The role of exercise intensity and contraction frequency in modulation of exercise response

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy is entirely my own work, and that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: _Linda Murphy_   (Candidate) ID Number: 10719611

Date: 08/12/2022

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Abbreviations

ACS	acyl-CoA synthase
ACSM	american college of sports medicine
ADP	adenosine di phosphate
AMP	adenosine monophosphate
AMPK	adenosine monophosphate activated protein kinase
ATP	adenosine tri phosphate
AUC	area under the curve
BMI	body mass index
Ca2+	calcium
CGM	continuous glucose monitoring
CHO	carbohydrate
CPT1	carnitine palmitoyltransferase
DEXA	dual energy x-ray absorptiometry
Eff	Gross efficiency
EMG	electromyography
EPOC	excess post exercise oxygen consumption
ETC	electron transport chain
FA	fatty acid
FAD	flavin adenine dinucleotide
Fatmax	relative maximum amount of fat utilised during exercise
FCC	freely chosen cadence
FFA	free fatty acid
G6P	glucose 6 phosphate
GIR	glucose infusion rate
H+	hydrogen
HIE	high intensity interval exercise
HIT	hight intensity interval training
H-MRS	h-magnetic resonance spectroscopy
HOMA-IR	homeostatic model assessment of insulin resistance

HR	heart rate
HRmax	heart rate max
HSL	hormone sensitive lipase
IDH	isocitrate dehydrogenase
iEMG	integrated raw electromyography
IMF	intermyofibrillar
IMP	inosine monophosphate
IMTG	intramuscular triglyceride
MICE	moderate intensity continuous exercise
MICT	moderate intensity continuous training
MPF	mean power frequency
MVC	maximal voluntary contraction
NAD	nicotinamide adenine dinucleotide
O_2	oxygen
OGTT	oral glucose tolerance test
PAS	periodic acid schiff
PCr	phosphocreatine
Pi	inorganic phosphate
PPO	peak power output
RER	respiratory exchange ratio
RMR	resting metabolic rate
RMS	root mean square
RPE	rate of perceived exertion
sEMG	surface electromyography
SIE	sprint interval exercise
SIT	sprint interval training
SR	sarcoplasmic reticulum
SS	subsarcolemmal
TCA	tricarboxylic Acid Cycle
VCO_2	volume of carbon dioxide produced

$\dot{V}O_2max$ maximal oxygen consumption

$\dot{V}O_2$ volume of oxygen consumed

Wmax   work performed at maximal exercise

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Abstract

High intensity exercise been shown to produce different acute exercise responses when compared to moderate intensity. Manipulating the rate of muscle contraction to alter intensity has received limited research. The primary purpose of this PhD was to investigate the potential mechanism that produces the acute differences observed when comparing high and moderate intensity exercise. Altering exercise intensity will be achieved through increased force and rate of contraction, as well as increased contraction rate alone.

Methods

In study 1, twelve recreationally active male participants completed 3 trials in random order consisting of 7x30second sprint cycles at 130%peak power output (PPO)(SIE), 60 minutes at 55% PPO (MICE), and a rest trial where no exercise was performed. In study 2 a separate nineteen recreationally active male participants cycled for 1-hr at 55% PPO at either 65-rpm or 95-rpm. In study 3 a separate eighteen recreationally active males completed a replica study similar to study 2 but with the addition of a 41minute submaximal trial at 95rpm at 55% PPO. Indirect calorimetry was used to measure a number of metabolic variables during exercise. Muscle biopsies were performed as part of study 1 and 3.

Results

Study 1: Significantly lower glycogen (94±24 vs 108±30 vs 125±31 mmol/kg/wt p=<.001) and increased insulin sensitivity (0.28±0.05 vs 0.25±0.04 vs 0.22±0.04 GIR p=<0.05) were observed 24 hours post SIE when compared to MICE. Study 2: Significantly greater carbohydrate utilisation (2.48±0.2 vs 1.97±0.2g/min p=<.01) was observed when exercising at 95rpm vs 65rpm at 55%PPO. Study 3: Greater total glycogen use per contraction was observed (-0.008±0.002 vs -0.006±0.002 mmol/kg/min p=<.05) at 95vs65rpm.

Conclusions

There were no fibre specific differences in glycogen use between trials. Increased glycogen use observed following SIE exercise is also observed following MICE at an increased contraction rate. The increase in glycogen use was not shown to occur as a result of increased type 2 fibre recruitment alone.

1. Chapter I Introduction

1.1. Introduction

The benefits of physical activity are well established, with regular exercise associated with a decreased risk of chronic illnesses such as cardiovascular disease, type 2 diabetes, and certain forms of cancer (Guthold *et al.*, 2018). Physical activity can also play an important role in preventing the progression of the overweight and obesity epidemic that has occurred over the past 25 years (Mitchell *et al.*, 2011). A single bout of exercise has the potential to stimulate a response that is considered metabolically beneficial, such as increased energy expenditure and insulin sensitivity (Mann *et al.*, 2014; Moniz *et al.*, 2020), and with chronic exposure, can reduce the risk of overweight and obesity related diseases (Ruegsegger and Booth, 2018). Aerobic exercise is one of the most frequently prescribed therapies for the prevention and treatment of metabolic conditions, to the extent that it has been suggested that exercise is medicine (EIM) (Vina *et al.*, 2012). The concept of exercise induced increases in health date back to ancient times with Hippocrates (460-370 B.C) often quoted as haven said "eating alone will not keep man well, he must also take exercise" (Berryman, 2010). As with all prescribed therapies, understanding the impact of the dose response is essential in providing the optimal benefits. Within the context of aerobic exercise, the dosage relates to the intensity and duration of a given bout as both will influence the acute metabolic response of an individual (Garber *et al.*, 2011). This thesis will seek to further our understanding of the acute metabolic response to exercise of different intensities as well as exercise of different muscle contraction rates.

1.2. Exercise Intensity

The intensity of an exercise bout will influence the metabolic response both during, and in the hours after exercise (Hargreaves, 2000). The relative intensity of exercise can be defined based on a known physiological maximal value. Commonly used variables include Heart rate max (HRmax), maximal oxygen consumption ($\dot{V}O_2$max), peak power output (PPO) and work performed at maximal exercise (Wmax), with a corresponding %max utilised to quantify the

relative exercise intensity (Norton *et al.*, 2010). Recently, a research focus on sprint interval training (SIT), has suggested that this form of exercise can be as beneficial in increasing $\dot{V}O_2$max (Gibala *et al.*, 2006), if not superior (Wisløff *et al.*, 2007), to traditional moderate intensity continuous training (MICT), although significantly less time is spent in active exercise (Norton *et al.*, 2010). Less is understood about the acute response to sprint interval exercise (SIE) compared to moderate intensity continuous exercise (MICE), with research comparing the impact of each form of exercise on the acute (0-24 hours post) response not clearly defined. Some studies have found increased energy expenditure in the 0-24 hours post SIE (Tucker *et al.*, 2016) while others have not (Malatesta *et al.*, 2009). SIE has also been proposed to provide comparative or superior benefits in relation to glucose metabolism, particularly in participants with insulin resistance or type 2 diabetes (Liu *et al.*, 2019), while less consistent findings have been produced when research was conducted in healthy individuals (Jelleyman *et al.*, 2015). The variation in the literature relating to the acute response to SIE and MICE and their impact on energy expenditure and glucose metabolism, provides a gap wherein further research is required.

1.3. Contraction force and rate

The rate of muscle contraction is another important, but relatively less studied variable that can influence the physiological response to a bout of exercise. Each muscle contraction can be measured in relation to the total force being produced as well as the rate at which the force is being generated per contraction. Research investigating the physiological response to manipulating cadence has consistently shown an increase in oxygen consumption at higher cadences during submaximal exercise (Coast *et al.*, 1986; Gotshall *et al.*, 1996; Zoladz *et al.*, 2000; Foss and Hallen, 2004; Bieuzen *et al.*, 2007; Brennan *et al.*, 2019). During incremental exercise, this increase diminishes as the exercise progresses until no difference is observed at maximal exercise (Coast *et al.*, 1986; Zoladz *et al.*, 2000). Additionally, research that

investigated gross efficiency, (ratio of total mechanical work to energy expenditure) has suggested that a J-shaped curve exists for the optimum cadence for a given submaximal %PPO, with lower efficiency observed when cycling above or below the optimum cadence (Ettema, 2009). An interesting yet unexplained finding from such research is that the cadence selected by trained cyclists (80-100-rpm) during submaximal cycling, is higher than the metabolically optimal or most efficient rates (50-70-rpm) for power outputs that are sustained for prolonged periods (Brisswalter *et al.*, 2000; Foss and Hallen, 2004; Brennan *et al.*, 2018). Such research has typically recruited trained cyclists with the study aimed at increasing cycling performance (Hansen and Sjogaard, 2007; Ansley and Cangley, 2009). As such, any findings from research on trained cyclists are not directly transferable to untrained populations as these athletes exhibit an optimised skeletal muscle recruitment pattern during cycling exercise as well as a significantly higher freely chosen cadence (FCC) when compared to untrained cyclists (Zorgati *et al.*, 2015). However, a consistent finding in both trained and untrained cyclists has been that adopting a high cadence will result in an increase in oxygen consumption at submaximal but not maximal exercise (Gaesser and Brooks, 1975; Coast *et al.*, 1986; Gotshall *et al.*, 1996; Zoladz *et al.*, 2000; Hill and Vingren, 2012; Zorgati *et al.*, 2015), as well as a reduction in cycling efficiency and increase in energy expenditure

A number of potential contributing factors have been suggested to explain such consistent findings, with an increase in type II muscle fibre recruitment at higher cadences routinely referenced (Hansen and Sjogaard, 2007; Ansley and Cangley, 2009; Ettema and Loras, 2009). Humans have adapted to have a range of skeletal muscle fibre types that can be recruited based on the force and speed of a contraction (Scott *et al.*, 2001). Research has shown that fibre type distribution has a direct effect on an individual's response to a given exercise intensity and will alter their energy expenditure and substrate utilisation for any given workload (Barstow *et al.*, 1996). However due to the invasive nature and high cost of muscle biopsies, few research

studies have taken fibre type into account when researching energy expenditure and substrate utilisation during exercise of varying intensities achieved through manipulating cadence. A potential explanation for the variation in efficiency and energy expenditure may relate to fibre type recruitment patterns, however very few studies have investigated this in detail. Those that have been conducted found no difference in fibre type recruitment at higher cadences (Gollnick *et al.*, 1974; Altenburg *et al.*, 2007) or a greater recruitment of type II fibres at lower cadences (Ahlquist *et al.*, 1992). Although potential methodological issues have been suggested as limiting the results from such studies.

1.4. Significance of this research

As further research is required relating to the acute impact of SIE, when compared to MICE, on post exercise metabolism, namely energy expenditure and glucose metabolism, this thesis will aim to provide further knowledge to this research topic.

Additionally, the role of contraction frequency on exercise intensity is relatively understudied when compared to force alone, with research to date focused on trained or elite cyclists and increasing their performance. A greater understanding of how contraction frequency affects energy expenditure in recreationally active healthy individuals may provide a novel way to exercise at a higher relative intensity without the need for additional external work. In the time poor world we live in, this may provide a novel way of increasing exercise intensity and reducing the time required to provide similar health benefits associated with longer duration lower intensity exercise. It may also be beneficial for individuals with chronic disease or those that cannot produce higher force to attain higher intensities of exercise, whereby they could gain additional benefits by cycling at a higher rate.

1.5. Thesis Aims, objectives and hypothesis

The purpose of this thesis was to investigate the role exercise intensity plays in determining energy expenditure during exercise in young recreationally active healthy males. The overarching aim is to investigate he role of exercise intensity and contraction frequency in modulation of exercise response. In the first instance, comparing how SIE and MICE impact the acute response relating to energy expenditure, substrate utilisation and insulin sensitivity was investigated (Experiment I). Secondly, the impact of high versus low contraction rate has on energy expenditure and substrate utilisation during exercise was investigated (Experiment II). Lastly, how contraction frequency impacts muscle fibre type recruitment during exercise was examined (Experiment III).

1.6. Experiment I: The effect of SIE and MICE on energy expenditure and glucose metabolism during, post (0-60 mins) and 24-hours post exercise.

1.6.1. Overview

Twelve recreationally active males completed three randomised trials separated by at least seven days. Each trial consisted of visiting the laboratory on two consecutive days. On the first day, participants undertook either MICE (60mins @ 60% $\dot{V}O_2$peak), SIE (7x130% PPO) or no exercise. The second day consisted of a resting metabolic rate test followed by two muscle biopsies of the Vastus lateralis pre and post a euglycemic hyperinsulemic clamp. Continuous glucose monitoring was performed throughout both days of each trial.

1.6.2. Aim

To compare the acute effects SIE and MICE have on energy expenditure, glucose metabolism and insulin sensitivity in 12 recreationally active young males.

1.6.3. Objectives

- To compare energy expenditure and substrate utilisation during, 1 hour post, and 24 hours post a single bout of SIE and MICE.

- To compare blood glucose as measured by continuous glucose monitoring in the 24 hours post a single bout of SIE and MICE.

- To compare insulin sensitivity 24 hours post a single bout of SIE and MICE.

- To compare whole muscle glycogen levels pre and post a euglycemic hyperinsulinemic clamp performed 24 hours post a single bout of SIE and MICE.

1.6.4. Hypothesis

- SIE will result in significantly greater energy expenditure 0-1 hours post exercise but not 24 hours post exercise when compared to MICE.

- SIE will significantly reduce muscle glycogen levels when compared to MICE 24 hours post exercise.

- SIE will significantly increase insulin sensitivity when compared to MICE 24 hours post exercise.

Schematic of experiment I
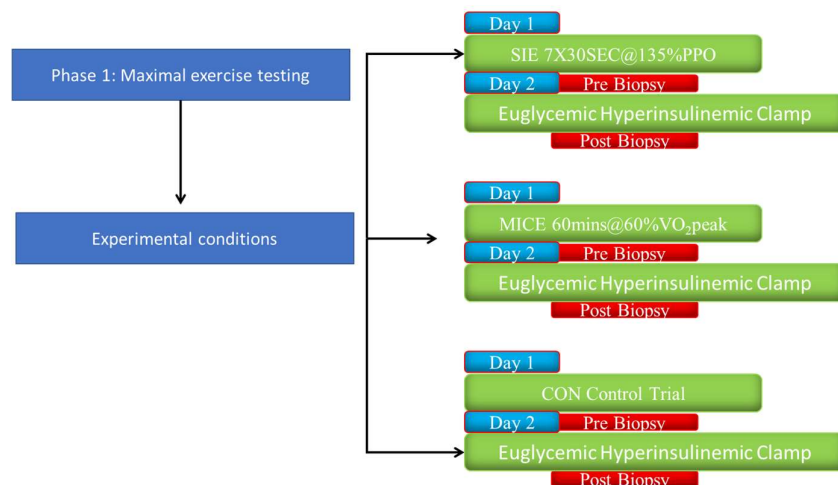
1.7. Experiment II : The impact of muscle contraction frequency on oxygen consumption during maximal and submaximal exercise.

1.7.1. Overview

Nineteen recreationally active young males completed two $\dot{V}O_2$peak trials randomised to 65- or 95-rpm. Subsequently they completed two randomised submaximal trials at 55% of PPO at 65- and 95-rpm respectively with at least 7 days between each trial.

1.7.2. Aim

To determine if the metabolic response in recreationally active participants is different when cycling at 95- versus 65-rpm during maximal and submaximal exercise intensities.

To examine the effect, the different contraction frequencies, have on the electrical activity in skeletal muscle.

1.7.3. Objectives

- To compare oxygen consumption, substrate utilisation and energy expenditure in recreationally active young men while cycling at 65- vs 95-rpm for 60mins@55%PPO.

- To compare iEMG activity of the vastus lateralis in recreationally active young men while cycling at 65- vs 95-rpm for 60mins@55%PPO.

1.7.4. Hypothesis

The rate of energy expenditure and muscle electrical activity will be significantly greater during the 95-rpm trial.

Schematic of experiment II
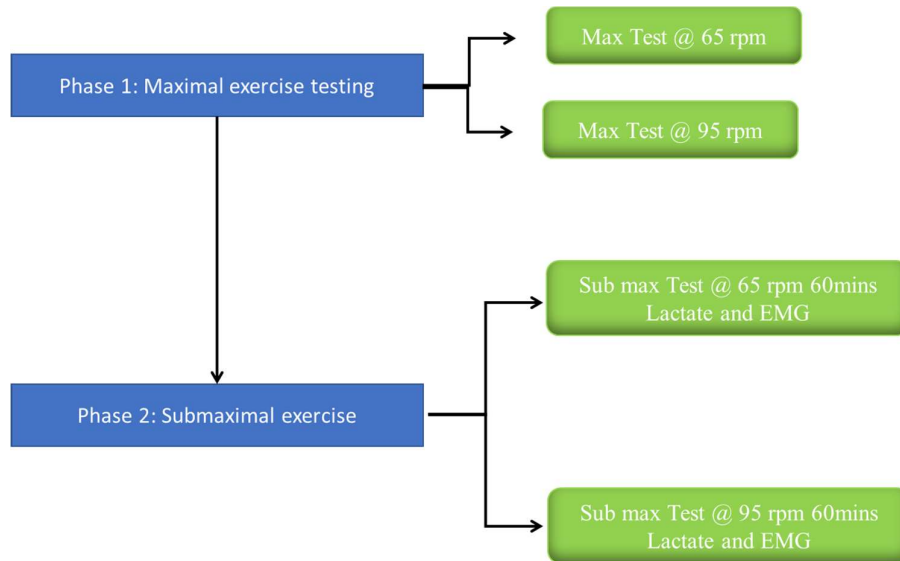
1.8. Experiment III: The impact of muscle contraction frequency during submaximal MICE on muscle fibre recruitment patterns.

1.8.1. Overview

Eighteen recreationally active non cyclist males completed two $\dot{V}O_2$peak trials randomised to 65- or 95-rpm. Subsequently they completed three randomised submaximal trials at 55% PPO at 65- and 95-rpm for 60-minutes and an additional trial at 95-rpm for 41-minutes. Muscle biopsies were taken from the Vastus Lateralis pre and post the submaximal trials. At least seven days elapsed between trials.

1.8.2. Aim

The aim of this study was to examine the relationship between the observed metabolic response and glycogen use in type I and type II muscle fibres during submaximal cycling exercise at 65- vs 95-rpm.

1.8.3. Objectives

- To compare oxygen consumption, energy expenditure, substrate utilisation and EMG on recreationally active young men while cycling at 65- vs 95-rpm for 60mins@55%PPO.

- To have participants perform cycling exercise at 95-rpm for 41-minutes and 5-seconds in order to perform the number of contractions equivalent to 65-rpm for 60-minutes but at the higher rate of 95-rpm.

- To compare glycogen use in type I and type II muscle fibres pre and post each submaximal exercise condition.

1.8.4. Hypothesis
- Glycogen use will be greater in type II fibres during cycling at 95-rpm.

- The rate of glycogen use in type II fibres will correlate with the rate of energy expenditure, iEMG and MPF (Mean Power Frequency) of the vastus lateralis.

Schematic of experiment III
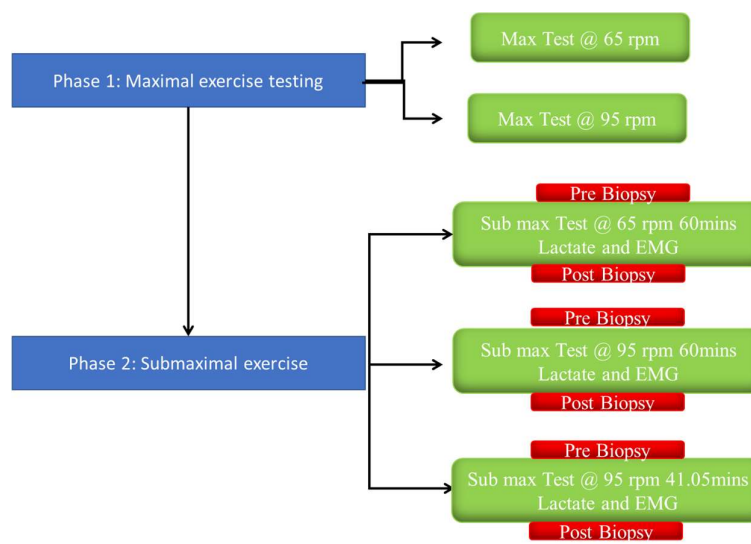
2. Chapter II Literature Review

2.1. Introduction

Physical activity was essential in human evolution as part of our hunter gatherer ancestors daily struggle to acquire sufficient nutrition to survive and reproduce (Eaton and Eaton Iii, 2003). Most modern humans do not face such struggles, however physical activity is now deemed essential in order to optimise health and prolong life span (Bull *et al.*, 2020). The metabolic pathways that provide energy to undertake physical activity are complex, and it is not fully understood how increased metabolic health is achieved or maintained. This literature review will focus on the acute metabolic response to structured exercise.

2.2. Introduction: An overview of Energy Metabolism during exercise

Performing repeated bouts of exercise has the potential to result in beneficial adaptations across multiple systems of the human body (Coffey and Hawley, 2007). Exercise can be broadly separated into two modes of activity, resistance exercise and aerobic exercise with divergent but overlapping adaptive responses to each form of exercise (Docherty and Sporer, 2000). Resistance exercise has been shown to have a significant impact on muscle size and strength (Kraemer *et al.*, 2002) while aerobic exercise leads to adaptations in the cardiovascular system such as increasing $\dot{V}O_2max$ (Garber *et al.*, 2011). Although evidence suggests a crossover in the mode of exercise and pathways of adaptation (Egan and Zierath, 2013), for the purpose of this thesis, focus will be kept on aerobic exercise.

Adenosine tri phosphate (ATP) is the energy currency of the muscle cell, with the energy stored being liberated through the hydrolysis of ATP by myosin ATPase, an essential reaction for muscular contraction to occur (Hargreaves and Spriet, 2020). Energy metabolism and substrate utilisation during exercise is complex, with the interaction between carbohydrate and fatty acid oxidation being dependent on the intracellular and extracellular environments, the exercise intensity and the duration of a given exercise bout (Spriet, 2014). The energy requirements of

working muscle are significantly higher than at rest with as much as 1000 fold increase in ATP production during high intensity exercise (Spriet, 1992). The concentration of ATP in resting skeletal muscle has been shown to be small, with approximately 5mmol/kg/WT present (Bergström *et al.*, 1967; Hultman *et al.*, 1967), and as a result its rapid resynthesis following hydrolysis is essential to meet the metabolic demands of the muscle cell, thus resulting in a balance between hydrolysis and resynthesis dictated by the rate of ATP degradation.

2.3. Energy systems during exercise

2.3.1. Anaerobic metabolism

2.3.1.1. Phosphocreatine system

The most readily available substrate that can be utilised for the resynthesis of ATP is phosphocreatine (PCr). Research in recreationally active young men suggest that there are sufficient quantities of PCr (~20mmol/kg/WT) stored in skeletal muscle to sustain 5-7 seconds of maximal ATP turnover rate (Harris *et al.*, 1976). Adenosine diphosphate (ADP) and inorganic phosphate (Pi) produced from the hydrolysis of ATP act as signalling molecules to activate creatine kinase, an enzyme that catalyses the reaction whereby ADP receives a Pi from PCr, therefore reforming ATP in the so called Creatine Phosphate system (Baker *et al.*, 2010b). A further supply of ATP can be generated by the activation of adenylate kinase by increasing levels of ADP, which catalyses 2 ADP molecules to form ATP and Adenosine monophosphate AMP (Spriet, 1992). AMP has been shown to be a potent stimulator of AMP activated protein kinase (AMPK), a sensor of the cells energy status and responsible for activating catabolic pathways and deactivating anabolic pathways within the cell (Gowans *et al.*, 2013). As ATP and PCr are only stored in small quantities, the active muscle must rely on other energy systems for energy production

2.3.1.2. Anaerobic Glycolysis

When exercise continues beyond a few seconds there is an increasing reliance on carbohydrate as a substrate for ATP generation, with a rapid increase in glycogen breakdown, producing Glucose-6-phosphate (G6P), which enters glycolysis resulting in ATP production. Increased levels of AMP, calcium, as well as Pi activate glycogen phosphorylase, the enzyme responsible for glycogen breakdown, with higher levels of these signalling molecules resulting in greater rates of glycolysis (Spriet, 1992). G6P undergoes 8 reactions in glycolysis resulting in the net production (2 ATP are required for glycolysis) of 2 or 3 ATP molecules, depending on if glucose of glycogen was the starting point, 2 pyruvate molecules, and 4 hydrogen molecules (H^+). During exercise, when insufficient oxygen (O_2) is present, pyruvate is converted to lactate through the action of the enzyme lactate dehydrogenase. The exercise intensity that elicits a large increase in blood lactate is referred to as the lactate threshold, and occurs when oxygen consumption is not occurring at a sufficient rate to meet the needs of aerobic metabolism (Svedahl and Macintosh, 2003).As glycogen levels are significantly higher than ATP and PCr, the capacity of anaerobic glycolysis to generate ATP is higher, however, the rate of ATP production is lower, resulting in a decrease in force produced by the muscle as the PCr system becomes depleted and there is an increase in the contribution from anaerobic glycolysis (Hultman and Spriet, 1986).

2.3.2. Aerobic metabolism

2.3.2.1. Aerobic Glycolysis

When sufficient O_2 is available (aerobic conditions), the H^+ is shuttled to the inner mitochondria via nicotinamide adenine dinucleotide (NADH) while pyruvate dehydrogenase (PDH) converts pyruvate to Acetyl CoA which enters the Tricarboxylic Acid Cycle (TCA)/Krebs Cycle also in the inner mitochondria (figure 2-1). The conversion rate of pyruvate to Acetyl CoA is ultimately dependent on the availability of oxygen for aerobic metabolism.

2.3.2.2. Lipolysis

Before discussing aerobic metabolism in detail, a brief description needs to be made as to the origins of fatty acids that enter into aerobic metabolism and the TCA cycle as Acetyl CoA in a similar fashion to the end products of aerobic glycolysis as previously discussed. Fat is by far the most abundant energy source available in the human body and is predominantly stored as triglyceride (TG) in adipocyte cells in adipose tissue (Jeukendrup, 2013). The release of free fatty acids (FFAs) from the triglyceride molecules in adipose tissue is called lipolysis and is controlled by an enzyme called hormone sensitive lipase (HSL). Insulin present in the post prandial state inhibits HSL allowing the uptake and storage of FFAs while epinephrine present during exercise activates HSL thus increasing the amount of FFAs in circulation (Kraemer and Shen, 2002). FFAs are also stored as intramuscular triglyceride (IMTG) in muscle cells with similar levels present in both well trained athletes as well as sedentary obese individuals however, the turnover rate and impact of IMTG is significantly different in each group (Moro *et al.*, 2008). The process of releasing the energy stored in the chemical bond of FFAs begins with beta oxidation.

2.3.2.3. Beta Oxidation

FFA transport into the muscle cell can occur through free diffusion as well as via transporting proteins present on the muscle cell surface (Bonen *et al.*, 2007). Once in the cell, FFA is acted upon by acyl-CoA synthase (ACS) to form fatty acyl-CoA which is transported to the inner mitochondria, mediated by carnitine palmitoyltransferase 1 (CPT1) (Stephens, 2018). Once in the mitochondria, fatty acyl-CoA is acted upon by acyl-CoA oxidase, the first enzyme in the beta oxidation pathway, that ultimately results in the production of Acetyl Co-A and 2 hydrogen atoms. The fatty acyl CoA can re-enter beta oxidation with the number of times it can re-enter determined by its length (Lundsgaard *et al.*, 2018). Oleic acid, a common long chain fatty acid, undergoing 8 cycles before its complete degradation, and produces 9

molecules of Acetyl CoA and 16 pairs of electrons (Hargreaves and Spriet, 2020) for the electron transport chain (ETC). The regulation of beta oxidation has been suggested not to be externally controlled and instead to be activated by the delivery of substrates (Eaton, 2002), suggesting the rate limiting step in fatty acid utilisation is controlled in another pathway.

2.3.2.4.   Tricarboxylic (TCA) Cycle/Krebs Cycle

Acetyl Co-A originating from aerobic glycolysis of carbohydrate sources, or beta oxidation of fat sources, enters the TCA cycle, combining with oxaloacetic acid having being acted upon by the enzyme citrate synthase, and initiates a pathway that ultimately produces electrons and carbon dioxide (CO_2) (Figure 2-1) (Martínez-Reyes and Chandel, 2020).
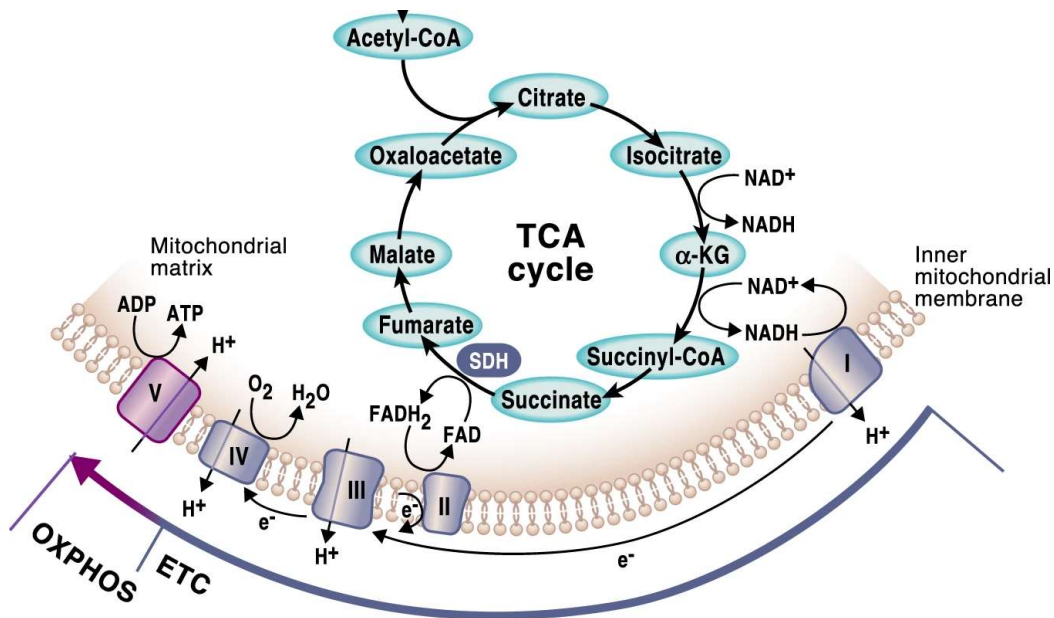
Figure 2-1: TCA/Krebs cycle in mitochondria. Electron transport chain also presented. Adapted from Martínez-Reyes (2020)

The TCA cycle is a tightly regulated energy producing pathway that has been shown to be a key regulator of whole muscle energy production during continuous aerobic exercise, with isocitrate dehydrogenase (IDH) acting as a rate limits step that is inhibited by ATP and

activated by ADP (Martínez-Reyes and Chandel, 2020). Ultimately each molecule of Acetyl Co-A that enters the TCA cycle will produce 1 ATP, 1 FADH and 3 NADH molecules with the ATP molecule being available for hydrolysis and FADH and NADH being transported to donate their electrons to the Electron transport chain (ETC) (Martínez-Reyes and Chandel, 2020).

2.3.2.5. Electron transport chain (ETC)

The ETC (Figure 2-1) is located on the inner mitochondrial membrane and is composed of 5 transmembrane protein complexes (complex I-V). Complex I-IV collectively generate a membrane potential between the matrix and intermembrane space of the mitochondrial. This potential is achieved by the pumping of protons received from FADH and NADH generated in aerobic glycolysis and the TCA cycle, out of the matrix and across the inner mitochondrial membrane as the electrons are shuttled from complex I-IV (Zhao *et al.*, 2019). Complex IV, cytochrome c oxidase, combines the electrons with O_2 and hydrogen to form water, a process that ensures electrons can continue to move along the chain. The build-up of hydrogen ions in the intermembrane space creates the energy potential that complex V of the ETC, ATP synthase, utilises to combine ADP and Pi to form ATP (Jonckheere *et al.*, 2012). Collectively the formation of ATP by the ETC is referred to as oxidative phosphorylation, with the rate of flow of electrons along the ETC being influenced by the delivery of NADH, FADH and the supply of oxygen. The complete oxidation of one glucose molecule will result in the formation of 36 ATP while the complete oxidation of the fatty acid palmitate will result in 130 ATP (Hargreaves and Spriet, 2020).

Ultimately the contribution of each energy system to ATP generation during a given exercise bout will be dependent on the ATP requirements of the muscle, which is largely dictated by the intensity and duration of the exercise bout as will be discussed in greater detail later in this thesis.

2.4. Muscle fibre composition and recruitment

The above sections relate to cellular respiration that takes place in all cells within muscles both at rest as well as during exercise. Human skeletal muscle cells are referred to as muscle fibres, with each fibre containing the contractile machinery responsible for muscle contraction. Different fibre types exist along a continuum, from slow to fast, defined as such by the speed at which a contraction (twitch) takes place following neural stimulation (Scott *et al.*, 2001). Classification of each fibre type by its myosin heavy chain isoform has been shown to be a reliable method for the identification of three primary fibre types in human skeletal muscle. Type I, slow twitch fibres have the slowest contraction speed and are highly fatigue resistant, type IIa fibres have a higher contraction speed then type I but are less fatigue resistant while type IIx fibres, produce the fastest contractions but are the least fatigue resistant (Plotkin *et al.*, 2021). Research using biochemical analysis on single muscle fibres has also suggested that further delineation of the fibre types can be made based on the relative contribution of the previously mentioned energy systems to ATP production. Type I fibres have a higher proportion of oxidative phosphorylation capacity and are thus referred to as slow oxidative fibres, type IIa fibres have an increased oxidative capacity when compared to type IIb fibres and are referred to as fast oxidative fibres, while type IIb fibres have the highest capacity to produce ATP from anaerobic energy pathways and are therefore referred to as fast glycolytic fibres (Pette *et al.*, 1999). Another differentiating factor that influences where on the speed spectrum a fibre sits relates to the size of the activating motor neuron that attaches to the muscle fibre from the nervous system. The smallest motor neurons have the lowest threshold for activating the muscle fibre with progressively larger motor neurons having a higher threshold of activation. The smallest motor neurons that innervate skeletal muscle do so with type I fibres, with progressively larger neurons associated with type IIa and type IIb fibres, in what is referred to as the Henneman size principle (1965). Hennemans original work was conducted in animal

models, and due to ethical reasons, similar research cannot be conducted on humans. However, using an intramuscular micro stimulation technique using microneedles allows for the activation of single motor units, and by measuring the force produced by the muscle it is possible to quantify the contraction response from different size motor neurons (Taylor and Stephens, 1976). Findings in agreement with the Henneman size principle have been found in humans during research using such micro-stimulation (Andreassen and Arendt-Nielsen, 1987) as well as voluntary contractions using microneedle electrodes to measure neural activity (Feiereisen *et al.*, 1997).
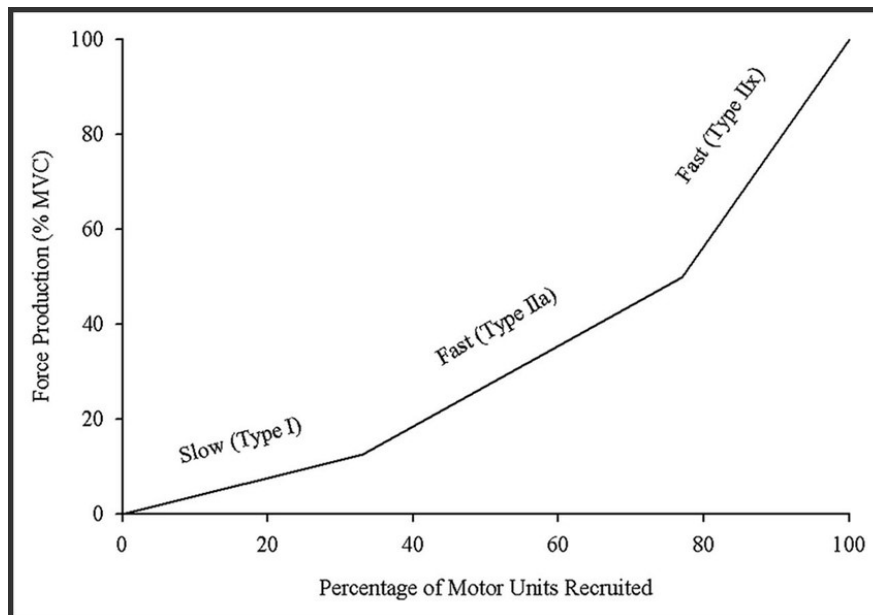
Figure 2-2 Graphical representation force produced relative to the percentage motor units recruited and the contribution of type I, type IIa, and type IIx, adapted from Gregory & Bickel (2005).

Skeletal muscle is typically classified into the three distinct categories mentioned, however, it is worth noting that strong evidence suggests that hybrid fibres can exist that have properties of type I and type IIa, as well as those that have properties of type IIa and type IIx (Pette *et al.*, 1999). Evidence exists that the proportion of each fibre type an individual may have is not a

fixed variable and that a shift in fibre type may be possible as an exercise training adaptation in some muscles. Luden et al (2012) performed muscle biopsies of the soleus and vastus lateralis muscles on recreationally active participants before and after 13 weeks of marathon training wherein participants increased their $\dot{V}O_2max$ by 9% over the training period. Fibre type analyses as well as a measure of oxidative capacity (citrate synthase) showed an increase in type I fibre distribution as well as increased oxidative capacity in the vastus lateralis muscle. Although no significant change was observed in the soleus muscle, previous research from the same research group using a similar study design did show changes in the gastrocnemius muscle, highlighting further the differences between muscle groups as well as muscles within the same muscle group (Trappe *et al.*, 2006). Both of these examples are consistent with research that suggests exercise training which targets a particular energy system or/fibre type, as endurance exercise does to oxidative phosphorylation/type I fibres, will result in an increase in an individual's ability to produce energy from that pathway/ increase in proportion of type I fibres (Wilson *et al.*, 2012).

Research comparing elite level athletes showed greater type I fibre type proportions in the vastus lateralis of runners compared to kayakers, while a greater type I fibre proportion was evident in the deltoid muscle of the kayakers, highlighting the sports specific relevance of fibre type distribution (Tesch and Karlsson, 1985). Similar findings have been found in sports associated with high speed and force with greater type IIb fibre proportions in the vastus lateralis of elite level weight lifters (Serrano *et al.*, 2019). Although the research into elite athletes is also suggestive of a fibre type shift as a result of a specific training, questions still remain as to how fibre type may influence an individual's response to a single bout of exercise.

Muscle biopsies performed pre and post an exercise bout that have been examined using single fibre analysis techniques, allow for the mechanical and biochemical analysis of the energy pathways within a single fibre. However, due to methodological difficulties it is possible that

different muscle fibres are being examined pre and post an intervention that might explain any potential differences observed. It is important to note here that the impact of an individual's fibre type distribution on their response to a given bout of exercise is being discussed currently while the impact of a given bout of exercise on fibre type recruitment will be discussed later in the review of literature. However, it is worth noting briefly that it has been consistently reported that the sequence of activation rates of the different fibre types during exercise is similar to the Henneman size principle with the intensity of the exercise replacing the force produced in the graph (figure 2-3). The impact of exercise intensity on muscle fibre type recruitment will be discussed in greater detail later in the review of literature.
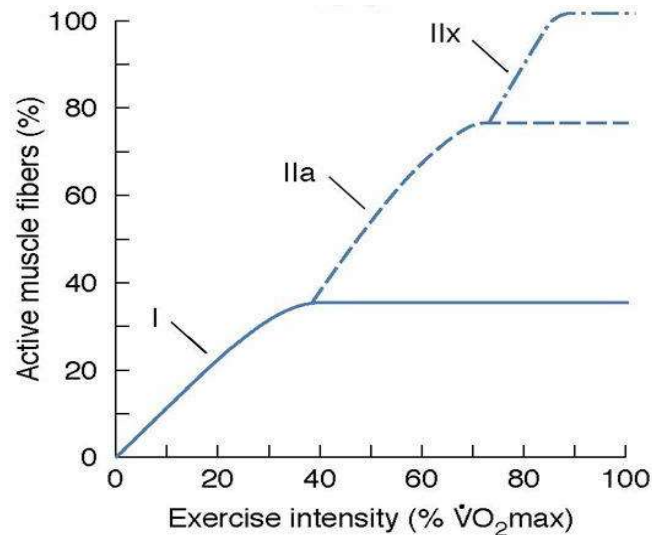
Figure 2-3 Activation pattern of skeletal muscle fibres with increasing exercise intensity, adapted from Sale et al (1987).

A common method of assessing how muscle fibre type can influence the response to acute exercise relates to correlating and individuals fibre type distribution with their metabolic

response to exercise, however research findings investigating this response have produced mixed results. When comparing well trained cyclists to untrained cyclists Jansson et al (1987) performed muscle biopsies of the vastus lateralis pre, during and post 60 minutes of exercise at 65%$\dot{V}O_2$peak. They found increased type I fibre type distribution (70% v 40%), increased oxidative enzyme activity (citrate synthase), increased fat utilisation and increased cycling efficiency in the trained cyclists. However, it is worth noting that this research was performed on very well-trained cyclist with $\dot{V}O_2$peak values 50% greater than the untrained group. Research by Mogensen et al (2006) produced similar findings when comparing recreational trained and untrained non cyclists when cycling at 80%$\dot{V}O_2$peak. Contrastingly, Hopker et al (2013) did not find a relationship between fibre type distribution and cycling efficiency when comparing trained cyclists versus untrained non cyclists (56 vs 46 ml/kg/min) when cycling at 60% $\dot{V}O_2$peak.

The degree of muscle activation from the central command motor neurons, in combination with the afferent neural feedback from the working muscle sets the activation level of sympathoadrenal activity during exercise (Christensen and Galbo, 1983). This activation level will dictate the degree of cardiovascular response and metabolic response mediated through increased levels of direct neural activation or indirect hormonal activation (epinephrine and norepinephrine) (Mastorakos *et al.*, 2005). The increased sympathetic neural activity has direct effects on metabolism by causing increased heart rate, cardiac output and blood pressure and therefore blood flow, increased lipolysis and therefore FFA in circulation, as well as increased glycogenolysis in the liver therefore increased glucose availability (Zouhal *et al.*, 2008). Similar to the differences in fibre type distribution in trained versus untrained, the sympathoadrenal response to exercise of different intensities has been shown to be different when comparing trained versus untrained individuals (Zouhal *et al.*, 2008). Given the systemic response to exercise, observing a response such as changes in efficiency and relating it to fibre

type alone may result in skewed findings that have not considered the potential influence of the sympathoadrenal activity response.

2.5. Maximal oxygen consumption and exercise intensity

2.5.1. Maximal oxygen consumption

During exercise, the energy requirements of working muscles is dependent on the intensity of the exercise being undertaken with greater energy demands as exercise intensity increases (Norton *et al.*, 2010). Oxygen consumption increases to fuel aerobic metabolism in order to meet this energy demand, however there is a limit to the amount of oxygen that can be consumed and utilised during exercise, referred to as an individual's $\dot{V}O_2max$ (Hill and Lupton, 1923). The concept of $\dot{V}O_2max$ is a long standing physiological phenomenon first introduced by AV Hill in 1923 (1923) and is well accepted in the scientific community. $\dot{V}O_2max$ refers to an individual's maximum ability to uptake and utilise oxygen and is traditionally measured during an incremental exercise session to exhaustion (Hill and Lupton, 1923). Measured in millilitres per kilogram per minute (ml/kg/min), it is a strong indicator of endurance performance (Lundby and Jacobs, 2016), as well as a key indicator of risk from all-cause mortality (Blair *et al.*, 1996). $\dot{V}O_2peak$ refers to the highest value achieved during an incremental exercise test and represents an individual's tolerance to exercise, which differs from $\dot{V}O_2max$ which is the physiological maximal value an individual is capable of achieving (Day *et al.*, 2003). The primary difference between $\dot{V}O_2peak$ and $\dot{V}O_2max$ is the presence of a plateau in the latter, with no increase in oxygen consumption observed with a corresponding increase in muscular work (Bassett and Howley, 2000). An accepted specific criteria for evidence of a plateau was suggested by Taylor et al (1955) who performed repeated incremental exercise tests on young (18-35yrs) healthy males who varied in training status from untrained to well trained, and suggested that an increase of $\dot{V}O_2 <150$ml/min was necessary for a plateau and $\dot{V}O_2max$ to be observed. Although a $\dot{V}O_2$ plateau is a desirable observation at the

end of an incremental test to exhaustion it is not routinely observed. Edvardsen et al (2014) performed treadmill exercise tests to exhaustion on 804 participants (390 women) and found that when applying the criteria of an increase of $\dot{V}O_2$ <150ml/min only 42% of the participants were said to have achieved $\dot{V}O_2$max. Exercise modality has also been shown to influence the likelihood of an individual achieving a plateau in oxygen consumption with significant differences existing between treadmill and cycle ergometer, the two most common modalities used in exercise science. Gordon et al (2012) had recreationally active young males complete four incremental exercise tests to exhaustion with two test performed on a treadmill and two on a cycle ergometer in random order and found that a $\dot{V}O_2$ plateau was present in 8% of cycle ergometer tests while it was present in 58% of the treadmill tests. Such findings are also related to the lower peak values observed in cycling incremental test when compared to treadmill incremental tests, with cycling producing 15-20% lower $\dot{V}O_2$peak values (Muscat *et al.*, 2015). Due to these accepted differences between exercise modalities, $\dot{V}O_2$max when referenced in the literature is typically referring to a treadmill exercise test while $\dot{V}O_2$peak is often referring to an exercise modality other than treadmill such as a cycling, although it should be noted that the two are interchangeable in the literature (Beltz *et al.*, 2016).

2.5.2. Exercise Intensity

The cardiovascular response to exercise is directly related to the workload and oxygen demands of the working muscle, with $\dot{V}O_2$ and HR increasing linearly with work rate until exhaustion (Hill and Lupton, 1923). The resting %HRmax and %$\dot{V}O2$max can vary between individuals, with trained individuals typically exhibiting lower values than untrained individuals (Jamnick *et al.*, 2020). Resting heart rate for young (approx. 20 years) recreationally active healthy individuals is typically 30-50%HRmax (60-100 bpm), while resting $\dot{V}O_2$ is typically 10-15% $\dot{V}O_2$max (resting =3.4ml/kg/min, $\dot{V}O_2$max= 40-50ml/kg/min) (Jung *et al.*, 2011). The difference during exercise is broadly suggested as the %HRmax being 10% higher than the

%$\dot{V}O_2$max at 60%$\dot{V}O_2$max and diminishing to 5% at 80%$\dot{V}O_2$max, with the American College of Sports Medicine suggesting that 60-80% %$\dot{V}O_2$max is equivalent to 70-85%HRmax in healthy individuals (American College of Sports, 2013). However research by Swain et al (1994) suggested that such broad ranges can lead to significant inter individual variation in the %$\dot{V}O_2$max achieved for a given HR. They had healthy men and women exercise to exhaustion and found that 40, 60, 80 and 85%$\dot{V}O_2$max resulted in 63, 76, 89 and 92%HRmax, suggesting that using the ACSM guidelines based on %HRmax may result in exercise at a lower %$\dot{V}O_2$max than desired. Additionally strong evidence exists that the mode of exercise and contraction rate of the exercising muscles will alter the %$\dot{V}O_2$max and %HRmax as will be discussed in greater detail later in this review of literature.

The work performed at the point of HRmax and $\dot{V}O_2$max during an incremental exercise test to exhaustion on a cycle ergometer is referred to as work max (Wmax) or Peak power output (PPO), with a %Wmax and %PPO interchangeably used to define exercise intensity in place of %$\dot{V}O_2$max or %HRmax (Norton *et al.*, 2010). The accuracy of PPO is increased when using an electronically braked cycle ergometer where the resistance applied can be independent of the pedalling rate used by the participant, and as such is preferentially used in populations with less cycling experience than trained cyclists (Myers and Bellin, 2000). The calculation of PPO is influenced by the protocol used and specifically the stage duration and increment with shorter/longer stage duration or smaller/greater stage increments resulting in lower/higher PPO (Zuniga *et al.*, 2012; Luttikholt and Jones, 2022). The accuracy of calculating PPO has been shown to be increased by using a prediction equation that takes into account the stage duration, stage increment and the point in the stage at which exhaustion occurred (Luttikholt *et al.*, 2006). Studies that do not use such a prediction model and instead use the power output of the last fully completed stage, may be underestimating the PPO and therefore the %PPO to elicit a %$\dot{V}O_2$max or %HRmax during submaximal exercise.

Unlike %$\dot{V}O_2$max or %HRmax, the %PPO can be fixed precisely during a submaximal exercise bout, however during continuous exercise the intensity of the exercise bout will determine the relationship between %PPO and %$\dot{V}O_2$max and HRmax. At the commencement of constant load/ fixed %PPO exercise that elicits an energy demand below the previously mentioned lactate threshold, oxygen consumption rises rapidly to reach a steady state (Whipp and Wasserman, 1972). Under such conditions the relationship between %PPO, %$\dot{V}O_2$max and %HRmax has been shown to be constant (Whipp and Wasserman, 1972), however the duration of the exercise bout will determine the length of time this constant state remains. When the %PPO is above the lactate threshold, no steady state is achieved and $\dot{V}O_2$ will continue to rise until $\dot{V}O_2$max is achieved . This $\dot{V}O_2$ increase is referred to as the $\dot{V}O_2$ slow component and results in the relationship between %PPO and %$\dot{V}O_2$max drifting apart during continuous exercise as %PPO does not change as %$\dot{V}O_2$max increases (Jones, 1976).

Establishing the exercise intensity of a given bout of continuous exercise that will result in a steady state has historically been achieved by anchoring the exercise intensity to the % of max for a given variable ($\dot{V}O_2$,HR or PPO), first reviewed by Katch et al (1978). They had men of a spectrum of fitness from untrained to trained ($\dot{V}O_2$peak = 37.8 - 68.8 ± 6.4 ml/kg/min) complete cycling exercise at 60%, 70% and 80% HRmax and found that exercise at the same intensity based on %HRmax produced a significantly different %$\dot{V}O_2$max response between individuals. Research by Baldwin et al (2000) highlighted the inter individual variation that can occur in response to exercise at an intensity using %$\dot{V}O_2$peak as a fixed maximal anchor. When trained and untrained cyclists exercised for 60 minutes at 70%$\dot{V}O_2$peak there was a significant difference between participants in their %$\dot{V}O_2$peak, %HRmax as well as lactate levels. Although such findings have been repeated and reviewed by research investigating the validity of using maximal anchors (Meyer *et al.*, 1999; Scharhag-Rosenberger *et al.*, 2010) such methods continue to be used to define exercise intensity.

2.5.2.1. Exercise intensity zones/ranges

The American College of Sports Medicine (ACSM) guidelines are broadly accepted as setting the level of exercise intensity that may be considered moderate as 60-80% $\dot{V}O_2max$ (Garber *et al.*, 2011). From a physiological perspective, to be considered moderate intensity researchers have suggested that a steady state of oxygen consumption should be present, indicating that ATP production is provided for via oxidative phosphorylation (Robergs *et al.*, 2004), that type I fibres are predominantly being recruited (Henneman *et al.*, 1965), that there is a low level of glycogen depletion rate (Gollnick *et al.*, 1974) and lactate levels are similar to baseline (Billat, 1996). Although this broad range assists in quantifying exercise intensity as moderate, it must be noted that significant individual variation exists in the physiological response to exercise at an intensity between 60-80% $\dot{V}O_2peak$. Lansley et al (2011) had 9 recreationally active untrained males ($\dot{V}O_2peak$ 48.6±8) perform an incremental exercise test to exhaustion on a cycle ergometer to determine their $\dot{V}O_2peak$. They subsequently had them exercise at 70%$\dot{V}O_2peak$ for 20 minutes and observed that four participants achieved $\dot{V}O_2peak$ before the 20 minutes had elapsed. Such findings, although in a small sample size, highlight the potential variation that can occur when individuals exercise at moderate intensity exercises.

Similar to the setting of moderate intensity, the ACSM guidelines set exercise intensities of 80-100% $\dot{V}O_2max$ as being heavy levels of physical activity (Garber *et al.*, 2011). During exercise between these levels of %$\dot{V}O_2max$ a slow component of $\dot{V}O_2$ consumption may be observed (Jones *et al.*, 2011), increased type II fibre recruitment levels occur (Hargreaves and Spriet, 2020), an increase in lactate above baseline that may plateau (Black *et al.*, 2017) and an increased rate of glycogen use when compared to exercise <80% $\dot{V}O_2max$ (Gollnick *et al.*, 1974; Black *et al.*, 2017). Exercise at intensities >100% $\dot{V}O_2max$ can only be sustained for very short durations of time, are considered maximal and are referred to as severe exercise intensity (Garber *et al.*, 2011). Physiological markers are suggested as being a slow component

in $\dot{V}O_2$ that does not reach a steady state (Jones *et al.*, 2011), a continual increase in blood lactate levels (Adeva-Andany *et al.*, 2014), and an increased contribution of type II muscle fibres compared to exercise intensity <100% $\dot{V}O_2$max (Henneman *et al.*, 1965; Gollnick *et al.*, 1974; Casey *et al.*, 1996). Unfortunately, the ACSM guidelines are not universally adopted with differential ranges used to define moderate, heavy and severe exercise. An example of this can be viewed in a review by Holloszy et al (1996) who suggests that moderate intensity be considered 50-70% $\dot{V}O_2$max, high intensity being 70-85% $\dot{V}O_2$max and vigorous intensity's being >85% $\dot{V}O_2$max .

Although these ranges of exercise intensities have the potential to assist in prescribing exercise to the general population as well as quantifying the exercise intensity in research trials, caution is required in the interpretation of results due to the variation that can occur in an individual's response to a given exercise intensity as well as the variation in the name given to a range of % $\dot{V}O_2$max used. For the purpose of this literature review, moderate intensity (MICE) will refer to exercise intensities of 60-80% $\dot{V}O_2$peak, high intensity (HIE) will refer to exercise intensities of 80-100% $\dot{V}O_2$peak while exercise intensity >100% $\dot{V}O_2$peak will be referred to as sprint interval exercise (SIE), with the specific range referenced in conjunction.

2.5.2.2.   Impact of exercise intensity on substrate utilisation

Since the advent of indirect calorimetry allowing for the quantification of substrate utilisation, it has been apparent that as exercise intensity increases there is a progressive shift from fat to carbohydrate oxidation (Hill and Lupton, 1923). Consistent evidence has shown that fat is the primary substrate utilised at rest with increased carbohydrate utilisation as exercise intensity increases to maximal intensity (100% $\dot{V}O_2$peak), however significant individual variation exists as to the exercise intensity that results in a shift from fat to carbohydrate as the predominant substrate utilised (Hawley *et al.*, 1998). This variation has been shown to be influenced by age,

gender and training status of an individual, all of which have been shown to affect substrate selection at a given exercise intensity (Baker *et al.*, 2010a).

Research by Romijn et al (1993) suggested that carbohydrate in the form of blood glucose and intramuscular glycogen accounts for 10-15% of total energy production when cycling at 30% $\dot{V}O_2$peak and increasing progressively to 70-80% total energy expenditure at 85%$\dot{V}O_2$peak. A similar study conducted by Van loon et al (2001) suggested that at 40%Wmax, carbohydrate accounted for 45% total energy expenditure and that this increased to 76% total energy expenditure when participants exercised at 75%Wmax (Figure 2-4).
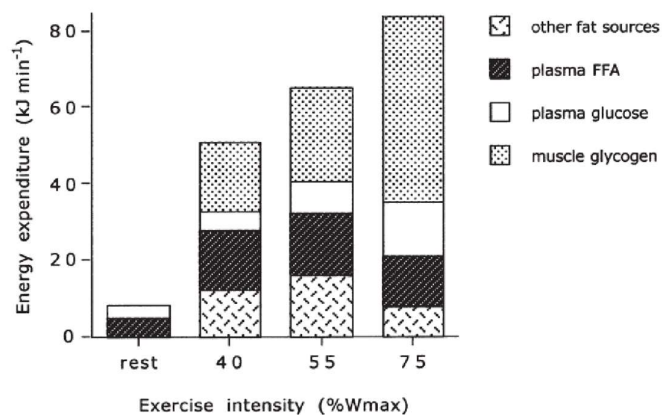
Figure 2-4 Energy expenditure, substrate utilisation and exercise intensity, adapted from Van Loon et al (2001).

While both groups used trained participants during cycling exercise, thus confining these results to that population and mode of exercise, these findings support the generally accepted results from a significant body of literature, that carbohydrate utilisation increases with increases in exercise intensity (Wasserman, 1995; Holloszy and Kohrt, 1996; Jensen and Richter, 2012; Hawley *et al.*, 2015).

Both Romijn et al (1993) and Van Loon et al (2001) also placed a significant emphasis on measuring fat utilisation during exercise at various intensities. Their findings were consistent with previous research on fat metabolism and showed that unlike carbohydrate utilisation, which increases continually with increased exercise intensity, fat utilisation initially increases but subsequently decreases. Romijn et al (1993) suggested that as exercise intensity increased from 25% $\dot{V}O_2$peak to 65% $\dot{V}O_2$peak , total fat contribution rates increased from 27 to 43 pmol/kg/min and decreased to 30 pmol/kg/min when participants exercised at 85% $\dot{V}O_2$peak. Similarly Van Loon et al (2001) estimated that the total fat oxidation was 0.68 g/min when exercising at 40%Wmax, 0.8 g/min at 55%Wmax while it decreased to 0.51 g/min at 75%Wmax. Such findings are generally accepted in the literature, however significant individual variation as to the intensity at which a corresponding increase or decrease in fat utilisation may occur.

The effect that moderate intensity continuous exercise and high intensity interval exercise has on substrate utilisation will be covered in greater depth later in this review of literature

2.6. Impact of Exercise duration on substrate utilisation

The duration of an exercise bout has also been shown to influence carbohydrate and fat utilisation during an exercise bout (Rothschild *et al.*, 2022). Bergman & Brooks (1999) had 7 trained ($\dot{V}O_2$peak 58ml/kg/min) and untrained ($\dot{V}O_2$peak 38ml/kg/min) non cyclists complete 2 hours at 20 and 40%$\dot{V}O_2$peak and 1.5 hours at 60%$\dot{V}O_2$peak on a cycle ergometer and showed a progressive decrease in carbohydrate utilisation with an accompanied increase in fat utilisation in both groups using indirect calorimetry. Watt et al (2002) had 7 trained males ($\dot{V}O_2$peak 62ml/kg/min) complete 4 hours of cycling exercise at 57% $\dot{V}O_2$peak and performed muscle biopsies at rest, 2 and 4 hour time points with indirect calorimetry and blood sampling performed throughout the exercise trial. They found similar findings to Bergman and Brooks (1999), in that carbohydrate oxidation decreased while fat oxidation increased over the 4 hours

(figure 2-5), with a greater contribution from fat towards energy expenditure observed from 120 minutes of exercise. Muscle glycogen was depleted at 2 hours and further at 4 hours while IMTG was decreased at 2 hours and only a slight further decrease was observed at 4 hours. As plasma FFA increased continuously over the 4 hours, it was suggested by the authors that the reliance on IMTG during continuous exercise is only evident up to 2 hours after which non-intramuscular fat deposits supply the FFA for substrate oxidation.
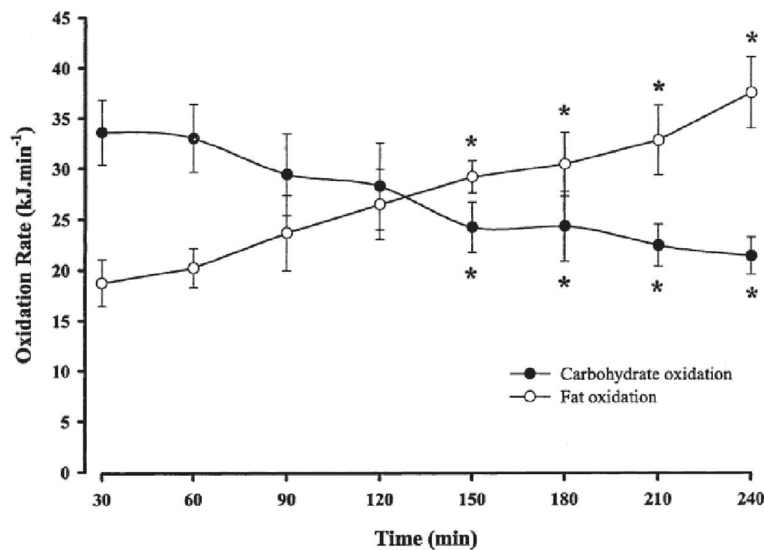
Figure 2-5 Whole body carbohydrate and fat oxidation rates during 4 hours of cycling exercise at 57%$\dot{V}O_2$peak, adapted from Watt et al (2002).

Romijn et al (1993) had 12 trained cyclists ($\dot{V}O_2$peak 67ml/kg/min) cycle for 120 minutes at 65% $\dot{V}O_2$peak and found that plasma derived oxidation of both fat and carbohydrate increasing over time, while muscle glycogen and intramuscular fat oxidation rates decreasing. Similar findings were observed by Van loon et al (2003) who additionally measured IMTG in type I and type II muscle fibres of 8 male cyclists ($\dot{V}O_2$peak 60ml/kg/min). They performed 2 hours of cycling exercise at 60% $\dot{V}O_2$peak with muscle biopsies performed before and after exercise and observed a decrease in IMTG in type I but not type II fibres. An interesting finding in all

three studies was the stable nature of blood glucose concentrations throughout the exercise trial, suggesting that hepatic derived glucose is a tightly regulated process during exercise. Such findings strongly suggest that as exercise duration increases a decrease in intramuscular carbohydrate and fat stores occurs, the rate of which is dependent on the exercise intensity, and that a greater reliance on hepatic glucose production and adipose tissue derived FFA can be observed.

2.7. Acute response to continuous aerobic exercise

2.7.1. Exercise effect: Acute Physiological Response to Moderate intensity continuous exercise (MICE)

The intensity of exercise has an inverse relationship with the duration of the exercise, with a higher intensity resulting in a decrease in the duration before fatigue occurs (Hargreaves, 2000; Ørtenblad *et al.*, 2013). Although variation exists as to what range of exercise intensity is considered moderate, for the purpose of this literature review, MICE will be considered continuous exercise at 60-80% $\dot{V}O_2$peak. A significant body of research exists relating to the acute response to exercise and how intensity, duration and changes in muscle contraction properties alter the metabolic and physiological response to a given bout, particularly in relation to substrate utilisation.

2.7.2. Substrate utilisation and Energy expenditure

As previously mentioned, exercise places a significant metabolic stress on the active contracting muscle fibres, with a large change in the energy demands when compared to resting. The first 1-2 minutes of continuous exercise are predominantly fuelled by anaerobic processes, due to the $\dot{V}O_2$ lag previously mentioned above (Hughson *et al.*, 2001). At low (<60% $\dot{V}O_2$peak) and moderate intensity (60-80% $\dot{V}O_2$peak) exercise, research suggests an equilibrium can be achieved whereby ATP use can be matched by ATP resynthesize through aerobic pathways in what is often referred to as the steady state concept (Ferretti *et al.*, 2017).

This concept, first described by Bock et al (1928), implies that the constant $\dot{V}O_2$ and heart rate observed at a constant low or moderate exercise work rate, can be inferred as a stable internal environment wherein energy consumption and production are at equilibrium. Although this concept is sometimes criticized for its simplification of complex and not yet fully understood pathways of control (Noakes, 1997), the ratio of oxygen consumption to carbon dioxide production, or respiratory quotient (RQ), is considered a valuable calculation in determining substrate utilisation (Jeukendrup and Wallis, 2005). In healthy individuals, the time to reach a steady state is intensity dependent, with greater time required as intensity increases (Wasserman *et al.*, 1967). The mechanism that delays $\dot{V}O_2$ consumption and achievement of a steady state is not specifically known. However the delay in increasing blood flow to the active muscle when transitioning from rest to exercise, a delay in the activation of the associated enzymes that control energy metabolism, as well as the time course for substrates to enter into the mitochondria for oxidation, have all been proposed as possible explanations (Hughson *et al.*, 2001).

Exercise dramatically increases blood flow to the muscle, increasing to as much a 10-15 fold in healthy untrained individuals when exercising at 60-80% $\dot{V}O_2$peak (Joyner and Casey, 2015), which significantly increases both FFA and glucose delivery to the muscle cell surface (Saltin *et al.*, 1998). Romijn et al (1993) showed that during MICE (65%$\dot{V}O_2$peak), FFA delivery from adipose tissue to the working muscle increased without a change in energy expenditure. Interestingly, they also showed that the absolute contribution of fat was the same at low intensity (25% $\dot{V}O_2$peak) as it was at high intensity (85% $\dot{V}O_2$peak). However, it should be noted that this study was performed in trained cyclists, on consecutive days, with each exercise bout lasting 2 hours, all of which limit the application of these results to other populations. It is also worth noting that the cadence in this population is also likely to be above that of untrained cyclists as will be discussed below, however it was not noted in the methods

as to what cadence was maintained for the duration of the exercise bout. Similar findings were shown by Van Loon et al (2001) using trained cyclists who performed exercise at 40, 55 and 75% Wmax, for 30 minutes each (90 minutes in total), as well as an additional trial with four participants who cycled continuously for 60 minutes at 40 and 55%Wmax, and a further 30 minutes at 75%Wmax. Using a stable isotope method as well as muscle biopsies, they found a decrease of 34±7% in fat oxidation at 75%Wmax when compared to 55%Wmax. They suggested that the decrease in fat oxidation rates at higher intensity were not as a result of availability but that the control was at a molecular level in the muscle cell. In order to review in greater detail the contribution of substrates to energy expenditure during exercise, a segregation will now be made and a focus on carbohydrate and fat independently will now be made.

2.7.3. Carbohydrate utilisation

Carbohydrate can be stored as glycogen in both the liver and skeletal muscle (Jue *et al.*, 1989). Skeletal muscle represents the larger glycogen store due to its relative size when compared to the liver, and has historically been the focus of research investigating glycogen use during exercise. However renewed interest in the liver, has highlighted its importance, particularly in elite endurance events lasting longer than 90 minutes (Gonzalez *et al.*, 2016). Although muscle glycogen represents the larger store, only glycogen in active muscles, and active muscle fibres is available during exercise (Greenhaff *et al.*, 1993). The relationship between pre exercise glycogen levels and time to exhaustion is well described, with early research by Bergstrom et al (1967) providing clear evidence that exercise capacity was greatly reduced when participants started MICE (75% $\dot{V}O_2$peak) in a glycogen depleted state. During MICE, as the duration of exercise increases, the continuous use of carbohydrate will result in a gradual decrease in glycogen stores, and given the relatively small storage of glycogen when compared to fat, it is not surprising that after prolonged MICE these glycogen stores will become limited and a great

proportion of energy supply will have to come from fat sources. Research by Hermansen et al (1967) found that the point of exhaustion during MICE (76%Wmax) was closely related to near glycogen depletion, but interestingly untrained participants were found to have slightly lower glycogen levels at the end of the exhaustive bout compared to well-trained participants (3.4 mmol/kg.wt versus 6.6 mmol/kg.wt). The specific mechanism by which glycogen depletion limits exercise capacity in MICE has not been discovered in part due to the many metabolic effects that occur in a state of reduced glycogen levels (Ørtenblad *et al.*, 2013). The primary theory relates to a global energy deficit within the muscle fibre and suggests that glycogen provides rapid essential ATP production that fuels the production of the intermediaries of the aerobic pathway of metabolism. Evidence for this concept is backed up by findings such as those from Norman et al (1988) which show an increase in IMP in glycogen depleted fibres following 60 minutes of MICE (70%$\dot{V}O_2$ peak) in recreationally active young men, suggesting a decrease in ATP regeneration rates. In contrast, studies investigating the role of glycogen in fatigue have brought into question the global energy deficit theory. Research such as that by Duhamel et al (2006) investigating the relationship between glycogen levels and Ca^{2+} release from the Sarcoplasmic reticulum (SR) following exercise at 70%$\dot{V}O_2$peak to exhaustion. They manipulated pre-exercise glycogen levels using glycogen depleting exercise followed by 4 days of a low or high carbohydrate diet, after which participants cycled to exhaustion at 70%$\dot{V}O_2$peak with muscle biopsies performed pre, during (time matched for exhaustion in the low glycogen condition) and post the exercise trial. They found no significant difference in ATP levels, however the low glycogen condition induced an earlier deterioration in the SR Ca^{2+} release. Further to this, in vitro research by Nielsen et al (2011) identified similar SR Ca^{2+} release deteriorations in isolated single fibres from rats that were low in glycogen yet ATP levels were maintained at normal levels. Findings such as these suggested that the fatigue associated with low glycogen might relate to its regulation of the contractile mechanism of

contraction rather than metabolic pathways of ATP resynthesis. During MICE lasting up to 60 minutes in healthy individuals, it is unlikely that glycogen depletion is going to occur unless a participant began the exercise bout in a glycogen depleted state through previous exercise, or by following a low carbohydrate diet, a technique routinely used in research to investigate the effects of exercise in a reduced glycogen state (Roepstorff *et al.*, 2005).

2.7.3.1. Muscle Fibre Specific carbohydrate changes

An interesting, and somewhat understudied aspect of glycogen metabolism relates to its fibre type specific storage and utilisation. Glycogen storage within muscle can be classified into three distinct subcellular regions of storage. These are intermyofibrillar (IMF), Intramyofibrillar and subsarcolemmal (SS) with IMF representing the majority (75%) of glycogen stored while SS and Intramyofibrillar can account for between 5-15% each (Nielsen and Ørtenblad, 2013). Type II muscle fibres can have between 16% (Vøllestad *et al.*, 1984) and 32% (Greenhaff *et al.*, 1993) greater total glycogen then type I fibres, however the distribution of glycogen across fibre type and the three subcellular regions does not appear to be different in sedentary or recreationally active individuals (Nielsen *et al.*, 2010). Interestingly, not only do well trained endurance athletes have an increased total glycogen level (Sherman *et al.*, 1981) but it would also appear that an adaptation to MICT is increases Intramyofibrillar and SS glycogen in type I fibres, and IMF in type II fibres, when compared to recreationally active healthy males or sedentary young men with obesity (Nielsen *et al.*, 2010). Another fibre type specific difference identified by researchers investigating glycogen localization within the muscle cell is that type I fibres have an increased Intramyofibrillar glycogen storage located at the I band while type II fibres have greater Intramyofibrillar glycogen storage located at the A band (Schmalbruch and Kamieniecka, 1974). Both of these findings have been suggested to confer that IMF glycogen may be associated with the speed of contraction while SS and intramyofibrillar might play a central role in endurance capacity,

however further research is required to confirm this association (Nielsen and Ørtenblad, 2013). Fibre type specific differences in the localisation of glycogen is a relatively understudied area with most exercise related studies investigating changes in elite athletes engaged in exhaustive exercise of a high intensity or long duration (Nielsen and Ørtenblad, 2013). Differences in localised depletion rates in different fibre types have been observed when comparing HIE and MICE, suggesting an intensity-dependant coupling of glycogen deposit sites with specific cellular functions such as energy homeostasis or calcium cycling (Vigh-Larsen *et al.*, 2022).

Research investigating fibre specific glycogen depletion patterns during MICE to exhaustion traditionally performs muscle biopsies during the exercise bout at various time points, where exercise is stopped briefly for the sample to be taken (Costill *et al.*, 1973; Gollnick *et al.*, 1974; Vøllestad *et al.*, 1984; Ball-Burnett *et al.*, 1991). Gollnick et al (1974) had 13 recreationally active males participants cycle at $64\%\dot{V}O_2$peak for 2 hours and performed muscle biopsies at pre, 20-, 60- and 120- minute time points. Their findings suggested that type I fibres were depleted first, with measurements at 20- and 60- minutes, after which type II fibres began to be depleted to a greater extent than up to that point in the trial. Vøllestad et al (1984; Vøllestad and Blom, 1985) produced similar findings in two separate studies using a similar protocol but with participants exercising at $75\%\dot{V}O_2$peak. Ball et al (1991), performed single leg cycling exercise on six active but not trained young males who exercised at $61\%\dot{V}O_2$peak until exhaustion. Muscle biopsies were performed at 15- and 60-minute time points and showed similar results to the previous studies, with type I fibres being depleted at an earlier stage and to a greater degree than type II fibres. Additional measurements of lactate, ATP, and creatine phosphate were made with significant differences between fibre types only existing at the 15-minute time point, with a significantly greater level of lactate existing in type II fibres. It is important to note that all of these studies found that type II fibres were not totally depleted of glycogen at the termination of exercise suggesting that glycogen levels alone are not an

exclusive factor in fatigue, as well as suggesting a sparing of this glycogen source for high intensity/contractions of a rapid nature, even when exhaustive fatigue has taken place (Vigh-Larsen *et al.*, 2021). Collectively the literature strongly suggests that during MICE lasting up to 60 minutes type I muscle fibres are recruited to a greater extent than type II fibres.

In summary, glycogen utilisation during MICE lasting up to 60 minutes appears to be a tightly regulated metabolic process with lowering glycogen levels having a direct and immediate effect on other metabolic pathways within the cell. Although it appears MICE will lower total glycogen levels in a consistent fashion, the divergent usage of fibre types as well as the usage of the localized storage sites of glycogen suggest a regulation of whole energy metabolism from specific muscle glycogen stores.

2.7.4. Fat utilisation

The control of fat utilisation during MICE has been well studied, with particular focus on the impact of intensity on fat utilisation rates for both health and performance reasons (Achten and Jeukendrup, 2004). This focus is in part driven by the significant potential benefit of being able to increase the relative contribution of fat at a given intensity, thereby in theory sparing glycogen levels with the performance aim of increasing the time to exhaustion (Venables *et al.*, 2005; Vigh-Larsen *et al.*, 2021). It is also of interest in the context of health, as increasing fat utilisation during exercise may aid in avoiding excess weight gain for healthy individuals or aiding those seeking to reduce their adipose fat levels (Miller *et al.*, 1997; Swift *et al.*, 2014).

Although it is consistently shown that fat utilisation is directly affected by the intensity of exercise (Coyle, 1995), similar to carbohydrate as previously described, significant variation exists between individuals as to the rate of fat use for a given intensity, with age, gender, training status and diet all having been shown to influence the fat utilisation rate (Bergman and Brooks, 1999; Jeukendrup and Achten, 2001; Maunder *et al.*, 2018). The relative maximum

amount of fat utilised during exercise, or Fatmax as coined by Jeukendrup et al (2001), marks the intensity of exercise where the relative contribution of fat as a substrate is at its highest. As exercise intensity increases there is a gradual shift from fat to carbohydrate as the primary substrate utilised, and therefore it is not surprising that the Fatmax for an individual would lie between low and high intensity exercise (Sidossis *et al.*, 1997; Amaro-Gahete *et al.*, 2019). Methodological issues have been highlighted that create differences in how Fatmax is calculated, and it has been suggested that this has influenced the range of exercise intensity that results in Fatmax for a given population. A recent systematic review by Amaro-Gahete et al (2019) highlighted these issues with particular focus on the influence of differences in ergometer used, stage duration and intensity, time selected for data collection, equations used to calculate substrate use, time of day exercise was performed and the timing of the last meal before the exercise trial. They also highlighted that differences in exercise protocols and variations in metabolic carts being used between studies as especially problematic (Amaro-Gahete *et al.*, 2019). Detailed studies that have measured substrate utilisation and also adopted the muscle biopsy technique in continuous exercise tend to do so using two (Bergman and Brooks, 1999) or three (Van Loon, L. J. *et al.*, 2001) distinct intensities that participants exercise at for a given period of time, so that a steady state is achieved (Wasserman *et al.*, 1967). Although this method gives a direct measure of the amount of fat being utilised at the level of the muscle, due to the cost associated with muscle biopsies, this is not a common method of calculating Fatmax, as the number of biopsies that would have to be performed would be significant. As indirect calorimetry has been shown to be an accurate and reliable indirect method for calculating substrate utilisation during steady state exercise (Jeukendrup and Wallis, 2005)), it is routinely used to measure Fatmax in research studies. A review by Maunder et al (2018) aimed at developing normative Fatmax values in healthy recreationally active individuals, suggested that the exercise intensity at which Fatmax was achieved ranged

from 43-59% $\dot{V}O_2$peak, with this range trending down as fat mass increased or trending up with increasing fitness levels. This finding is backed up by a detailed experiment by Jeukendrup et al (2003) who found that the Fatmax for trained male cyclists ranged from 48-64% $\dot{V}O_2$peak. It was noted within this study that $\dot{V}O_2$peak in cycling exercise is not $\dot{V}O_2$max, suggesting that the range for Fatmax is likely to be higher than stated when cycling exercise is used. The observed increase in Fatmax with increasing fitness levels is associated with strong evidence that increasing $\dot{V}O_2$peak through regular exercise will increase the amount of fat utilised for a given exercise intensity (Hurley *et al.*, 1986).

Fat is stored in several places around a healthy human body with the primary site being in adipose tissue, with smaller deposits within muscle and in circulation in the blood (Jeukendrup and Achten, 2001). However, questions remain as to the contribution of these deposits to fat utilisation during MICE. During exercise, the previously described increased blood flow increases the delivery of FFA from adipose tissue to the working muscle (Saltin *et al.*, 1998), with evidence that the oxidation rate of these FFA increases as the duration of MICE (65%$\dot{V}O_2$peak) increases (Romijn *et al.*, 1993). Using indirect tracer infusion methods it has been estimated that up to 50% of the total fat utilised during 2 hours of MICE (63%$\dot{V}O_2$peak) was not plasma based and therefore potentially IMTG (Martin 3rd *et al.*, 1993). However, it is important to note this study was on well-trained athletes cycling for 2 hours, a period of time not associated with exercise durations in untrained individuals. The contribution of IMTG to total fat utilisation during MICE is of interest for a number of reasons. Increased IMTG levels are associated with obesity and insulin resistance in sedentary individuals, yet also associated with well-trained insulin sensitive endurance athletes (Goodpaster *et al.*, 2001). Individuals with obesity have been shown to have reduced IMTG oxidation during MICE (Simoneau *et al.*, 1995; Standley *et al.*, 2017) while trained individuals appear to have increased IMTG oxidation (Phillips *et al.*, 1996). During 60 minutes of MICE (65%$\dot{V}O_2$peak) using an infused

tracer method in combination with indirect calorimetry, Friedlander et al (1999) found that IMTG accounted for 27% total fat oxidation in untrained healthy males. Direct methods of assessing IMTG contribution utilising the biopsy technique or more recently H-magnetic resonance spectroscopy (H-MRS) have shown that IMTG can decrease by up to 28% by 45 minutes during MICE (50%$\dot{V}O_2$peak) with further reductions as exercise duration increases (Egger *et al.*, 2013). Given that IMTG have been found to be in close proximity to the mitochondria, it is not surprising that they supply FFA for oxidation during exercise (Van Loon, 2004). Research has also shown that in untrained individuals, type I fibres have similar, though slightly lower glycogen levels to type II fibres, however have 2-3 times the IMTG content (Essén *et al.*, 1977). The relative contribution of IMTG or plasma FFA to MICE fat oxidation rates is highly individual with significant variation present in the literature (Loher *et al.*, 2016), highlighting the metabolic flexibility that exists in relation to fats contribution to substrate utilisation during MICE.

In summary, fat utilisation during MICE is a highly individual process with significant inter individual variation existing. An individual's ability to uptake and utilize oxygen is a key variable that dictates the increase or decrease in the relative contribution of fat during MICE (Maunder *et al.*, 2018). Although some disagreement exists in the literature as to the exact relative contribution from plasma FFA and IMTG (Watt, Heigenhauser and Spriet, 2002), it is accepted that both contribute to fat utilisation in healthy individuals during MICE (Goodpaster *et al.*, 2001).

2.8. High Intensity Interval Exercise/ Training
2.9. Interval exercise/ Training terminology

Interval exercise refers to an intermittent form of exercise whereby periods of relatively low intensity exercise are interspersed with periods of high intensity exercise (Gibala and Little, 2020). Although leading researchers in the field of interval exercise have attempted to

introduce standardised definitions of the various intensities of interval exercise, a definitive consensus has not yet been achieved (Biddle and Batterham, 2015). A review by MacInnis & Gibala (2017) encapsulated the physiological adaptations that occur as a result of interval exercise as well as providing a concise definition of the various interval definitions. In order to review the literature, they segregated the various exercise types based on the intensity used as well as the duration of active exercise (see figure 2-6). They suggested that high intensity interval exercise (HIE) should consist of intense exercise that elicits >80% of an individual's maximal heart rate, with exercise periods lasting up to 5 minutes in duration. Sprint interval exercise (SIE) consists of maximal or "all out" exercise periods typically <60 seconds, that use a workload greater than that used to achieve maximal oxygen consumption in an incremental exercise test. Both HIE and SIE when used as part of regular exercise sessions are referred to as high intensity interval training (HIT) and sprint interval training (SIT) respectively, with the number of repetitions of intervals placing the bout in low or high volume respectively (Macinnis and Gibala, 2017).
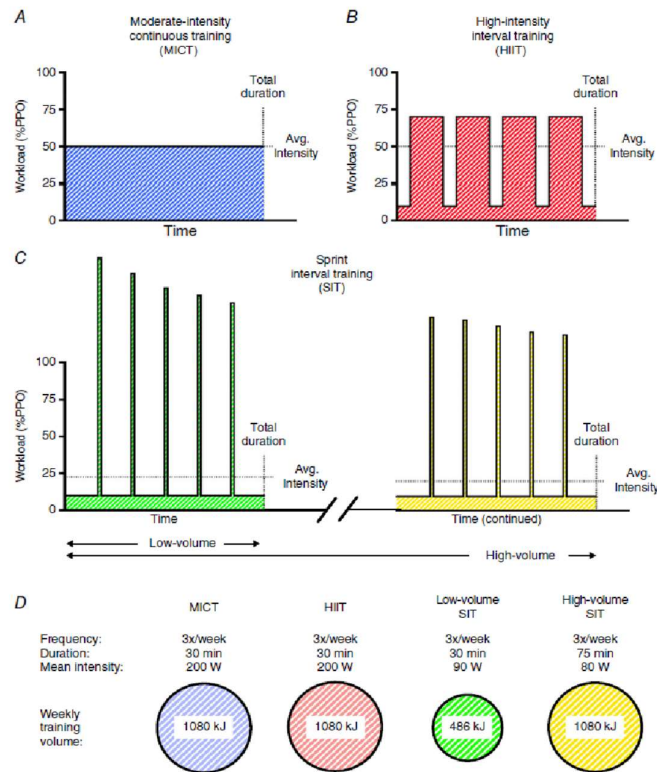
Figure 2-6 A graphical representation of differences in the main types of aerobic exercise adapted from MacInnis & Gibala (2017).

Further to this, Bishop et al (2018) have suggested that high intensity training (HIT) be defined as including intervals performed above 75% of the maximum power achieved during a graded exercise test. This should include both high intensity interval exercise and training (HIE and HIT respectively), as well as including sprint interval exercise and training (SIE and SIT).

2.10.   Research models of interval exercise

Although the previous examples by Macinnis et al (2017) and Bishop et al (2019) assist in delineating between HIE and SIE, researchers investigating the potential health related benefits of HIT and SIT have typically adopted three potential models of exercise, that vary in intensity duration and total volume of exercise. Such models are similar when in use in healthy individuals as well as in diseased populations such as those with cardiovascular disease (Ito,

2019). The three models include, low volume HIT, medium volume HIT and high volume HIT and are outlined below.

2.10.1. Low volume HIT

In low volume HIT, the focus is on keeping the time period spent at intense exercise low, typically <30 seconds for each interval with the intensity of each effort >95% of $\dot{V}O_2$peak (Tabata *et al.*, 1996; Gibala *et al.*, 2006; Wisløff *et al.*, 2009). The recovery period between efforts can be as low as 10 seconds (Tabata *et al.*, 1996) to significantly longer, with recent research showing that the length of time between exercise efforts can be hours, yet still produce similar findings to traditional HIT where rest periods are minutes rather than hours (Little *et al.*, 2019). In research studies investigating HIT with intensities >100% of $\dot{V}O_2$peak, a modified Wingate test is often used. The Wingate test consists of a 30 second "all-out" effort that is considered the gold standard for establishing maximum anaerobic power in athletes (Maud and Shultz, 1989). Research by Gibala et al (2006) has reignited the interest in low volume HIT with a focus on the time efficiency of this form of exercise. Total exercise time can be as low as 2 minutes (4x30second Wingate's) as part of a low volume HIT protocol (Gibala *et al.*, 2006; Hazell *et al.*, 2010), however the intensity of the exercise is supramaximal, typically being an "all-out" sprint effort.

2.10.2. Medium volume HIT

Exercise efforts consisting of "all out" efforts or maximal efforts lasting >30 seconds but <60 seconds are considered medium volume. Research studies incorporating modified Wingate tests >30 but <60 as part of HIT generally involve 8-10 repetitions with 1-2 minutes recovery, thus maintaining relatively low total exercise time with exercise intensities generally 85-100% of $\dot{V}O_2$peak (Little *et al.*, 2011). Total active exercise times of 8-12 minutes (Little *et al.*, 2010) adopted in medium volume HIT is relatively longer than low volume HIT, yet are still significantly shorter when compared to MICT.

2.10.3. High volume HIT

HIT that consists of interval exercise lasting >60 seconds results in a relatively high total exercise time when compared to bouts of <30 seconds. Intervals can be up to 4 minutes in duration with exercise intensities between 85-95% of $\dot{V}O_2peak$, with participants generally completing 4-6 repetitions with recovery periods at least matching the exercise time (Wisløff *et al.*, 2009). High volume HIT has demonstrated positive effects on cardiovascular function when compared to work matched MICT (Wisløff *et al.*, 2009).

2.11.   Operational Definitions

Even though the above broad guidelines assist in defining interval exercise protocols, it has been highlighted in the literature that inconsistencies in defining interval exercise in research publications has resulted in a reduced ability to draw concise conclusions and a generalisability from research findings (Viana *et al.*, 2018). This is due in part to the almost infinite possible variations that can be made of the variables that contribute to an interval exercise bout. These variables include the exercise mode, intensity, duration, repetitions and the rest duration and intensity if active recovery is adopted, all of which will affect the physiological response to the exercise bout (Buchheit and Laursen, 2013).

Therefore, for the purpose of this thesis, HIE will refer to any interval exercise consisting of efforts >80% of a specified max while SIE will refer to exercise performed at intensities >100% of max. For both HIE and SIE, where necessary, specific reference will be made to the % of max used, work to rest ratio as well as the number of repetitions used when appropriate. Additionally HIT and SIT will refer to studies where interval exercise was utilised as part of a training study.

2.12. Exercise effect: Acute Physiological Response to interval exercise.

2.12.1. Rate of force production

A signature of HIE and SIE is that it places a significant metabolic demand on the working muscle (predominantly anaerobic) involving rapid force generation in the initial 5-10 seconds (Kilpatrick *et al.*, 2014). It is not clear whether it is the initial high force generation or the effort in maintaining the high force that results in the subsequent health benefits associated with interval exercise. There is no consensus on the contribution of the two parts however it has been suggested by some (Hazell *et al.*, 2010), that this initial high force generation at the start of the effort is responsible for the metabolic benefits observed in the acute post exercise phase. Hazell et al (2010) had 48 recreationally active males ($\dot{V}O_2$peak 47 ml/kg/min) perform a randomised controlled trial with 2 groups performing 2 weeks of training consisting of 6 sessions of 5x30 second sprints or 5x10 second "all out" cycling sprints. A 5km cycling time trial and $\dot{V}O_2$peak were performed pre and post training. The increase in 5km time trial performance and $\dot{V}O_2$peak was not significantly different between groups, suggesting that the increase was due to the work performed in the first 10 seconds. This hypothesis is supported by Bogdanis et al (1996) who reported that 45% of the total work done in a 30 second bout is done in the first 10 seconds. However, two studies performed by Burgomaster et al (2005; 2006) also used 5x30 second "all out" efforts during 6 sessions over 2 weeks but did not find a significant difference in $\dot{V}O_2$peak. As only eight participants took part in the exercise group, a lack of statistical power may be responsible for the lack of a significant increase in $\dot{V}O_2$peak. It should also be noted that in both studies the short duration (2 weeks) of the training program may be a limitation of the findings as a longer training program might produce significant differences between groups. Although the high rate of force development is an integral part of HIE and SIE, further research is required to determine its relative contribution to adaptations as a result of HIT and SIT.

2.12.2. Substrate utilisation

Given the variations in definitions and protocols which exist between studies, interpretation of the research investigating the acute physiological response to interval exercise is challenging. That said, it is generally accepted that due to the rapid force generation, and therefore energy demand within the muscle, SIE lasting <15 seconds, relies chiefly on the ATP-PCr and anaerobic glycolysis pathways to supply the energy required for the exercise effort while SIE lasting >15 seconds will rely on both the anaerobic and aerobic pathways (Bogdanis *et al.*, 1996). As there is a broad spectrum of possible intensities of exercise, as well as work to rest ratios adopted in interval exercise, the reliance on any particular energy system will be dependent on the particulars of the exercise undertaken. However, it is accepted that interval exercise should aim for intensities that place a predominant stress on the anaerobic pathways during the active exercise interval (Macinnis and Gibala, 2017). As anaerobic pathways are the principal supplier of ATP during interval exercise, it is not surprising that significantly greater glycogen reductions can be observed following a bout of HIE or SIE when compared to a work-matched bout of MICE. Research investigating fibre type specific glycogen utilisation suggests that both HIE and SIE will result in a pronounced fibre type specific glycogen degradation, with type II fibre glycogen utilised in the initial stages of exercise (Gollnick *et al.*, 1974; Greenhaff *et al.*, 1994; Vigh-Larsen *et al.*, 2021). However, as exercise duration increases and the number of intervals performed increases, the disparity between type I and type II fibre glycogen utilisation is less apparent with a greater reliance on type I fibre glycogen stores compared to the initial stages of the exercise bout (Vigh-Larsen *et al.*, 2022).

Although the research relating to fibre type specific glycogen utilisation during HIE and SIE suggests a higher reliance on type II fibres during the initial stages of the exercise bout, the literature relating to how interval exercise influences the subcellular regions of storage is less precise with relatively little research to date. Gejl et al (2017) had 10 elite skiers complete

4x4minute @95%HR max with 45 minutes recovery between bouts with muscle biopsies performed on the triceps brachii muscle pre and post the first and last intervals. They found an even reduction in IMF and SS in type I and Type II fibres while Intramyofibrillar were reduced significantly in type I fibres alone, however the long duration of the interval resulted in a greater contribution of the aerobic energy system than traditionally observed in HIE and SIE. Vigh-Larsen et al (2022) had 18 moderately and well trained males ($\dot{V}O_2$peak 51-66 ml/kg/min) complete 4x10x45sec@105%PPO intervals with 135 seconds rest between repetitions and 10 minutes rest between sets. Their findings suggested that both Intramyofibrillar and IMF were preferentially used in type II fibres during the initial set of intervals while an even reduction between regions was observed during the last set of intervals. This finding is similar to the glycogen depletion patterns previously mentioned (initial preferential type II utilisation) and can in part be expected due to IMF representing the largest subcellular glycogen store. Given the relatively little research conducted on the influence of HIE and SIE on subcellular glycogen utilisation patterns it is difficult to determine their specific role in the metabolic response to this form of exercise, however, as research using MICE has shown a definite role for each region during exercise as previously mentioned, it is possible the same may exist during interval exercise. Further research is required to determine the specific role subcellular glycogen stores have during HIE and SIE.

2.12.3. Energy expenditure

Indirect calorimetry is traditionally used to calculate energy expenditure during exercise (Hill and Lupton, 1923), however this method will tend to underestimate energy expenditure when the exercise has a large contribution from glycolytic pathways (Scott, 2005). Calculating additional measurements relating to $\dot{V}O_2$ kinetics during exercise (Krogh and Lindhard, 1920) and EPOC (Excess post exercise oxygen consumption) (Gaesser and Brooks, 1984) will increase the accuracy of indirect calorimetry at calculating energy expenditure during an

interval exercise bout, however this method is time consuming and not commonly performed in interval exercise related research. As energy expenditure calculations using indirect calorimetry require a steady state to exist (Consolazio *et al.*, 1963), which is not present during interval exercise, precise energy expenditure measurements are not possible (Seiler *et al.*, 2013). However, it is accepted that energy expenditure from a single bout of interval exercise is typically lower than MICE due to the significantly reduced time spent exercising (Gibala and Little, 2020). To this extent, the mechanical work produced is routinely used in studies comparing interval exercise and MICE (Macinnis and Gibala, 2017). Although useful, mechanical work significantly underestimates the internal work performed during interval exercise and can skew the interpretation of energy expenditure when comparing interval exercise and MICE (Scott, 2005). Similar to energy expenditure, mechanical work performed during a bout of HIE or SIE is generally lower due to the significantly lower time spent in active exercise (Gibala *et al.*, 2006)

For a given bout of exercise, researchers who have matched MICE, HIE and SIE by way of the mean intensity of the bout, have shown similar energy expenditure, carbohydrate and fat metabolism when averaged over the trial (Essen, 1977; Cochran *et al.*, 2014; Zafeiridis *et al.*, 2016). However, some researchers who have also matched trials in a similar fashion did not show similar findings (Gosselin *et al.*, 2012). Should energy expenditure be similar, it would align with research that shows similar acute cellular signalling changes yet different chronic changes when comparing interval exercise and MICE (Cochran *et al.*, 2014). Further work is required to increase the accuracy of calculating both the aerobic and anaerobic components of interval exercise, which will increase our understanding of the mechanisms underpinning the adaptive response to this form of exercise.

2.12.4. Fibre type recruitment

Skeletal muscle fibre type recruitment is proportional to the intensity of the exercise bout, with type I fibres predominantly recruited at low and moderate intensity, with the additional recruitment of type II fibres as exercise intensities (Henneman *et al.*, 1965) or duration (Gollnick *et al.*, 1974) increase. Interval exercise typically involves brief, high intensity exercise, and as such, should rely on the additional recruitment of type II fibres to meet the force requirements of the exercise (Henneman *et al.*, 1965). Research from Casey et al (1996) clearly showed a significant contribution of type II fibres to interval exercise, with significant reductions in ATP and PCr when compared to type I fibres. However, it should be noted they used only 2 "all out" Wingate intervals to show their findings, which may not be applicable to protocols of shorter duration or lower intensities. Further work from Esbjornsson et al (1999) did show a fibre type specific metabolic response to a single Wingate test as well as gender related differences in fibre type specific glycogen use. Acutely, Kristensen et al (2015) did show a significantly greater use of type II fibres in interval cycling exercise (6x90sec@100$\dot{V}O_2$peak) when compared to MICE (30mins@70%$\dot{V}O_2$peak), showing increased glycogen use and AMPK expression in these fibres. Vigh-Larsen et al (2022) had 18 moderately and well trained males ($\dot{V}O_2$peak 51-66 ml/kg/min) complete 4x (10x45sec)@105%PPO intervals with 135 seconds rest between repetitions and 10 minutes between sets. Additionally, participants performed 5x6sec maximal sprints at baseline as well as immediately after each set of intervals. Muscle glycogen levels were significantly lower in type II fibres following the first set of intervals with a significant reduction in sprint ability. Muscle glycogen reductions were not different between fibre types following the 3rd set of intervals although a further reduction in sprint performance was observed. This research suggests that type II fibre glycogen use is preferential during interval exercise, however type I fibres are recruited when type II glycogen levels become lowered. However, other researchers

have found no difference in fibre type changes following an acute bout of HIE (Scribbans *et al.*, 2014; Tan *et al.*, 2018). The differences in results may be as a result of the difference in protocols used within each study with Kristensen et al (2015) using high volume interval exercise while Scribbans et al (2014; 2018) used low volume interval exercise.

Should specific fibre type differences exist in response to interval exercise, this may explain the exercise-type specific, acute adaptations that can be observed (increased insulin sensitivity) when comparing SIE and MICE (Kristensen *et al.*, 2015).

2.13.   Physiological Benefits of HIT

2.13.1. $\dot{V}O_2$peak

A significant body of evidence exists to support the use of HIT in athletic training programs to increase $\dot{V}O_2$ peak levels, with benefits shown in both endurance athletes (Billat, 2001) as well as field based sports involving intermittent high intensity exercise (Bangsbo, 1994). Research has also been conducted to investigate the use of HIT in clinical populations to increase $\dot{V}O_2$peak levels and reduce the risk of disease (Kilpatrick *et al.*, 2014). These studies have shown consistently that high volume HIT can produce significantly greater improvements in $\dot{V}O_2$peak when compared to the same volume and time commitment of MICT (Wisløff *et al.*, 2009).

Research in 2006 by Gibala et al (2006) showed similar changes in exercise capacity and selected muscle adaptations when comparing SIT and MICT over a 14-day period. They had 8 recreationally active men ($\dot{V}O_2$peak 51 ml/kg/min) perform 6 sessions over the 14 day period that consisted of SIE (5x30sec@250%$\dot{V}O_2$peak) or MICE (105mins@65%$\dot{V}O_2$peak) and found similar increases in muscle oxidative capacity as measured by the maximal activity of cytochrome C oxidase as well as the decrease in the time to complete a 750kj cycling time trial. This was especially noteworthy given a 90% lower training volume in the SIT group. It was

acknowledged by the authors that this was a relatively short training program (2 weeks) and that the rapid changes that occurred from SIT may be overtaken by the slower adaptations that occur from MICT. Subsequent research showed similar findings in sedentary young men following 12 weeks of training using a similar protocol (Gibala, 2016). Research by Helgerud et al (2007) showed superior effects from 8 weeks of low volume HIT and high volume HIT at increasing $\dot{V}O_2$peak when compared with work matched exercise at 70% of $\dot{V}O_2$peak and 85% $\dot{V}O_2$peak in moderately trained subjects ($\dot{V}O_2$peak 55ml/kg/min). Low volume HIT consisted of 15 seconds running at 95%$\dot{V}O_2$peak with 15 seconds rest with 47 repetitions performed while high volume HIT consisted of 4x4 minutes at 90-95%$\dot{V}O_2$peak with 3 minutes active recovery between intervals. Both the 70%$\dot{V}O_2$peak and 85%$\dot{V}O_2$peak groups performed continuous running protocols. Both HIT groups significantly improved $\dot{V}O_2$peak compared to the continuous exercise groups with no difference observed between HIT groups. Wisloff et al (2007) also showed similar findings in heart failure patients following 12 weeks of similar exercise training.

The mechanism by which HIT increases $\dot{V}O_2$peak is not fully understood, however in studies of <6 weeks, both MacPherson et al (2011) and Jacobs et al (2013) suggested that adaptations related to peripheral changes may contribute to the increase in $\dot{V}O_2$peak. In these shorter duration studies, strong evidence exists that changes in skeletal muscle are the main drivers of the benefits of HIT on $\dot{V}O_2$peak with an increase in mitochondrial content as the main facilitator of improvements in tissue oxygenation, increasing maximal oxygen consumption and ultimately endurance capability. In training studies greater than 6 weeks, increases in red blood cell volume and stroke volume also contributed to the increases in $\dot{V}O_2$peak in untrained (Warburton *et al.*, 2004) and trained individuals (Wisløff *et al.*, 2007), further suggesting that the initial benefits of HIT are driven by changes in skeletal muscle. A number of potential pathways of adaptation in skeletal muscle may explain the similar increase in $\dot{V}O_2$peak

observed from HIT. Mitochondria biogenesis is a core adaptation that occurs from exercise training and is strongly associated with improvements in $\dot{V}O_2$peak. It has also been shown to be an early adaptive response to exercise with significant increases observed in as little as 5 days (Starritt *et al.*, 1999). Mitochondrial biogenesis has been suggested as being a key potential pathway activated by HIT that can explain the initial significant increase in $\dot{V}O_2$peak (Daussin *et al.*, 2008), however a review by Bishop et al (2018) highlighted the need for a better understanding of the factors that regulate exercise induced mitochondrial biogenesis .

The high force and intensity associated with SIE activates type II muscle fibres to a greater degree than traditional MICE (Casey *et al.*, 1996)**.** This has led to studies investigating fibre specific differences that may explain the variation in the time requirement of the exercise needed to produce a measurable change in variables such as $\dot{V}O_2$peak. AMPK, as mentioned previously, is a key signalling pathway that regulates metabolism during exercise as well as the adaptive response to training (Herzig and Shaw, 2018). It has been shown that certain isoforms of AMPK are activated to a greater degree in type II fibres as a result of SIE when compared to MICE (Kristensen *et al.*, 2015). Such fibre specific changes may also explain in part the initial changes in $\dot{V}O_2$peak observed as a result of SIT. It is important to note that both mitochondrial biogenesis, and fibre type specific adaptations are methodologically difficult markers of exercise adaptation to measure and given the previously mentioned difficulties in matching SIT and MICT, caution should be observed when interpreting results.

In summary, low volume HIT programs <6 weeks have been shown to be a time efficient means of increasing $\dot{V}O_2$peak. Training programs that are longer than 6 weeks should incorporate HIT to maximise the potential adaptations that will increase $\dot{V}O_2$peak (Wen *et al.*, 2019).

2.13.2. Time efficiency benefits

A significant driver for the increase in research in recent decades relates to the potential time saving benefits of HIT, in what is often referred to as a "time poor" world, as a lack of time is often quoted as a reason for those who struggle to exercise regularly. HIT can produce desirable physiological changes that are associated with an increase in $\dot{V}O_2peak$ and reduced risk for chronic disease with relatively low training volumes when compared to traditional MICT (Little *et al.*, 2010). Although this claim has been a staple of modern research, it is important to note that there is variation in the time spent exercising in the different forms of HIT, as well as the supramaximal nature of some of the protocols, a level of effort that some individuals may not be able to attain. This has led to debate as to whether HIT can be used as an effective tool in population level recommendations for health enhancing physical activity (Biddle and Batterham, 2015).

2.14.   Muscle Contraction Frequency/Cycling cadence
2.14.1. Introduction

The physiological response to an acute bout of exercise is dependent on a number of factors and the rate of force development, is the most commonly studied variable. A greater rate of force development will increase cardiac output, minute ventilation, energy expenditure and substrate utilisation during exercise (Van Loon, L. J. C. *et al.*, 2001; Lucía *et al.*, 2002; Siebenmann *et al.*, 2015). In addition to the rate of force development, the frequency of muscle contraction is another important contributor to the response to an acute bout of exercise but has not been studied to the same extent. The literature relating to muscle contraction frequency is not consistent with variation in the literature as to the observed physiological responses that occur when contraction frequency is manipulated during exercise.

2.14.2. Freely chosen cadence (FCC)

During cycling, the power output is achieved by a combination of the force applied to the pedals (torque) as well as the rate at which the individual is pedalling , which is referred to as cadence. An increase in cycling cadence will reduce the torque applied to the pedals, and vice versa, for any given power output when using an electrically braked cycle ergometer (Ettema and Loras, 2009) and has been shown to influence the physiological response to exercise (Ansley and Cangley, 2009). Although in theory a broad range of possible cadences exists, it is interesting that individuals typically adopt a rate that is influenced by their previous exposure levels to cycling (Mater *et al.*, 2021) which is referred to as their freely chosen cadence (FCC).

2.14.3. Population level differences in cycling cadence

During cycling exercise the ratio of energy production to the work being performed is referred to as cycling efficiency (Abbiss *et al.*, 2009). A greater cycling efficiency has been associated with higher level of performance in individuals engaged in endurance sport (Coyle, 1995). An interesting phenomena relating to cycling cadence is that even though the most economical cycling cadence on a stationary cycle ergometer during MICE (60-80% $\dot{V}O_2$peak) is 50-80-rpm, trained cyclist typically adopt a cycling cadence of 90-105- rpm (Gaesser and Brooks, 1975; Horowitz *et al.*, 1994) while untrained cyclists typically adopt the most efficient cadence (Coast, J. R. and Welch, H. G., 1985; Ansley and Cangley, 2009). Although the specific reason for the differences between trained and untrained cyclists has not been identified, it would appear that the increase observed in the trained group is a product of cycling experience rather than an intrinsic physiological or biomechanical adaptation (Marsh *et al.*, 2000a). It has been suggested that as trained cyclists often cycle for prolonged periods, by adopting a higher cycling cadence, the resistance per rotation is decreased resulting in less exposure to high force as would be the case if a lower cadence was adopted (Patterson *et al.*, 1983). It has also been suggested that the lower force per pedal at the higher cadence may result in a reduced

recruitment of both slow and fast muscle fibres therefore resulting in a glycogen sparing effect. This theory was supported by research by Ahlquist et al (1992) who had 4 trained cyclists and 4 trained runners who also cycled regularly, cycle at 85%$\dot{V}O_2$peak for 30 minutes at 50- rpm and 100- rpm, and found a greater utilisation of glycogen in both type I and type II fibres at the lower contraction rate. To date, no research has definitively found a reason for the higher cadences in trained cyclists, however it has been suggested that it may be as a result of a number of contributing factors (Ansley and Cangley, 2009). These findings cause difficulties with taking results from experiments conducted on trained cyclists and applying them to untrained populations. That said, there are numerous physiological observations made that are present in both trained and untrained cyclists as will be detailed further in this review of literature.

2.14.4. Physiological response to manipulating cadence.

2.14.4.1. Oxygen consumption

A consistent finding in research investigating the physiological response to manipulating cadence has been higher oxygen consumption at higher contraction frequencies. The primary purpose of the majority of these studies has been to increase performance in trained cyclists through identifying the most efficient cycling cadence for them to use during competitive events. Early work by Gaesser et al (1975) had 8 well-conditioned males ($\dot{V}O_2$peak not reported) cycle at a range of power outputs at 40-, 60-, 80- and 100- rpm and found that oxygen consumption for any power output was higher at the higher contraction rate. Similar finding were observed by Coast et al (1985) who had 5 trained cyclists ($\dot{V}O_2$peak 66ml/kg/min) complete an incremental $\dot{V}O_2$peak test at 40-, 60-, 80-, 100- and 120- rpm and found significantly elevated oxygen consumption at all stages except at maximal exercise. Such results have been repeated in trained cyclists (Gotshall *et al.*, 1996; Zoladz *et al.*, 2000; Foss and Hallen, 2004; Bieuzen *et al.*, 2007; Vercruyssen *et al.*, 2009; Brennan *et al.*, 2019) as well as untrained but active populations. Research by Zorgati et al (2015) had 9 untrained

participants ($\dot{V}O_2$peak 47ml/kg/min) and 9 trained cyclists ($\dot{V}O_2$peak 63ml/kg/min) complete cycling exercise to exhaustion at 90% $\dot{V}O_2$peak at 40- and 100- rpm. For untrained participants a higher oxygen consumption was observed at 100- rpm as well as a decreased time to exhaustion, while trained participants had a higher oxygen consumption at 100- rpm but no difference in time to exhaustion. Formenti et al (2015) also showed increased oxygen consumption with 10 healthy non cyclists ($\dot{V}O_2$peak 48ml/kg/min) while cycling at intensities between 30-50%PPO at cadences of 50-, 70-, 90- and 110- rpm. Similar findings were observed by Shastri et al (2019) who had 10 sedentary but healthy males ($\dot{V}O_2$peak 29ml/kg/min) complete cycling exercise at 70 and 90%$\dot{V}O_2$peak at cadences 30-, 50-, 70-, 90- and 110- rpm and found higher oxygen consumption at all cadences except 110- rpm.

2.14.4.2. Cycling efficiency

The amount of ATP produced per litre of oxygen consumed will depend on whether carbohydrate or fat is the substrate being utilised (Consolazio and Pecora, 1963; Jonckheere *et al.*, 2012). As both have been shown to be oxidised during submaximal exercise, research on cycling efficiency typically converts oxygen consumption values using standard equations into energy expenditure (Consolazio and Pecora, 1963). They had 12 well-conditioned males complete exercise at 40-, 60-, 80- and 100- rpm at 5 submaximal exercise intensities 0, 200, 400, 400, 800 kgm/min and calculated efficiency using the 4 commonly used calculations. Gross efficiency is calculated by comparing energy expenditure as measured by indirect calorimetry and the total mechanical work completed by the individual. Net efficiency = Gross efficiency – resting energy expenditure and has been suggested as being a more accurate representation of exercise related efficiency as it takes account for the resting energy expenditure that would be additional to exercise related energy expenditure. Work efficiency = Gross efficiency - energy expenditure during unloaded cycling at a given cadence and has been suggested to account for the higher inertia generated at increased contraction rates. Delta

efficiency = change in efficiency between two cadences when power output remained constant, and has been suggested as the most accurate method of identifying the difference in efficiency between two contraction rates (Gaesser and Brooks, 1975). Although each calculation has been criticized as having artifacts relating to precise calculations across a cadence range, their use, and subsequent interrogation must be done in the context of the research question being asked (Hopker *et al.*, 2013). Gross efficiency is the most basic of the calculations and due to the methodological ease of its use is the preferred method of calculating cycling efficiency (Ansley and Cangley, 2009; Ettema and Loras, 2009). Values typically range form 18-24% during MICE in both trained and untrained cyclists (Marsh *et al.*, 2000; Moseley *et al.*, 2004). Using gross efficiency, a J shaped increase exists between the most efficient cadence for a given power output with cadence ranging from 50-80- rpm at power outputs from 100-300 watts (Coast, J. Richard and Welch, Hugh G., 1985). This is an interesting finding as it suggests trained cyclists do not cycle at the most efficient cadence, and that a training effect has occurred that results in them cycling at a less efficient rate during prolonged exercise. Also of interest with this finding is that an untrained or novice cyclist will select the most efficient cadence for a given power output, suggesting an internal mechanism that senses, and results in the participant adapting to the power output required by changing the FCC for that level of power output (Coast, J. Richard and Welch, Hugh G., 1985; Marsh and Martin, 1997). Manipulating cadence above or below the most efficient rate has shown consistently that cycling efficiency will decrease (Coast, J. Richard and Welch, Hugh G., 1985; Coast *et al.*, 1986; Marsh and Martin, 1997; Marsh *et al.*, 2000) , particularly at extremes of <60- rpm or >100- rpm (Brisswalter *et al.*, 2000a; Zoladz *et al.*, 2000). Many studies have used short duration (3-8 mins) and/or incremental exercise bouts (Gaesser and Brooks, 1975; Coast, J. R. and Welch, H. G., 1985; Gotshall *et al.*, 1996; Chavarren and Calbet, 1999; Zoladz *et al.*, 2000; Foss and Hallen, 2004; Hansen and Sjogaard, 2007; Vercruyssen *et al.*, 2009; Formenti *et al.*, 2015;

Zorgati *et al.*, 2015; Brennan *et al.*, 2019) where it was possible to test range of cycling cadences.

2.14.4.3. Hemodynamic Response

2.14.4.3.1. Heart Rate

The strong relationship between heart rate and $\dot{V}O_2$ consumption typically observed during exercise (Albouaini *et al.*, 2007) is interestingly not observed when contraction frequency is manipulated, with HR values being higher at higher and lower contraction rates above and below the most efficient rate (Coast, J. Richard and Welch, Hugh G., 1985). Although some studies have shown a linear relationship between HR and $\dot{V}O_2$ when cycling at a constant power output but different cadences (Hagberg *et al.*, 1981), a general consensus is that there is curvilinear relationship with greater differences observed at submaximal levels and the differences diminishing as $\dot{V}O_2$ and heart rate approach max (Hagberg *et al.*, 1981; Coast, & Welch., 1985; Gotshall *et al.*, 1996; Zoladz *et al.*, 2000). Although clearly defined, it has not been established why the divergence between $\dot{V}O_2$ and HR occurs with increases in cycling cadence. A study by Gotshall et al (1996) investigated this phenomena and suggested increased efferent neural activity at higher cadences as a possible mechanism whereby the heart is stimulated to increase its rate relative to the increased efferent activity required to activate the working muscle. They also stated that the efferent neural activity alone would not be sufficient to increase heart rate to the observed level and that other possible mechanisms may exist.

2.14.4.3.2. Blood Flow

Blood flow during exercise is a tightly regulated physiological process that delivers oxygenated blood to the working muscle while also controlling the rate of flow of de-oxygenated blood back to the heart (Joyner and Casey, 2015). The increased heart rate observed at increased contraction frequencies will increase the rate of blood traveling to the working muscles. It has been shown that each muscle contraction will transiently reduce the ability of blood to flow

into the muscle during the contraction phase and increase it during the relaxation phase. Once passed through the capillary bed of the muscle, the skeletal muscle pump is the primary mechanism by which blood flow back to the heart is controlled and is a system that is confined to the rhythmical nature of cycling exercise with higher cadences resulting in a greater muscle pump venous return (Gotshall *et al.*, 1996). Although findings by Takaishi et al (2002) suggested arterial blood flow to the legs was restricted at higher cadences, and could possible counteract any potential increase of the muscle pump venous return, it is generally accepted that there is a greater net benefit of higher cadences in relation to blood flow and may be another potential reason to explain the increased cadences observed in trained cyclists.

2.14.5. Muscle Recruitment

2.14.5.1. Electromyography

It is possible to measure the level of activation of individual working muscles using bipolar surface electrodes that are able to pick up the low-level voltage change that occurs as a muscle fibre depolarises to initiate a contraction. This method is referred to as Electromyography (EMG) and has been a primary method of establishing the involvement, as well as the activation level of various muscles during pedalling exercise of various cadences (Ericson *et al.*, 1985). Difficulties exist in interpreting EMG data as it can produce a significant amount of artefact due to methodological and physiological differences. Methodological differences include variations in the degree to which the preparation of skin underneath the electrode between data collection timepoints. Additionally it is virtually impossible to ensure the exact same placement site of electrode between data collection timepoints with additionally variation in sweat during exercise also unavoidable. Physiological differences such as variation in fat thickness superficial to the muscle under the electrode can also not be controlled. Collectively these methodological and physiological differences result in variation in EMG data collection between trials and participants. This is also compounded by differences between individuals

in the depth and location of motor units activating the muscle (Farina *et al.*, 2004). Additionally, variation in the software utilised and calculations of EMG raw signal also exist in the literature (Hug and Dorel, 2009) Given these difficulties it is not surprising that variation exists in the literature with regard to the effect of increased contraction frequencies on EMG data (Mater *et al.*, 2021). That said, research has shown consistently that the total activation level of a particular muscle, is elevated at higher contraction frequencies during short (<20 minutes) (Tetsuo *et al.*, 1996; Bessot *et al.*, 2008) and long (>50 minutes) exercise (Macintosh *et al.*, 2000; Farina *et al.*, 2004; Sarre and Lepers, 2005; Vercruyssen *et al.*, 2009; Kounalakis and Geladas, 2012; Brennan *et al.*, 2019). Sarre et al. (2005) found a small, but significant, increase in neuromuscular activity of the vastus lateralis when cycling at 110-rpm compared with 50-rpm at 65%PPO for 1-hr. However, there was no difference between the 50-rpm and a self-selected cadence of ~88-rpm indicating the difference in neural activity may not be linear. Kounalakis & Geladas (2012) also report a greater muscle activation while cycling up to 55-mins at 60% $\dot{V}O_2$peak at 80- vs. 40-rpm. There were no significant differences from 55- 90-mins of exercise and this may indicate a fatigue related impact with longer duration bouts of exercise. Muscle activation has also been shown to have a greater upward drift at cadences ±20% FCC suggesting an increased impairment of the muscles ability to produce force resulting in additional motor units being recruited to maintain the power output (Taylor and Gandevia, 2008). In summary, although variation exists in the literature, the predominant finding is that there is a J shaped curve in EMG data as contraction rates increase (Mater *et al.*, 2021)

The mean power frequency (MPF) of the raw EMG has been shown to reflect the action potential velocity in motor units (Hagg, 1992), and considering the action potential velocity is higher in fast motor units, an increase in MPF has been suggested to show an increase in the recruitment of fast motor units (Borrani *et al.*, 2001; Sarre and Lepers, 2005). However,

research investigating the effect of contraction frequency on MPF has not been consistent. Kounalakis & Geladas (2012) found a greater median frequency in the vastus lateralis when cycling at 40- vs. 80- rpm but neither Sarre et al (2005) nor Vercruyssen et al (2009) reported differences at 50- vs. 110-rpm. However, like most studies, the participants were highly trained cyclists and this may explain differences in the findings. Although MPF is a common calculation in EMG analysis, it is important to note that it is not the most reliable method of calculating the action potential velocity during dynamic exercise such as cycling (Farina *et al.*, 2004)

2.14.6. Fibre Type Recruitment

An increase in oxygen consumption, a decrease in efficiency and an increase in EMG activity has been shown to take place as a result of exercising at a cadence above the FCC. An increased recruitment of type II muscle fibres at higher cadences has been suggested as a possible contributor to the changes observed. Due to the invasive nature of muscle biopsies, a number of studies that have suggested a fibre type shift have drawn this conclusion from observations made during non-invasive experiments such as those that utilise isokinetic dynamometry or EMG spectrum analysis. Lepers et al (2001) recorded maximal isometric and concentric knee extension torque's before and after 30 minutes of continuous cycling at 80% PPO at FCC-20%, FCC and FCC+20% which calculated as 69-, 86- and 103- rpm respectively however, no effect of cadence was present on changes in the maximal torque post cycling. Sarre et al (2005) showed that the maximal voluntary contraction (MVC) of the knee extensor muscles was decreased after 30 minutes of continuous exercise at 65% PPO at FCC-43% and FCC+25% equating to 50- and 110- rpm respectively. This later study suggests an increase of at least 25% is required to invoke a difference in recruitment patterns during steady state exercise; however, both studies were performed on well-trained cyclists. As previously mentioned, the MPF of an EMG signal has been shown to reflect the action potential velocity in motor units (Hagg, 1992)

and has been suggested as a possible measurement of changes in fibre type recruitment based on the slower and faster action potential velocity observed in type I and type II fibres. Borrani et al (2001) identified a significant increase in MPF of the vastus lateralis of well-trained runners during prolonged exercise that corresponded with the $\dot{V}O_2$ slow component and suggested that this may be evidence of an increase in type II fibre recruitment as type I fibres become fatigued. Komi et al (2000) found higher MPF at higher contraction velocities when comparing elbow flexion at various speeds and inferred the use of MPF as a marker of the recruitment of type II fibres. Scheuermann et al (2001) found no change in MPF of the vastus lateralis when comparing moderate intensity to high intensity cycling at a fixed cadence close to FCC, suggesting that fibre type changes observed through MPF are only evident when the velocity of the contraction/cadence is increased. Osbourne et al (2006) compared cycling at 70% PPO in a control and type I muscle fibre depleted state achieved through 140 minutes of low intensity cycling followed by a dietary fast until the exercise trial the following day. They found MPF was significantly higher during heavy exercise when type I fibres were depleted suggesting MPF can be used to measure the activation of type II fibres during cycling exercise. Sarre et al (2005) showed the MPF of the vastus lateralis was significantly higher at FCC and +25% FCC as well as increasing over 60 minutes of 65% PPO at both these cadences, however it should be noted that MPF decreased at -43% FCC as well as not following the same pattern in several other leg muscles involved in generating force during cycling. Collectively these studies have been used to suggest MPF as a valid marker of fibre type recruitment as well as being suggestive of a fibre type recruitment shift from slow type I to fast type II fibres at higher contraction frequencies.

Few studies have employed the muscle biopsy technique to investigate the recruitment pattern of muscle fibres during cycling exercise of varying cadences, but those that have, have not been consistent in their findings. One of the first comprehensive assessments of fibre type

recruitment and cadence using the biopsy technique was Gollnick et al (1974) who performed muscle biopsies during continuous exercise at 31, 64 and 84%$\dot{V}O_2$ peak at 30-, 60-, 80- and 120- rpm. They found no significant effect of cadence on glycogen use in the two distinct fibre types, however, their method of visually rating glycogen use has since been criticised as being too subjective and not sensitive enough to possible subtle changes (Vøllestad *et al.*, 1984). Subsequent research by Ahlquist et al (1992) found a greater decrease in type II muscle fibre glycogen content following 30 minutes cycling at 50- vs. 100- rpm. There was no significant difference in the depletion of type I fibres leading the authors to conclude that the greater force production at the lower cadence led to a greater recruitment of type II fibres. However, it should be noted that participants in this study were exercising at 85% $\dot{V}O_2$peak for the 30 minutes, an intensity that previous research would suggest a cadence above 100 rpm would have been required to observe possible type II fibre recruitment (Coast, J. Richard and Welch, Hugh G., 1985). Altenburg et al (2007) showed that non-cyclist who cycled for 45 minutes at 75% $\dot{V}O_2$ peak at 90- rpm recruited both type I and type II fibres evenly throughout the 45 minutes of exercise. In similar conclusions from the results to Vøllestad et al (1984), they suggested that this finding was divergent from previous findings which suggested that type I were recruited first then and type II additional recruited as the type I fibres fatigued. They further suggested that previous research using the Periodic Acid Schiff stain method to identify fibre type recruitment, although useful, was not as sensitive as the PCr/Cr ratio method they used. Although significant research exists to suggest a possible fibre type shift at higher contraction frequencies that may explain the observed increases in oxygen consumption, heart rate and decreased efficiency, further research is required to concisely identify the role fibre type may play. It is worth noting to the best of our knowledge that no research since Ahlquist et al (1992) has sought to investigate, in a similar fashion, the role of fibre type may play in contraction frequencies even with the advance in optical density techniques.

2.15.   Post exercise response

The beneficial effects of exercise training are as a result of frequent repeated exposure to exercise which act as a potent stimulus for beneficial adaptive changes across multiple systems in the human body (Coffey and Hawley, 2007; Egan and Zierath, 2013). This longer term adaptive response as a result of the repeated stress of exercise is highly variable and involves a vast array of cellular and molecular changes across the human body that are not fully understood (Zierath and Wallberg-Henriksson, 2015). Many of the adaptive benefits of regular physical activity that reduce the risk of diseases also have an acute benefit that can be observed in the hours following a given exercise bout. However, variation exists in the literature with some studies suggesting SIE may result in similar changes to energy expenditure, substrate utilisation and insulin sensitivity in the 0-24 hours post exercise (Moniz *et al.*, 2020).

2.16.   Excess Post Exercise oxygen consumption (EPOC)

Excess post exercise oxygen consumption (EPOC) is the increased oxygen consumption, when compared to pre-exercise resting levels, that can be observed after an exercise bout of sufficient duration and intensity (Moniz *et al.*, 2020). Both the duration and the intensity of an exercise bout will influence the magnitude and duration of EPOC (Børsheim and Bahr, 2003). Although increased oxygen consumption in itself is not considered beneficial, it is accepted as a marker of the level of recovery necessary from a given bout of exercise.

Studies that have compared the effects of MICE, HIE and SIE on EPOC have produced varying results with similar (Malatesta *et al.*, 2009) and sometimes greater (Tucker *et al.*, 2016) EPOC as a result of HIE and SIE compared to MICE. Malatesta et al (2009) had 12 recreationally active ($\dot{V}O_2$peak 52ml/kg/min) male non cyclist complete MICE cycling (60mins@60%$\dot{V}O_2$peak) and HIE cycling (60sec@80%Wmax active recovery

60sec@40%Wmax) with the work between MICE and HIE matched for the mechanical work performed during the MICE bout. They found no significant difference between trials in EPOC measured up to 3 hours post exercise. Contrastingly, Matsuo et al (2012) had 10 recreationally active males ($\dot{V}O_2$peak 52 ml/kg/min) complete MICE (40mins@65%$\dot{V}O_2$peak), HIE (3x3mins@90%$\dot{V}O_2$peak 2mins active recovery 50%$\dot{V}O_2$peak) and SIE (7x30sec@120%$\dot{V}O_2$peak 15sec rest) cycling exercise. They found no difference in EPOC between MICE and HIE but significantly greater EPOC following SIE in the 3 hours post exercise period. These findings were replicated by Tucker et al (2016) who had 10 recreationally active males ($\dot{V}O_2$peak 46 ml/kg/min) complete MICE (30mins@80%HRmax), HIE (4x4min@95%HRmax 3mins recovery) and SIE (6x30secwingate 4mins active recovery@60%HRmax). They found that EPOC was significantly higher in the 3 hours post exercise following SIE when compared to both MICE and HIE conditions. Divergent findings between studies is not uncommon, however the differences in HIE protocols appear to explain the differences between research studies (Moniz *et al.*, 2020). A consistent finding in the literature relates to research that suggests SIE, when compared to HIE and MICE, produces significantly higher EPOC in the first hour post exercise (Moniz *et al.*, 2020). These results are considered particularly interesting due to the relatively short time spent exercising, be it at greater exercise intensities. Williams et al (2013) had 18 recreationally active ($\dot{V}O_2$peak 41ml/kg/min) males complete MICE (60mins@65%$\dot{V}O_2$peak) and SIE (4x30sec wingate (7.5%bw-kg) 4.5mins active recovery). When recordings were analysed in 15-minute periods only significantly increased EPOC as a result of SIE was observed in the first 30-minutes post exercise. The physiological mechanisms that explain EPOC post exercise are well understood and described and ultimately relate to the body's restoration of resting levels ATP, PCr, ph, temperature, circulation and ventilation (Laforgia *et al.*, 2006). This process is often called the "fast component" of recovery, as many of these variables return to baseline within 1- hour of

exercise cessation. It is suggested that similar mechanisms are responsible for the significantly greater EPOC relating to SIE, however further work is required to understand this phenomenon.

Compared to 1-3 hours post exercise and the so-called fast component, less is understood about the effects of MICE, HIE and SIE on metabolism up to 24-hour post exercise often called the "slow component" of recovery. However, although relatively small when compared to the 1-hour post exercise phase, in rare cases EPOC can still be present up to 24 hours post exercise. Greer et al (2015) had 10 recreationally active ($\dot{V}O_2$peak 35ml/kg/min) males perform continuous cycling exercise (43mins@40%$\dot{V}O_2$peak) and SIE (10x30sec@90%$\dot{V}O_2$peak 3mins recovery) and measured EPOC at 12 and 22 hours post exercise. They found significantly greater EPOC at both timepoints following the SIE trial when compared to the MICE trial, however it should be noted that the participants were classed as low to moderate fitness as well the low intensity of the continuous exercise trial may contribute to the significance of the results. However, Hazell et al (2012) had 8 recreationally active ($\dot{V}O_2$peak 53ml/kg/min) perform MICE (30mins@70%$\dot{V}O_2$peak) and SIE (4x30sec wingate (10%bw-kg) 4mins active recovery) and did not find a significant difference in EPOC at any time point over a 24 hour period post exercise. Skelly et al (2014) also found no significant difference in EPOC when comparing MICE (50mins@70%HRmax) and SIE (10x60sec@90%HRmax 60sec active recovery) in 8 recreationally active men ($\dot{V}O_2$peak 46 ml/kg/min). Although no significant difference was noted it was of interest that SIE produced similar EPOC although requiring significantly less time exercising to achieve this (20-mins VS 50-mins active exercise). Given that many physiological variables are elevated as a result of exercise (body temperature, blood lactate, blood pH) have returned to baseline, or near baseline levels 1- hour post exercise, it is unlikely that they play a significant role in the EPOC up to 24 hours post exercise (Laforgia *et al.*, 2006). Instead EPOC relating to SIE over a 24-hour period is thought to relate to cellular

related processes whose homeostasis is disrupted to a greater degree by interval exercise when compared to MICE (Moniz *et al.*, 2020).

Collectively the inconsistent reporting of elevated EPOC as result of HIE and SIE when compared to MICE may be as a result of differences in protocol. However, studies that have used the same protocol also have not been consistent with some studies reporting greater EPOC when compared to MICE in the 0-3 hours period (Malatesta *et al.*, 2009) while others reported no difference (Tucker *et al.*, 2016). A consistent finding that is generally accepted in the literature relates the to the elevated EPOC observed in the initial hours (0-3) post exercise from SIE when compared to MICE (Laforgia *et al.*, 2006; Matsuo *et al.*, 2012; Townsend *et al.*, 2013; Skelly *et al.*, 2014; Tucker *et al.*, 2016) .

2.17. Substrate utilisation post exercise

Exercise increases energy expenditure with both the intensity and duration influencing the rate of energy expenditure as well as the relative contribution of fat and carbohydrate oxidation to meet the energy demands of a bout of exercise (Van Loon, L. J. *et al.*, 2001). The influence of exercise intensity and duration on acute post exercise substrate utilisation are well studied with clear evidence that a single bout of exercise can alter substrate utilisation when compared to pre-exercise (Børsheim and Bahr, 2003).

2.18. Carbohydrate

Exercise has been shown to be a powerful modulator of carbohydrate metabolism in the hours post exercise. Ivy et al (1988) had 12 recreationally active males who cycled regularly ($\dot{V}O_2$peak 60ml/kg/min) perform MICE (70mins@70%$\dot{V}O_2$peak) interspersed with HIE (6x2min@88%$\dot{V}O_2$peak) with the protocol aimed at depleting glycogen levels. They found that a carbohydrate drink (2g/kg body wt) provided immediately post exercise resulted in increased glycogen restoration when compared to the same drink provided at 2-hours post

exercise. This suggests that the impact of exercise on glycogen resynthesis is time dependent, with the highest levels of glycogen resynthesis possible immediately post exercise and diminishing as time extends. However, although diminished, the findings from Mikines et al (1988) suggest increased glycogen synthase activity up to 48 hours post exercise. They had 7 untrained but active men ($\dot{V}O_2$peak 44 ml/kg/min) perform MICE (60mins@64%$\dot{V}O_2$peak) with muscle biopsies performed immediately and 48-hours post exercise. They found that although glycogen synthase activity was highest immediately post exercise it was still elevated when compared to a rest trial where no exercise was performed. The observed increase in glycogen resynthesis post exercise is directly determined by the reduction in glycogen that occurred during the exercise bout, which as previously described is related to the intensity and duration of the exercise undertaken (Hawley *et al.*, 2015). Although glucose uptake by skeletal muscle is increased post exercise, oxidation rates have been shown to be decreased (Børsheim and Bahr, 2003), highlighting that the restoration of glycogen is prioritised in the post exercise period as will be discussed in greater detail below.

2.19.   Insulin sensitivity

The action of insulin increases the uptake of glucose from the blood stream by skeletal muscle, inhibits the release of glucose from the liver and inhibits the release of FFA from adipose tissue (Kraemer *et al.*, 2002). The glucose insulin clamp technique, first developed by Defronzo et al (1979), is considered the gold standard of measuring insulin and glucose metabolism (Muniyappa *et al.*, 2008). The hyperglycemic euinsulinemic clamp technique acutely raises and maintains the elevated plasma glucose levels in order to assess beta cell sensitivity to glucose as well as measuring the amount of glucose metabolised following a hyperglycemic stimulus. The euglycemic hyperinsulinemic clamp acutely raises insulin levels and controls glucose levels by adjusting the infusion rate in order to test the whole body tissue sensitivity to insulin (Defronzo *et al.*, 1979). The sensitivity of the clamp technique has allowed for detailed

investigations into how both MICE, HIE and SIE exercise can modulate insulin and thus carbohydrate metabolism post exercise. However, it is a highly invasive, costly and time-consuming technique which has resulted in the development of less invasive methods of calculating insulin sensitivity, which have been validated against the clamp technique, and allow for the indirect measure of insulin sensitivity. These include but are not limited to the homeostatic model assessment of insulin resistance (HOMA IR) which can be calculated from a glucose and insulin value obtained from a fasting blood sample as well as the glucose and insulin response in the 2 hours following a known oral load of glucose or oral glucose tolerance test (OGTT) (Mann *et al.*, 2014).

Research utilising the various methods of measuring insulin sensitivity have consistently found that exercise can increase insulin sensitivity (Mann *et al.*, 2014). Richter et al (1989) had 6 healthy males perform 60-minutes of continuous single leg extension exercise and utilising the clamp technique showed evidence of increased insulin sensitivity in the exercised leg. Mikines et al (1988) utilised the clamp technique and demonstrated that a single bout of MICE exercise (60mins@64%$\dot{V}O_2$peak) increased insulin sensitivity up to 48-hours post exercise in 7 untrained but active men ($\dot{V}O_2$peak 44 ml/kg/min). Magkos et al (2008) demonstrated that the energy expenditure of a given bout of MICE had a direct effect on the level of insulin sensitivity observed 24-hours post exercise. They had 48 recreationally active untrained males ($\dot{V}O_2$peak 42ml/kg/min) perform MICE (30-120mins@60%$\dot{V}O_2$peak) and measured fasting glucose and insulin levels 24-hours later. They found a significant negative correlation between energy expenditure during the exercise bout and HOMA IR. Such findings suggest energy expenditure to be the key determinant of the magnitude of change in insulin sensitivity. However, research that has controlled for energy expenditure has found that higher intensity exercise increases the insulin sensitivity to a larger degree then lower intensity exercise. Kang et al (1996) found that 14 consecutive days of performing cycling exercise (50mins@70%$\dot{V}O_2$peak) increased insulin

sensitivity to a greater degree than the same energy matched exercise at a lower intensity (70mins@50%$\dot{V}O_2$peak) suggesting that the intensity at which exercise is performed will also influence the degree of insulin sensitivity observed.

Exercise at intensities <80%$\dot{V}O_2$peak result in a greater glycogen utilisation then exercise performed at lower intensities (Van Loon, L. J. *et al.*, 2001). The increase in insulin sensitivity post exercise has been strongly correlated with a decrease in glycogen levels. Bogardus et al (1983) had 13 recreationally active males (no $\dot{V}O_2$peak recorded) perform HIE (2mins@80-90%HRmax 3mins rest) until exhaustion. They performed a hyperinsulinemic euglycemic clamp 12-hours later and found a significant increase in insulin sensitivity that correlated with glycogen synthase activity. Although this acute effect is similar to MICE as previously stated, this was in exhaustive exercise consisting of on average 9 sets of 2-minute intervals that resulted in a 40% reduction in glycogen. When using more traditional protocols that are not exhaustive, as little as 2-weeks of HIT has been shown to be an effective means of increasing insulin sensitivity with studies utilising SIT exercise suggesting potentially superior increases in insulin sensitivity when comparing SIT to MICT. Hovanloo et al (2013) had 8 recreationally active males ($\dot{V}O_2$peak 34ml/kg/min) perform 6 SIE (5x30sec@7.5%bw-kg) or 6 MICE (105mins@65%$\dot{V}O_2$peak) over 2-weeks and found a greater increase in insulin sensitivity as measured by HOMA IR following SIT. However, Cocks et al (2013) who had 16 recreationally active males ($\dot{V}O_2$peak 42ml/kg/min) perform MICE (60mins@65%$\dot{V}O_2$peak) or SIE (30sec@7.5% bw-kg) for 6-weeks did not find a significant difference between training methods, suggesting the benefits of MICE on insulin sensitivity are manifested over a longer period of time then the initial increase as shown by Hovanloo et al (2013). However, Richards et al (2010) demonstrated that a single bout of SIE (4x30sec wingate (7.5%bwkg)) did not induce increased insulin sensitivity 24-hours post exercise while 6 SIE sessions (4-7X30sec(7.5%bwkg)) over 2-weeks was sufficient to increase insulin sensitivity 72-hours post

the final exercise bout. Such studies highlight that beneficial increases in insulin sensitivity can be gained even with significantly less time spent in active exercise. A meta-analysis by Jelleyman et al (2015) described how similar, if not slightly greater increases in insulin sensitivity can be made in healthy individuals from HIT when compared to MICE, particularly when supramaximal SIT style exercise is used.

Insulin sensitivity has been inversely associated with IMTG in inactive individuals (Pan *et al.*, 1995), and the IMTG lowering effect of MICE has been suggested as a possible mechanism for increased insulin sensitivity post MICE (Cartee, 2015). Interestingly the opposite can be observed in well trained endurance athletes who have been found to have increased levels of IMTG while also having increased insulin sensitivity (Amati *et al.*, 2011). As increased insulin sensitivity is positively associated with the level of glycogen depletion that a given bout of exercise induced (Bogardus *et al.*, 1983). The predominant reliance on anaerobic pathways, and therefore glycogen utilisation, observed during HIE and SIE (Gibala and Little, 2020), is thought be the primary pathways by which insulin sensitivity is increased in the acute post exercise period (Cartee, 2015).

2.20. Fat Metabolism

The effect of exercise on fat substrate utilisation post exercise is well studied, with a large body of literature existing on the topic (Lundsgaard *et al.*, 2020). It is well accepted that post exercise, the relative contribution of fat oxidation to whole body energy expenditure is increased with a corresponding decrease in relative carbohydrate oxidation rates (Gaesser and Brooks, 1984). Increased fat oxidation is classically measured by a decreased respiratory exchange ratio (RER) (ratio of carbon dioxide production to oxygen consumption) determined by indirect calorimetry and can be present for a number of hours post exercise. In a study by Henderson et al (2007) had 10 recreationally active males ($\dot{V}O2peak$ 56ml/kg/min) perform

MICE (60mins@65%$\dot{V}O_2$peak) and found a significantly decreased RER 24-hours post the exercise bout when compared to no exercise (0.78 vs 0.86). However, not all findings are in agreement. Melanson et al (2002) had 8 recreationally active males ($\dot{V}O_2$peak 45ml/kg/min) perform MICE (400kcal@70%$\dot{V}O_2$peak) and did not find an increase in fat oxidation in 24-hours post exercise. Such a finding that would be in contradiction to the majority of scientific literature may possibly be explained by methodological issues such as the use of a whole room indirect calorimetry which may not be sensitive enough for subtle changes in RER. HIE has also been shown to increase fat oxidation rates in the 24-hours post exercise. Greer et al (2015) had 10 recreationally active men ($\dot{V}O_2$peak 35ml/kg/min) perform HIE (9x30sec@90%$\dot{V}O_2$peak) and found a significantly decreased RER 24-hours post exercise when compared to no exercise demonstrating increased fat utilisation. The increase in fat oxidation in skeletal muscle during the recovery period post exercise has been shown to be regulated at several steps within muscle and ultimately allows for the increase in glucose uptake to be directed towards the resynthesis of muscle glycogen rather than being oxidised for energy (Gaesser and Brooks, 1984). Ultimately, the post exercise response to a given bout of exercise will be dependent on the intensity and duration of the exercise bout with the response predominantly associated with restoring the physiological equilibrium that existed pre-exercise.

2.21. Conclusions

It is clear from the literature that exercise has the capacity to significantly alter metabolism, with both the intensity and duration of the exercise itself being central to the metabolic response that can be observed (Bergman and Brooks, 1999; Van Loon, L. J. C. *et al.*, 2001). Not only does exercise lead to significant perturbations in metabolism during exercise itself, but it is also evident that variation away from the metabolic baseline can be observed in the hours following

exercise (Moniz *et al.*, 2020).   Although interval exercise has long been a staple of athletic training programs, the interest in this form of exercise, particularly that of a supramaximal nature, has found a resurgence in recent years, with the time saving benefits of this form of exercise, attractive to the time strapped general public. Although SIE has been shown to provide similar, and potentially greater benefits when compared to MICE when used as part of an exercise training program (Gibala and Little, 2020), questions remain in relation to the acute response when comparing these forms of exercise. From reviewing the literature, it is particularly evident that agreement does not exist when comparing SIE and MICE and their effect on energy expenditure, glucose metabolism and insulin sensitivity in the 0-24 hours post an exercise bout.

Altering contraction frequency as a means of altering exercise intensity is a relatively understudied area when compared to altering force, and the literature generally focuses on improving cycling performance of well-trained cyclists (Ansley and Cangley, 2009). However, an interesting yet unexplained phenomenon that appears to be present in both trained and untrained cyclists is related to increased oxygen consumption at a fixed power output when cycling at a high versus low cadence. Although a number of potential reasons for the observation have been suggested, it is still unclear as to why this occurs. It has been suggested that additionally type II fibre recruitment at the higher cadence may explain this increase, however the limited studies that have been performed hare not in agreement with some finding no difference in fibre type recruitment patterns (Gollnick *et al.*, 1974; Altenburg *et al.*, 2007) and others finding greater type II recruitment at lower rather than higher cadences (Ahlquist *et al.*, 1992). From reviewing the literature, it is clear that further work is required to provide additional evidence related to cadence-dependant fibre type recruitment in non-cyclists

3. Chapter III Experiment I

The effect of SIE and MICE on energy expenditure and glucose metabolism during, post (0-60 mins) and 24-hours post exercise.

Preface

As highlighted in the literature review, controversies exist relating to acute metabolic response to exercise of different intensities. Experiment I sought to create high quality data to investigate this area of exercise physiology.

3.1. Introduction

Previous research has suggested that SIT can produce similar (Gibala *et al.*, 2006) if not superior (Helgerud *et al.*, 2007) increases in $\dot{V}O_2peak$ when compared to traditional MICT. This has created renewed interest in the effect of altering exercise intensity, as such results are intriguing considering that significantly less time is spent in performing SIE. The impact of a single bout of SIE when compared to MICE is relatively less studied, with variation in the literature relating to the acute metabolic effect of SIE when compared to MICE (Sloth *et al.*, 2013). Some evidence suggests SIE may induce greater EPOC and fat oxidation in the hours post exercise (Tucker *et al.*, 2016) while others do not (Malatesta *et al.*, 2009). Additionally it has been suggested SIE will result in increased insulin sensitivity when compared to MICE (Jelleyman *et al.*, 2015), while others found no difference (Richards *et al.*, 2010). Due to this variation this study was designed to investigate the impact of SIE versus MICE on the acute metabolic response (0-24 hours) to a bout of this form of exercise.

3.2. Overview of experimental design

This study was a randomised control crossover study with 3 experimental conditions. Each condition required the participants to attend the laboratory on 2 consecutive days with at least 10 days wash out period between each condition. On the first day of each condition, participants were randomised to perform MICE (60mins@60%$\dot{V}O_2peak$), SIE (7x30seconds@130%PPO), or no exercise (control). The following day consisted of a resting metabolic rate test followed

by two muscle biopsies of the vastus lateralis pre and post a euglycemic hyperinsulinemic clamp.

3.3. Methodology

The study received ethical approval from the Dublin City University Research Ethics Committee (appendix 1). Participants were recruited for the study via a University wide email sent to all undergraduate and postgraduate student distribution lists in Dublin City University (DCU). Those who expressed interest in taking part in the study were sent a plain language statement of what the study involved, and those who still wished to partake were asked to sign an informed consent. Participants were screened using a general health questionnaire and undertook an electrocardiogram to ensure it was safe for them to partake in the study. All participants partook in recreational level physical activity 2-3 times per week. Sixteen healthy males were included to take part in the study, however 4 participants did not wish to continue due to difficulties with repeating the biopsy procedure. Twelve young males completed an incremental cycling exercise test to exhaustion before partaking in the randomised crossover trial (Table 3-1).

3.3.1.   Dual energy x-ray absorptiometry (DEXA) scan

Body fat levels were determined by a DEXA scan (DEXA, Stratos, DMS Imaging, France). Briefly, this scan takes approximately 10 minutes with the subject lying on a bench. Two x-rays with high and low photon energies are emitted and the measurable variation in the attenuation of the x-rays is caused by the variation in the density and chemical composition of fat, bone and lean tissue. The radiation exposure is low; 1.0-3.6 μSv. The level of radiation of a regular chest X-Ray, is approximately 100 μSv. The software will calculate fat mass, fat free mass as well as bone mass for each participant. The percentage fat is presented along with the other physical characteristics in Table 3-1.

Table 3-1: Summary of Physical Characteristics

Age (yrs)	22.1±3.5
Height (m)	183.6±6.9
Body mass (kg)	81.4±8.1
BMI ($kg \cdot m^{-2}$)	24±1.6
Body fat (%)	15.3±1.9
$\dot{V}O_2$peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	48.3±3.7
Peak power output (Watts)	306±44

Data presented as mean ± standard deviation.

3.3.2. $\dot{V}O_2$ Peak Test

The incremental tests were performed on an electronically braked cycle ergometer (E100 P/K COSMED Srl, Middlesex, UK) with the test starting at 70 W and increasing by 30 W every 3-mins until exhaustion. $\dot{V}O_2$peak was determined by indirect calorimetry using a breath-by-breath metabolic system (Quark CPET, Cosmed Srl, Rome, Italy). The metabolic system was calibrated following the manufacturers guidelines within 10 minutes of the initiation of the exercise test. Briefly, a flow loop calibration was performed using a 3 liter syringe in order to calibrate the flow sensor for rate and volume of air flow during the test. A air and gas calibration was performed using a reference tank of known gas percentage. Heart rate was recorded throughout using a Garmin HR Bluetooth Chest Strap (Garmin Ltd, Southampton, UK). Rate of perceived exertion (RPE) was recorded at the end of each 3-minute stage. A test was deemed maximal if three of the following criteria were satisfied: (i) volitional exhaustion by being unable to maintain a cycling cadence above 60-rpm; (ii) a levelling off in oxygen consumption

(<200ml.min-1) with increasing workload; (iii) a respiratory exchange ratio >1.1; (iv) an RPE >18. PPO was calculated with the following equation (American College of Sports, 2013).

$$PPO = P+(T/(SL \times W)) \qquad \text{(Equation 1)}$$

where P is the power output from the last 3-min stage that was fully completed; T is the time in seconds completed in the stage when exhaustion occurred; SL is the duration of each stage in seconds and W is the increment in Watts for each stage (Lutttikohlt *et al.*, 2006).

3.3.3. Physical activity and Dietary Control

Participants were asked to refrain from intense exercise 72-hours before each trial and any exercise 48-hours before each trial. Participants were also asked to refrain from caffeine or alcohol for 24-hours before each trial. Participants were asked to maintain a similar sleep routine the night before each trial. Participants were asked to complete a food diary for the day before the first trial and to repeat this the day before subsequent trials. Participants were also asked to record a food diary after leaving the lab following the day of each condition and to repeat this for the subsequent conditions.

3.3.4. Day 1

Participants were asked to attend the laboratory for 7am following an overnight fast and a non-active commute to DCU. Participants height and body mass were taken before lying in a bed to rest for an hour.

3.3.5. Resting Metabolic Rate (RMR)

The metabolic system was calibrated following the manufacturers guidelines within 10 minutes of the initiation of the resting metabolic rate test. A plastic canopy was placed over their head while they remained in a lying position. The canopy consisted of an inlet valve that allowed room air to enter the canopy while an outlet valve was connected to the metabolic analyser

(Quark RMR, Cosmed Srl, Rome, Italy) via a plastic tube. The tube allowed the analyser to draw fresh air into the canopy while extracting the expired air within the canopy to be analysed. The Quark metabolic cart analysed the expired air for oxygen and carbon dioxide and calculated how much oxygen is consumed and carbon dioxide is produced by the participant at rest. These values were then used to calculate energy expenditure and substrate utilisation using an equation (Consolazio, 1963) (see section 3.3.14 below).

3.3.6. Continuous Glucose monitoring (CGM)

Following the RMR, a CGM sensor probe (DEXCOM with G4 Platinum Sensor, DEXCOM, San Diago, CA, USA) was inserted into the subcutaneous fat tissue on the abdomen of each participant (see figure 3-1). Once inserted, the CGM recorded interstitial fluid glucose concentrations every 5-minutes until the device was removed at the end of the clamp the following day (worn for 26-28 hours). Following the initial calibration when the device was turned on, the DEXCOM was calibrated every 4-hours to capillary blood glucose levels collected following a finger stick and measured on a portable handheld glucometer (OneTouch® Vita™ Blood Glucose Monitoring System, LifeScan, Inc., Milpitas, CA). While the participant was in the lab, the DEXCOM was calibrated by research personnel. Each participant was given verbal and written instructions on how to calibrate the DEXCOM while at home. The data was downloaded from the DEXCOM following each trial and stored for later analysis.

Figure 3-1 Image of CGM probe and recording device.

3.3.7. Blood draw

A cannula was inserted in an antecubital fossa vein from which blood samples were to be taken, pre, post and 1-hour post exercise. Serum samples were left to sit at room temperature for 20-minutes before being centrifuged. Samples containing EDTA and fluoride were centrifuged directly after being drawn. Both serum and plasma samples were aliquoted before being stored at -80C. The cannula was removed before participants left the laboratory on the first day and a new one was inserted the following morning following the RMR. On the first day, a blood sample was taken before participants warmed up at 40% $\dot{V}O_2$peak for 10-minutes.

3.3.8. Experimental Conditions

For the MICE trial, participants cycled @60%$\dot{V}O_2$peak for 60-minutes while maintaining a contraction frequency of 70-80rpm (Figure 3-1). For the SIE trial, participants completed 7x30-sec@130%PPO while not exceeding 130-rpm with 4.5 minutes rest between intervals (Figure 3-2). Oxygen consumption and carbon dioxide production was determined by indirect calorimetry using a breath-by-breath metabolic system (Quark CPET, Cosmed Srl, Rome, Italy). During the MICE trial, calorimetry was recorded at 6-18, 20-32, 34-46 and 48-60

minutes during the trial. A gas calibration was performed during the break periods to increase the accuracy of the metabolic analyser. During the SIE trial, calorimetry was recorded throughout. During the MICE trial, the rate of perceived exertion RPE was recorded at 10-minute intervals while for the SIE trial it was collected after each interval. A blood sample was collected immediately post exercise and 1-hour post exercise. Once the exercise was complete, participants were asked to return to lying in the bed where another RMR test was conducted. Participants left the laboratory following the RMR with all instructions needed until the following morning.
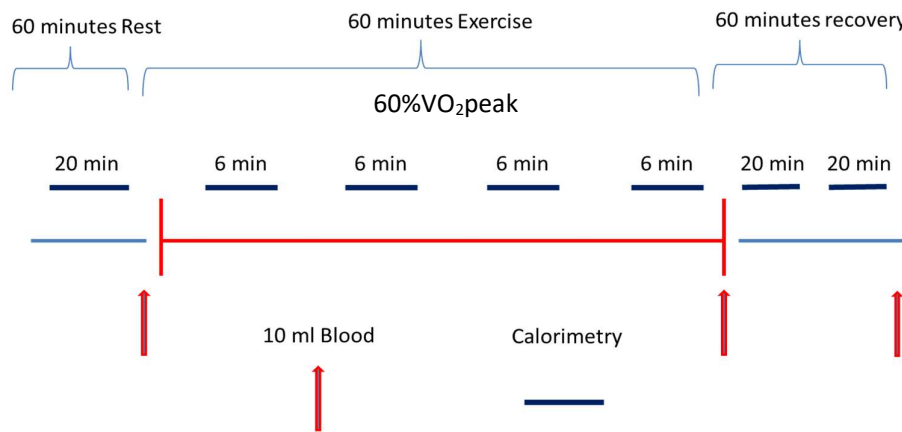
Figure 3-2 Schematic representation of MICE exercise trial
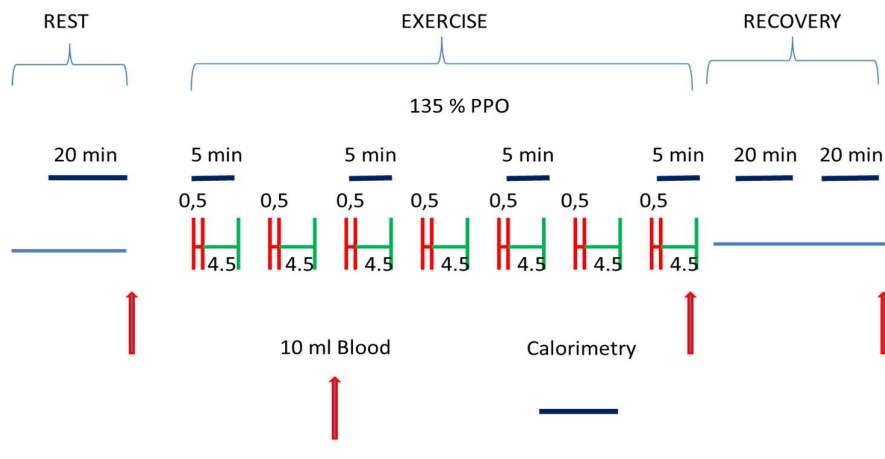
Figure 3-3 Schematic representation of SIE trial

3.3.9. Day 2

On the following morning, participants attended the laboratory following an overnight fast and an inactive commute to the University, replicating the previous morning. Their height and body mass were recorded before a RMR test was performed, after which a cannula was inserted, and a blood sample taken.

3.3.10. Muscle Biopsy

A muscle biopsy was taken from the vastus lateralis by experienced medically trained personnel using a percutaneous muscle biopsy needle (Bergstrom, 1962). The site of the biopsy was marked as being 2/3rds of the way down the midline ranging from the anterior spina illicia, superior to the lateral side of the patella and in the belly of the muscle. Once identified the site was cleaned with antiseptic solution (Videne) and alcohol. The site was then anaesthetised with 1% Lidocaine hydrochloride (Braun, Melsungen AG). The lidocaine was allowed time to act (5-minutes) after which a small (0.5-1cm) incision was made with a scalpel after which a biopsy needle was used to remove small pieces (100-200 mg) of muscle. The tissue was dabbed with non fibre gauze to remove blood before being snap frozen. Once sufficient muscle was obtained, the incision was closed using steri-strips and a compression bandage applied.

3.3.11. Euglycemic Hyperinsulinemic Clamp

Following the biopsy, a 20-minute period elapsed where 3 blood samples were taken at 0-, 10- and 20-minutes. During this period, a polyethylene dual port cannula was placed in the antecubital vein of the left arm through which glucose and insulin could be infused. A second cannula was placed in a vein on the back of the right hand in a retrograde fashion. The right hand was placed in a warm glove and heated to 55°C. A 5ml blood sample was taken at -30, -20 and -10 minutes before the start of the clamp. To commence the clamp, insulin infusion began at 45 ml/h and reduced every minute for the first 10-minutes to reach a rate of 15ml/h,

after which it did not change. The glucose infusion rate was changed during the next 120-minutes depending on the participant's blood serum glucose levels, which were monitored every 5-minutes. During the last 20-minute period of the clamp, blood draws were made in similar fashion to the 20-minutes before the start of the clamp. Once the clamp had finished, a second muscle biopsy was performed on the opposite leg to the first, after which the participant received a meal and was monitored for the subsequent hours to ensure non-adverse effects occurred as a result of the insulin infusion or muscle biopsies. An image depicting a participant during a clamp procedure can be seen in figure 3-4. A schematic of the clamp technique and measure time points mentioned can be viewed in figure 3-5.

Figure 3-4 Image of participant during clamp procedure.
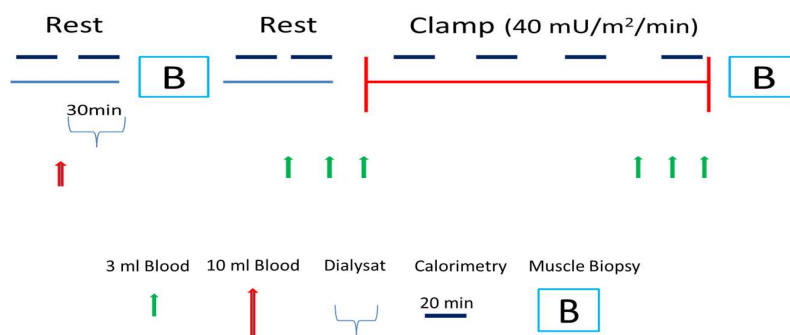
Figure 3-5 Schematic of euglycemic hyperinsulinemic clamp.

3.3.12. Glucose analysis

Serum glucose was measured using an automated analyser (YSI 2300 Stat Plus, Yellow Springs Instruments, Ohio, USA)

3.3.13. Insulin analysis

Serum insulin was quantified using an immunoassay method on the Cobas R 8000 modular analyser (module e602, Roche Diagnostics, Indianapolis, USA).

3.3.14. Glycogen analysis

Muscle glycogen levels were determined in 10mg (wet weight) homogenised in water using a mechanical handheld homogeniser which mixed the sample for approximately 15-seconds. The homogenizer was washed between samples first in ethanol followed by cold water to avoid overheating of the device tip. The samples were then boiled for 5-minutes at 100C in a water bath followed by centrifugation at 13,000 x g to remove insoluble material. The supernatant was then extracted, and glycogen was measured using an endpoint colorimetric assay (Cat# MAK016: Sigma Aldrich, St Louis, MO, USA).

3.3.15. Data Analysis

Energy expenditure: The rate of energy expenditure was estimated from oxygen consumption and carbon dioxide data obtained by indirect calorimetry (Equation 2) using the Consolazio equation (Consolazio and Pecora, 1963). The rates of non-protein carbohydrate (Equation 3) and fat (Equation 4) oxidation were also calculated from the same source.

$3.78 * \dot{V}O_2$ (L.min-1)$+1.16 * VCO_2$ (L.min-1)   (Equation 2)

$4.115 * VCO_2$ (L.min-1)$-2.909 * \dot{V}O_2$ (L.min-1))   (Equation 3)

$1.689 * \dot{V}O_2$ (L.min-1))$-1.689 * VCO_2$ (L.min-1))   (Equation 4)

3.3.16. Statistical Analysis

All data was analysed using SPSS (IBM SPSS Statistics for windows version 26, Armonk NY) and GraphPad Prism (Version 4.7 for Windows, GraphPad Software, San Diego, California, USA) and expressed as mean ± standard deviation. A Shapiro-Wilk test was used to determine the normality of the data. A paired samples t-test was used to compare the mechanical work performed between exercise trials. A one-way ANOVA with pairwise comparison was used to determine differences in the effect of each trial on energy expenditure, CGM, glycogen and GIR and a Student Newman-Keuls post-hoc test was used to differentiate between trials and time when significant differences were detected. A two-way (trial x time) repeated-measures ANOVA was used determine differences in substrate utilisation and energy expenditure and a Bonferroni post-hoc test was used to differentiate between trials and time when significant differences were detected. The alpha level for statistical significance was set at $\alpha = 0.05$.

3.4. Results

The mechanical work performed in the SIE and MICE trials is presented in Figure 3-4. The work performed in the MICE trial was significantly greater than the SIE trials (567 ±83.6 v 85.6 ± 12 kj p<.001) (Figure 3-4).
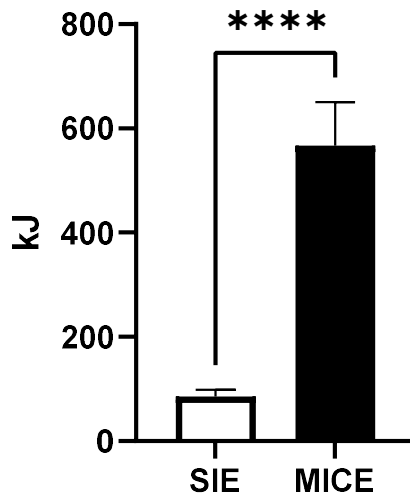
Figure 3-6 Work performed during exercise. Data presented as mean ± standard deviation. ****
denotes significance p<.001.

3.4.1. Substrate utilisation

Indirect calorimetry allowed for the calculation of substrate utilisation using the Consolazio equation (Consolazio and Pecora, 1963) at 3 time points for each trial, pre, 30- and 60- minutes post the exercise trial. Carbohydrate utilisation rates were significantly decreased at 30-minutes post SIE and although increased at 60-minutes remained significantly decreased when compared to pre-exercise (0.14±0.03 – 0.03±0.03 – 0.07±0.05 p<0.05). Fat oxidation rates were significantly elevated at 30-minutes post both exercise conditions when compared to the control trial (Con30-MICE30 = 0.08±0.02 – 0.12±0.02 p<0.01, Con30-SIE30 = 0.08±0.02 - 0.19±0.04 p=<0.001). However, significant within-trial differences were only observed following SIE (Pre-30 = 0.1±0.02 – 0.2±0.04 p=<0.001, 30-60= 0.2±0.04 - 0.14±0.03 p=<0.005). Additionally the increase in fat oxidation at 30minutes post was significantly higher following SIE when compared to MICE (0.2±0.04 -0.12±0.02 p=<0.001).
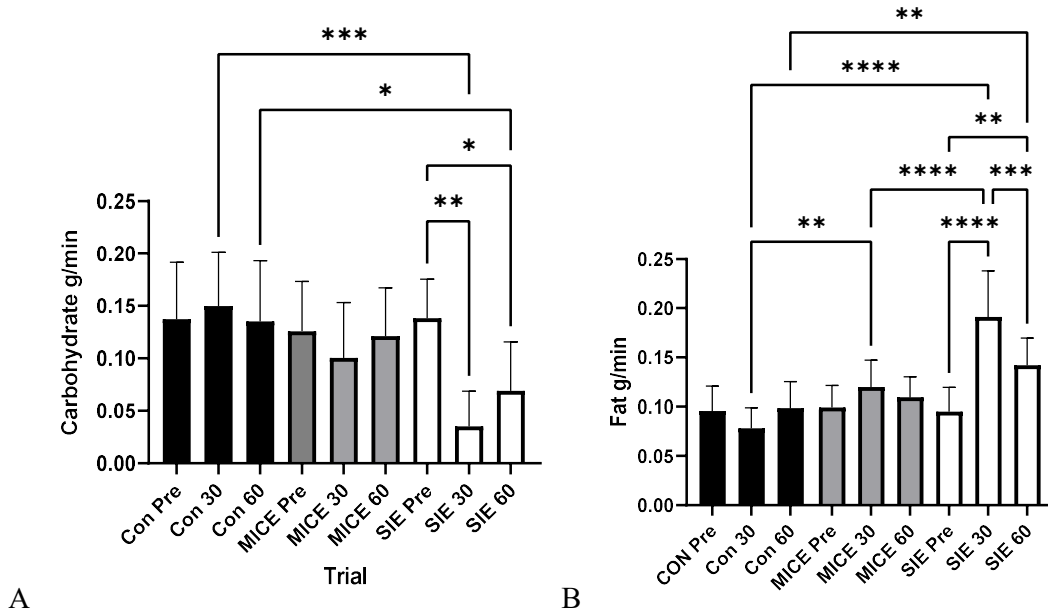
Figure 3-7 Carbohydrate (A) and fat (B) utilisation rates pre, 30-minutes and 60-minutes post each experiment. Data presented as mean ± standard deviation. * denotes significance p<.05, ** denotes significance p<.01, *** denotes significance p<.005, **** denotes significance p<.001.

3.4.2. Energy expenditure

Indirect calorimetry allowed for the calculation of energy expenditure at 3 time points for each trial, pre, 30- and 60-minutes post SIE, MICE and a control trial (Figure 3-6). using the Consolazio equation (Consolazio and Pecora, 1963). Energy expenditure was significantly elevated at 30-and 60-minutes post SIE (Figure 3-6) while no significant differences were observed post MICE.
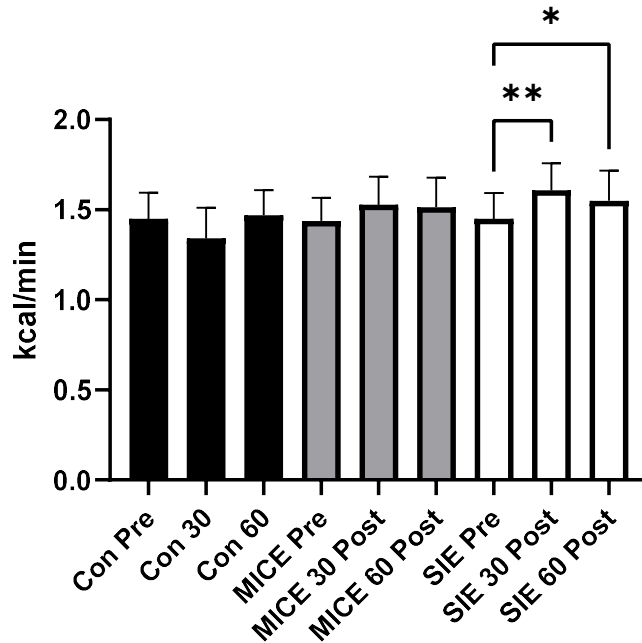
Figure 3-8 Energy expenditure pre, 30- and 60-minutes post SIE and MICE. Data presented as mean ± standard deviation. * denotes significance p<.05, ** denotes significance p<.005.

3.4.3. Glycogen

Resting muscle glycogen levels as assessed in muscle biopsy samples taken pre and post the euglycemic hyperinsulinemic clamp 24-hours post SIE, MICE and a control trial (Figure 3-7). Glycogen was significantly lower 24-hours post SIE and MICE when compared to the control trial (94.8±23.9 vs 108.2±31vs 124.5±9 mmol/kgWT p=<.05,). The pre to post clamp increase in muscle glycogen was also significantly greater following SIE when compared to MICE and control trials (45.9±8 v 27.2±10 v 18.1±2 mmol/kg WT P=<.001).
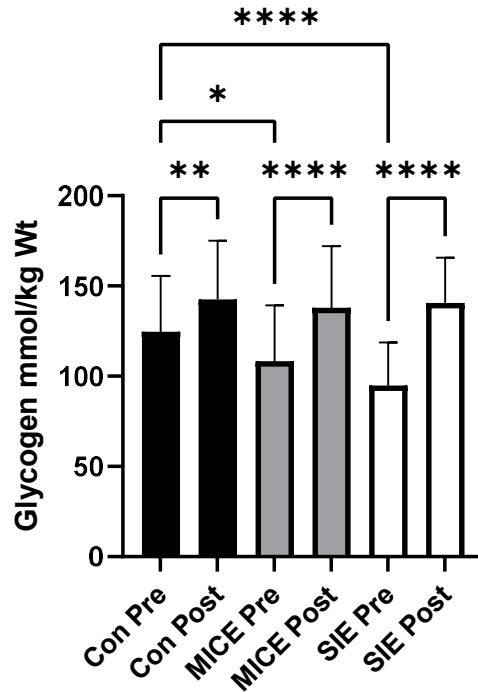
Figure 3-9 Pre clamp resting glycogen levels 24 hours post exercise. Change in glycogen post clamp. Data presented as mean ± standard deviation. * denotes significance p<.05, ** denotes significance p=<.01, *** denotes significance p<.005, **** denotes significance p=<.001.

3.4.4. Insulin sensitivity

Insulin sensitivity as assessed using a euglycemic hyperinsulinemic clamp 24-hrs post SIE, MICE and a control trial (Figure 3-8). Glucose infusion rate was significantly higher 24 hrs post SIE when compared to MICE and control trials (0.281±.01 v 0.25±.01 v 0.23±.01 mg/min/kg p=<.001) .
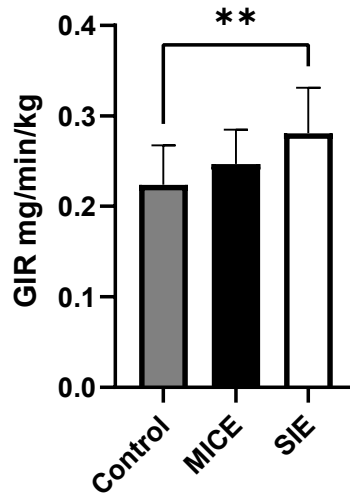
Figure 3-10 Glucose infusion rate during the steady state period of the euglycemic hyperinsulinemic clamp relative to body mass. Data presented as mean ± standard deviation. ** denotes significance p=<.01.

3.4.5. Continuous Glucose Monitoring (CGM)

Interstitial glucose concentrations as measured using CGM during (Figure 3-9 A) and 60-minutes post Figure 3-9B SIE, MICE and a control trial There was a significant difference during exercise in the interstitial plasma glucose levels between both the MICE and SIE trials and Control trial as measured by Area under the curve from a CGM system (AUC CGM) (35.7±0.8 v 39±2.1 v 40.6±2.1 mmol/L p<.001) (Figure 3-9 A). Interstitial plasma glucose levels were significantly lower during the 1-hour post exercise period when comparing the MICE trial to both the SIE and control trials respectively (46.1±2 v 54.6±2.1 v 55.7±2.1 mmol/L, p=<.001) (Figure 3-9B). No significant differences in Nocturnal AUC (12pm-6am) CGM values were observed between SIE, MICE and the control trial (383±24.6 v 393.6±17.6 v 405.8±14.9 mmol/L).
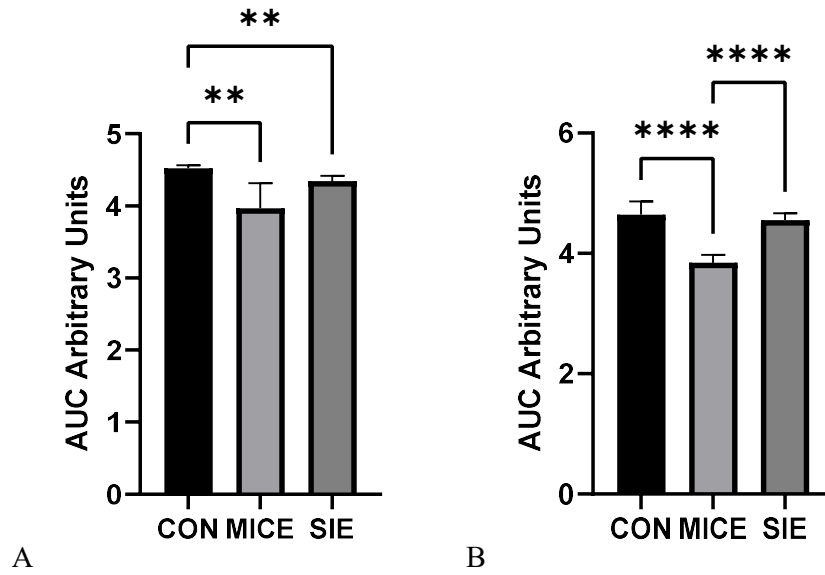
Figure 3-11 AUC CGM during exercise (A) and the 60 minutes post exercise (B). Arbitrary Units presented to represent Area under the glucose curve. Data presented as mean ± standard deviation. * denotes significance p<.05, ** denotes significance p<.01, *** denotes significance p<.005, **** denotes significance p<.001.

3.5. Discussion

This experiment sought to compare the effects of SIE and MICE on metabolism during and up to 24-hours post exercise with a focus on energy expenditure, glucose metabolism and insulin sensitivity. Increased energy expenditure was observed following SIE at 30- and 60-minutes but not at 24-hours post exercise. By performing measurements of indirect calorimetry pre and post exercise in experiment I, it was shown that SIE has the capacity to significantly increase energy expenditure up to 60-minutes post exercise when compared to MICE, a finding that is in agreement with literature relating to the impact of SIE versus MICE on excess post exercise oxygen consumption (EPOC) (Matsuo *et al.*, 2012; Tucker *et al.*, 2016; Moniz *et al.*, 2020). Previous research suggests that SIE may increase EPOC up to 24 hours when compared to MICE, however this may only to be present in less well trained individuals as shown by Greer et al (2015) ($\dot{V}O_2$peak 35ml/kg/min). The participants in this experiment were recreationally active males with a much higher $\dot{V}O_2$peak then would be observed in an untrained individual ($\dot{V}O_2$peak 48 ml/kg/min), and may explain the lack of a significant difference in EPOC observed at the 24 hour time point.

Significant differences in interstitial glucose during exercise were observed in this experiment, with both SIE and MICE resulting in a decrease in glucose concentrations when compared to the control trial. Additionally the decrease was significantly greater during MICE when compared to SIE. In healthy individuals, exercise has the potential to decrease blood glucose concentrations (Erickson *et al.*, 2017). Evidence also suggests that vigorous exercise of a supramaximal nature, has the potential to increase blood glucose in healthy individuals which is suggested to be as a result of increased hepatic glucose release stimulated by counter regulatory hormones during the exercise bout (Kjaer *et al.,* 1990). It is worth noting that research suggests the accuracy of CGM devices is reduced during exercise, with changes in temperature, pH and blood flow suggested as potential reasons for the reduced accuracy

(Muñoz Fabra *et al.*, 2021). No further differences were observed in the remaining 24-hour period. This suggests that the glucose uptake by the working muscle during MICE was not matched by hepatic glucose release, while during SIE, either glucose uptake was not increased to the same level, or hepatic glucose release was increased. A glucose lowering effect of exercise up to 24 hours post exercise has previously been reported when assess using CGM in healthy individuals (Dubose *et al.*, 2021) using a larger sample size (N=153). The relative smaller sample size as well as lack of specific control on carbohydrate intake post exercise may have contributed to the lack of statistical difference observed in this experiment.

A principal finding from this experiment was that insulin stimulated glucose disposal was significantly higher 24-hours post SIE when compared to MICE and the control trial. To the best of my knowledge, this is the first time this has been reported in healthy recreationally active males using a hyperinsulinemic euglycemic clamp. Previous research has suggested that insulin sensitivity can be significantly increased as a result of SIE when compared to MICE following two weeks of training (Hovanloo et al., 2013). Additionally Richards et al (2010) demonstrated that 6 SIE sessions (4-7X30sec(7.5%bw(kg))) over 2-weeks was sufficient to increase insulin sensitivity 72-hours post the final exercise bout, however the initial session (4x30sec(7.5%bw(kg))) was not sufficient to induce a change in insulin sensitivity. This suggests that a threshold exists between 4- and 7- repetitions (as used in experiment I) which provides sufficient stimulus to induce change in insulin sensitivity that are superior to MICE 24-hours post exercise. The cost and invasive nature of the clamp technique has resulted in others interested in measuring insulin sensitivity using less sensitive methods. Metcalfe et al (2016) found no change in insulin sensitivity in healthy individuals 16-hours post SIE when compared to MICE utilising an oral glucose tolerance test. However this is a less sensitive test for changes in insulin action and is typically adopted to assess glucose tolerance in individuals where poor glucose control is suspected, such as those with type 2 diabetes (Little and Francois,

2014). The increase in glycogen utilisation rates during SIE, when compared to MICE, has been suggested as a possible mechanism by which SIE produces greater increases in insulin sensitivity (Gibala and Little, 2020). The results from his experiment suggested glycogen levels were significantly reduced 24-hours post exercise for SIE vs control, MICE vs control but not SIE vs MICE. Although the changes in glycogen levels observed in experiment I may have instigated the significantly different changes in insulin sensitivity, we did not find a correlation between the change in glycogen and change in insulin sensitivity.

Collectively, our findings provide strong evidence for the adoption of SIE as part of exercise programs for individuals who list lack of time as a barrier to meeting the recommended amount of physical activity. Additionally, our results would suggest SIE may be particularly beneficial to individuals who have developed insulin resistance and would benefit from an exercise induced increase in insulin sensitivity. Although noteworthy, this results must be viewed in the context of being produced in laboratory based exercise sessions which are not applicable to a real world exercise conditions. As noted by others (Gray *et al.*, 2016), the evidence supporting the benefits of HIT is well defined, however this research has typically been performed in laboratory settings which limits its application in public health guideline. Training studies that support the similar benefit HIT may provide compared to MICT, exist up to 12 weeks, with limited research establishing the long term differences that might exist. Additionally, the intense nature of SIT exercise may be perceived as to hard for sedentary individuals not familiar with regular exercise (Hardcastle *et al.*, 2014), although it is not common practice to have sedentary individuals perform solely SIT. Evidence exists that exercise at 80% $\dot{V}O_2$max results in higher psychological distress (Blanchard *et al.*, 2001) and displeasure (Hall *et al.*, 2002) when compared to exercise at 50% $\dot{V}O_2$max. However, others have reported similar enjoyment when completing MICE, HIE and SIE as well as a preference to complete HIT over MICT and SIT (Jung *et al.*, 2015). Although strong evidence exists as to the time efficiency potential of

HIT and SIT, randomised control trials, with greater participant numbers and of longer duration, are required to determine the long term health outcomes as well as how it impacts key psychological constructs that influence adherence to regular physical activity (Biddle and Batterham, 2015).

In conclusion, these findings suggest that a SIE has the potential to significantly increase energy expenditure up to 60 minutes post exercise and insulin sensitivity up to 24 hours post exercise when compared to MICE. The increased insulin sensitivity is of note as our participants were healthy individuals with normal insulin sensitivity as well as being accustomed to regular physical activity. SIE may provide a time efficient means of increasing insulin sensitivity acutely for individuals at risk, or who have insulin resistance.

4. Chapter IV Experiment II

The impact of muscle contraction frequency on oxygen consumption during maximal and submaximal exercise.

Preface

In experiment I, both contraction rate and % PPO were manipulated during SIE exercise and to a lesser degree during MICE. Experiment II will focus on contraction rate during both incremental and continuous cycling exercise.

4.1. Introduction

The impact of contraction frequency on the metabolic cost of exercise is relatively understudied when compared to the impact of exercise intensity or duration. Previous research has typically focused on cycling exercise, using well trained cyclists, with the aim of increasing cycling performance (Hansen and Sjogaard, 2007). Findings from such studies are not directly translatable to untrained populations due to training adaptations that trained cyclists display when compared to non-cyclists (Zorgati *et al.*, 2015). Additionally, previous research has typically adopted incremental exercise to exhaustion (Coast *et al.*, 1986; Zoladz *et al.*, 2000) or submaximal exercise of short duration (3-10 minutes) when performing experiments (Coast and Welch, 1985; Foss and Hallen, 2004; Brennan *et al.*, 2019). As such, it is unclear in the literature what impact contraction frequency has on the metabolic cost of cycling in untrained individuals.

4.2. Experimental design

This study was a randomised crossover study with 2 sets of experimental conditions with 7-10 days between experiments. On the first two visits, participants completed an incremental exercise test to exhaustion on a cycle ergometer while maintaining a cadence of either 65- or 95-rpm, with the order of trials randomly assigned. On the third and fourth visits, participants were randomised to perform MICE (60mins@55%PPO) at 65- and 95-rpm.

4.3. Methodology

The study received ethical approval from the Dublin City University Research Ethics Committee (appendix 2). Participants were recruited for the study via a University wide email sent to all undergraduate and postgraduate student distribution lists in Dublin City University. Those who expressed interest in taking part in the study were sent a plain language statement of what the study involved and those who still wished to take part were asked to sign an informed consent. Participants were screened using a general health questionnaire and undertook an electrocardiogram to ensure it was safe for them to take part in the study. Nineteen recreationally active healthy young men who did not follow a structured exercise plan were recruited to take part in the study. Participants were excluded if they cycled regularly (> 2 times per week or >20km per week), followed a structured exercise plan or had a peak oxygen consumption ($\dot{V}O_2$peak) less than 40 ml/kg/min or greater than 55 ml/kg/min.

A familiarisation session was provided to become accustomed to the environment, equipment, and testing procedures prior to the first exercise visit. Prior to completing the first incremental exercise test, participant's height without shoes was measured to the nearest 0.001m using a stadiometer (Seca Leicester stadiometer, Seca Vogel, Hamburg, Germany). Body mass in light shorts and a t-shirt was measured to the nearest 0.1kg using a digital weight scale (Seca 875, Seca Vogel, Hamburg, Germany). Each participant's % bodyfat was determined using a DEXA scan (DEXA, Stratos, DMS Imaging, France) (see section 3.3.1).

Table 4-1   Physical Characteristics

Age (yrs)	22..2±1.4
Height (m)	181±0.07
Weight (kg)	76.9±9.2
BMI ($kg \cdot m^{-2}$)	23.5±1.6
Body fat (%)	20.6±3.4

Data presented as mean ± standard deviation.

The incremental exercise tests as well as the submaximal exercise trials were performed at the same time of day with 7-10 days elapsing between trials. Participants were asked to refrain from caffeine, alcohol, and strenuous exercise for 24-hrs before each trial. In addition, they recorded their food intake for 24 hours before the first trial and were asked to follow a similar nutrient intake pattern for subsequent trials.

4.3.1. $\dot{V}O_2$ Peak Test

The incremental exercise tests to exhaustion were performed on an electronically braked cycle ergometer (E100 P/K COSMED Srl, Middlesex, UK) with the test starting at 70 W and increasing by 30 W every 3 minutes until volitional exhaustion. Participants wore the same footwear for each trial and were not strapped to the pedal. Measurements were taken to ensure the position of the foot and height of the saddle were consistent in each trial. Participants maintained a pedalling rate of 65- or 95-rpm during the incremental tests. $\dot{V}O_2$peak was determined by indirect calorimetry using a breath-by-breath metabolic system (Quark CPET, Cosmed Srl, Rome, Italy). Blood lactate was measured in whole blood obtained from the ear lobe at baseline and within 30 seconds of the end of the test using a handheld lactate analyser (Lactate Pro 2, LT-1730, Carlton, NSW, Australia). Heart rate was recorded throughout using

a Garmin HR Bluetooth Chest Strap (Garmin Ltd, Southampton, UK). Rate of perceived exertion (RPE) was recorded at the end of each 3 minute stage using the BORG RPE scale (Borg, 1970). A test was deemed maximal if three of the following criteria were satisfied: (i) volitional exhaustion by being unable to maintain the cycling cadence; (ii) a levelling off in $\dot{V}O_2$ consumption (<200ml.min-1) with increasing workload; (iii) a respiratory exchange ratio >1.1; (iv) post-test lactate (earlobe measurement) >8 mmol.L-1; (v) an RPE >18 (Borg scale 6-20) (American College of Sports, 2013). PPO was calculated with the following equation

$$PPO = P+(T/(SL))×W \qquad \text{(Equation 1)}$$

where P is the power output from the last 3-min stage that was fully completed; T is the time in seconds completed in the stage when exhaustion occurred; SL is the duration of each stage in seconds and W is the increment in Watts for each stage (Lutttikohlt *et al.*, 2006).

4.3.2. Submaximal exercise trials

On the morning of each submaximal exercise trial, participants reported to the lab following an overnight fast and cycled at either 65- or 95-rpm for 60mins@55%PPO. The cycling cadence was displayed in front of the participant and was constantly monitored by the research team to ensure it remained ±3 rpm of the target value. A surface electromyography (sEMG) sensor was placed on the vastus lateralis of the dominant leg (SENIAM, Enschede, The Netherlands). The skin surface was shaved, light abrasion tape was used to remove dead skin cells and the site was cleaned with alcohol wipes. Once dry, the sEMG sensor was placed on the belly of the muscle two-thirds of the distance between the anterior spina iliaca superior to the lateral side of the patella in the direction of the muscle fibres and secured with adhesion tape. sEMG signals were recorded for 1-min every 20-mins during the trials (Delsys Trigno Wireless EMG System, Boston, MA, USA). Indirect calorimetry was recorded and averaged over 6-minutes to measure oxygen consumption and carbon dioxide production at 20-, 40- and

60-minute time points. Heart rate was measured continuously and blood lactate as well as RPE were recorded every 10 minutes.

4.3.3. Data analysis

Energy expenditure: The rate of energy expenditure was estimated from oxygen consumption and carbon dioxide data obtained by indirect calorimetry (Equation 2) using the Consolazio equation (Consolazio and Pecora, 1963). The rates of non-protein carbohydrate (Equation 3) and fat (Equation 4) oxidation were also calculated from the same source.

$3.78*\dot{V}O_2$ (L.min-1)+$1.16*VCO_2$ (L.min-1)   (Equation 2)

$4.115*VCO_2$ (L.min-1)-$2.909*\dot{V}O_2$ (L.min-1))   (Equation 3)

$1.689*\dot{V}O_2$ (L.min-1))-$1.689*VCO_2$ (L.min-1))   (Equation 4)

Gross efficiency was calculated as the power output (watts) relative to the energy expended during the 1-hr submaximal exercise trials. The Wattage at 55%PPO was converted to kilocalories and divided by the energy expended, as measured by indirect calorimetry, and expressed as a percentage efficiency.

Gross efficiency (%) = (Watts x 0.000238845897 x60)/(kcal/min)   (Equation 5)

Torque: An electrically braked cycle ergometer keeps the work rate (watts) constant by varying the force required in relation to the pedalling frequency. The angular velocity was calculated taking the revolutions per minute (RPM) into account (Equation 6). At 65- and 95-rpm the torque (force that causes rotation) will differ. In order to calculate Torque (τ), measured in Newton-meters (Nm), we used the following equation (Equation 7) where P is power (Watts) and ω is angular velocity (radians).

Angular velocity (radians) $\omega=2\pi RPM/60$ (Equation 6)

Torque (Nm) $\tau=P/\omega$ (Equation 7)

sEMG analysis was performed using the Delsys EMGworks Analysis software version 4.3.1 (Boston, MA, USA). The raw sEMG data was sampled at a rate of 1296Hz for 1-min after 20-, 40- and 60-minutes of exercise. Each sEMG recording was assessed for signal quality using peak-to-peak values during the non-contraction phase of the recording with an inclusion criteria of<25 µV. The root mean square (RMS) of the sEMG signal was then calculated and subsequently integrated to give an estimation of 'total' muscle activation (iEMG) over the 60-second period. This method allows for the RMS calculation of each contraction to be integrated and represented as an average RMS for the 60 second recording. The Mean Power Frequency (MPF) was calculated from the power spectral density function using a Fourier transformation to assess the rate of neural drive.

4.3.4. Statistical Analysis

All data was analysed using GraphPad Prism (Version 4.7 for Windows, GraphPad Software, San Diego, California, USA) and expressed as mean ± standard deviation. A Shapiro -Wilk test was used to determine the normality of the data. A paired samples t-test was used to compare $\dot{V}O_2$peak, PPO, heart rate and lactate max following the incremental tests at 65- and 95-rpm. A two-way (trial x time) repeated-measures ANOVA was used to compare the submaximal trials at 20-, 40- and 60-minutes and a Student Newman-Keuls post-hoc test was used to differentiate between trials and time when significant differences were detected. The alpha level for statistical significance was set at $\alpha = 0.05$.

4.4. Results

4.4.1. $\dot{V}O_2$peak tests at 65- and 95- rpm

Peak responses as measured at the point of exhaustion during the incremental exercise test performed at 65- and 95-rpm are presented in Table 4-2. Additionally, the average oxygen consumption for the final minute of each stage is presented in Figure 4-1. There was a linear increase in oxygen consumption at each stage of the 65- and 95-rpm tests with a significant difference between tests at each increment including $\dot{V}O_2$peak ($p<0.001$). There was a small but significant difference in PPO ($p<0.001$) and heart rate max ($p<0.001$), but no differences between trials in peak blood lactate levels or the peak RER.

Table 4-2. Peak responses to maximal exercise test performed at 65- vs 95-rpm

$\dot{V}O_2$peak tests	65-rpm	95-rpm
$\dot{V}O_2$peak (mL·kg^{-1}·min^{-1})	42.6±3.4	44.3±3.6*
RER at $\dot{V}O_2$peak	1.17±0.06	1.15±0.06
Peak power output (Watts)	244±29	235±35*
Heart rate peak (bpm)	189±12	194±11*
Peak lactate (mmol·L^{-1})	8.4±3.9	9.7±3.7

Data are presented as mean±SD. *significantly different ($p<0.05$).
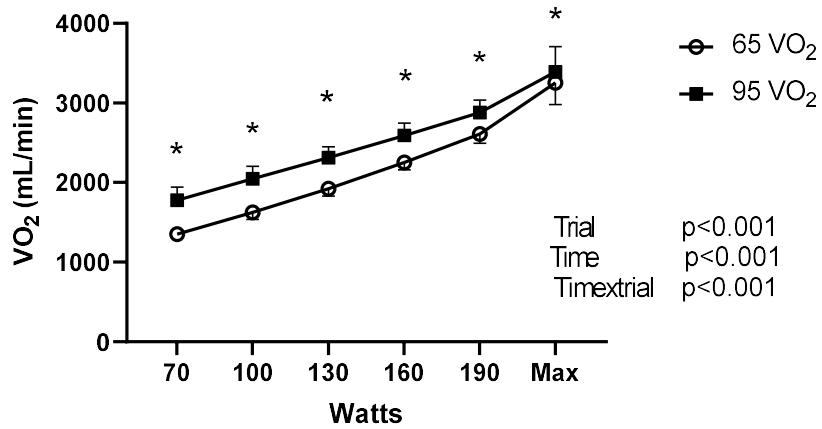
Figure 4-1: Absolute oxygen consumption as measured during the incremental tests to exhaustion. Data are presented as mean±SD. * significant difference (p<0.05).

4.4.2. Submaximal exercise trials

4.4.2.1. Oxygen consumption

Oxygen consumption during cycling exercise performed at 95- vs 65-rpm at 55% PPO (figure 4-2). Oxygen consumption was significantly higher (15-17%) when cycling at 95- vs 65-rpm (2080±192 vs 2424±208 ml/min p<0.001) with a significant effect of time observed in both trials (p<0.001).
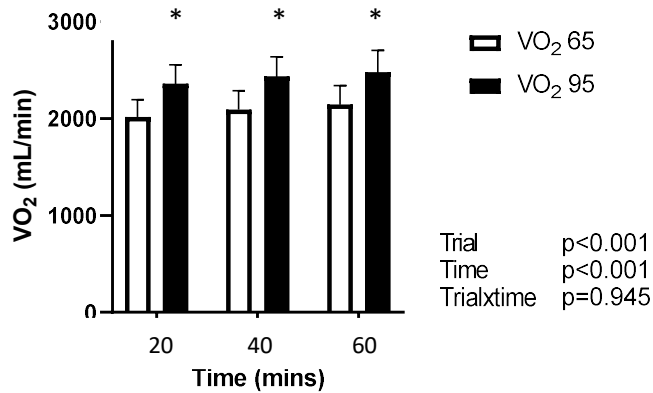
Figure 4-2 Absolute oxygen consumption as measured by indirect calorimetry during submaximal exercise at 55%PPO at 65- vs 95-rpm. Data are presented as mean±SD.

4.4.2.2.   Energy Expenditure and Gross efficiency

Energy expenditure during exercise at 95- vs 65-rpm at 55% PPO as calculated from indirect calorimetry using the Consolazio equation (Consolazio and Pecora, 1963) (figure 3). Energy expenditure (figure 4-3A) was significantly higher during the 95-rpm trial (10.14±0.9 vs 11.85±0.98 kcal/min p<0.001) with a significant increase over time observed in both trials (p<0.001). Gross efficiency (Eff) (figure 4-3B), (energy expenditure/mechanical work) was significantly higher in the 65-rpm trial (19±1.4% vs 16.2±1.4 % p<0.001) with a significant decrease over time observed in both trials (p<0.001).
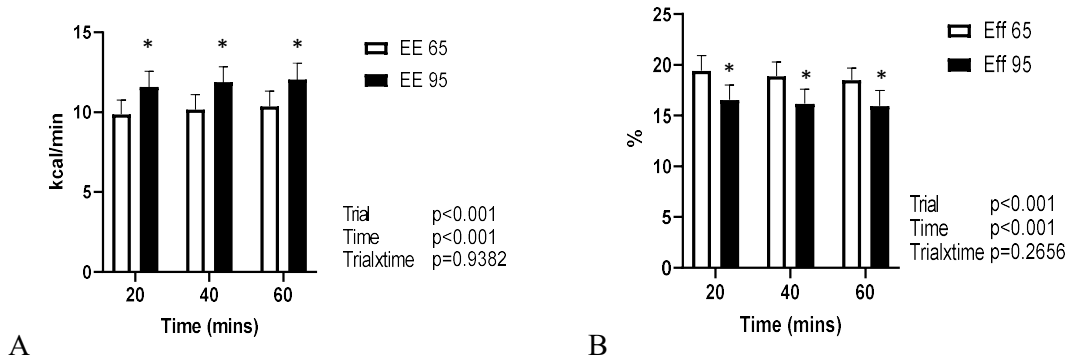
Figure 4-3 Energy expenditure (A) and gross efficiency (B) as calculated during submaximal exercise at 55%PPO at 65- vs 95-rpm. Data are presented as mean±SD.

4.4.2.3. Substrate utilisation

Substrate utilisation during cycling exercise at 95- vs 65-rpm at 55% PPO (figure 4-4) was calculated from indirect calorimetry data using the Consolazio equation (Consolazio and Pecora, 1963). The rate of carbohydrate oxidation (Figure 4-4A) was significantly higher during the 95-rpm trial (1.97±0.18 vs 2.48±0.22 g/min p<0.001). The rate of fat oxidation (Figure 4-4B) was similar between trials (0.22±0.1 vs 0.19±0.11 g/min). For both trials there was a significant effect of time with carbohydrate rates decreasing and fat oxidation rates increasing over the 60 minutes of exercise (p<0.001).
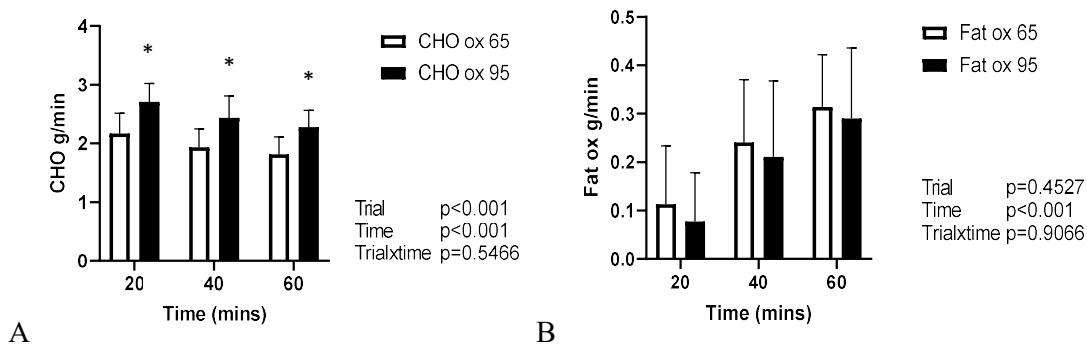
Figure 4-4 Carbohydrate (A) and Fat (B) oxidation rates as calculated from indirect calorimetry during submaximal exercise at 55%PPO at 65- vs 95-rpm. Data are presented as mean±SD.

4.4.2.4. RPE and Blood Lactate.

The RPE (figure 4-5A) as measured by the Borg scale (Borg, 1970) at 20-, 40- and 60-minute timepoints during exercise at 65- vs 95-rpm. The RPE was significantly higher during the 95-rpm trial at all time points (12.6 ± 1.7 vs 14.5 ± 2.1 $p<0.001$) as well as significantly increasing over time ($p<0.001$). Blood Lactate (figure 4-5B) as measured in whole blood from the earlobe using a handheld lactate analyser. Blood lactate was significantly higher during the 95-rpm trial (1.8 ± 0.3 vs 2.7 ± 0.5 mmol/L $p<0.001$), as well as significantly decreasing over time ($p<0.005$) with the difference also decreasing with time as shown by the time x trail effect ($p<0.05$).

A   B
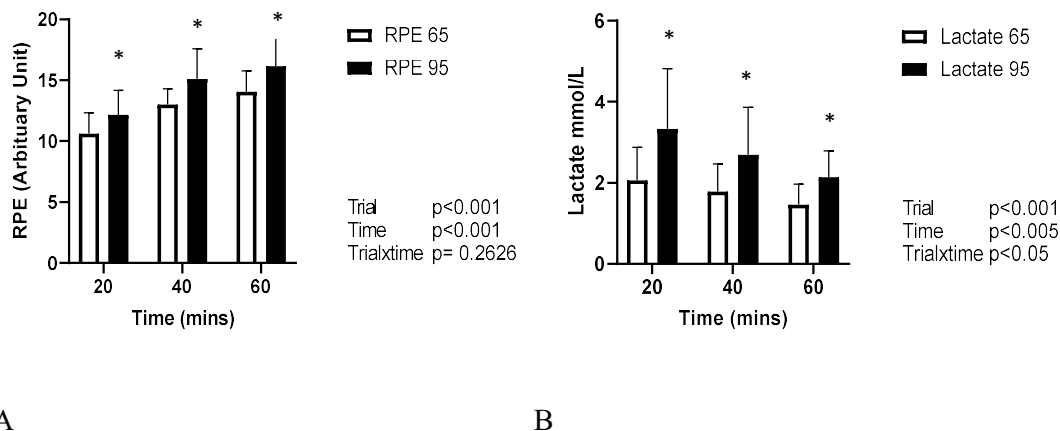
Figure 4-5 The RPE as measured using the Borg scale (A) and blood lactate levels (B) during submaximal exercise at 55%PPO at 65- vs 95-rpm. Data are presented as mean±SD.

4.4.2.5. Integrated Electromyography (iEMG)

The electrical activity of the vastus lateralis, an indicator of the total neural activity of the muscle, measured using iEMG (figure 4-6A) as measured by a surface electrode at 20-, 40- and 60-minute timepoints during cycling exercise at 95- vs 65-rpm at 55% PPO. iEMG was

significantly higher at 95-rpm (1965±663 vs 2115±701 µV p=0.016) with both trials significantly increasing with time (p=0.007). The iEMG per contraction (figure 4-6B) (iEMG/number of contractions per minute) was significantly higher in the 65-rpm trial (30.2±10.2 vs 22.3±7.4 µV/Con p=0.007) as well as both trials significantly increasing over time (p=0.003).
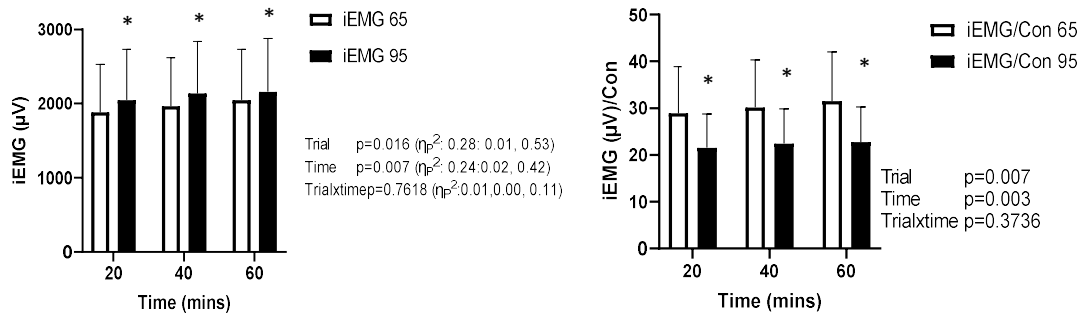
A   B

Figure 4-6 The iEMG (A) and iEMG/Con (B) of the vastus lateralis during submaximal exercise at 55%PPO at 65- vs 95-rpm. Data are presented as mean±SD.

4.4.2.6.   Mean Power Frequency (MPF)

The MPF of the iEMG, representing the average motor unit action potential conduction velocity during cycling exercise at 95- vs 65-rpm at 55% PPO is presented in figure 4-7. MPF (figure 4-7A) was significantly greater during the 65-rpm trial (98.2±14.6 vs 91.5±12 Hz p=0.006) with both trials significantly increasing over time (p<0.001). The MPF per contraction (figure 4-7B) was significantly greater during the 65-rpm trial (1.51±0.2 vs 0.96±0.1 Hz/Con p<0.001) with both trials significantly increasing with time (p<0.001).
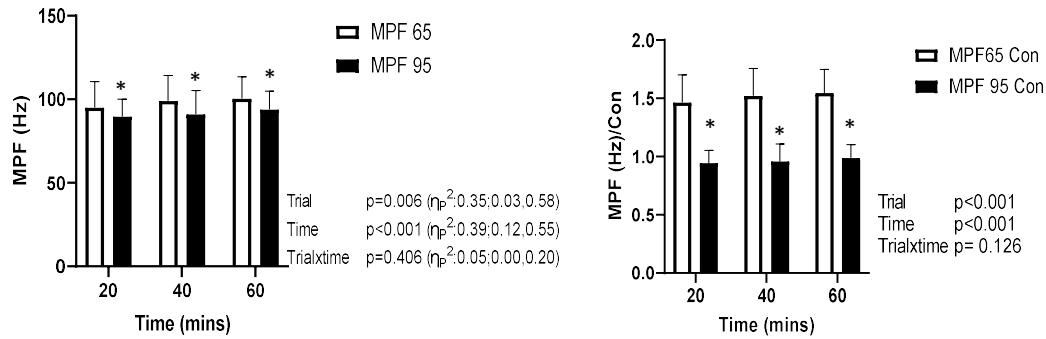
Figure 4-7 The MPF of the iEMG (A) and the MPF of the iEMG per contraction (B) during submaximal exercise at 55%PPO at 65- vs 95- rpm. Data are presented as mean±SD.

4.5. Discussion

The aim of this experiment was to determine if the metabolic response in untrained participants is different when cycling at 95- versus 65-rpm during maximal and submaximal exercise intensities. The main finding from this study was that cycling at a higher cadence while exercising at a moderate intensity can increase energy expenditure ~15% in untrained individuals. Additionally, we observed significantly higher oxygen consumption at all stages of the incremental exercise test to exhaustion at 95- vs 65-rpm. The power output (watts) during both submaximal exercise trials in this study was the same but the relative percentage of $\dot{V}O_2$peak was significantly greater while pedalling at 95-rpm. The relative intensity was ~60% $\dot{V}O_2$peak while cycling at 65-rpm but increased to ~70% $\dot{V}O_2$peak at 95-rpm and consequently, the energy cost of cycling at 95-rpm was significantly greater and gross efficiency was lower. These results are supported in the literature where increased oxygen consumption has been reported using cycling cadences between 40-120 rpm (Gaesser and Brooks, 1975; Coast, J. R. and Welch, H. G., 1985; Chavarren and Calbet, 1999; Zoladz *et al.*, 2000; Foss and Hallen, 2004; Sarre and Lepers, 2005; Formenti *et al.*, 2015; Brennan *et al.*, 2019) and at varying

exercise intensities (Chavarren and Calbet, 1999; Foss and Hallen, 2004; Formenti *et al.*, 2015). A strength of the current study was that V̇O2peak was determined at both cycling cadences and we found a significant difference in absolute oxygen consumption at all increments up-to and including peak oxygen consumption. Contrastingly, a significantly higher PPO was achieved when cycling at 65-rpm. The rationale for choosing 65- and 95 rpm was based on previous research that suggests untrained cyclist will adopt an FCC of 50-80- rpm during MICE while trained cyclist will adopt a cadence of 90-105 -rpm (Ansley and Cangley, 2009). Additionally the optimum cadence when cycling is influenced by the power output and the experience of the participant (Welch, 1985). We chose 55% PPO as a moderate exercise intensity and cadence of 65- and 95-rpm to allow enough separation between the trials but still remain in a range that would be feasible for active, non-cyclists.

Cycling cadence has been shown to impact on oxygen consumption during submaximal exercise with increased contraction rate associated with increased oxygen consumption for a given power output (Welch, 1985). During submaximal exercise (55% PPO), higher oxygen consumption and energy expenditure weas observed when cycling at 95-rpm. Other studies have shown that efficiency is affected even more if the cadence is <60 rpm or >100 rpm (Brisswalter *et al.*, 2000b; Zoladz *et al.*, 2000). In addition, many studies used short duration (3-8 mins) and/or incremental exercise bouts (Gaesser and Brooks, 1975; Welch, 1985; Brisswalter *et al.*, 2000a; Formenti *et al.*, 2015; Zorgati *et al.*, 2015; Brennan *et al.*, 2019) where it was possible to test a range of cycling cadences. A consistent finding amongst short (Foss and Hallen, 2004; Formenti *et al.*, 2015) or long (Sarre and Lepers, 2005; Kounalakis and Geladas, 2012) duration bouts of exercise is a greater oxygen consumption as the cadence increases. Kounalakis & Geladas (2012) compared cycling at 40- and 80-rpm for 90-min @60%V̇O2peak and reported greater oxygen consumption at the higher cadence. They also found significant differences in cardiovascular drift between the two cycling cadences, with a

higher rate of drift observed at 80-rpm. We observed drift in most of the physiological parameters over the 60-min trials but did not find a difference between 65- and 95-rpm. The difference in cadence used in both studies and the duration of the trials may explain these differences. The greater energy expenditure at 95-rpm as well as the increased rate of carbohydrate oxidation suggest the motor unit activation or the number of muscle fibres recruited may be greater at 95-rpm, leading to increased rates of glycogenolysis. However, the energy cost per muscle contraction was greater at 65-rpm, possibly reflecting the greater torque at the lower cadence. Therefore, it is important to distinguish between the metabolic and neuromuscular factors contributing to the increase in energy expenditure.

Total neural activity of the vastus lateralis, estimated using the iEMG, was greater while cycling at 95-rpm. One interpretation could be that a greater number of muscle fibres were recruited at the higher cadence and this accounts for the increased energy expenditure and the respiratory drive to increase oxygen consumption. The increased iEMG is consistent with other published studies (Sarre and Lepers, 2005; Kounalakis and Geladas, 2012; Brennan *et al.*, 2019). Sarre et al. (2005) found a small, but significant, increase in neuromuscular activity of the vastus lateralis when cycling at 110-rpm compared with 50-rpm @65%PPO for 1-hr. However, there was no difference between the 50-rpm and a self-selected cadence of ~88-rpm indicating the difference in neural activity may not be linear. Kounalakis & Gelades (2012) also report a greater iEMG while cycling up to 55-mins at 60% $\dot{V}O_2$peak at 80- vs. 40-rpm. There were no significant differences from 55- 90-mins of exercise and this may indicate a fatigue related impact with longer duration bouts of exercise. In addition, the average motor unit action potential conduction velocity, estimated by the MPF was significantly greater at 65-rpm. Kounalakis & Gelades (2012) also found a greater median frequency in the vastus lateralis when cycling at 40- vs. 80-rpm but neither Sarre et al. (2005) nor Vercruyssen et al. (2009)

reported differences at 50- vs. 110-rpm. However, like most studies, the participants were highly trained cyclists and this may explain differences in the findings.

Collectively, our data suggest that a greater number of muscle fibres are recruited per contraction at 65-rpm but the overall electrical activity of the muscle was greater at 95-rpm due to the greater number of contractions. Definitive conclusions about the extent of type II muscle fibre recruitment cannot be made and further research is required to ascertain if there are differences in recruitment patterns. There was an effect of time on iEMG and MPF suggesting a greater number of fibres were recruited as the exercise trial progressed. However, Ahlquist et al (1992) found a greater decrease in type II muscle fibre glycogen content following 30-min cycling at 50- vs. 100-rpm. There was no significant difference in the depletion of type I fibres leading the authors to conclude that the greater force production at the lower cadence led to a greater recruitment of type II fibres. This data supports our findings and suggest that a greater muscle fibre recruitment does not explain the increase in oxygen consumption at 95-rpm. The iEMG activity may also help explain why gross efficiency was greater and carbohydrate oxidation was lower at 65-rpm. If a greater number of muscle fibres are being recruited per contraction, the workload may be dispersed to a greater number of fibres and the metabolic stress per muscle fibre reduced (Hoffman *et al.*, 1996).

A change in muscle fibre recruitment patterns may explain some of the differences in energy expenditure but other mechanisms may also be playing a role. Glycogen lowering itself has been shown to impact iEMG activity during heavy exercise, while exercising under glycogen lowering conditions, oxygen consumption is higher and there is a greater rate of increase in MPF (Osborne and Schneider, 2006). We found greater carbohydrate oxidation rates at 95-rpm but further studies will be required to determine if there are differences in fibre-specific muscle glycogen content. However, previous research (Ahlquist *et al.*, 1992) has shown no difference in glycogen depletion of type I fibres following 30-min of cycling at ~85% $\dot{V}O_2$peak at either

50- or 100-rpm, while there was greater depletion in type II fibres at the lower cadence. These findings suggest that differences in muscle glycogen are unlikely to explain the differences in energy expenditure between the trials. It is possible that other physiological systems contribute to the increase in energy expenditure and the contribution of respiratory and smooth muscle activation, while small, should not be discounted (Lorenzo and Babb, 2012).

In conclusion, these results report that greater oxygen consumption, energy expenditure and carbohydrate oxidation when cycling at 95 vs 65 -rpm. Additionally differences in neuromuscular activity were observed that suggest a fibre type recruitment difference between the trials. Collectively the data may suggest that fibre type recruitment may play a role in explaining the observed differences between trials, however further research is required.

5. Chapter V Experiment III

The impact of muscle contraction frequency during submaximal MICE on muscle fibre recruitment patterns.

Preface

Based on the results of experiment II, experiment III was designed to investigate further the role of contraction rate in the metabolic response observed. This experiment will build on the methods previously adopted as well as the addition of muscle biopsy sampling and laboratory muscle analysis techniques.

5.1. Introduction

The results from experiment II suggested a higher metabolic cost when cycling at 55% PPO but adopting a cadence of 95-rpm rather than 65-rpm. The higher contraction rate resulted in elevated $\dot{V}O_2$ consumption, heart rate, lactate levels, and iEMG as well as energy expenditure that persisted for the duration of the exercise bout (60-minutes). Although matched for power output (55%PPO), a key difference between the trials was the number of contractions taking place over the 60-minutes with approximately 3900 contractions occurring during the 65-rpm trial and approximately 5700 during the 95-rpm trial. Although not a fixed number of contractions, this represents approx. Forty six percent more contractions performed over the 60 minutes. The increased efficiency during the 65-rpm trial as well as the increased carbohydrate oxidation rates during the 95-rpm trial, could be suggestive of a difference in recruitment of muscle fibres based on the metabolic characteristic of type I and type II fibres previously described.

5.2. Experimental design

Due to the similarities of this experiment to experiment II, where possible the duplication of information will be avoided and a reference to experiment II will be made.

This experiment was a randomised crossover study with two randomised incremental exercise tests performed at 65- and 95-rpm and two submaximal 60-minute MICE@55%PPO trials randomised to be performed at 65- or 95-rpm. An additional randomised submaximal trail was also performed where participants cycled at 95-rpm@55%PPO for 41-mintues and 5-seconds (41 trial) in order to have performed the same number of contractions during the 65-rpm trial, but at the higher 95-rpm contraction rate (65-rpmX60mins = 3900 contractions, 95-rpmX60mins = 5700 contractions ∴ 41.5mins@95-rpm=3900 contractions).

5.3. Methodology

The study received ethical approval from the Dublin City University Research Ethics Committee (appendix 3). The recruitment of participants followed the same procedure as experiment II. Eighteen recreationally active young men were recruited to take part in the study. The same exclusion criteria and familiarisation session as experiment II were used in this experiment. Height and body mass were measured, however no DEXA scan was performed on this group. The physical characteristics of this group can be viewed in Table 5-1.

Table 5-1: Physical Characteristics

Age (yrs)	27.3±3.5
Height (m)	180±6.7
Body mass (kg)	77.6±11
BMI ($kg \cdot m^{-2}$)	21.5±2.7

Data presented as mean ± standard deviation.

5.4. Incremental exercise tests

The methods for the incremental exercise tests were the same as those used in study II (page94).

5.4.1. Submaximal exercise Trials

On the morning of each submaximal exercise trial, participants reported to the lab following an overnight fast. A muscle biopsy was performed before participants completed each submaximal exercise trial. A surface electromyography (sEMG) sensor was placed on the vastus lateralis of the leg that was not having biopsies performed. The placement and analysis of data from the EMG sensor was described in study II. Participants cycled for 60-mins@55%PPO at 65- or 95-rpm as well as 41mins 5 seconds @55%PPO at 95-rpm, after which a second muscle biopsy was performed. Indirect calorimetry was recorded and averaged over 6-minutes to measure oxygen consumption and carbon dioxide production at 20-, 40- and 60-minutes respectively. Heart rate was measured continuously and blood lactate, 1-minute iEMG, as well as RPE were recorded every 10-minutes.

5.4.2. Muscle Biopsy

A muscle biopsy was taken from the vastus lateralis by experienced medically trained personnel using a percutaneous muscle biopsy needle (Bergstrom, 1962). The sites of the biopsies were marked as being 2/3rds of the way down the midline ranging from the anterior spina illicia, superior to the lateral side of the patella and in the belly of the muscle ±2cm (2 sites per trial). This resulted in a gap of 4cm between biopsy sites within which the sEMG sensor was placed on the subsequent trial, ensuring no scar tissue was present on the site of EMG data collection. Once identified, the sites were cleaned with antiseptic solution (Videne) and alcohol. The sites were then anaesthetised with 1% Lidocaine hydrochloride (Braun, Melsungen AG).

The lidocaine was allowed time to take action (5-minutes) after which a small (0.5-1cm) incision was made with a scalpel after which a biopsy needle was used to remove small pieces (100-200 mg) of muscle. The blood and connective tissue were removed from the sample before being snap frozen or mounted for immunohistochemistry as described below. Once sufficient muscle was obtained, the incision was closed using steri-strips and a compression bandage applied. Sufficient lidocaine was administered to ensure the post exercise biopsy could be performed immediately upon the cessation of exercise. Once the exercise trial was finished, participants lay down on a bed directly beside the cycle ergometer in order for the second biopsy to be performed. The time of exercise cessation to the first piece of muscle being snap frozen was on average 90-seconds.

5.4.3. Muscle analysis
5.4.3.1. Whole muscle Glycogen analysis

The method of analysing whole muscle samples for glycogen was described in chapter III (page 101).

5.4.3.2. Muscle Fibre type

A piece of muscle sample that was deemed suitable for fibre type analysis (>50ug with muscle striation visible) had any visible blood and connective tissue removed before being embedded in Tissue Tek OCT compound (Sakura Finetek, LA, USA) and rapidly frozen in liquid nitrogen cooled isopentane (2-methyl-butane, Sigma-Aldrich, St Louis, MO, USA), before being stored at -80C for later analysis. Serial cryosections (10um) were made using a Leica CM3050 Cyrostat (Leica Microsystems, Newcastle Upon Tyne, UK), and were thaw mounted on uncoated pre-cleaned glass slides. Three serial sections were mounted per slide with three slides per biopsy generated, resulting in 9 sections per biopsy in total for each participant. The slides were air dried for 15-minutes at room temperature before being processed for immunohistochemistry. All of the following immersions were performed at room temperature

unless otherwise noted. In order to maintain contact between the muscle sections and the solutions containing antibodies and washes, a line was drawn around the samples using a hydrophobic pen. Samples were first immersed for 15-minutes in an antibody dilution buffer consisting of phosphate buffered saline (PBS) containing 1% Bovine serum albumin (BSA) (Sigma Sigma-Aldrich, St Louis, MO, USA). A wash cycle consisted of draining the solution that the samples were immersed in (dilution buffer in this case) and re-immersing the samples in PBS for 5-minutes, before draining and re-immersing again in PBS two more times. Following the first wash cycle, samples were immersed in a blocking buffer (PBS, 5% Goat Serum (IgG, Sigma Aldrich, St Louis, MO, USA) for 30-minutes after which a wash cycle was performed. A solution containing the primary antibody cocktail associated with laminin, type I and type II fibres diluted in antibody dilution buffer (PBS1%BSA) was applied to the samples which were stored at 4C overnight (12-16 hours). This solution contained the antibodies 2E8, a mouse IgG1 monoclonal antibody that is directed at human laminin, a basement membrane protein present in muscle cell membranes, BA:F8, a mouse IgM monoclonal antibody raised against human myosin heavy chain type I fibres and SC-71 a mouse IgM monoclonal antibody raised against human myosin heavy chain type IIa/IIx fibres (Developmental Studies Hybridoma Bank (DSHB), Iowa, IA, USA). The following day a wash cycle was performed before a solution containing the secondary antibody cocktail was applied for 2-hours and placed in a cardboard box to avoid exposure to light. This solution contained the BA-F8 secondary antibody Alexa Fluor 350 IgG2b, SC-71 secondary antibody Alexa Flour IgG 594 and the Laminin secondary antibody Alexa Fluor IgG FITC (DSHB, Iowa, IA, USA) (table 5-2).

Table 5-2 Primary and secondary antibody dilution ratios

Primary Antibody Cocktails and Concentrations	MHC Reactivity	Secondary Antibody Cocktails and Concentrations
BA-F8 (1:25)	I	Alexa Fluor 350 IgG2b 1:500 (blue)
SC-71 (1:50)	IIa/x	IgG 594 1:500 (red)
Laminin (1:50)		IgG FITC 1:500 (green)

Primary and related secondary antibodies with dilution ratios as utilised in quantification of skeletal muscle fibre type.

Following this 2-hour period, a wash cycle was performed, and samples were immersed in PBS before being viewed using a Leica DM1L fluorescence microscope with Leica image capturing software (Leica Microsystems, Newcastle Upon Tyne, UK). The microscope had light filters to capture Green (Excitation 440-470), Blue (Excitation 312-365) and Red (Excitation 525-545) enabling the illumination of secondary antibodies associated with Laminin, type I and type II fibres respectively. To ensure a valid computer assisted analysis of sections the images were captured using identical camera settings with the exposure time, offset and gain identical between captured images. Images were processed using Image J software (National Institute for Health, Bethesda, MD, USA) with the quantification of fibre type completed by overlaying the images of Laminin, type I and type II fibres and counting the respective fibre types using the Image J cell counter plugin. As 9 sections in total were assessed per biopsy, and six biopsies performed per participant, 54 sections in total were assessed for fibre type analysis. The total number of fibres counted per participant was 1888±360. Although a considerable amount of time was spend optimising the dilution ratios and exposure times, we were not able to ensure SC-71 only stained type II fibres and a co-stain of type I fibres occurred. As type I fibre identification was possible using BA-F8, we decided to proceed to periodic acid schiff (PAS) staining only using the overlay image for type I for fibre type analysis. An example of a section

with type I fibres shown in blue can be viewed in figure 5-1. All black fibres are not type I fibres.
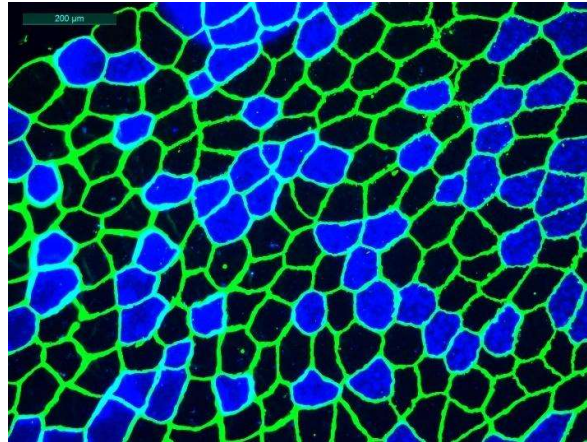
Figure 5-1. Cross section of muscle biopsy from vastus lateralis stained for laminin (green) and type I fibres (blue) as viewed on fluorescence microscope.

5.4.3.3.   Periodic acid Schiff Staining (PAS)

Following the capture of the images relating to immunohistochemistry, the sections were stained for glycogen using a PAS staining kit (Sigma Sigma-Aldrich, St Louis, MO, USA). Firstly, the sections were immersed in Periodic acid solution for 5-minutes to oxidise the glycols present to aldehydes, after which a wash cycle was performed. The samples were then immersed in Schiffs reagent for 15-minutes in order to stain the aldehydes created in the first reaction, after which a wash cycle was performed. Following this period, the sections were immersed in Hematoxylin Solution for 90-seconds to counter stain the Schiff's reagent stain formed, before a final wash cycle was performed. Sections were viewed using the same microscope used for capturing the fibre type classification images but using the bright field light to capture the images. To ensure a valid computer assisted analysis of sections, the images were captured using identical camera settings with the exposure time, offset and gain identical between captured images. Glycogen content was measured using image J software (National

Institute for Health, Bethesda, MD, USA). The full colour image of sections stained with PAS was converted to 8-bit grey scale, with a threshold of 256 pixels set and kept constant between samples. Type I and type II fibres were manually delineated using the overlay fibre type image of the sections, with measurements of glycogen achieved using a multi measurements plug in that allowed for the calculation of mean grey value (pixels) per fibre counted. Due to poor image quality related to fractured adhesion of the sections to the slide, only 14 participants produced images deemed suitable for quantification of the PAS stain using grey scale. At the fracture point of a cell on the slide, a pooling of PAS stain was observed that resulted in significantly more pixilation that increased the pixel quantification. Due to this, only whole cells devoid of fractures were suitable for PAS staining. For the included participants, each biopsy resulted in 42±15 fibres where PAS staining and fibre type assessment was categorical. An example of fibre type classification (Figure 5-2 A), PAS staining with glycogen stained in purple (Figure 5-2 B) and PAS picture converted to grey scale (Figure 5-2 C) of the same section can be viewed below.
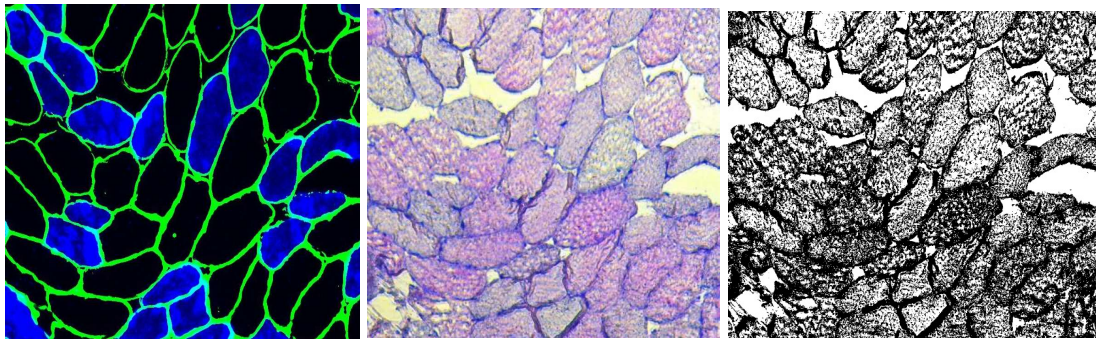
A   B   C

Figure 5-2 Laminin stained green and type I fibres stained blue on cross section of muscle biopsy from vastus lateralis as viewed on fluorescence microscope (figure 5-2 A). PAS stain performed on the above cross section. Glycogen is stained purple in this image (figure 5-2 B). Image of PAS stain after conversion to grey scale (figure 5-2 C).

5.4.4. Data analysis

Indirect calorimetry, energy expenditure, efficiency, torque and EMG analysis was all performed as was detailed in the methods section of Chapter IV (page 115).

5.4.5. Statistical analysis

All data was analysed using GraphPad Prism (Version 4.7 for Windows, GraphPad Software, San Diego, California, USA) and expressed as mean ± standard deviation. A Shapiro -Wilk test was used to determine the normality of the data. A paired samples t-test was used to compare $\dot{V}O_2$peak, PPO, heart rate and lactate max following the incremental tests at 65- and 95-rpm. A two-way (trial x time) repeated-measures ANOVA was used to compare the submaximal trials at 20-, 40- and 60- mins and a Student Newman-Keuls post-hoc test was used to differentiate between trials and time when significant differences were detected. A one-way ANOVA with pairwise comparison was used to determine differences between energy expenditure and the change in whole muscle glycogen and PAS levels between trials. The alpha level for statistical significance was set at $\alpha = 0.05$.

5.5. Results

5.5.1. $\dot{V}O_2$peak tests at 65- and 95-rpm

Peak responses as measured at the point of exhaustion during the incremental exercise tests performed at 65- and 95-rpm are presented in Table 5-3. PPO was significantly higher when cycling at 65-rpm (p<0.05)

Table 5-3 Peak responses to maximal exercise test performed at 65- vs 95-rpm

$\dot{V}O_2$peak tests	65 rpm	95 rpm
$\dot{V}O_2$peak (mL·kg^{-1}·min^{-1})	46.3±4.2	46.7±4.9
RER at $\dot{V}O_2$peak	1.17±0.04	1.15±0.03
Peak power output (Watts)	275±35	259±35*
Heart rate peak (bpm)	189±7	191±8
Peak lactate (mmol·L^{-1})	9.1±2.8	9.4±3

Data are presented as mean±SD. *significantly different (p<0.05) 65- vs 95-rpm trial.

5.5.2. Submaximal exercise trials

5.5.2.1. Oxygen consumption

Absolute oxygen consumption as measured by indirect calorimetry at 20- and 40-minutes during all submaximal trials (figure 5-3A) and 20-, 40- and 60- minutes at 65- and 95-rpm (figure 5-3B). Oxygen consumption was significantly higher during the 60-minute and contraction matched 95- vs 65- rpm at 55%PPO ($\dot{V}O_2$65 vs $\dot{V}O_2$41 vs $\dot{V}O_2$95 = 2183±216 vs 2402±120 vs 2522±253 p<0.001) with both the 41 and 95- rpm trial significantly increasing over time (p<0.001).
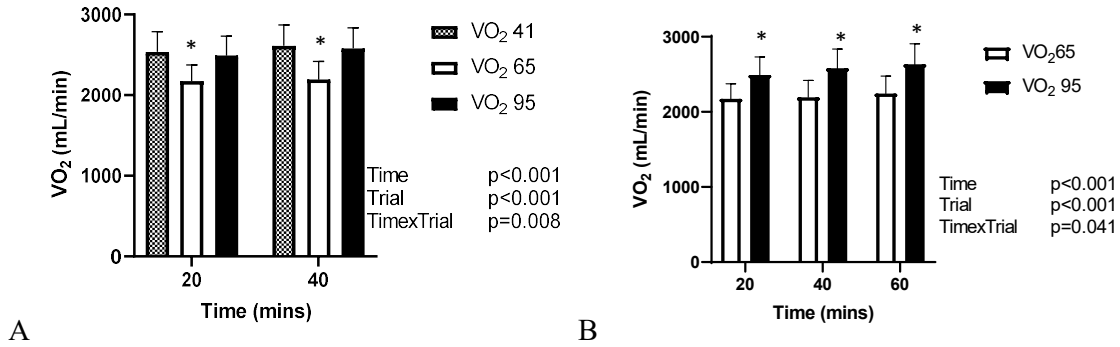
Figure 5-3 Absolute oxygen consumption comparing all trials at 20- and 40- minutes (A) and 20-, 40- and 60- minutes at 65- and 95-rpm (B) as measured by indirect calorimetry during submaximal exercise at 55%PPO at 65- vs 95- rpm. Data are presented as mean±SD.

5.5.2.2.   Energy expenditure

Energy expenditure at 20- and 40- minutes during all submaximal trials (figure 5-4A) and 20-,40- and 60-minutes at 65- and 95- rpm (figure 5-4B) calculated from indirect calorimetry using the Consolazio equation (Consolazio and Pecora, 1963). Energy expenditure (figure 5-4A) was significantly higher during the 95-rpm trials (EE65 vs EE41 vs EE95 =10.6±1.1 vs 12.4±0.5 vs 12.3±1.2 kcal/min p<0.001) with both the 95-rpm trials significantly increasing over time (p<0.001).
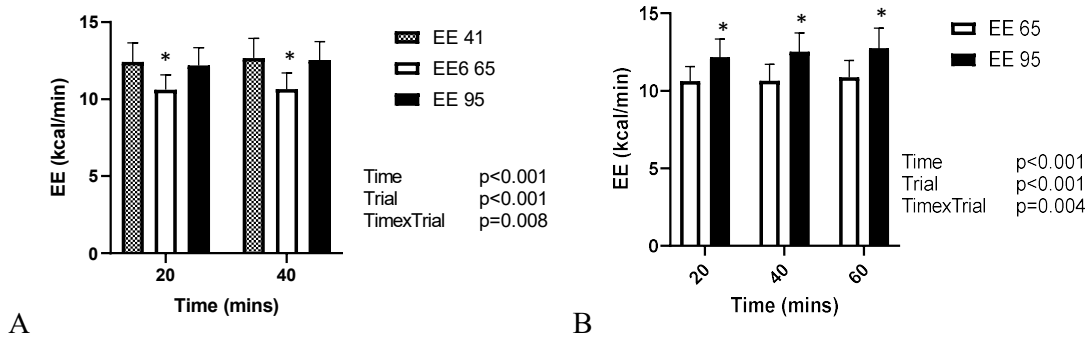
Figure 5-4 Absolute energy expenditure comparing all trials at 20- and 40- minutes (A) and 20-, 40- and 60-minutes at 65- and 95- rpm (B) as measured by indirect calorimetry during submaximal exercise at 55%PPO. Data are presented as mean±SD.

5.5.2.3. Total energy expenditure

The total energy expenditure (Figure 5-5A) was significantly different between all trials (TotalEE41 vs TotalEE65 vs TotalEE95 = 515±51 vs 643±61 vs 749±72 p=<0.001). When adjusted to be per contraction that took place in each trial (Figure 5-5B), there was significantly greater energy expenditure when cycling at 65-rpm with no significant difference observed between the 95-rpm trials (EE/Con 65 vs EE/Con 41 vs EE/Con 95 = 0.17±0.01 vs 0.13±0.01 vs 0.13±0.01 p<0.001).

A   B
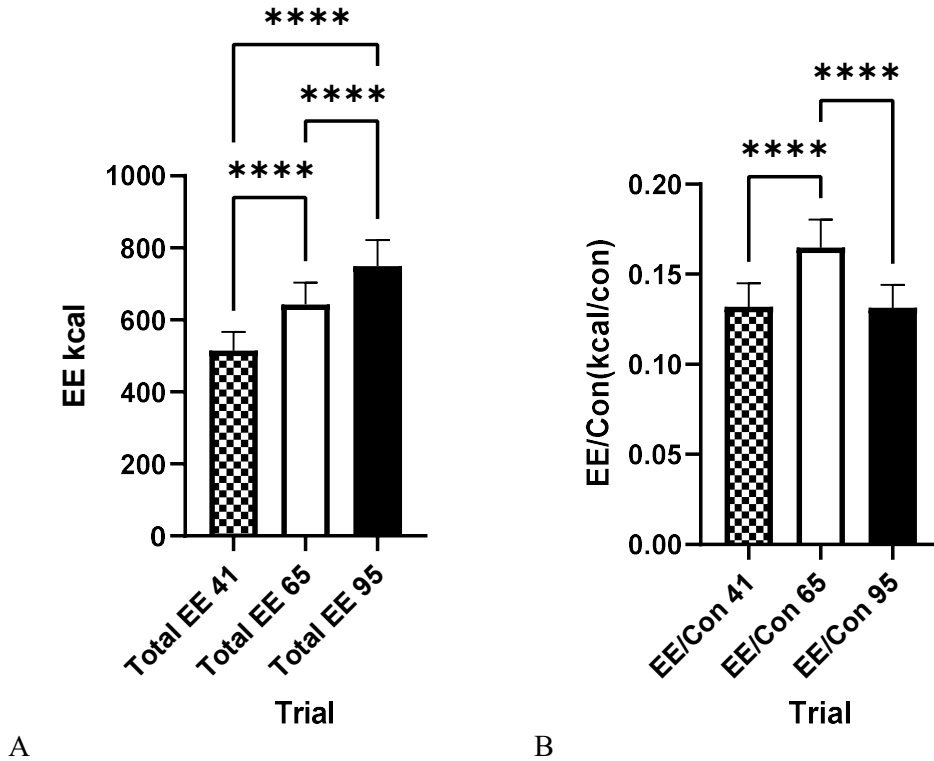
Figure 5-5 Total energy expenditure expended during each submaximal trial as measured by indirect calorimetry (A). Energy expenditure per contraction (B). Data are presented as mean±SD. **** significantly different (p<0.001).

5.5.2.4. Substrate utilisation
5.5.2.4.1. Carbohydrate oxidation

Carbohydrate oxidation as calculated from indirect calorimetry data using the Consolazio equation (Consolazio and Pecora, 1963). The rate of carbohydrate oxidation at 20- and 40-minute time points for all trials (figure 5-6A) and 20-, 40- and 60-minutes at 65- and 95- rpm (figure 5-6B). Carbohydrate oxidation was significantly higher during both 95-rpm trials (CHOox65 vs CHOox41 vs CHOox95 = 2.14±0.3 vs 2.52±0.2 vs 2.52±0.3 g/min p<0.001) with all trials significantly decreasing over time (p<0.001).
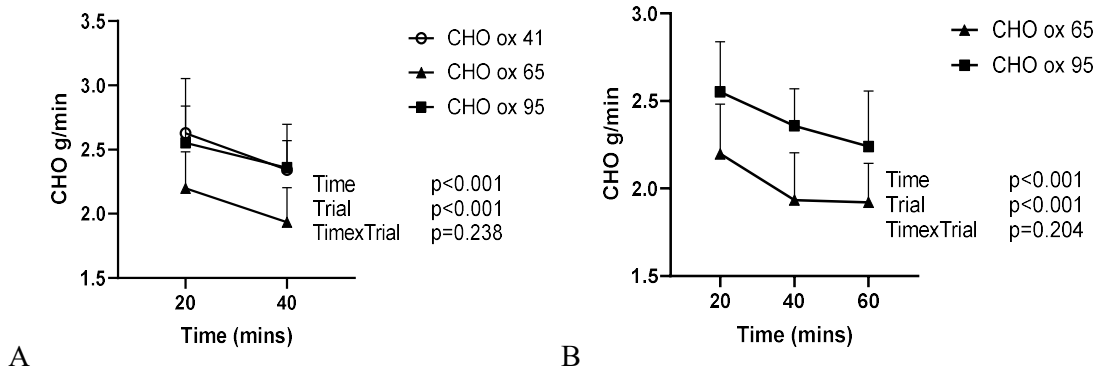
Figure 5-6 Carbohydrate oxidation in all trials at 20- and 40- minutes (A) and 20-, 40- and 60- minutes at 65- and 95- rpm (B). Data are presented as mean±SD.

5.5.2.4.2. Fat oxidation

Fat oxidation as calculated from indirect calorimetry data using the Consolazio equation (Consolazio and Pecora, 1963). The rate of fat oxidation at 20- and 40- minute time points for all trials (figure 5-7A) and 20-, 40- and 60- minutes at 65- and 95-rpm (figure 5-7B). There was no significant difference in fat oxidation rates between all trials (FATox65 vs FATox41 vs FATox95 = 0.21±0.1 vs 0.23±0.1 vs 0.22±0.1 g/min $p<0.001$) with all trials significantly increasing over time ($p<0.001$).
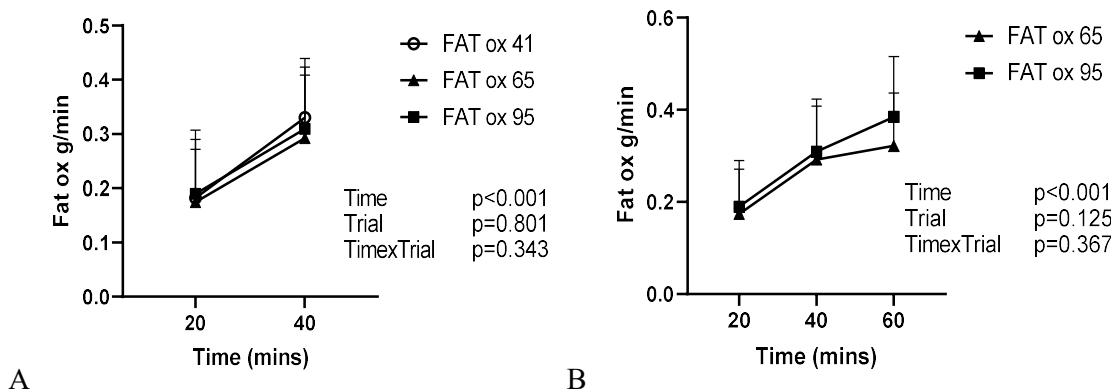
Figure 5-7 Fat oxidation in all trials at 20 and 40 minutes (A) and 20-, 40- and 60- minutes at 65- and 95- rpm (B). Data are presented as mean±SD.

5.5.2.5.   Integrated Electromyography (iEMG)

The electrical activity of the vastus lateralis, an indicator of the total neural activity of the muscle, measured using iEMG at 20- and 40- minute time points for all trials (figure 5-8A) and 20-, 40- and 60- minutes at 65- and 95- rpm (figure 5-8B). Although the iEMG was higher when cycling at 95- rpm there were no significant differences observed between trials. The iEMG per contraction at 20- and 40- minute time points for all trials (figure 5-8C) and 20-, 40- and 60-minutes at 65- and 95-rpm (figure 5-8D). The iEMG per contraction was higher at 65-rpm however no statistical differences were observed between trials.
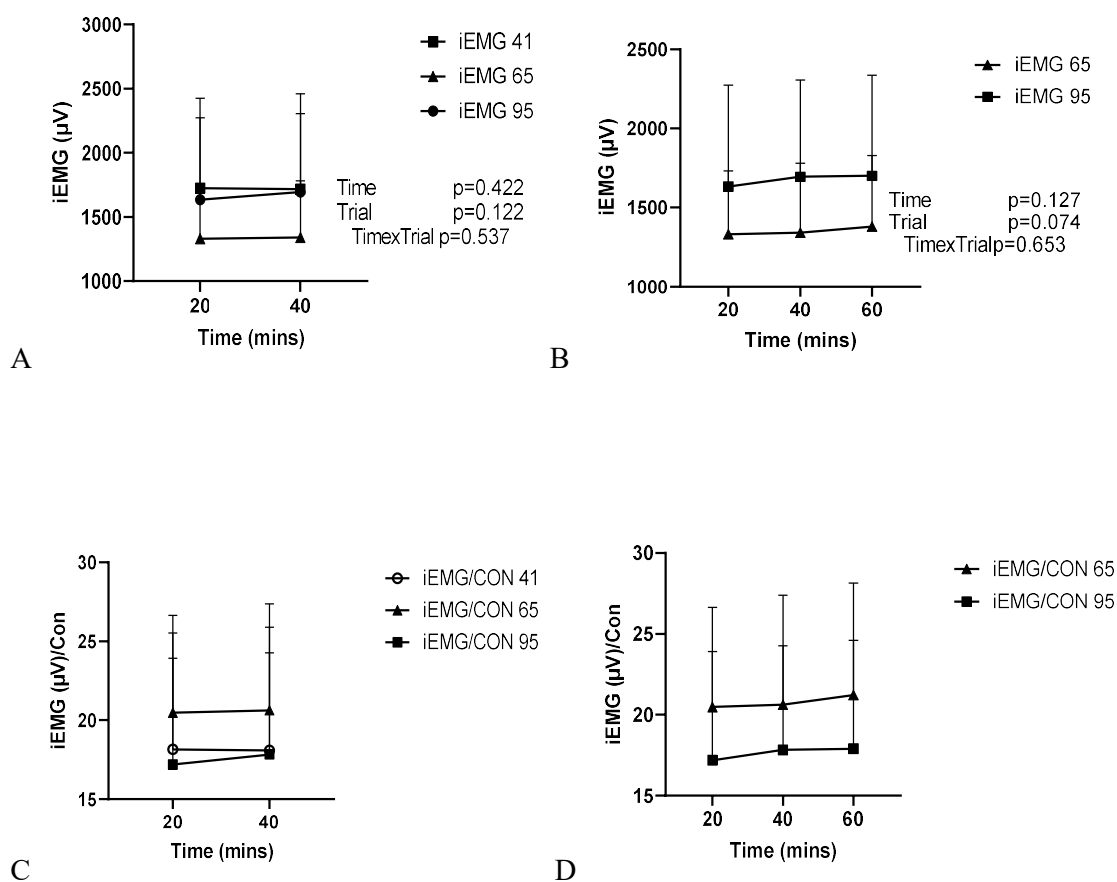
Figure 5-8 The iEMG in all trials at 20- and 40- minutes (A), 20-, 40- and 60- minutes at 65- and 95- rpm (B), iEMG /contraction in all trials at 20 and 40 minutes (C), and 20-, 40- and 60- minutes at 65- and 95- rpm (D). Data are presented as mean±SD.

5.5.2.6. Mean Power Frequency (MPF)

The MPF of the iEMG, representing the average motor unit action potential conduction velocity at 20- and 40- minute time points for all trials (figure 5-9A) and 20-, 40- and 60-minutes at 65- and 95-rpm (figure 5-9B). There were no significant differences observed between trials.
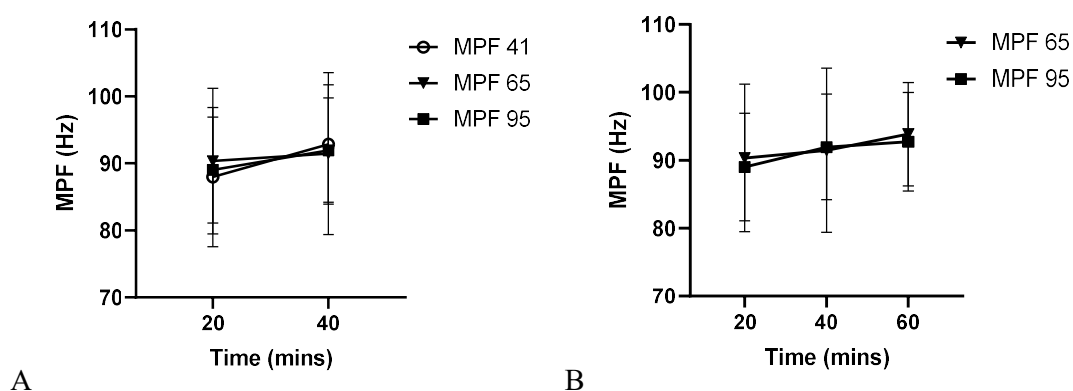
Figure 5-9 The absolute MPF of the iEMG of the vastus lateralis in all trials at 20- and 40-minutes (A) and 20-, 40- and 60- minutes at 65- and 95- rpm (B). Data are presented as mean±SD.

5.5.2.7. Glycogen

5.5.2.7.1. Whole muscle glycogen

Muscle glycogen as measured by an endpoint colorimetric assay in whole muscle biopsy samples of the vastus lateralis pre and post exercise. The absolute change (figure 5-10A) in glycogen pre to post each trial. Significant differences were observed between the 60-minute 95-rpm trial and all other trials (95 vs 65 vs 41 = -48.1±11.4 vs -23.3±6.8 vs -29.6±8.7 mmol/kgwt $p<0.001$). Additionally, there was significantly greater glycogen use during the 41min@95-rpm trial when compared to the 65-rpm trial (-29.6±8. vs -23.3±6.8 mmol/kgwt

p<0.05). When controlled for the number of contractions that took place in each trial (Figure 5-10B) (60mins@65-rpm = 3900, 60mins@95-rpm = 5700, 41.05mins@95-rpm = 3900) there was significantly greater glycogen use per contraction when cycling at 95-rpm (95 vs 65 vs 41 = -0.008±0.002 vs -0.006±0.002 vs -0.008±0.002 mmol/kgwt /con, p<0.05).

A   B
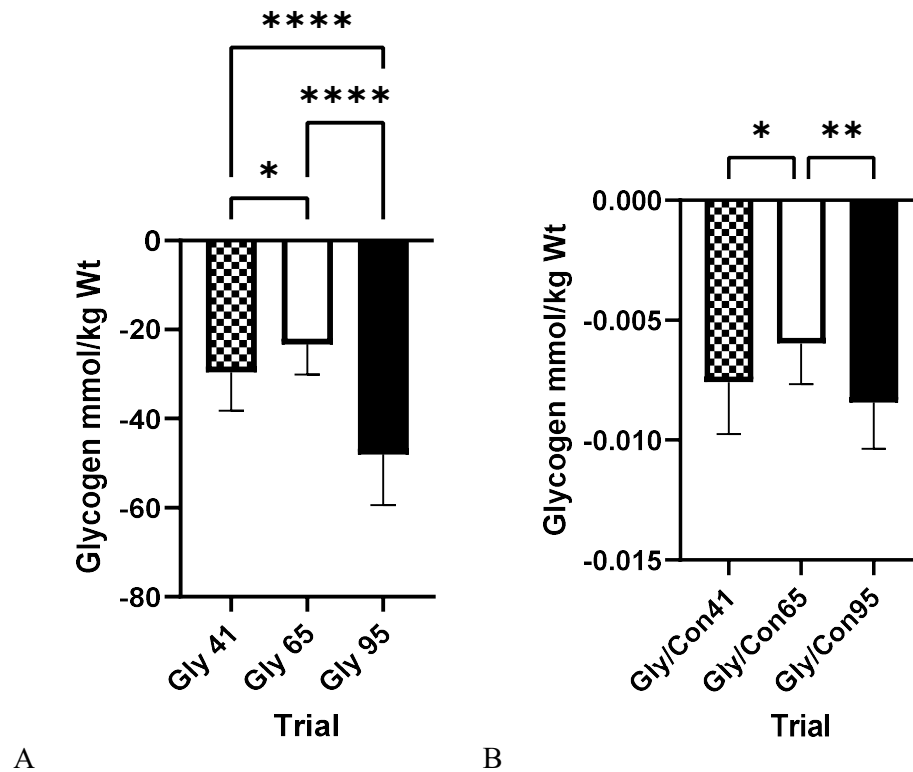
Figure 5-10 The absolute change in glycogen pre to post each trial (A). The absolute change in glycogen per contraction that occurred in each trial (B). Data are presented as mean±SD. * significantly different (p<0.05), **** significantly different (p<0.001).

5.5.2.7.2.   Type I and type II muscle fibre glycogen utilisation

The glycogen utilisation in type I and type II muscle fibres was assessed using a PAS staining technique to quantify glycogen, in combination with immunohistochemistry to identify type I and type II fibres pre and post exercise (figure 5-11). There was a significant decrease in

glycogen in all trials in type I and type II fibres (41typeI pre-post =3637±890 - 2688±593 pixels, p<0.01), (41typeII pre-post = 2822±706 - 1949±401 pixels, p<0.05), (65typeI pre-post = 3584±993 - 2696±595 pixels, p<0.01), (65typeII pre-post = 2822±2096±563 pixels, p<0.05), (95typeI pre-post = 3485±959 - 2360±754 pixels, p<0.005), (95typeII pre-post = 3078±1004 - 1931±626 pixels, p<0.005). A time x trial effect was also observed (p=0.038).
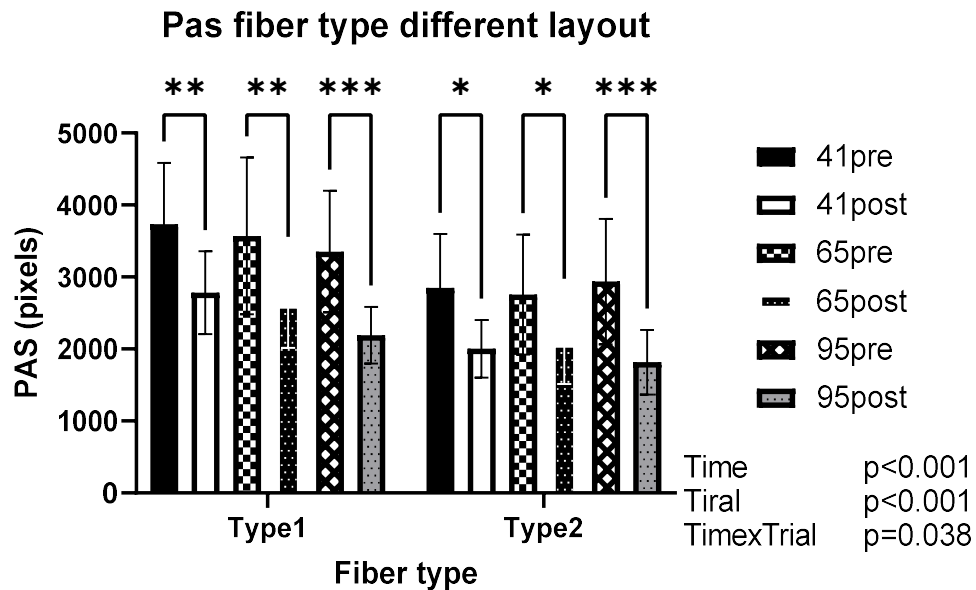
Figure 5-11 Muscle glycogen as measured using PAS staining presented as average pixels when converted to greyscale (n=14). Data presented as mean ± standard deviation. * denotes significance p<.05, ** denotes significance p=<.01, *** denotes significance p<.005.

5.5.2.7.3. Change in glycogen in type I and type II fibres

There was no significant difference in the absolute change in glycogen in type I fibres (figure 5-12A) as assessed by PAS staining pre and post exercise. A significantly greater reduction in glycogen in type II as assessed by PAS staining occurred in the 60min@95-rpm trial when compared to the 60min@65-rpm trial (figure 5-12B) (-661±518 vs -1122±632 pixels, p<0.05).
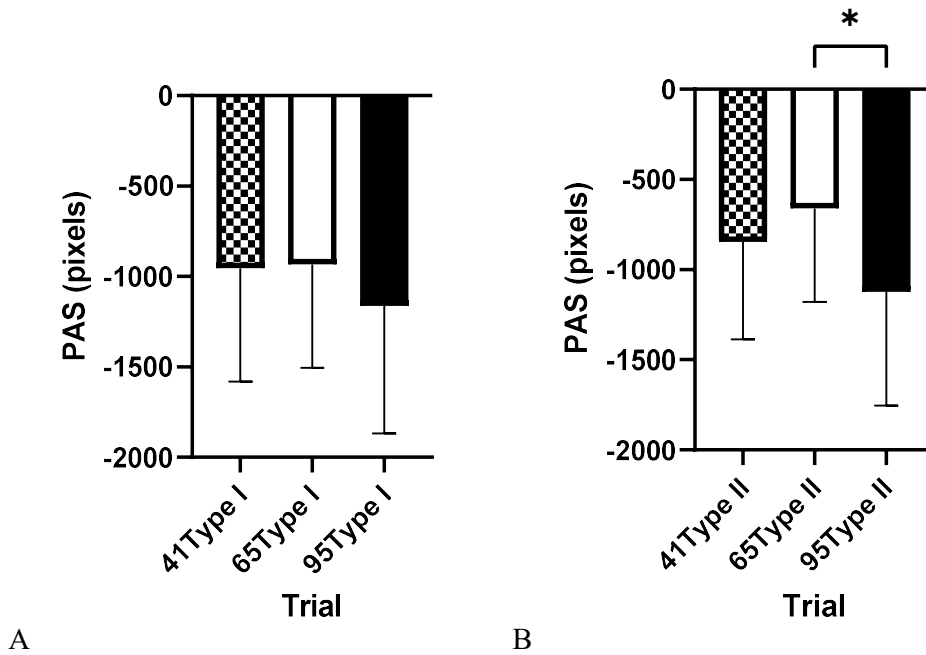
Figure 5-12 Change in muscle glycogen in type I (A) and type II (B) muscle fibres as measured using PAS staining presented as average pixels when converted to grey scale (n=14). Data are presented as mean±SD. * significantly different (p<0.05).

5.5.2.7.4.   Change in glycogen in type I and type II fibres per contraction

The change in glycogen as measured by PAS staining in type I fibres (figure 5-13A) and type II fibres (figure5-13B) pre and post exercise. The was no significant differences observed between trials.
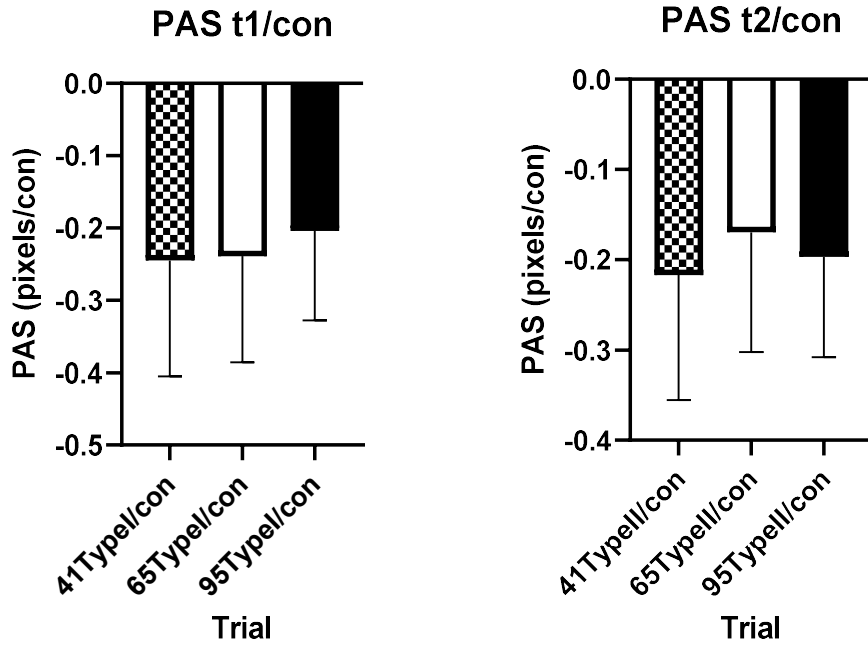
Figure 5-13 Change in muscle glycogen per contraction in type I (A) and type II (B) muscle
fibres as measured using PAS staining presented as average pixels when converted to grey
scale and divided by the number of contractions performed during the trial. Data are
presented as mean±SD.

5.6. Conclusions

The aim of this study was to examine the relationship between the observed metabolic response and glycogen use in type I and type II muscle fibres during submaximal cycling exercise at 65- vs 95-rpm. We found a significant decrease in absolute PAS pre to post each trial in both type I and type II fibres. There was no significant difference in the rate of change in type I fibres between trials, however a significantly greater change was observed in type II fibres when comparing the 95-rpm to 65-rpm 60-minute trial. However, when the number of contractions was controlled for, the absolute rate of change in each fibre type (decrease in PAS per contraction) was not significantly different between trials. This finding is in agreement with others who have investigated fibre type recruitment patterns during submaximal cycling at different cadences (Gollnick *et al.*, 1974; Vøllestad *et al.*, 1984).

Ahlquist et al (1992) found a greater decrease in type II muscle fibre glycogen content following 30-min cycling at 50- vs. 100-rpm with no difference in type I fibres, however participants in this study were exercising at 85% $\dot{V}O_2$peak for the 30 minute period. As previously stated, the metabolic response to exercise @85% $\dot{V}O_2$peak would be expected to different then that at 55%PPO as was our exercise intensity. Additionally differences in fibre type recruitment would also be expected with a greater total number of fibres being recruited based on the activation pattern of skeletal muscle fibres with increasing exercise intensity (Sale, 1987). Although consistent with others, our findings do not definitively show that fibre type recruitment does not explain, in part, the observed differences in metabolism. It is important to note that it is not possible to ensure the exact same fibres were biopsied pre and post exercise, as well as the number of fibres measured only representing a fraction of all fibres in the vastus lateralis. Although the vastus lateralis is an ideal muscle to biopsy when conducting this type of research, the relative contribution of the other 11 prime mover muscles associated with cycling should be investigated in relation to contraction frequency (Ericson *et al.*, 1985).

However, performing a muscle biopsy on the muscle selected for EMG analysis was deemed paramount for this study and thus only the vastus lateralis was chosen. Additionally, it is possible that the 30-rpm difference in cycling cadence, or the elevated rate of 95-rpm adopted was not sufficient to provide significant differences in fibre type recruitment between trials. Our findings are not consistent with Ahlquist et al (1992) who utilised PAS staining in combination with fibre type analysis and found greater type II fibre recruitment at 50- vs 100-rpm after participants cycled for 45-minutes at each rate. However, this research was conducted in well-trained cyclists and runners who were exposed to considerably more force during each exercise trial (335 watts), a greater difference in cadence (50-rpm) as well as exercising at 85%$\dot{V}O_2$peak, therefore not MICE. PAS staining is a semi quantitative method of muscle fibre glycogen quantification and as such is not an exact method of quantifying muscle fibre glycogen use. Muscle fibre isolation techniques that allow for direct measurement of glycogen in whole muscle fibres dissected from a muscle biopsy would allow for a greater accuracy in glycogen measurement and possible insight into true fibre type glycogen use. However, this technique was not possible for this thesis and PAS staining was chosen instead. Although strict protocols established for the PAS staining technique, small differences in the application time of reagents may lead to differences in the final quantification of glycogen and as such reduces the accuracy of this technique (Fairchild and Fournier, 2004). Additionally, high quality cross sections of muscle tissue are required for the image quantification software which resulted in a reduced number of fibres being considered suitable for glycogen quantification. While the change in whole muscle glycogen pre to post was significantly different between all trials however this is performed on a muscle sample that contains both type I and type II fibres. However, this enzymatic technique allows for greater accuracy in glycogen quantification but only at a whole, mixed fibre, muscle level.

During submaximal exercise (55% PPO), higher oxygen consumption, energy expenditure, and carbohydrate oxidation were observed when cycling at 95-rpm. As mentioned in the discussion of experiment II, this finding is in agreement with other studies that have shown greater oxygen consumption at elevated cycling cadences (Gaesser and Brooks, 1975; Welch, 1985; Brisswalter *et al.*, 2000a; Zoladz *et al.*, 2000; Sarre *et al.*, 2003; Foss and Hallen, 2004; Kounalakis and Geladas, 2012; Formenti *et al.*, 2015; Brennan *et al.*, 2019). However, the findings from the EMG of this experiment are divergent to experiment II as no significant difference between trials in absolute iEMG or when contraction number was controlled, as well as no differences in the MPF of the iEMG observed. Sarre et al (2005) observed a significant increase in neuromuscular activity of the vastus lateralis when cycling at 110-rpm when compared to 50-rpm at 65% PPO for 1 hour, however no differences were observed when comparing 50-rpm to the FCC for the same period. As we did not control for each participants FCC, it is possible that participants in the experiments FCC was closer to 95-rpm then participants in experiment II, contributing to the lack of statistical significance. Another important consideration relates to the site of each muscle taking place above and below the location of the EMG sensor. Although at least 7 days elapsed between exercise trials, it is plausible that scar tissue as a result of the previous muscle biopsy may have influenced the EMG recording, which would not have occurred in experiment II.

In conclusion, although difference in glycogen use in type I vs type II were observed when cycling at 65- vs95-rpm for 60 minutes @55%PPO, per contraction no significant differences were observed. We did not observe significant difference between trials in EMG data and no interpretation can be made relating to EMG activity and fibre type recruitment. Our results suggest that differences in fibre type recruitment may contribute to the increased energy expenditure observed when cycling at an elevated contraction rate.

6. Chapter VI Summary, Conclusions and Recommendations

6.1. Introduction

This thesis sought to compare the effects of SIE and MICE on metabolism during and up to 24--hours post exercise. Additionally, it sought to investigate the impact of altering contraction frequency on the acute response to cycling exercise, both incremental exercise as well as continuous exercise. The main findings of this thesis are

- SIE when compared to MICE significantly increases energy expenditure up to 60-minutes post exercise, as well as greater insulin sensitivity 24-hours post exercise in healthy young recreationally active males.

- Elevated cycling cadence has the capacity to alter $\dot{V}O_2peak$ and PPO during incremental exercise in healthy young non-cyclists.

- Elevated cycling cadence resulted in increased oxygen consumption as well as energy expenditure during submaximal exercise in non-cyclists, with significantly greater carbohydrate utilisation rates at the higher cadence.

- Per contraction, fibre type specific glycogen utilisation does not appear to be significantly different between cycling at a 65- vs 95-rpm during submaximal exercise.

The findings from this research suggest that alterations in both the force and contraction frequency have the potential to produce significant acute metabolic perturbations in young healthy individuals. SIE appears to provide a time efficient means of increasing energy expenditure post exercise (0-1hr) as well as increasing insulin sensitivity 24-hours post exercise. Increasing cycling cadence during MICE may present a novel method of increasing energy expenditure during exercise for recreationally active individuals.

6.2. Impact of exercise intensity induced alterations in metabolism

The contribution of the three primary energy systems (ATP-PCr, anaerobic and aerobic systems) to metabolism during exercise is a tightly regulated process that ensures ATP production in a sequential yet overlapping fashion, that is dictated by exercise intensity as well as duration (Baker *et al.*, 2010a). Additionally, exercise has the potential to increase energy expenditure post exercise with the magnitude of this increase dictated by the intensity and duration of the exercise undertaken (Børsheim and Bahr, 2003). This thesis sought to explore the impact of alterations in force and contraction frequency on metabolism, with specific focus on energy expenditure and substrate metabolism.

6.3. Energy expenditure

By performing measurements of indirect calorimetry pre and post exercise in experiment I, it was shown that SIE has the capacity to significantly increase energy expenditure up to 60-minutes post exercise when compared to MICE, a finding that is in agreement with literature relating to the impact of SIE versus MICE on excess post exercise oxygen consumption (EPOC) (Matsuo *et al.*, 2012). The 60-minutes post exercise or "fast component" of exercise recovery ultimately relates to the body's restoration of metabolism to the pre-exercise state, such as resting ATP levels, PCr levels, pH as well as temperature (Moniz *et al.*, 2020). Therefore, it is predictable that the level of such a "fast component" would be directly related to the increase in energy expenditure during a bout of exercise. SIE typically involves "all out" maximal effort for short periods of time, interspersed with periods of recovery, with the active phase aiming to achieve maximal levels of effort (Gibala and Little, 2020). As such, this should in theory produce the maximal deviation from the resting state, albeit for short periods of time. Therefore, as shown within this thesis and elsewhere in the literature, when SIE is performed

with sufficient repetitions of high force production, the recovery needs and post exercise energy expenditure, are significantly greater than MICE up to 60-minutes following exercise cessation.

The high force associated with SIE during cycling exercise is often accompanied by increased cycling cadence. Participants are routinely told to cycle "as fast as possible" as well as "as hard as possible" in an "all out" effort. Our results from experiment II and II strongly suggest that cycling cadence can alter the metabolic response to maximal exercise independent of force. However, it is important to note that the differences observed in maximal exercise, were following incremental increases to max in exercise lasting minutes, rather than the sudden maximal force production associated with SIE that lasts seconds. Additionally, other researchers did not find that cadence impacted maximal values during an incremental exercise test in trained (Coast *et al.*, 1986; Brickson *et al.*, 2022) or untrained individuals (Swain and Wright, 1997; Beneke and Alkhatib, 2015; Hill and Vingren, 2020), which would suggest that cadence may only impact on interval exercise utilising submaximal intensity for repeated bouts. An additional consideration is that significant differences in $\dot{V}O_2peak$ were only observed in experiment II, with significant differences in PPO observed in both experiment II and III. It is unclear as to why this occurred, however future research that aims to investigate the influence of cadence on VO_2peak alone may look to perform additional incremental tests to ascertain if a true impact of cadence on VO_2peak exists. The participants in experiment III had a slightly higher $\dot{V}O_2peak$ than those in experiment II, and as deviations in submaximal $\dot{V}O_2$ are less as a result of cadence alterations in trained individuals (Zorgati *et al.*, 2015), this may explain the lack of a significant result in maximal exercise. Additionally, using a % of $\dot{V}O_2peak$ or PPO alone as an anchor for exercise intensity has previously been criticised by others as when exercise is performed at a given % of a maximal value, significant differences in oxygen kinetics and lactate concentrations have been observed between similarly trained individuals (Meyer *et al.*, 1999; Scharhag-Rosenberger *et al.*, 2010). Were a % of $\dot{V}O_2peak$ alone to be

used in future research, a verification bout of exercise performed prior to the exercise trials may be beneficial to establish the individuals % $\dot{V}O_2$peak a given PPO elicits (Jamnick *et al.*, 2018). Additionally, monitoring oxygen consumption during the exercise bout and adjusting the power output as necessary, would increase the ability of research's to ensure a participant is exercising within the desired % $\dot{V}O_2$peak (Jamnick *et al.*, 2020). The lack of such verification bouts, or monitoring of intensity in experiment II and III may explain in part the divergent results between experiments with regard to $\dot{V}O_2$peak and EMG.

Of note from our results was the 15-17% increase in energy expenditure over 60-minutes of continuous exercise when exercising 95- vs 65-rpm. When controlling for the number of contractions, 25% greater energy expenditure per contraction was observed at the lower contraction speed. However, for a given period, a higher contraction rate will induce greater total energy expenditure, as a greater number of contractions are performed. Within experiment II and II, the absolute power was consistent between each trial, however the force per contraction was significantly higher when exercising at the lower contraction rate, a well-established effect of altering cycling cadence at a fixed power output (Ettema and Loras, 2009). This is a consistent finding from the limited research conducted on untrained individuals related to elevated energy expenditure at higher cadences. However short (3-10 minutes) or increment exercise performed at different cadences has typically been adopted (Gaesser and Brooks, 1975; Coast, J. Richard and Welch, Hugh G., 1985; Marsh *et al.*, 2000b; Foss and Hallen, 2004; Saltvedt *et al.*, 2015; Zorgati *et al.*, 2015; Brennan *et al.*, 2019). Although this may provide an insight into energy expenditure at a given exercise intensity, extrapolating results from short duration bouts of exercise to bouts of longer duration can be problematic due to the effects of cardiovascular drift, which has been shown to be exaggerated by cycling exercise (Nassis and Geladas, 2002) as well as elevated cycling cadence (Kounalakis and Geladas, 2012). Additionally, it is possible that other physiological systems contribute to the increase in energy

expenditure and the contribution of respiratory and smooth muscle activation, while small, should not be discounted (Lorenzo and Babb, 2012). Future studies may look to control for, or quantify the contribution of auxiliary muscles to energy expenditure when performing cycling exercise at different cadences.

As health-maintaining moderate intensity aerobic exercise is recommended to be at least 30-minutes in duration 5-times per week (Bull *et al.*, 2020), it is surprising how few studies have manipulated cadence in untrained individuals during exercise bouts lasting at least 30-minutes. Energy expenditure and cycling cadence have long been studied in cyclists (Ettema and Loras, 2009), and although physiological and biomechanical differences exist between trained cyclists and untrained individuals, our findings suggest that similarities exist between the groups. Our findings suggest that the elevated energy expenditure observed by others during short duration exercise when untrained cyclists cycle at high versus low cadence, persists up to at least 60-minutes of exercise. The results from experiment II and III also suggest that recreationally active participants were most efficient at the lower cadence, which is a well-established observation in cyclists (Ettema and Loras, 2009). The magnitude of the decrease in efficiency at 95-rpm may have been less had a higher %PPO been utilised during the submaximal exercise, as Coast et al (1985) demonstrated a linear increase in the most efficient cycling cadence with increasing workloads. Collectively, our findings may present a novel method for recreationally active young healthy individuals to increase energy expenditure during exercise. This is particularly relevant should a limited amount of time be available for them to exercise, as our results show total energy expenditure will be higher when cycling for 60-minutes at 95-rpm when compared to 65-rpm. As untrained cyclists have been shown to adopt a cadence of 50-80-rpm when cycling at moderate intensity (Ansley and Cangley, 2009), adopting a cadence of 95-rpm will possible be a novel experience for them during this form of exercise.

6.4. Substrate utilisation

Our findings suggest that the increase in energy expenditure observed when cycling at 95-rpm appears to be as a result of an increase in carbohydrate oxidation, with no significant difference in fat oxidation rates when compared to cycling at 65-rpm. This increase was observed in combination with higher blood lactate levels at all time points during the submaximal exercise trial. From our results it is apparent that increasing cycling cadence at a fixed power output will result in an increase in exercise intensity (%$\dot{V}O_2$peak). A large body of research exists that supports the concept whereby carbohydrate utilisation will increase as exercise intensity increases (Romijn *et al.*, 1993; Van Loon, L. J. *et al.*, 2001; Hawley *et al.*, 2015). However, this is traditionally achieved with increases in force rather than contraction frequency, wherein the force per pedal stroke is less at a fixed power output as can be achieved when using an electronically braked cycle ergometer. Increasing the contraction rate simultaneously decreases the force but increases the contraction speed. It has been suggested by previous research that this increased contraction speed will result in increased recruitment of type II fibres (Osborne and Schneider, 2006). However, the results from experiment III suggest that per contraction, fibre type differences in glycogen utilisation do not exist when cycling at 95- versus 65-rpm. Although this finding is in agreement with others (Gollnick *et al.*, 1974; Vøllestad *et al.*, 1984), however our findings do not definitively show that fibre type recruitment does not explain, in part, the observed differences in metabolism. Previously mentioned methodological issues need first been considered as the reduced number of fibres counted due to poor image quality undoubtedly limited our ability to answer our research question in this regard.

Significant differences in interstitial glucose during exercise were observed in experiment I, with both SIE and MICE resulting in a decrease in glucose concentrations when compared to the control trial. Additionally the decrease was significantly greater during MICE when compared to SIE. In healthy individuals, exercise has the potential to decrease blood glucose

concentrations (Erickson *et al.*, 2017). Evidence also suggests that vigorous exercise of a supramaximal nature, has the potential to increase blood glucose in healthy individuals which is suggested to be as a result of increased hepatic glucose release stimulated by counter regulatory hormones during the exercise bout (Kjaer et al., 1990). It is worth noting that research suggests the accuracy of CGM devices is reduced during exercise, with changes in temperature, pH and blood flow suggested as potential reasons for the reduced accuracy (Muñoz Fabra *et al.*, 2021).

Due to the previously mentioned methodological issues relating to the reduced accuracy of indirect calorimetry during exercise with a large glycolytic component (Scott, 2005), it was not possible to calculate substrate utilisation during exercise in experiment I.

6.5. Post exercise metabolism

The impact of exercise intensity and duration on energy metabolism in the hours post exercise is well studied in healthy individuals (Moniz *et al.*, 2020). EPOC, represents the recovery level required post exercise and has been shown to be influenced by the intensity and duration of an exercise bout, with higher intensity or longer duration eliciting greater EPOC (Moniz *et al.*, 2020). The renewed interest in interval exercise, particularly exercise of a supramaximal nature such as SIE, has been driven by the suggested similar, if not potentially greater increase in $\dot{V}O_2$peak, that can potentially be gained from SIT when compared to MICT (Macinnis and Gibala, 2017). Our research sought to investigate the acute metabolic response, as inconsistencies exist in the literature relating to energy metabolism in the 24-hours post SIE when compared to MICE in healthy individuals. The findings from experiment I of this thesis suggests that SIE has the potential to significantly increase energy expenditure up to 60-minutes post exercise, however no differences were found 24-hours post exercise. Such findings are in agreement with others who showed evidence of greater EPOC in the 0-60

minutes post SIE when compared to HIE or MICE (Matsuo *et al.*, 2012; Tucker *et al.*, 2016; Moniz *et al.*, 2020). As previously described, SIE should aim for intervals of sufficient intensity as to rely predominantly on anaerobic metabolism, with repeated efforts taxing the glycolytic pathways and resulting in greater glycogen depletion when compared to MICE of a similar total duration (Macinnis and Gibala, 2017). Our findings of decreased carbohydrate oxidation and increased fat oxidation following SIE when compared to MICE, suggest that the fast component of recovery was being fuelled by fat metabolism and that glycogen resynthesis was being prioritised. However post exercise glucose levels as assessed using CGM suggest that MICE will significantly decrease glucose levels post exercise (0-60 minutes) when compared to SIE. This may in part be as a result of a lag in the decrease of the counter regulatory hormones associated with SIE that result in increased hepatic glucose production (Kjaer *et al.*, 1990). Additionally, the glucose lowering effect of MICE has been shown to be present for up to 24 hours post exercise in healthy individuals when assessed using CGM (Dubose *et al.*, 2021). However, we observed only significant differences at 60 minutes post exercise with no significant differences for the remaining time the CGM was worn.

6.6. Insulin sensitivity

Exercise has previously been shown to be a potent method to increase insulin sensitivity (Mann *et al.*, 2014), however, the impact of SIE vs MICE in healthy individuals on acute changes in insulin sensitivity are unclear (Jelleyman *et al.*, 2015). I found that insulin sensitivity in healthy individuals was significantly higher 24 hours post SIE when compared to MICE, when measured using a hyperinsulinemic euglycemic clamp to assess insulin stimulated glucose disposal. Although previous research has shown that insulin sensitivity can be increased following 2 weeks of SIT versus MICT (Richards *et al.*, 2010; Hovanloo *et al.*, 2013), to the best of our knowledge, at the time of writing, this is the first time a single bout of SIE have significantly increased insulin sensitivity 24 hours post exercise when compared to MICE.

Although we believe this is a noteworthy finding, it must be highlighted that this was exercise performed in a laboratory setting with research providing supervision and motivation for participants. Additionally the participants were healthy individuals who were familiar with high intensity exercise as part of performing recreational exercise. While both considerations need to be acknowledge, future research may aim to utilise SIE to potentially increase insulin sensitivity in individuals who have developed insulin resistance. That said, research that has utilised HIT and found it beneficial has typically not been performed in real world settings (Gray *et al.*, 2016). Additionally some participants have found HIT to cause greater discomfort, displeasure and psychological distress when compared to MICT (Blanchard *et al.*, 2001; Hall *et al.*, 2002), while others found increased enjoyment and a preference for HIT (Jung *et al.*, 2015). While healthy participants have been used in the past as well as in this study, future research relating to insulin sensitivity should target populations where the impact of such findings would carry the most weight, such as individuals with pre diabetes, as well as randomised control trials of longer duration to assess if the acute response translated to a decrease risk of disease progression.

6.7. Muscle activation:

EMG analysis allows for the quantification of the level of activation of a muscle through surface electrodes and is regularly used to assess the contribution of various muscles to cycling exercise (Ericson *et al.*, 1985). For both experiment II and III of this thesis, EMG analysis was performed during the submaximal exercise bouts with the activation level of the vastus lateralis presented as iEMG. For both studies, the iEMG was higher when cycling at 95-rpm but only reached statistical significance in experiment II (experiment III p=0.074). Small but significantly greater muscle activation as assessed using iEMG, is a consistent finding when cycling at higher contraction frequencies (Tetsuo *et al.*, 1996; Farina *et al.*, 2001; Sarre and Lepers, 2005; Bessot *et al.*, 2008). When calculated per contraction, the neuromuscular activity

was greater when cycling at 65-rpm which could be explained by the longer duration of each pedal stroke at that rate, wherein the torque exposure is greater. Therefore, the total activation being higher at the 95-rpm rate may be as a result of a greater number of contractions being performed over the course of the 60-minute trial.

In addition to the total activation of the vastus lateralis during exercise, we quantified the MPF of the iEMG, which has been suggested as an estimate of the motor unit action potential velocity within the active contracting muscle (Hagg, 1992; Borrani *et al.*, 2001). In experiment II, we observed significantly greater MPF during the 65-rpm trial, while no significant differences were observed in experiment III. It is unclear as to why divergent results are present between studies, however subtle differences in the participants used in each study may, in part, explain the results. Participants in experiment III had a muscle biopsy performed before each submaximal exercise trial, with the EMG recording being performed on the opposite leg. This may have led to a greater activation of the leg that was not biopsied during each trial which may have skewed the iEMG results when compared to experiment II. A limitation of both experiment II and III is that iEMG analysis was only performed on one leg during exercise, and although the analysed leg was randomised for each participant and trial, potential error may have occurred in the iEMG data collected in experiment III. Although only observed in experiment II, significantly higher MPF at the lower cycling cadence is in agreement with others, studies which found greater MPF when healthy non cyclists cycled at 40- vs 80-rpm @60%$\dot{V}O_2$peak (Kounalakis and Geladas, 2012). Such a finding in non-cyclist is divergent from results in well trained cyclists which suggest no difference in MPF when cycling at 50- vs 110-rpm (Sarre and Lepers, 2005).

The absolute change in PAS stain was not significantly different between trials in type I fibres but type II fibres were significantly reduced following the 95- rpm trial when compared to the 65 -rpm trial. However, per contraction no significant difference existed in the PAS stain,

suggesting that the greater total number of contractions performed when cycling at 95-rpm produced the significant difference observed. Additionally, when the same number of contractions were performed at 65- rpm and 95-rpm (41mins@95-rpm vs 65- rpm trial), although glycogen use was greater at 95-rpm, it did not reach a level of statistical significance.

The lack of statistical difference in experiment III in relation to MPF and the PAS stain of type I and type II fibres presents challenges for the data interpretation. We had hypothesised that the significantly different iEMG data from experiment II would be replicated in experiment III, as the same participant demographics and exercise protocol were being performed. As this was not the case, it is not possible to establish if the change in fibre type utilisation of glycogen patterns when cycling at 65- or 95-rpm was related to the iEMG data recorded during the trial. Had the same participants repeated experiment III, it may have been possible to establish why differences were observed in the iEMG data, however, muscle biopsies being performed was a deterrent for a number of participants from that experiment. Additionally, as previously stated, the additional muscle biopsies may have produced differences in how participants recruited the muscle during the exercise trial in comparison to experiment II.

6.8. Conclusions

Small amounts of physical activity have been shown to be beneficial for health with the current minimum recommendation of 150-minutes per week, representing a mere 1.5% of the minutes in a week. Even so, 40% of adults do not meet this recommendation (Marques *et al.*, 2015), with a "lack of time" routinely stated as a reason for not exercising regularly. The key finding of this thesis, has provided evidence that the time saving form of exercise that is presented by SIE can have beneficial effects on energy expenditure and insulin sensitivity in healthy individuals. Additionally, increasing cycling cadence appears to be a novel method of increasing energy expenditure during a bout of cycling exercise of a fixed duration. Both

findings established through a study design incorporating the use of robust physiological measures provide strong evidence that the results may be applicable to similar healthy populations. In this time poor modern world, any and all time saving exercise options may be of interest to individuals who wish to maximise the time they choose to spend being physically active as it may promote greater physical activity engagement and adherence.

6.9. Limitations

There are several limitations of this thesis that warrant consideration relating to the findings observed as well as their interpretation. These include limitations relating to research design and methodology that place a constraint on the conclusions and implications of each study's findings.

A limitation of this research is that only male participants were studied in each experiment. The recruitment of participants for experiment I, II and III was open to females, however, only 4 well trained females expressed interest in study II. The lack of female participants limits the interpretation of the results to just males, as although similarities exist between females and males, extrapolating our findings to females is not a sound method and similar research performed on females is required. Throughout each study of this thesis, indirect calorimetry was utilised to investigate the impact of alterations in exercise intensity on the metabolic response, and allowed for the calculation of energy expenditure as well as substrate utilisation (Hill and Lupton, 1923). A notable limitation of the use of indirect calorimetry is its underestimation of energy expenditure during exercise that has a large contribution from glycolytic pathways (Scott, 2005). As highlighted in detail previously, traditional calculations of substrate utilisation from indirect calorimetry measurements during exercise, are less accurate when the exercise has a large anaerobic component, as is intended during SIE. It is a limitation of this thesis that additionally measures were not performed to establish the

metabolic work performed during the SIE bout. However, the focus of experiment I was to investigate the impact of SIE when compared to MICE on post exercise metabolism, as this is an area of research that has not been well characterised.

Additionally, a limitation of experiment I relates to the unmatched nature of the SIE and MICE exercise trials, with mechanical work performed presented to demonstrate the significantly different time spent in active exercise during SIE compared to MICE. However, it is generally accepted that low volume SIE, as was performed within experiment I, will result in lower exercise energy expenditure when compared to MICE (Gibala and Little, 2020). This may potentially skew the findings to demonstrate an acute increase in energy expenditure that when calculated over a longer duration may not exist. An additional limitation of experiment I is a lack of precise control of participants diet between trials, with participants asked to create a food diary from 24-hours before, to the end of the first trial, and replicate this for subsequent trials. This may have resulted in variation between trials with participants consuming more or less carbohydrate following exercise, which may have altered glycogen resynthesis rates (Hargreaves and Spriet, 2020).

It is a limitation of this thesis that the iEMG data was not normalised to a maximal voluntary contraction (MVC) or a standardised bout of continuous cycling exercise. This limits our ability to interpret the EMG data and future studies should aim to normalise EMG during data processing. Although a considerable amount of time was spent optimising the antibody dilution ratios for fibre type identification, we were only successful in identifying type I fibres. Although this did not hinder our fibre type quantification process and the delineation between type I and type II, we feel it is important to note as possible differences between type IIa, type IIx and potential hybrid fibres was not established as part of this thesis. Subtle differences in hybrid fibre recruitment may provide insight into interpreting the difference in energy expenditure and future research should aim to prioritise this aspect of the fibre type analysis.

Additionally, in relation to PAS staining to quantify glycogen use in type I and type II fibres, it is a limitation of experiment III that only 42 ± 15 fibres were deemed suitable for glycogen quantification. Future research may wish to prioritise the mounting of biopsy samples in tissue tek gel (as described in the methods of experiment III) to ensure the striation of the muscle is aligned correctly (possible aided by a microscope). Additionally, using a single fibre dissection technique and quantifying total glycogen use in each fibre type may also be investigated. As PAS staining is semi quantitative, adopting such quantitative methods may provide greater accuracy in determining true glycogen use in each fibre type.

6.10.   Recommendations

When performing exercise, adopting cycling exercise results in an almost infinite amount of potential exercise protocols that can vary in the force, contraction rate, intensity of exercise, intervals, rest periods and total time of exercise performed. Although we believe this thesis has contributed to the existing knowledge relating to the acute response to exercise of different intensities and contraction frequencies, it is evident that further research is required. While it appears that SIE exercise has the potential to produce similar, if not superior changes in health-related outcomes, this form of exercise is challenging and may not be tolerated by all as part of a regular exercise regime. Further work may aim to investigate interval exercise of lower intensity than SIE, but with a greater number of repetitions, to mimic the acute changes observed within this study. Additionally, the free-living nature of our participants in experiment I allowed for "real world" data to be collected 24-hours post the exercise bout, however it would be interesting to view results had the food intake of participants been controlled in detail.

The divergent results relating to iEMG from experiment II and III suggest that further work is required to assess the potential use of iEMG as a marker for muscle fibre recruitment patterns

during cycling exercise. It would be interesting to conduct research where iEMG analysis and muscle biopsies were performed during exercise on alternate days to avoid the biopsied leg potentially influencing the bout of exercise. Although this would increase the study involvement of participants and scope of the study, significant findings in relation to the MPF of the iEMG data, as observed in experiment II, coupled with fibre type recruitment assessment may provide a definite answer to iEMG and fibre type recruitment measurement. Additionally, future work may wish to focus on the significantly greater impact cycling at 95-rpm on reducing glycogen content post exercise. As changes in glycogen have been shown to be correlated with insulin sensitivity (Bogardus *et al.*, 1983) this may be a novel area of research whereby elevated contraction frequency may provide a time efficient means of increasing insulin sensitivity. Finally, contraction frequency in the context of this thesis was confined to cycling and similar novel studies may be performed using other traditional modes of exercise such as running or rowing.

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Appendices

Appendix 1: Ethical approval Experiment I

Ollscoil Chathair Bhaile Átha Cliath
Dublin City University

DCU

Dr Donal O'Gorman
School of Health and Human Performance

21st May 2014

REC Reference: DCUREC/2014/098

Proposal Title: **Crosstalk between muscle and adipose tissue: effect of
exercise intensity**

Applicants: **Dr Donal O'Gorman, Dr Francois Crampes, Dr Isabelle De
Glisezinski**

Dear Donal,

Further to review, the DCU Research Ethics Committee approves this research
proposal. Materials used to recruit participants should note that ethical approval for
this project has been obtained from the Dublin City University Research Ethics
Committee. Should substantial modifications to the research protocol be required at a
later stage, a further submission should be made to the REC.

Yours sincerely,

DCU
Research & Innovation

Dónal O'Mathúna

Dr. Donal O'Mathuna
Chairperson
DCU Research Ethics Committee

Appendix 2: Ethical approval Experiment II

DCU

Dr Donal O'Gorman
School of Health and Human Performance

3rd May 2016

REC Reference:	**DCUREC/2016/054**
Proposal Title:	**The influence of contraction frequency on oxygen uptake and power output**
Applicant(s):	**Dr Donal O'Gorman, Mr Enda Murphy, Dr Javier Monedero & Mr John Noone**

Dear Donal,

Further to expedited review, the DCU Research Ethics Committee approves this research proposal.

Materials used to recruit participants should note that ethical approval for this project has been obtained from the Dublin City University Research Ethics Committee.

Should substantial modifications to the research protocol be required at a later stage, a further amendment submission should be made to the REC.

Yours sincerely,

Dónal O'Mathúna

Dr Dónal O'Mathúna
Chairperson
DCU Research Ethics Committee

DCU
Research & Innovation

Appendix 2: Ethical approval Experiment III

Ollscoil Chathair Bhaile Átha Cliath
Dublin City University

Dr Donal O'Gorman

School of Health and Human Performance

28th June 2017

REC Reference:	**DCUREC/2017/087**
Proposal Title:	**The influence of contraction frequency on muscle cell signalling and mitochiondrial function**
Applicant(s):	**Dr Donal O'Gorman,** Dr Javier Monedero, Dr Noel McCaffery, Mr Enda Murphy, Mr John Noone, Ms Rajan Priyadarshini

Dear Colleagues,

Further to a full committee review, the DCU Research Ethics Committee approves this research proposal.

Materials used to recruit participants should note that ethical approval for this project has been obtained from the Dublin City University Research Ethics Committee.

Should substantial modifications to the research protocol be required at a later stage, a further amendment submission should be made to the REC.

Yours sincerely,

Dr Dónal O'Gorman
Chairperson
DCU Research Ethics Committee

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