The Influence of Low-Load High-Repetition Resistance Exercise and Sex on the Metabolic Properties of Skeletal Muscle

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.

I understand that my thesis may be made electronically available to the public

Abstract

INTRODUCTION. The findings of exercise trials have shown that women are better able to initiate aerobic metabolism and are less reliant on anaerobic energy stores. The effect that LL-HR resistance exercise and sex has on substrate oxidation and metabolic species remains unknown. **PURPOSE**. The purpose of this study was to characterize anaerobic fuel utilization patterns during a bout of LL-HR resistance exercise and to assess whether sex influences fuel utilization during LL-HR. **METHODS**. Twenty young, healthy participants (n=10 men and women) were recruited and matched for VO_{2peak} relative to fat-free mass and habitual resistance training. The LL-HR bout consisted of a circuit of chest press, leg extension, lat pulldown, hamstring curl, shoulder press, and leg press for 25-35 repetitions at 30% of 1RM with 30s rest between each exercise and 2 minutes rest between circuits. Western blot analysis was completed for relevant metabolic enzymes; GP, PFK, LDH (H and M), PDHE1α, PDHK4, CK, AMPD2, MCT (1 and 4), and phosphorylated CK and PDHE1α. Content of metabolic species was analyzed before and after the exercise bout and included measures of creatine, ATP, lactate, P_i, pyruvate, and glycogen. MHC staining was used to determine the distribution of fibre type between men and women. Blood lactate was measured and area under the curve (AUC) calculated. **RESULTS**. Women had a greater type I muscle fibre content than men ($p=0.007$). Baseline content of all enzymes of interest were similar between men and women ($p>0.05$). Men had higher concentrations of muscle glycogen, lactate, ATP, and P_i than women ($p=0.001$, $p=0.019$, $p=0.01$, and $p=0.007$, respectively). Phosphorylation of CK and PDHE1α, and content of glycogen and ATP all decreased in men and women with exercise ($p<0.001$). Phosphorylation of CK decreased more in women than men ($p=0.023$); whereas creatine concentration increased during exercise to a greater extent in men than women ($p=0.026$). Blood lactate increased during exercise ($p=0.023$), with no differences between the sexes ($p = 0.235$). **CONCLUSION**. Men rely to a greater extent on the HEPT system to produce ATP during a bout of LL-HR resistance exercise than women. Interestingly, this occurred despite a greater decrease in phosphorylated CK in women. Overall these findings are supportive of the hypothesis that men rely to a greater extent on anaerobic fuel sources during exercise, which may be in part due to their greater content of type II muscle fibres.

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“I honestly believe that sometimes, my genius, it generates gravity.”
– Jeremy Clarkson (Grand Tour, Season 3 Episode 8)

Chapter One: Literature Review

1.0 Introduction

The population of Canada, as well as most industrialized nations, is aging[1]. Aging is associated with the development of numerous diseases[2–4], such as cardiovascular disease, type 2 diabetes and sarcopenia. The higher proportion of older adults highlights the need for more streamlined interventions to prevent and combat the development of these diseases. Exercise has been prescribed to this population as an intervention meant for either the prevention or the rehabilitation of age-associated diseases.

Of the possible exercise modes prescribed, moderate intensity continuous training (MICT) is the most studied and can improve cardiorespiratory fitness[5], blood pressure[6], lipid profile[7], and insulin sensitivity[8], reducing the risk/severity of cardiovascular disease[7] and type 2 diabetes[9], but as it has little effect on muscle mass and strength[10,11], not influencing the risk of sarcopenia. Alternatively, heavy resistance training induces gains in muscle mass, strength, function and quality[12,13], decreasing the risk/severity of sarcopenia[13–15]. A novel and less studied mode of exercise is low-load, high-repetition (LL-HR) resistance training, which has been shown to increase muscle mass, strength, and function to a comparable extent as heavy resistance training[16–18], thereby possessing the ability to decrease the risk of sarcopenia and type 2 diabetes[12]. However, since the intensity is lower and the sets take longer to complete, it is more aerobic in nature and may also induce benefits to the cardiovascular system, as well as insulin sensitivity similarly to MICT. Hypothetically, LL-HR may be able to slow/reverse sarcopenia to a comparable extent as heavy RE while also providing the cardiovascular and insulin sensitizing benefits that are typically associated with aerobic exercise. Indeed, this time-efficient exercise modality could hypothetically provide

synergistic adaptations and therefore benefits to a number of populations that would otherwise be at risk for sarcopenia or metabolic disorders. The first step to understanding the potential adaptations induced by LL-HR is to characterize the fuel utilization pattern during an acute bout of whole-body LL-HR.

There are well known sex differences in the prevalence of aging-associated diseases such as type II diabetes and sarcopenia[19,20]. Indeed, prior to menopause women have a lower risk of cardiovascular disease and type 2 diabetes than men, due to the protective effects of estrogen[21,22]. Similarly, women have a lower risk of sarcopenia than men[20]; however, whether estrogen plays a role in the etiology of sarcopenia is less understood. Previous work has shown that women and men respond to exercise interventions differently[23–25], which again may be mediated by estrogen and the effects estrogen has on fuel metabolism. Since the fuel utilization pattern during acute bouts of moderate intensity continuous exercise (MICE) differs between men and women as a result of estrogen[26–28], it is important to characterize fuel utilization during LL-HR in both men and women in order to completely understand muscle metabolism during this mode of exercise and to generate hypotheses related to long-term benefits of LL-HR in relation to health. Thus, the research conducted in this thesis will characterize the anaerobic fuel utilization pattern during an acute bout of LL-HR resistance exercise and examine whether it differs between men and women.

1.1 Overview of fuel utilization during exercise

At the onset of exercise energy is supplied first by using stored ATP (enough to support ~2s) and then through anaerobic energy pathways – high energy phosphate transfer system and glycolysis. However, as exercise progresses the muscle demands energy that is provided from macronutrient substrates: carbohydrates, fats, and proteins. Carbohydrates and fats, stored as glycogen and triglycerides (TG) respectively, are the principle substrates used for energy in human muscle tissue with ~95-98% of energy coming from these two sources[29,30]. Protein plays an integral role in recovery and tissue synthesis following exercise[31], but only contributes ~2 and 5% of total energy production during exercise in men and women respectively[30,32]. An overall summary of the energy yielding pathways in skeletal muscle is found in Figure 1. The relative contribution of carbohydrate and fat to energy metabolism during exercise is dependent on the intensity and duration of the exercise bout[29,33]. Contribution from carbohydrates is highest for exercise bouts that are of high intensity and short duration, whereas the relative contribution of fat is highest for exercise bouts that are of low intensity and longer duration[29,33]. Biological sex has also been shown to influence fuel utilization during aerobic exercise, with women relying on fat stores to a greater extent to produce ATP during exercise at a given intensity as compared with men[34,35,26,32]. Additionally, sex has been demonstrated to influence the utilization of anaerobic energy systems, with men relying more heavily on anaerobic energy production[36]. However, while sex differences in metabolism have been well established during aerobic exercise, whether sex influences fuel utilization during different modes of exercise where the fuel utilization pattern is different than during aerobic exercise remains unexamined.

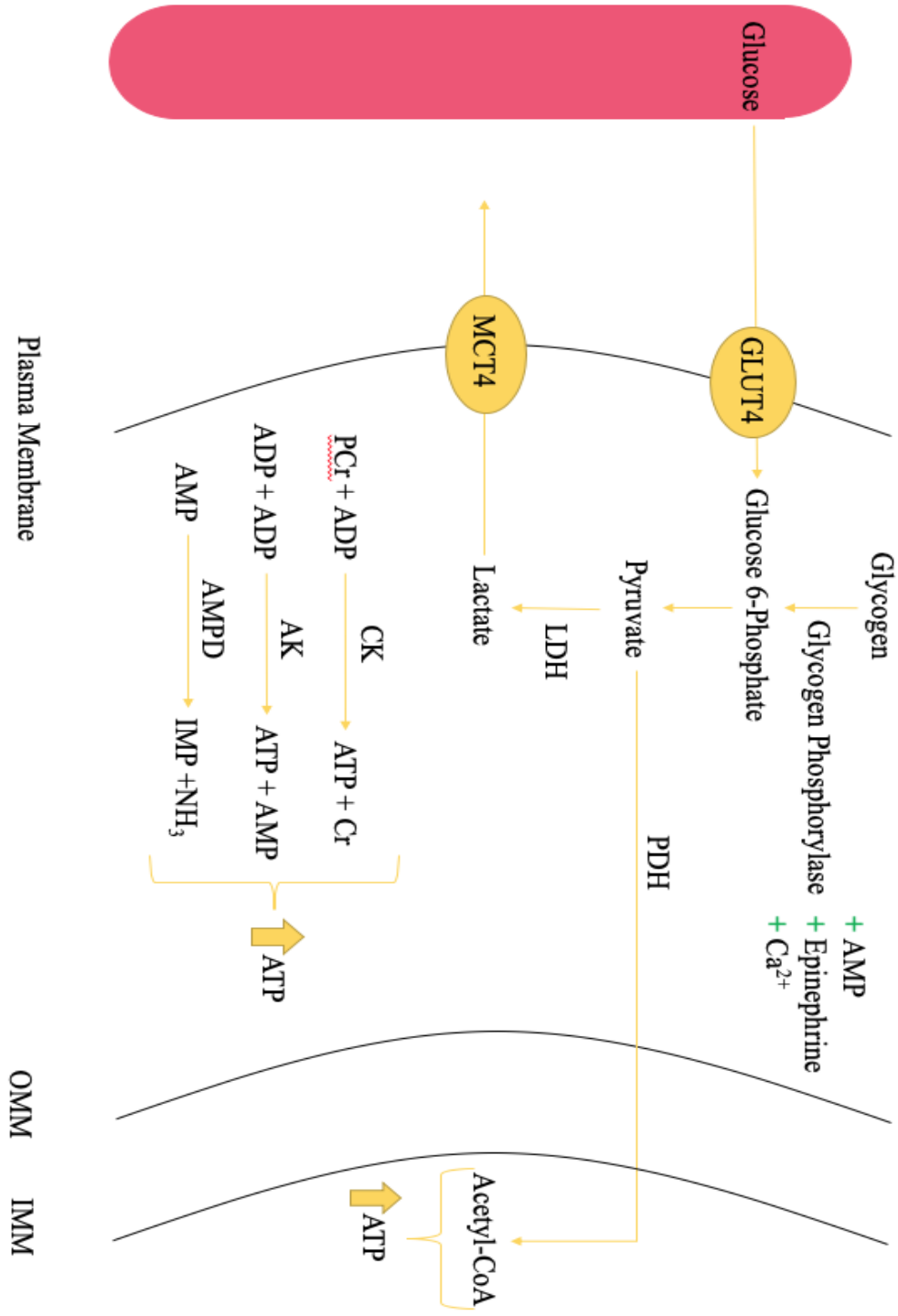

Figure 1: General overview of aerobic and anaerobic glycogen utilization during exercise. Additionally, an overview of phosphocreatine breakdown and HEPT system synthesis of ATP.

1.1 Effects of Exercise Mode on Substrate Utilization During Exercise

Different modes of exercise induce different patterns of fuel utilization due to differences in intensity and duration and whether or not they are continuous or intermittent.

1.1.1 Substrate Utilization During Moderate Intensity Continuous Exercise (MICE)

Much of what we know about the effects of exercise duration and intensity on substrate oxidation come from studies employing moderate intensity continuous exercise (MICE) protocols of increasing intensity and duration. Substrate utilization during exercise is heavily dependent on the intensity at which the exercise is performed[33]. At low exercise intensities fat oxidation predominates, but as exercise intensity increases carbohydrate oxidation also increases and becomes the predominant fuel source at higher exercise intensities[29,33]. While the relative contribution of fat stores to energy production decreases with increasing exercise intensity, the absolute rate of fat oxidation increases with increasing exercise intensity up until ~60-65% VO_{2peak}, at which point fat oxidation is limited[29,33]. The reason for this is that during bouts of high intensity exercise, cardiac output is unable to meet the oxygen demands of contracting muscle thereby resulting in an increase in anaerobic glycolysis as a means of generating ATP within the muscle[37]. As a consequence of anaerobic glycolysis, lactate and H^+ are generated[37]. The increase in H^+ and lactate inhibit the action of hormone sensitive lipase (HSL), thereby reducing the breakdown of TG to FFA and therefore reducing the availability of FFA as a fuel source as exercise intensity increases[38].

Furthermore, while the oxidation of plasma fuel sources (glucose and FFA) predominate at lower exercise intensities[29,33], the diversion of cardiac output towards contracting skeletal muscle as exercise intensity increases reduces adipose and hepatic blood flow, which in turn reduces FFA and glucose release, resulting in a lower contribution from

plasma fuel sources as exercise intensity increases[37]. Indeed, as exercise intensity increases to levels >65% $VO_{2\ max}$, fuel utilization begins to shift towards relying predominately on muscle sources of energy (glycogen and IMCL) stores[29]. To summarize, as exercise intensity increases there is an increased reliance on CHO, as opposed to FFA, to produce ATP. Furthermore, there is an increased reliance on muscle, instead of plasma, fuel stores.

Substrate utilization during exercise is also largely dependent on the duration of the exercise bout[29]. At the onset of exercise, muscle sources of fuel are oxidized primarily as the system requires sources of energy that are immediately available[29]. Muscle glycogen and IMCL are present at the site of contraction and are therefore able to be utilized immediately[29]. As exercise duration increases and muscle fuel stores are depleted, there is an increased reliance on the plasma sources of fuel that are derived from hepatic glucose release (due to increased glycogenolysis and gluconeogenesis) and adipocyte TG breakdown into FFAs[29].

Additionally as exercise duration increases, there is a shift from a reliance on glucose to a dependence on fat oxidation to support energy needs[29]. This shift occurs as glycogen stores in the muscle and liver begin to diminish, and an alternate fuel source must be utilized to generate ATP[37,39]. TG storage in adipocytes offer a much larger energy source that can be utilized during exercise bouts of increasing duration[29]. The increase in fat oxidation as exercise duration increases is mediated by decreases in insulin[37]. Insulin inhibits lipolysis by activating PKB/Akt, which in turn phosphorylates phosphodiesterase 3B[37,39]. Phosphodiesterase 3B converts cAMP to AMP, reducing cAMP concentration, which in turn reduces HSL activity[37,39]. As exercise duration increases and glycogen stores diminish, plasma glucose levels are lower, and thus insulin levels decrease, reducing the inhibition on lipolysis[37,39]. The shift from carbohydrate to fat oxidation is able to spare glycogen in the

muscle and liver, and is thought to prolong an individual's time to exhaustion[40]. In summary, as exercise duration increases there is a shift from muscle fuel sources to plasma-derived substrates as well as a shift away from carbohydrate oxidation to fat oxidation to support muscle energy needs.

As such, we see that with increasing intensity of MICE there is an increased reliance on glycogen stores and with increasing duration there is a greater reliance on fat stores[29,33]. Since MICE is performed below VO_{2peak}, the majority of ATP being produced is done so via aerobic metabolism.

1.1.2 Substrate Utilization During Resistance Exercise

Substrate utilization differs between resistance exercise (RE) and moderate intensity continuous exercise (MICE) due to the higher exercise intensity, shorter duration and intermittent nature of the RE bout[29,33]. For heavy RE, defined as lifting a heavy load (~80% 1RM) for ~ 8 – 12 repetitions, individual sets last approximately 20s with ~ 2-3 minutes of rest between sets[41]. The high energy phosphate transfer (HEPT) system can provide the body with ATP for ~20s[39], suggesting that for heavy RE, other methods of ATP synthesis are not as crucial[39] compared to exercises with longer durations. Additionally, the replenishment of the PCr pool typically takes ~2-3 minutes which is typically the duration of the rest periods[37]. During heavy RE, glycogen stored in skeletal muscle also serves as a substrate for ATP synthesis, albeit to a lesser extent[37]. The breakdown of glycogen through glycogenolysis will allow for anaerobic glycolysis to meet the ATP demand of the muscle[37]. During heavy RE, glycolysis will be second to HEPT for ATP provision, with a lesser dependence on oxidative metabolism[42]. The lesser reliance on oxidative metabolism during heavy RE is due to a slower/blunted rise in skeletal muscle blood flow, as well as the higher intensity of the

exercise bout, thus the majority of the pyruvate formed during glycolysis will be fluxed towards lactate production as a means of synthesizing ATP[37,39], thus highlighting the anaerobic nature of the exercise bout.

Interestingly, there is evidence suggesting that lipolysis of IMCL and FFA oxidation may also contribute to substrate utilization during heavy RE[43]. A single study noted the increase in muscle FFA and glycerol following heavy resistance exercise, highlighting that lipolysis may provide energy during heavy-resistance exercise despite the relatively short duration and high intensity[43].

1.1.3 Low Load High Repetition Resistance Exercise (LL-HR)

A comparatively unique and less studied exercise mode, low-load, high-repetition (LL-HR) RE, is becoming increasingly of interest since it induces lower mechanical strain on joints[44]. LL-HR is described as lifting a load corresponding to ~30% one repetition max (1RM) to volitional failure [43]. Recent literature has shown that acute LL-HR resistance exercise is able to evoke a greater increase in skeletal muscle hypertrophic factors such as myofibrillar protein synthesis (MPS) and phosphorylation of hypertrophic signalling molecules as traditional RE[18]. Furthermore, training trials have shown that LL-HR induces comparable increases in muscle mass and strength as traditional RT[45,13,17]. Given the lower mechanical joint strain associated with LL-HR and the fact that it can induce favourable effects on muscle mass and strength, this mode of exercise may be particularly beneficial for older adults or those who have or are recovering from joint injuries, who are at an increased risk of joint instability and injury.

With the exception of how LL-HR RE influences muscle protein synthesis, the acute effects of this exercise modality on muscle metabolic pathways is largely unknown. As a

consequence of the duration of the exercise, the circuit style manner in which it is utilized, and the lower intensity compared to heavy RE, substrate utilization during this mode of exercise is hypothesized to follow a pattern more similar to MICE, but that anerobic metabolism (HEPT, glycolysis) would still be significant contributors. Indeed, three studies that utilized a LL-HR RE protocol consisting of 3 sets of plantarflexion at 20% of 1RM for 30 repetitions separated by 1 minute of rest in 12 young, healthy males[46] and females[47,48] found that there were no changes in PCr or muscle pH during the LL-HR bouts[47]. This could be due to the very low intensity of the exercise, coupled with the fact that they did not exercise to volitional failure. These studies suggest that at a very low resistance exercise intensity (20% one repetition max) HEPT may not contribute to ATP production. However, since exercise was not performed to failure, it is not clear if all muscle fibres were recruited. Previous work has shown that LL-HR resistance exercise performed at 30% 1RM to failure recruits type II muscle fibres as evidenced by LL-HR training increasing type II muscle fibre size[45]. Since, type II muscle fibres are more anaerobic, it could be that HEPT did not contribute to ATP production during LL-HR at 20% 1RM because type II muscle fibres were not recruited, due to the lower intensity and/or that the set did not go to failure. As recruitment of type II fibres is important to maximizing muscle hypertrophy during training, further work is needed to characterize the fuel utilization pattern during a bout of LL-HR where type II fibres are recruited to determine the role that the HEPT system plays in producing ATP during LL-HR RE.

Given the lower resistance exercise intensity of LL-HR, it is also speculated that there will be a shift towards more oxidative metabolism during LL-HR as the circuit continues with glycolysis producing pyruvate that is converted to acetyl CoA, and increased fat

oxidation from adipose and muscle TG breakdown. Oxidative metabolism would also be greater during LL-HR compared to traditional RE due to the decreased intensity and prolonged activation of type I skeletal muscle fibres that contain a higher concentration of mitochondria compared to their type II counterparts[13]. To the best of our knowledge, no study has examined the contribution of muscle glycogen, muscle lipids or aerobic metabolism to ATP production during LL-HR and this should be an area of future investigation.

1.2 Sex Differences in Muscle Metabolism

Numerous studies have shown that fuel utilization during MICE differs between men and women[49]. Specifically, women have a lower respiratory exchange ratio (RER) than men during MICE [50], indicating that women have a lesser reliance on carbohydrates and an increased reliance on fat to support muscle energy needs[49]. These initial findings have prompted much research examining the sites of glycogen sparing (hepatic vs. skeletal muscle) and increased reliance on lipids (adipose vs intramuscular TG) as well as whether there are sex differences in the dependence on anaerobic metabolism for energy production.

1.2.1 Sex Differences in CHO Metabolism

The lower reliance on CHO sources during MICE is supported by findings that women have a lower glucose rate of appearance, rate of disappearance and metabolic clearance rate during MICE, as compared with men, indicating a decreased reliance on hepatic glucose stores[34,26,51,27]. Whether this decreased reliance on hepatic CHO is due to decreased hepatic muscle glycogenolysis and/or gluconeogenesis in humans is unexamined due to ethical concerns regarding liver biopsies in humans. However, studies conducted in animals have shown that males have an increased dependence on hepatic glycogen to meet

exercise demands[52]. Additionally, a study in oophorectomized female rats found a dose dependant effect of estradiol to decrease liver and muscle glycogen utilization during exercise[53]. Together, these findings suggest that the decreased reliance on CHO during exercise in women is, at least in part, due to a decreased reliance on liver CHO stores, with animal studies suggesting that this is due to decreased hepatic glycogenolysis.

In skeletal muscle whether sex influences muscle glycogen utilization during MICE is contentious. Numerous studies have reported no effect of sex on skeletal muscle glycogen utilization[26,23,27,54,55] while other studies have reported a glycogen sparing effect in women[34,49,56]. There is a multitude of possibilities for the discrepancies between these studies including exercise intensity, exercise mode, training status of the participants, and failing to control for menstrual cycle phase. For instance, it has been reported that no difference in muscle glycogen utilization exists between men and women during cycling at 75% VO_{2peak}[26,23]. This may be explained by the intensity being too high during this exercise bout that thus prevented lipid breakdown and glycogen sparing in women[51], since maximal fat oxidation has been show to occur at ~60-65% VO_{2peak}[51]. However, amongst the trials conducted at 65% VO_{2peak}[26,54,55] where fat oxidation should be at or near its maximal rate, only one study[49] involving a 15.5km (90-101minutes) running protocol found that women used less muscle glycogen (25%) than men[49]. All of the other trials conducted at 65% VO_{2peak}[22,32,52,55] involved cycling and thus these findings could provide insight into the role exercise mode could have on muscle glycogen utilization, as a running protocol forces the recruitment of more muscle mass as the participant has to carry their body weight. Furthermore, running protocols are shown to recruit the vastus lateralis muscle to a greater

extent than cycling as well as using both concentric and eccentric contractions to perform the activity[58].

Alternatively, differences in the training status of the participants in the cycling trials as compared with the running trial could also contribute to the differential findings. The trial that utilized the running protocol involved highly trained individuals as participants [VO_{2peak} (ml*kg FFM^{-1}* min^{-1}): men = 75.5±5.1, women = 65.2±4.9][49], whereas the cycling protocols involved recreationally active participants [VO_{2peak} (ml*kg FFM^{-1}* min^{-1}): men = 52.4±1.7, women = 49.8±2.1][26,55,57,59]. A higher training status would mean that the runners would rely to a lesser extent on carbohydrate stores during exercise at a given intensity[59] and would spare glycogen, thus despite being of similar duration to the cycling protocols sex-based differences were still evident because muscle glycogen stores were less depleted[60].

The duration of the exercise bout could be one of the main reasons that sex differences in muscle glycogen (and IMCL) utilization are hard to detect. While both muscle and hepatic/adipose fuel stores support ATP production throughout a bout of MICE, muscle fuel store breakdown predominates at the onset of exercise[29,59], with plasma sources taking over once muscle stores have significantly diminished[29,59]. Therefore, the effects of sex on muscle substrate utilization may be masked by the long duration of the exercise bout since reliance on muscle sources to produce ATP decreases as exercise duration increases[29,59]. Thus, to examine whether there are inherent differences in the reliance on muscle fuel stores during exercise, shorter bouts of exercise should be employed.

Additionally, inconsistencies in the effects of sex on muscle glycogen utilization during exercise are likely due to failing to control for sex hormone fluctuations throughout the menstrual cycle, as studies comparing fuel utilization in women during the follicular

phase (FP) to that in the luteal phase (LP) have shown that in the LP there is a lesser reliance on muscle glycogen during exercise[26]. Furthermore, while not significant, there was a trend ($p = 0.1$) for LP women to use less muscle glycogen during exercise as compared with men[26]. However, there was no difference in muscle glycogen utilization during exercise between FP women and men[26]. These findings suggest that muscle glycogen utilization can vary across the menstrual cycle and highlights the importance of controlling for menstrual phase when conducting sex comparative trials. However, controlling for phase of the menstrual cycle has inherent problems as well since the concentration of estradiol and progesterone can significantly vary between women during the same period of the menstrual cycle[61,62]. Of the studies that did control for menstrual cycle[25,53], only one[27] reported estrogen concentrations.

Sex differences in CHO metabolism are underpinned by sex differences in mRNA, protein content and enzyme activities of enzymes involved in CHO metabolism. Muscle glycogen breakdown is facilitated by the enzyme glycogen phosphorylase[26]. It has been shown that glycogen phosphorylase has a lower maximal activity in women compared to men suggesting that the process of glycogenolysis is reduced in women[39]. Additionally, phosphofructokinase (PFK) activity, the rate-limiting enzyme of glycolysis that converts fructose-6-phosphate to fructose-1,6-bisphosphate, is lower in women compared to men[35]. Together these findings demonstrate that women have a dampened capacity to breakdown and oxidize CHO within the muscle. However, women do produce ATP through glycolysis during high intensity exercise without compromise, suggesting that the decreased glycolytic enzyme activity in women is not a performance detriment, but that women are simply less dependent on carbohydrate sources to meet energy demands during moderate intensity exercise.

1.2.2 Sex Differences in High Energy Phosphate Transfer

To the best of my knowledge, only three trials have examined sex differences in HEPT metabolism. In one study, young, healthy, untrained participants' forearm flexor muscles underwent a rest-exercise-recovery protocol until exhaustion, which was meant to mimic a bout of resistance exercise to failure. It was reported that men utilized more of their phosphocreatine (PCr) stores during exercise, however when this usage was normalized to power output it was shown that women had a greater PCr breakdown per unit of power[63]. This study also found that at the end of exercise, women preferentially produced ATP using aerobic pathways as evidenced by a higher amount of ATP produced by aerobic phosphorylation, whereas men relied more heavily on anaerobic energy production as evidenced by greater depletion of PCr stores and a lesser increase of ATP produced by aerobic phosphorylation[63]. It is unclear, however, whether men and women were equally trained, and the exercise protocol employed is not reflective of a typical exercise bout, and thus the results are difficult to interpret and require verification. However, these findings suggest that women are better able to switch from anaerobic to aerobic energy production and are better able to use aerobic cellular respiration to meet energy demands. Consequently, women exhibit an inefficient anaerobic fuel utilization through the depletion of PCr stores, which could explain the lower maximal power output produced by women compared to men[63]. These findings suggest that women have a lesser ability to perform single bouts of movements requiring high power outputs such as a one repetition max. However, it also implies that for exercise bouts requiring a lesser load for a longer time (i.e. LL-HR resistance exercise) women may be able to perform more repetitions than males before reaching muscular fatigue, a hypothesis supported by several trials[64–69].

To the best of our knowledge, no other studies have examined sex-based differences in HEPT metabolism during resistance exercise. However, two studies have investigated these differences during sprint interval training (SIT). While SIT differs from RE due to its mode of exercise and relative intensity, it is similar to RE in terms of duration. Therefore, understanding how sex influences HEPT metabolism during SIT can help in our understanding of how exercise mode and duration influence sex differences in metabolism. The two SIT studies were conducted to examine anaerobic metabolism during either a single 30s Wingate[56] or repeated 30s Wingates (3 sprints separated by 20 minutes rest)[57]. Both studies found that glycogen utilization in type I, but not type II, muscle fibres was less in women than men during the exercise bout[56,57]. Additionally, while there was no effect of sex on changes in ATP or ATP by-products during exercise[56,57], the study involving repeated Wingates found that ATP was higher and IMP was lower in women, as compared with men at the onset of the 3rd, but not the 1st sprint[57]. These differences were the result of sex-based differences in the recovery response as during the 20-minute recovery period there was a greater decrease in IMP in women, suggesting that women were better able to resynthesize ATP from the reanimation of IMP during recovery. Collectively, the findings of these two studies suggest that women rely to a lesser extent on glycolysis, further supporting the hypothesis that women are better able to initiate aerobic metabolism during exercise. Additionally, these findings highlight that the sex-based differences in glycolysis during HIIT are not solely due to differences in muscle fibre type distribution as absolute differences in metabolism were seen within type I fibres. Furthermore, while men and women rely to a similar extent on HEPT during sprint exercise, women have a greater capacity to remove

ATP by-products during rest, allowing them to maintain peak power output during subsequent sprints, whereas peak power declines in men[57].

1.2.3 Estrogen-Mediated Differences in Metabolism

Sex differences in substrate utilization during exercise are at least partly due to differences in estrogen (E2) between men and women. Animal studies have reported that male rats have higher storage and utilization of liver glycogen as compared with female rats[52]. Furthermore, in oophorectomized female rats there is a dose-dependent response of E2 on the extent of glycogen sparing in both muscle and liver, peaking at 10μg E2[70]. Moreover a 10μg dose of E2 in male rats induced glycogen sparing in muscle, liver, and heart, while consequently they were able to run longer while completing more work[53]. The decrease in glucose oxidation for total energy demands must be compensated from another oxidative source in order to meet metabolic demands. To accompany the sparing in glycogen, the supplemented rats demonstrated a greater affinity for fat oxidation to meet energy demands. Male rats supplemented with 10μg of E2 demonstrated an increase in circulating plasma FA as well as a decrease in blood lactate[53]. These findings, coupled with the evidence surrounding glycogen utilization, indicate that E2 treatment shifts the dependence of fuel utilization from glycolysis to FFA oxidation to meet exercise energy demands.

Estrogen supplementation trials have also been conducted in humans with conflicting findings[27,32,71–73]. Differences in findings may be related to differences in dosing strategies (low dose to mimic the follicular phase vs high dose to mimic the luteal phase of the menstrual cycle) or participants (men vs amenorrhoeic women). In a study where men were supplemented with luteal phase equivalents (2 mg/d) of E2 for 8 days [71], CHO oxidation decreased by 5-16%, leucine oxidation decreased by 16% and lipid oxidation increased by 22

– 44% at rest and during exercise at 65%VO_{2peak}[72]. Correspondingly, there was a decrease in glucose Ra and Rd during exercise compared to placebo[27]. The trial also noted that E2 lowered proglycogen and total glycogen concentration compared to placebo, without an associated effect on net muscle glycogen use during exercise[27]. A similar trial administered 3mg/day of E2 to men for 8 days and found a reduction of glucose Ra and MCR, as well as an increase in circulating plasma glucose following 90-minutes of cycling at 65%VO_{2peak}, suggestive of a lesser dependence on glucose for oxidation[27]; however, there was no difference in whole-body carbohydrate or fat oxidation during exercise. Furthermore, lower dose estrogen supplementation, meant to mimic levels seen in the follicular phase, to men for 11 days did not influence whole body substrate utilization or muscle glycogen utilization during exercise[73]. However, when low-dose E2 was given to amenorrhoeic females it was determined that, although the contribution of plasma glucose and muscle glycogen to total energy expenditure was similar across all groups, there was a decrease in glucose rate of appearance and rate of disappearance[71]. The study noted that a decrease of gluconeogenesis, epinephrine secretion, and glucose transport were some of the variables that would account for the effect of E2 on glucose metabolism[71]. Together, these findings indicate that E2 can modulate fuel utilization during exercise, but that consideration must be given to the dose administered as well as the sex of the participants.

Importantly, the findings of the E2 supplementation trials are unable to determine whether it is the increase in E2 or the corresponding decrease in testosterone induced by E2 that is causing changes in muscle metabolism. Braun et al[28] was able to determine that it is indeed estrogen that is mediating these effects by conducting a trial where they tested exercise substrate metabolism in young men at physiological, low (pharmacological

testosterone ablation), and high (testosterone treatment) concentrations[32]. The study found that testosterone had very little effect on substrate utilization during exercise in young men[28]. Therefore, these findings confirm that it is indeed estrogen that is mediating the observed sex differences in metabolism during exercise.

Estrogen has also been found to influence the mRNA and protein content of key metabolic enzymes involved in lipid and glucose metabolism[28]. E2 supplementation to men has been shown to increase both PPARα and PPARδ compared to baseline[35]. An increase in both these nuclear hormone transcription factors will upregulate enzymatic species related to metabolic function and will therefore enhance fatty acid oxidation[39]. E2 has also been shown to increase β-oxidation through increasing the expression of TFP-α[35]. Mitochondrial FA transport has also been increased in men with administration of E2, through an increase in the expression of CPTI[35]. IMCL synthesis is also affected by E2, through an increase in the expression of SREBP-1c and mtGPAT[35]. Finally, cellular glucose uptake is thought to increase with E2 through the increased expression of GLUT4[35]. Additional studies are needed to elucidate the full extent that E2 is responsible for sex-based differences in metabolism, as well as uncover potential sex based-differences that are mediated by E2.

In summary, the findings of sex comparative trials have shown that women are more reliant on fat oxidation during exercise, as compared with men, which results in a sparing of endogenous CHO stores. Additionally, women are better able to metabolize fuels through aerobic metabolism at the onset of exercise as compared with men and are thus less reliant on anaerobic energy production. Whether sex differences in fuel utilization persist during resistance exercise, particularly LL-HR exercise, has yet to be examined.

Chapter Two: Rationale, Purpose and Hypotheses

2.0 Study Rationale

The population of Canada, as well as most industrialized nations, is aging[1]. Aging is associated with the development of numerous diseases[2–4], such as cardiovascular disease, type 2 diabetes and sarcopenia. The higher proportion of older adults highlights the need for more streamlined interventions to prevent and combat the development of these diseases. Exercise has been prescribed to this population as an intervention meant for either the prevention or the rehabilitation of age-associated diseases; however, different modes of exercise are needed to induce specific adaptations. For example, MICT is known to improve cardiorespiratory fitness[5] and insulin sensitivity[8], decreasing the risk for cardiovascular disease and type 2 diabetes[74]; however, MICT has little effect on muscle mass, strength and function. Alternatively, heavy resistance training can increase muscle mass, strength and function[17,18,31], but is less effective at improving insulin sensitivity and cardiorespiratory fitness. Thus, in order for older adults to prevent/ameliorate aging-associated diseases they have to perform multiple modes of exercise, which is time consuming and may decrease participation.

A relatively novel resistance exercise mode, LL-HR resistance exercise, has been an area of active interest and it has been shown to induce greater increases in skeletal muscle hypertrophic factors[20], comparable increases in muscle mass and strength[21-23], and impart less mechanical strain on joints[19] compared to heavy RE. Therefore, this mode of exercise may be especially beneficial for older adults as it may prevent/attenuate the development of sarcopenia[13]. Additionally, given that LL-HR requires sets that are longer in duration along with less rest and lower intensity, it is likely more aerobic in nature. Aerobic adaptations may

be able to manifest during LL-HR to a comparable extent as MICT. Since aging is associated with the development of sarcopenia[75], T2D[76], and CVDs[77], LL-HR may represent an optimal mode of exercise to prevent "aging-associated" diseases. Physical activity recommendations for older adults include doing both aerobic and resistance exercise[74], and LL-HR may allow for both modes of exercise to be completed in one, time-efficient session. Since time is one of the biggest barriers to physical activity, LL-HR could have a profound effect on meeting physical activity guidelines.

There are well-established sex-based differences in fuel utilization during exercise, which are thought to underpin some of the sex-based differences in training-induced adaptations (i.e. insulin sensitivity)[23,27,49,59]. In general, women rely to a lesser extent on carbohydrate sources to fuel endurance exercise compared to men, as indicated by the finding that women have a lower respiratory exchange ratio (RER)[49]. The lower RER is at least in part due to the finding that women have a lower glucose Ra, Rd, and MCR, suggestive of a lesser reliance on hepatic glucose stores[26,27,49,51,52]. The lower RER may also be due to sex-based differences in muscle glycogen utilization; however, sex differences in muscle glycogen utilization is contentious as some [8,30,33-35], but not all[49], studies have found no difference in muscle glycogen utilization between men and women during exercise Differential findings with respect to muscle glycogen utilization may be due to differences in the menstrual phase women were tested[8,60-62], the mode of exercise employed[8,29,30,33-35], and the training status of the participants.

Anaerobic sources also contribute substantially at the onset of exercise and during exercise bouts that are of higher intensity and/or are intermittent in nature[9,10]. During forearm flexor exercise performed to failure, meant to mimic resistance exercise, men used a greater

amount of PCr than women[36]. Although when expressed relative to the unit of power produced, women used a greater amount of PCr than men[63]. These findings suggest that during resistance-type exercise women require more PCr to perform identical feats of power, thereby suggesting an inefficient utilization of the stored energy source compared to men[63]. Alternatively, studies examining sex differences in anaerobic metabolism during sprint interval exercise found that while men had a greater decrease in muscle glycogen and a greater increase in muscle lactate in type I muscle fibres than women, there were no differences in HEPT metabolism during spring interval exercise[56,57]. While collectively these findings suggest that men are more reliant on anaerobic systems during exercise irrespective of exercise mode, while women are better able to initiate aerobic metabolism, the differential findings between studies suggest that sex differences in fuel utilization during exercise may be related to exercise mode.

Men and women do not always respond similarly to exercise training. Indeed, sprint interval exercise robustly improves insulin sensitivity in men[78], this response is blunted or absent in women[79]. As sex differences in metabolism may underpin differences in training adaptations between the sexes, and exercise mode may influence the extent of sex differences in metabolism during the exercise bout, it is important that we characterize the fuel utilization pattern during LL-HR RE in both men and women to get an understanding of potential adaptations this mode of exercise may induce. Therefore, the research conducted in this thesis will serve to 1) identify the anaerobic fuel utilization pattern during an acute bout of LL-HR resistance exercise and 2) examine the differences in substrate utilization during LL-HR resistance exercise between men and women.

2.1 Purpose

The purpose of the research conducted in this thesis was to characterize the anaerobic fuel utilization pattern during an acute bout of LL-HR resistance exercise and determine whether sex influenced the fuel utilization pattern.

2.2 Objectives

1. To determine the contribution of the HEPT and glycolytic systems to energy production during LL-HR RE.
2. To identify how LL-HR RE influences the phosphorylation status of enzymes involved in aerobic and anaerobic metabolism.
3. To examine whether sex influences the contribution of the HEPT and glycolytic systems during LL-HR RE.
4. To determine if sex differences in HEPT and glycolysis are underpinned by differences in protein content of relevant enzymes.

2.3 Hypotheses

1. The concentration of creatine, inorganic phosphate, and lactate will increase, and muscle glycogen will decrease during LL-HR resistance exercise.
2. Phosphorylated CK and phosphorylated PDHe1α will decrease during LL-HR RE.
3. Glycogen will decrease and creatine, inorganic phosphate, and lactate will increase to a greater extent in men than women during LL-HR RE. ATP will decrease to a lesser extent in men than women during LL-HR RE.
4. Men will have a higher content of anaerobic glycolytic enzymes (GP, PFK, LDH-M, PDHe1α, and PDHK4) as well as HEPT enzymes (CK, and AMPD2) compared to women. Men will also have a greater content of transporters responsible for lactate

expulsion (MCT4) while women will have a higher content of transporters responsible for lactate uptake (MCT1) as well as the ability to convert that lactate to pyruvate (LDH-H).

Chapter Three: Methods

3.0 Participants

Young, healthy men and women (n = 10/sex) participants were recruited. Men and women were appropriately matched for habitual training status and maximum aerobic capacity relative to fat-free mass [$VO_{2\ peak}$ (mL $kgFFM^{-1}$ min^{-1})]. Men were excluded if they have a VO_{2peak} of 51mL$kg^{-1}min^{-1}$ or higher. Women were excluded if they have a VO_{2peak} of 44mL$kg^{-1}min^{-1}$ or were pregnant or suspected they may be pregnant. The VO_{2peak} limits excluded people with excellent VO_{2peak} scores. Exclusion criteria pertaining to both sexes included the presence of chronic health conditions, the inability to complete the single exercise session, regular participation in cardiovascular (>3 session $week^{-1}$) or resistance (>2 sessions $week^{-1}$) exercise, having an allergy to local anesthetic, having undergone a barium swallow or an infusion of contrast agent in the past 3 weeks, taking prescription anti-coagulant or anti-platelet medications, the inability to exercise as dictated by the Get Active Questionnaire, or having a body mass index of >27kgm^{-2}. Prior to commencing the trial all participants had the study explained to them, including advisement of the risks and benefits of participation. This study received ethics approval by the University of Waterloo Research Ethics Board (REB# 2277) and conformed to all standards for ethical engagement of human subjects in research set out in the Canadian tricouncil research policy (http://www.pre.ethics.gc.ca/pdf/eng/tcps2/TCPS_2_FINAL_Web.pdf).

3.1 Study Design

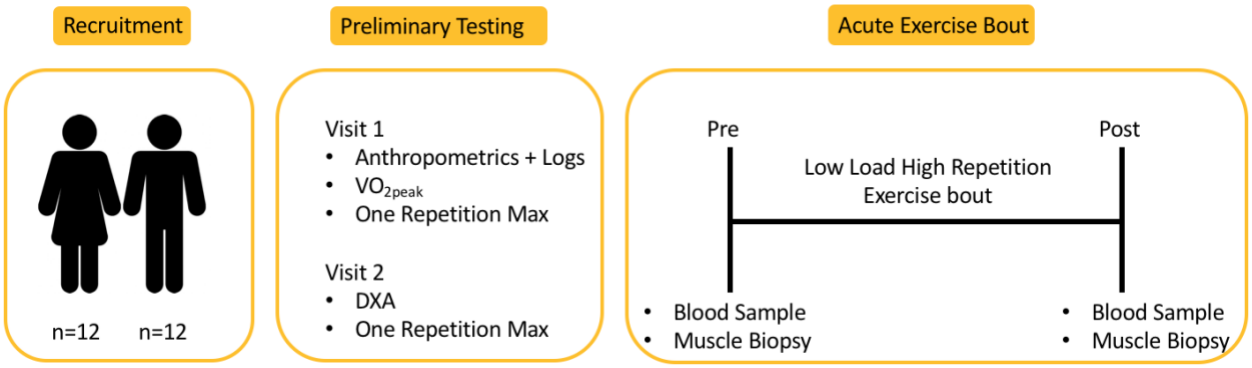

Figure 2: General chronological overview of study design

3.1.1 General Study Outline

Upon enrollment into the study, participants underwent a VO_{2peak} test on a cycle ergometer. This test served as a normative value to appropriately match men and women with respect to aerobic power and thus enable the ability to elucidate sex-based differences. In order to appropriately match men and women based on training status the VO_{2peak} data was normalized relative to fat-free mass[80]. In order to determine fat-free mass participants underwent a measure of body composition using a full-body dual energy x-ray absorptiometry (DXA) scan using a Hologic Discovery W (Hologic, Mississauga, ON, CA). Once the scan was completed, the image was analyzed using QDR APEX software (Version 4.5.3, Hologic, Mississauga, ON, CA) by a trained technician. Upon completion of the VO_{2peak} test, participants were given 3-day food logs, as well as in depth physical activity log for seven days along with a pedometer to track steps within the day. The physical activity logs allowed for assessment of habitual physical activity. The food logs allowed for comparison of habitual dietary intake between the sexes, by comparing macro and micronutrient profiles.

On two separate occasions participants underwent assessment of their one repetition maximum (1-RM) with each session separated by at least 72 hours. 1-RM was assessed for

bench press, leg extension, lat pulldown, hamstring curl, shoulder press, and leg press. Official 1RMs were the maximal lift between the 2 attempts. These values served to determine the appropriate load for the low-load, high-repetition exercise bout.

On the morning of testing, at least 72 hours after performing any moderate-vigourous physical activity, participants arrived at the lab following an overnight fast (12 h). A muscle biopsy was taken from the *vastus lateralis* and a blood sample collected from the antecubital vein. After a general aerobic warmup of five minutes, the low-load high repetition exercise began. Once the exercise culminated, the participant immediately had another muscle and blood sample collected.

3.1.2 Detailed study procedures

3.1.2.1 VO_{2peak}

Maximal oxygen uptake was measured using a Vmax system (Vyaire Medical, Mettawa, IL, USA) on a cycle ergometer (Ergoline, Bitz, Germany). Participants began by warming up at 50W for 2 minutes. A finger prick was administered using a lancet and lactate was analyzed every minute throughout the test using a Lactate Scout Plus (EKF, Penarth, England). Heart Rate was collected every 30 seconds using a Polar Heart Rate monitor (Polar, Lachine, QC, CA). A rating of perceived exertion (RPE), using a Borg scale (6-20) was also collected every minute of the test. Programmed into the ergometer was our ramp protocol consisting of a 1W increase every 2 seconds, beginning at 50W and ongoing until the participant reached volitional failure. Breathe-by-breathe analysis was collected by the Vmax system and averaged for every 30 seconds in order to determine VO_{2peak}.

3.1.2.2 One Repetition Max Testing

Each participant completed the 1-RM testing on 2 separate occasions separated by at least 72 hours, and the highest weight achieved between the attempts was used to calculate the exercise weight. Participants underwent 1-RM testing for the following resistance exercises; bench press, leg extension, lat pulldown, hamstring curl, shoulder press, and leg press (Life Fitness, Rosemont, IL, USA). The session began with a five-minute general aerobic warmup before transitioning into the resistance exercises. Heart rate was gathered after every set of resistance exercise, using a polar heart rate monitor. RPE was determined at the same time intervals. Each exercise began with a conservatively chosen warmup weight, based on the participant's training familiarity, for a 10-repetition warmup. Based on the RPE following the first warm up set, 10-20% of weight was added for the subsequent set, which served as a second warmup and consisted of 3-5 repetitions. Following this final warmup set, additional weight was added as the participant worked towards their 1-repetition max. There was 2 minutes of rest between each of the sets to ensure PCr pool replenishment. A qualified spotter was present to ensure the safety of the participant as well as the quality of the repetitions being performed. 1-RM was determined within 5 attempts and was used to determine the load for each exercise that was performed during the low-load high-repetition exercise bout.

3.1.2.3 LL-HR Protocol

The low-load high repetition resistance exercise bout consisted of lifting a load corresponding to 30% 1-RM for 20-25 repetitions for each exercise in a circuit format. Participants completed 3 sets of every given exercise in a circuit format (Chest Press, Leg Extension, Lat Pulldown, Hamstring Curl, Shoulder Press, Leg Press), with each exercise

being separated by 30 seconds of rest and each round of the circuit separated by 2 minutes of rest. The final set of each exercise continued to volitional failure. At rest and throughout the exercise session a finger prick blood sample was taken every 3 minutes for determination of blood lactate concentration using a Lactate Scout Plus (EKF, Penarth, England).

3.1.2.4 Muscle Sample Collection

Before and after the LL-HR protocol participants underwent a muscle biopsy of the *vastus lateralis* using a custom suction-modified Bergstrom needle. The first sample obtained was immediately placed in liquid nitrogen prior to removing the sample from the needle to allow for determination of muscle glycogen and metabolite concentrations. The second sample collected was dissected free of fat and connective tissue and one piece was snap frozen in liquid nitrogen and stored at -80$_{o}$ C for analysis of protein content of enzymes related to anaerobic and aerobic energy metabolism. The other piece was mounted for determination of muscle fibre type.

3.2 Analysis

3.2.1 Western Blot Analysis

Muscle samples for Western Blot analyses were homogenized 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)). Muscle samples were transferred into a prechilled homogenization Biopur Eppendorf (Eppendorf, Mississauga, ON, Canada) and homogenization buffer was added at a ratio of 10µL buffer to 1mg of muscle. A homogenization bead (Qiagen, Toronto, ON, Canada) was added to the Eppendorf, and samples were homogenized using TissueLyser II (Qiagen, Toronto, ON, Canada) run at 20 cycles seconds^{-1} for 40 seconds. Once the sample were sufficiently homogenized, samples

were spun at 10,000G for 10 minutes at 4$_{o}$C. Supernatant was separated and allocated into a prechilled Eppendorf, while the pellet was frozen for potential use in analyses. A bicinchoninic acid (BCA) assay was used to determine the total protein content of each sample. Subsequently, samples were prepared in Laemmli buffer (0.5M Tris-HCl, glycerol, 10% SDS, 1% bromophenol blue, β-mercaptoethanol, and ddH_2O) and stored at -80$_{o}$C until western blotting analysis began.

Using SDS-PAGE and western blotting, principle metabolic proteins were analyzed. An equal amount of protein (10 μg) of each sample was run on 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) were run on every gel to induce comparative power. Proteins were transferred to a PVDF membrane using the Trans-Blot Turbo Transfer System (BioRad, Hercules, CA, USA). Total protein and visual confirmation of protein transfer was done pre and post membrane transfer, respectively, using a Chemidoc MP (BioRad, Hercules, CA, USA). Blocking of the membranes was done for 2 hours in either 5% bovine serum albumin (BSA) in 1X Tris-buffered saline and Tween 20 (TBST) or 5% skim milk in 1X TBST to ensure the optimization of the blocking, detailed in Table 1. Membranes were kept at 4°C for 12 hours in primary antibody at 1:1 000 – 10 000 dilutions as optimal for each antibody in 1X TBST (Table 1). After the 12-hour period, the membranes were washed 5 times for 3 min with 1X TBST to remove excess primary antibody before the appropriate secondary antibody (1: 20,000) was added. Secondary antibody was incubated with the membrane at room temperature for 1 hour. Afterwards, the membrane was imaged one more time using chemiluminescence Super-Signal West Dura Extended Duration Substrate (Thermo Fisher,

Scientific, Waltham, MA, USA) on the Chemidoc imaging system (BioRad, Hercules, CA, USA). Bands were quantified using ImageJ (Version 1.51a, National Institute of Health, USA) and protein content was normalized using the standard curve obtained from the gel. The list of antibodies and their specific information can be found in Table 1.

Table 1. 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
GP	Invitrogen	5% Skim Milk in 1X TBST	1:2 000	1:5 000
PFK	Abcam		1:10 000	1:10 000
LDH-H	Abcam		1:1 000	1:5 000
LDH-M	Abcam		1:4 000	1:5 000
PDH-E1α	Invitrogen		1:1 000	1:5 000
PDHK4	Abcam		1:1 000	1:5 000
CK	Abcam		1:1 000	1:5 000
AMPD2	Santa Cruz		1:2 000	1:10 000
MCT1	Abcam		1:1 000	1:5 000
MCT4	Abcam		1:2 000	1:5 000
Phospho-CK	Abcam	5% BSA in 1X TBST	1:4 000	1:10 000
Phospho-PDHE1α	Abcam		1:3 000	1:10 000

3.2.2 Muscle Glycogen and Metabolite Assays

Muscle glycogen and metabolite concentrations were determined using commercially available kits (Table 2). In order to determine muscle metabolite and glycogen concentrations, muscle samples were homogenized in 25mM Tris buffer with 0.05% Triton X using TissueLyser II (Qiagen, Toronto, ON, Canada). Immediately after homogenization, samples were centrifuged for 10 minutes at 15,000g at 4$_{o}$C, and the supernatant was separated. A deproteinization procedure was then completed. 4M PCA was added to each sample until a concentration of 1M PCA was achieved, samples were centrifuged for 5

minutes at 15,000g at 4ºc, and the supernatant removed. 2M KOH was added to each sample until the KOH was 34% of the final volume of the supernatant, samples were centrifuged for 3 minutes at 15,000g at 4ºc and the supernatant successfully deproteinized.

Each metabolite assay was done in an identical manner with the differences being the enzyme reagent mix and the enzyme reaction mix that was used. The composition of each of these mixes remains unknown, as the proprietary blends are the creation of the manufacturer. Each sample well was loaded with 10µl of processed muscle sample and brought to volume with 40µl of enzyme reagent mix. 50µl of enzyme reaction mix was added to each of the wells to begin their respective reactions. After a 30-minute incubation period, each plate was read at their respective wavelength and metabolite concentration analyzed. Each metabolite kit, and their respective wavelength is summarized in table 2.

Table 2. Metabolite of interest, assay kit utilized, and wavelength used for reading

Metabolite of Interest	**Colourimetric Assay Kit Used**	**Wavelength**
Glycogen	Glycogen Assay Kit II (ab169558)	450nm
Pyruvate	Pyruvate Assay Kit (ab65342)	570nm
Lactate	L-Lactate Assay Kit (ab65331)	450nm
Creatine	Creatine Assay Kit (ab65339)	570nm
ATP	ATP Assay Kit (ab83355)	570nm
Phosphate	Phosphate Assay Kit (ab65622)	650nm

3.2.3 – Immunohistochemical Staining

Using muscle samples preserved in OCT, serial sections of 10µm thick samples were cut using a cryostat (Thermo Electronic, MA, USA) and mounted on slides to be analyzed. Blocking solution (10% goat serum, 90% 1X PBS) was aliquoted over the sections (100µl) and allowed to incubate at room temperature for 1 hour. After the incubation, 100µl of each 1_o antibody (table 3) was aliquoted to cover the cross sections. Muscle samples with 1_o antibody were allowed to incubate overnight in the dark (~18hours). Samples underwent 3 x 5-minute washes using 1X PBS in the dark. Samples were allowed to dry and subsequently incubated with the appropriate 2_o antibodies diluted 1:500 in 10% goat serum and 1x PBS for 1 hour in the dark. At the end of the incubation, slides were once again washed using 3 x 5-minute washes using 1X PBS in the dark. Slides were allowed to dry, and 15µl of Prolong was applied over all of the sections and mounted with a #1 coverslip. In order to reach peak fluorescence, slides were allowed to sit in the dark for 18 hours. The slides were imaged in the dark under the microscope (Zeiss, Oberkochen, Germany) on the highest intensity setting and were captured using the Zen System (Zeiss, Oberkochen, Germany) computer program using the 'Image Processing' tab for analysis. Muscle fibre type and cross-sectional area were determined using Image J with an average of 500 fibres/sample analyzed.

Table 3. 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	Absence of Colour
IIx	6H1	1:100	IgM	1:500	Red

3.3 Statistical Analyses

Baseline differences between groups was assessed using a non-paired t-test in SPSS (version 25, IBM, Armonk, NY, USA). 2-way mixed model ANOVA with sex as the between variable (2 levels, male/female) and time (2 levels, pre/post exercise) as the within variables were used to determine the effects of sex and exercise on all other experimental variables using SPSS. Post-hoc analyses were conducted using a Tukey's HAD test where appropriate. Linear regression analysis was used to determine the influence that muscle fibre type distribution had on the metabolic changes found in the study using GraphPad Prism (version 8.3.0, San Diego, CA, USA). Significance was set at $P < 0.05$.

Chapter Four: Results

4.1 Participant Characteristics

Baseline characteristics were different between the two groups with men having a greater height (p=0.0014, table 4), weight (p=0.0169, table 4), and VO_2 peak relative to total body mass (p=0.0017, table 4) compared to women. Women had a body fat percentage compared to men (p=0.0002, table 4). Importantly, when expressed relative to fat-free mass, men and women had comparable VO_2 peak values (p=0.2839, table 4).

Table 4. Participant characteristics

	Men	**Women**	**p Value**
Age (y)	22 ± 1	21 ± 1	0.68
Height (cm)	178.4 ± 2.4	164.3 ± 2.9	0.001
Weight (kg)	74.7 ± 3.2	62.6 ± 3.2	0.017
BMI (kg/cm$_2$)	23.8 ± 1.0	23.7 ± 1	0.926
% BF	22.2 ± 1.6	33.5 ± 1.7	0.001
VO_{2peak} (ml O_2/min/kg)	43.7 ± 1.8	35.6 ± 1.2	0.002
VO_{2peak} (ml O2/min/kg FFM)	59.7 ± 1.7	56.8 ± 2.0	0.284

Data are means ± SEM. BMI - body mass index, % BF - percent body fat, FFM – fat free mass.

Baseline one repetition max measurements revealed that men had significantly higher 1RMs than women for chest press ($p = 0.002$, table 5), shoulder press ($p = 0.002$, table 5), lat pulldown ($p < 0.001$, table 5), knee extension ($p = 0.007$, table 5), and hamstring curl ($p < 0.001$). Additionally, men had a strong trend to have a higher leg press 1RM ($p = 0.062$). These differences continued to exist when 1RM was normalized to fat free mass for chest press ($p = 0.008$), shoulder press ($p = 0.029$), and lat pulldown ($p = 0.046$). However, there was no significant difference for 1RM/FFM for leg press ($p = 0.621$), knee extension ($p = 0.643$), and hamstring curl ($p = 0.103$).

Table 5. Average 1 RM values for 10 men and 10 women participants

		Men	Women	p Value
Chest Press	**Absolute (lbs)**	147 ± 17	76 ± 8	0.002
	Relative to (FFM)	1.20 ± 0.1	0.89 ± 0.1	0.008
Shoulder Press	**Absolute (lbs)**	149 ± 18	79 ± 7	0.002
	Relative to (FFM)	1.23 ± 0.1	0.94 ± 0.1	0.029
Lat Pulldown	**Absolute (lbs)**	167 ± 12	102 ± 10	0.001
	Relative to (FFM)	1.39 ± 0.1	1.20 ± 0.1	0.046
Leg Press	**Absolute (lbs)**	377 ± 37	278 ± 33	0.062
	Relative to (FFM)	3.11 ± 0.2	3.26 ± 0.3	0.621
Knee Extension	**Absolute (lbs)**	209 ± 15	143 ± 16	0.007
	Relative to (FFM)	1.74 ± 0.1	1.67 ± 0.1	0.643
Hamstring Curl	**Absolute (lbs)**	160 ± 12	103 ± 7	0.001
	Relative to (FFM)	1.33 ± 0.1	1.22 ± 0.1	0.103

Data are means ± SEM.

Nutritional information from the 3-day food logs revealed no statistical differences between men and women for absolute energy and macronutrient intake (table 6). There was a strong trend however, for men to consume more energy ($p = 0.07$) than women. Additionally, there was a strong trend for men to consume more protein than women ($p = 0.06$). However, when normalized to % of daily energy intake and to body weight, protein intake was similar between men and women ($p = 0.41$ and $p = 0.68$, respectively) There was no difference in % carbohydrate or fat intake relative to energy intake.

Table 6. Dietary intake for 10 men and 10 women participants

		Men	Women	p Value
Energy (kcal)		1791 ± 104	1550 ± 67	0.07
Protein	**Absolute (g)**	85 ± 9	65 ± 3	0.06
	% of Daily Kcal	17 ± 1	19 ± 2	0.41
	g/kgBW/d	1.61 ± 0.1	1.75 ± 0.1	0.68
Fat	**Absolute (g)**	72 ± 7	61 ± 5	0.18
	% of Daily Kcal	35 ± 2	36 ± 2	0.71
Carbs	**Absolute (g)**	207 ± 16	194 ± 11	0.51
	% of Daily Kcal	50 ± 2.0	47 ± 3	0.3

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

4.2 LL-HR Acute Exercise Bout

During LL-HR there was a trend for women to complete more repetitions during the final set of each exercise compared to men ($p = 0.094$, figure 3). However, when the total work of exercise was standardized to fat-free mass, both men and women completed an identical volume of work ($p = 0.774$, figure 3). Lactate at the point of failure did not differ between men and women ($p=0.183$).

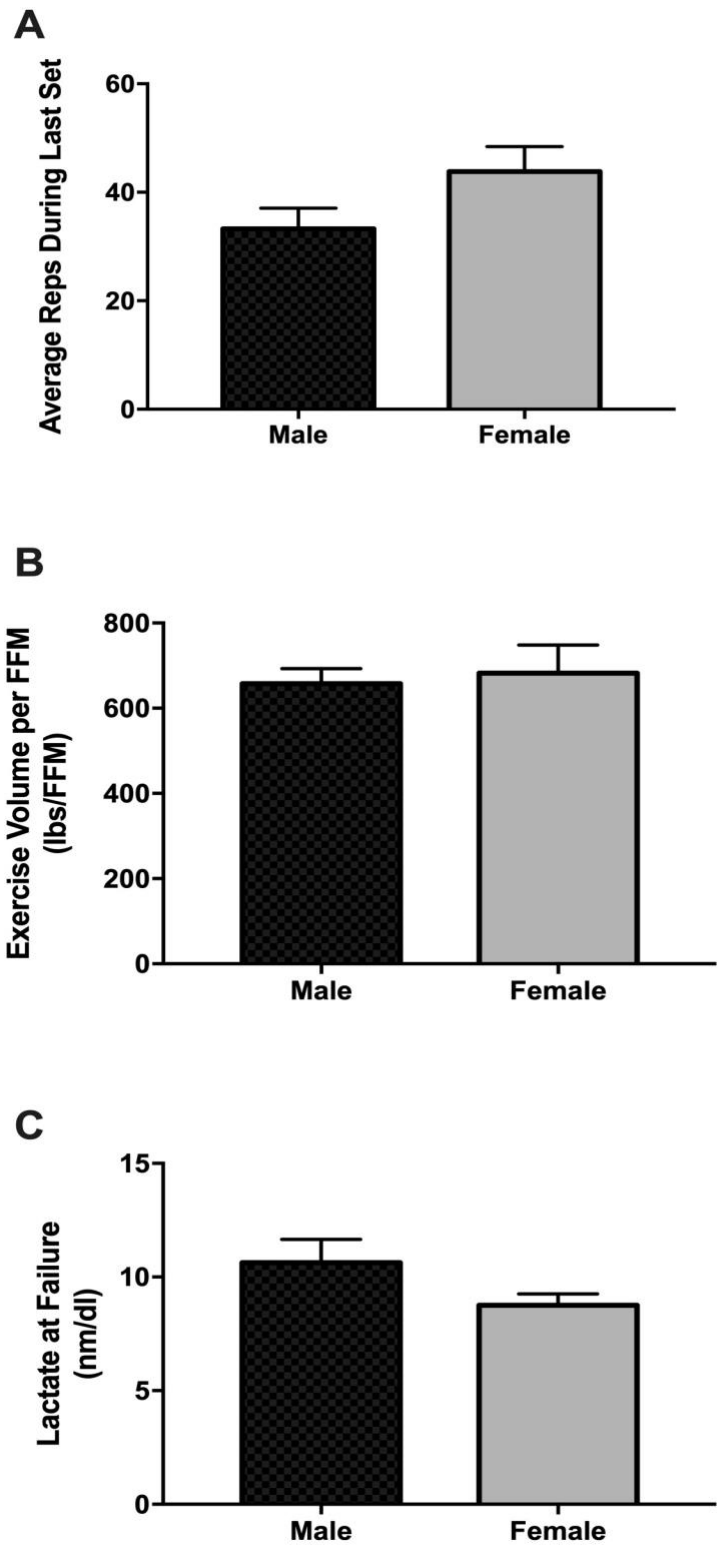

Figure 3: Representation of A) number of reps to failure during the last set B) exercise volume per kg of fat-free mass, and C) lactate concentration at failure during an acute bout of low load, high repetition resistance exercise. Data are reported as the mean ± SEM. Analysis done by independent T-Test.

4.3 Changes during LL-HR Exercise Bout

As expected, there was an increase in heart rate ($p < 0.001$, figure 4 A), RPE ($p < 0.001$, figure 4 B), and lactate ($p < 0.001$, figure 4 C) over the course of the exercise bout with no differences between men and women. Additionally, there was no difference in the AUC for heart rate ($p = 0.928$), RPE ($p = 0.445$), or lactate ($p = 0.235$) between men and women. The percentage of heart rate max was calculated for each set of the circuit and was found that men worked at 65%$_{HRmax}$, 67%$_{HRmax}$, and 70%$_{HRmax}$ during sets 1, 2, and 3, respectively. With respect to women, they worked at 69%$_{HRmax}$, 80%$_{HRmax}$, and 85%$_{HRmax}$ during sets 1, 2, and 3, respectively. Therefore, women worked at a significantly higher percentage of their heart rate max for set 2 ($p = 0.002$) and set 3 ($p = 0.003$), but not set 1 ($p = 0.26$) than men.

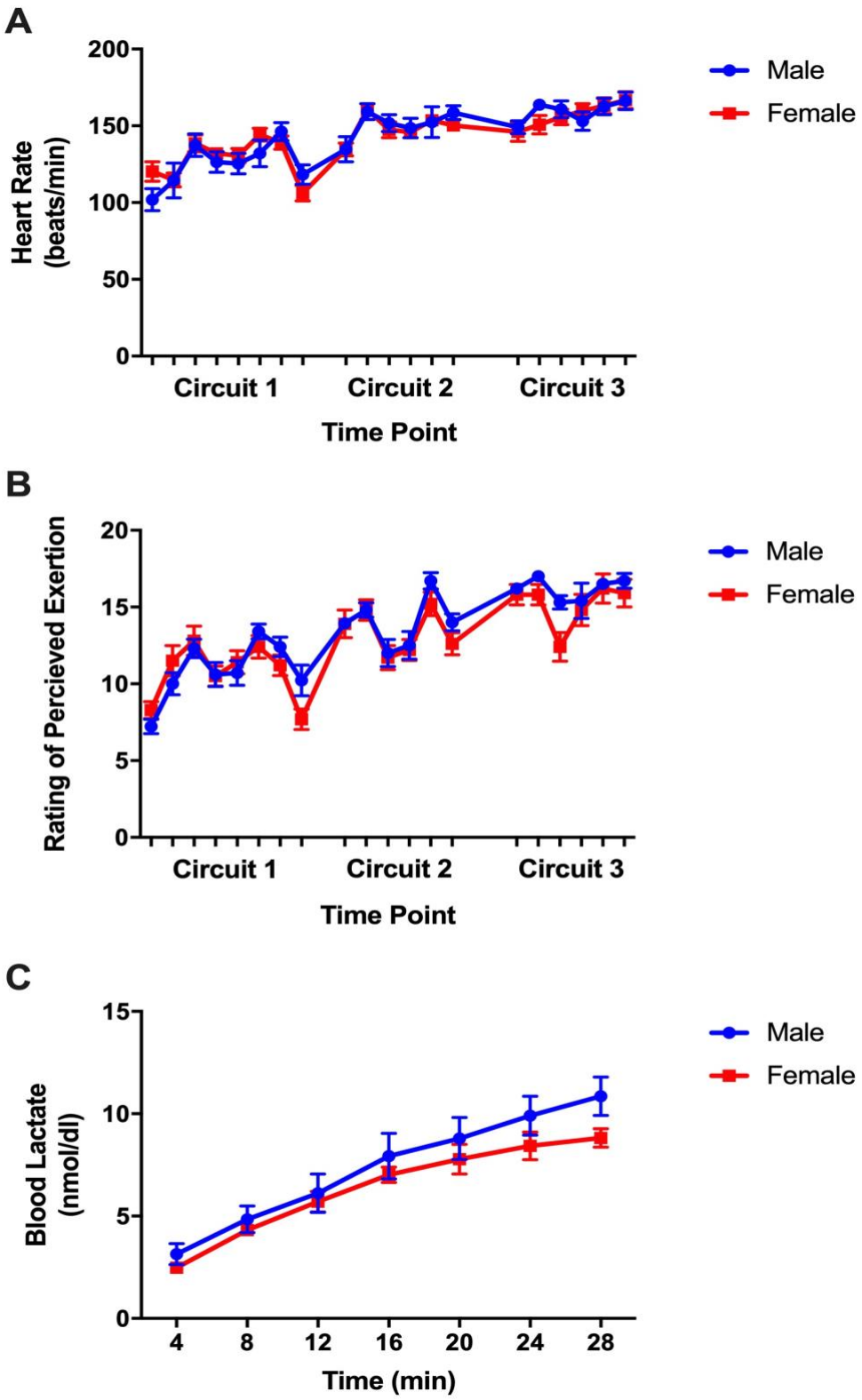

Figure 4: A) Heart rate, B) rating of perceived exertion, and C) blood lactate over the course of a LL-HR exercise bout. Data are reported as the mean ± SEM for 20 participants. Analysis done by AUC calculation

4.4 Contribution of HEPT to energy production

4.4.1 Sex differences in the change in HEPT metabolites

Creatine

At baseline creatine concentration did not differ between men and women ($p = 0.747$, figure 5 A). Creatine increased during LL-HR in men, but not women ($p = 0.026$, figure 5 A). When expressed relative to baseline creatine content, creatine increased by 22% in men and decreased by 3% in women ($p = 0.05$).

ATP

Overall men had a higher ATP concentration than women ($p = 0.01$, figure 5 B). During LL-HR ATP concentration decreased in both men and women ($p < 0.001$, figure 5 B), with no difference in the extent of the decrease between the sexes ($p = 0.352$, figure 5 B). However, when expressed relative to baseline ATP content there was a tendency for the percent change in ATP to be greater in women (-39%) than men [-18%, ($p = 0.09$)].

Inorganic phosphate

Inorganic phosphate levels were higher in men than women ($p = 0.007$, figure 5 C). Phosphate levels did not change significantly with exercise in either sex ($p=0.492$, figure 5 C).

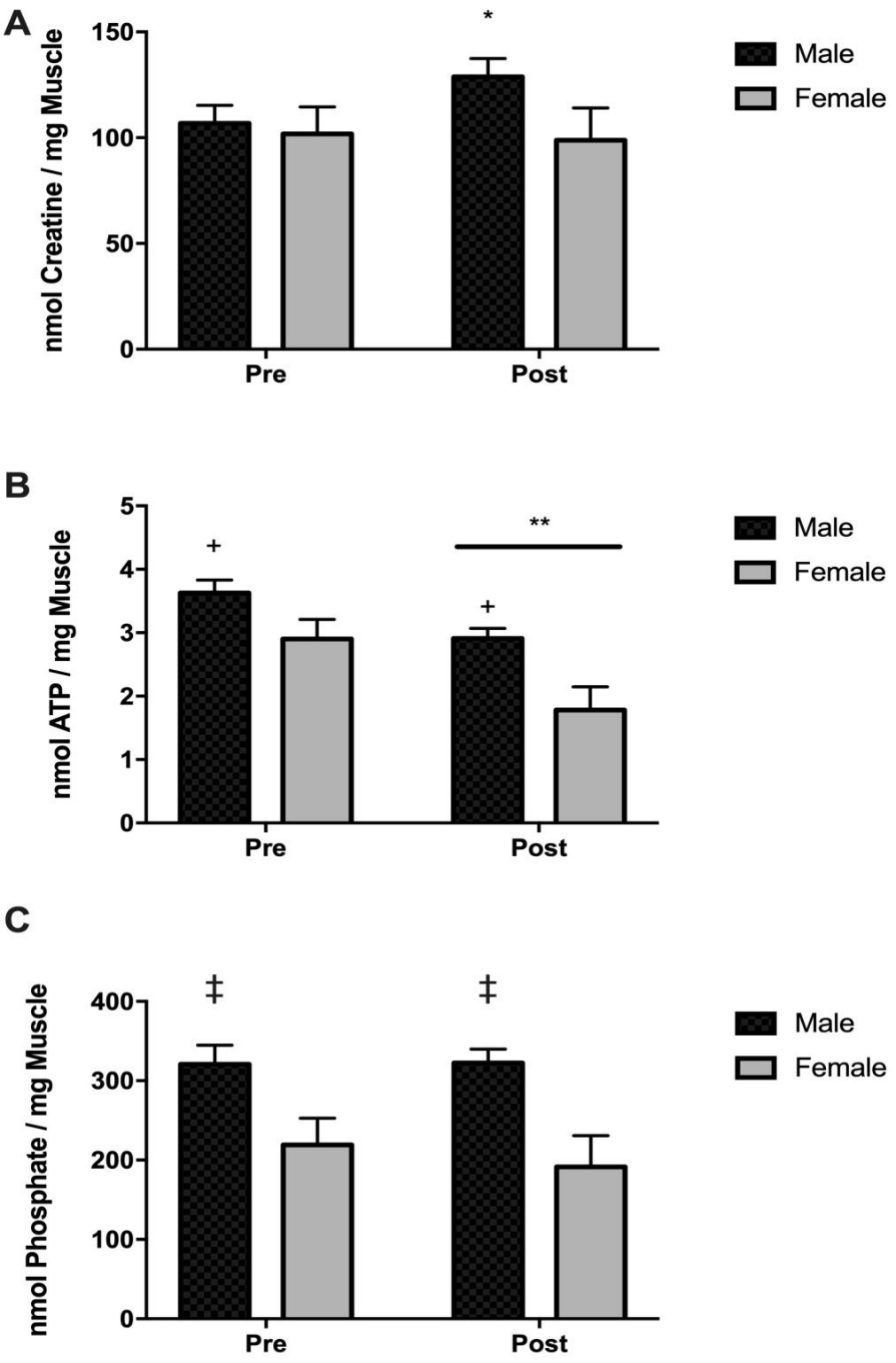

Figure 5: A) Creatine, B) ATP, and C) inorganic phosphate content of skeletal muscle in male and female participants prior to and following LL-HR exercise. Data are reported as the mean ± SEM for 20 participants. Data analysis by mixed model ANOVA* $p = 0.026$, ** $p < 0.001$, + $p = 0.01$, and ‡ $p = 0.007$.

4.4.2 Sex differences in protein content of HEPT enzymes

We also examined whether there were differences in the protein content of enzymes involved in HEPT metabolism. At rest there were no differences between men and women in the protein content of creatine kinase ($p = 0.370$, figure 6 A) or AMP deaminase 2 ($p = 0.159$, figure 6 B). At baseline phosphorylated CK was similar between men and women ($p = 0.422$, figure 6 C). The content of phosphorylated CK decreased following LL-HR in both men and women ($p<0.001$, figure 6 C)., but to a greater extent in women as compared with men ($p=0.023$, figure 6 C).

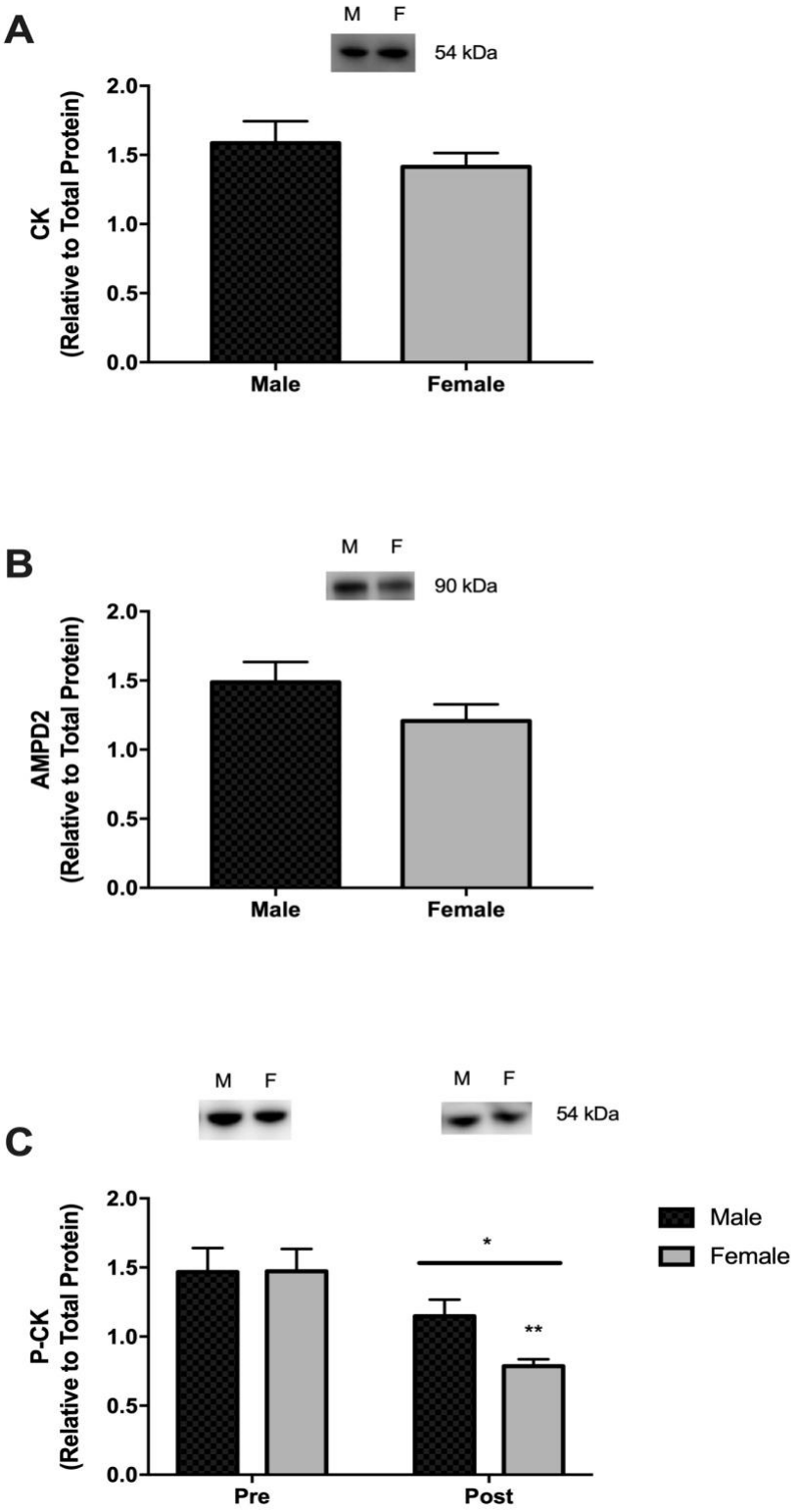

Figure 6: A) CK, B) AMPD2, and C) Phosphorylated CK protein expression of skeletal muscle from male and female participants. Data are reported as the mean ± SEM for 20 participants. Analysis by independent T-test for CK and AMPD2. Analysis by mixed model ANOVA for phosphorylated CK. * $p < 0.001$ and ** $p = 0.023$.

4.5 Contribution of glycolysis to energy production during LL-HR

4.5.1. Sex differences in glycolytic metabolites

Muscle glycogen

Muscle glycogen content was higher in men than woman ($p = 0.001$, Figure 7 A). With respect to glycogen breakdown, exercise decreased muscle glycogen content in both men and women ($p < 0.001$, figure 7 A), with men tending to utilize more glycogen than women ($p=0.08$, figure 7 A). However, when expressed as a percent change from baseline muscle glycogen content there was no difference in glycogen utilization during exercise.

Muscle pyruvate

Pyruvate was not different between men and women ($p=0.234$, figure 7 B), nor was there a significant change during LL-HR ($p=0.672$, figure 7 B). Additionally, there was no difference in the change in pyruvate during exercise between men (593%) and women [420% ($p=0.766$)].

Muscle lactate

Muscle lactate was greater in men as compared with women ($p = 0.019$, figure 7 C). There was no overall effect of LL-HR on muscle lactate concentration ($p=0.339$, figure 7 C); however, there was a trend for muscle lactate to increase in men, but not women during LL-HR ($p = 0.09$, figure 7 C). Furthermore, when expressed relative to baseline muscle lactate content there was a tendency for the percent change in lactate to be greater in men (35%) than women [-9%, ($p = 0.07$)].

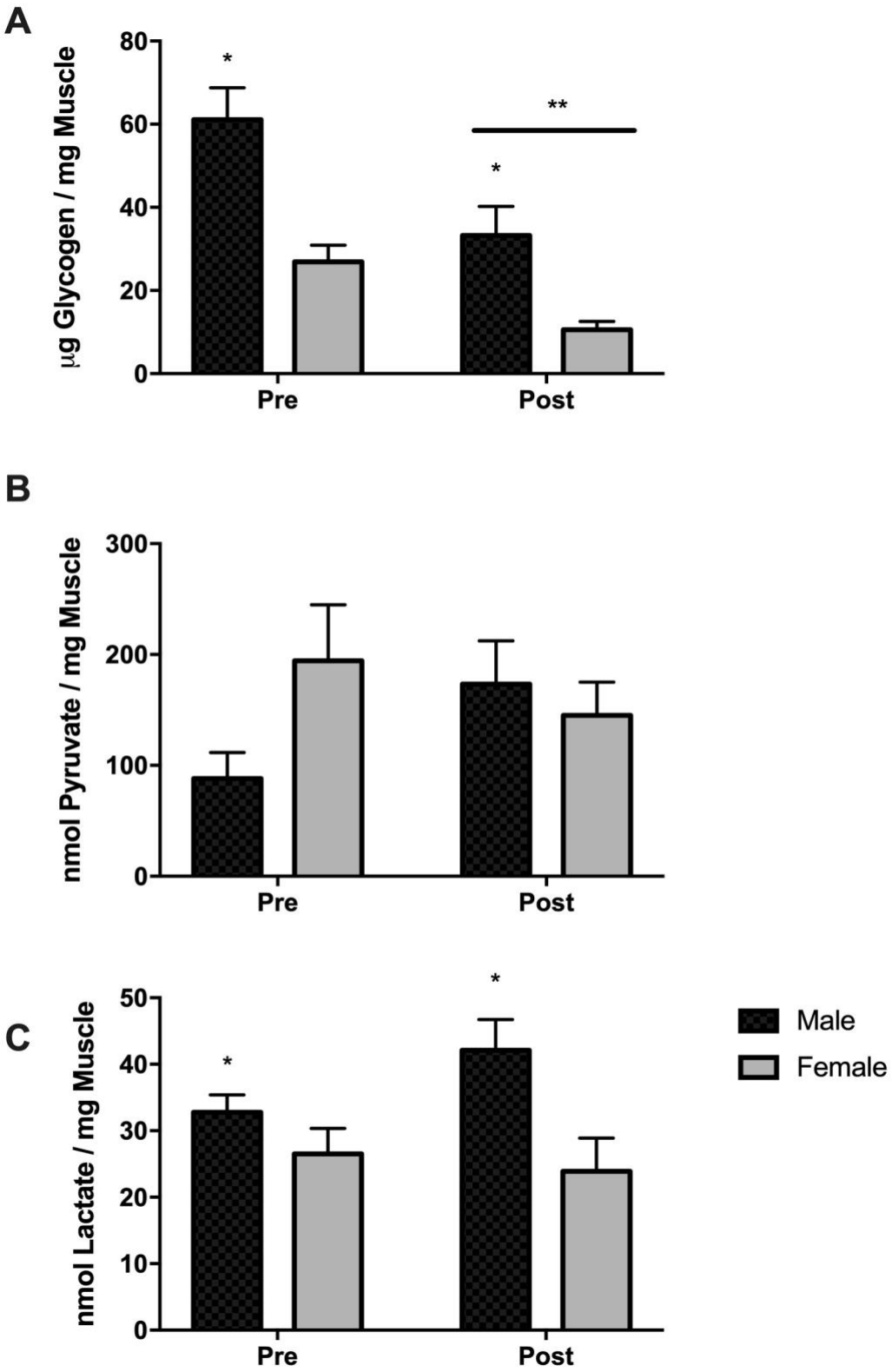

Figure 7: Glycogen, pyruvate, and lactate content of skeletal muscle from male and female participants from both pre and post LL-HR exercise bout. Data are reported as the mean ± SEM for 20 participants. Analysis by mixed model ANOVA. * $p = 0.001$ and ** $p < 0.001$.

4.5.2 Sex differences in the protein content of glycolytic enzymes

As with the HEPT system we also probed for differences in protein content of enzymes related to glycogen/glucose metabolism (Figure 8). There were no differences in the protein content of glycogen phosphorylase ($p = 0.517$, figure 8 A) or phosphofructokinase ($p = 0.372$, figure 8 B). We also determined the protein content of enzymes related to the metabolic fate of pyruvate. There were no differences in the protein content of PDHK4 ($p = 0.180$ figure 8, C), PDHE1α ($p = 0.741$, figure 8 D), LDH-M ($p = 0.415$, figure 8 E), or LDH-H ($p = 0.525$, figure 8 F), MCT-1 ($p = 0.241$, figure 8 G) or MCT-4 ($p = 0.940$, figure 8 H). Furthermore, there was no difference between men and women in the phosphorylation status of PDHE1α ($p = 0.72$, Figure 8 I), but phosphorylated PDHE1α did decrease during LL-HR in both men and women ($p < 0.001$, figure 8 I) with no difference between the sexes ($p = 0.45$, figure 8 I).

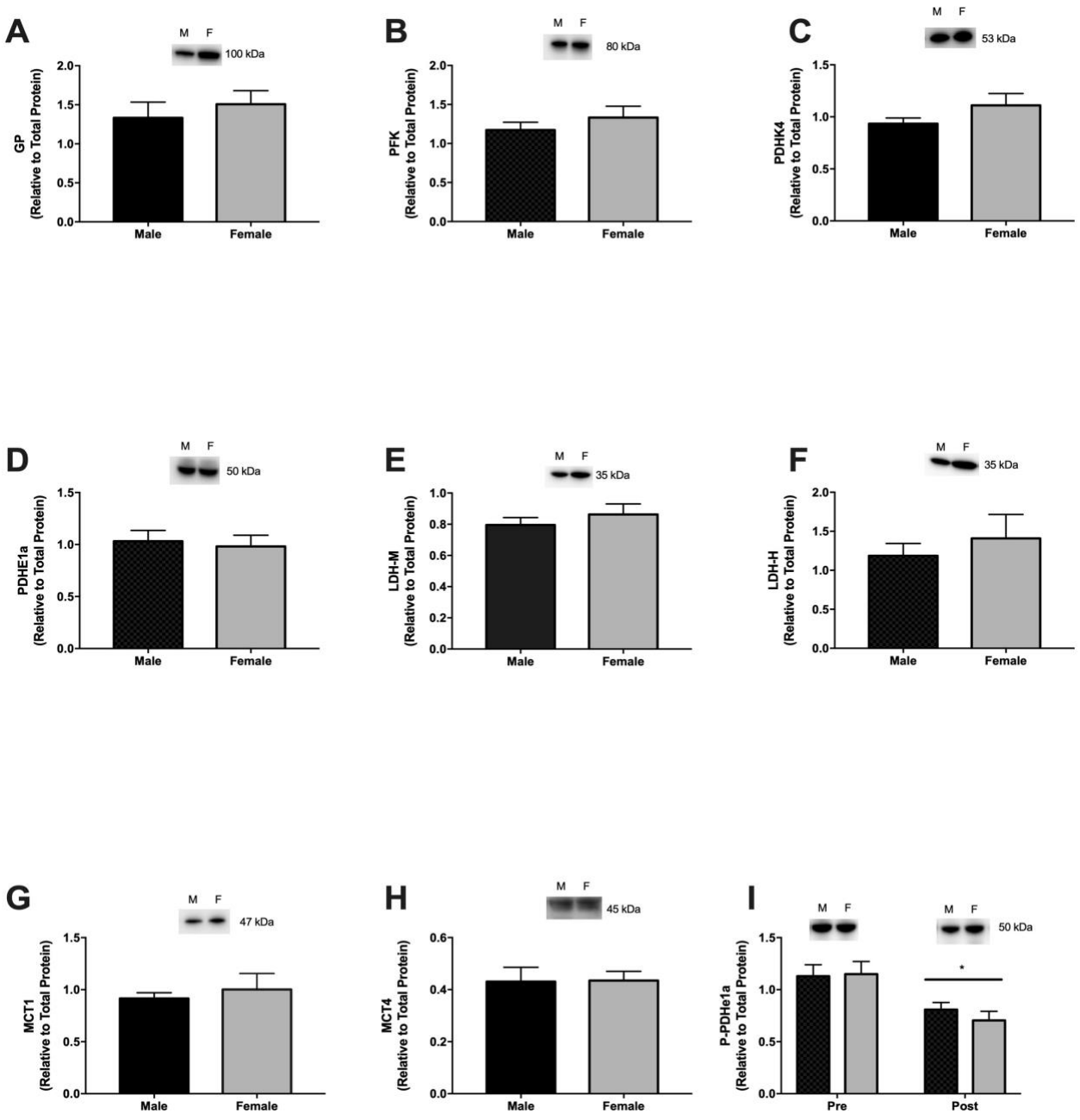

Figure 8: A) GP, B) PFK, C) PDHK4, D) PDHE1a, E) LDH-M, F) LDH-H, G) MCT1, H) MCT4, and I) Phosphorylated P-PDHE1a protein expression in skeletal muscle from male and female participants. Data are reported as the mean ± SEM for 20 participants. Analysis by independent T-tests except for P-PDHE1α which was analyzed by mixed model ANOVA. * denotes a significance of $p < 0.001$.

4.6 Sex differences in muscle fibre type

Women had a significantly greater percentage of type I muscle fibres as compared with men $p = 0.07$, figure 9 A). Men had a significantly greater percentage of type II muscle fibres as compared with women ($p = 0.09$, figure 9 A); however, this was not due to a specifically greater amount of type IIa ($p = 0.10$, figure 9 B) or type IIx fibres ($p = 0.19$, figure 9 B). A representative image is seen in figure 9, C.

Correlational analyses was conducted to examine the influence of fibre type distribution on muscle metabolism during LL-HR The absolute ($r = -0.50$, $p = 0.02$, figure 10 A) and relative ($r = -0.53$, $p = 0.02$, figure 10 B) changes in lactate were negatively correlated with type I fibre content. Additionally, the absolute ($r = -0.52$, $p = 0.02$, figure 10 C) relative ($r = -0.48$, $p = 0.03$, figure 10 D) decreases in P-CK were also negatively correlated with type I fibre content. Finally, the absolute change ($r = -0.53$, $p = 0.02$, Figure 10 E), but not the relative change in pyruvate was negatively correlated with type I fibre content. Additionally, there was a negative relationship between type I fibre content and the content of PDHK4 ($r = -0.49$, $p = 0.02$, figure 10 F) and the content of MCT1 ($r = -0.47$, $p = 0.04$, figure 10 G). There was no relationship between type I fibre content and any of the other metabolites (Figure 11 A-D) or proteins measured in the current trial (Figure 11 E-N).

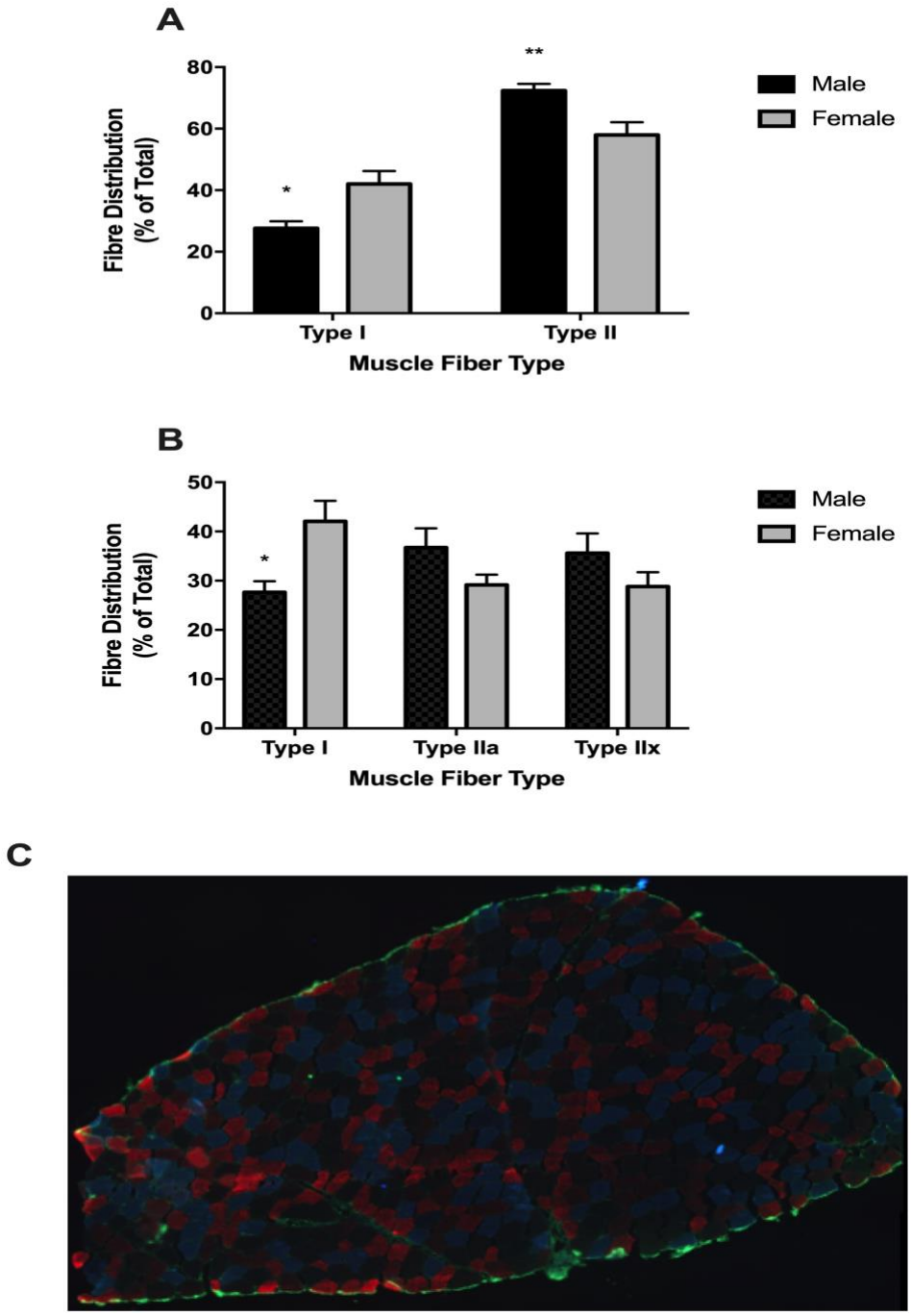

Figure 9: A) Relative distribution of type I and II muscle fibers expressed as a percentage of total number, B) Relative distribution of type I, IIa and IIx muscle fibres expressed as a percentage of total number. Data are reported as the mean ± SEM for 20 participants. Analysis by independent T-test. C) Representative image of muscle cross section. * p = 0.007 ** p = 0.009.

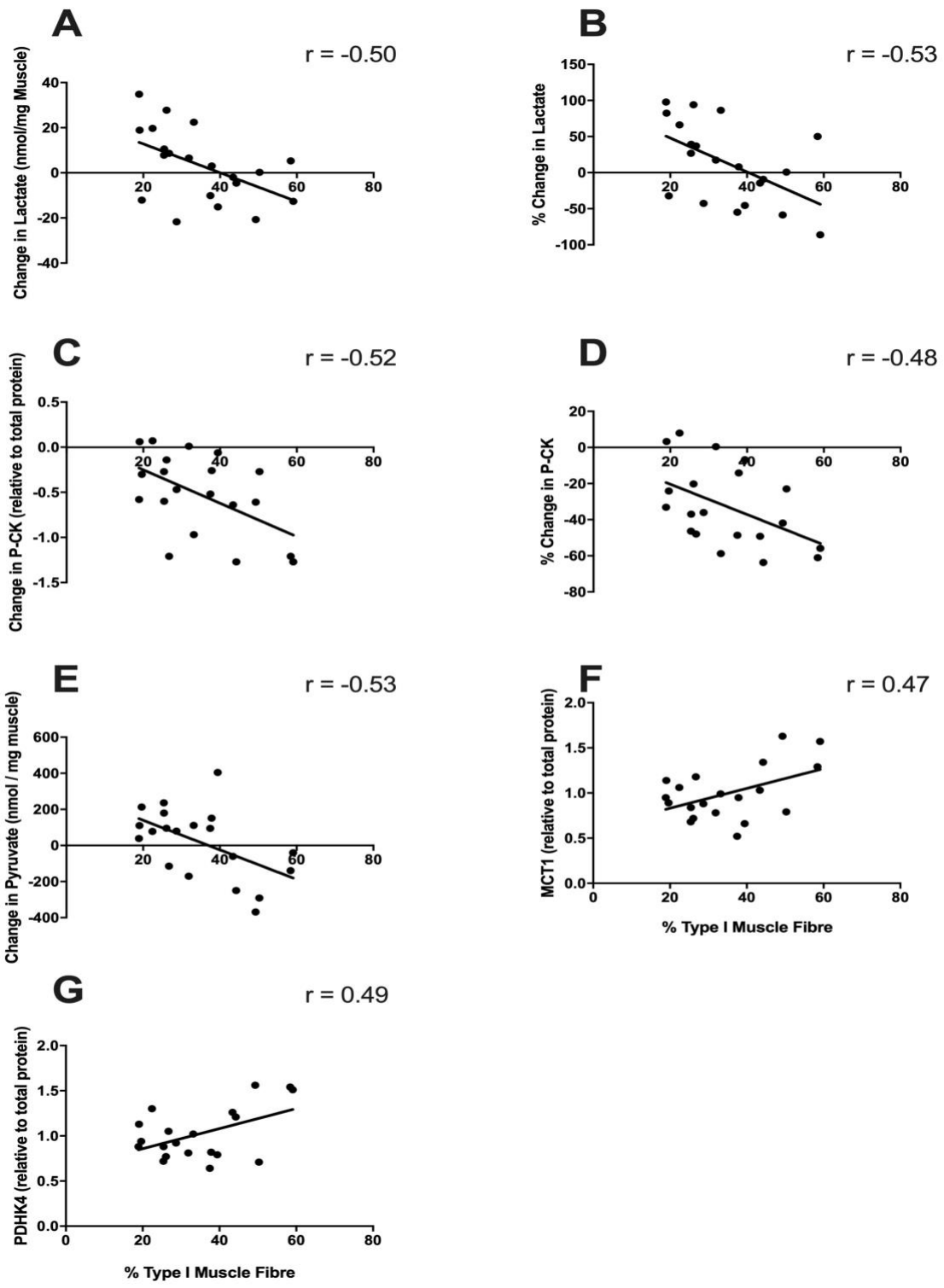

Figure 10: Linear regression analysis showing the relationship between type I muscle fibre content and A) Absolute change in lactate concentration (r = -0.50, p = 0.02), B) relative change in lactate concentration (r = -0.53, p = 0.02), C) absolute changes in P-CK content (r = -0.52, p = 0.02), D) relative changes in P-CK content (r = -0.48, p = 0.03), and E) absolute changes in pyruvate concentration (r = -0.53,p = 0.02).

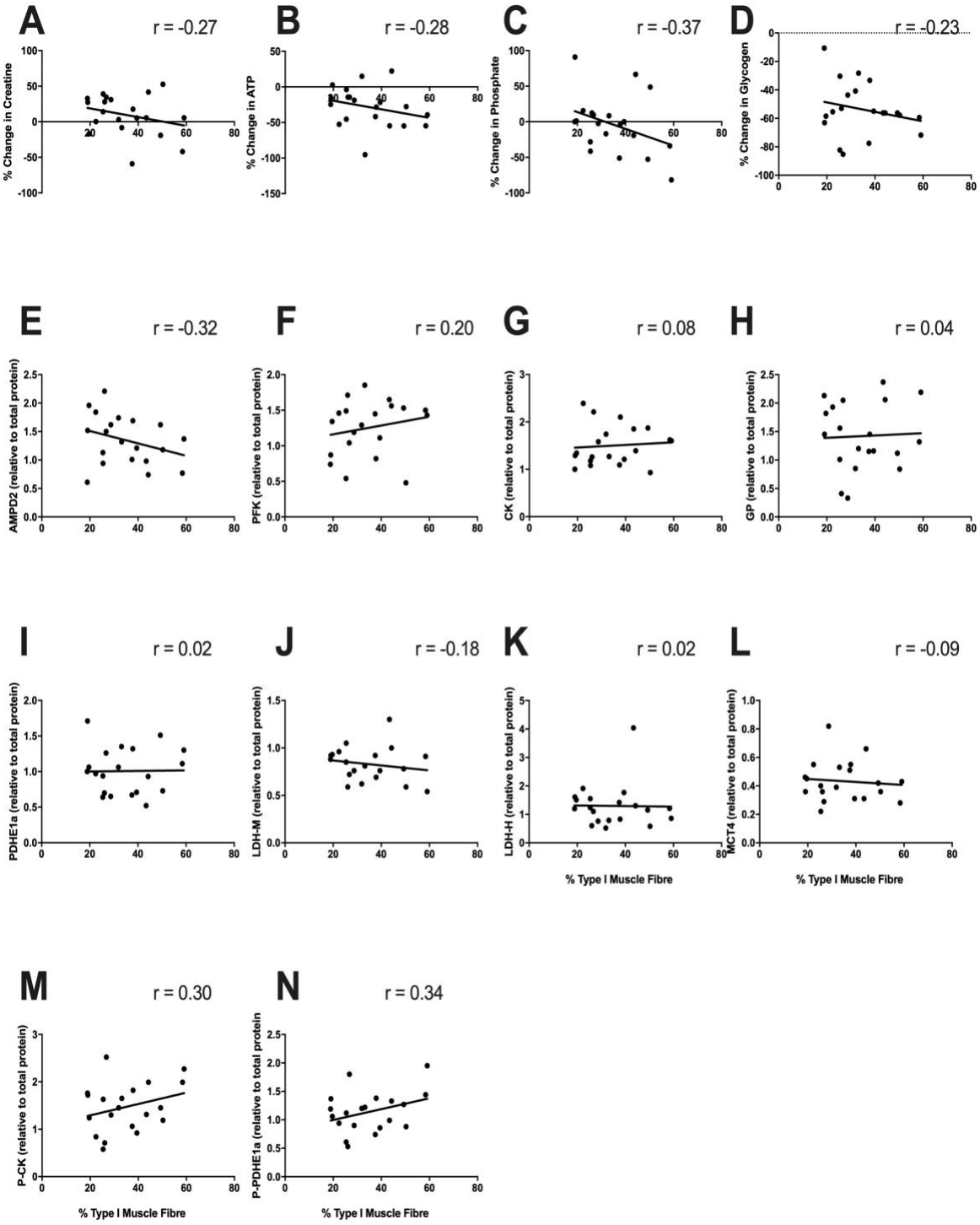

Figure 11: Depiction of linear regression analysis. Relative changes in A) creatine (r = -0.27, p = 0.25), B) ATP (r = -0.28, p = 0.24), C) phosphate (r = -0.37, p = 0.11), D) glycogen (r = -0.23, p = 0.13), E) AMPD2 (r = -0.32, p = 0.17), F) PFK (r = 0.20, p = 0.40), G) CK (r = 0.08, p = 0.73), H) GP (r = 0.04, p = 0.85), I) PDHE1a (r = 0.02, p = 0.95), J) LDH-M (r = -0.18, p = 0.45), K) LDH-H (r = 0.02, p = 0.94), L) MCT4 (r = -0.09, p = 0.69), M) P-CK (r = 0.30, p = 0.21), and N) P-PDHE1a (r = 0.34, p = 0.15).

Chapter Five: Discussion

5.1 Overall Summary

We found that after an acute bout of LL-HR resistance exercise creatine increased in men, but not women, and muscle glycogen decreased in both men and women with a tendency for this decrease to be greater in men. Furthermore, muscle lactate was higher in men than women. Additionally, the phosphorylation status of both CK and PDHe1α decreased during LL-HR, with women having a greater decrease in the phosphorylation of CK. Together these findings suggest that men had a greater reliance on anaerobic energy systems while women were able to initiate aerobic metabolism to meet the demands of this exercise.

5.2 Reliance on HEPT and glycolysis during LL-HR resistance exercise

5.2.1. Reliance on the HEPT system during LL-HR resistance exercise

Creatine concentration increased in men during LL-HR, suggestive of the utilization of phosphocreatine stores to meet energy demands. This is in disagreement with previous work done in the field that found no changes in creatine or phosphocreatine concentration during LL-HR resistance exercise performed at 20% 1RM[46–48], suggesting minimal contribution from the HEPT system. Importantly, these three studies all involved protocols at a lower exercise intensity (20% 1RM) and did not go to volitional failure, thus the exercise stimulus may have been too low to induce meaningful changes in HEPT metabolite concentrations. We also found that LL-HR RE a reduction of the phosphorylation status of creatine kinase, which further indicates that the HEPT system was upregulated during LL-HR resistance exercise at 30% 1RM. Thus, while the HEPT system does not seem to contribute

to energy production during LL-HR resistance exercise performed at 20% 1RM, it does contribute significantly, at least in men, at 30% 1RM. Thus 30% 1RM may represent an intensity threshold whereby HEPT contributes to exercise energy production during resistance exercise.

Alternatively, it may be that RE needs to be performed to volitional failure in order for the HEPT system to contribute to energy production during LL-HR resistance exercise. The Henneman size principle highlights the sequential recruitment of skeletal muscle fibers to complete a given task, with smaller motor units being recruited first and larger motor units being recruited as the force requirement to complete a task increases[81–84]. At the onset of LL-HR resistance exercise type I muscle fibers are recruited first as they are the smallest[84]. Type I fibres are fatigue resistant but will fatigue over time. Thus, as the set progresses and type I fibres begin to fatigue, type II fibres will be recruited. While the HEPT system produces ATP in both type I and type II fibres, the contribution of HEPT may be more readily observed when type II muscle fibers are recruited. In the current study since the bout went to volitional failure it can be assumed that type II muscle fibres were recruited[45]. Indeed, findings from a training study using the same training regime (30% 1RM to failure) that was used in the current trial found that there was hypertrophy of both type I and type II muscle fibers thereby suggesting that both types of muscle fiber were recruited for the completion of the exercise bout[17,45]. Since type II muscle fibers are more anaerobic, it follows logic that their recruitment may be necessary to observe the contribution of the HEPT system. Using an exercise protocol that had a slightly higher intensity coupled with

exercising to volitional failure are likely the factors that lead to us observing an effect of LL-HR RE on HEPT metabolites; whereas this was not found in the previous trials[46–48].

5.2.2. Reliance on muscle glycogen during LL-HR resistance exercise

Muscle glycogen also contributed substantially during the LL-HR resistance exercise bout. Studies investigating muscle glycogen utilization during MICE[23,26,49,54,55,57,85] and heavy RE[42,43,86] have found that glycogen is significantly diminished after the exercise bout. Interestingly, MICE studies have found that the extent of glycogen depletion appears to reach a plateau at approximately 30-50% of its initial concentration[23,26,49,54,55,57,85]. This is likely a function of substrate utilization changing from myocellular stores of substrate (skeletal muscle glycogen) to peripheral sources of substrate (liver glycogen) as the muscle sources are significantly lowered[29,59]. In fact, our study found that during our ~30-minute resistance exercise bout, skeletal muscle glycogen stores were decreased by an average of 54%. Therefore, although studies using MICE protocols were longer (~90 minutes) than the exercise bout employed in the current study, muscle glycogen stores were similarly depleted. The similar utilization of muscle glycogen in our 30-minute protocol as compared to 90 minutes of MICE suggests that this mode of exercise is more glycolytic than MICE.

With respect to glycogen utilization during heavy RE, studies have found that glycogen utilization ranges from 26-33% of initial glycogen stores[42,43,86]. Interestingly heavy RE appears to have a lesser reliance on muscle glycogen than LL-HR as in our study we found that participants used ~54% of initial muscle glycogen content. It is likely that the reason for the lesser reliance on muscle glycogen during heavy RE pertains to differences in the amount of rest between sets employed in our study as compared with the

aforementioned heavy RE studies. The heavy RE protocols employed in these trials had participants lift ~75% of their 1RM for 8-12 repetitions with 60-90 seconds of rest between bouts for 30 minutes[42,43,86]. It is likely that participants reached volitional failure during these exercises, but rest times were significantly longer than what was employed in our trial, thus it is likely that more PCr replenishment occurred between sets and that HEPT was relied on to a greater extent to produce ATP. Moreover, total body exercise volume was greater during the LL-HR bout performed in the current trial compared with the heavy RE trials. Therefore, due to the continuous nature and greater exercise volume, LL-HR appears to induce greater muscle glycogen utilization than heavy RE. Although, it would be interesting for further studies to compare glycogen utilization per set between LL-HR and heavy RE.

The LL-HR resistance exercise bout is aerobic in nature as evidenced by the finding that the content of phosphorylated PDHe1α was significantly lower after the exercise bout. To the best of my knowledge, the phosphorylation status of PDHe1α has not been investigated during heavy RT therefore we are unable to draw comparisons between LL-HR and heavy RE in this regard. When dephosphorylated, PDHe1α removes its inhibition on the PDH thereby allowing for more pyruvate to flux to acetyl-CoA where it can undergo complete oxidation. Therefore, since there was an increase in the content of dephosphorylated PDHe1α, more pyruvate could flux towards aerobic metabolism. These findings further support the aerobic nature of LL-HR. However, since lactate concentration tended to increase in both muscle and blood, it is apparent that this mode of exercise was not completely aerobic. Interestingly, heavy RE results in an on average 7-fold increase in

muscle lactate concentrations[42,43,86], while MICE results in an on average 1.5-fold increase in muscle lactate[23,26,49,54,55,57,85]. Our findings using LL-HR RE resulted in an average 1.2-fold increase in muscle lactate, therefore LL-HR may be as aerobic an exercise mode as MICE. Given these findings it is apparent that future trials need to examine aerobic metabolism during LL-HR to test this hypothesis.

5.3 Sex Based Differences in Metabolism

5.3.1 High Energy Phosphate Transfer System

With respect to the reliance on the HEPT system, we found that men had a greater increase in Cr during exercise than women, suggesting that they utilized more PCr than women. This is in agreement with two other studies that investigated sex-based differences in anaerobic energy systems[36,56] and found that men are more reliant on PCr to meet the demands of the exercise than women. However, our findings are in contrast with two studies involving sprint exercise that found no sex differences in the extent of PCr utilization during exercise[56,57]. Taken together the findings of these studies suggest that exercise mode may influence whether sex affects HEPT metabolism as both studies that involved resistance exercise protocols found a sex difference; whereas the two studies that involved sprint cycling did not.

Interestingly, while phosphorylated CK decreased in both men and women during exercise, it decreased to a greater extent in women. When dephosphorylated, CK prefers to use PCr to produce Cr and ATP[87], thus the finding that phosphorylated CK decreased to a greater extent in women suggests that women have a greater capacity to utilize PCr to produce ATP during exercise than men. This finding is in direct contrast with the finding that Cr increased to a greater extent in men than women. However, the attenuated decline in

phosphorylated CK in men may be related to their greater reliance on the HEPT system during LL-HR, as the increase in dephosphorylated CK would permit increased ATP production, while the preservation of phosphorylated CK would allow for more rapid synthesis of PCr during rest periods. A single study found no difference in the rate of PCr synthesis between bouts of sprint cycling[56], however, in this study there were no differences in fibre type distribution between men and women. This is important to consider since in our study women had a greater % of type I muscle fibres than men and P-CK was negatively associated with type I muscle fibre content. Thus, while no difference in PCr synthesis was observed in the previously conducted trial, this may be due to the fact that muscle fibre type did not differ between the sexes. Overall, the greater increase in Cr concentration following LL-HR suggests that men are more reliant on the HEPT system to produce ATP than women. Further work examining sex differences in the kinetics of these reactions and the rate of replenishment of PCr is needed.

5.3.2 Glycogen Utilization

Men had greater muscle glycogen content than women, a finding that is in agreement with previous literature[27,71,72]. Accompanying this finding, there was a strong tendency for men to utilize more glycogen than women during the exercise bout. This is in agreement with findings from sprint interval[56] and running[34] trials which found that men utilize more glycogen than women[57]. The tendency for men to rely to a greater extent on muscle glycogen during exercise in the current trial is not surprising given that men had a greater muscle glycogen content at rest. During exercise at a given intensity, the extent of muscle glycogen depletion is a function of initial glycogen storage. The literature is clear that men store more glycogen than women[27,71,72]. Therefore, sex differences in glycogen utilization could be

explained by men simply having higher muscle glycogen stores than women and thus using more of it during exercise.

Another potential reason that men may rely to a greater extent on muscle glycogen during exercise is due to them having a greater proportion of type II muscle fibres, which are more glycolytic in nature. However, correlational analyses in the current study did not find a relationship between muscle glycogen utilization and muscle fibre type. Furthermore, previous work has shown that muscle glycogen utilization in type I fibres is greater in men than women during sprint exercise[56], suggesting that it is inherent differences in the aerobic capacity of skeletal muscle that is contributing to sex differences in muscle glycogen utilization.

While not directly measured, of the muscle glycogen that was utilized, it is likely that more of it was directed towards aerobic metabolism in women as compared with men. While phosphorylation of PDHe1α decreased similarly in men and women, indicating removal of the inhibition on PDH and thus a greater capacity to synthesize acetyl-CoA from pyruvate, the finding that muscle lactate was lower in women as compared with men suggests that women were better able to flux the pyruvate produced during glycolysis towards aerobic metabolism. The mechanism for women being able to flux more substrate towards aerobic metabolism is likely 2-fold. Pre-menopausal women possess a greater endothelial cell function than men[88], meaning that oxygenated blood flow can reach the working muscle faster[89]. Indeed, occluding tension is higher in women than men and thus blood flow to muscle is maintained to a greater extent in women than men, which is at least in part responsible for the increased fatigue-resistance in women[90–93]. Maintenance of blood flow during resistance exercise it thought to be, at least in part, responsible for the greater reliance

on aerobic metabolism by women[94]. Additionally, women have a greater amount of type I muscle fibres for a given level of fitness and therefore have a greater inherent aerobic capacity of skeletal muscle. Thus, at a given exercise intensity women have greater blood flow to the muscle, thus greater oxygen delivery, as well as, a greater capacity to utilize the delivered oxygen due to the greater proportion of type I muscle fibres.

5.3.3 ATP Preservation

Interestingly, women had a tendency to lower their ATP stores to a greater extent than men. This could suggest that since men were able to utilize anaerobic energy stores more efficiently than women that they were able to prevent a disturbance in the metabolic concentrations of ATP since the HEPT and glycolytic systems have a greater power to produce ATP than aerobic metabolism[63]. The energy disturbance caused by the decrease of ATP in women would then serve to upregulate aerobic metabolism by removing inhibition on PFK, pyruvate dehydrogenase, isocitrate dehydrogenase, and α ketoglutarate dehydrogenase[37,39]. Thus, the greater decline in ATP during exercise in women may be one of the mechanisms by which women are better able to initiate aerobic metabolism at the onset of exercise.

According to the data, men were able to produce more ATP by means of HEPT system as well as from glycogen than women. This finding uncovers the possibility that women were able to produce their ATP from a different substrate, and the potential for an increased reliance on fat oxidation could be the missing link in explaining where women receive additional ATP compared to men. As women rely to a greater extent on lipid during aerobic exercise[27,51,72] it would not be surprising to find that they also rely to a greater extent on lipid during resistance exercise. Importantly, during heavy resistance exercise IMCL

breakdown occurs[43] and fatty acid oxidation is upregulated[95]. Furthermore, comparable 24 h nutrient oxidation has been reported following acute bouts of heavy RE and aerobic exercise[96]. Future investigations should determine how fat metabolism differs between men and women during LL-HR RE in order to determine whether there is a greater reliance on IMCL and/or plasma fatty acids by women during this mode of exercise.

5.3.4 Differences in Fiber Type Distribution

Our findings in relation to muscle fibre distribution are consistent with the findings in the literature[97,98]. Women had a higher proportion of type I fibres while men had a higher proportion of type II fibres. These differences in fibre type distribution could explain the sex differences in substrate oxidation that were observed in this study. However, correlational analyses suggest that sex differences in muscle fibre type are only partially responsible for the observed sex differences in metabolism. For example, the negative relationship between type I muscle fibre content and lactate can explain the finding that lactate was higher in men than women and is supportive of men having a greater reliance on the anaerobic metabolism by fluxing pyruvate to lactate. Furthermore, P-CK was also negatively related with type I fibre content, suggesting that the observed differences in HEPT metabolism are related to differences in muscle fibre type distribution. Interestingly, muscle glycogen utilization was not related to fibre type composition. While the implications of this finding are discussed more fulsomely above, it is important to note that since muscle lactate accumulation was associated with muscle fibre type distribution, but muscle glycogen was not, it appears as though sex differences in muscle glycogenolysis and glycolysis are independent of muscle fibre type distribution, but that the metabolic fate of pyruvate is dependent on muscle fibre type distribution.

Since men have a higher amount of type II fibres, it would carry that men also have a higher power for anaerobic systems to meet the demands of working muscle. Indeed, men had a greater increase in Cr content following the exercise bout, suggestive of a greater decrease in PCr stores. Furthermore, while glycogen utilization only tended to be higher in men, muscle lactate was higher in men than women, suggesting that the glycogen that was utilized by men tended to flux towards the production of lactate while women tended to flux towards the synthesis of acetyl-CoA. This is in line with women having more type I fibres, which are more oxidative, and previous studies showing that women have shown a greater capacity for oxidative metabolism than men[23,26,80].

5.3.5 Sex Differences in the Physiological Response to LL-HR Resistance Exercise

Men and women had a similar response in both heart rate and rating of perceived exertion during the LL-HR resistance exercise bout, suggesting that the bout was both physiologically and perceptually of similar intensity. Interestingly, despite a similar heart rate response to the exercise bout, women were working at a higher percentage of their heart rate max and heart rate reserve compared to men during the second and third round of the circuit. A linear relationship between VO_2 and heart rate has been established[99], meaning that working at a higher percentage of one's heart rate max would correspond to working at a higher percentage of one's VO_{2max}. Indeed, this would suggest that women were working at a higher relative VO_2 than men in the current study, which may be another reason that we did not observe a sex difference in muscle glycogen utilization as glycogen utilization increases with increasing exercise intensity. However this assumption has only been shown to be true during steady-state aerobic exercises, and the assumptions may not be able to be carried over

to resistance exercise[77]. For this reason, it is difficult to offer a meaningful interpretation of these findings as they relate to the relative oxygen consumption of the participants.

Men and women have been shown to deliver blood differently to skeletal muscle during resistance exercise. Men have a greater amount of hemoglobin than women[100], therefore they are able to deliver the same amount of oxygen to the muscle with a lesser increase in blood flow. Conversely, since women have less hemoglobin[100] they must increase blood flow to a greater extent than men as a means of providing the muscle with sufficient oxygen during exercise. A mechanism that allows for this to occur is women's ability to dilate their smooth muscle[101,102], thus allowing more blood to be supplied. However, the decrease in the peripheral resistance of the arterioles would cause a hypotensive state in women[77] therefore causing a cascade of negative performance outcomes. As a compensatory mechanism to prevent this hypotension, women increase their heart rate to a greater extent[77]. Therefore, although women were working at a higher percentage of heart rate max/reserve, there is not necessarily a difference in the $\%VO_{2max}$ that they were working at during this mode of exercise. Future work should measure oxygen consumption during the LL-HR resistance exercise bout in order to compare the aerobic intensity of LL-HR resistance exercise between men and women. Furthermore, examining how blood flow is altered during LL-HR resistance exercise in both men and women should also be examined. Given the information available, the physiological response to LL-HR seems to be similar between men and women although direct measure of oxygen uptake would be needed to solidify the aerobic intensity at which men and women were working.

During the exercise bout, we observed that women were able to complete a higher number of repetitions during the last set of each exercise, which went to volitional failure.

This is likely due to the fact that women had a higher amount of type I muscle fibres as compared with men, that would therefore enable them to initiate and rely on aerobic energy systems to a greater extent and thus fatigue less rapidly, particularly during resistance exercise that involves lifting a lighter load. Indeed, correlational analysis demonstrated that repetitions to failure tended to be related to the abundance of type I muscle fibers. Furthermore, while not significant, muscle lactate concentration tended to increase in men, but not women, during the LL-HR bout, suggesting that men were relying to a greater extent on anaerobic glycolysis than women. The literature has consistently shown that women are more fatigue resistant during dynamic exercise[64–69], which is true for both upper and lower body muscle groups. Collectively, the studies suggest that it is the larger accumulation of metabolic products in men that interferes with contractile processes and induces clear sex-based differences in the fatigue. Our findings are in agreement with the literature as the men in our study had a greater concentration of creatine, ATP, inorganic phosphate, and a trend for a greater concentration of lactate than women, which lead to a greater fatigability of the muscle in men. This was manifested as men completing fewer repetitions than women during the final exercise set to failure. However, even though women completed more repetitions during the final set, the total volume of work throughout the exercise bout per unit of fat free mass was the same between men and women. This is likely a consequence of men having significantly higher 1RM/FFM than women for upper body exercises, therefore the total volume of the exercise balances out with men lifting more weight for less reps and women lifting lower weight for more reps. Although, this is only true for the upper body exercises. Therefore, the exercise volume is the same between men and women but the means of achieving that volume is marginally different.

5.3.6 Participant Matching

Importantly the sex differences in metabolism observed in this study were found when men and women were appropriately matched for training status[80]. The training status of an individual greatly influences their pattern of substrate utilization during exercise[54,60,103], with greater rates of fat oxidation occurring in the trained state. Since women have a greater amount of fat mass than men it is not appropriate to match men and women based on VO_{2peak} relative to total body weight. Instead men and women should be matched for training status based on VO_{2peak} relative to fat-free mass, the metabolically active tissue. Previous work has shown that when men and women are matched in this manner that training histories align and the lactate threshold occurs at the same % of VO_{2peak}[80]. Furthermore, matching men and women for VO_{2peak}/FFM has been utilized and verified in numerous trials[26,34,51,103–105]. As men and women were matched in this manner in the current trial, we are confident that any differences seen between men and women are the result of sex-based differences and not differences in training status.

Baseline strength measurements showed that men had a higher 1RM for all exercises utilized in the LL-HR bout, which is to be expected. However, when normalized to FFM, the strength differences were eliminated for the lower body exercises but maintained for the upper body exercises. Again this is to be expected as population-based data has shown that for a given level of resistance training strength relative to mass is similar between the sexes for lower body exercises, but greater in men for upper body exercises[106]. From a resistance training perspective, the pattern of upper and lower body strength between the men and women in this study suggests that our participants were equally resistance trained, which is

an important consideration as the influence of resistance training on muscle metabolism has not been extensively investigated.

In addition to accounting for training status of the participants, women were tested during the mid-follicular phase (FP) of their menstrual cycle when circulating estrogen levels are at their lowest and therefore the sex differences we observed likely represent a minimum difference in fuel utilization between the sexes[27]. Estrogen fluctuations across the menstrual cycle are known to influence fuel utilization during exercise[26,32,107]. Therefore, differences in metabolism between men and women may be greater in the luteal phase (LP) of the menstrual cycle when women have higher levels of estrogen[26,27]. Indeed previous research has shown that women in the LP have a decreased reliance on carbohydrate stores than both women in the FP and men[26,27]. However, irrespective of phase, women have a lower reliance on carbohydrate stores during exercise than men[26].

Nutritional analyses revealed no differences in energy or macronutrient intake between men and women, which is important since macronutrient intake can influence the fuel utilization pattern during exercise[108,109]. While, there was a strong trend for men to have ingested more protein and energy as compared with women, this would be expected given their higher body weight. However, when protein intake was normalized to body weight the trend was eliminated. In addition, macronutrient intake normalized to total energy intake was identical between men and women. Therefore, we can confidentially conclude that since macronutrient profiles were the same between men and women, there was negligible influence of habitual dietary intake on the findings of the study.

5.4 Limitations

The main limitation of this study was the inability to determine the activity of relevant enzymes in both the HEPT and glycolytic systems. The literature has demonstrated that men have increased activity and content of glycogen phosphorylase[39] as well as phosphofructokinase[35]. These findings, as they relate to the protein content of the enzymes were not found in our study. While enzyme activity has been found to be greater in men than women[110], other studies have found no difference in the expression or content of these species[107,111]. Unfortunately, due to tissue constraints we were unable to determine whether sex differences in the activity of these enzymes existed during LL-HR resistance exercise. Further work should be conducted to examine this potential difference as it could be that, despite there not being a difference in protein content, there is a difference in the metabolic activity of these enzymes between the sexes. Lower activities of glycolytic enzymes suggest that women have a lower capacity to breakdown carbohydrate within the muscle. However, women do produce ATP through glycolysis during high intensity exercise without compromise suggesting that the decreased glycolytic enzyme activity in women is not a performance detriment, but that women are simply less dependent on carbohydrate sources to meet energy demands during moderate intensity exercise.

We were also unable to measure oxygen consumption during the exercise bout and thus were unable to determine whether men and women were working at the same percentage of VO_{2peak}. By design our trial wanted to examine fuel utilization during a bout of LL-HR resistance exercise at 30% of 1RM, thus it was not necessarily expected that this load would correspond to the same $\%VO_{2peak}$. However, it would have been interesting to utilize metabolic measures in order to determine oxygen consumption and whether 30% 1RM

equates to a similar %VO_{2peak} in men and women. Furthermore, using metabolic measurements would have also allowed us to determine whole body fuel utilization to determine the contribution of carbohydrates and fats during the LL-HR bout, as well as determine whether the fuel utilization pattern differed between men and women. While systems that allow for portable measurements of VO_2 do exist, there are discrepancies in their reportings[112,113] and therefore could cause erroneous findings within our study. New, more valid, technology[114] is becoming available and could serve as a beneficial addition to future studies within this area.

Finally, our study would have benefitted from recruiting a larger sample size. Power calculations revealed that we had 50% power to detect a difference in muscle glycogen utilization between men and women and indicated that we would have needed n=17 participants per group to detect a significant difference. Importantly, while our findings related to sex differences in muscle glycogen utilization were not statistically significant, they may be physiologically significant as we found men to utilized 41% more muscle glycogen than women. A larger sample would have served to better validate these findings.

5.5 Future Directions

Additional areas of investigation are needed to completely uncover the mechanisms that underpin sex-based differences in metabolism during LL-HR resistance exercise. Future studies would benefit from employing a training model, so that changes over a longer period of time can be tracked and adaptations that may be induced during this type of training would have sufficient time to manifest themselves. Furthermore, investigating aerobic sources of fuel utilization as well as anaerobic would serve to achieve a more complete idea of the fuel utilization patterns during LL-HR exercise, as fat seems to play an important role[43].

Moreover, given the more aerobic nature of LL-HR, as compared with heavy resistance exercise, studying the cardiorespiratory effects of LL-HR training in comparison to aerobic training could provide additional evidence of LL-HR's ability to elicit synergistic effects to both resistance and aerobic training. Additionally, comparing men to women in both the luteal phase and follicular phase could serve to identify the influence of estrogen on metabolism during LL-HR. Finally, investigation into the activity of the enzymes involved would identify the metabolic processes that underpin these differences. Although there is still a paucity of data in the literature surrounding LL-HR, specifically surrounding substrate utilization, we believe that our findings have helped open up the area for additional research.

5.6 Conclusion

Much of the previous work in the area has shown that sex-based differences do exist, but this work has predominately occurred during MICE[23,26,29,33,80]. Additionally, they have predominantly focused on carbohydrate and fat metabolism during bouts of exercise that are longer in duration[23,26,29,33,80]. Here, we have focused on anaerobic metabolism during a more aerobic form of resistance exercise to characterize whether LL-HR relied heavily on HEPT and anaerobic glycolysis and whether these patterns differentiated between the sexes. Our findings appear to be in agreement with the findings of sex-based differences during MICE in that men relied more heavily on glycogen stores and the HEPT system to meet the energy demands of working muscle. It is difficult to comment on the aerobic utilization of substrates as that was outside the scope of our study, however accounting for the lesser synthesis of ATP from anaerobic sources in women we are able to speculate that women were more readily able to initiate aerobic metabolism. The extent of aerobic carbohydrate and fat utilization remains to be investigated.

In conclusion, both the HEPT and glycolytic pathways contributed to energy production during LL-HR resistance exercise. Interestingly, the extent of muscle glycogen breakdown and lactate accumulation during LL-HR resistance exercise suggest that it is more similar metabolically to MICE than heavy RE. There were clear sex-based differences in metabolism during LL-HR resistance exercise with men relying to a greater extent on PCr, as evidenced by a greater increase in Cr, and tending to utilize more muscle glycogen than women. While these differences may be in part due to the greater type I fibre content in women, correlational analyses suggest that inherent differences in metabolism must exist as there was no correlation between muscle fibre type content and the extent of glycogen depletion. Overall, these findings suggest that men rely to a greater extent on anaerobic metabolism during LL-HR RE than women.

Chapter Six: Significance of the research

The main allure of LL-HR is its potential to induce similar adaptations as both aerobic and resistance exercise, without the practicality issues of adding more time to each exercise session. Previous research has shown that despite its lower load, LL-HR RE can acutely induce similar or greater increases in muscle protein synthesis as 'traditional' heavy-load resistance exercise[18], which overtime leads to similar gains in muscle mass and strength[17]. In the current study we wanted to characterize the anaerobic fuel utilization pattern and determine whether it was similar between men and women. Understanding the utilization patterns of this mode of exercise would allow us to characterize the normal physiological response, which can then be compared to the influence that LL-HR resistance exercise/training has when it is employed in other populations. Additionally, since men and women do not respond to interventions identically[23,26,80], a finding which may be due to differences in the metabolic response to exercise; it is important to characterize metabolism during this exercise mode in both sexes. Furthermore, with an enhanced understanding of LL-HR RE, we can begin to employ training trials to examine long term effects and determine whether LL-HR RE can act as an optimal mode of exercise that can induce both RT and aerobic adaptations.

It is important to understand the basic anaerobic fuel utilization patterns during LL-HR resistance exercise because it can allude to the potential adaptations that would occur during LL-HR RE training in a healthy population. Furthermore, by characterizing the fuel utilization pattern in young, healthy individuals we now understand the normal physiological response to this mode of exercise, which can then be compared to that of other populations (i.e. aging, metabolic disorders) to understand how these disturbances influence metabolism

during LL-HR RE. These findings can then be used to improve our understanding of how LL-HR training can improve various health outcomes in different populations. Moreover, this study served to characterize the anaerobic utilization patterns as well as investigate the influence that sex has on these findings. It has been consistently shown that men and women do not respond to exercise interventions in the same way[24,25,51], which may be due to differences in metabolism. Thus, should future trials examine the effects of LL-HR resistance training in men and women and find a differential response, differences in metabolism during LL-HR may be able to explain these differences.

One population in which LL-HR may be especially beneficial is aging adults. Due to its lesser strain on joints[44], LL-HR resistance exercise may be a preferential mode of resistance exercise that older adults can use to slow or potentially reverse sarcopenia[16–18]. Aging is also associated with the development of insulin resistance, type II diabetes and cardiovascular disease[2–4]. Given the more aerobic nature of LL-HR, this mode of exercise may also induce favourable adaptations in insulin sensitivity and cardiorespiratory fitness, thus combating three of the most common aging-associated conditions with one concise mode of exercise. Given that insulin resistance speeds the loss of muscle mass[115], improving insulin sensitivity would also slow/prevent the loss of muscle mass[75,116], therefore LL-HR RE may be particularly beneficial because it can improve muscle mass and insulin sensitivity together. Therefore, this exercise mode could be ideal for populations afflicted with sarcopenia, T2D/CVD, and aging. Essentially, LL-HR resistance training could provide a more streamlined intervention to populations afflicted by multiple health disorders by attempting to correct for multiple underlying issues simultaneously.

In order to fully understand the potential applications of LL-HR resistance exercise, further investigations must be done. Particularly, employing a training study over a much longer course of time would allow for determination of the effects of LL-HR on insulin sensitivity, cardiorespiratory fitness and other metabolic markers of disease and disease risk (i.e. CRP, cholesterol, etc.). Investigating the aerobic substrate oxidation pattern, as well as the influence of LL-HR on the cardiovascular system would help substantiate the claims of LL-HR truly being able to elicit the adaptations that are typically seen with aerobic exercise. Ultimately, LL-HR may be able to induce the greatest health benefit for numerous populations in a time-efficient manner.

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