinary uric acid, but urinary Hx was significantly increased post exercise from rest ($p<0.05$; see figure 7.37), and no differences observed. Together these results suggest that in the female group, purine loss was similar leading to parallel amount of energy loss for both HIIE protocols. There may have been significant differences in urinary uric acid excretion if between if collection was longer than 90 mins. There was a significant increase from rest and significant difference between the two HIIE trials in the plasma sample collected at 90 mins post HIIE, the 24:36 HIIE trial exhibiting greater concentrations compared to the 8:12 HIIE trial. If collection was for a longer duration, the plasma containing these significant concentrations may have been filtered at the glomerulus and excreted in the urine, potentially increasing urinary concentrations.

The significantly increased plasma uric acid after the 24:36 trial compared to 8:12 trial may have been due to increased blood flow resulting in increased conversion of Hx to uric acid via xanthine oxidase. Xanthine oxidase is an enzyme that converts Hx to uric acid is

found in the epithelium of vessels, thus increased blood flow would take greater amounts of Hx in the blood through the vessels and it would be converted to uric acid. There also is the possibility of quicker reamination of Hx accounting for the difference in uric acid. A greater proportion of Hx may have been reaminated to IMP in 8:12 HIIE trial, hence lower concentrations of plasma uric acid accumulation. Nonetheless, these results suggest that equal in the metabolic responses in the female gender at elevating energy loss and potential energy expenditure. This was not expected as there was a clear difference between trials observed in the male group in purine and lactate responses.

It was hypothesised that in the female group there would be an increased reliance on fat substrate during HIIE due to the rest periods of the mode of exercise, however the combined results (plasma glycerol and FFA) indicates this is unlikely. During HIIE in both 8:12 and 24:36 trials there was a significant decrease in plasma FFA from rest, further decreased in the 24:36 HIIE trial (see figure 7.13; $p<0.05$). Plasma glycerol, reflective of lipolysis, was not elevated during the 10 mins of HIIE (see figure 8.7); together these results suggest that fat utilisation may have been depressed during HIIE. This may be due to inhibited action of CPT1 from altered muscle pH caused by lactate production, a validated metabolic response section 2.7.2. Plasma lactate in this study peaked at just under 15mmol/L at the end of the 10 min 8:12 and 24:36 HIIE bouts. In a study employing males and RSA exercise, blood lactate peaked at 9mmol/L whilst muscle pH decreased to 6.89 (Bishop *et al.,* 2004). In an additional study also investigating muscle pH and lactate production, after exhaustive exercise muscle pH was 6.69 (Juel *et al.,* 2003). If 9-10mmol/L equates to a muscle pH of 6.69-6.89, it may be assumed that 15mmol/L will correlate to a lower muscle pH hence

potentially limiting FFA uptake into the mitochondria due inhibition of CPT1. Interestingly this effect was observed in the female participants but not in the males, despite similar peak lactate levels. It is possible that the oxidative rest periods of HIIE may have been fuelled by IMTG, of which females have significant stores (section 2.9.2.1) and circulating FFA were reesterified (see section 2.5.1). However as there was no increase in plasma glycerol during HIIE this remains unclear.

In the 90 min following HIIE, fat utilisation may have increased, but one trial not more so than the other. Plasma glycerol increased post exercise in both HIIE trials and plasma FFA returned to resting concentrations, possibly indicating greater lipolysis and hence opportunity for FFA to be taken up into the skeletal muscle cell for utilisation. Lipid utilisation post exercise is an established metabolic outcome of high intensity exercise, as this substrate is used to fuel restorative processes (see section 2.9.2.3). This is further supported by RER results, where RER in both trials post exercise is closer to 0.7, indicative of greater fat contribution to substrate utilisation.

7.4.5 Metabolic gender differences during and post maximal effort HIIE

It could be argued that females show similar metabolic patterns as males as there were minimal significant differences observed between both HIIE trials; but the extent of metabolic stress was not the same in relative or absolute terms to stress the metabolic pathways as there were significant differences between the genders in RPE and VO_2 (see appendix D; $p<0.05$). The male cohort showed a significantly greater purine loss compared to the female group and across both 24:36 HIIE and 8:12 HIIE trials trial (see appendix D; $p<0.05$), equating in a greater energy loss. Interestingly plasma Hx in the 8:12 females group

was significantly higher than the males (see appendix D; $p<0.05$), however this pattern not reflected in the urinary results. This may be due to a smaller cross sectional area of muscle, therefore a shorter diffusion area/distance in women than men, facilitating an easier recovery (Esbjornsson-Liljedahl *et al.,* 2002).

Further exacerbating this energy loss in the male group after the 24:36 HIIE trial was the heightened urinary lactate excretion observed, which was significantly greater compared to the 8:12 HIIE trial. This would most likely be due to greater muscle mass and absolute strength with males maintaining workload for the longer duration bout (24 s compared to 8 s) and fibre type distribution (see section 2.10), hence producing greater lactate accumulations and purine loss. Regardless energy loss was apparent with females, but may be more exacerbated in males in the 24:36 HIIE trial. This may be a potential reason why women find it harder to lose weight than males.

7.4.6 Conclusion

The 8:12 and 24:36 HIIE protocols employed in this study are based on earlier work which indicated that fat loss may be more dominant with HIIT compared to CONT due to greater reliance on fat metabolism (Trapp *et al.,* 2007, Trapp *et al.,* 2008) with no mention of the potential favourable effect of high intensity exercise at increasing energy expenditure. The differences between this study and the current study are the exercise intensity and HIIE duration; 70% of power achieved at VO_{2peak} intermittently for 20 mins Trapp *et al.* (2007) study compared to "all out" maximal effort intensity for 10 mins. It can be speculated that exercising at power output achieved at 70% VO_{2peak} may not induce metabolic disturbances to the same extent as maximal all out HIIE. Therefore lactate loss

and ATP degradation will not be as severe; hence the energy deficit may not as extensive as the less intense exercise study (Trapp *et al.,* 2007).

In the male group, the 24:36 maximal effort HIIE induces significantly more energy loss via lactate and purine base excretion compared to the 8:12 HIIE trial while loss was similar in the female group. In addition, the results from the male group indicate that the losses via the urine are a significant factor that may not necessarily be reflected in increased VO_2 post exercise. The replacement may be simply via dietary uptake and do not reflect any 'cost' of exercise due to it being accounted for in the energy intake calculation.

Cumulative evidence from this chapter suggests that in the male group, the 24:36 HIIE trial is better at heightening energy expenditure and decreasing adiposity compared to the 8:12 model of HIIE however either may be preferable for the female group. Therefore while all out maximum effort HIIE causes energy deficit in both genders, it may be more suited to the male gender. Furthermore, as discussed in the previous chapter, the 24:36 HIIE trial may induce energy loss and potential energy expenditure in the male group compared to the 8:12 HIIE group.

Chapter eight: Conclusions and future directions

8.1 Conclusions

This series of exercise studies provides analysis of various aspects skeletal muscle metabolism such as purine nucleotide and substrate metabolism, during different modalities of HIIE. Furthermore, this thesis provides insight into why HIIT may be a better protocol than CONT at decreasing adiposity, first observed by Tremblay *et al.* (1993). Moreover it provides evidence as to what protocol may be best at inducing fat loss and increasing energy expenditure in healthy untrained males and females.

This thesis challenges conventional considerations of changes in energy balance with high intensity exercise, specifically HIIE. Results from this thesis have established a form of energy loss, urinary lactate, which has never been incorporated for in the energy balance equation, thus never attributed to energy expenditure. This is an important theory as optimising the loss of lactate may provide a rapid and safe way to produce an energy deficit and reduce adiposity. Furthermore, this chapter discusses the extent of the calculated purine loss may only play a minor role in energy loss compared to lactate excretion and hence indicates that the energy cost of restorative may explain accelerated fat loss with HIIT compared to CONT. This series of studies, particularly chapter 6 and 7, demonstrated that urinary lactate plays an even greater role in energy loss and that supramaximal intensities may elevate this energy loss and all out maximal all out HIIE heightening this even further. The table below displays the estimated equivalent of fat loss that has been calculated from the energy required to restore urinary purine loss, measured in $\mu mol.hr^{-1}$ and the lost energy equivalent to urinary loss, measured in $mmol.hr^{-1}$. These calculations use the

assumptions of energy cost of losing purines and lactate in the urine and the Δ G of ATP rephosphorylation. For an example of how these numbers were generated, please refer to appendix C.

Table 8.0 Estimated equivalent of fat loss calculated from energy required to restore purine loss and equivalent to energy loss

Difference in excretion from basal to 90 mins post exercise	Estimated equivalent fat loss calculated from energy required to restore urinary purine base loss following exercise (g)	Estimated equivalent fat loss calculated from energy equivalent to urinary lactate loss following exercise (g)
50% CON		1.14
150% HIIE	0.53	10.49
8:12 maximal HIIE	0.89	23.05
24:36 maximal HIIE	2.99	60.35
Magnitude of the difference of excretion between the 50% CON and 150% HIIE		9.35
Magnitude of the difference of excretion between the 8:12 HIIE and 24:36 HIIE in males	0.95	37.30

It should be noted that there are purine results for the 50% CON trial as this has not been measured in this thesis. Regardless, the differences between purine and lactate are great, demonstrating that urinary lactate loss plays a more significant role in energy loss compared to purine loss.

This body of work demonstrated that maximal effort HIIE (exercising for 10 mins at intervals of 24 s at maximal effort followed by 36 s rest) is the most favourable protocol out of the 8 protocols tested increasing energy expenditure. Chapter 7 shows that significant

elevation in end products of purine and lactate metabolism above the 8:12 HIIE protocol, hence elevating energy loss. Additionally, chapter 7 proposed that in the male group, the 24:36 all out HIIE protocol may have utilised FFA as an energy source. Hence the cumulative effects of these metabolic shifts may contribute to heightening energy expenditure and inducing a negative energy balance when exercise is performed on a regular basis, hence potentially decreasing adiposity. When diet is controlled and physical activity performed regularly, a difference in 7MJ a day may be observed (Dionne *et al.,* 1997). Therefore if employing an exercise model that increases energy loss with each exercise session such as HIIE compared to CON and specifically the 24:36 maximal effort HIIE, the elevations in energy expenditure may be increased even more than 7MJ per day and even greater reductions in adiposity may appear. Small imbalances induced regularly such as with HIIT may induce significant fat loss; hence this thesis provides substantial evidence behind the rapid fat loss observed in earlier studies where HIIT decreases adiposity faster than CONT.

The results of this thesis may lead to future studies (see section 8.2.3 training studies) with the future results wielding possible important implications for the overweight/obese, insulin resistant and type II diabetic populations. HIIE creates the metabolic environment to utilise fat during exercise (in the rest periods) but may also produce significant energy loss in the form of purines and lactate. In the past, HIIE has been shown to improve hyperglycaemia however the HIIE protocols were very different in duration and intensity to those used in this study, most recently ten 60 s cycling bouts at 90% of maximum heart rate followed by 60 s rest (Whyte et al., 2010; Richards et al., 2010; Little *et al.* 2011; Gillen *et al.,* 2012). Given the metabolic changes observed in the HIIE protocols of this thesis, once tested in a training program and if proved to induce fat loss

faster than CONT, this type of HIIT may show significant improvement for those metabolically compromised.

Lastly, this thesis showed that male and female participant groups seemed to exhibit similar metabolic pathways except that the male cohort had a significantly exacerbated glycolytic response. In the male group there was a greater lactate and purine loss after the 24:36 HIIE bout compared to the 8:12 HIIE bout, whereas excretion between the two bouts was similar in females. Therefore as opposed to the males, metabolic processes in the female group were not heightened by either HIIE protocol, which may have been due to the female group not being able to maintain the workload. Future research is required with different HIIE models to find the optimal protocol for females.

8.1.2 Limitations

Although providing a comprehensive analysis of different HIIE types, much of the evidence is from plasma samples and thus needs to be extrapolated to infer what may be happening intracellularly.

Initially the plan was to complete a study comparing acute workload matched CON and HIIE using isotopic tracers (palmitate and glucose) to show precisely what substrate was utilised at each point during exercise and recovery of both exercise bouts, but specifically providing insight into the role of fat during HIIE. This would have been beneficial to provide greater clarity on substrate partitioning but also provide insight into excess post exercise oxygen consumption metabolic restorative processes. Unfortunately the facilities were not available at the time for palmitate tracers as it is known this type of isotopic tracer can

induce a hypersensitivity reaction in some individuals, thus a hospital setting for trial days would have been required.

Furthermore, results obtained in chapter 5 were restricted due to a limited number of participants consenting to muscle biopsies. Per exercise trial day, four muscle biopsies were performed thus twelve biopsies in total, which was a substantial deterrent in recruitment and participants continuing on with participating in the study.

Moreover it would have been beneficial to analyse all plasma samples for plasma epinephrine and norepinephrine. These catecholamine's stimulate lipolysis and have been shown to increase post exercise along with plasma glycerol concentrations and the increases in fat mass loss associated with HIIT compared to CONT attributed to this increase (Trapp *et al.,* 2007; Trapp *et al.,* 2008). However due to cost and other metabolic markers being predominant in this thesis, epinephrine and norepinephrine were not analysed. A commercially made enzyme linked immunoassay (ELISA) plate for plasma epinephrine was tested however this method did not produce viable results and due to cost, it was not repeated.

Lastly, changes in mRNA and protein expression in skeletal muscle pertaining to metabolism have been found after single bouts of HIIE. This may have been the result of exercise intensity as a study utilising all out wingate exercise found more rapid upregulation of proteins (Little *et al.,* 2011) than another study where the exercise intensity was 90% VO_{2max} (Perry *et al.,* 2008).

It would be interesting to investigate changes in PGC1α, AMPK, FABPpm, FAT/CD36 via western blotting between the 150%, 200% and 300% trials of this thesis. This was not

completed and included in this thesis, while interesting the results do not enhance any theories specific to substrate utilisation and energy expenditure. They will be analysed at a later date but it would be more advantageous in analysing these changes after successive weeks of HIIT using these HIIE models, in order to determine whether the increased intensity yet same total work completed, induces any skeletal muscle metabolism changes over time as upregulation of mRNA from one bout of exercise does not generally lead to changes in protein levels (Vogel and Marcotte, 2012).

8.2 Future directions

There are numerous future directions for this research, such as isotopic tracers, innovative metabolomics technologies, training studies, correlating fibre typing and genetics to substrate profiles of HIIE and expanding these protocols to other participant populations.

8.2.1 Isotopic tracers to elucidate pathways of substrates and metabolites

As mentioned in limitations, employing isotopic tracers are needed to determine the pathway of fat and carbohydrate oxidation during and post HIIE. A study comparing workload-matched HIIE and CON needs to be completed with glucose and palmitate tracers to observe substrate partitioning and to determine the level of contribution of each substrate.

Furthermore, identifying the pathways of Hx and lactate metabolism using isotopic tracers is desirable. It is speculated, but currently unknown, whether the one way

transporter by which Hx effluxes the working skeletal muscle is in fact 'one way'. Thus isotopically labelling Hx and then completing ATP depleting exercise to determine whether Hx can be taken back up by the muscle and re-salvaged is an important next step in research regarding purine nucleotide metabolism. It also would be noteworthy to employ a lactate tracer to determine the amount lost in the urine and taken up by other tissues such as the liver, kidney and myocardium and how this relates to overall lactate production.

8.2.2 Metabolomics

Metabolomics is a new innovative technology that can provide a complete metabolic profile of HIIE. This technology can investigate metabolic flexibility of different exercise intensities by examining a large range of metabolic changes to plasma and muscle in response to an exercise challenge. These methodologies can be costly, thus is presently used sparingly.

8.2.3 Training Programs

This thesis employed single bouts of exercise to investigate substrate metabolism of HIIE. However, training programs utilising the 150%HIIE and 300%HIIE need to be employed for two reasons. Firstly, there were no differences in muscle, plasma or urine substrate between the two HIIE bouts. It needs to be determined if repeated sessions of these HIIE protocols will induce muscle and mitochondrial protein changes which may in fact lead to significant substrate differences during the work and rest periods. Secondly, if there are changes in protein content, specifically those associated with carbohydrate and FFA intake

into the mitochondria, whether there will be differences in fat loss between the 150% and 300% HIIE trials over the longer term.

In addition, it would be valuable to employ the 24:36 maximal HIIE protocol in a training regimen and compare the results to continuous training or an additional mode of HIIT that has been shown to induce reductions in adiposity to investigate whether it really is optimal HIIT program for weight loss.

8.2.4 Population groups

Perhaps most interesting is employing HIIE models to treat metabolic diseases and other illness where adiposity and hyperlipidaemia become a major problem.

Using single bouts of HIIE, and subsequent HIIT, in a number of disease groups is required as HIIE may be a favourable treatment option before resulting to medications that induce side effects or invasive surgery. Along with decreasing adiposity and hyperlipidaemia, HIIT improves insulin sensitivity, elevates energy expenditure and increases VO_{2max} whilst maintaining muscle mass, thus utilising it in exercise programs for those inflicted by obesity, type II diabetes, coronary heart disease, and cancer (where adiposity and sarcopenia are prevalent) is supported from this research.

A substantial amount of research regarding HIIT exists, the majority pertaining to cardiometabolic diseases such as Type II Diabetes, coronary artery disease, congestive heart failure, metabolic syndrome, obesity and atherosclerosis.

Various types of HIIT have induced invaluable physiological changes to cohorts of each disease group, such as improved respiratory fitness in peak oxygen uptake (VO_{2peak})

(Rognmo *et al.,* 2004; Warburton *et al.,* 2005; Wisloff *et al.,* 2007; Amundsen *et al.,*2008), increased maximal strength (Stensvold *et al.,* 2010) improved cardiovascular measures such as heart rate at rest and during exercise (Wisloff *et al.,* 2007), lower blood pressure (Guimareas *et al.,* 2010; Stensvold *et al.,* 2010), improved endothelial function (Rabowchuk *et al.,* 2009; Guimareas *et al.,* 2010), ventricular morphology (Wisloff *et al.,* 2007) stroke volume (Helgerund *et al.,* 2010) ejection fraction (Wisloff *et al.,* 2007) and better insulin sensitivity (Tjonna *et al.,*2009; Little *et al.,* 2012). However, for significant metabolic results, HIIT may need to be longer than two weeks duration, as no changes in metabolic function have been observed in an obese population (Skleryk *et al.,* 2013). However one study has shown that a single session of HIIT, totalling 10mins of exercise reduced postprandial hyperglycaemia in participants with type II diabetes (Gillen *et al.,* 2012).

However most of these HIIT used are relatively quite low exercise intensities compared to those employed by healthy individuals and the studies which have shown the most beneficial results. Many research groups and medical practitioners state that high intensity exercise may be unsafe, however two recent studies recruited cohorts of patients with coronary artery disease, the HIIE intensity being 100% of peak power output. Both studies reported no safety issues or concerns as well as favourable and successful physiological adaptations (Guirand *et al.,* 2011; Meyer *et al.,* 2012). In addition to this, supra-maximal exercise intensities have been previously dispelled due to the cardiovascular safety concerns of such intensities. However, our group has previously compared 150% HIIE to continuous exercise at 50% VO_{2peak} (matched for workload) and demonstrated similar heart rate responses thus exercising at 50% continuously is no different to exercising at 150% intermittently in respect to cardiovascular stress. In addition to this when comparing the BPM of 90% of maximum heart rate to those continuously recorded during the three

HIIE trials in the current study, heart rates remained significantly below 90%. This validates that supra-maximal exercise is safe if performed at the correct intervals, although should be verified before utilisation. Continued use of HIIE may prove a less invasive treatment where the participants does less physical work (kJ) but the end result is significant energy loss and fat utilisation contributing to shifting energy balance negative with the resultant decreasing adiposity.

8.2.5 Single fibres, genetics and Fibre typing

It would be incredibly interesting to obtain muscle biopsies and then isolate single muscle fibres from individuals undertaking HIIT to determine upregulation of carbohydrate and fat transporters and whether they pertain to a specific fibre type. Also, if specific skeletal muscle proteins are upregulated in response to different work to rest combinations and intensities of HIIE as well as comparing these changes between type I and type II fibres across the genders.

Correlating an individual's substrate utilisation during HIIE to their genetic profile would also be the next step in programming an individual's exercise regimen. Some people may respond better to a particular intensity and type of HIIE, thus correlating their metabolic makeup to HIIE would be significantly beneficial.

Chapter nine: List of references

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Appendices

Appendix A

The effect of continuous *vs* intermittent exercise on substrate utilization during exercise and recovery in healthy adults

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Introduction The incidence of obesity and type II diabetes is reaching epidemic proportions in today's society and are associated with long-term ill health and reduced quality of life. As such, it has been recommended that effective weight loss strategies be developed. Carbohydrate and lipids are the primary substrates utilized for energy during exercise. Lipids provide the majority of fuel supply to the exercising skeletal muscle during steady state, moderate intensity exercise (SSMIE), with an increasing supply from carbohydrate sources with increasing intensities (Romijn *et al.*, 2000). However, recent studies have demonstrated an increased capacity for high intensity intermittent exercise (HIIE) to evoke decreases in adiposity compared with SSMIE, without providing metabolic mechanisms (Trapp *et al.*, 2008; Tremblay *et al.*, 1994). Therefore the purpose of this study was to examine plasma and respiratory indicators of lipid and carbohydrate metabolism during and after a single bout of HIIE compared with SSMIE in order to explain decreased adiposity witnessed during HIIE.

Methods This study obtained approval from Victoria University, Human Research Ethics Committee (HRETH 07/281) and all experiments conformed to the National Statement on Ethical Conduct in Human Research. Participants (8 males) performed two exercise bouts, SSMIE (50% VO_2 peak), and HIIE (20s sprint: 40s rest) for 30 minutes on two separate occasions in randomised order. The HIIE bout was designed to be three times the workload of SSMIE, performed for a third of the time, such that the two exercise bouts required the same amount of mechanical work. Blood was taken during exercise and one hour of recovery, and was analysed for glucose, lactate, glycerol and free fatty acids (FFA). Respiratory gas exchange data was also obtained.

Results There was no significant difference in oxygen consumption between the bouts, indicating similar aerobic requirements of SSMIE and HIIE. Both exercise bouts increased lipid oxidation as measured by increased plasma glycerol concentrations during exercise and in recovery. However, RER values were significantly lower ($p < 0.05$) during recovery after HIIE than SSMIE, indicating an increased reliance on lipid oxidation. HIIE also showed a significant decrease in plasma FFA at the end of exercise ($p < 0.05$), suggesting increased uptake by the muscle to support lipid oxidation. Lactate concentrations rose over the 30 minutes, and were significantly higher in HIIE ($p < 0.05$), mostly due to the anaerobic breakdown of glycogen, as plasma glucose concentrations remained the same.

Discussion It is feasible that HIIE creates a 'substrate shuttle' whereby there are repeated shifts from anaerobic to aerobic energy sources. ATP and PCr are partly depleted during the high intensity work phases, with their resynthesis during the rest periods occurring via oxidative pathways (Essen *et al.*, 1977), leading to increased energy expenditure during rest periods of HIIE. Glycogen, although depleted, does not appear to be resynthesised during the rest periods, with ongoing diminution over the HIIE session (Bangsbo *et al.*, 1991). As such, restoration of glycogen stores is of high metabolic priority during recovery, contributing to increased energy expenditure and a negative energy balance after HIIE. Differences in metabolism during rest and recovery from HIIE may explain decreases in adiposity observed, and further investigation of exercise specifically designed for fat loss is required.

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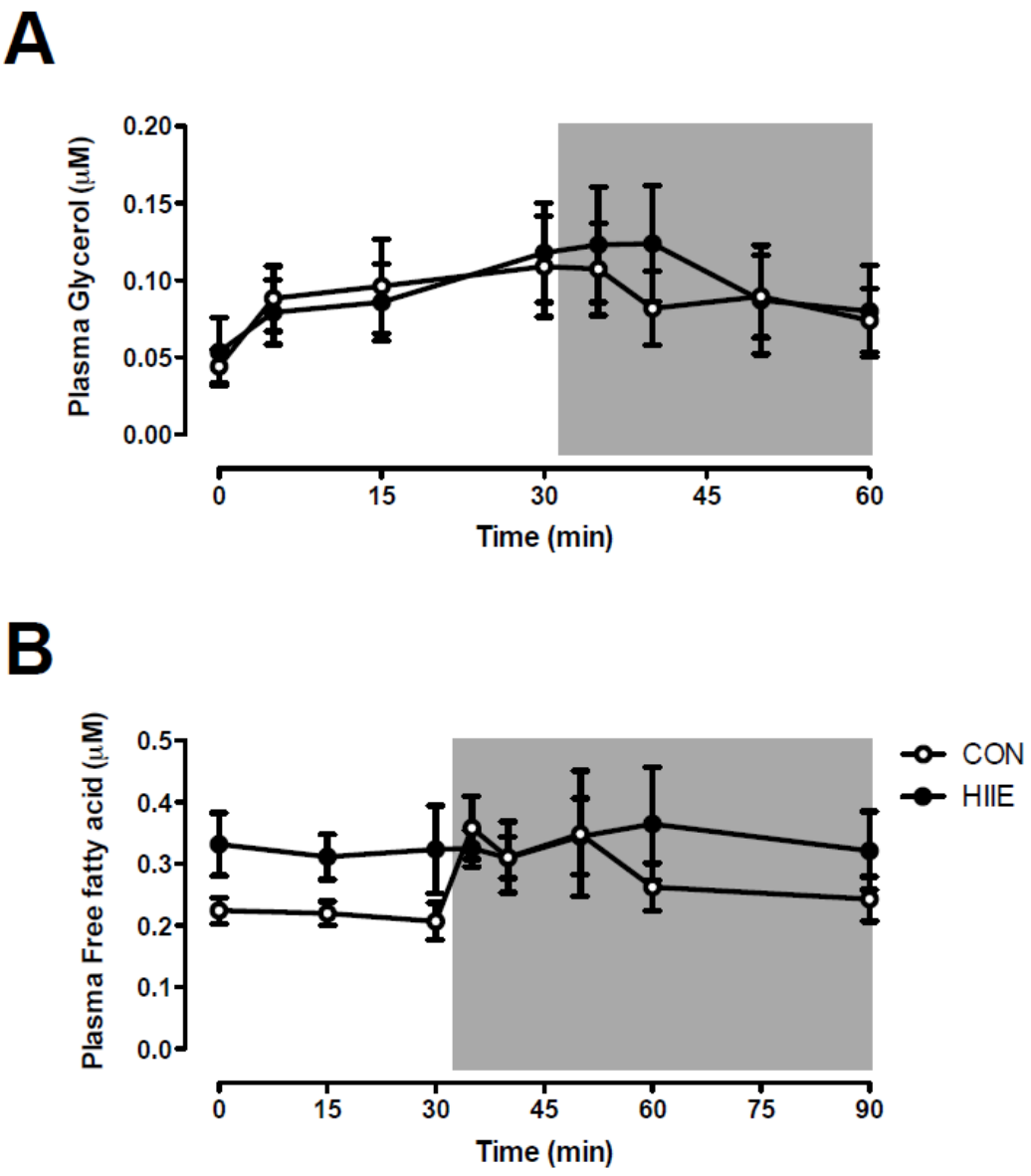
- **Please see Appendix B for figures pertaining to this conference proceeding**

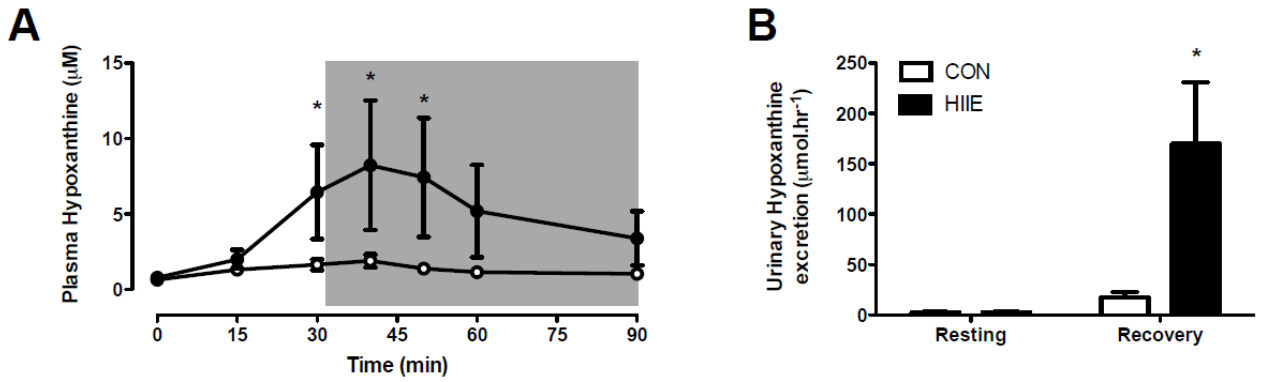
Appendix B

Appendix B contains result figures from the above abstract concerning the plasma concentrations of FFA, glycerol, hypoxanthine and uric acid. Furthermore, figures of urinary hypoxanthine and uric acid excretion are also included.



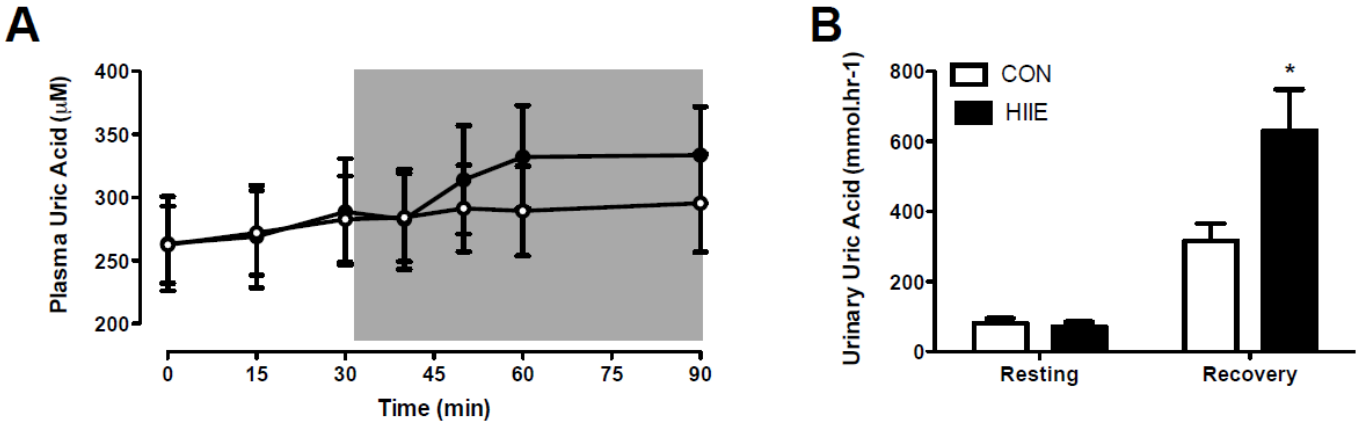
Plasma glycerol and FFA concentrations during and post CON and HIIE

(A) Plasma glycerol and **(B)** Plasma free fatty acid
Data are means ± SEM; n=8 per group; CON, white circles; HIIE black circles; Grey box represents the recovery period.
* $p<0.05$ CON vs. HIIE



Plasma and urinary hypoxanthine concentrations during and post CON and HIIE

(A) Plasma hypoxanthine **(B)** Urinary hypoxanthine excretion
Data are means ± SEM; n=8 per group; CON, white circles/bars; HIIE, black circles/bars; Grey box represents the recovery period.
* p<0.05 CON vs. HIIE at a given time point



(A) Plasma uric acid **(B)** Urinary uric acid excretion
Data are means ± SEM; n=8 per group; CON, white circles/bars; HIIE, black circles/bars; Grey box represents the recovery period.
* p<0.05 CON vs. HIIE at a given time point

Appendix C

- Subtract the difference of each participant's rest value from post exercise value
- Average the differences for each exercise protocol (If calculating purine loss, sum Hx, uric acid, xanthine and inosine to get total purines
- Lactate concentrations are measured in mmol.hr-1 and purine concentrations are measure in µmol.hr^{-1}

<u>To calculate estimated equivalent fat loss from lactate</u>

X multiplied by 16 = X mmol of ATP

X mmol ATP / 1000 = X mol ATP

(30514 multiplied by X mol ATP) / 37

= X g/fat

- *30514 is Δ G of ATP rephosphorylation of high energy bond (measured in kJ)*
- *37 is number of kJ per gram of fat*

<u>To calculate estimated equivalent fat loss from purine excretion and loss</u>

X multiplied by 5 = ***X*** mmol ATP

X mmol ATP / 1000000 = X mol ATP

(30514 multiplied by X mol ATP) / 37

= X g/fat

Appendix D

Figures where there is statistical significance between males and females in the 8:12 and 24:36 HIIE trials.

A: Rating of Perceived Exertion

RPE was significantly different between the male and female groups at the end of exercise (E10).

Figure 1: RPE at E5 and end of exercise in 8:12 HIIE and 24:36 HIIE in males and females.
Results expressed as mean ± SEM; Males N=8; Females N =7
# denotes significant difference between male and female 24:36 trial

B: Oxygen consumption (VO_2)

VO_2 was significantly elevated from rest in both genders in both trials. Five mins post exercise; the male 8:12 HIIE trial was significantly greater than both female HIIE trials. The male 24:36 trial was also elevated significantly above the female 24:36 HIIE trial and this remained at 20 mins post exercise.

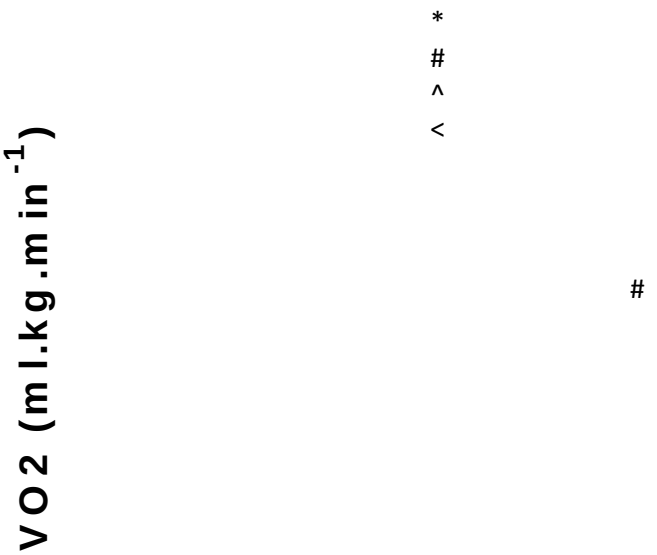


Figure 2: VO_2 at rest and post 8:12 HIIE and 24:36 HIIE in males and females.
Results expressed as mean ± SEM; Males N=8; Females N =7
* denotes significant difference between male and female 8:12 trial
# denotes significant difference between male and female 24:36 trial
^ denotes significant difference between male 24:36 trial and female 8:12 trial
< denotes significant difference between male 8:12 trial and female 24:36 trial

C: Plasma glycerol

There were no significant differences between the genders in plasma glycerol except at 75 and 90 mins post exercise where the male 8:12 group was significantly greater than the female 24:36 HIIE group.

Figure 3: Plasma glycerol at rest, during and post 8:12 HIIE and 24:36 HIIE in males and females. Results expressed as mean ± SEM; Males N=8; Females N =7
< denotes significant difference between male 8:12 trial and female 24:36 trial

D: Plasma hypoxanthine

Plasma Hx was significantly greater upon completion of HIIE in the female 8:12 HIIE compared to the male 8:12 HIIE trial. No other differences between the two genders were detected.

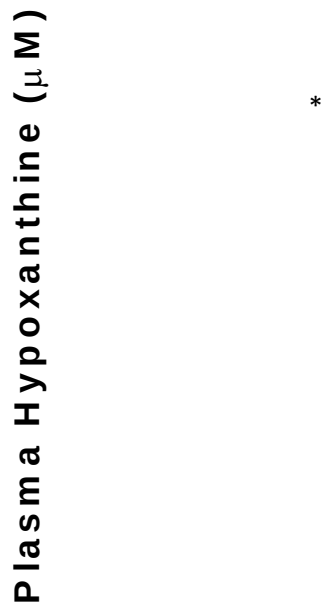


Figure 4: Plasma hypoxanthine at rest, during and post 8:12 HIIE and 24:36 HIIE in males and females.
Results expressed as mean ± SEM; Males N=8; Females N =7
* denotes significant difference between male and female 8:12 trial

E: Plasma uric acid

Upon completion of HIIE and at every recovery time point, differences between genders were detected. At the end of exercise (E10), the male 8:12 HIIE trial was significantly greater than the female 8:12 trial. The male 24:36 HIIE trial was also higher compared to female 8:12 trial as well as male 8:12 greater than the female 24:36 HIIE trial. At R10 and R15, the male trials were significantly greater than the female. At R30 and R60 the male 8:12 and 24:36 HIIE trials were significantly higher than the female concentrations over both HIIE trials; except between the male 8:12 trial and female 24:36 trial. Finally at 90 mins post exercise, the only difference between genders was observed between the 24:36 male trial and the 8:12 female trial.

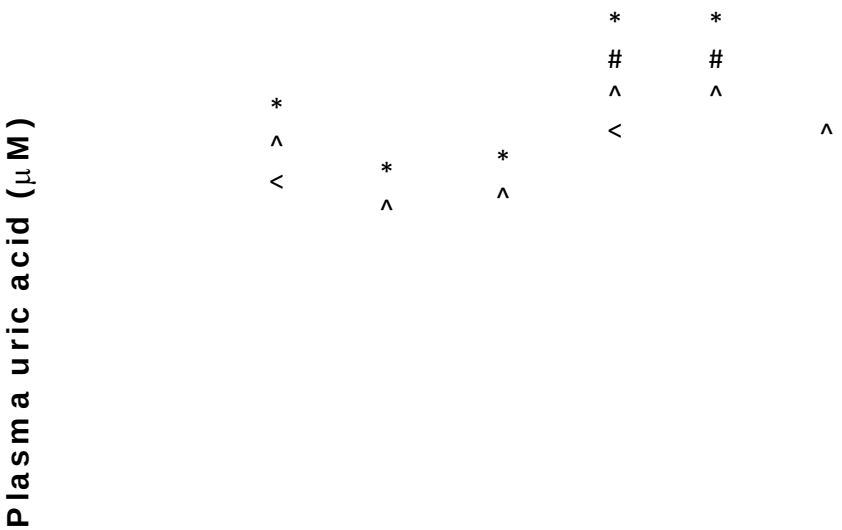


Figure 5: Plasma uric acid at rest, during and post 8:12 HIIE and 24:36 HIIE in males and females. Results expressed as mean ± SEM; Males N=8; Females N =7
* denotes significant difference between male and female 8:12 trial
# denotes significant difference between male and female 24:36 trial
^ denotes significant difference between male 24:36 trial and female 8:12 trial
< denotes significant difference between male 8:12 trial and female 24:36 trial

Table 1: Urinary purines at rest and post 8:12 HIIE and 24:36 HIIE in males and females.

The male 24:36 HIIE was significantly greater than the female 8:12 HIIE trial in urinary Hx and uric acid. Additionally, the male 24:36 HIIE urinary Hx concentration was higher than the female 24:36 HIIE trial.

	Rest (umol.hr^{-1})	**90 mins post exercise (umol.hr^{-1})**
Hx		
Males 8:12	6.0	88.9
Males 24:36	8.2	119.3 #^
Females 8:12	1.8	37.8
Females 24:36	4.7	67.1
Uric acid		
Males 8:12	177.4	299.1
Males 24:36	143.0	433.3 ^
Females 8:12	112.0	148.5
Females 24:36	169.0	218.5

Results expressed as mean ± SEM; Males N=8; Females N =7
# denotes significant difference between male and female 24:36 trial
^ denotes significant difference between male 24:36 trial and female 8:12 trial