Characterizing Sex Differences in Skeletal Muscle Anaerobic Metabolism during an Acute Bout of High Intensity Interval Exercise

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis including any required final revisions, as accepted by my examiners.

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Abstract

INTRODUCTION: Exercise is known to improve insulin sensitivity (IS); however, its effectiveness may be absent or blunted in women, particularly following high intensity interval (HII) training. Sex differences in anaerobic metabolism during an acute bout of HII exercise have not yet been established and may underpin the lack of effect of HII training on IS in women. Furthermore, it is unclear whether the standardized HII exercise protocol induces similar exercise intensities in men and women which may confound the research findings. **PURPOSE:** The purpose of the proposed research was to examine whether sex influences the exercise response and/or anaerobic metabolism during an acute bout of high intensity interval exercise. **METHODS:** Twenty-four young, healthy, recreationally active males (n=12) and females (n=12) were recruited for this study. Men and women were matched according to their maximum aerobic capacity (VO_{2peak}) relative to their fat-free mass ($mL\ O_2 \cdot KgFFM^{-1} \cdot min^{-1}$). The exercise protocol consisted of a 5-min warm up, followed by 10 intervals of 60 sec at high intensity (90% HR_{max}), interspersed with 60 sec at low intensity (50 Watts) and ended with a 5 min cool down. Throughout the exercise bout, heart rate (HR) and rating of perceived exertion (RPE) using the Borg Scale were recorded every minute and blood lactate was recorded every 3 minutes. Muscle biopsies were taken from the *vastus lateralis* muscle before and after the exercise bout. Muscle samples were analyzed for protein content of metabolic enzymes [phosphorylated pyruvate dehydrogenase (PDH) E1α, and phosphorylated creatine kinase (CK)] and Periodic Acid Schiff (PAS) stained for determination of muscle glycogen utilization. Indirect calorimetry was used to compare indices of exercise intensity and whole-body fuel utilization between men and women during the exercise bout. **RESULTS:** Sex had no effect on HR (p=0.17) or RPE (p=0.66) throughout the exercise bout; however, %target HR during the high intervals was higher in

women (p=0.002). Women exercised at a higher relative %VO_{2peak} during the warm up (p<0.0001), high intervals (p=0.011) and cooldown (p=0.005), but not during the low intervals (p=0.25). Women also worked at a higher wattage during the low intervals when expressed relative to kg lower body fat-free mass (LB FFM) (p<0.0001). During the high intervals, men worked at a higher absolute wattage (p<0.001) and relative wattage when expressed relative to kg LB FFM (p=0.02). Men also expended more energy during exercise (p=0.001) but when expressed relative to kg FFM, there was no difference in energy expenditure between the sexes (p=0.13). Sex had no effect on blood lactate throughout the exercise bout (p=0.35) however lactate AUC was higher in men (p=0.007). Glycogen utilization and phosphorylation status of PDHE1α and CK decreased in both men and women during exercise (p<0.0001, p=0.002 and p=0.002, respectively), with no difference between the sexes. Elevated RER values obtained from indirect calorimetry resulted in invalid whole-body substrate oxidation estimates.

CONCLUSION: Markers of HEPT and glycolysis did not differ between men and women, however the greater lactate AUC in men suggests that men relied to a greater extent on anaerobic metabolism during exercise. Importantly, using the standard HII exercise protocol caused women to exercise at a higher %VO_{2peak}, which was mostly likely due to the difference in the relative intensity of the low intervals. Future work should consider using a relative workrate during low intervals to better equate exercise intensity between the sexes.

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Chapter One: Literature Review

1.0 Introduction

The prevalence of type 2 diabetes (T2D) is at epidemic levels worldwide and is expected to continue to increase in both adults and children[1]. In 2018, the projected prevalence of diabetes globally was estimated to reach 642 million people by 2040, with 90% of those cases being T2D[2]. The main recommendation to prevent the development of T2D is adopting a healthy lifestyle through a combination of diet and exercise[1]. However, a large majority of the research that has investigated the effectiveness of exercise training at eliciting improvements in IS has not considered sex and/or only included women in their sample. The small number of studies that have controlled for sex have mostly involved interval training programs, and their findings suggest that men and women may respond differently to interval training. More specifically, improvements in IS seem to be blunted in women following interval training[3–5].

The link between sex differences in IS and interval training may be related to sex differences in anaerobic metabolism, specifically the high energy phosphate transfer (HEPT) system metabolites ADP and AMP. Increased concentrations of ADP and AMP in the muscle can activate the enzyme AMPK[6]. AMPK is involved in both contraction-mediated insulin-independent and contraction-mediated insulin-dependent GLUT 4 translocation[6–8]. Sex differences in AMP production during interval exercise have not been widely examined. With women being able to initiate aerobic metabolism more quickly at the onset of exercise[9,10] and relying to a greater extent on fat substrates during exercise[11–20], the likelihood that sex may influence AMP and/or the ratio of AMP:ATP during exercise is possible. Specifically, if women have a lesser reliance on the HEPT system at the onset of exercise there may be less of a disturbance in the AMP:ATP ratio leading to a lesser activation of AMPK. Over time, a lesser

activation of AMPK may result in less improvement in skeletal muscle insulin signalling and whole-body IS. Support for this theory comes from previous research showing that AMPK activation during aerobic exercise is higher in men than women[21].

The predominant form of interval training used in clinical/pre-diabetic/diabetic populations is HII training. Therefore, examining the effect of sex on fuel metabolism during a single bout of HII exercise may improve our understanding as to why IS does not improve in women following a period of HII training. Thus, this thesis was conducted to examine how sex influences anaerobic fuel utilization and metabolism during HII exercise to gain insight into possible explanations for the blunted improvements in IS seen in women in response to HII training.

1.1 Fuel Utilization during Exercise

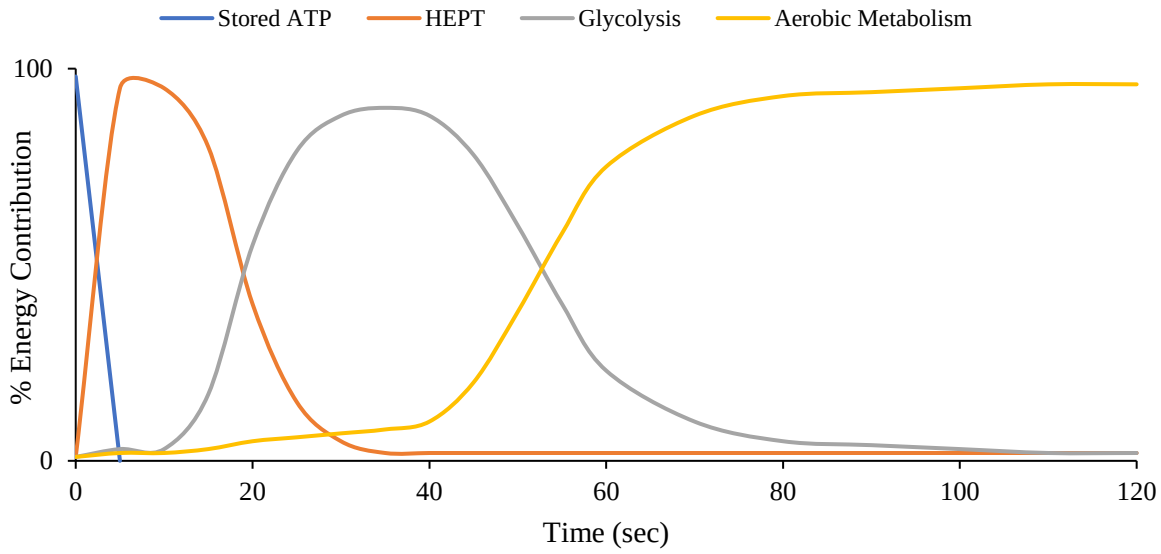
Energy is supplied to working skeletal muscle primarily by combustion of carbohydrates (CHO) and fats[22]. The relative contribution of each substrate is influenced by both the duration and intensity of the activity, with higher exercise intensity increasing the reliance on CHO metabolism[23] and longer exercise duration at a given intensity increasing the reliance on fat metabolism[22–24]. To efficiently support the energy requirements of the muscle, CHO are stored as glycogen in the liver and muscle[22], and fat is stored as intramyocellular lipids (IMCL)[22] in the muscle [with the majority of lipids in IMCL being triglycerides (TG)[25]], and as triglycerides in adipose tissue[22,26]. Protein is the third and final energy yielding substrate, but it contributes only a very small amount (typically 2-5%)[22] to energy production during exercise as its main role is to help the muscle repair and recover[22,26]. While CHO and fat are the main contributors to energy production during exercise, at the onset of exercise and/or at the onset of each interval when oxygen is limited, anaerobic fuel metabolism is the main mechanism for generating ATP[22,27]. Anaerobic metabolism can sustain ATP production until the body has had the time to make

sufficient physiological vascular changes to adapt to the exercise stimulus. These vascular changes allow for oxygenated blood to be shunted towards the muscle to a greater extent compared at to the onset of exercise. The increased delivery of oxygen to the muscle will then allow for aerobic metabolism to become the primary mechanism of ATP production[22].

1.1.1 Order of Predominating Energy Generating Pathways Upon the Onset of Exercise

At the onset of exercise, the muscle has a very small amount of stored ATP which is rapidly utilized[22], necessitating other ATP-generating systems to upregulate and sustain ATP production during exercise. These energy-generating pathways are the HEPT system, glycolysis, and aerobic metabolism (fatty acid oxidation, tricarboxylic acid cycle, and electron transport chain). The relative contributions of each of these pathways depends on many factors including exercise intensity[22–24,27–30], exercise duration[22,23,27,28] and biological sex[12,20,31,32].

It has been well established that the three main energy generating pathways (HEPT system, glycolysis and aerobic metabolism) each predominate at different time points throughout a moderate intensity continuous (MIC) exercise bout. Figure 1 below provides a simplified overview of when each ATP generating pathway predominates during the first 120 seconds of a MIC exercise bout at ~65%VO_{2peak}. Once steady state is reached around the 2-min mark, the relative contributions shown at that time point can be sustained for the remaining duration of the exercise bout[22] (typically ~90 minutes).



***HEPT Reactions**

$$ADP + PCr + H^+ \xleftrightarrow{\text{Creatine Kinase}} ATP + Cr$$

$$ADP + ADP \xleftrightarrow{\text{Adenylate Kinase}} ATP + AMP$$

$$AMP \xrightarrow{\text{AMP Deaminase}} IMP + NH_3$$

****Glycolysis Reactions**

$$Glucose + 2NAD^+ + 2ADP + 2P_i \longrightarrow 2Pyruvate + 2NADH + 2H^+ + 2ATP$$

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

*****Aerobic Metabolism Reactions**

$$NADH + 3ADP + \frac{1}{2}O_2 + 3P_i + H^+ \longrightarrow NAD^+ + 3ATP + H_2O$$

$$FADH_2 + 2ADP + \frac{1}{2}O_2 + 2P_i \longrightarrow FAD + 2ATP + H_2O$$

Figure 1: Simplified overview of when each ATP generating pathway predominates during the first 120 seconds of a MIC exercise bout at ~65%VO_{2peak} and the reactions that occur within in each system. This is subject to slight changes based on the intensity and mode of exercise as well as training status of the individual.

1.1.2 Fuel Utilization During MIC Exercise

Substrate utilization patterns during MIC exercise have been well established and are influenced by both the intensity and duration of the exercise[22]. At low-to-moderate exercise intensities (aerobic conditions), TG from adipocytes and IMCL stores are the main source of fuel[22–24,27–30]. Fat oxidation and exercise intensity are positively associated up until ~65% VO_{2peak} after which fat oxidation rates plateau and decline[22–24,29]. Fat oxidation is limited at higher exercise

intensities due to lactate accumulation that can lead to inhibition of adipose tissue lipolysis[33]. Consequentially, CHO (from glucose and glycogen) utilization predominates at higher exercise intensities[22–24,27]. Additionally, as exercise intensity increases there is a concomitant increase in blood flow peripherally towards the muscle, and therefore a reduced blood flow to the liver[23] and adipose tissue[23]. As a result, there is an increased reliance on substrates stored within skeletal muscle (i.e. muscle glycogen and IMCL) and a decreased reliance on substrates originating from the liver (i.e. glucose), adipose or other tissues[23].

Duration of exercise also plays a role in substrate utilization. At a given exercise intensity, fuel stores in the muscle (phosphocreatine, muscle glycogen, and IMCL) are the preferred substrate sources due to their proximity to the site of muscle contraction[23]. As exercise duration increases, muscle glycogen and IMCL stores diminish, eventually causing there to be a greater reliance on free fatty acids (FFA) derived from adipocyte lipolysis and glucose derived from hepatic glycogenolysis and gluconeogenesis[22,23,34]. As glucose from the plasma is taken up by the muscle, hormones are secreted to prevent the development of hypoglycemia by maintaining glucose concentrations at homeostatic levels. This mechanism is known as the counterregulatory response and the secreted hormones (epinephrine, norepinephrine, cortisol, growth hormone, and glucagon) act to ensure blood glucose levels are maintained during, and post exercise[35]. Although glucose contributes significantly to ATP production during MIC exercise, given that FFA are more energy dense than glycogen[22,26] and TG stores are substantial[22,26], once a steady state during MIC exercise has been established, fat represents an almost limitless fuel supply to support exercising muscle energy demands.

1.1.3 Fuel Utilization and Metabolism during Interval Exercise

Recently, interval exercise has increased in popularity as research suggests that it can elicit similar health benefits to MIC exercise in much less time. There are two main types of interval exercise: HII exercise and sprint interval (SI) exercise[36]. Both HII and SI exercise consist of a series of repeated bouts of intense exercise, interspersed with periods of rest or active recovery at very low intensities. More specifically, HII exercise protocols involve performing multiple, near maximal intensity intervals interspersed by periods of low intensity active recovery with similar work:recovery ratio durations[36]. SI exercise protocols however, involve performing multiple supramaximal intensity intervals of shorter duration interspersed with longer recovery intervals[36]. The durations of HII and SI work intervals range anywhere from 8 seconds to 4 minutes. Most SI exercise studies use work intervals of ~20 seconds and most HII exercise studies use intervals of ~60 seconds[37].

1.1.3.1 Substrate Utilization and Metabolism During HII Exercise

Like MIC exercise, the ATP required to sustain HII exercise is generated through the HEPT, glycolytic and aerobic metabolism systems; however, fuel utilization during HII exercise has not been studied as extensively as fuel utilization during MIC exercise. While more of a resistance-type protocol, not HII exercise, one study involving 30 seconds of repeated plantar flexion exercise at ~30% maximal voluntary contraction with a 40-degree range of motion at a rate of 2-3 Hz (maximal rate) was conducted[38]. This study is able to give insight into the time course of metabolic changes that occurred at the muscular level both during and post intense exericse[38]. This study found that phosphocreatine (PCr) concentration immediately decreased at the onset of exercise and within 20 seconds, less than 20% of original PCr content was left[38]. Furthermore, ADP, AMP and P_i also increased immediately and leveled off within 10-15 seconds, which

coincided with a peak in both glycolytic and glycogenolytic rates at the 15-second mark, suggesting exhaustion of the HEPT system, and a shift towards glucose (glycogen) utilization at this point[38]. The progressive decrease in pH throughout the 30 seconds likely contributed to the slight decrease in glycogenolytic and glycolytic rates seen towards the end of the exercise due to inhibition of PFK[22,26]. Overall, it was determined that within 9 seconds of the onset of exercise, aerobic ATP supply reached ~85% of its maximal capacity and remained at this level for the remainder of the exercise[38]. Although this study did not include subsequent intervals, post-exercise metabolic measures revealed that within 2 minutes of recovery, PCr concentrations were almost at their original levels whereas it took much longer (~8 min) for pH to return close to normal[38]. These findings outline how the HEPT system is the most active during the first 5-10 seconds and then glycolysis takes over.

Adequate recovery duration between intervals is essential for full PCr resynthesis for the HEPT system to be used to its full capacity on subsequent intervals. Recovery periods that are too short in duration, or too high of an intensity can impede PCr resynthesis[39,40] and can hinder the HEPT system's ATP contribution since the re-amination of IMP to ATP does not occur during the intervals[41–43], but during the recovery periods only[41,43]. Adequate recovery periods are also essential for lactate clearance since shorter recovery periods are associated with a decrease in the amount of lactate cleared[40,44,45]. Therefore, during HII exercise, the duration of rest or active recovery periods influences the relative contribution of anaerobic and aerobic metabolism to ATP production.

Importantly, the HEPT system can only supply a limited amount of ATP during each interval, therefore, glycogen is also significantly relied upon during HII exercise. The studies that have measured glycogen utilization during HII exercise have found muscle glycogen to decrease by

~40-50% post exercise[46–48]. Additionally, in animals, one group found that HII exercise upregulates the transcription factor *Hif*-1α[49] which is known to increase expression of proteins related to glycolysis and lactate transporters[50], increasing the capacity for glycogen breakdown. Together these findings demonstrate that muscle glycogen is heavily relied upon during HII exercise.

Interestingly, the notion that fat oxidation is negligible at intensities above ~85% VO_{2max}[51,52] (or ~90% VO_{2max} for runners[52]) was challenged by a study that found that fat oxidation during interval exercise at 85-95% VO_{2max} contributed ~16% and ~33% of ATP production in untrained and endurance trained individuals, respectively[47]. Other studies involving longer work interval durations (i.e. 4 min) have also found that fat contributes substantially to energy production at intensities above 85% VO_{2peak}[47,53]. Together, the findings of the studies discussed above suggest that the HEPT system, anaerobic glycolysis and aerobic (fat) metabolism all contribute substantially to ATP production during HII exercise.

Compared to MIC exercise, HII exercise induces greater increases in catecholamines (ACTH, epinephrine and norepinephrine)[54], which stimulate both lipolysis[55–57] and CHO[58] metabolism during exercise. Overall fat oxidation during HII exercise however is lower, and CHO oxidation is higher than MIC exercise[54]. Indeed, a study that had participants exercise at the same intensity of a typical HII interval bout (~85%VO_{2peak}) but for 30 min continuously[59], found that compared to the group working at 65%VO_{2peak} continuously for the same duration, FFA uptake and fat oxidation were lower and glucose uptake and carbohydrate oxidation were higher[59]. These findings support the notion that exercising at higher intensities results in a greater reliance on CHO stores. However, in humans and horses, when high intensity exercise is performed in an interval format overall glycogen utilization is similar to MIC exercise[60–62]. The similar reliance

on muscle glycogen during HII exercise as compared with MIC exercise is likely due to the fact that while the rate of glycogen breakdown and utilization is greater during HII exercise during the high intensity intervals, it is lower than MIC exercise during the low intensity intervals. Thus, over the total exercise bout, a similar amount of glycogen is used. What has been found to differ between the two types of exercise is the pattern of fibre-specific muscle glycogen utilization with greater glycogen utilization in type II than type I fibres during HII versus MIC exercise[61]. Overall, these findings indicate that during the intervals, CHO utilization is higher than MIC, however since CHO oxidation is so low during the recovery periods, there is no apparent difference in muscle glycogen utilization.

There is only one study to my knowledge that compares SI to HII exercise[63]. Although it did not directly measure PCr or glycogen degradation, based on the fact that blood lactate increased to a greater extent following SI exercise compared to HII exercise we can speculate that, unsurprisingly, SI exercise is more anaerobic than HII exercise[63]. Additionally, RER was lower throughout the entire exercise bout during HII compared to SI exercise, which also indicates that there was a greater reliance on fat oxidation during HII exercise compared to SI exericse[63].

1.1.3.2 Fuel Utilization and Metabolism during SI Exercise

During SI exercise, participants are exercising at very high intensities and therefore deplete their PCr stores and exhaust their HEPT system quickly. Assuming the sprint intervals last ~20 seconds, the majority of ATP would therefore be supplied by the HEPT system, and anaerobic glycolysis (glycogen) would supply the rest[22,26,64]. Indeed, an acute bout of SI exercise (2 to 6 bouts of ~20-30s ‘all out sprints’ interspersed with 2-4 min active recovery) induces significant decreases in muscle glycogen[65–68], and PCr stores[65], and increases in lactate[9,66–70]. Furthermore, similar to that seen during HII exercise, SI exercise trials have found that glycogen utilization is

greater in type II than type I muscle fibres[9,10,61]. Given that type II fibres are less aerobic than type I fibres, and that type II fibres are recruited to a greater extent during higher intensities, it is not surprising that lactate increases substantially during SI exercise. Accumulation of lactate inhibits glycolysis by inhibiting the rate limiting enzyme of glycolysis, PFK[22]. Removal of lactate during the rest intervals is necessary to remove some of that inhibition for the subsequent interval. SI exercise protocols with inadequate rest interval duration (i.e. <30 sec) do not provide enough time for PCr regeneration[71] whereas a rest interval of 2-4 min is long enough to regenerate most PCr stores and for glycolytic enzymes to be more readily available so that exercise can be performed more effectively[22].

Glycogen contributes substantially to ATP production during both MIC and SI exercise; however, whether one mode is more reliant on glycogen is controversial. One study found that glycogen utilization was similar during both MIC (30 min at 65% VO_{2peak}) and SI exercise (8 x 20s at ~170% VO_{2peak} with 10 sec of rest between intervals) in both type I and IIA fibres, despite significant differences in exercise intensity[72]. However, another study found that glycogen utilization was higher during MIC (50 minutes at 70% VO_{2peak}) compared to SI exercise (6 x 20sec 'all out' sprint with 120 sec recovery or 18 x 5 sec 'all out sprint' with 30 sec recovery)[73]. These studies differ in both their SI and MIC exercise protocols, which could likely explain the difference in their findings. The participants in the first study[72] exercised at a higher intensity, the overall combined duration of the sprints was longer and only had 10s of rest before the next interval which all placed a greater metabolic demand on the muscle, resulting in greater utilization of glycogen. Regardless, the findings from these studies indicate that glycogen can contribute substantially to ATP production during SI exercise despite its short duration.

1.2 Sex Differences in Muscle Metabolism

The MIC exercise trials that have been conducted in women, have found that women have a lower respiratory exchange ratios (RER) compared to men at a given moderate exercise intensity. A lower RER is indicative of an greater reliance on fat oxidation[20,11–19,21], which has led to substantial research examining the sites of increased fat and decreased CHO oxidation as well as the mechanisms by which this occurs. Human and animal estrogen supplementation trials have revealed that estrogen may contribute to this sex difference in fuel utilization during exercise. Estrogen supplementation has been found to increase fat oxidation during exercise and alter the expression, content and/or activity of key metabolic enzymes to favour fat metabolism[74–82].

1.2.1 Sex Differences in Carbohydrate Metabolism During Exercise

Women rely less on CHO during MIC exercise as evidenced by a lower RER as compared to men[11–20]. RER does not tell us where the exact sites of fuel oxidation are, but rather a proportion of whole-body CHO and fat oxidation[22]. However, measurement of glucose rate of appearance (Ra) and disappearance (Rd) in plasma and glycogen content in muscle tissue can provide evidence of whether the decreased reliance on CHO during exercise is the result of decreased liver or muscle glycogen utilization, respectively.

It has been consistently shown that women have a lower glucose Ra and Rd as compared with men during 90 minutes of MIC exercise[11,13,18,83]. A lower glucose Ra is indicative of a lower rate of hepatic glucose release. Unfortunately, since it is not possible to obtain liver biopsies from humans, it is unclear whether the decreased glucose Ra is due to decreased hepatic gluconeogenesis, hepatic glycogenolysis, or a combination of the two. However, animal studies have found that male rats have higher liver glycogen content[84,85], and greater expression of gluconeogenic genes[85]. Furthermore, when supplemented with estrogen, liver glycogen

utilization was attenuated in male and oophorectomized female rats[74–76]. The effect of estrogen on hepatic glucose release has been confirmed by several studies that found that glucose Ra, Rd and metabolic clearance rate (MCR) was lower in men following estradiol treatment[78–80]. Regardless of whether it is a decrease in liver glycogenolysis or gluconeogenesis, the fact that there is less glucose entering the plasma (decreased Ra), and that the muscle itself is taking up less glucose from the plasma (decreased Rd) provides mechanistic support for the finding that liver CHO oxidation is lower in women than men during MIC exercise. To date, no study has investigated whether the sex difference in hepatic glucose metabolism persists during HII or SI exercise.

Whether there is a sex difference in muscle glycogen utilization during MIC exercise is contentious as some studies suggest that there is no difference[11,21,86,87], whereas others suggest that men have greater glycogen depletion[20,88]. Furthermore, SI exercise trials have also found both attenuation[9,10] and no attenuation[89] of muscle glycogen depletion in women during exercise. Although the male and female groups within each of these studies were equally matched, perhaps the differences in exercise protocols, mode and/or training status of the participants between the studies contributed to the discrepant findings in effect of sex on glycogen utilization during MIC and SI exercise.

Inconsistencies in exercise protocols may influence whether a sex difference in metabolism is found. Both intensity and duration of the MIC exercise trials ranged greatly from 58%-75% VO_{2peak} for 25 minutes - 3 hours[11,17,20,86,88] . The studies that did not find a sex difference in glycogen utilization seemed to be those on the upper[17] and lower[11] ends of the intensity range (i.e. 58% VO_{2peak} and 75% VO_{2peak}) as well as the longest in duration[86] (i.e. 3 hours). Although there was no difference in overall glycogen utilization during the 3-hr trial at 65% VO_{2peak},

perhaps it was that women did not use as much muscle glycogen during the first part of the exercise, but to sustain ATP levels for 3 hours they eventually needed to tap into their glycogen stores, resulting in no apparent difference in overall glycogen utilization. The idea that the timing of muscle glycogen utilization is different between men and women is supported by a shorter MIC exercise study that found men used more glycogen during just 25 minutes at ~68% VO_{2peak}[88], whereas studies of longer duration[11,21,86] (i.e. 90 minutes - 3 hours) tend to report no difference in glycogen utilization between the sexes.

Differences in the exercise intensity at which the exercise bout is conducted could also explain the inconsistencies found in the literature. Peak fat oxidation occurs around 65% VO_{2peak}[23,29], therefore sex differences in fat and CHO oxidation would theoretically be the most prominent at this intensity. MIC exercise trials above[17] or below[11] peak fat oxidation rates (75 and 58% VO_{2peak}, respectively) did not find a sex difference in glycogen utilization but trials at this intensity[20,88] (65% and 68% VO_{2peak}) found that men used more glycogen during exercise. The differing intensities and results of these trials suggest that for a sex difference in glycogen utilization to be detected during MIC exercise, perhaps participants must exercise at ~65% VO_{2peak}. Similarly, in SI exercise trials, the trial that did not find a sex difference in muscle glycogen utilzation[89] involved a lower intensity (5% vs 7.5% kg•body weight), and shorter interval duration (20 sec vs 30 sec) than the other two studies[9,10]. The higher intensity and longer duration would suggest a greater reliance on glycogen stores and since this is not a glycogen depleting exercise, sex differences in glycogen utilization might be more likely to be detected. Overall, the protocols used in the two types of exercise studies above were inconsistent in terms of their duration and intensity, both of which strongly influence fuel utilization during exercise[22].

Therefore, the differences in exercise protocols described above could be a potential factor for the discrepant findings in glycogen utilization during exercise.

When comparing between trials, it is also important to consider the training status of the participants. All[11,17,21,86,88] but one[20] of the sex comparative MIC exercise trials used recreationally trained participants. It is the study that used trained participants that found that women spare muscle glycogen during exercise. Endurance trained athletes have a greater capacity to produce ATP aerobically, rely less on CHO stores (glycogen), and more on fat at a given, relative intensity compared to those less trained[22]. As well, training has shown to increase the VO_2 at which peak fat oxidation occurs[90]. Although the duration of the one study that used endurance trained individuals[20] was quite long (i.e. 90 minutes), training adaptations allowed the participants to optimize fat utilization and use glycogen more sparingly. The effect of sex therefore becomes more apparent in this study with highly trained athletes because they are using more fat and their rate of glycogen depletion is slower, making the sex difference in muscle glycogen utilization detectable because muscle glycogen is not depleted at the end of the exercise bout. For recreationally trained participants, the rate at which they utilize glycogen is higher since they are less efficient at using fat, resulting in more substantial glycogen depletion, making it harder to detect a sex difference in glycogen utilization during longer bouts of MIC exercise. Similarly, in the SI exercise studies, there was also a difference in the training status of participants between the studies. Participants in the SI exercise trial that found no difference in glycogen utilization were untrained[89]. On the other hand, the SI trials that found an effect of sex on muscle glycogen utilization used recreationally active participants[9,10]. The fact that glycogen sparing was found during SI, but not MIC exercise in recreationally active participants likely relates to the fact that since this is not a glycogen depleting bout of exercise, due to its shorter

duration. Together, the findings of these trials highlight the importance of considering training status when examining sex differences in metabolism.

Exercise mode is another factor that can influence glycogen utilization during MIC exercise trials. Cycling, for example, recruits mostly lower body muscles, whereas for running (which is a weight-bearing exercise), there is a greater distribution of muscle fibre recruitment. Running therefore requires greater oxygen uptake overall to sustain ATP levels throughout the exercise because of the increased amount of muscle required to run. As well, most muscle biopsies are taken from the *vastus lateralis*, a muscle that is heavily relied upon during cycling whereas with running, the recruitment of lower body muscles are more spread out. Therefore, the *vastus lateralis* muscle during cycling may be more likely to become depleted, making a sex difference difficult to detect. Indeed, all of the MIC exercise trials that did not find a sex difference in glycogen utilization were cycling protocols[11,17,86], whereas the one running protocol[20] found men to use more glycogen during the exercise. However, the limited sample of running studies make it difficult to come to a full conclusion regarding effect of mode on potential sex difference in muscle glycogen utilization during MIC exercise.

In addition to sex differences in muscle glycogen utilization during exercise, sex differences in the metabolic fate of pyruvate during SI exercise have also been found. Both SI exercise trials that found a sex difference in muscle glycogen utilization[9,10] also found that lactate was higher post exercise in men than women, supportive of the hypothesis that men flux more pyruvate towards lactate than women[9,10]. These studies however only controlled for habitual physical activity level between the groups and did not consider the gold standard of matching based on maximal aerobic capacity relative to FFM (ml•O2/min/kg FFM). Therefore, we cannot rule out the possibility that undetected differences in training status between men and women may have

influenced these results. Unfortunately, lactate was not measured in the other SI study that found no difference in muscle glycogen utilization between the sexes[89]. Together, these findings suggest men may favour anaerobic metabolism to a greater extent during SI exercise than women, however more SI studies with adequate matching for sex is required before we can definitively determine if this is the case.

Sex differences in other glycolytic enzymes have also been found. Hexokinase II (HKII) mRNA increased to a greater extent in women during SI exercise[89], suggesting that women have a greater capacity to initiate glycolysis once glucose enters the muscle as compared with men. Elevated HK II protein content in women at rest has also been noted[91]. On the contrary, other studies looking at glycolytic enzyme activity at rest found that men had higher PFK[92,93], pyruvate kinase (PK)[92], glycogen phosphorylase (GP)[92], lactate dehydrogenase (LDH)[92,93] and HK[92,93] activity, all of which suggest that men rely to a greater extent on glycolysis and anaerobic metabolism, whereas women had higher β-HAD activity[92], which suggests that women have a greater capacity for oxidative and fat metabolism. Although not all SI exercise studies show that men use more muscle glycogen, it is evident that sex differences in CHO metabolism do persist at the molecular level. Overall, women use less CHO during exercise compared to men and this is believed to be at least partly due to a decreased reliance on hepatic glycogen. The effect of sex on muscle glycogen utilization during MIC and SI exercise appears to be intensity, training status, and potentially mode specific, with women using either similar or lesser amounts of muscle glycogen than men. However, to the best of my knowledge no trial has examined whether sex differences in muscle glycogen utilization persist during HII exercise.

1.2.2 Mechanisms of Sex Differences in CHO Utilization during Exercise

The mechanisms by which sex differences in CHO oxidation occur can in part be explained by differences in epinephrine. Women have lower epinephrine concentrations compared to men during exercise[10,20,83]. Epinephrine acts as a signal to promote hepatic gluconeogenesis and glycogenolysis[22], leading to increases in glucose Ra which we know increases less in women during exercise[11,13,18,83]. Epinephrine also influences muscle glycogen utilization due to its ability to stimulate glycogenolysis, which could explain why some studies showed that men used more muscle glycogen during exercise compared to women[20,88]. Contrary to its ability to stimulate glycogen breakdown, epinephrine also stimulates whole-body lipolysis[94]. This effect is strongest at very low intensities and as exercise intensity increases to moderate and high intensities, the effect that epinephrine has at increasing whole-body lipolysis lessens[95]. Or rather, the effect that epinephrine has on increasing CHO contribution to energy expenditure surpasses the effect that it has on lipolysis, which explains why men use CHO to a greater extent during exercise.

Sex differences in muscle fibre type can also explain the observed differences in muscle metabolism during exercise. Men typically have a higher proportion of type II muscle fibres[96]. One study, where it was confirmed that the male group had greater proportion of type IIA muscle fibres found that after a 30 second cycle sprint, glycogen reduction was the same in both men and women in type II fibres but women used less glycogen in their type I fibres[9]. Similarly, in another study involving 3 x 30 cycle sprints, women were found to use less glycogen in their type I fibres but no sex differences in glycogen utilization were noted in the type II fibres[10]. The authors suggested that this could perhaps be explained by the fact that type I fibres have a greater proportion of β-adrenergic receptors, giving them a greater capacity to respond to epinephrine binding and stimulate glycogenolysis[97]. Therefore, since men have higher concentrations of

epinephrine and more β-adrenergic receptors on their type I fibres, they might be better able at initiating glycogenolysis, despite women having greater type I fibre content. This could potentially explain the sex differences in fibre type specific glycogen utilization however, this hypothesis is speculative and has yet to be tested. As well, type II fibres are more anaerobic in nature so if men have a greater proportion of type II fibres, they are therefore more likely to rely on their anaerobic fuel pathways compared to women[9]. This is supported by two SI exercise trials showing that blood lactate concentration during[10] and post-exercise[9,10] is greater in men. MIC[18,20] (90 min at ~65% VO_{2peak}) and HII[98] (6 x 4 min intervals with 1-4 min recovery) exercise trials however show no effect of sex on blood lactate accumulation. Greater increases in blood lactate suggests a greater reliance on CHO during exercise since more glucose is fluxing through glycolysis (producing lactate). Since SI exercise is more intense than MIC and HII exercise, it would therefore recruit more type II fibres[22]. Since men have more type II fibres to begin with, and they would get recruited more during intense exercise (i.e. SI exercise), it may be that this sex difference only becomes apparent during supramaximal intensity exercise. Overall, the findings of these studies suggest that differences in muscle fibre type between men and women may be at least partially responsible for the observed differences in CHO metabolism during exercise. These findings are summarized in Table 1.

Table 1: Overall summary of potential mechanisms that underpin sex differences in CHO utilization during exercise.

Mechanism	Sex Difference	Action	Result	Effect on CHO utilization
Epinephrine	↑ Concentrations in men[10,20,83]	↑ Hepatic Gluconeogenesis ↑ Hepatic Glycogenolysis ↑ Muscle Glycogenolysis	↑ Hepatic glucose production ↑ Hepatic glycogen utilization ↑ Muscle glycogen utilization	↑ CHO utilization ↑ CHO utilization ↑ CHO utilization
Fibre Type Distribution	↑ %Type II fibres in men[96]	↑ Capacity for anaerobic metabolism ↑ Capacity to exercise at a higher intensity	↑ Anaerobic glycolysis + lactate production ↑ Recruitment at high intensities	↑ CHO utilization ↑ CHO utilization
β-Adrenergic Receptors	↑ Expression in type I fibres in men[97]	↑ Capacity for epinephrine to bind	↑ Effects of epinephrine	↑ CHO utilization in type I fibres

1.2.3 Sex Differences in the High Energy Phosphate Transfer System

To the best of my knowledge, no studies have examined sex differences in HEPT metabolism during HII exercise. However, two SI exercise trials and one MIC exercise trial have investigated how HEPT metabolism differs between men and women during exercise. One of the SI exercise studies that involved a single 30 sec all-out sprint found that neither sex nor muscle fibre type affected any of the HEPT system muscle metabolites that were measured (ATP, ADP, IMP, or PCr)[9]. The other SI exercise study from the same group involved 3 x 30s all-out sprints with 20 min recovery between sprints and once again no sex differences in the change in HEPT system muscle metabolites (ATP, ADP. IMP, PCr or hypoxanthine) was detected in either type I or type

II fibres[10]. However, they did find that ATP was higher and IMP and inosine were lower in women during the third sprint in type II muscle fibres and that inosine was lower in women during the third sprint in type I muscle fibres[10]. These sex differences in metabolite concentration were attributed to differences that occurred during the recovery period. In both fibre types IMP decreased to a greater extent in women during the recovery period between sprint 1 and sprint 2[10]. Furthermore, in type II muscle fibres, inosine content was lower in women as compared with men at the start of sprint 2[10]. Together these sex differences in metabolite clearance led to men having a higher concentration of inosine, IMP and a lower concentration of ATP at the onset of the third sprint and suggest that women have a greater ability to re-aminate IMP back to ATP.

Unfortunately, AMP was not measured in either of the SI exercise trials that have been conducted thus far. Therefore, it is unclear whether AMP accumulation differs between men and women during SI or HII exercise. Thus, we are unable to speculate if this is a potential mechanism that may contribute to sex differences in IS during exercise. However, as demonstrated in the latter SI exercise trial[10], women were better able to maintain ATP concentration during repeated sprints, suggesting that the AMP:ATP ratio may be better preserved in women and thus the potential remains that AMPK activation may be lower in women during SI and/or HII exercise. Taken together the findings of these two studies demonstrate that there are sex differences in the HEPT system during a bout of repeated sprints; however, further work, including examining how sex influences HEPT metabolism during HII exercise is required.

While sex differences in AMP metabolism have not been examined during interval exercise, they have been examined during MIC exercise. Following a 90 min bout of MIC exercise at 60% $VO_{2peak,}$ both creatine (Cr) and AMP concentration and the ratio of AMP:ATP increased

significantly in men but not women[21]. Furthermore, both AMPK Thr[172] and α_2AMPK activity increased in men, but not women[21]. Given the role that AMPK plays in improving insulin-independent and insulin-dependent GLUT4 translocation[6,7], greater activation of AMPK in men may over time lead to a greater improvement in IS. This finding is important because it draws the link between anaerobic metabolism and a potential sex difference in improvements in IS. However, more trials need to corroborate these findings before we can definitively say if this is the case, as well as determine how this might be affected by other factors such as training status, exercise intensity and exercise mode. The studies discussed in this section indicate that sex differences in the HEPT system during exercise exist, however they may be intensity-dependant and/or protocol specific. More research to better understand how sex influences HEPT metabolism (particularly AMP) may help us to better understand the mechanisms that underlie sex differences in IS improvement.

1.2.4 Sex Differences in Exercise Metabolism are Mediated by Estrogen

Sex differences in muscle metabolism during exercise have been shown to be the result of estrogen (E2). The effects of estrogen supplementation on muscle metabolism during exercise are summarized in Table 2 at the end of this section. In humans, estrogen supplementation has been found to decrease glucose Ra[78–81], Rd[78–80] and MCR[80], indicating sparing of hepatic glycogen. In some of these studies however, the decrease in glucose Ra was seen without a change in CHO oxidation as evidenced by no change in RER[79–81]. Under these circumstances where liver glycogen is being spared, a compensatory increased reliance on muscle glycogen utilization during exercise is therefore expected, however no effect of E2 supplementation on muscle glycogen utilization has been observed[12,78]. E2 supplementation did however reduce total resting muscle glycogen (specifically proglycogen) levels prior to exercise[78]. The findings

from estrogen supplementation trials are in line with the findings from sex comparative trials where sex/estrogen affect hepatic, but not skeletal muscle glycogen utilization during MIC exercise.

When E2 is given to men it induces a reduction in testosterone. As such, it is important to determine whether it is the increase in E2 or the decrease in testosterone that is inducing the reduction in carbohydrate utilization during exercise. To my knowledge, only study to date has investigated the effect of exercise on muscle metabolism in young men under conditions of altered testosterone [99]. In this study, muscle metabolism during exercise was examined under conditions of low testosterone (induced by suppressing endogenous testosterone production with gonadotrophin-releasing hormone antagonist), physiological testosterone (no intervention) or at supraphysiological testosterone levels (induced by administering testosterone through a skin patch)[99]. The findings showed that testosterone had no effect on substrate utilization during a bout of MIC exercise[99], leading to the conclusion that it is in fact estrogen, (rather than testosterone) that influences substrate utilization during exercise.

One mechanism by which estrogen is thought to influence substrate utilization is through the alteration of mRNA expression of certain metabolic proteins. One study that supplemented men with E2 (2mg/day for 8 days) investigated changes in mRNA of those proteins after 90 min of MIC exercise compared to placebo[82]. E2 increased TFP and CPT-1 mRNA expression both pre and post exercise[82], suggestive of an increase in the capacity for fat oxidation. Lastly, the E2 group had higher SREBP-1c and mtGPAT mRNA expression both pre and post exercise which are critical in the formation of IMCL. E2 however did not influence any CHO metabolism related genes[82]. Another study found that after 8 days of estrogen supplementation in men (1 mg/day for 2 days, then 2 mg/day for 6 days), there was an increase in PGC-1α mRNA content[100]

(a key regulator in FA oxidation[101]). The increase in PGC-1α mRNA was thought to potentially be a mechanistic explanation for the increased protein content of medium chain acyl-dehydrogenase that was also found[100]. Together, these findings suggest that estrogen upregulates the expression of genes and content of proteins that promote fat metabolism related to FA breakdown, β-oxidation, lipid uptake and lipid storage.

In summary, sex differences in muscle metabolism and fuel utilization seem to be at least partly mediated by estrogen. Specifically, estrogen is thought to play a role in decreasing glucose Ra, Rd and MCR, enhancing β-oxidation capacity and lipid storage, and potentially decreasing glycogen utilization during a bout of exercise. The mechanism that underpins these changes is suggested to be mediated by upregulation of certain genes relating to these metabolic processes. It is important to be aware that the majority of these studies were MIC exercise trials, most of which used a protocol of 90 min at 65% VO_{2peak}. It is difficult to apply these findings to other types of exercise that are not mainly aerobic. Future studies investigating the effects of estrogen on the anaerobic energy generating pathways (i.e during interval exercise) would allow us to identify common trends between the aerobic and anaerobic energy generating pathways, as well as identify any other sex differences that may have not yet been identified in the anaerobic systems.

Table 2: Summary of the effect of estrogen supplementation on muscle metabolism during exercise

Study	Participants	Supplementation dose and length	Exercise Protocol	E2 supplementation:
Devries (2005)[78]	11 men	1mg/d for 2 days, then 2mg/d for 6 days (mimics LP)	90 min cycling at 65% VO_{2peak}	-↓RER -↓ glucose Ra and Rd -No change in glycogen utilization
Ruby (1997)[79]	6 amenhorrheic females	1mg/d for 72hr and 144hrs (mimics FP)	90 min treadmill running at 65% VO_{2peak}	-↓glucose Ra and Rd during exercise -No change in RER -No change in glycogen utilization
Carter (2001)[80]	4 males	~3mg/d for 8 days (higher than LP)	90 min cycling at 60% VO_{2peak}	-↓ glucose Ra and Rd -No change in RER
Tarnopolsky (2001)[81]	11 males	100μg/d for 3.5 days, then 200μg/d for 3.5 days, then 300μg/d for 4 days (mimics FP)	90 min of cycling at 60% VO_{2peak}	-no change in muscle glycogen utilization -no change in RER -no change in concentration of plasma glucose and lactate -no change in CHO and fat oxidation rates
Fu (2009)[82]	12 males	2mg/d for 8 days (mimics LP)	90 min cycling at 65% VO_{2peak}	-↑TFP mRNA expression -↑ CPT-1 mRNA expression -↑SREBP-1c mRNA expression -↑mtGPAT mRNA expression
Maher (2010)	10 males[100]	1mg/d for 2 days, then 2mg/day for 6 days (mimics LP)	90 min cycling at 65% VO_{2peak}	-↑MCAD protein content -↑PCG-1α mRNA content

Abbreviations: CPT-1: carnitine palmitate transferase-1, FP: follicular phase, LP: luteal phase, MCAD: medium chain acyl-coenzyme A dehydrogenase, mtGPAT: mitochondrial Glycerol-3-Phosphate Acyltransferase, PGC-1α: peroxisome proliferator-activated receptor-γ coactivator, Ra: Rate of appearance, RER: respiratory exchange ratio, Rd: Rate of disappearance, SREBP-1c: Sterol regulatory element-binding protein-1c, TFP: trifunctional protein.

Chapter Two: Rationale, Purpose, Objectives, Hypotheses

2.0 Study Rationale

The prevalence of T2D is increasing worldwide and it is estimated that the prevalence will reach 200-300 million people globally by 2025[1]. In most cases, T2D is preventable and even treatable with a healthy diet and exercise[1]. An acute bout of exercise is able to increase glucose uptake for up to 72 hours after the exercise bout[8,102,103] . Furthermore, exercise training can increase the content of insulin signalling proteins[104–107], enhancing insulin sensitivity[5,105–107]. A new form of exercise that has gained popularity and is preferred by many over traditional MIC training is interval training. Interval training is effective at eliciting similar adaptations to MIC training (i.e. increased mitochondrial content, capillary density and oxidative capacity) in a much more time efficient manner[36]. However, the findings from sex-comparative interval training studies suggest that exercise may not be as effective at improving IS in women compared with men[3–5]. More specifically, 6 weeks of interval training (3 sessions•wk^{-1}) improved glycemic control in men, but not women[4,5]. One of these studies also found that GLUT 4 protein content increased in both men and women post training, however GLUT 4 protein content was significantly higher in men compared with women post training[4]. These findings suggest that exercise-induced improvements in insulin signaling are blunted in women following interval training; however, why this occurs is unknown.

Sex differences in fuel utilization and metabolism during MIC exercise have been extensively researched and may offer insight into the potential mechanisms responsible for the blunted improvements in IS seen in women. MIC exercise trials consistently show that women rely to a lesser extent on CHO as a fuel source, as indicated by a lower RER[11–20]. Specifically, sex comparative trials have found women to have a lower glucose Ra and Rd; suggesting less of a

reliance on hepatic glycogenolysis and/or gluconeogenesis[11,13,18,83]. The effect of sex on muscle glycogen utilization during exercise is contentious as some[20,88], but not all[11,21,86,87], studies have found skeletal muscle glycogen utilization to be attenuated in women during exercise. If men rely on CHO to a greater extent during exercise, their post-exercise glucose uptake would likely be greater since greater muscle glycogen utilization is associated with a greater post-exercise glucose uptake[108,109]. While not studied during HII exercise, sex differences in muscle glycogen utilization during sprint interval have been reported[9,10]. Furthermore, following a bout of MIC exercise where estimated muscle glycogen utilization was greater in men than women, post-exercise glucose uptake was also higher in men for 120 minutes post exercise[110]. Thus, differences in muscle glycogen utilization during HII exercise may help explain the blunted effect of HII exercise on improving IS in women; however, this has yet to be examined.

Another potential link between sex differences in IS and interval training may be related to sex differences in the anaerobic HEPT system metabolite AMP. An increase in the ratio of AMP:ATP in the muscle activates the enzyme AMPK[6]. AMPK is involved with both post-exercise insulin-independent and insulin-dependent GLUT 4 translocation to the membrane, increasing glucose uptake[111]. Sex differences in AMP during interval exercise have not been examined. However, one MIC exercise study did find that men had higher levels of AMP after 90 minutes of exercise at 60%VO_{2peak}, along with greater AMPK Thr^{172} phosphorylation and activity of α2AMPK [21]. Additionally, a sprint interval study found that men had a lesser decrease in the metabolite IMP (a direct precursor to AMP) compared with women during recovery periods between intervals[10]. These findings suggest that a sex difference in HEPT metabolism during HII exercise is possible. Theoretically, an increased reliance on HEPT metabolism suggests the potential for a greater increase in AMP, which in turn could induce greater

activation of AMPK and eventually a greater improvement in IS; however, this remains to be examined.

Many interval training trials have been designed such that participants exercise at a given relative intensity (i.e. 90% HR_{max} for HII exercise)[3,107,112,113] during the high intensity intervals, but at a fixed intensity (i.e. 50W) for the low-intensity intervals [3,107,113]. In fact, numerous sex comparative interval training studies have used an absolute wattage for the recovery periods[3,4,66,89,113,114]. None of those studies however have considered the fact that an absolute wattage during the recovery periods would result in the female participants exercising at a relatively higher intensity during the low-intensity recovery intervals even if men and women were matched appropriately for aerobic fitness. Furthermore, whether this exercise stimulus induced similar physiological responses to the exercise bout in men and women was not examined. Given that women would be working at a higher relative intensity during the low-intensity recovery intervals this is an important consideration, particularly since two interval exercise trials where men and women worked at the same relative intensity during the low-intensity recovery periods found that HR response to interval exercise may be different between men and women[98,115]. One study used a 6 x 4 min HII cycling bout at ~85-90%VO_{2peak} and found that women reached a higher %HR_{max} during the intervals despite exercising at a slightly lower %VO_{2peak}[98]. Another trial that used a SI exercise bout found that on the fourth 30s treadmill sprint at 110% VO_{2peak}, women were exercising at a higher %HRmax[115]. Thus, if women achieve a higher %HR_{max} when the recovery intensities are relatively equal, then when the recovery intensities are relatively higher in women, sex differences in the HR response may be even more pronounced. This is important because many HII exercise and training studies conducted in clinical populations[3,107,112,113] have used an absolute workrate of 50W during the recovery

periods and ~90% HR_{max} during the intervals. Therefore, if the overall stimulus is not the same between men and women, this could also give us more insight into why women may not respond in a similar manner to men in response to HII training.

As noted above, HII exercise and training trials typically include warm ups, low interval and cooldowns at a fixed wattage. Wattage allows the metabolic demand of the exercise bout to easily be calculated, however to control for differences in body composition between men and women, wattage should be expressed relative to muscle mass. It is important to note that it is not muscle mass, but fat-free mass that is typically measured when determining body composition. Whole body assessments of FFM include not only muscle mass, but organ mass as well. However, FFM can be expressed as whole body or regional values and since cycling predominately involves recruitment of lower body muscle mass it is more appropriate to express wattage relative to lower body fat-free mass (LB-FFM). Furthermore, since whole body estimates of fat-free mass include tissues other than muscle, expressing exercise workrate relative to lower body fat circumvents the issue related to including organ mass. Therefore, during high intensity interval cycling wattage relative to LB FFM would be the most representative of the work done by the muscles being recruited during cycling, and furthermore, since a given wattage requires a standard energy output, the metabolic demand per kg of lower body muscle can also be determined. If the metabolic demand of the intervals is the same per kg of LB FFM between the sexes, then any differences in energy expenditure would reflect a sex difference in the metabolic pathways. Therefore, by determining if the exercise intensity of a standardized HII exercise bout is similar between men and women when expressed as wattage/LB FFM will allow us to determine if this specific protocol elicits similar exercise intensities between the sexes. Thus, the purpose of the research conducted in this thesis was to

examine whether sex influences the exercise response and/or anaerobic metabolism during an acute bout of HII exercise. Furthermore, we examined whether the standardized workrate of a typical HII exercise bout differed between men and women to infer whether differences found were the result of sex or different exercise intensity.

2.1 Study Purpose

The purpose of the proposed research was to examine whether sex influences the exercise response and/or anaerobic metabolism during and acute bout of high intensity interval exercise.

2.2 Objectives

1. To determine whether sex influences glycolytic metabolite concentrations during HII exercise.
2. To determine whether sex influences HEPT system metabolite concentrations in the muscle during HII exercise.
3. To identify how HII exercise influences the phosphorylation status of enzymes involved in anaerobic metabolism.
4. To determine whether sex influences the phosphorylation status of enzymes involved in anaerobic metabolism.
5. To examine whether the exercise response to a standardized bout of HII exercise is similar between men and women.

2.3 Study Hypotheses

We propose the following hypotheses for this study:

1. Men will rely on glycolysis to a greater extent than women as evidenced by a greater increase in muscle lactate and pyruvate and a greater decrease in muscle glycogen.
2. Men will rely to a greater extent on the HEPT system as evidenced by a greater decrease in PCr and ATP, and a greater increase in Cr, ADP, AMP, IMP and Pi
3. Phosphorylated CK and PDHE1α will decrease during HII exercise.
4. Phosphorylated CK and phosphorylated PDHE1α will decrease to a greater extent in women as compared with men during HII exercise.
5. **a)** Women will work at a significantly higher $\%VO_{2peak}$ and select a higher RPE score during the low intervals but not the high intervals compared with men.

   b) There will be no sex difference in the HR response to HII exercise.

Chapter Three: Methods

Due to circumstances regarding the COVID-19 global pandemic, access to laboratory facilities were restricted for a number of months. Unfortunately, I was unable to gain access to the laboratory facilities to perform the intended metabolite assays. Therefore, the methods described in this section reflect the laboratory measures that were performed before lockdown.

3.0 Participants

Twenty-four young, healthy, recreationally active males (n=12) and females (n=12) were recruited for this study. Men and women were matched by group, according to their maximum aerobic capacity (VO_{2peak}) relative to their FFM. Participants were between 18-30 years of age and were excluded if they had any chronic health conditions (i.e. metabolic, cardiovascular, respiratory or digestive disorders), were unable to complete a single exercise session, or regularly participated in cardiovascular or resistance training >3 or >2 times•week^{-1}, respectively. Individuals who had an allergy to local anesthetic, had undergone a barium swallow or an infusion of a contrast agent in the 3 weeks leading up to the trial, were taking anti-coagulant or anti-platelet prescription medications, had a BMI >27kgm^{-2}, or were unable to exercise as suggested by the Get Active Questionnaire were also excluded from the trial. Female participants who were pregnant, suspected that they may be pregnant, were breastfeeding, or were taking monophasic oral contraceptives were also excluded from the trial. Male and female participants with a relative VO_{2peak} above 51 and 44 mL•kg^{-1}·min^{-1}, respectively, were excluded from the study. Each participant had the opportunity to read a detailed outline of the procedures, risks and benefits associated with the study. This was also verbally explained to them by the researcher prior to the exercise trial. This study was reviewed and received ethics clearance from the University of Waterloo Research Ethics Committee (ORE# 22477).

3.1 Study Design

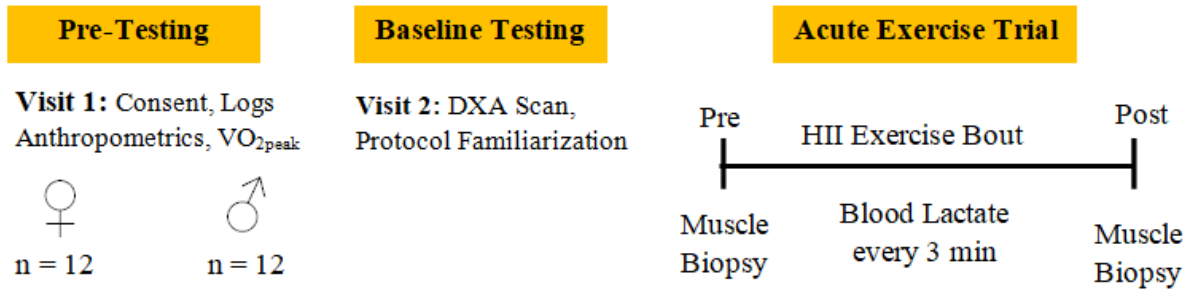


Figure 2: General Overview of Study Design

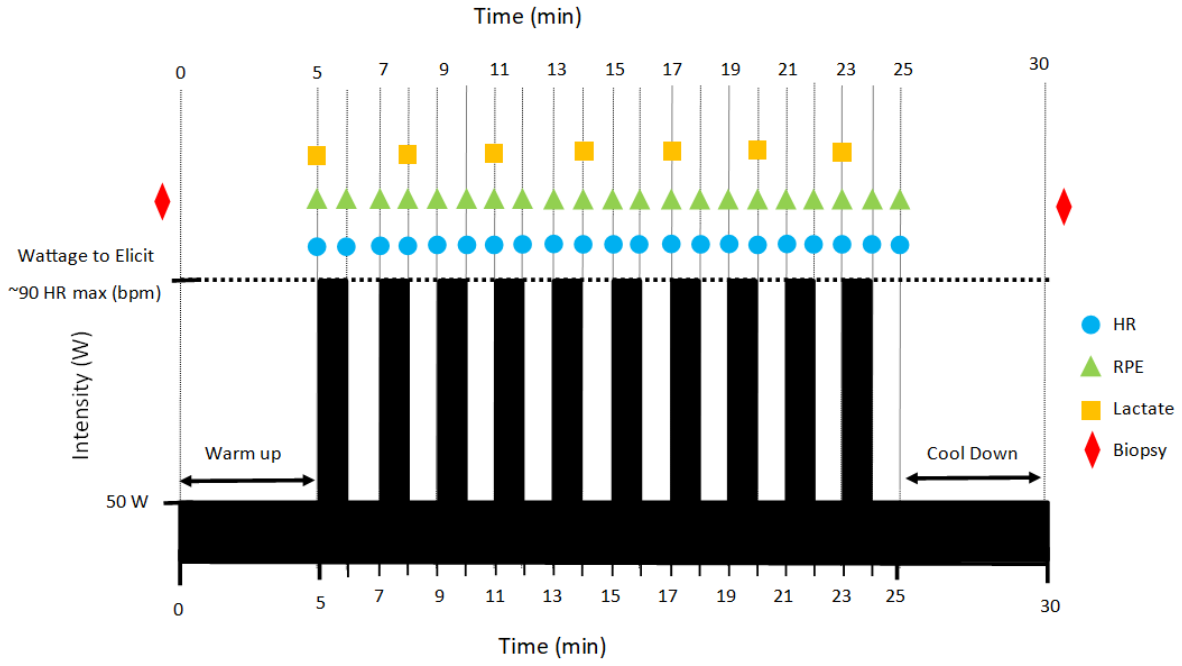


Figure 3: Detailed overview of the acute HII exercise protocol.

3.1.1 General Study Outline

Visit 1: Informed Consent, Anthropometrics, Food and Activity Logs, VO_{2peak}

Participants completed a health screening questionnaire, the Get Active Questionnaire (GAQ), and provided written, informed consent to participate in the study. They were then given instructions on how to complete a 3-day food log and 7-day physical activity log. In addition to the exercise logs, participants were given a PiexoX^{TM} pedometer (StepsCount, Deep River, ON, Canada) to record their step count on the days they logged their physical activity. Anthropometric measurements (i.e. height and weight), were also recorded during this visit.

Lastly, the participants underwent a VO_{2peak} test using a ramp protocol (start at 50W, increase by $1W \cdot 2sec^{-1}$) on a cycle ergometer (Ergoline, Bitz, Germany) attached to a metabolic cart (Vmax, Vyaire Medical, Mettawa, IL, USA) to determine their relative maximum aerobic power ($mL \bullet kg^{-1}min^{-1}$). Participants started with a 2-minute warm up at 50W, followed by an increase of 1 Watt every 2 seconds. Participants were instructed to maintain a minimum speed of 60 $revolutions \cdot min^{-1}$. During the warm-up, a finger prick was performed using a Lactate Scout Plus (EKF, Penarth, England) to analyze blood lactate. Lactate measures were taken every minute throughout the test. A Polar Heart Rate Monitor (Polar, Lachine, QC, CA) was used to record HR every 30 seconds. Rating of perceived exertion (RPE) was also measured every minute based on the Borg Scale (6-20). The test was terminated when the participant reached volitional failure, or when they were unable to maintain a cadence of 60 rpm for $\geq$ 3 seconds. The breath-by-breath data from the Vmax system was time averaged on 10-second intervals. The highest achieved VO_2 ($mL \bullet kg^{-1}min^{-1}$) out of each of the averaged 10 second intervals was taken as VO_{2peak}.

Visit 2: DXA Scan, Familiarization

A full body dual x-ray absorptiometry (DXA) scan was done using the Hologic Discovery W (Hologic, Mississauga, ON, CA). Each image was analyzed with QDR APEX software (Version 4.5.3, Hologic, Mississauga, ON, CA) by a trained technician to obtain each participant's body composition and FFM. A measurement of VO_{2peak} relative to FFM averaged across each group served as a baseline characteristic to ensure similar training status between groups[12].

To familiarize each participant to the exercise trial protocol, and to ensure that they were exercising at the desired intensity (90% of max HR achieved during VO_{2peak} test), they underwent a familiarization test on the cycle ergometer. The familiarization started with a warm-up (50W), followed by 3-6 x 60 sec intervals at the intensity calculated to elicit the target HR (90% HR_{max}). Each interval was interspersed with 60 second of low intensity cycling at 50W. If target HR was not achieved by the third interval, the wattage on the bike was adjusted accordingly. If the target HR was not close to being achieved after 6 intervals, an additional familiarization session on a different day was conducted in order to determine the appropriate testing wattage.

Visit 3: HII Exercise trial

Participants came in the morning of the HII exercise trial having fasted for 12 hours (except for water) and having refrained from moderate-vigorous exercise for at least 72 hours. Females were tested during the mid-follicular phase of their menstrual cycle (days 4-9, with the first day of menses being day 1). Prior to beginning the exercise, they had a muscle biopsy taken from their *vastus lateralis* which was either immediately frozen in liquid nitrogen or mounted in OCT and frozen in liquid nitrogen for subsequent analysis. They then got on the cycle ergometer where

they did a 5-min warm up (50W), followed by 10 intervals of 60 sec at 90% HR_{max} (wattage for this determined during the familiarization), interspersed with 60 sec at low intensity (50W). This was followed by a 5 min cool down (50W). Breath samples were collected during the warm up (minutes 0 – 5), intervals (minutes 12 – 17) and cooldown (minutes 25-30) using a metabolic cart (Vmax, Vyaire Medical, Mettawa, IL, USA) to allow for determination of whole body substrate utilization. Within a few seconds of exercise completion, a second muscle biopsy was taken to the same leg, 1-2 cm above or below the first biopsy and processed as described above. Starting at the onset of the first interval (minute 5), HR and RPE were measured every minute, and blood lactate was measured every 3 minutes until the end of the final low interval (minute 24) using the same measurement tools as the VO_{2peak} test. An overview of the HII exercise bout including when all measurements were taken can be found in Figure 3.

3.1.2 Sample Collection

Prior to, and immediately after the exercise bout, a muscle biopsy was performed on the *vastus lateralis* muscle using a custom suction-modified Bergstrom needle. Two muscle samples were taken during each biopsy. The first sample was immediately placed in liquid nitrogen to be used for analysis of muscle metabolites. The second muscle sample was processed (removal of any fat or excess tissue) and sectioned into different pieces; each placed in a different, labeled cryotube or mounted in OCT. The samples were then be submerged in liquid nitrogen and stored at -80^{o}C for analysis of protein content of enzymes related to anaerobic metabolism.

3.2 Analysis

3.2.1 Western Blot Analysis

Homogenization of muscle samples for western blot analyses was performed in ice cold 25mM Tris buffer [25mM Tris, 0.5% (v/v) Triton X-100, and protease/phosphatase inhibitor tablets

(Roche Diagnostics, Laval, QC, Canada)]. The samples were placed into pre-chilled homogenization Biopur Eppendorfs (Eppendorf, Mississauga, ON, Canada) where a homogenization buffer was then added at a ratio of 10μL of buffer to 1mg of muscle. Samples were homogenized upon the addition of a homogenization bead (Qiagen, Toronto, ON, Canada) to the Eppendorf using TissueLyser II (Qiagen, Toronto, ON, Canada) and run at 20 cycles•sec^{-1} for 40 seconds. To determine total protein content in the homogenized sample, the sample were spun in a centrifuge at 10 000G for 10 minutes at 4°C. The supernatant was transferred into additional pre-chilled Eppendorfs and the pellet was frozen for future analysis. A bicinchoninic acid (BCA) assay was used to determine total protein content.

Once total protein content was known, samples were prepared for Western Blot analysis in a Laemmli buffer (0.5M Tris-HCl, glycerol, 10% SDS, 1% bromophenol blue, β-mercaptoethanol, and ddH_2O), and stored at -80°C. SDS-PAGE and Western Blot techniques were performed to identify the proteins of interest. 10μg of protein from each sample was loaded and run on the 4-15% Criterion TGX Stain-Free protein gels (BioRad, Hercules, CA, USA) for 45 minutes at 200 volts. Protein ladders (precision Plus Protein Standard, BioRad, Hercules, CA, USA) and a standard curve pooled from all samples was run. Using the Trans-Blot Turbo Transfer System (BioRad, Hercules, CA, USA), the proteins were transferred to a PVDF membrane. To ensure protein transfer was successful, the membranes were viewed using the Chemidoc MP (BioRad, Hercules, CA, USA) to confirm protein presence. Membranes were blocked for 2 hours in 5% bovine serum albumin (BSA) in 1X Tris-buffered saline and Tween 20 (TBST) to optimize the blocking (outlined in Table 1). Blocked membranes were stored overnight at 4°C in primary antibody (see Table 1 for specific detail regarding each protein) on shaker plate. To remove excess primary antibody, membranes were then washed 5 times for 3 minutes each with 1X

TBST. The membranes were then incubated in the appropriate secondary antibodies for 1 hour at room temperature at a dilution ratio of 1:10 000. To remove excess secondary antibody, membranes were then washed again 5 times for 3 minutes each with 1X TBST. At this point, Clarity ECL Western Blotting Substrates (BioRad, Hercules, CA, USA)) was added to the membrane and the membrane was viewed using the Chemidoc MP imaging system (BioRad, Hercules, CA, USA). To remove the Clarity ECL, membranes were washed again 5 times for 3 minutes with 1X TBST. Antibodies were then stripped from the membrane by washing the membranes with a mild stripping buffer (Glycine, SDS, Tween20 and ddH_2O at pH 2.2) two times for 15 minutes. Membranes were then washed with 1X PBS ($Na_2HPO_4 \bullet 7H_2O$, $Na_2H_2PO_4$, NaCl and ddH_2O at pH 7.2) two times for 10 minutes, then two more times for 5 minutes with 1X TBST. Membranes were then reprobed with 1^o antibody for the other protein. ImageJ (Version 1.51a, National Institute of Health, USA) was used to quantify the protein bands. Total protein content from the stain-free blot (supplementary Figure 4) was also determined using ImageJ (Version 1.51a, National Institute of Health, USA) allowing for the protein of interest to be expressed relative to total protein content. For the complete list of antibodies and their information, see Table 3.

Table 3: Antibodies for western blot analysis with the specifics for blocking, primary antibody, and secondary antibody incubations.

Antibody	**Provider**	**Blocking Agent**	**Primary Antibody Dilution**	**Secondary Antibody Dilution**
Phospho-CK	Abcam	5% BSA in 1X TBST	1:8 000	1:10 000
Phospho-PDHE1α	Abcam		1:3 000	1:10 000

*All primary and secondary antibodies are diluted in 5% BSA with 1X TBST

3.2.2 Immunofluorescence and Histochemical Staining

3.2.2.1 Slide Mounting

Prior to staining, 10µm thin sections from each muscle sample were cut using a cryostat (Thermo Electronic, MA, USA) and mounted on glass slides. Slides were wrapped in tin foil and stored at -80^{o}C until analyzed. When ready to be analyzed, slides were removed from the freezer and allowed to air dry for 5-10 minutes at room temperature.

3.2.2.2 Muscle Fibre Type Determination

Muscle fibre type was determined using myosin heavy chain immunofluorescence staining. 100µL of blocking solution (10% goat serum, 90% 1X PBS) was added to each slide and slides were incubated at room temperature while shaking at 220 rpm for 1 hour. While slides were blocking, the 1^{o} antibody working solution was made (see Table 3 for dilution ratios).

The blocking solution was shaken off and the calculated amount of appropriate1^{o} antibody was added immediately. The solution was then incubated overnight at room temperature while shaking. The next day, slides were placed back-to-back and washed for 3 x 5 minutes in a Columbia jar wrapped in tin foil (to protect from light) with 1X PBS. The slides were blotted dried, and then incubated in the appropriate 2^{o} antibodies diluted 1:500 in blocking solution for 1 hour at room temperature in the dark. Slides were placed back-to-back and washed again for 3 x 5 minutes in a Columbia jar wrapped in tinfoil with 1X PBS. Slides were blotted dry and 15µL of Prolong was applied to each slide and mounted with a #1 coverslip. This step was performed in the microscope room in the dark. The corners of the coverslip were tacked down with nail polish and placed in a labeled, light-proof slide box. Slides were then be imaged the next day.

The slides were imaged in the dark under the microscope (Zeiss, Oberkochen, Germany) on the highest intensity setting and captured using the Zen System (Zeiss, Oberkochen, Germany)

computer program using the 'Image Processing' tab for analysis. Proportion and sizes of type I, IIa and IIx fibres were determined.

Table 4: Antibodies, dilution factors, volumes to be added and the immunofluorescent colour of each antibody for the fibre typing protocol

MHC	1° Antibody	1° Dilution Factor	2° Antibody	2° Dilution Factor	Colour
I	BA-F8	1:50	IgG2b	1:500	Blue
IIa	SC-71	1:600	IgG1	1:500	Green
IIx	6H1	1:100	IgM	1:500	Red

*All primary and secondary antibodies are diluted in blocking solution (10% goat serum, 90% 1X PBS)

3.2.3 Indirect Calorimetry Calculations

Breath-by-breath indirect calorimetry information that was obtained from the metabolic cart during minutes 0-5 (warm up), minutes 12-17, and minutes 25-30 (cooldown) was used to calculate an estimate of whole-body substrate utilization.

3.2.3.1 %CHO and Fat contribution to TEE

An average RER from the breath data during the warm up, high intervals, low intervals, and cooldown was determined to calculate an estimate of %CHO and fat oxidation. Although the breath sample from minutes 12-17 included three low intervals and two high intervals, only two low intervals were used to calculate the average RER for that time period to maintain consistency with the high intervals. The following modified stoichiometric equation (also used by Peric et al. (2016)[116] and developed by Elia and Livesey (1992)[117]) that assumes negligible protein oxidation was used to estimate %CHO and fat oxidation:

$$\%CHO = \frac{[(5.045 * RQ) - 3.582]}{[(0.36 * RQ) + 1.103]}$$

$$\%Fat = 1 - \%CHO$$

3.2.3.2 Determination of Total Exercise Energy Expenditure and Absolute rates of CHO and Fat Oxidation

Average oxygen intake (VO_2, L/min) for warm up, high intervals, low intervals, and cooldown were multiplied by their duration (min) to determine oxygen consumption during each time period. Oxygen consumption for each time period was subsequently multiplied by 5 to determine the energy cost (kcal) for each measurement period.

To determine the total grams of each substrate that was oxidized during each time period, the CHO and fat percentages were multiplied by the energy expenditure of their respective time periods and subsequently divided by either 4 (for CHO) or 9 (for fat). The total grams of each substrate was lastly divided by the duration (min) of each respective time period to obtain the rate of CHO and fat oxidation in g/min.

3.2.4 Periodic Acid Schiff (PAS) Staining for Muscle Glycogen Determination

Muscle samples were mounted on slides as described in section 3.2.2.1. Slides were then incubated in PAS Fixative (32mL EtOH, 6.0mL Chloroform, 2.0mL Glacial Acetic Acid) for 7 min at 25^{o}C in foil in a Columbia Jar on a shaker set at 200rpm. After that, slides were dipped 15 times in a large beaker containing ddH_2O and then incubated in periodic Acid (0.2g period acid powder, 40mL ddH_2O) for 8 min at 25^{o}C in foil in a Columbia jar on shaker set at 200rpm. Slides were then dipped another 15 times in a larger beaker containing ddH_2O. Next, the slides were incubated in Schiff's Reagent in an incubator for 15 min at 37^{o}C in foil in a Columbia jar on a shaker set at 200rpm. Slides were then be rinsed for 10 min in ddH_2O water in a Columbia jar while shaking at 200rpm. The samples were then serially dehydrated in Columbia jars

containing increasing concentrations of alcohol (80%, 90%, then 100% alcohol) for 2 minutes in each jar. Upon completion, slides were rinsed in Xylene for 2 minutes and mounted with 20µL heated Permount on a hotplate set at 100^{o}C then removed and let to dry on number#2 coverslips. Slides were viewed using the Cytation 5 automated microscope (BioTek Instruments, Inc., VT, USA). Brightfield images were captured with the Cytation 5 built-in camera (BioTek Instruments, Inc., VT, USA) and serial stitched using Gen5 software (BioTek Instruments, Inc., VT, USA). Muscle glycogen content of each muscle fibre was analyzed by outlining each fibre and measuring the average greyscale value (ranging from 0 to 255; with 0 being all black and 255 being all white) using ImageJ.

3.3 Statistical Analyses

A non-paired t-test was used to assess differences between the sexes in baseline characteristics, total energy expenditure during exercise, high intensity interval wattage, %target HR, lactate AUC and average RPE, HR and lactate during high and low intervals. A 2-way mixed model ANOVA with sex being the between variable (2 levels: male/female), and time being the within variable (2 levels: pre/post exercise) was used to determine the effect of sex and exercise on all other experimental variables. A Tukey's HSD test was conducted when necessary. Significance was set at $p<0.05$.

Chapter Four: Results

4.1 Participant Characteristics

Men were taller (p=0.0001, Table 5), weighed more (p=0.002, Table 5), had a lower % body fat (p<0.0001, Table 5) and a higher relative VO_{2peak} (p<0.001, Table 5). However, when expressed relative to total body and lower body FFM, there was no difference in VO_{2peak} between the groups (p=0.19 and p=0.27, respectively, Table 5). Men also had fewer type I muscle fibres than women (p=0.007, Table 5).

Table 5: Participant Characteristics

	Men	**Women**	**P Value**
Age (y)	22 ± 2	21 ± 1	0.46
Height (cm)	177.9 ± 2.0	162.7 ± 2.5	0.0001
Weight (kg)	75.6 ± 2.4	62.6 ± 3.1	0.002
BMI (kg/cm^2)	23.7 ± 0.8	22.9 ± 0.6	0.45
% BF	20.9 ± 1.6	32.9 ± 1.3	<0.0001
VO_{2peak} (ml O_2/min/kg)	44.3 ± 1.6	35.3 ± 1.5	<0.001
VO_{2peak} (ml O2/min/kg FFM)	61.3 ± 1.5	58.3 ± 1.6	0.19
VO_{2peak} (ml O2/min/kg LB FFM)	185.4 ± 10.9	172.1 ± 4.2	0.27
Daily Activity (steps/day)	8307 ± 466	9679 ± 856	0.22
Fibre Type Distribution (% of total)			
Type I	28 ± 13.2	42 ± 7.1	0.007
Type IIa	37 ± 12.4	29 ± 6.6	0.10
Type IIx	36 ± 12.6	29 ± 9.2	0.19

Data are means ± SEM. % BF: percent body fat, BMI: body mass index, FFM: fat free mass, LB FFM: lower body fat free mass.

Habitual dietary intake determined from 3-day food logs revealed that men consumed more energy (p=0.01, Table 6), which was due to a greater consumption of fat (p=0.016, Table 6) and protein (0.016, Table 6), but not CHO (p=0.07, Table 6). However, when protein intake was expressed relative to kg body weight there was no difference between the sexes (p=0.26, Table 6). Furthermore, when expressed as a percentage of total energy intake there was no significant difference in CHO (p=0.42, Table 6), fat (p=0.33, Table 6), or protein (p=0.43, Table 6) intake between men and women.

Table 6: Nutrient Profile

	Men	**Women**	**P Value**
Daily Energy (kcal)	2008 ± 171	1477 ± 78	0.01
Protein			
Absolute (g)	96 ± 11	65 ± 4	0.02
% of Daily Kcal	19 ± 2	17 ± 1	0.43
g/kgBW/d	1.27 ± 0.2	1.07 ± 0.1	0.26
Fat			
Absolute (g)	79 ± 7	56 ± 5	0.02
% of Daily Kcal	36 ± 2	33 ± 2	0.33
Carbohydrate			
Absolute (g)	234 ± 23	186 ± 11	0.07
% of Daily Kcal	47 ± 2	50 ± 2	0.43

Data are means ± SEM. BW: body weight, d: day, g: gram.

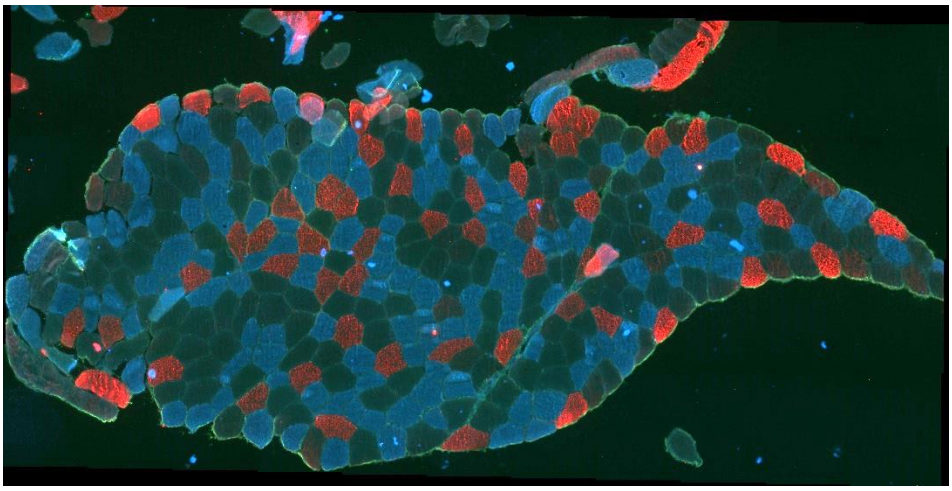


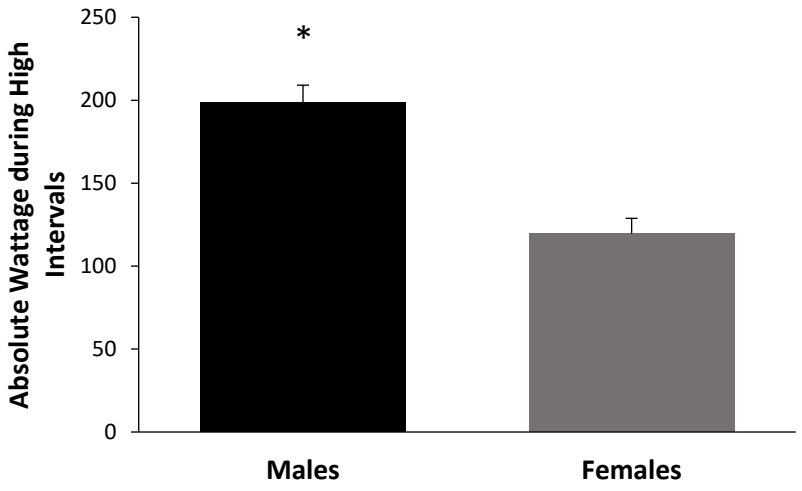
Figure 4: Representative image of muscle cross section used for fibre typing.

4.2 Measures of Intensity during HII exercise

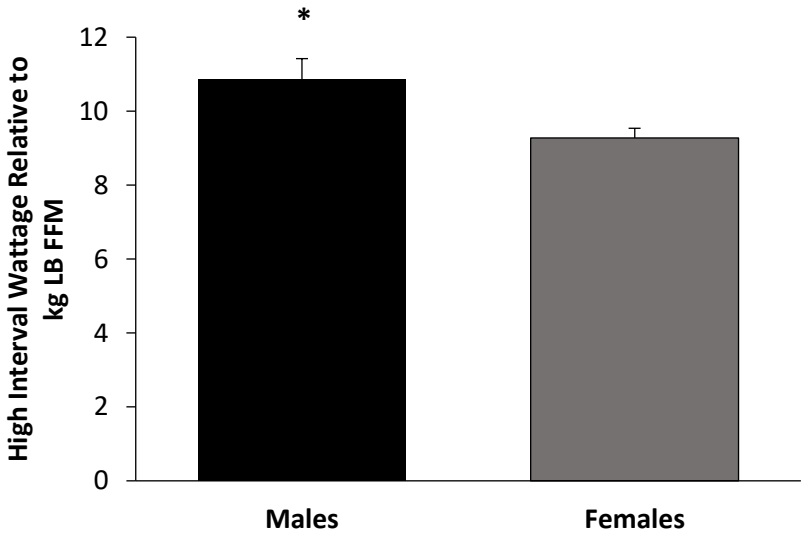
4.2.1 Wattage during HII exercise

Men worked at a higher absolute wattage during exercise than women ($p<0.0001$, Figure 5A). Furthermore, when expressed relative to lower body (LB) FFM, the male group exercised at a higher wattage on the cycle ergometer during the high intervals compared to their female counterparts ($p=0.02$, Figure 5B). During the low intervals, when expressed relative to LB FFM, the female group exercised a higher relative wattage compared to their male counterparts ($p=0.0002$, Figure 5C). Additional comparisons of relative wattage can be found in supplementary Figure 1.

A


Absolute Wattage during High Intervals
250
200
150
100
50
0
*
Males
Females

B


High Interval Wattage Relative to kg LB FFM
12
10
8
6
4
2
0
*
Males
Females

C

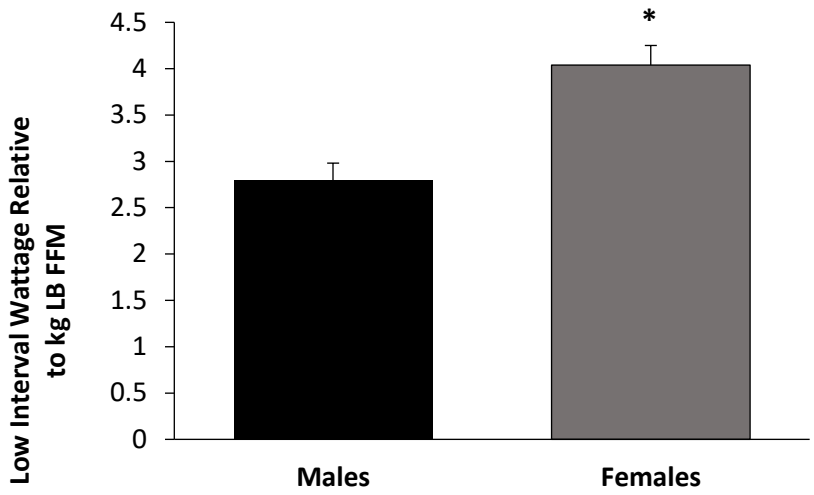
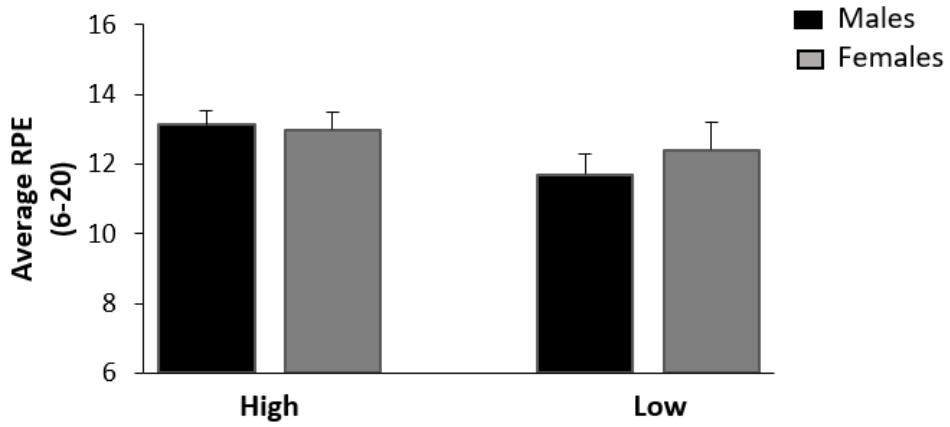


Figure 5: A) Absolute wattage during high intervals, *greater than females, p<0.0001. **B)** Wattage during high intervals expressed relative to kg LB FFM, *greater than females, p=0.02. **C)** Wattage during low intervals expressed relative to kg LB FFM, *greater than males, p=0.0002. Data are reported as the mean ± SEM.

4.2.2 Rating of Perceived Exertion during HII exercise

There was no effect of sex on RPE (p=0.66, Figure 6B). RPE increased significantly during exercise (p<0.0001, Figure 6B) with no difference between the sexes (p=0.15, Figure 6B). There was no sex difference in the average RPE values during both the high and low intervals (p=0.81 and p=0.49, respectfully, Figure 6A).

A



B

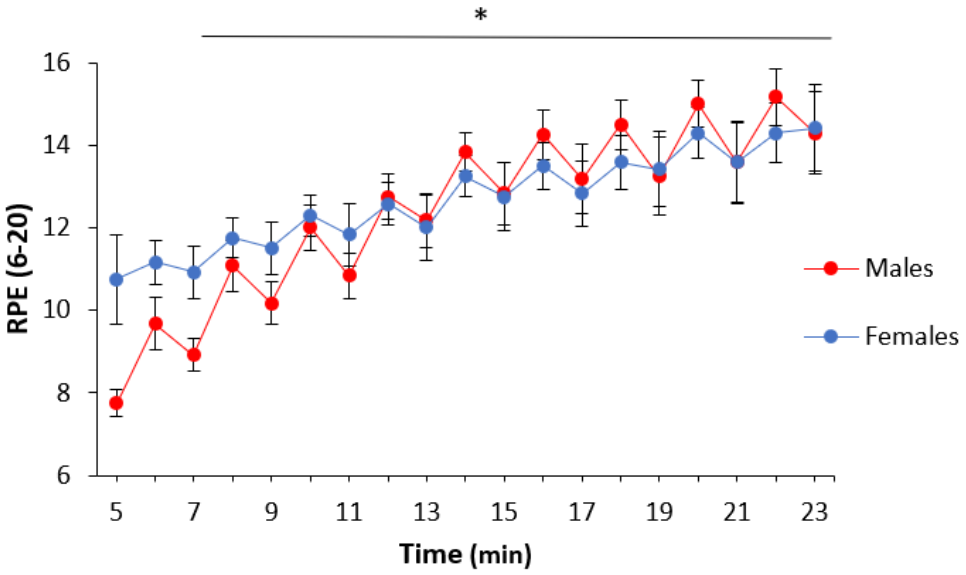
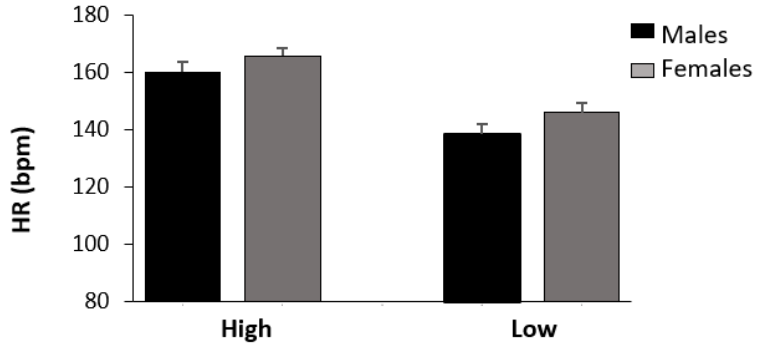


Figure 6: A) Average RPE during high and low intervals. **B)** RPE during exercise, *greater than minutes 5-7, p<0.0001. Data are reported as the mean ± SEM.

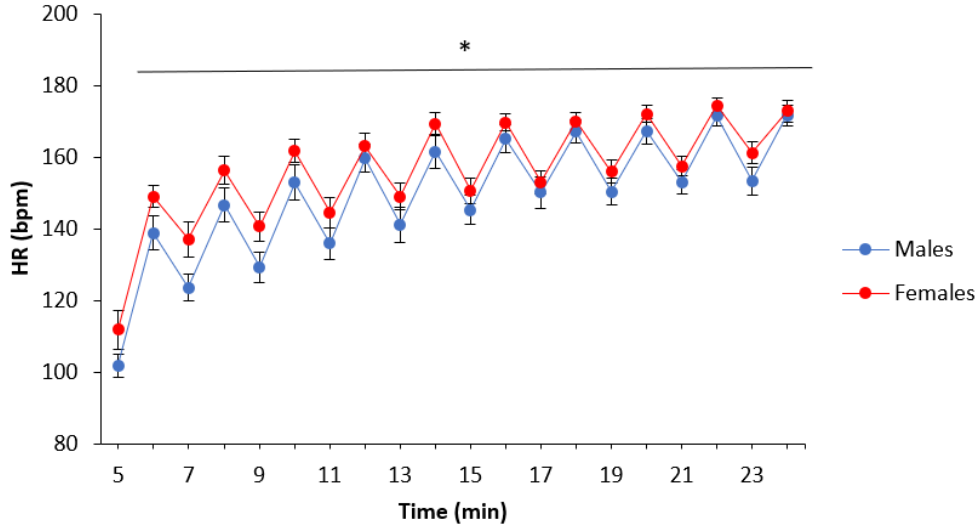
4.2.3 Heart rate during HII exercise

There was no effect of sex on HR (p=0.17, Figure 7B). HR increased significantly during exercise (p<0.0001, Figure 7B) with a tendency for a difference between the men and women (p=0.06, Figure 7B). There was no sex difference in the average HR values during both the high and low intervals (p=0.24 and p=0.13, respectively, Figure 7A). The average %target HR during the high intervals, and peak HR achieved during exercise was higher in females than males (p=0.002, and p=0.002, respectively, Figure 7C). Interestingly, when comparing the HR response to the target HR, if average HR during the high-intensity intervals was used then women were right at target and men were below target; however, if peak HR during the high-intensity intervals was used, men were right at target and women were above target (Figure 7C).

A


180
160
140
120
100
80
HR (bpm)
Males
Females
High
Low

B


200
180
160
140
120
100
80
HR (bpm)
*
5
7
9
11
13
15
17
19
21
23
Time (min)
Males
Females

C

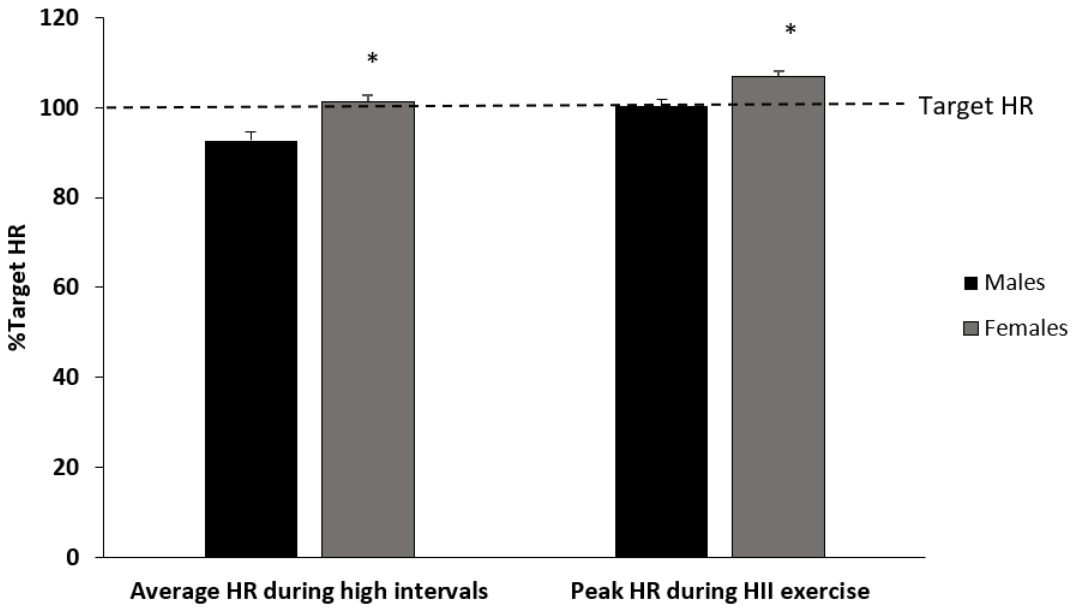


Figure 7: A) Average HR (bpm) during the high and low intervals. **B)** HR (bpm) during exercise, *greater than minute 5, p<0.0001. **C)** %Target HR based on averaged HR during high intervals and peak HR during HII exercise, *greater than males, p=0.002. Data are reported as the mean ± SEM.

4.2.4 %VO_{2peak} during HII exercise

There was a main effect of time such that %VO_{2peak} was higher during the high and low intervals compared to the warm up and cooldown (p<0.0001, Table 7). Furthermore, irrespective %VO_{2peak} was higher during the cooldown than the warm up (p<0.0001, Table 7). Lastly, there was no difference in %VO_{2peak} between the high and low intervals (Table 7).

There was a main effect of sex such that, women exercised at a higher %VO_{2peak} during the warm up, high intervals and cooldown (p=0.001, p=0.011 and p=0.005, respectively, Table 7)., However, %VO_{2peak} was not significantly different between men and women during the low intervals (p=0.25, Table 7). Additional comparison of %VO_{2peak} can be found in supplementary Table 1.

Table 7: $\%VO_{2peak}$ during warm up, high intervals, low intervals and cool down

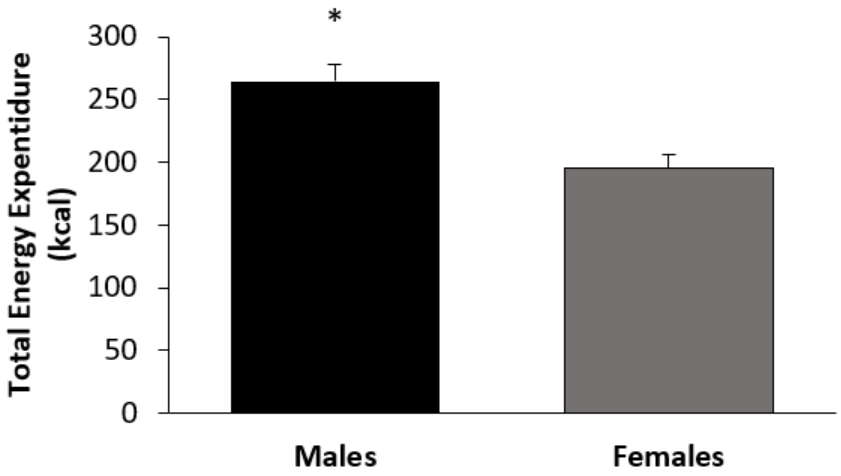
	Warm Up	High Intervals	Low Intervals	Cool down	P Values
$\%VO_{2peak}$					
Men	30.3 ±1.5[e]	55.8 ± 3.6[a,b,c]	62.4 ± 3.2[a,b]	37.0 ± 2.3[a,d]	Time: <0.0001
Women	42.3 ± 2.4	68.9 ± 3.2[a,b]	67.2 ± 2.6[a,b]	51.5 ± 3.4[a]	Sex: 0.008
					S x T: 0.006

P Vales are presented in order of main effect of time, main effect of sex, and interaction of sex and time. Data are means ± SEM. [a] different than warm up, p<0.0001; [b] different than cooldown, p<0.0001; [c] different than women, p=0.011; [d] different than women, p=0.005; [e] different than women, p=0.001.

4.3 Energy Expenditure during HII exercise

Men expended more energy overall during the exercise bout compared to women (p=0.001, Figure 8A). However, energy expenditure relative to kg FFM was not different between the sexes (p=0.13, Figure 8B).

A



B

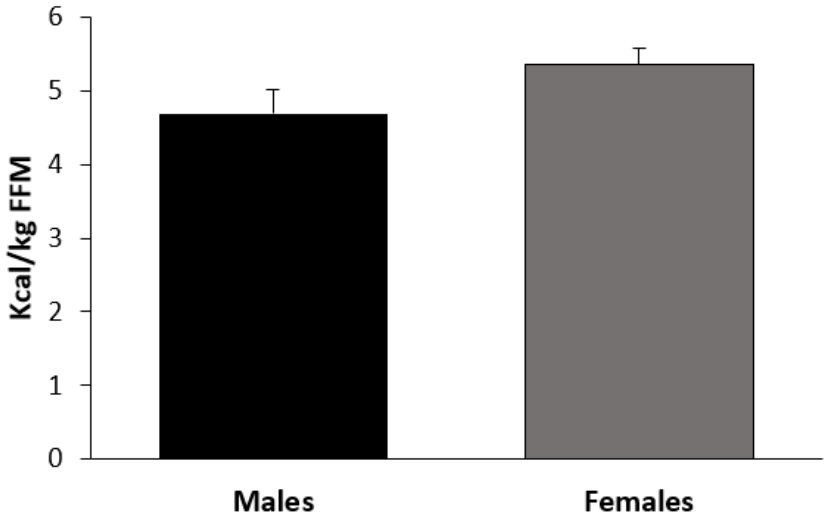


Figure 8: A) Total energy expenditure (kcal) during HII exercise, *greater than females, p=0.001. **B)** Total energy expenditure (kcal) relative to kg FFM. Data are reported as the mean ± SEM.

4.4 Metabolic Response to HII Exercise

4.4.1 Whole-body Substation Utilization during HII Exercise

4.4.1.1 RER during HII Exercise

There was a main effect of time such that during the high and low intervals, RER was higher than both the warm up ($p<0.0001$, Table 8) and cooldown ($p<0.0001$, Table 8). Furthermore, during the cooldown, RER was higher than the warm up ($p<0.0001$, Table 8); however, there was no difference in RER between the high and low intervals ($p=0.27$, Table 8). Sex had no effect on RER at any time point during exercise ($p=0.55$, Table8), nor did sex influence the change in RER throughout the exercise bout ($p=0.29$, Table 8).

4.4.1.2 Whole Body CHO Oxidation during HII Exercise

Irrespective of how it was expressed, there was a main effect of time such that CHO oxidation was higher during the high and low intervals compared to warm up and cooldown ($p<0.0001$, Table 8 and Table 9). Furthermore, irrespective of how it was expressed CHO oxidation was also higher during the warm up than the cooldown ($p<0.0001$, Table 8 and Table 9). Additionally, when expressed as rate of oxidation (g/min), absolute g oxidized, or g oxidized relative to BW or FFM, CHO oxidation was higher during the low intervals compared with the high intervals ($p=0.002$, $p=0.002$, $p=0.003$ and $p=0.007$, respectively, Table 9). However, there was no difference in % CHO contribution to TEE between the high and low intervals ($p=0.28$, Table 8).

There was a main effect of sex such that the rate of CHO oxidation, the absolute number of grams of CHO oxidized and grams of CHO oxidized expressed relative to BW was higher in men than women. ($p<0.0001$, $p<0.0001$ and $p=0.046$, respectively, Table 8 and 9). There was no effect of sex on the % CHO contribution to TEE or grams of CHO oxidized expressed relative to FFM ($p=0.56$ and $p=0.40$, respectively, Table 8 and Table 9).

There was a significant sex x time interaction such that the rate of CHO oxidation and absolute grams of CHO oxidized was higher in men than women during the high intervals ($p=0.001$, Table 8 and Table 9), low intervals ($p<0.0001$, Table 8 and Table 9), and cooldown ($p=0.001$, Table 8 and Table 9), but not the warm up ($p=0.65$, Table 8 and Table 9). There was also a significant interaction of sex x time on the grams of CHO oxidized expressed relative to BW where men oxidized more CHO during the low intervals ($p=0.001$, Table 9) than women. There were no other sex x time interactions for any of the other CHO oxidation measures (Table 8 and Table 9).

Whether expressed in absolute terms or relative to BW men oxidized more CHO than women ($p<0.0001$, $p=0.046$, respectively, Table 11) overall during the exercise bout. However, when expressed relative to fat-free mass, the sex difference was eliminated ($p=0.40$, Table 11).

4.4.1.3 Whole Body Fat oxidation during HII Exercise

Irrespective of how it was expressed, there was a main effect of time ($p<0.0001$, Table 8 and Table 9) such that fat oxidation during the warm up was higher than all other time points. Additionally, the % fat contribution to TEE and rate of fat oxidation were higher during the cooldown than both the high and low intervals ($p<0.0001$, Table 8). Furthermore, fat oxidation was higher during the cooldown than the low intervals when expressed as absolute grams of fat oxidized ($p=0.006$, Table 9) or grams of fat oxidized relative to body weight ($p=0.003$, Table 9) and FFM ($p=0.004$, Table 9). Lastly, during the high intervals, the rate of fat oxidization, the absolute grams of fat oxidized and grams of fat oxidized relative to BW and FFM were higher than the low intervals ($p=0.016$, $p=0.016$, $p=0.014$ and $p=0.019$ respectively, Table 8 and Table 19). There was no difference in %fat contribution to TEE between the high and low intervals ($p=0.28$, Table 8).

There was no effect of sex or a sex x time interaction for fat oxidation during the exercise bout irrespective of how it was expressed. Furthermore, when totalled across the entire exercise bout there was no difference in the number of grams of fat oxidized expressed in absolute terms ($p=0.94$, Table 9), or relative to BW ($p=0.48$, Table 9) or FFM ($p =0.19$, Table 9).

Table 8: RER, %CHO and %Fat Contribution to TEE and Rate of CHO and Fat Oxidation (g/min) during Warm Up, High Intervals, Low Intervals and Cooldown

	Warm Up	High Intervals	Low Intervals	Cooldown	P-Value
RER					
Men	0.84 ± 0.2	0.99 ± <0.01[a,b]	0.99 ± <0.01[a,b]	0.96 ± 0.01[a]	Time:<0.0001
Women	0.86 ± 0.02	0.97 ± 0.01[a,b]	0.98 ± 0.01[a,b]	0.93 ± 0.01[a]	Sex: 0.55
					S x T: 0.29
% CHO Contribution to TEE	49.2 ± 7.8	95.4 ± 1.5[a,b]	96.8 ± 1.1[a,b]	85.9 ± 3.2[a]	Time: <0.0001
Men	54.7 ± 7.6	91.8 ± 1.9[a,b]	93.5 ± 2.1[a,b]	77.0 ± 3.9[a]	Sex:0.56
Women					S x T: 0.31
% Fat Contribution to TEE	50.8 ± 7.8	4.6 ± 1.5[a,b]	3.2 ± 1.1[a,b]	14.1 ± 3.2[a]	Time: <0.0001
Men	45.3 ± 7.6	8.2 ± 1.9[a,b]	6.5 ± 2.1[a,b]	23.0 ± 3.9[a]	Sex: 0.56
Women					S x T: 0.31
CHO Oxidation Rate (g/min)					
Men	0.61 ± 0.10[b]	2.35 ± 0.14[a,b,e]	2.63 ± 0.13[a,b,d,f]	1.39 ± 0.09[,a,e]	Time: <0.0001
Women	0.56 ± 0.07	1.69 ± 0.10[a,b,e]	1.69 ± 0.10[a,b,d,f]	1.02 ± 0.04[a,e]	Sex: <0.0001
					S x T: <0.0001
Fat Oxidation Rate (g/min)					
Men	0.27 ± 0.03	0.05 ± 0.02[a,b]	0.04 ± 0.01[a,b,c]	0.10 ± 0.02[a]	Time: <0.0001
Women	0.22 ± 0.04	0.07 ± 0.02[a,b]	0.03 ± 0.01[a,b,c]	0.15 ± 0.03[a]	Sex:0.98
					S x T:0.17

P Vales are presented in order of main effect of time, main effect of sex, and interaction of sex and time. Data are means ± SEM. [a] different than warm up, p<0.0001; [b] different than cooldown, p<0.0001; [c] different than high intervals, p=0.016; [d] different than high, p=0.002, [e] different than women, p=0.001; [f] different than women, p<0.0001.

Table 9: Absolute and Relative Grams of CHO and Fat Oxidized during Warm Up, High Intervals, Low Intervals, Cooldown and Total

	Warm Up	High Intervals	Low Intervals	Cooldown	P-Value	Total
CHO (g)						
Men	3.1 ± 1.6	23.5 ± 1.4[a,e,m]	26.3 ± 1.3[a,e,k,n]	7.0 ± 0.4[a,m]	Time: <0.0001	59.8 ± 3.2[n]
Women	2.8 ± 0.3	16.9 ± 1.0[a,e]	16.9 ± 0.9[a,e,k]	5.1 ± 0.2[a]	Sex: <0.0001	41.7 ± 1.8
					S x T: <0.0001	
Fat (g)						
Men	1.3 ± 0.2	0.5 ± 0.2[a]	0.4 ± 0.1[a,b,g]	0.5 ± 0.1[a]	Time: <0.0001	2.8 ± 0.5
Women	1.1 ± 0.2	0.7 ± 0.2[a]	0.3 ± 0.1[a,b,g]	0.7 ± 0.1[a]	Sex:0.94	2.8 ± 0.5
					S x T:0.10	
g CHO/kg BW						
Men	0.04 ± 0.01	0.31 ± 0.02[a,e]	0.34 ± 0.02[a,e,j,m]	0.09 ± <0.01[a]	Time: <0.0001	0.84 ± 0.07[l]
Women	0.05 ± 0.01	0.27 ± 0.01[a,e]	0.27 ± 0.01[a,e,j]	0.08 ± 0.01[a]	Sex: 0.046	0.68 ± 0.03
					S x T: 0.02	
g Fat/kg BW						
Men	0.02 ± 0.01	<0.01 ± <0.01[a]	0.01 ± <0.01[a,d,h]	0.01 ± <0.01[a]	Time: <0.0001	0.04 ± <0.01
Women	0.02 ± <0.01	0.01 ± <0.01[a]	<0.01 ± <0.01[a,d,h]	0.01 ± <0.01[a]	Sex: 0.49	0.04 ± 0.01
					S x T: 0.13	
g CHO/kg FFM						
Men	0.05 ± 0.01	0.42 ± 0.03[a,e]	0.47 ± 0.03[a,e,i]	0.12 ± 0.01[a]	Time: <0.0001	1.14 ± 0.12
Women	0.08 ± 0.01	0.46 ± 0.02[a,e]	0.45 ± 0.02[a,e,i]	0.14 ± 0.01[a]	Sex: 0.40	1.13 ± 0.05
					S x T: 0.26	
g Fat/kg FFM						
Men	0.02 ± <0.01	0.01 ± <0.01[a]	0.01 ± <0.01[a,c,f]	0.01 ± 0.01[a]	Time: <0.0001	0.05 ± 0.01
Women	0.03 ± <0.01	0.02 ± <0.01[a]	0.01 ± <0.01[a,c,f]	0.02 ± <0.01[a]	Sex: 0.19	0.07 ± 0.01
					S x T:0.12	

P Vales are presented in order of main effect of time, main effect of sex, and interaction of sex and time. Data are means ± SEM. [a] different than warm up, p<0.0001; [b] different than cooldown, p=0.006;[c] different than cooldown, p=0.004; [d] different than cooldown, p=0.003; [e] different than cooldown, p<0.0001; [f] different than high, p=0.019; [g] different than higher p=0.016; [h] different than high p=0.014; [i] different than high, p=0.007; [j] different than high, p=0.003; [k] different than high, p=0.002; l different than women , p=0.046; [m] different than women, p=0.001; [n] different than women, p<0.0001.

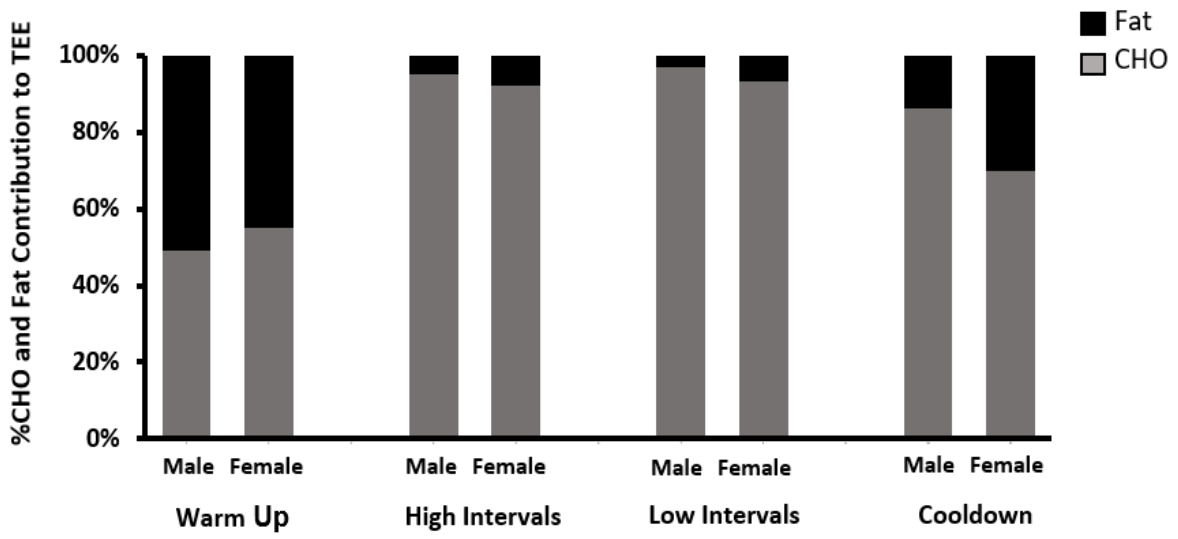
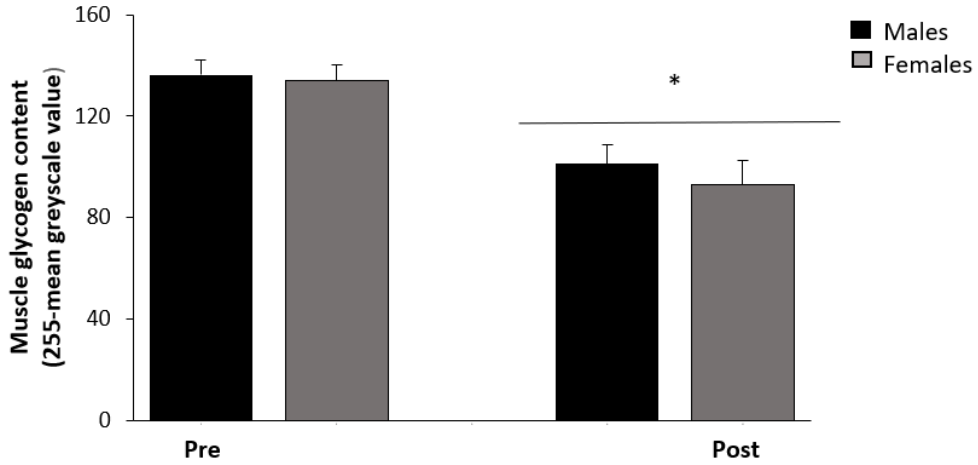


Figure 9: %CHO and %fat contribution to TEE during HII exercise.

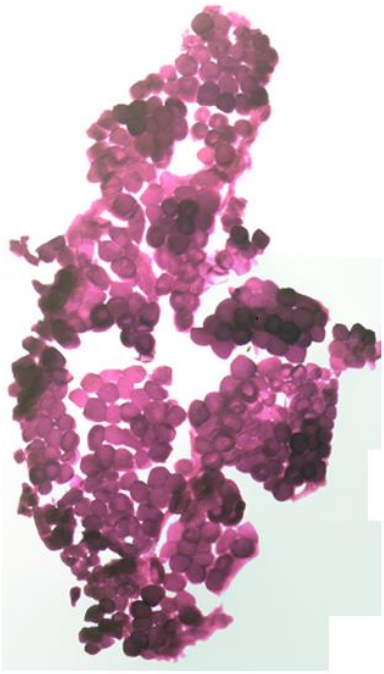
4.4.2 Muscle Glycogen Utilization during HII exercise

Muscle glycogen concentration was similar between men and women at rest and post exercise ($p=0.54$, Figure 10A). Muscle glycogen decreased from pre to post exercise ($p<0.0001$, Figure 10A) with no difference in the decrease between the sexes ($p=0.63$, Figure 10A).

A



B



`C

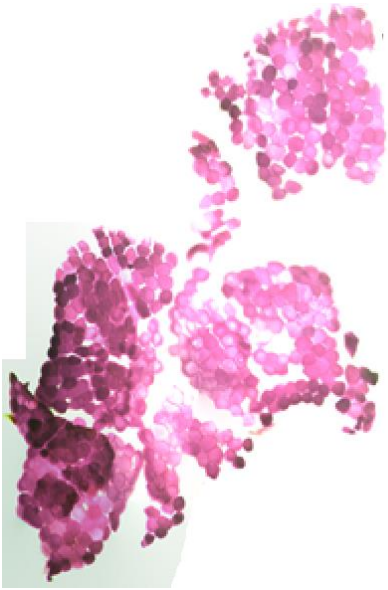
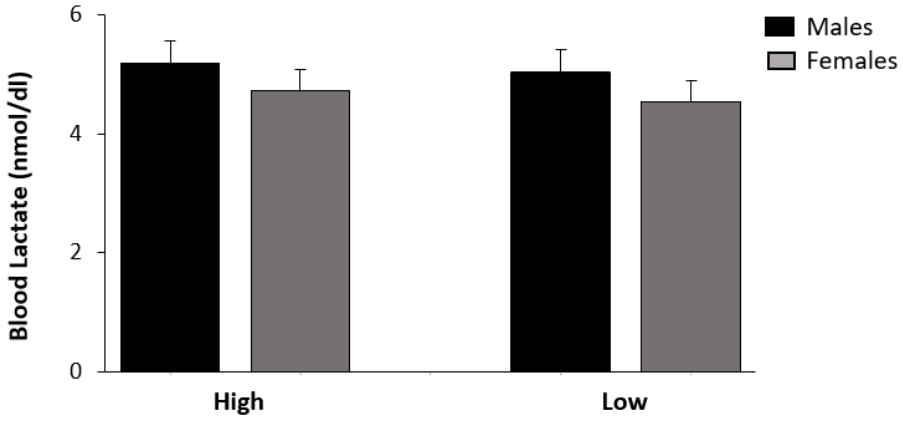


Figure 10: A) Muscle glycogen utilization during HII exercise, *less than pre, p<0.0001. **B)** Representative PAS stained pre-exercise image. **C)** Representative PAS stained post-exercise image (same participant as B).

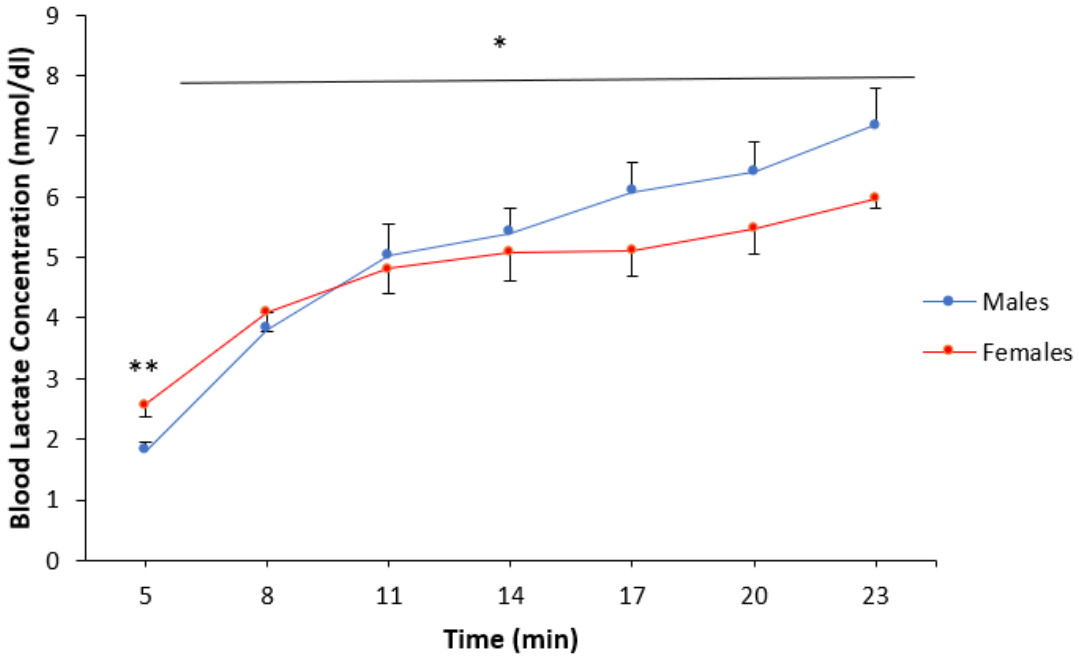
4.4.3 Blood Lactate Concentration during HII Exercise

Blood lactate concentration was not significantly different between men and women at rest or during exercise (p=0.35, Figure 11B). Blood lactate increased significantly during exercise in both men and women (p<0.0001, Figure 11B) with the increase being greater in men than women (sex x time interaction, p=0.02, Figure 11B). Post hoc test revealed that at the end of warm up, blood lactate concentrations were greater in women than men (p=0.007, Figure 11B). There was no sex difference in peak blood lactate concentration (p=0.17, Figure 11C) or the average blood lactate concentration during the high and low intervals (p=0.36 and p=0.35, respectively, Figure 11A). When resting blood lactate levels were controlled for, blood lactate AUC was higher in men than women (p=0.007, Figure 11D); however, this was not found when resting blood lactate levels were not controlled for (p=0.32, Figure 11E).

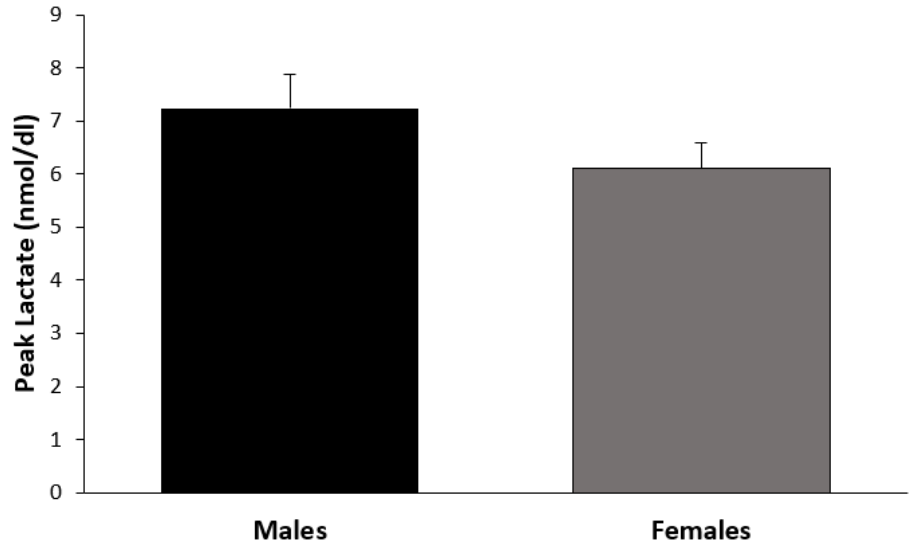
A



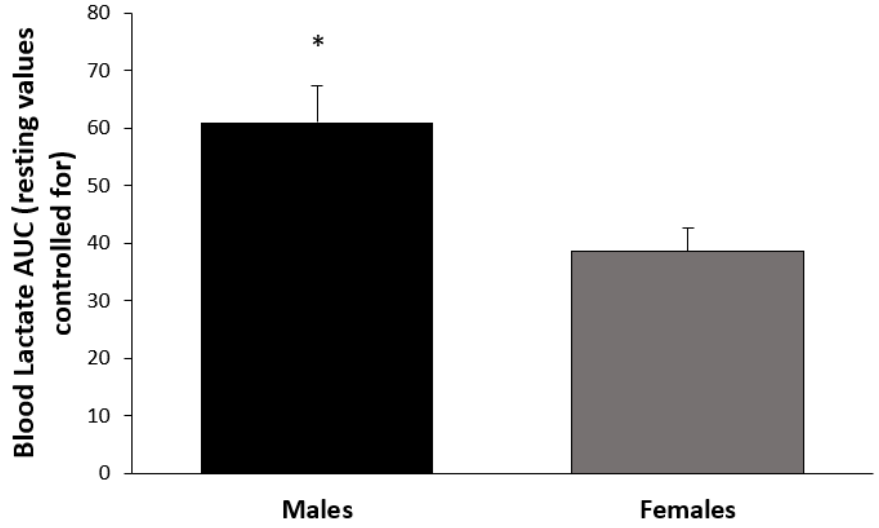
B


Blood Lactate Concentration (nmol/dl)
Time (min)
Males
Females
*
**
0
1
2
3
4
5
6
7
8
9
5
8
11
14
17
20
23

C


Peak Lactate (nmol/dl)
Males
Females
0
1
2
3
4
5
6
7
8
9

D



E

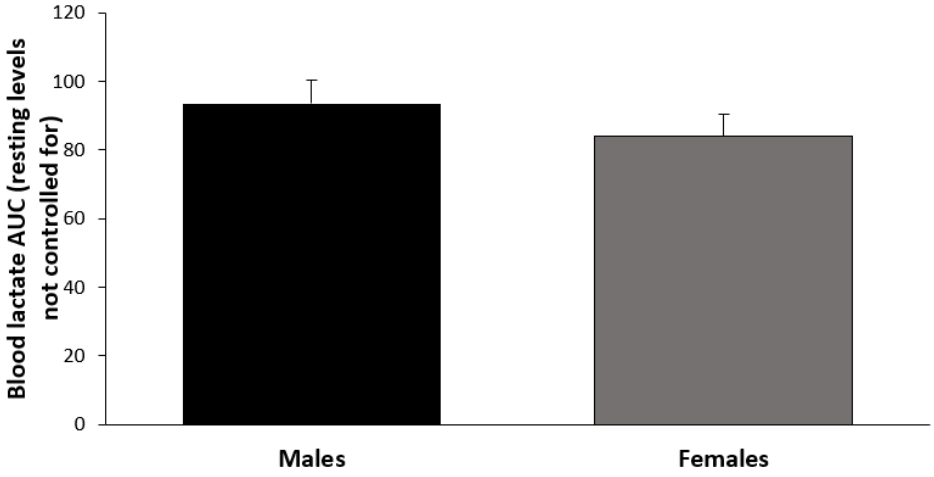


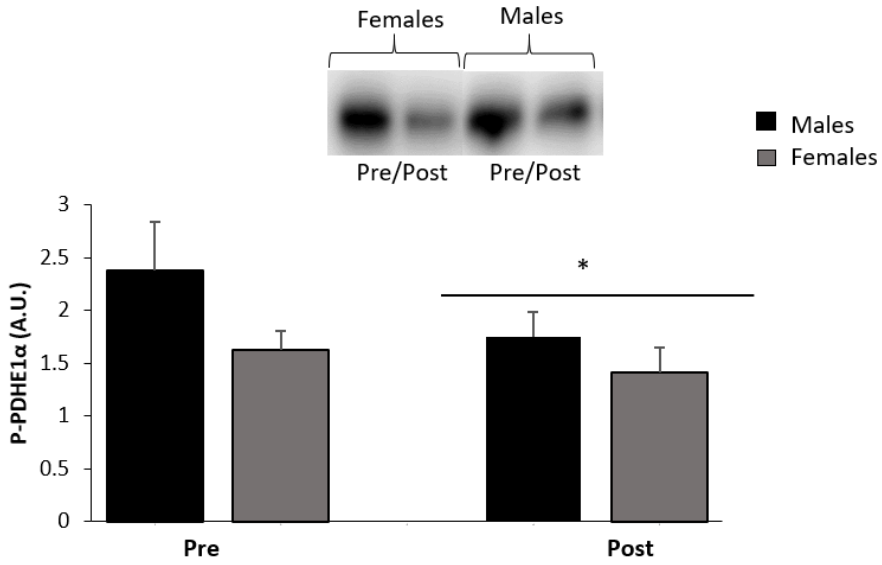
Figure 11: A) Average blood lactate concentration during high and low intervals. **B)** Blood lactate concentration during HII exercise, *greater than minute 5, $p<0.0001$; **greater in females than males, $p=0.007$. **C)** Peak blood lactate concentration. **D)** Blood lactate AUC with resting values controlled for, *$p=0.007$. **E)** Blood lactate AUC with resting values not controlled for ($p=0.32$). Data are reported as the mean ± SEM.

4.4.4 Change in Protein Phosphorylation Status during HII Exercise

There was no difference in P-PDHE1α between men and women (p=0.31, Figure 12A). PDHE1α phosphorylation decreased during exercise (p=0.002, Figure 12A) with no difference between the sexes (p=0.18, Figure 12A). Western blot images for all participants for PDHE1α can be found in supplementary Figure 2.

There was no difference in P-CK between men and women (p=0.23, Figure 12B). CK phosphorylation decreased during exercise (p=0.002, Figure 12B) with no difference between the sexes (p=0.243, Figure 12B). Western blot images for all participants for P-CK can be found in supplementary Figure 3.

A



B

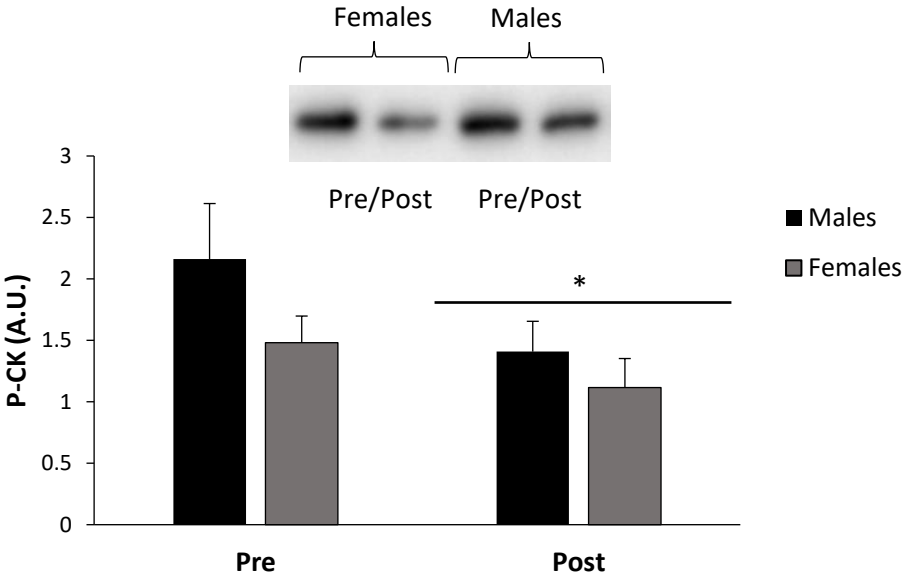


Figure 12: A) Phosphorylation status of pyruvate dehydrogenase E1α normalized to total protein content, *less than pre, p=0.002. **B)** Phosphorylation status of creatine kinase normalized to total protein content, *less than pre, p0.002. Data are reported as the mean ± SEM.

Chapter Five: Discussion

5.1 Overall Summary

We found similar decreases in muscle glycogen, phosphorylation status of glycolytic enzyme PDHE1α, and phosphorylation status of HEPT system enzyme CK. However, despite a similar decline in muscle glycogen and PDHE1α phosphorylation, lactate AUC was higher in men than women. Interestingly, this all occurred despite women exercising at a slightly higher relative $\%VO_{2peak}$ and achieving a higher average %target HR during the high intervals. Unfortunately, given that steady state during exercise was not achieved due to the interval nature of the exercise bout leading, it is difficult to make any conclusions regarding sex differences in whole body substrate utilization. Overall, these findings suggest that during an acute bout of HII exercise with fixed intensity low intervals, men may rely on their anaerobic glycolytic pathways more than women, however the reliance on muscle glycogen and the HEPT system is similar.

5.2 Activation of HEPT and Glycolysis during HII Exercise

5.2.1 Activation of HEPT during HII Exercise

The phosphorylation status of the HEPT system enzyme CK was used in the present study to give insight into the activation of the HEPT system during an acute bout of HII exercise. Considering that phosphorylation of CK inhibits its activity[118,119], our finding that phosphorylated CK decreased pre to post exercise suggests that the HEPT system was upregulated during exercise. The mechanism by which CK is phosphorylated however is unclear with previous research suggesting that there is likely that more than one mechanism responsible for the regulation of CK phosphorylation[118,119]. In one study using rat heart muscle[118], protein kinase C (PKC) was found to phosphorylate CK. However, in another study using rabbit skeletal muscle[119] it was found that AMPK phosphorylated CK. The latter study also suggested that

AMPK can be activated by a drop in PCr:Cr ratio, whereby it phosphorylates CK as a preservation mechanism for PCr breakdown[119]. This would refute our findings since we know that AMPK increases with exercise[22], this would therefore theoretically result in an increase in P-CK. However, if phosphorylating CK inhibits CK activity, then that would suggest the HEPT system is not activated during exercise (or at least not through this mechanism). In agreement with the present findings, another trial from our lab found P-CK to decrease after an acute bout of low-load, high repetition exercise[120], implying that that the HEPT system is upregulated through dephosphorylation of CK during exercise by an unknown mechanism, or group of mechanisms. Future research in human skeletal muscle is needed to identify the underpinning mechanisms responsible for phosphorylating this enzyme and how it may be influenced by exercise.

5.2.2 Activation of Glycolysis during HII Exercise

The increase in glycolytic activation that is seen during intense exercise results in an increased flux of glycogen to pyruvate[22,26]. Pyruvate is then converted either to lactate under anaerobic conditions or acetyl-CoA under aerobic conditions[22,26]. Importantly, the contribution of pyruvate flux to each of these pathways is relative, and not an 'all or none' phenomenon[22]. The phosphorylated state of the enzyme PDHE1α inhibits the conversion of pyruvate to acetyl-coA[121]. P-PDHE1α can be dephosphorylated however to remove this inhibition when oxygen becomes more readily available[121]. Our findings showed that indeed, there was a reduction in phosphorylated PDHE1α from pre to post exercise, suggesting an increase in aerobic metabolism from rest since the ability to convert pyruvate to acetyl-coA had less inhibition. Moreover, anaerobic metabolism also seems to be upregulated as there was an increase in blood lactate concentration from pre to post exercise. Blood lactate increases during MIC

exercise[11,13,17,21,23,86,88], but to a greater extent during HII[47,48,61,122] and SI[9,10,65,66] exercise, highlighting the anaerobic aspect of interval exercise. Other exercise trials have corroborated our findings that phosphorylation of PDHE1α decreases during 20 minutes of knee extensor exercise at 65-70%$Watt_{max}$[123], during 3 hours of knee extensor exercise at 50% $Watt_{max}$[124], and during 30 minutes of cycling at 70% $Watt_{max}$[125]. The fact that both aerobic and anaerobic aspects of the glycolytic system are upregulated during our exercise bout would suggest that both aerobic and anaerobic glycolytic pathways are relied upon significantly during HII exercise.

5.2.2.1 Glycogen Utilization during HII Exercise

Although the exact concentration of muscle glycogen was not determined in the present study, we were able to obtain a relative measure of glycogen utilization using PAS staining. We found a significant decrease in muscle glycogen of ~34%. Acute MIC[11,17,18,20,86] and interval[9,10,122,46–48,61,65,66,68,89] exercise trials have also shown that glycogen contributes significantly to energy expenditure during exercise. The extent of glycogen utilization during MIC exercise seems to plateau around 30-50% depletion[11,17,18,20,86]. Our findings are within this range, emphasizing the efficiency of interval exercise as it shows that only 10 minutes of intense exercise (interspersed with low intensity cycling) was able to elicit similar amounts of muscle glycogen utilization to 90 minutes of continuous cycling at a moderate intensity (i.e ~60-65%$VO_{2peak.}$). In the present study, the glycolytic system must have therefore been activated to a greater extent than MIC exercise trials during the high intervals since similar degrees of muscle glycogen degradation was achieved in much less time; confirming HII exercise to be more glycolytic in nature than MIC exercise.

In other acute HII exercise trials, glycogen seems to be depleted by about ~40-50%[46–48,61,122], which is slightly higher than what our study found. Most of these HII exercise studies however,

used protocols of 6-8 intervals that were 4-5 minutes long, with 1-2 minutes rest, at 85-100% aerobic capacity[46–48,122]. Therefore, the difference between our findings and these HII exercise trials is expected since the overall time spent exercising was longer and the intensity was slightly higher in the other HII exercise trials compared to our exercise protocol.

Glycogen also contributes significantly to TEE during SI exercise[9,10,65,66,68], with the extent of overall glycogen depletion to be around ~20-30%[66,68]. This is slightly less than what our study found, and this difference could partially be attributed to the difference in work:rest ratios between SI and HII exercise protocols. For SI protocols, a longer rest duration provides ample time for the HEPT system to replenish PCr, providing a greater capacity for the muscle to use PCr during each sprint and resulting in less of a demand for glycogen. Some SI exercise studies that have measured fibre-specific muscle glycogen utilization have found that the extent of glycogen degradation was higher in type II than type I fibres[9,10,65]. Additionally, the one HII exercise study to my knowledge that investigated fibre-specific glycogen utilization corroborated these findings[61]. Thus, the results of the present study where muscle glycogen decreased by ~34% indicates that our HII exercise trial relied on glycolytic systems greater than MIC exercise, equally to other HII exercise, and equally, or slightly more than SI exercise.

5.3 Whole Body Substrate Oxidation during HII Exercise

Indirect calorimetry data revealed that on average, CHO accounted for 94% of total energy expenditure (TEE) during the high intervals, and 95% during the low intervals; whereas fat contributed to 6% of TEE during the high intervals and 5% during the low intervals (assuming negligible protein oxidation). During the exercise bout, CHO oxidation rates averaged to be ~2.09g/min (2.02 g/min during the high intervals and 2.16g/min during the low intervals); whereas for fat, the oxidation rate was~0.05g/min (0.06g/min during high intervals and

0.03g/min during low intervals). Importantly however, given that it takes ~2-3 minutes to reach steady state during exercise and the intervals in the current trial were only 1 minute in duration, we cannot assume that these results are valid. Indeed the finding that CHO oxidation was higher during the low intervals compared to the high intervals supports that these data are invalid as with increasing exercise intensity, there is an increased demand for CHO[22]. Therefore, the validity of the results derived from the indirect calorimetry measurements are in question and due to this, we unfortunately cannot make any definitive conclusions or speculations about whole-body substrate utilization.

5.4 Sex differences during HII Exercise

5.4.1 Exercise Intensity

At an absolute level, men expended more energy throughout the exercise bout. However, when expressed relative to FFM, the effect of sex was eliminated. This is in accordance with the fact that men have a higher body mass and therefore require a greater energy output during exercise at the same relative intensity compared to women who have a lower body mass[126].

Given differences in body weight and body composition, sex comparative exercise trials are conducted such that men and women work at the same relative exercise intensity so that the fuel utilization pattern can be accurately compared. In the present study, which used a standardized HII exercise protocol, the intensity during the warm up, low intervals and cooldown was a fixed intensity of 50W, which resulted in women working at a higher relative intensity throughout the exercise bout (avg 68%VO_{2peak} for women vs. avg 59% VO_{2peak} for men). Additionally, when the workrate was expressed relative to LB FFM, in order to account for differences in leg muscle mass between the sexes, women worked at a higher workrate than men during the warm up, low intervals and cooldown (avg workrate of 4.0 Watts/LB-FFM for women vs avg workrate 2.8

Watts/LB-FFM for men). Furthermore, despite using the same relative workrate for the high intervals, when workrate was expressed relative to LB-FFM for the high intervals, workrate was higher in men than women (avg workrate of 9.3Watts/LB-FFM for women vs avg workrate 10.9 Watts/LB-FFM for men). Given that men and women were exercising at different intensities throughout the exercise bout, the sex differences (or lack thereof) in metabolism that were found in the current trial are hard to interpret as they may reflect true sex differences in metabolism or differences due to exercise intensity. Importantly, however, is that the intent of the current trial was to examine how sex influenced fuel metabolism during a standardized bout of HII exercise since that is what is commonly used in HII training trials that have found a blunted effect of training on insulin sensitivity in women. Thus, the differences in metabolism observed in the current trial should be examined further to determine whether they may underpin the blunted effect of HII training on insulin sensitivity in women.

Women exercised at a higher absolute and relative intensity during much of the exercise bout. When exercising at a higher intensity there is a greater reliance on CHO substrates[22]. Therefore, the higher exercise intensity at which women were working may have masked a sex difference in muscle glycogen utilization and we cannot necessarily say that muscle glycogen does not differ between men and women during HII exercise when performed at the same relative intensity. Additionally, since women were working at a higher relative intensity during the low intervals their ability to recover between intervals may have been compromised compared with men. Together these findings suggest that men and women may adapt differently in response to a period of HII training and that, since the stimulus is greater in women, women may respond more favourably to HII training than men. This is in contrast to what is seen in response to HII training as physiological and metabolic adaptations are reported to be similar between men and

women[4,5,127,128] and women respond less favourably than men when it comes to the ability of HII training to improve insulin sensitivity[3–5]. However, perhaps men and women adapt similarly to HII training due to the fact that men work at a higher relative workrate during the high intensity intervals. Indeed, if the relative energy requirement during the high intervals is greater in men, then they may be using their HEPT system to a greater extent as well during those intervals compared with women. If this is the case, perhaps the ratio of AMP:ATP is also greater in men, which would result in greater activation of AMPK, which in turn may explain why insulin sensitivity improves to a greater extent in men than women. Furthermore, perhaps it is the intensity of the high intervals that drives the improvement in insulin sensitivity. Indeed, increased reactive oxygen species following exercise have been suggested as a mechanism by which training induces adaptations[129,130] and reactive oxygen species generation is intensity-dependent[131]. Thus, perhaps it is the difference in relative intensity between men and women during the high intervals that results in a blunted/absent improvement in insulin sensitivity in women following HII training.

Despite men working at a higher wattage during the high intervals relative to LB FFM, women exercised at a significantly higher $\%VO_{2peak}$ than men during the high intervals (women: 69% vs men: 55%), but not during the low intervals (women: 67% vs men: 62%,). When the high and low interval VO_2 measures were averaged, women exercised at a higher $\%VO_{2peak}$ (women: 68% vs men: 59%) than men. The apparent difference in relative exercise intensity between the sexes can most likely be attributed to differences in relative intensity during the low intervals since all participants exercised at 50W during the low intervals. To determine the $\%VO_{2peak}$ that 50W elicits in each group without the influence of EPOC, the VO_2 from the final two minutes of warm up (representing steady state at 50W) was averaged and it was found that 50W corresponded to

42% VO_{2peak} in women and only 30%VO_{2peak} in men. Therefore, it is of no surprise that the relative %VO_{2peak} for women during the overall exercise was higher, since they exercised at a higher relative intensity during the low intervals.

Interestingly, despite the fact that participants underwent a familiarization visit to ensure that the workrates they were cycling at equated to 90% HR_{max} (target HR), females achieved 101% of the target HR on average during the high intervals, whereas males only achieved 93%. When we look at peak HR achieved during the exercise bout however, men achieved 100% of their target HR whereas women overshot their target and achieved 107%. Therefore, whether men or women were working at the targeted HR was dependent on how we analyzed the data. Regardless, in both instances, women achieved a higher % target HR. This finding was unexpected since we assumed that the familiarization visit confirmed that the appropriate HR response would be achieved during the acute trial. Furthermore, although our participants were fasted for the HII exercise trial, and fed during the familiarization visit, being in a fed vs fasted state does not influence HR response to exercise[3,132,133]. Our HR findings are in line with some literature, including one HII exercise trial that found women had a higher %HR_{max} on average, during 6 x 4-min intervals, despite working at a slightly lower relative %VO_{2peak}[98]. As well, in men and women over the age of 60, women had higher HR values for a given submaximal workload[134]. Lastly, after only 4 x 30sec sprints at a treadmill speed set to elicit 110% VO_{2peak}, females achieved a higher %HR_{max} than their male counterparts[115]. The findings from these studies suggest that at the same, or lower relative intensity, women reach a higher %HR_{max}. This somewhat supports our finding that women had a significantly higher relative HR than men during exercise, however it does not address the fact that the familiarization visit failed to bring this issue to light. Although our female participants were working at a higher intensity during the

low intervals, the findings from the two aforementioned interval studies[98,115] found that women had higher relative HRs during the high intervals even when the recovery period intensity was the same between the sexes. Therefore, we cannot definitively conclude that the increased relative HR females experience in our trial was due to differences in intensity during the recovery periods.

It was also found that although women exercised at a higher relative $\%VO_{2peak}$ and $\%HR_{max}$, there was no difference in RPE throughout the exercise bout. As well, average RPE during the high and low intervals were similar between men and women, despite women working at a greater relative workrate during the low intensity intervals. This lack of a sex difference in RPE is not surprising considering that research has suggested that at a given relative workrate, women select a lower RPE score[135–138].

The current trial utilized the standard HII exercise mode used in numerous HII training trials[3,107,113]. The finding that the relative intensity during this specific HII exercise protocol differs between men and women is important as it highlights that the training stress induced by this mode of training differs between men and women. The finding that the exercise intensity during HII exercise is higher in women than men makes it difficult to determine whether sex differences in metabolism during HII exercise are due to sex, exercise intensity or both. However, this specific HII protocol is what is used in clinical populations[3,107,113] and has shown a lack of effect of HII training on IS improvements in women[3]. Thus, if differences in metabolism underpin the lack of effect of HII training on IS it is important to study sex differences in metabolism during HII exercise even if the exercise intensity differs between the sexes.

5.4.2 Metabolism

5.4.1 Whole-body Substrate Oxidation

There was no difference in RER between men and women during HII exercise. Consequentially, there was no difference in % contribution of substrate to TEE at the whole-body level at any time point. Although carbohydrates contributed 96% of TEE in males and only 93% in females when the high and low intervals were averaged together, this difference was not significant. However, when expressed absolutely (g oxidized), relative to total body mass (g/kg) or as a rate (g/min), men oxidized a greater amount of CHO throughout the exercise bout. When expressed relative to FFM however, CHO oxidation was not different between men and women. There was no sex difference in any measure of fat utilization during HII exercise. As previously mentioned however, due to the questionable validity of the indirect calorimetry measurements, we cannot definitively say if this is in fact the case or interpret these results any further.

5.4.2 CHO Utilization

Muscle glycogen utilization during HII exercise was similar between the sexes, which is in agreement with the findings of one SI exercise study that did not find overall muscle glycogen utilization to differ between the sexes[89]. However, two other SI exercise studies reported sex differences in muscle fibre-type specific glycogen utilization with women using less muscle glycogen in type I fibres, but no difference in muscle glycogen utilization in type II fibres[9,10]. Unfortunately these two studies did not determine whether the sex difference in type I muscle glycogen utilization equated to an overall effect of sex on muscle glycogen utilization; however, the one study noted that overall muscle glycogen utilization was ~20% lower in women[10], but did not indicate whether this was statistically significant. Together these findings suggest that it

may be difficult detect sex differences in muscle glycogen utilization during interval training if fibre-type specific glycogen utilization is not determined.

Despite muscle glycogen utilization being similar between men and women, lactate AUC (representing anaerobic glycolysis) was higher in men. This would suggest that there was a greater flux of the pyruvate produced during exercise towards anaerobic metabolism in men than women and that men used their anaerobic glycolytic systems to produce ATP more than women. The greater reliance on anaerobic glycolysis in men was seen without a sex difference in the change in phosphorylation status of PDHE1α during exercise. This would suggest that men and women had equal capacity to flux pyruvate towards aerobic metabolism. To my knowledge, the only other sex comparative HII exercise trial that measured blood lactate concentration did not find any sex differences in blood lactate throughout the exercise bout[98]. This group however, only analyzed blood lactate throughout the exercise bout and did not determine lactate AUC[98], which may be better able to detect slight differences during exercise between the sexes.

5.4.3 HEPT system

There was no difference in the extent that phosphorylated CK decreased between the sexes during HII exercise. As mentioned previously, phosphorylation of CK inhibits its ability to break down PCr[118,119]. Therefore, it would make sense that P-CK would decrease during intense exercise to allow for less inhibition of CK and increased breakdown of PCr. Consequentially, a similar decrease of P-CK between the sexes suggests that the removal of CK inhibition and capacity for PCr breakdown was the same in men and women. If PCr breakdown was the same, we could speculate that the downstream HEPT system metabolite AMP would increase and subsequently, ATP would also decrease similarly in men and women. This supposed increase in AMP:ATP ratio would imply that the extent of AMPK activation, and therefore AMPK-

dependent GLUT 4 translocation would be comparable between the groups, resulting in similar improvements in IS. This refutes our hypothesis and suggests that sex differences in AMPK activation are not responsible for the differential insulin sensitizing effect of HII exercise in men and women. Importantly, future studies should measure HEPT metabolites in order to confirm or refute this hypothesis.

Although we did not measure if PCr was broken down similarly in men and women during HII exercise, two other SI exercise trials found no difference in PCr breakdown between men and women during the exercise bout[9,10]. However, one of these trial found that women accumulated less IMP and experienced less of a reduction of ATP compared to men during the recovery periods between intervals[10]. Unfortunately, this study did not measure AMP concentrations during the SI exercise bout[10]. However, the fact that IMP is formed directly from AMP[22] would suggest that if women have lower concentrations of IMP, they most likely had a lower concentration of IMP's precursor AMP. Thus, if the concentration in AMP during SI exercise is lower in women, then this could lead to a difference in AMPK activation. In support of this hypothesis, AMPK activation has also been shown to be lower in women during MIC exercise[21]. Therefore, future HII exercise studies need to measure AMP levels and AMPK activation to determine if a sex influences AMPK activation during HII exercise. This would allow for a better mechanistic understanding of sex differences in AMPK activation and in turn, IS improvements during HII exercise.

In contrast to the finding that sex does not influence PCr utilization during SI exercise, another study from our lab found that Cr increased to a greater extent in men than women during an acute bout of low-load, high repetition resistance (LLHR)[120], suggestive of a greater extent of PCr breakdown and reliance on HEPT metabolism. This study also found an attenuated decrease

of P-CK in men compared to women during the exercise bout. This second finding would suggest that women have a greater capacity to breakdown PCr since there is less P-CK to inhibit the reaction, which is in contrast to our findings. Regardless, our findings align more with those from SI studies[9,10] indicating no sex difference in activation of the HEPT system, which could theoretically result in equal concentrations of downstream HEPT system metabolites ATP and AMP and equal activation of AMPK. However, more research into establishing and understanding the basic sex differences within the HEPT system during HII exercise are required before we can draw full conclusions as to if men and women utilize their HEPT systems equally during intense exercise.

5.5 Limitations

The main limitation of this study was that a measure of HEPT system metabolites was not obtained. These metabolites, specifically the ratio of AMP to ATP, are the hypothesized link between blunted improvements in IS in women and exercise. MIC[21] and SI[10] exercise studies have shown that men have greater increases in HEPT system metabolite concentrations, however no HII exercise study to date has investigated this. Unfortunately, due to time restraints and lack of laboratory access due to COVID-19, the originally intended muscle metabolite assays were unable to be seen through. A measure of HEPT system metabolite concentrations would give us a more definitive indication as to if the observed sex differences in IS is linked to this ATP:AMP ratio mechanism.

With the intervals used in our protocol only lasting only 1 minute in duration, an exercise steady state was not reached, making it difficult to estimate substrate oxidation. Indeed, the RER values during the intervals were often greater than 1.0 (most likely due to hyperventilation resulting in greater volumes of CO_2 being exhaled). Perhaps an additional visit where participants exercise at

their high intensity wattage for a duration where steady state could be reached (i.e. 5-8 min) would allow for a more exact determination of VO_2 and RER during the high intervals. This would therefore allow for whole-body substrate oxidation calculations to be conducted with a higher degree of validity.

The HII exercise protocol used in the current study was meant to mimic protocols used in clinical trials examining sex differences in improvements in IS and used an absolute workrate for the low-intensity intervals[3,107,113]. In terms of study design, having a relative recovery period workrate, rather than an absolute one would allow us to more accurately determine whether there are sex differences in metabolism during a bout of HII exercise. However, since the purpose of the trial was to examine whether sex differences in metabolism during HII exercise could be responsible for the differential effect of HII training on IS it was important to use the HII exercise protocol that has been used in these previous trials. Our findings raise the question of the validity of this protocol when determining the effect of sex. Numerous other sex comparative interval studies have used an absolute wattage during their recovery periods[3,4,66,89,113,114] although this concern was never discussed. Moving forward, in order for future studies to ensure that their results reflect a true sex difference in metabolism, a relative recovery period would be necessary.

The timing of our muscle biopsies could also be considered a limitation as it is unclear how the phosphorylation of CK and PDHE1α would change during the cooldown and thus by including the cooldown, we may have missed sex differences in the HEPT and glycolytic pathways. It would be ideal to obtain a muscle biopsy immediately after the tenth high interval, as well as immediately after the cooldown (as was done in this trial), in order to determine not only how the HII exercise bout as a whole influenced the phosphorylation status of these enzymes, but how the high intensity intervals influenced them as well in both sexes.

5.6 Future Directions

To fully identify and understand the underpinning mechanisms responsible for sex-based differences in metabolism during HII exercise, additional research is needed. Future studies should investigate the content and activity of key proteins, metabolites and enzymes that are involved in the metabolic response of the muscle and fuel utilization during HII exercise. Sex differences at the molecular level could provide insight into which mechanism(s) may be contributing to differences in IS improvements. Although we hypothesized that the mechanisms responsible for sex differences in IS are anaerobic, investigation into the aerobic pathway would also be beneficial since there still is an aerobic component to HII exercise. As well, some MIC training trials, which are more aerobic in nature, have also found limited improvements in IS in women[139,140], suggesting that this sex difference is not necessarily limited to primarily anaerobic exercise. Additionally, either comparing luteal phase to follicular phase women or supplementing men with estrogen, would allow for the role of estrogen in mediating fuel utilization during HII exercise to be further understood. Future HII exercise trials could also benefit from including a greater representation of females and untrained individuals in their sample since the majority of HII exercise studies are done in endurance trained men[47,53,116,122,141–143]. Although there are still many gaps in the literature surrounding sex-based differences in the metabolic response to HII exercise, the present study sets the foundation for which future research can be conducted and compared to.

5.7 Conclusion

The findings from the current study differ from previous SI exercise trials in that we did not find an effect of sex on muscle glycogen utilization during exercise. However, these previous trials only found a sex difference in type I muscle glycogen utilization, not total glycogen utilization.

Importantly, we show that despite similar muscle glycogen utilization, men relied on anaerobic glycolysis to a greater extent than women (as evidenced by a greater lactate AUC), suggesting that more of the pyruvate produced during HII exercise was fluxed towards aerobic metabolism in women. However, this occurred despite no sex difference in the change in phosphorylation status of PDHE1α. While not completely explored, our finding that phosphorylation of CK changed similarly in men and women is in agreement with previous research from SI exercise trials finding minimal differences in HEPT metabolism between men and women. Importantly, these differences (or lack of differences) occurred despite women working at a higher exercise intensity than men, which occurred by design. Future work should consider comparing sex differences in metabolism during HII exercise using relative workrates during the low-intensity intervals to truly understand sex differences in metabolism. Overall, while limited in scope, the findings from our study suggest that there are sex differences in the anaerobic contribution of CHO, but not HEPT metabolism during HII exercise; however, further work in this area is needed.

Chapter Six: Significance of the Research

Interval training is capable of eliciting similar improvements in aerobic capacity as MIC training[36], despite having a larger anaerobic component. However, the normal physiological response of the muscle to an acute bout of interval exercise in a healthy population has not yet been established. As well, interval training studies have suggested that improvements in IS may be blunted in women[3–5]. The hypothesized link between anaerobic metabolism and improvements in IS is the HEPT system metabolite AMP. A rise in the ratio of AMP:ATP activates the enzyme AMPK which can influence both insulin-independent and insulin-dependent GLUT4 translocation[6]. Thus, in the present study, we wanted to determine if sex influences the metabolic response of the muscle and/or the anaerobic fuel utilization pattern during an acute bout of HII exercise. Unfortunately, due to time constraints and restricted laboratory access from the COVID-19 global pandemic, we were unable to determine if a sex influenced HEPT metabolites. We did however find that the change in phosphorylation status of the HEPT system enzyme CK is similar between men and women. Equal phosphorylation of CK suggests that the capacity for PCr breakdown during HII exercise is also the same between the sexes. From this finding, we could speculate that if the reliance on the HEPT system is the same, then the concentrations of downstream metabolite AMP may also be similar. Very few acute exercise trials have investigated sex differences in the HEPT system, making the finding that sex did not affect CK phosphorylation status novel. This result is important because it can act to support future research investigating sex differences in the HEPT system. Therefore, our findings narrow the gap in our understanding on this topic and creates a reference for future studies to be compared to.

We specifically chose the HII exercise protocol employed in this trial because it is what is commonly used for training trials in clinical populations[3,107,113]. The results obtained from this study can therefore be compared and applied to those previously conducted trials. This is important because it allows any differential response observed in a clinical population to be easily identified as the normal response in a healthy population has been extensively characterized. As well, this specific protocol is more likely to be used recreationally by the general population compared to SI exercise, making our findings more applicable to the general population of untrained, yet healthy individuals. The findings from this trial also provide important information on how this specific HII exercise protocol induced different exercise responses in men and women, likely due to using an absolute workrate of 50W for the low interval in both men and women, which is a higher relative workrate for the women. Going forward it is important to use a relative intensity during the low intervals during future HII exercise trials, rather than an absolute intensity to ensure that both groups are exercising at a similar intensity.

The findings of the research conducted in this thesis shows that men may rely on anaerobic glycolysis, but not the HEPT system, to a greater extent than women during HII exercise. However, more extensive research is needed to substantiate and replicate the findings from the current study before we can definitively say that this is in fact the normal physiological response of the muscle to HII exercise. Additionally, ensuring that females were all tested during the mid-follicular phase of their menstrual cycle allows us to be confident that our results are not confounded by elevated levels of estrogen, and represent a minimal inherent sex difference that exists within the muscle when estrogen levels are low. The novel findings from this study set a foundation for future research to be conducted so that eventually the sex differences during HII

exercise are well understood and hopefully provide insight into the mechanism(s) responsible for blunted improvements in IS in women during interval training.

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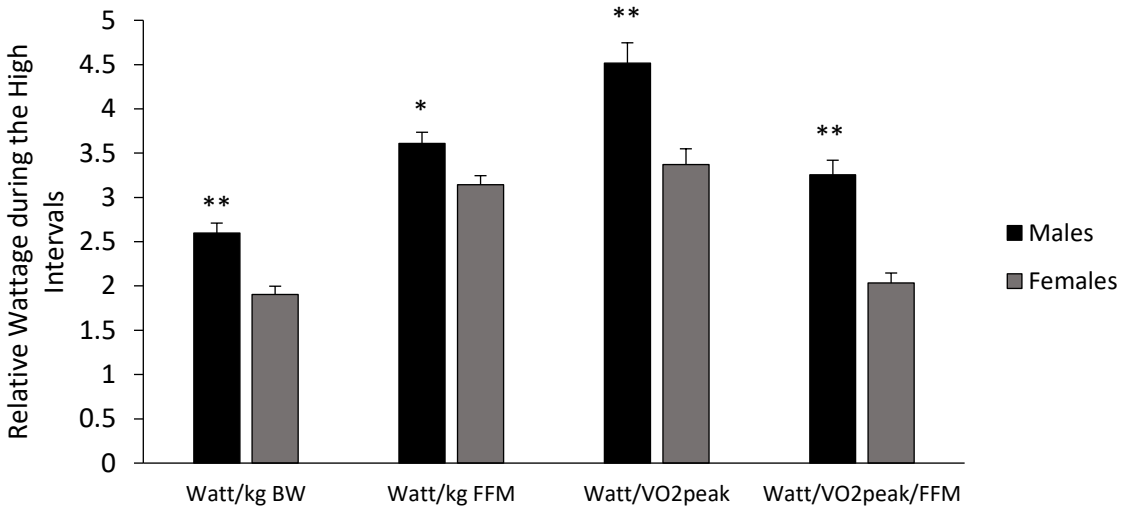
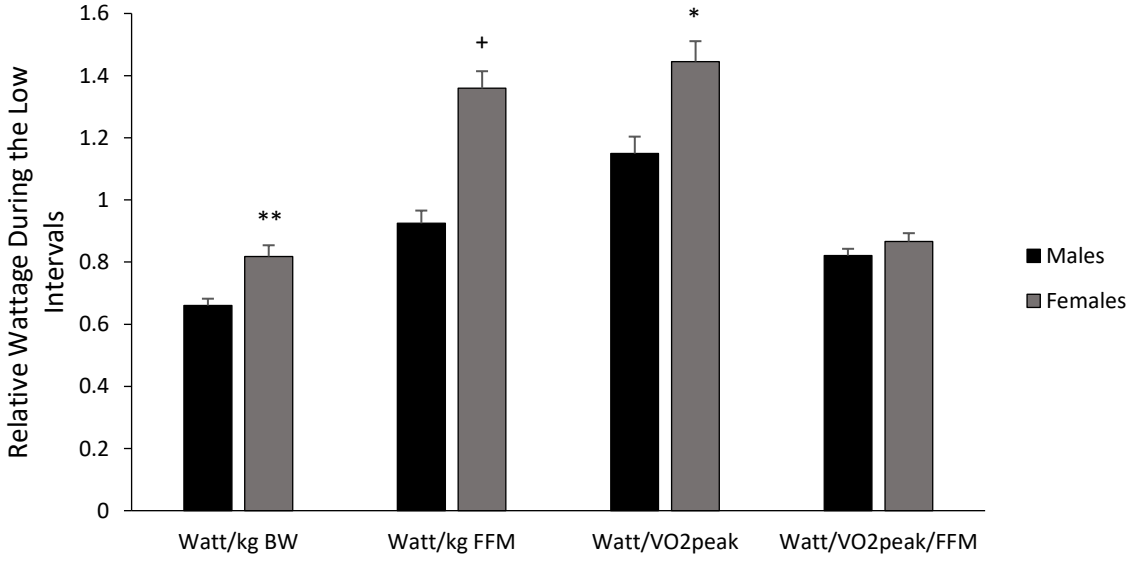
Appendix

A



B



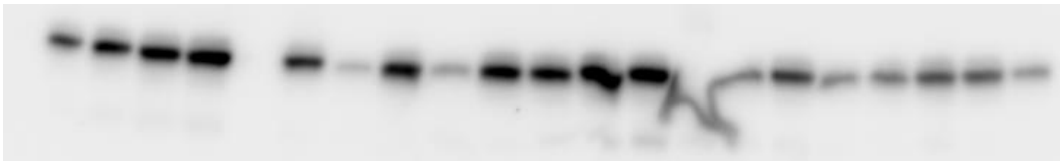
Supplementary Figure 1: A) Relative Wattage during the high intervals expressed relative to kg BW, kg FFM, VO_{2peak} and VO_{2peak}/FFM, *greater than women, p=0.009; **greater than women, p<0.0001. **B)** Relative Wattage during the low intervals expressed relative to kg BW, kg FFM, VO_{2peak} and VO_{2peak}/FFM, *greater than women, p=0.001; **greater than women, p=0.0002; +greater than women, p<0.0001.

Supplementary Table 1: $\%VO_{2peak}$ relative to kg FFM and LB FFM during warm up, high intervals, low intervals and cool down

	Warm Up	High Intervals	Low Intervals	Cool down	P Values
$\%VO_{2peak}$/kg FFM					
Men	0.54 ± 0.04[e]	0.98 ± 0.07[a,b,e]	1.12 ±0.10[a,b,e]	0.66 ± 0.05[a,e]	Time: <0.0001
Women	1.20 ± 0.10	1.90 ± 0.13[a,b]	1.86 ± 0.13[a,b]	1.44 ± 0.13[a]	Sex: <0.0001
					S x T: 0.004
$\%VO_{2peak}$/kg LB FFM					
Men	1.70 ± 0.22[d]	3.08 ± 0.41[a,b,d]	3.45 ± 0.35[a,b,c]	2.09 ± 0.27[a,d]	Time: <0.0001
Women	3.57 ± 0.34	5.66 ± 0.44[a,b]	5.53 ± 0.44[a,b]	4.28 ± 0.42[a]	Sex: 0.001
					S x T: 0.021

P Vales are presented in order of main effect of time, main effect of sex, and interaction of sex and time. Data are means ± SEM. [a] different than warm up, p<0.0001; [b] different than cooldown, p<0.0001; [c] different than women, p=0.003; [d] different than women, p=0.001; [e] different than women, p<0.0001.

A



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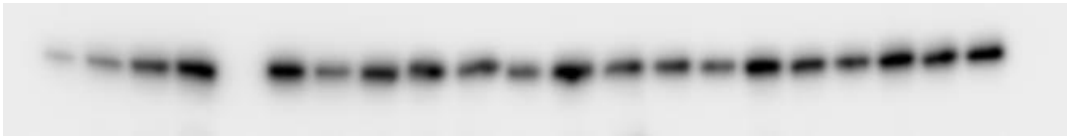
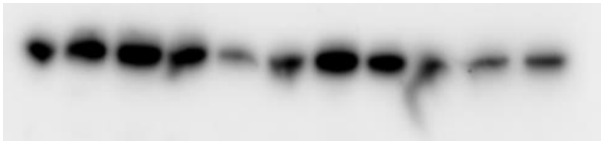


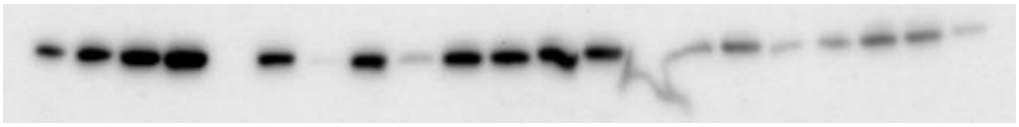
C

D



Supplementary Figure 2: Western Blot Image Blots for P-PDHE1α. Bands follow the pattern of female (pre, post) then male (pre, post). First 4 bands of blots A-C are standards. First 3 bands of blot D are standards. 5th participant in blot A and 2nd-4th participants in blot C were re-analyzed on blot D.

A



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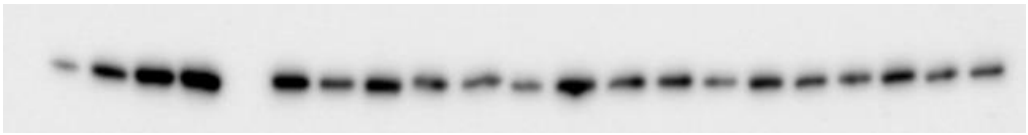
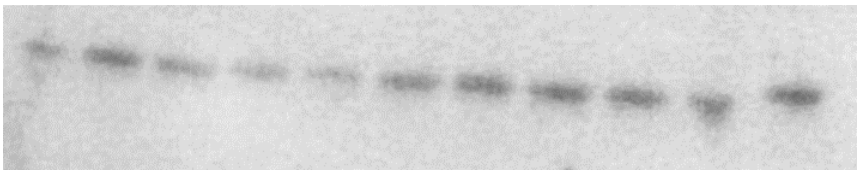


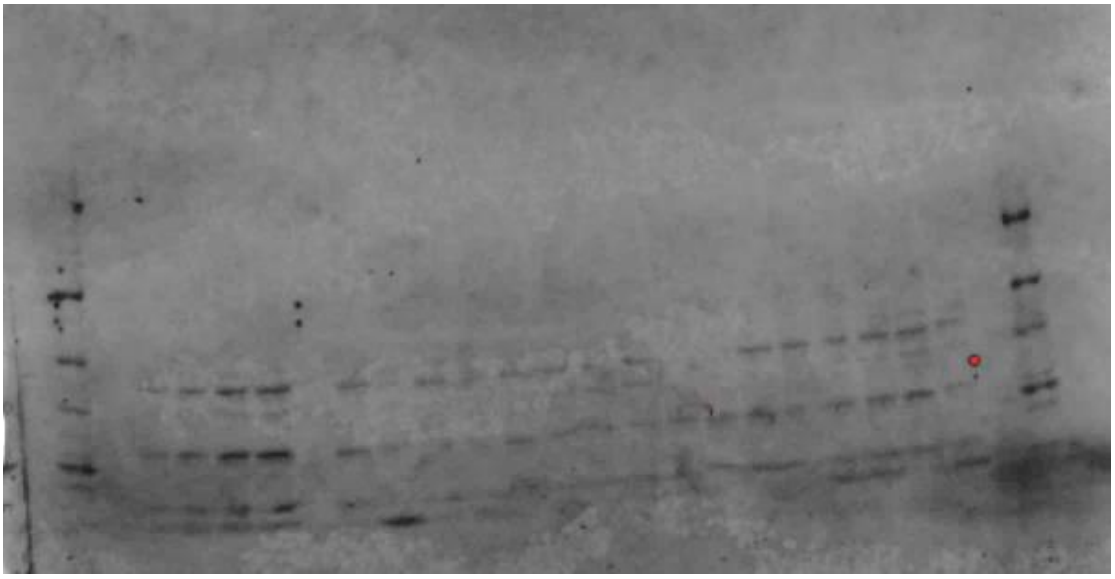
C

D



Supplementary Figure 3: Western Blot Image Blots for P-CK. Bands follow the pattern of female (pre, post) then male (pre, post). First 4 bands of blots A-C are standards. First 3 bands of blot D are standards. 5th participant in blot A and 2nd-4th participants in blot C were re-analyzed on blot D.

A



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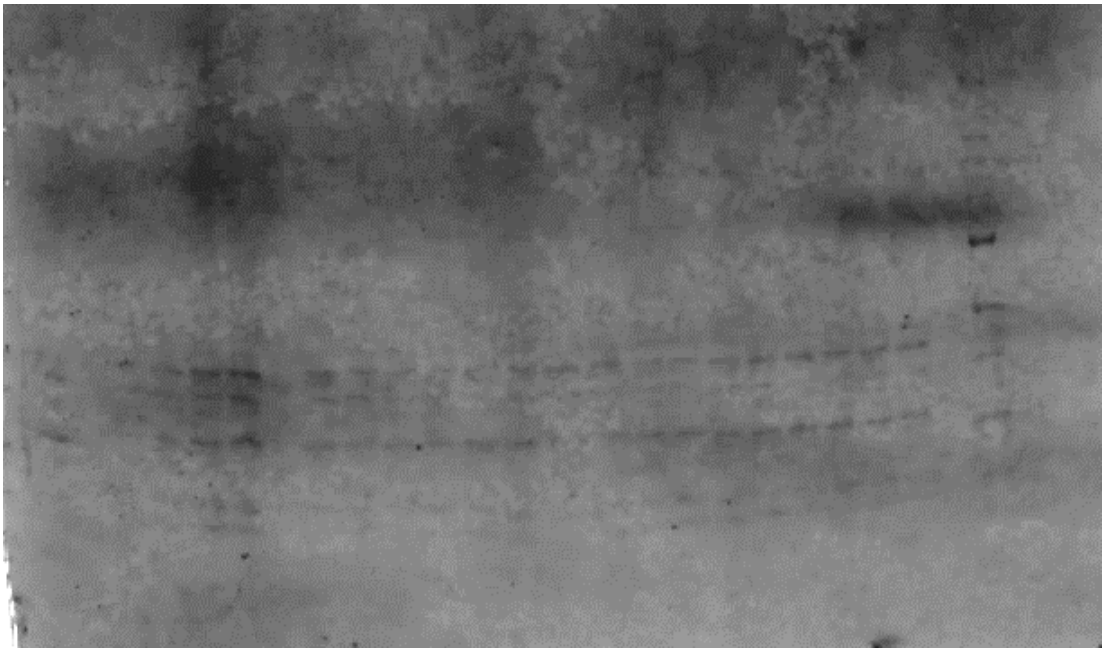

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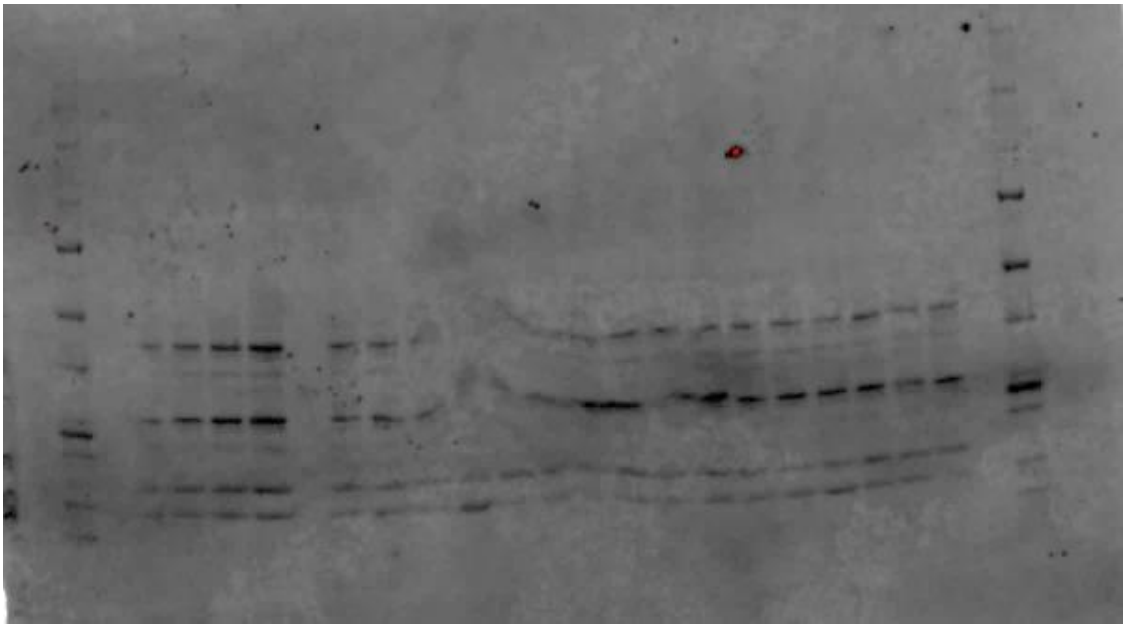
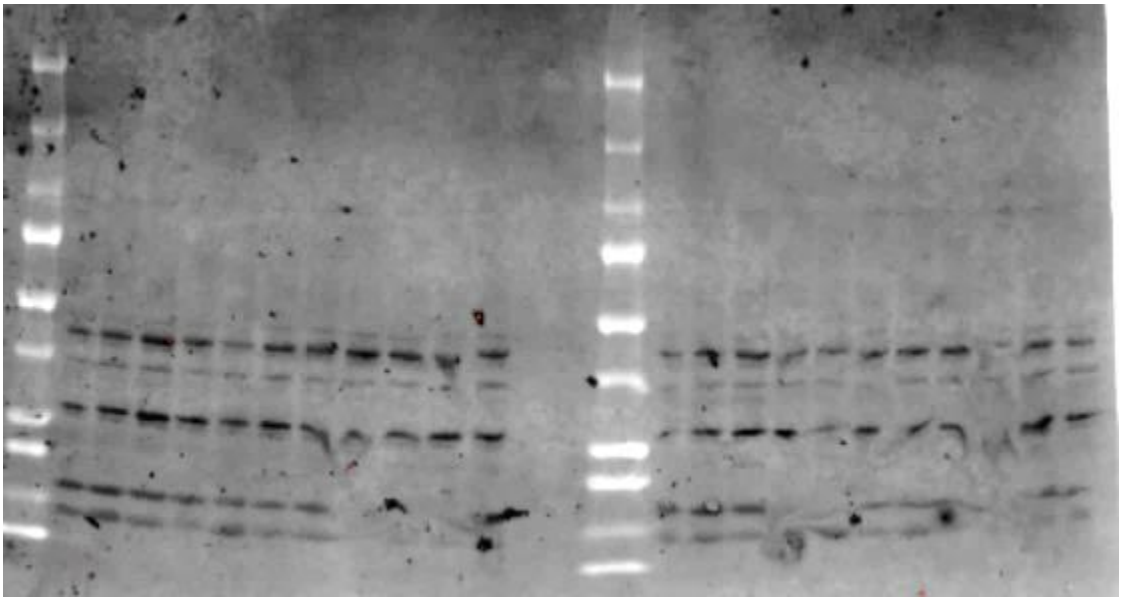


D



Supplementary Figure 4: Stain-Free Blots (SFB) representing total protein content. SFB D-right used for P-CK and SFB D-left used for P-PDHE1α.