Does sex or exercise modality influence post-exercise glucose handling or insulin sensitivity?

by

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Author's Declaration

This thesis consists of material all of which I authored or co-authored; see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Statement of Contributions

Thesis Chapter 4: TBC1D1 Ser^{237} phosphorylation, but not insulin sensitivity, is higher following a bout of high-intensity interval exercise in healthy males as compared with females

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Thesis Chapter 5: Acute high intensity interval exercise increases plasma markers of inflammation with no sex-based differences in recreationally active males and females

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Thesis Chapter 6: Low-load, high-repetition resistance exercise improves post-exercise glucose handling to a greater extent than moderate-intensity continuous exercise in young, recreationally active, males and females

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Abstract

Exercise has long been recognized for its beneficial effects on glucose handling in both insulin sensitive and insulin resistant populations. However, there is conflicting evidence in regards to the best type and duration of exercise to elicit the greatest benefit on glucose metabolism. Additionally, biological sex is known to influence the metabolic response to both acute and chronic bouts of exercise. Therefore, the purpose of this thesis was to examine the acute effects of three different modes of exercise on post-exercise glucose handling in young, healthy males and females.

In Study 1, twenty-four recreationally active males and females (n=12/sex) completed an acute bout of high intensity interval exercise (HIIE, 10x1min at 90% HR_{max}), to evaluate whether sex influenced the physiological effects of HIIE on post-exercise glycemic control during an oral glucose tolerance test (OGTT). We also examined whether sex differences in post-exercise glycemic control were related to sex differences in muscle metabolism and/or insulin signaling proteins. HIIE increased post-exercise insulin sensitivity in both sexes characterized by the Matsuda and homeostatic model assessment for insulin resistance (HOMA-IR) indices. We also found that HIIE lowered insulin concentration during the OGTT compared to the control OGTT. When normalized for glucose dose relative to lean body mass, glucose area under the curve (AUC) was lower in females than males. While we did not find any difference in total insulin signaling protein content, muscle glycogen utilization or AMPK activation during exercise between the sexes, we found that TBC1D1 Ser^{237} phosphorylation increased in males, but not females, post-exercise. Overall, these findings indicate that when the glucose dose is normalized for differences in body composition glycemic handling is better

in females and that an acute bout of HIIE improves insulin sensitivity equally in healthy males and females.

In study 2, we sought to examine the effect of sex on markers of oxidative stress and inflammation following an acute bout of HIIE and during an OGTT. Twenty-four (12/sex) males and females matched for aerobic fitness ($\dot{V}O_{2peak}$ relative to lean body mass) had muscle biopsies taken at rest and following an acute bout of high-intensity interval exercise (HIIE, 10x1min at 90% HR_{max}) and blood taken at rest, post exercise, 90 minutes post exercise (immediately prior to an OGTT) and at 60 min during the OGTT. Muscle biopsies were analyzed for markers of oxidative stress/oxidant generation (NOX2, 4HNE, p38 MAPK) and antioxidant status (TRX1, glutathione reductase, GPX1) and blood samples were analyzed for inflammatory markers (IL-6 and TNFα). Following exercise there was an increase in plasma concentrations of IL-6 and TNFα, with IL-6 continuing to increase during the OGTT, with no differences between the sexes at rest or following exercise. We did not see any effect of sex on antioxidant or oxidative stress status; however, we did find that females had higher p38 MAPK phosphorylation at rest compared to males. Overall, these findings suggest that antioxidant status, ROS production and inflammation do not differ between males and females at rest, following an acute bout of HIIE or during a post-exercise glucose challenge.

In study 3, twenty-four (n=12/sex) participants completed acute bouts of MIC exercise (30mins at 65% $\dot{V}O_{2peak}$) and LLHR (3 circuits, 6 exercises/circuit, 25-35 repetitions/exercise/ circuit) to compare the acute effects of each of these exercise bouts on post-exercise glycemic control and insulin sensitivity. Blood glucose concentrations were lower during a post-exercise OGTT following LLHR RE compared to MIC exercise in males and females. Glucose AUC was also

lower in both sexes. In males and females phosphorylated ACC Ser^{79} increased following MIC exercise only, with no changes following LLHR RE. These findings suggest that LLHR RE is a feasible exercise modality to improve post-exercise glycemic control in males and females.

In conclusion we found that LLHR RE decreased blood glucose concentrations to a greater extent than MIC exercise. Additionally, we found that acute HIIE was able to improve post-exercise IS in both sexes. While absolutely glucose concentrations did not differ between males and females, we found that once blood glucose concentrations were normalized to body weight and lean body mass, females had lower glucose concentrations compared to males. The collective results from this thesis indicate that there are some slight differences between the sexes in the metabolic response to acute exercise that may influence pathways responsible for glucose uptake. Overall, the work done in this thesis provides necessary proof of concept for future sex comparative research done in the area of exercise and glucose handling in individuals with insulin resistance.

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List of Abbreviations

4HNE	4-Hydroxynonenal
ADP	Adenosine diphosphate
AE	Aerobic exercise
AICAR	5-aminoimidazole-4-carboxamide ribonucleoside
AMP	Adenosine monophosphate
AMPK	Adenoisine-5'-monophosphate protein kinase
ATGL	Adipose triglyceride lipase
ATP	Adenosine triphosphate
BW	Body Weight
Ca^{2+}	Calcium
CAMK	Ca^{2+}/calmodulin (CaM)-dependent protein kinase
DAG	Diacylglycerol
DPI	Diphenyleneiodonium
E2	17β-estradiol
FA	Fatty Acid
FABP	Fatty Acid Binding Protein
FAT/CD36	Fatty acid translocase/CD36
FFA	Free fatty acid
FP	Follicular phase
GDP	Guanosine diphosphate
GLUT4	Glucose transporter type 4
GPAT	Glycerol-3-phosphate acyltransferase
GPX1	Glutathione peroxidase 1
GR	Glutathione reductase
GS	Glycogen Synthase
GSIS	Glucose stimulated insulin secretion
GTP	Gaunosine-5'triphosphate

HbA1c	Glycosylated hemoglobin
HEPT	High-energy phosphate transfer
HIIE	High intensity interval exercise
HOMA	Homeostatic model assessment
HSL	Hormone sensitive lipase
IL-6	Interleukin-6
IMCL	Intramyocellular lipids
IR	Insulin resistance
IRS-1	Insulin receptor substrate 1
IS	Insulin sensitivity
ITT	Insulin tolerance test
JNK	c-Jun N-terminal kinase
Kg	Kilogram
LBM	Lean Body Mass
LLHR	Low-load, high-repetition resistance exercise
LP	Luteal phase
MAPK	Mitogen-activated protein kinase
MIC	Moderate intensity continuous exercise
mRNA	Messenger ribonucleic acid
NOS	Nitric oxygen species
NOX	NADPH oxidase
OGTT	Oral glucose tolerance test
PI3K	Phosphoinositide 3-kinases
PKB	Protein kinase B
PKC	Protein kinase C
PLIN2	Perilipin 2
R_a	Glucose rate of appearance
Rac1	Ras-related C3 botulinum toxin substrate
R_d	Glucose rate of disappearance

RE	Resistance exercise
RER	Respiratory exchange ratio
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
Ser	Serine phosphorylation
SIE	Sprint interval exercise
SOCS-3	cytokine signaling 3
SREBP-1c	Sterol regulatory element-binding protein 1c
T2D	Type 2 diabetes
Thr	Threonine phosphorylation
TNF	TNFα
TRX1	Thioredoxin 1
Tyr	Tyrosine phosphorylation
XO	Xanthine oxidase
ZMP	Ribofuranosyl imidazole-4-carbocaide monophosphate

Chapter 1 Introduction

1.1 Rationale

The prevalence of pre-diabetes (defined as fasting blood glucose concentrations 5.6 – 6.9 mmol/L) and type II diabetes (T2D; defined as fasting blood glucose ≥ 7.0mmol/L) has risen dramatically in recent years affecting approximately 31% of Canadians (1, 2). Furthermore, the prevalence of diabetes is estimated to increase nearly 50% in Canada from 2015 until 2025 (3). T2D is characterized by elevated blood glucose concentrations caused by an impairment in glucose tolerance from the development of insulin resistance (IR) and relative insulin deficiency (4). IR or impaired insulin sensitivity (IS) reduces the ability of body tissues, including muscle, to take up and store both glucose and triglycerides (4). Furthermore, insulin resistance also attenuates the suppression in muscle protein breakdown following a meal (5). Elevated blood glucose concentrations resulting from insulin resistance increase the risk for developing cardiovascular disease, hypertension, and microvascular disorders such as retinopathy, nephropathy, and neuropathy, which further worsen health and quality of life (6-8).

Exercise has long been recognized for its beneficial effects on glucose handling in a variety of populations such that a single bout of exercise can markedly increase post-exercise glucose handling up to 20-fold for 2-72 hours, depending on exercise type, intensity and duration (9-14). However, when looking at the effects of exercise on post-exercise glucose handling there is conflicting evidence in regards to the best type and duration of exercise to elicit the most beneficial impact on blood glucose concentrations (15). There is some evidence that exercise elicits a dose-response effect such that higher energy expenditures and exercise

intensities will elicit a greater response on glucose handling and IS, whereas others state that a combined effect of different exercise modalities (aerobic and resistance exercise) is the best approach (15). While recent studies have compared different modes of exercise to elucidate the best exercise prescription for glucose control, few studies have compared the effects of different acute bouts of exercise on glucose control to examine the molecular mechanisms that underpin these effects. Understanding the underlying mechanisms by which exercise modalities influence glucose control is critical in determining the most effective lifestyle strategies to prevent and manage IR and T2D.

Biological sex is known to influence the metabolic response to acute and chronic bouts of exercise (16-18). It has been widely reported that females tend to oxidize more fat and less carbohydrates compared to males during a bout of acute exercise (19-22), which may influence the insulin sensitizing effects of exercise. This has been seen particularly with interval training, which has been shown to be more efficacious in males compared to females (16-18). While we know that these inherent sex differences in metabolism exist, what is currently unknown is how these differences are related to post-exercise IS and glucose control in healthy individuals. Determining how sex influences the metabolic response to exercise in the absence in pathology is a critical first step for providing effective exercise recommendations to prevent and/or manage IR and T2D in both sexes.

1.2 Statement of the problem

Exercise attenuates the development and progression of pre-diabetes and T2D; however, its effectiveness may differ between the sexes, which may be related to sex-based

differences in metabolism. There is a need for an increased understanding of how exercise influences post-exercise insulin sensitivity and glucose control in relation to sex-based differences in metabolism in the absence of pathology ahead of examining how it is affected by pathology. Furthermore, muscle metabolism differs during different modes of exercise, thus any sex-based differences in post-exercise insulin sensitivity and glucose control may differ based on exercise modality. Therefore, the purpose of the research conducted in this thesis was to examine the acute effects of three different modes of exercise on post-exercise glucose handling in young, healthy males and females.

Chapter 2: Review of the literature

2.1 Regulation of blood glucose concentration

2.1.1 Functions of insulin and metabolic consequences of insulin resistance

Understanding the role of insulin and its wide array of physiological effects has significant implications for our understanding of T2D (23). Insulin is a peptide hormone produced by the β-cells of the pancreatic islets of Langerhans and it maintains normal blood glucose concentration by facilitating cellular glucose uptake through binding with its receptor, which initiates the insulin signaling cascade culminating with GLUT4 translocation to the cell membrane and glucose uptake (23, 24). Insulin also regulates carbohydrate, lipid, and protein metabolism by increasing glycogen synthesis, decreasing glycogen breakdown, stimulating fatty acid synthesis in adipose tissue and liver, and suppressing protein breakdown. (23, 25). Furthermore, insulin, via activation of mitogen-activated protein kinase (MAPK), also has mitogenic effects, promoting cell division and growth (23). When an individual has IR, insulin signaling in muscle, liver and other tissues is impaired resulting in impaired insulin-mediated glucose disposal (23), an inability to suppress muscle protein breakdown (26), increased unstimulated lipolysis (27), and an inability to suppress hepatic exogenous glucose release. Ultimately this results in sustained elevated blood glucose levels, increased adipose tissue depots, an enlargement of adipocytes and a subsequent decrease in muscle mass (26, 27).

2.1.2 Regulation of blood glucose concentration by insulin and glucagon

Blood glucose concentrations are tightly regulated by the hormones insulin and glucagon (23). Insulin is secreted from the pancreatic β-cells when blood glucose concentrations increase, promoting blood glucose uptake into body tissues, and thus lowering blood glucose concentrations (23). The process of insulin being secreted from the pancreas is called glucose stimulated insulin secretion (GSIS) (23). Alternatively, glucagon is secreted from the pancreatic α-cells when blood glucose concentrations are low, stimulating hepatic glycogenolysis, gluconeogenesis and glucose release from the liver, and thus increasing blood glucose concentration (23).

Following the consumption of food, blood glucose concentrations rise resulting in GSIS. The circulating blood glucose is taken up into the pancreas by facilitative diffusion via glucose transporter 2 (GLUT2), which is located on the surface of pancreatic β-cells (28). Once glucose is within the cell, it undergoes glycolysis thereby generating ATP and increasing the ATP/ADP ratio (28). This altered ratio will lead to the closing of the ATP-sensitive K^+ channels, depolarizing the membrane followed by an opening of the voltage-dependent Ca^{2+} channels (28). This increase in the intracellular calcium concentration triggers the fusion of insulin-containing granules with the membrane thereby releasing their contents (28). Insulin is secreted in a biphasic manner that includes a rapid “first phase” and a slower more prolonged “second phase”. The initial rapid “first phase” of insulin release involves secretion of preformed insulin and occurs within 1 minute of increased blood glucose concentration, peaks at 3-5 minutes and lasts ~10 minutes overall (23, 29). The slower phase or “second phase” of insulin release begins shortly after the first phase but is not apparent until approximately 10 minutes

after stimulation and involves secretion of newly synthesized insulin. This second phase lasts the entire duration of hyperglycemia and is proportional to basal glucose concentration (23, 29). In insulin sensitive individuals blood glucose concentrations will return to baseline levels ~1-2 hours after consumption of a meal (30); whereas this is delayed in those who are insulin resistant. Furthermore, in insulin sensitive individuals' peak glucose concentration following a meal is lower than that seen in insulin resistant individuals as insulin can act more rapidly to begin clearing glucose from the circulation (30, 31).

2.2 Glucose uptake is facilitated by glucose transporters

Insulin regulates skeletal muscle metabolism by promoting glucose uptake, glycogen synthesis, lipogenesis, and supressing protein breakdown (32). The uptake of glucose from the blood stream is a tightly regulated function that includes the delivery of glucose from the blood to the interstitial space, the transmembrane transport from the interstitial space to the inside of the muscle cell and then ending with the intracellular metabolism of glucose (12). The major mechanism by which exogenous glucose load can be disposed is insulin-stimulated glucose transport into skeletal muscle, which is facilitated by a family of specialized transporter proteins named glucose transporters (GLUT) (33). GLUT4 is one of 23 sugar transporter proteins which includes GLUT1-GLUT12, GLUT14 and HMIT that catalyzes hexose transport across cell membranes through a facilitated diffusion mechanism (34, 35). The expression and location of the GLUT transporters are summarized in Table 2.1 below. The GLUT proteins act as shuttles to move glucose across the cell surface by forming an aqueous pore across the membrane through which glucose can move (36). In some tissues, such as the brain, which have a continuously high

glucose requirement there are transporters that are always at the cell surface. Other tissues, such as skeletal muscle and adipose tissue, have highly sensitive and specialized glucose transport systems that allow activity to be rapidly upregulated (36). This system allows the rate of glucose transport to be increased 10-40 fold within minutes of exposure to a stimulus (i.e during exercise or in the post-prandial period), to facilitate the rapid storage of glucose in muscle and adipose tissue to prevent large fluctuations in blood glucose concentrations (36).

Skeletal muscle blood glucose uptake is facilitated predominately by GLUT1 and GLUT4. GLUT1 is localized almost exclusively to the sarcolemma and is thought to be the predominate transporter responsible for basal/non-insulin stimulated glucose uptake (37-39). Alternatively, GLUT4 is the main transporter responsible for promoting glucose uptake in the insulin-stimulated and exercise states. In contrast to GLUT1, GLUT4 resides both on the cell surface (approximately ~20% of total GLUT4 protein) and in GLUT4 storage vesicles within the cell (~80% of total GLUT4 protein) (40, 41). In response to insulin and exercise stimulation, GLUT4 translocates to the cell membrane and t-tubules of skeletal muscle (and adipose tissue) to allow for glucose uptake into the cell by different, but convergent pathways, which will be discussed in detail below (34). There is evidence that GLUT4 may exist in two separate intracellular pools of GLUT4 that respond to insulin and contraction, respectively. This existence of two pools of GLUT4 could also illustrate the additive effect of both insulin and contraction (42-45). Translocation of GLUT4 from the cytosol of the cell to the cell membrane is a multi-step process that involves intracellular sorting, vesicle transport to the plasma membrane and finally docking, priming and fusion of GLUT4 storage vesicles within the cell membrane (46), as discussed in detail below.

Table 2.1: GLUT transporters locations and expression adapted from Litwack et al, (35) and Evans et al, (30)

Glucose Transporter	Location	Glucose Transporter	Location
GLUT1	Erythrocytes, brain (blood–brain barrier), skeletal muscle	**GLUT8**	Testis, brain (neuronal), adipocytes
GLUT2	Liver, islet cells, kidney, small intestine	**GLUT9**	Liver, kidney
GLUT3	Brain (neuronal), testis, skeletal muscle	**GLUT10**	Liver, pancreas
GLUT4	Adipocytes, skeletal muscle	**GLUT11**	Pancreas, kidney, placenta, muscle
GLUT5	Testis, intestine, muscle	**GLUT12**	Heart, prostate, breast cancer
GLUT6	Brain, spleen, peripheral leukocytes	**GLUT14**	Testis
GLUT7	Intestine, testis, prostate	**HMIT**	Brain

2.3 Insulin and contraction-mediated glucose uptake

2.3.1 Insulin-stimulated glucose uptake

Insulin-signalling begins at the insulin receptor and is summarized in Figure 2.1. The insulin receptor is a glycoprotein composed of two alpha subunits and two beta subunits linked together by disulfide bonds that is embedded within the plasma membrane (47). Insulin binds to the insulin receptor which induces a conformational change of the receptor, resulting in autophosphorylation and activation of receptor tyrosine kinases, which in turn recruits and stimulates members of the insulin receptor substrate (IRS) family of proteins, including IRS-1 (46). The IRS family (IRS-1 to through IRS-6) act as a framework to organize and mediate signaling complexes. IRS-1 is found predominantly in skeletal muscle and IRS-1 knockout

animals have impaired insulin action (48). IRS-2 is found only in selective tissues such as neurons and islet cells, in rodents IRS-3 is found most abundantly in adipocytes, liver and lungs (48). In humans, IRS-3 is a pseudogene, so no protein in produced at all (48). IRS-4 is present in skeletal muscle, liver, heart, brain and kidney and can be phosphorylated by insulin and IGF-1 and promote the same biological actions as insulin (49). Both IRS-3 and IRS-4 appear to play a redundant role in the IRS signaling system (50). IRS-5/IRS-6 have limited tissue expression and are relatively poor insulin receptor substrates (48).

The critical pathways that link IRS proteins to the metabolic actions of insulin in skeletal muscle is the phosphoinositide 3-kinase (PI3K) and Akt pathway (48). IRS-1 binds to the regulatory subunit PI3K via SH2 domains, which leads to the activation of PI3K (46). Activation of PI3K in turn results in the activation of its p110 catalytic subunit, which will rapidly phosphorylate phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate phosphatidylinositol (3,4,5)-triphosphate (PIP_3) (48). PIP_3 then activates the 3-phosphoinositide-dependent protein kinase -1 (PDK1) resulting in activation and recruitment of Akt/protein kinase B (PKB) and atypical protein kinase C (PKC) to the plasma membrane (32, 46, 48). The PKC isoforms are one of the major modulators of insulin effects on glucose and lipid metabolism through association with PDK1(46). The Akt proteins consist of three different isoforms of serine/threonine protein kinases that are encoded by different genes, however it is Akt2 that is the essential isoform to ensure normal glucose homeostasis (46). Once activated, Akt phosphorylates TBC1D4 (AS160) and TBC1D1,which are closely related Rab-GTPase-activating proteins (GAP) (46). In their active forms, TBC1D1 and TBC1D4 promote the hydrolysis of gaunosine-5'triphosphate (GTP) to guanosine diphosphate (GDP) on Rab proteins and restrain GLUT4 translocation (46).

Phosphorylation of TBC1D1 and TBC1D4 inactivates TBC1D1 and TBC1D4 and therefore allows for GLUT4 translocation to the plasma membrane (46, 51).

The translocation of GLUT4 storage vesicles occurs in four main steps: release from internal retention, translocation using cytoskeletal-mediated processes, tethering, and docking to the cell membrane and finally fusion with the plasma membrane (52). Upon fusion glucose can then enter the cell, where it can then be phosphorylated to glucose-6-phosphate by hexokinase II to then be utilized in glycolytic pathways or incorporated into glycogen by glycogen synthase (GS) (46). Using intravital imaging it has been shown that with increasing insulin concentration insulin binds to its receptor at the sarcolemma and initiates PI3K signalling within 1-2 minutes leading to rapid GLUT4 translocation to the sarcolemma (53). However, it takes longer for insulin to diffuse into the t-tubules and thus insulin binding to receptors in the t-tubules is not seen until ~10 minutes after binding at the sarcolemma, where it then activates local PI3K leading to GLUT4 translocation to the t-tubules as well (53). When insulin concentrations decline, GLUT4 gradually re-internalizes in proportion to the declining insulin concentration (53). Similar to what is seen with GLUT4 translocation to the sarcolemma and t-tubules, there is an ~10-minute difference in GLUT4 re-internalization between the sarcolemma and t-tubules upon declining insulin concentrations, again likely due to it taking longer for insulin to diffuse out of the t-tubules (53). Upon complete removal of insulin, GLUT4 will completely re-internalize within 10 minutes (54).

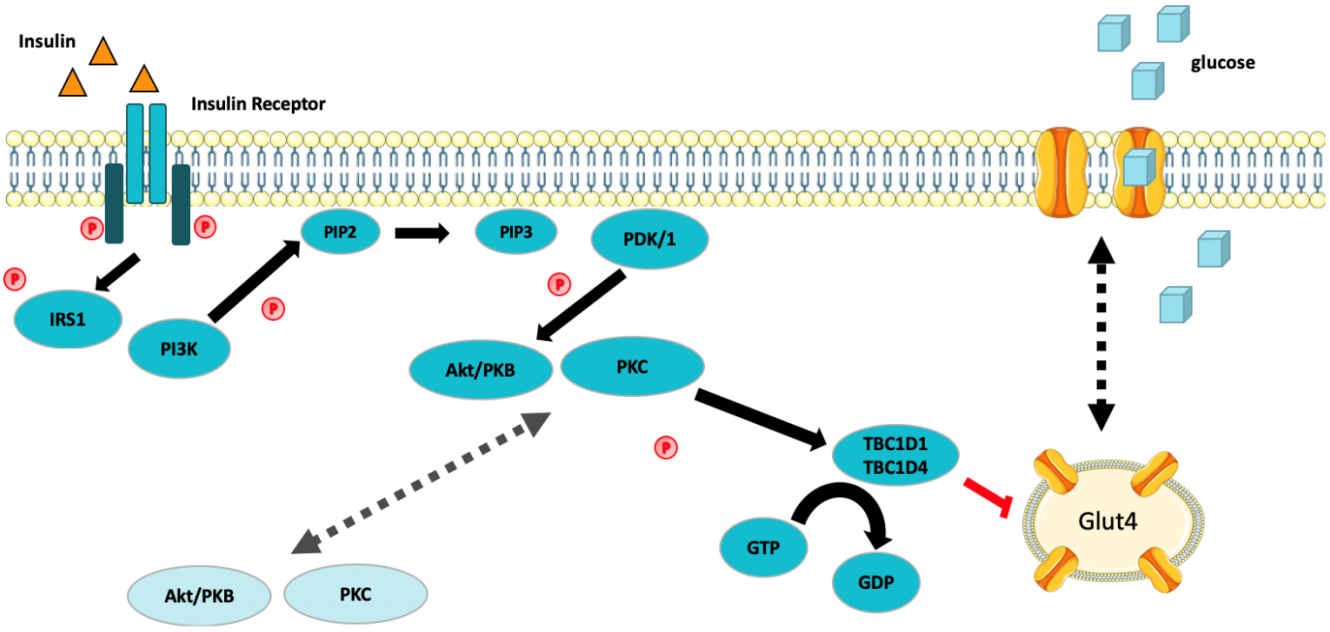

Figure 2.1 Skeletal Muscle Insulin-Dependent Signaling Cascade. Insulin binds to the insulin receptor, which leads to autophosphorylation and activation of receptor tyrosine kinases, which in turn recruits and stimulates IRS-1. IRS-1 then activates PI3K which phosphorylates PIP_2 to generate PIP_3. PIP_3 will then activate PDK1 resulting in activation and recruitment of Akt/PKB and PKC to the plasma membrane. Akt kinase activation leads to the phosphorylation and inactivation of TBC1D1 and TBC1D4 and allows GLUT4 translocation to the plasma membrane.

2.3.2 Contraction-stimulated/insulin-independent glucose uptake

Exercise also induces GLUT4 translocation and muscle glucose uptake via an insulin-independent pathway (33). GLUT4 translocates to the plasma membrane within 15secs-2mins of TBC1D4 phosphorylation and inactivation (55). Upon cessation of exercise GLUT4 re-internalization is completed by ~85 minutes after the cessation of exercise (53). Understanding the mechanisms behind insulin-independent glucose disposal is important because 1) skeletal muscle accounts for ~80% of glucose disposal and 2) muscle IR is a key defect in the progression to T2D (52). While not fully understood it is thought that increased calcium release from the sarcoplasmic reticulum, production of muscle metabolites, and/or increased reactive oxygen

species (ROS) activate various kinases (i.e., CaMK, AMPK), which phosphorylate TBC1D1 and TBC1D4 (46, 51). Phosphorylation of TBC1D1 and TBC1D4 inhibits Rab GTPase activity, promoting GTP binding to Rabs and allowing GLUT4 translocation (46, 51). These mechanisms are activated at the onset muscle contractions as part of a metabolic feedback signal, and generally increase with the increased energy demand and metabolic flux from exercise (12). The insulin-independent pathway is crucial to allow for increased glucose uptake despite having decreased insulin levels during exercise (56). Insulin-independent signaling to promote glucose uptake is rapidly reversed (within minutes to hours) upon the cessation of exercise (57) However, the effects of exercise on IS persists for up to 48-72h (58, 59).

There are several main intracellular mechanisms that have been suggested to explain contraction-dependent/insulin-independent glucose transport. The first mechanism is based on the metabolic strain imposed on skeletal muscle during exercise, which therefore causes changes in high-energy phosphates (33, 60). The second is based on the depolarization of the sarcolemma and T-tubule membranes via calcium-mediated messengers (33, 60). However, because muscle contraction leads to changes in both high-energy phosphates as well as an increase in Ca^{2+}, these effects are additive and work synergistically to increase GLUT4 translocation to the plasma membrane during exercise (61). Additional mechanisms include mitogen-activated protein kinases such as p38 MAPK. Exercise results in an increase in p38 phosphorylation that lasts for hours and in turn increases glucose transport activity 2-3-fold (62). However, the full role of p38 MAPK and its role as regulator of contraction-induced glucose uptake is not fully elucidated. Nitric oxide synthase (NOS) and ROS have also been

previously shown to increase during muscle contraction and play a role in the control of contraction-induced glucose uptake (45, 63).

2.3.2.1 Metabolism-dependent mechanisms

In order to meet the energy demands of exercise muscle metabolic pathways are activated, leading to the production of various metabolites including ADP and AMP (64). The increase in ADP and AMP leads to activation of adenosine-5'-monophosphate protein kinase (AMPK), which regulates metabolic processes and energy homeostasis by attenuating ATP consuming pathways such as fatty acid and cholesterol synthesis and increasing ATP generating processes such as glucose and fatty acid uptake and oxidation (64, 65). Increased AMPK activity during exercise is also thought to mediate GLUT4 translocation during exercise through interaction with TBC1D1 and TBC1D4 (33, 64), suggesting that the insulin-dependent and insulin-independent pathways converge at TBC1D1/TBC1D4. To support this, several studies have used AICAR or 5-aminoimidazole-4-carboxamide ribonucleoside to increase glucose uptake and increase TBC1D4 activation (61). AICAR is taken up by cells and converted to the AMP analog ribofuranosyl imidazole-4-carbocaide monophosphate (ZMP) which can then activate AMPK (33, 61). A study using AMPK KO transgenic mice demonstrated that TBC1D4 phosphorylation is impaired in these mice in the presence of AICAR and contraction, which suggests a role for AMPK in TBC1D4 activation (66). There are several studies that have found increases in AMPK following acute exercise in both humans and mice (67-72). Musi et al (71), demonstrated that a 45-min cycling protocol at 70% of maximum workload in both diabetic and healthy subjects increased AMPK α2 activity 2.7-fold. However, a recent review paper

published by McConell (73) calls into the question the role of AMPK and contraction stimulated glucose uptake. During a 120 min bout of exercise at ~65% $\dot{V}O_{2peak}$ in well trained endurance athletes there was no activation of skeletal muscle AMPK, despite a significant increase in skeletal muscle glucose uptake at this intensity (74). It is important to note that the lack of activation may be due to measuring total AMPK activity rather than activation of separate AMPK subunits. However, similarly, Wojtaszewski et al (75), found an increase in skeletal muscle leg glucose uptake in young healthy males during the first hour of moderate intensity cycling yet there was no significant increase in AMPK $\alpha 2$ and AMPKα Thr^{172} phosphorylation until near the end of the 3.5-hour exercise bout. These studies call into question the notion that AMPK is required to induce GLUT4 translocation and glucose uptake during exercise, however there is likely redundancy repetition in the signalling for contraction stimulated glucose uptake that may explain the differences in AMPK activation during exercise (73). Additionally, there is emerging evidence that supports AMPK activation as being important for glucose uptake post-exercise, but not during exercise (76, 77).

2.3.2.2 CAMKII and calcium-mediated messengers

During exercise, there is also an increase in cytosolic calcium (Ca^{2+}) triggered by sarcolemmal depolarization during muscle contraction that leads to enhanced GLUT4 translocation to the plasma membrane (78). There have been several studies that have shown that there is an increase in glucose transport into the cell when there is an increase in cytoplasmic calcium concentrations independent of insulin or any changes in energy status in animals and humans (79-81). Previous studies have shown that a Ca^{2+} releasing agent, such as

caffeine, releases Ca^{2+} from the sarcoplasmic reticulum into the cytosol and significantly increases glucose uptake into cells (81). However, subsequent trials determined that it is unlikely that it is Ca^{2+} itself that is increasing glucose uptake, rather that the energy demand of Ca^{2+} reuptake by sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) altered cellular energy status and resulted in greater AMPK activity [reviewed by (45)]. However, increased Ca^{2+} may play a role in increased glucose uptake through activation of AMPK by CaMK kinase [CaMKK, (82-84)]. CaMKK is an important mediator of $Ca^{2+,}$ and in the cytoplasm there is extensive crosstalk between cAMP-dependent kinase (PKA), ERk-1/2 and Akt (85). Originally, TBC1D4 was thought to be the convergence point between CaMK and insulin independent signaling, however a study by Witczak et al, (86) demonstrated that an increase of TBC1D4 phosphorylation has not been observed with CaMKK overexpression suggesting that CaMKK triggers metabolic reactions independent of TBC1D4. Additionally, further research has found that CaMKK mediated glucose uptake occurs independent of AMPK activation (86). Unpublished data from Jensen et al [reported in review by Richter and Hargreaves, (45)] indicate that there is no impairment of AMPK Thr^{172} phosphorylation or glucose uptake during electrical stimulation in CaMKK knock out mice. Thus, the role of CaMKK in mediating glucose uptake is unclear. There are two other proteins of interest identified: Ca^{2+}/calmodulin-dependent protein kinase (CaMK), and protein kinase C (PKC) that may be related to Ca^{2+}-mediated enhanced glucose uptake (33).

2.3.2.3 p38 MAP kinase

Mitogen-activated protein (MAP) kinases ERK1, ERK2, p38 and JNK become activated during exercise (87-90). p38 MAPK has been implicated in contraction induced glucose uptake

(91). When p38 MAPK α and β isoforms are inhibited, glucose uptake is subsequently decreased in mice (91). p38 MAPK mediates the stimulation of glucose uptake during exercise in part by elevating the expression levels of GLUT4 and increasing PGC1α activity (92). Conversely, the γ isoform of p38 MAPK overexpression tended to decrease contraction-stimulated glucose uptake. However, these results were also accompanied by an overall decrease in GLUT4 expression which may explain the decrease in glucose uptake. Therefore, the full role of p38 MAPK on glucose uptake at rest and during exercise remains to be elucidated.

2.3.2.4 Rac1

Ras-related C3 botulinum toxin substrate (Rac1), is a Rho family GTPase that also has a role in exercise-stimulated glucose uptake (45). Rac1 is activated by exercise and increases GTP activation in muscle (93). Additionally, inhibition of Rac1 in muscle partly impairs contraction-induced glucose uptake in animals (93). Rac1 regulates various cellular processes, including ROS production (94, 95) and reorganization of the actin cytoskeleton (96-98). Sylow et al, (99) have shown that in Rac1 knockout mice GLUT4 translocation to the plasma membrane was impaired. In muscle cells, Khayat et al, (100) found that Rac1-dependent actin remodeling was necessary for GLUT4 docking to the plasma membrane. Therefore, it seems Rac1 appears to play an important role in GLUT4 translocation in muscle and may be a convergence point for insulin and contraction signalling (99).

2.3.3 Mechanisms of enhanced insulin-dependent glucose uptake following acute exercise.

Acute exercise sessions act on several areas of the insulin-dependent signaling cascade, which results in increased IS for up to 72h post exercise (59). While these mechanisms aren't fully elucidated, acute exercise bouts act on different components of the insulin-dependent signaling cascade, enhancing IS. Acute moderate intensity continuous exercise of 45-60min in duration at 65-75% $\dot{V}O_{2peak}$ leads to higher insulin-stimulated rates of tyrosine phosphorylation of the insulin receptor and IRS1/2 as well as increased activity of PI3K in muscle of untrained healthy and insulin resistant individuals (101-104). AMPK may also influence the insulin-dependent signaling cascade and thus enhance IS; however, there is some discussion as to what portion of the insulin signaling cascade AMPK acts upon (33). In vivo studies have shown that PI3K expression is decreased several minutes after exercise (51, 105), suggesting that any effects of exercise on the insulin-signaling cascade occur beyond PI3K action (33).

In animal models, there is concrete evidence that aerobic exercise increases Akt activation in the fasted and insulin-stimulated state (106-109). Using a treadmill running protocol both an intermediate and high-intensity session significantly increased Akt activity and phosphorylation in rat skeletal muscle (106). This is also confirmed in humans, where a single bout of endurance exercise increased Akt Ser^{473} phosphorylation by 1.8-fold in fasted trained cyclists (110). Interestingly however, while there was a significant change in Akt Ser^{473} phosphorylation, there were no changes in Akt Thr^{308} phosphorylation post-exercise (110). This was confirmed by Creer et al, (111) who demonstrated that Akt phosphorylation was elevated 10min after resistance exercise at 70% of 1RM in cyclists in the insulin stimulated state. In contrast however, it has been reported that after 10 sets of 10 leg extensions at 80% of 1RM in

healthy young males, phosphorylation of Akt/PKB on both Ser^{473} and Thr^{308} decreased immediately post exercise in the fasted state (112). At 24 hours post-exercise however, phosphorylation of Akt/PKB on Ser^{473} had returned to baseline levels, whereas phosphorylation on Thr^{308} was still depressed (112). The authors speculated that the differences in Akt phosphorylation post-exercise could be due to a difference in exercise intensities as well as type of contraction (concentric versus eccentric) (112). Similarly, Deshmukh et al (110), found increases in Akt Ser^{473} phosphorylation after endurance exercise but not after resistance exercise in endurance trained cyclists, suggesting that endurance exercise may be a more potent stimulus to increase Akt phosphorylation compared to resistance exercise.

TBC1D1 and TBC1D4 are also potent stimulators to increase IS after exercise (52, 113). While TBC1D1 and TBC1D4 share a similar structure and both regulate GLUT4 translocation and glucose uptake in response to exercise and insulin, they have diverse phosphorylation sites which are targeted by different kinases (114-116). Furthermore, while TBC1D4 is expressed in several tissues, TBC1D1 is mostly expressed in skeletal muscle, suggesting a specialized role of TBC1D1 in skeletal muscle (117).

Both insulin- and contraction-induced glucose uptake in skeletal muscle have been shown to depend, at least partially, on TBC1D4 (118). A study by Kramer et al, (118) suggested that TBC1D4 phosphorylation mediates around 50% of glucose uptake in response to insulin in mouse muscle. Contraction also results in phosphorylation of TBC1D4 on multiple sites in a temporal manner in both human and animal skeletal muscle (113). Endurance exercise increases TBC1D4 Thr^{642} phosphorylation, however this appears to be a delayed effect as it appears to only be increased after 40-60min of cycling at 67-70% $\dot{V}O_{2peak}$ (119, 120).

Furthermore, increased TBC1D4 phosphorylation is seen immediately post-endurance exercise and it remains elevated for 3-4 hours after exercise, at least on some phosphorylation sites (120-122). Increased TBC1D4 phosphorylation is also found in humans after a bout of resistance exercise; however, with a different temporal pattern than what is seen with endurance exercise (123). While TBC1D4 phosphorylation tended to decrease immediately following resistance exercise, there was a significant increase at 1 hour and 2 hours into recovery (123). The increase in basal phosphorylation of TBC1D4 which occurs during recovery acts synergistically to the effect of insulin stimulation (124). In a rat model, 27 hours of fasting post-exercise increased both insulin action and TBC1D4 Thr^{642} phosphorylation (121), suggesting that TBC1D4 has a role in regulating increased IS post-exercise. However, the role of TBC1D4 in mediating exercise-induced glucose uptake is less clear. Inhibition of PI3K with wortmannin prevented TBC1D4 phosphorylation during exercise but did not impact glucose uptake during exercise (125), suggesting that phosphorylation of TBC1D4 is not necessary for contraction-induced glucose uptake.

Similar to TBC1D4, TBC1D1 is also phosphorylated in response to both insulin and skeletal muscle contractions in animal and human skeletal muscle (126, 127). While TBC1D4 has several insulin responsive sites, TBC1D1 has several contraction/AICAR response sites and therefore could be the main driver behind contraction induced glucose uptake (127). Jessen et al, (117) demonstrated that exercising for 30mins at 70% $\dot{V}O_{2max}$ increased TBC1D1 Ser^{231} and Ser^{660} and this increase mirrored the increase in AMPKα2 activity. Further evidence that TBC1D1 is phosphorylated by AMPK comes from a study in rats where contraction-stimulated activation of TBC1D1 was prevented when an AMPK-inhibitor (compound C) was added (125).

The decreased TBC1D1 activation with compound C was accompanied by reduced AMPK phosphorylation and glucose uptake (125) indicating that TBC1D1 is important for contraction-mediated glucose uptake via AMPK. Treebak et al, (128) also found immediate phosphorylation of TBC1D1 Ser^{660} phosphorylation post-exercise in both the fed and fasted state. In contrast, a recent paper by Tobias et al, (129) demonstrated a delayed phosphorylation of TBC1D1 at Ser^{660} in the fasted state, with increased phosphorylation only occurring at 180 min post-exercise after a HIIT protocol. However, another study found increased Ser^{237}, but not Thr^{596}, TBC1D1 phosphorylation immediately post-exercise following either an acute bout of low and high volume HIIT (30s or 2 min intervals) or moderate-intensity continuous [(77% VO2peak, 20 min (130)). Together these data suggest that overall, there is an increase in TBC1D1 phosphorylation during exercise, but that intensity, time, and exercise protocol may influence TBC1D1 phosphorylation during and post-exercise. Therefore, while there seems to be a role for TBC1D1 during exercise for contraction induced glucose uptake, the role of TBC1D1 in regulating increased IS post-exercise is less clear (124).

2.3.4 Exercise-induced ROS and inflammatory mediators as signaling molecules in relation to glucose uptake

Repetitive muscle contraction, such as in exercise, results in the accumulation of ROS (131). ROS include all unstable metabolites of molecular oxygen that have a higher reactivity (132). ROS are generated under normal physiological conditions as a by-product of aerobic metabolism, however excessive ROS, and reactive nitrogen species (RNS) production leads to

oxidative stress that can lead to negative health consequences (132, 133). Acute bouts of exercise transiently increase ROS production, which act as key signaling molecules to induce exercise training adaptations (134). ROS are generated predominantly by contracting skeletal muscle during exercise. At rest, the majority of ROS are produced by the mitochondria through the process of generating ATP through oxidative phosphorylation processes (132). Other sources of ROS include: peroxisomal oxidases (135), cytochrome *P*-450 enzymes (136), NADPH oxidases (NOX) (137) and xanthine oxidase (XO) (138). Under normal physiological conditions, ROS are released as by-products of cellular respiration by the mitochondria and can be observed in both resting and exercising muscle (139, 140). During this process, O_2 can lose an electron which may lead to the formation of $O_2^{\cdot}$ or H_2O_2 which can then be converted to other ROS species (132). During exercise, NOX in particular is a key ROS generator and contributes a large extent of the cytosolic reactive oxygen species (141, 142). NOX is a multi-component enzyme that is located on skeletal muscle fibres and NOX-induced ROS in the t-tubules can directly activate ryanodine receptor type 1 to enhance Ca^{2+} release and muscle contractions during exercise (143, 144). XO also contributes to the production of extracellular $O_2^{\cdot}$, particularly during isometric muscle contraction and plays a critical role in muscle force generation (145, 146). Aerobic exercise is commonly known to induce ROS and RNS overproduction due to enhanced metabolism which can lead to oxidative stress (134, 145). During aerobic exercise there can be a 1-3-fold increase of $O_2^{\cdot}$, with mitochondria only accounting for a small portion of the $O_2^{\cdot}$ generated (139). Additionally, elevated lipid peroxidation and DNA oxidative damage has been observed following a single bout of aerobic exercise (131). Regular aerobic exercise however will help enhance the cellular ability to

detoxify ROS over-accumulation (147). Similarly, exercise-mediated adaptation can also increase myocellular antioxidant capacity which helps to lower ROS levels (148, 149).

ROS also act as messengers to stimulate insulin-signaling. ROS-induced signaling is one of the proposed mechanisms by which acute exercise increases glucose uptake (150). ROS significantly enhance the level of tyrosine phosphorylation of the insulin receptor and IRS proteins, thereby promoting GLUT4 translocation to the plasma membrane (47). Catalase treatment (an antioxidant enzyme) had no effect on basal tyrosine phosphorylation but reduced the insulin-stimulated autophosphorylation of the insulin receptor and the IRS proteins by ~48% and ~43%, respectively (151). Furthermore, ROS has a prominent role in increasing PI3K/Akt signaling (150). Cellular treatment with diphenyleneiodonium (DPI) completely inhibited the activation of PI3K activity by insulin and reduced insulin-induced activation of serine phosphorylation of Akt by 49% (152). Overall, preventing ROS generation with DPI decreased insulin-stimulated glucose uptake and GLUT4 translocation (152).

Similarly, cytokines also have a role in glucose uptake. Interleukin-6 (IL-6) has been found to have a direct role in promoting glucose uptake both at rest (153) and following exercise (154). IL-6 in response to exercise can also mediate glucose uptake through activation of both AMPK and PI3K pathways (155). Tumor necrosis factor alpha (TNFα) has also been shown to up-regulate glucose uptake in culture human muscle cells (156). However, most findings indicate a role of TNFα in the pathogenesis of IR and impairment of glucose disposal, as discussed below. These data overall demonstrate a prominent role of exercise-induced increases in ROS and inflammatory cytokines in promoting post-exercise insulin-signaling.

2.4 Insulin resistance

2.4.1 Development of insulin resistance

IR is defined clinically as the inability of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in a normal population (157). IR is a hallmark characteristic of T2D, which is often also characterized by an increase in hyperglycemia (158). However, many individuals with IR maintain normal glycaemia due to compensatory increases in pancreatic insulin secretion (159, 160). The hyperglycemia in T2D is a consequence of insulin deficiency (157). Early in the disease progression of T2D, pancreatic β-cells secrete sufficient insulin to compensate for insulin resistance which maintains euglycemia (158). Ultimately, however continuous hyperglycemia will lead to β-cell dysfunction therefore exacerbating the disease progression of T2D (158).

2.4.2 Insulin resistance and the insulin signalling cascade

IR is broadly defined as a reduced ability of insulin to stimulate glucose uptake in peripheral tissues such as skeletal muscle (46). In normal circumstances about 80% of a glucose load is taken up by skeletal muscle, therefore it is not surprising that skeletal muscle is one of the key sites of IR in T2D (24). In T2D and IR individuals' skeletal muscle GLUT4 content is normal, therefore impaired glucose uptake in response to insulin most likely results from the inability of insulin to induce GLUT4 translocation to the plasma membrane (161). In contrast however, the protein expression of GLUT1 in type 2 diabetes is reduced and could contribute to impaired basal glucose uptake (162). Additionally, several studies have demonstrated that

GLUT4 translocation and glucose transport is stimulated normally by muscle contraction, confirming that it is insulin signalling that is depressed and exacerbating IR (24). In animal models of T2D, it has been found that activity of the insulin receptor, IRS-1 tyrosine phosphorylation and PI3K activity are all lower in response to insulin stimulation (163-165). Most studies support the notion that skeletal muscle IR is likely due to downstream defects of the insulin receptor and the IRS proteins (166). In models of obesity and T2D there is inhibitory serine/threonine phosphorylation of the insulin-receptor tyrosine kinase (167), which is also found with the IRS proteins (166). The IRS proteins can be phosphorylated on more than 20 identified phosphorylation sites including both serine/threonine and tyrosine phosphorylation sites, which leads to a complex regulation of IRS activity (168). In skeletal muscle of obese and T2D individuals, IRS-1 tyrosine phosphorylation is diminished (101). Furthermore, studies in rodents have demonstrated that the JNK-mediated phosphorylation of IRS-1 Ser^{307}, equivalent to Ser^{312} in humans, promoted IR by impairing the ability of IRS-1 to bind to the activated insulin receptor (169, 170). Additionally, obese and T2D patients have higher skeletal muscle IRS-1 Ser^{312} phosphorylation, which effectively hinders the rest of the insulin signalling cascade (171).

There are also components further down the insulin signalling cascade that are hindered or downregulated in skeletal muscle in obesity and T2D (172). Specifically, increased FA uptake into skeletal muscle in obesity/TD2 leads to dysfunction in PI3K/Akt signaling (32). In normal physiological conditions, FFAs enter skeletal muscle through fatty acid transporters including fatty acid translocase/CD36 (FAT/CD36) and fatty acid binding protein (FABP). In the muscle the fatty acids are linked to coenzyme A and are directed towards TG synthesis or enter into the

mitochondria for oxidation (173). In obesity, rates of fatty acid transport into skeletal muscle are higher due to a permanent relocation of FAT/CD36 to the plasma membrane, but not an increase in FAT/CD36 expression (24). Higher rates of fatty acid uptake are highly correlated with an increase in TG (intramyocellular lipid) accumulation, but not an increase in rates of fatty acid oxidation (24), resulting in lipid oversupply in the muscle. This excessive fatty acid uptake and impaired disposal promotes the development of IR in skeletal muscle via several mechanisms. In relation specifically to the IRS protein and the PI3K-Akt pathways, the accumulation of intramyocellular lipid by-products such as diacylglycerol (DAG's) and ceramides both impair insulin signaling, but by different mechanisms. DAG's activate serine kinases, which phosphorylate and inhibit the signal transduction capacity of IRS-1 whereas ceramides disturb insulin signaling at Akt, leading to a reduction of glucose transport (32). Additionally, there is an inverse relationship between the plasma membrane content of FAT/CD36 and GLUT4 ($r = -0.91$), which can further exacerbate IR (174). The subcellular locations of both GLUT4 and FAT/CD36 are juxtaposed such that when FAT/CD36 is relocated to the plasma membrane, GLUT4 is retained within its intracellular depots (24). Together, the current research indicates that insulin signaling is impaired in obese and T2D individuals at multiple points in the signaling cascade.

2.4.3 Role of reactive oxygen species and inflammation in the development of insulin resistance

Chronic inflammation and elevated oxidative stress have been implicated in the pathogenesis of a myriad of diseases including IR and T2D (175, 176). Increased ROS and oxidative stress can lead to the progression of T2D through several pathways. Firstly, chronic

exposure of pancreatic β-cells to ROS results in a suppressed insulin biosynthesis and secretion (177). In obese diabetic mice, antioxidant treatment led to significantly larger β-cell mass and insulin content (178). Additionally, activation of the c-Jun N-terminal kinase (JNK) pathway is involved in pancreatic β-cell dysfunction. The activation of the JNK pathway by ROS results in a decrease of insulin gene expression which over time leads to a reduction in insulin production (177). Secondly, in addition to their role in inducing β-cell dysfunction, ROS are also involved in the progression of IR (179). Chronic elevations in ROS can disrupt insulin-induced cellular redistribution of IRS-1 and PI3-K and therefore impair GLUT4 translocation to the plasma membrane (180, 181). Under diabetic conditions, free fatty acids (FFAs), inflammatory cytokines and endoplasmic reticulum (ER) stress are increased which also leads to the activation of the JNK pathway (182, 183). Activation of the JNK pathway results in serine phosphorylation of IRS-1 which inhibits insulin-stimulated tyrosine phosphorylation of IRS-1 (169).

Obesity-induced chronic inflammation is also a key component in the pathogenesis of IR (184). TNFα is a pro-inflammatory cytokine secreted predominantly by monocytes and macrophages and has a wide range of biological effects (184). Several studies have demonstrated links between TNFα and insulin resistance (185-187). In vitro it has been shown that by activating IKKB, TNFα leads to serine phosphorylation of IRS1 thereby blocking the insulin signaling cascade (187). Additionally, overproduction of TNFα can lead to a reduced expression of the insulin receptor, IRS1 and GLUT4 genes thereby leading to a decrease in insulin stimulated glucose uptake and contributing to insulin resistance (24, 25). Similarly, IL-6 when acting as a proinflammatory cytokine is also involved in the development of IR through

the activation of various pathways (188). IL-6 induces the expression of suppressor of cytokine signaling 3 (SOCS-3), which inhibits insulin signaling (188). Additionally, IL-6 causes impaired phosphorylation of both the insulin receptor/IRS1 and activates JAK signaling which leads to further inhibition of insulin signaling (189, 190).

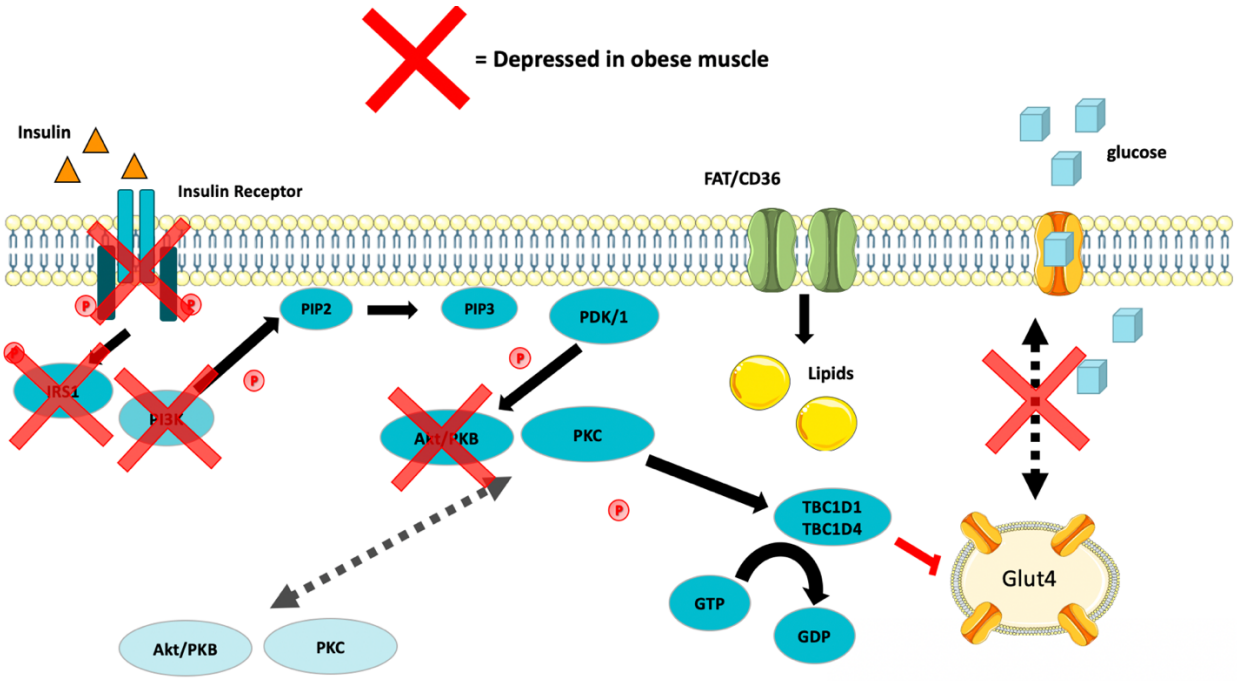

Figure 2.2: Disruptions in the insulin signaling cascade in obese/T2D muscle

2.4.4 Acute and chronic exercise effects on insulin-dependent signaling and insulin sensitivity

Exercise results in key improvements to overall metabolic health in two main phases which include: I) the events that occur during exercise and in the hours to days following exercise and II) the adaptations that occur following long-term repeated exercise training (191). The first phase, which occurs during exercise, occurs due to the large increase in energy

utilization and occurs via insulin-independent mechanisms and is therefore quite efficacious in lowering blood glucose in insulin resistant individuals (192). The elevation in insulin-independent glucose uptake during and post-exercise is mostly reversed approximately 2-3 hours post exercise (122, 193-197), with GLUT4 completely reinternalizing by 85 minutes after the cessation of exercise (53). The second phase includes the effects following an acute exercise bout and involves the increase in muscle IS for 48-72 hours after exercise in healthy participants (58, 59) and for >15 hours in participants with T2D (198). The increase in IS in the exercised muscles can improve whole body post exercise insulin-stimulated glucose disposal by several mechanisms. (195, 198, 199).

One of the main reason's IS is enhanced post exercise is to allow for the replenishment of muscle glycogen stores that were lowered during the exercise bout (52, 200). Several studies in humans have confirmed that the improvement in IS following exercise is proportionate to the extent of glycogen depletion (70, 102, 199, 200). In humans approximately 80% of the glycogen in the body is stored within skeletal muscle (201). While hepatic glycogen stores are used to maintain physiological blood glucose concentration and as a substrate during exercise, skeletal muscle does not release glucose into the circulation, and thus when glycogen is broken down it provides a local energy substrate for ATP production during exercise (202) that needs to be replenished once exercise is completed. Insulin activates GS by dephosphorylating GS via PKB-mediated phosphorylation of GSK3 (203-205). GS is also activated by glucose 6-phosphate and allosteric activation is necessary for normal rates of glycogen synthesis (204, 205). As discussed above, exercise training enhances IS and is associated with adaptions in skeletal muscle such as increased expression of key insulin signalling proteins, hexokinase II and GS all involved in

insulin-stimulated glucose metabolism (206). The intensity of the exercise bout as well as duration of the bout will determine the amount and type of substrate used (202). Studies have reported a glycogen concentration from anywhere from 7-20 mmol/kg in the vastus lateralis muscle after cycling to exhaustion, with glycogen depletion being most pronounced at workloads of ~75% $\dot{V}O_{2max}$ (207-209). This is compared to the usual 80-150 mmol/kg of glycogen concentration in vastus lateralis muscle at rest (208, 210).

A secondary trigger for the post-exercise increases in IS could be the extent of AMPK phosphorylation during exercise (52). As previously mentioned, at the onset of exercise energy consumption can lead to a 100-fold increase in energy demands as it is a potent stimulus for AMPK activation (211). In general, AMPK tends to be activated at intensities involving a minimum of 60% $\dot{V}O_{2peak}$, unless the exercise bout is performed until exhaustion then AMPK activation has been noted at intensities of 30-40% $\dot{V}O_{2peak}$ (75). However, several studies have demonstrated that AMPK activation is typically reversed 30-60 min post-exercise (212-215), therefore it may not be AMPK per se, but a downstream target activated by AMPK that is responsible for greater post-exercise insulin sensitivity such as TBC1D1 and TBC1D4 (52, 216). Recent work has indicated that AMPK is not required for glucose uptake during exercise and contraction, but rather plays an important role in glucose permeability following exercise (76).

2.5 Sex-based differences in metabolism

Historically females are underrepresented in the literature and as a result we have a paucity of information related to how females specifically respond to any given intervention (217). What we do know is that the risk of T2D is lower in females than males (218), which has

been attributed to the hormone estrogen (219). However, research has suggested that females may not respond as readily to exercise interventions when it comes to improvements in insulin sensitivity (16-18). There has been quite a bit of research indicating that fuel storage and metabolism during exercise differs between the sexes (220, 221) and across the menstrual cycle (20, 222, 223). Sex-based differences in high-energy phosphate transfer (HEPT) systems, glycogen and fat metabolism (18, 224, 225) during exercise can lead to different concentrations of signaling molecules within the muscle that overtime could lead to the differential adaptations in IS between males and females. Sex differences in metabolism are mediated by the sex hormone estrogen (22, 226-230). As estrogen fluctuates in females across the menstrual cycle, timing of when females are tested during the menstrual cycle may also influence underlying metabolism, insulin signaling and IS and needs to be carefully controlled for.

2.5.1 Females are underrepresented in the literature

There is increasing attention to the underrepresentation of females in biological research (217). Generally, sex differences are often disregarded in research design, study implementation and scientific reporting despite there being considerable evidence that indicated that sex affects the presentation, outcome of treatment and progression of many disease states (231-234). This underrepresentation appears to stem from the variability in females due to monthly hormonal cycles. Due to the underrepresentation of females in physiological research we do not fully understand inherent differences between the sexes and

therefore our understanding of health and disease is incomplete because we do not know whether females will respond in a similar manner to males in response to a given intervention.

2.5.2 Sex based differences in T2D prevalence, impaired fasting glucose, and impaired glucose tolerance

There is an inherent sex difference in T2D prevalence with 14 million more males having T2D than females (235). Furthermore, biological sex affects glucose homeostasis and parameters of insulin sensitivity such that females have a lower incidence of developing T2D later in life (236-238). This enhanced IS and reduced incidence of T2D compared with age-matched males is thought to be due to circulated estrogen (219). Estrogen has been demonstrated to have a protective effect in the regulation of glucose homeostasis (219). Individuals are classified for risk and presence of type 2 diabetes based on fasting plasma glucose and 2-hour glucose concentration during an OGTT (239). It has been repeatedly found that males are more frequently classified as having impaired fasting glucose, whereas females are classified as having impaired glucose tolerance due to elevated glucose concentrations at the end of the OGTT (239-241), suggesting potential differences in disease pathogenesis. Furthermore, recent trials have shown that 2-hour glucose concentration, but not fasting glucose concentration, is negatively associated with body surface area and height (240, 241) and thus the greater prevalence of impaired glucose tolerance in females is likely due to the fact that the relative dose of glucose consumed during the OGTT is greater. The effect of anthropometrics, which differ between the sexes make our current understanding of impaired

glucose tolerance flawed in females because they are predisposed to having higher 2-hour glucose compared to males, due to the higher relative glucose dose.

2.5.3 The menstrual cycle

The menstrual cycle is a tightly regulated process that occurs in females once they reach sexual maturation (242). The menstrual cycle is driven primarily by the ovaries which serve two main roles; 1) to mature and release eggs for reproduction and 2) to synthesize sex hormones (243). The main sex hormones synthesized by the ovaries are oestrogens (17β- estradiol and estrone) and progesterone (243). The typical menstrual cycle lasts 28-32 days and consists of follicular and luteal phases that are separated by ovulation (243). The menstrual cycle begins with the follicular phase which encompasses the time between the first day of menses and ovulation. The follicular phase lasts approximately 12-14 days and consists of low levels of oestrogens and progesterone (243). This is followed by ovulation which is the release of the egg from the ovary mid-cycle. Ovulation lasts approximately 1 day and is preceded by a surge of oestrogen (243). Following ovulation is the luteal phase which is the time between ovulation and before the start of menstruation when the body prepares for a possible pregnancy. The luteal phase lasts approximately 12-14 days and during this time there are high levels of oestrogen and progesterone, which both peak and then drop to signal the start of menses (243). It is important to note that the follicular phase is characterized by low estrogen, but a high estrogen:progesterone ratio whereas the luteal phase is characterized by high estrogen, but a low estrogen:progesterone ratio.

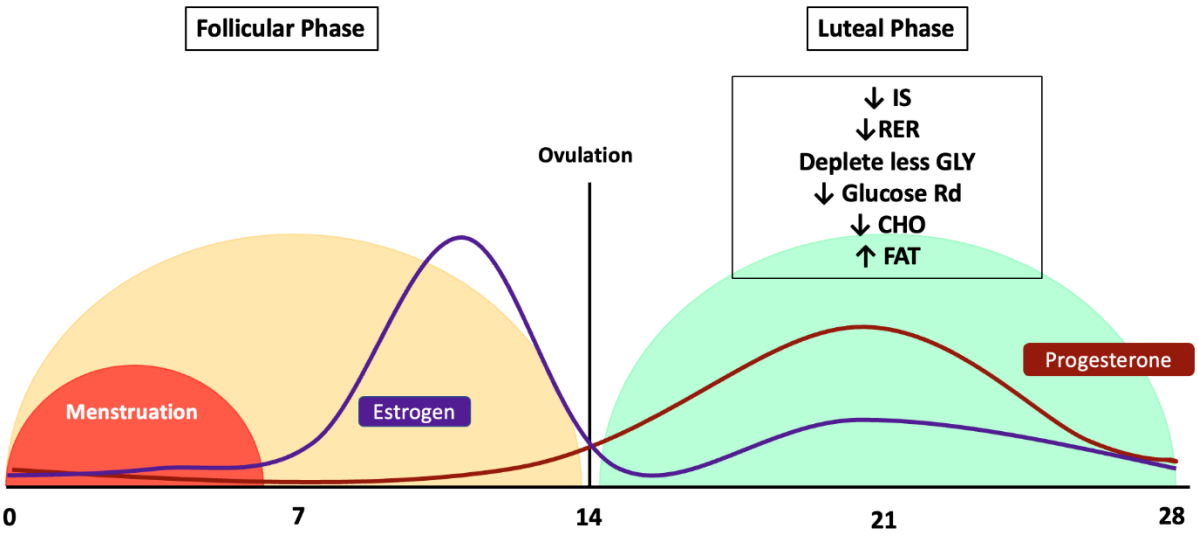

Figure 2.3: Phases of the Menstrual Cycle adapted from Ray (244).

2.5.4 Sex differences in metabolism

As noted above, to date most of the research that acts as a foundation for metabolism and substrate use during exercise has been based on studies using predominantly males as males represent a stable physiological milieu. As a result, there is a bias within the literature where females are underrepresented and it is unclear how exercise influences metabolism and health in females. We now know that sex plays a large role in exercise physiology and substrate use during exercise. In response to endurance exercise, females have a lower respiratory exchange ratio (RER) (19, 20, 22, 245), which is indicative of less reliance on whole-body carbohydrate oxidation. Similarly, females have been consistently found to have a lower reliance on liver carbohydrate stores through a decreased glucose rate of appearance, rate of disappearance and metabolic clearance rate (19, 20, 246) compared to males. It is not known however if this is a result of lower liver glycogenolysis, gluconeogenesis or both. Whether

females spare muscle glycogen during endurance exercise is currently controversial. While some studies have found no effect of sex on muscle glycogen utilization during endurance exercise (225, 247, 248), there are some studies that have found that females utilize 25% (22) and 50% (18) less muscle glycogen during endurance exercise compared to males. These differences could be due to exercise modality, as the studies that have found differences in glycogen utilization used running and a high-intensity bike sprint protocol respectively, as compared to endurance cycling where no differences were found. Furthermore, these differences could be related to differences in exercise intensity and/or duration as both of these factors are known to influence the extent of muscle glycogen utilization during exercise (245, 249).

There are also sex-based differences in lipid metabolism during exercise as evidenced by a higher glycerol rate of appearance, indicative of greater whole-body lipolysis, during exercise compared to males (19). Females also have a greater capacity to store and oxidize fat within skeletal muscle evidenced by a greater intramyocellular lipids (IMCL) content compared to males (21, 225, 250, 251). The greater ability of female muscle to metabolize lipids is also evidenced by metabolic pathways related to muscle fatty acid (FA) uptake, IMCL synthesis and degradation and FA transport into mitochondria and oxidation being upregulated in females (252). Specifically, females have a greater mRNA expression and protein content of FABP and FAT/CD36 compared to males (253-255) which is indicative of greater FA transport and utilization within the mitochondria. Females also have higher mRNA content of sterol regulatory element-binding protein 1c (SREBP-1c) and mitochondrial glycerol-3-phosphate acyltransferase (GPAT) (254) and perilipin 2 (PLIN2) protein content (256), which is suggestive

of a greater capacity for IMCL synthesis. Furthermore, females also have a greater adipose triglyceride lipase (ATGL) activity (257) and hormone sensitive lipase (HSL) mRNA expression (but not content) (258) which is indicative of a greater IMCL lipolytic capacity. Whether IMCL use during exercise is greater in females is contentious, however. While some studies have found that females have greater IMCL use during exercise (225, 250, 258), others have found a lesser reliance (248) or equal reliance (21, 259). Differences in the findings of these studies could be explained by limitations in the methodologies used as well as differences in the training status of participants used.

Lastly, there are also sex differences in protein oxidation during exercise. Compared to males, females oxidize less leucine during endurance exercise (260-262). Additionally, previous research has shown that non-oxidative leucine disposal which is indicative of whole-body protein synthesis is greater in females than males during endurance exercise (262).

2.5.5 Substrate utilization across the menstrual cycle

The sex-differences in substrate utilization during exercise is influenced by the fluctuations of hormones across the menstrual cycle. During the luteal phase (LP) as compared to the follicular phase (FP), females have a lower RER (222, 223), deplete less muscle glycogen (222, 223), have a lower glucose rate of disappearance (R_d) (263) and oxidize less carbohydrate (223, 263) and more lipids (263) (Figure 2.3). Additionally, when females are tested in the luteal phase compared to the follicular phase, there was a lesser reliance on proglycogen (the dynamic storage form of glycogen) during a 90-min cycling bout at 65% $\dot{V}O_{2peak}$ (20). Overall, these studies indicate that during the LP of the menstrual cycle, when estrogen is high, there is

a reduction in carbohydrate utilization during endurance exercise and that any apparent sex differences may be more pronounced when performing studies during the LP (20).

Insulin sensitivity is also influenced by the menstrual cycle. A study investigating IS in 12 healthy eumenorrheic females found that IS was higher in the follicular phase and decreased in the luteal phase, thereby decreasing over the course of the menstrual cycle (264). This is also evidenced by females having a significantly higher HOMA-IR during the luteal phase of the menstrual cycle as compared to the follicular phase (265). Within the aforementioned study, HOMA-IR was positively associated with estradiol and progesterone, both of which are higher during the luteal phase (265). These findings indicate that insulin sensitivity and insulin resistance exhibit menstrual cycle variability and therefore menstrual cycle should be considered when conducting sex comparative research.

2.5.5 Role of estrogen in metabolism

Sex differences in metabolism are due predominantly to 17β-estradiol (E2). Animal research has shown that supplementation with E2 significantly influences substrate utilization during endurance exercise, such that there is a decreased reliance on carbohydrates and increased reliance on lipids (226-230). Furthermore E2 supplementation in male or oophorectomized female rats improves several markers of exercise capacity including exercise performance (226, 229), muscle and liver glycogen utilization during exercise (226, 229, 230) and free fatty acid (FFA) availability (226, 228, 230). E2 supplementation trials in humans have found similar results (19, 266-269). Specifically, a study by Devries et al, (268) found that supplementing recreational active young males with E2 for 8 days decreased RER during

exercise, signifying a decrease in carbohydrate utilization and an increase in lipid utilization. The decreased carbohydrate utilization was the result of lower hepatic glucose release, not decreased muscle glycogen utilization; however, the source of increased lipid (i.e., adipose vs. skeletal muscle) was not determined (268). Importantly, E2 supplementation induces a concomitant decrease in testosterone (268), so it is important to determine whether the effects seen with E2 supplementation are the result of increased E2 or decreased testosterone. Braun et al (270), compared muscle metabolism in 9 healthy, physically active young males at normal, low (<1 ng/ml, induced using subcutaneous injections of 3 mg cetrotide) or high (>8 ng/ml, induced using 5-mg transdermal testosterone patches) testosterone levels in order to determine if testosterone influences fuel utilization during exercise. Regardless of suppression or the replacement of testosterone there was very little impact on the utilization of blood glucose, glycogen, or lipid during exercise in males (270) suggesting that it is E2, not testosterone, that influences fuel utilization during exercise. Taken together, these sex differences in metabolism during exercise may lead to different signaling within muscle that may explain why males and females differ when it comes to the insulin sensitizing effects of exercise training; however, this area of research is not well studied and requires further examination.

2.5.6 Sex Differences in oxidative stress and inflammation

Biological sex has been shown to influence physiological levels of oxidative stress and inflammation such that males have greater levels of oxidative stress (271-277) and greater inflammation compared with females (278, 279). Males have greater expression and increased

subunits of NADPH oxidase (NOX) (271, 280, 281), a major oxidative stress generator in cells (282), particularly during exercise (141, 142). The potential that NOX activity is greater in males than females is important as it suggests that ROS generation during exercise may be greater in males than females. As ROS are important signaling molecules during exercise (283) and have been found to influence the extent of adaptation following training (283), differences in ROS generation during exercise between the sexes may be related to sex-based differences in the adaptation to training. Additionally, males have greater homocysteine levels compared to females which is indicative of a greater level of oxidative stress (274, 284, 285). Similarly, males have increased levels of pro-inflammatory cytokines such as IL-6 and TNFα (278, 279). Furthermore, females have been found to have a lesser inflammatory response to exercise compared to males in response to eccentric exercise (286). However, there are other studies that have shown there are no differences reported in serum IL-10, IL1ra, IL-6, and IL-8 between males and females immediately post and 1.5 hours into recovery following a marathon (287). Additionally, 90-mins of cycling at 65% $\dot{V}O_{2peak}$ did not show any sex-differences in serum IL-6 levels (288). Similarly, in-vitro production of IL-1, IFN-γ, and IL-4 cultured in whole blood following continuous incremental cycling at 55%, 70%, and 85% $\dot{V}O_{2peak}$ did not differ between males and females (289). Therefore, it seems as though the cytokine response to exercise is not markedly different between the sexes. Importantly, these sex differences in oxidative stress and inflammation are thought to be due to estrogen. Estrogen up-regulates the expression of antioxidant enzymes via intracellular signaling pathways thereby giving it antioxidant properties (276). Furthermore, estrogen has an inhibitory effect on the gene expression of inflammatory markers (278, 279), leading to a decreased level of inflammation in females compared to males.

Given the important role of ROS and inflammation in both promoting positive adaptation to training and the development of T2D, examining how ROS and inflammation are influenced by exercise in both sexes is necessary to further our understanding of how sex influences the development of T2D and how exercise can prevent its development.

2.6 Effects of different exercise modes on insulin sensitivity with a focus on sex-based differences in response

In metabolic disease such as IR and T2D where insulin-stimulated glucose uptake by muscle is often severely impaired, the exercise-stimulated glucose uptake response remains intact reinforcing the importance of exercise in these populations (191). Moderate to high levels of aerobic physical activity and higher levels of cardiovascular exercise are associated with reductions in morbidity and mortality in males and females with T2D (290). Physical activity induces both acute and chronic improvements in IS (15). It has been previously shown that with each additional 500kcal/week energy expenditure from exercise, the risk of T2D decreases by approximately 9% (291). Current recommendations state that individuals with diabetes should accumulate a minimum of 150 minutes per week of moderate or 75 minutes of vigorous aerobic exercise each week with additional muscle-strengthening activities at least 2 days per week and no more than 2 consecutive days without exercise (292). Although the beneficial effects of exercise are well known in relation to T2D and all-cause mortality, individuals with T2D are among the least likely population to exercise and the adherence rates to physical activity are exceptionally low (293). Some barriers to exercise in these individuals

include poor health, lack of company, lack of interest and lack of time (293). Other barriers include the perception that exercise will induce pain, a sensation of being tired, as well as the presence of altered mood states, which outweigh the beneficial effects of exercise (293). As such, it is important to understand how different exercise modes influences IS and insulin signaling as different people will enjoy different modes of exercise and enjoyment is important for exercise adherence. While aerobic exercise is the most commonly studied exercise modality for its beneficial effects on glucose handling and IS, there is evidence that the combination of both aerobic and resistance exercise will induce maximal improvements in glycemic control and thus may be most beneficial in combination rather than either exercise mode alone, which may be mediated by a greater training volume (15). Furthermore, recent trials have explored the effects of different exercise modalities, namely aerobic exercise (AE) and resistance exercise (RE), and their influence on glucose handling and IS, and they have all been shown to improve IS and glucose control in a variety of populations (4, 15, 294).

Understanding whether the insulin sensitizing effects of exercise differ between the sexes is also imperative to provide the most efficacious exercise recommendations to prevent IR in both sexes. While epidemiological research shows that increasing exercise decreases the risk of developing IR and T2D in both sexes (295), current literature does suggest that there is an effect of sex on the effectiveness of the given exercise modalities to improve IS and glucose handling (17, 296-298), although the mechanisms that underpin this sex difference are not fully understood (16). Determining how sex influences the effectiveness of exercise training on IS and glucose handling is rendered difficult by studies not including proper protocols such as not matching males and females for habitual training status and aerobic capacity relative to lean

body mass (220, 255), as well as a lack of control for phase of menstrual cycle or use of oral contraceptives and how they may influence energy metabolism and glucose handling. Further complicating matters is the fact that many of the studies that do include both males and females in the study sample do not include a comparison of the effectiveness of the exercise modality between the sexes (298-300).

2.6.1 Aerobic exercise

Increasing physical activity and/or exercise is considered a fundamental treatment for the prevention and management of T2D. Of the different exercise modes, AE is the most studied exercise modality (301). There are several different modalities that can all be classified as AE. The most studied include *moderate-intensity continuous exercise* (MIC)*, high-intensity interval exercise* (HIIE) *and sprint-interval exercise* (SIE). While there is no universal definition to describe the appropriate intensities for each of the given types of AE, definitions set forth by Gibala et al (302) describe HIIE as relatively intense bouts of intermittent exercise that elicit ≥80% of maximal heart rate (302). SIE on the other hand is a form of AE that includes either 'all-out' intervals or intensities that exceed the workout load required to elicit maximal oxygen uptake [%$\dot{V}O_{2max}$, (302)]. MIC exercise on the other hand tends to be longer in duration and continuous in nature at a lower intensity [~60-80% $\dot{V}O_{2max}$, (303-305)].

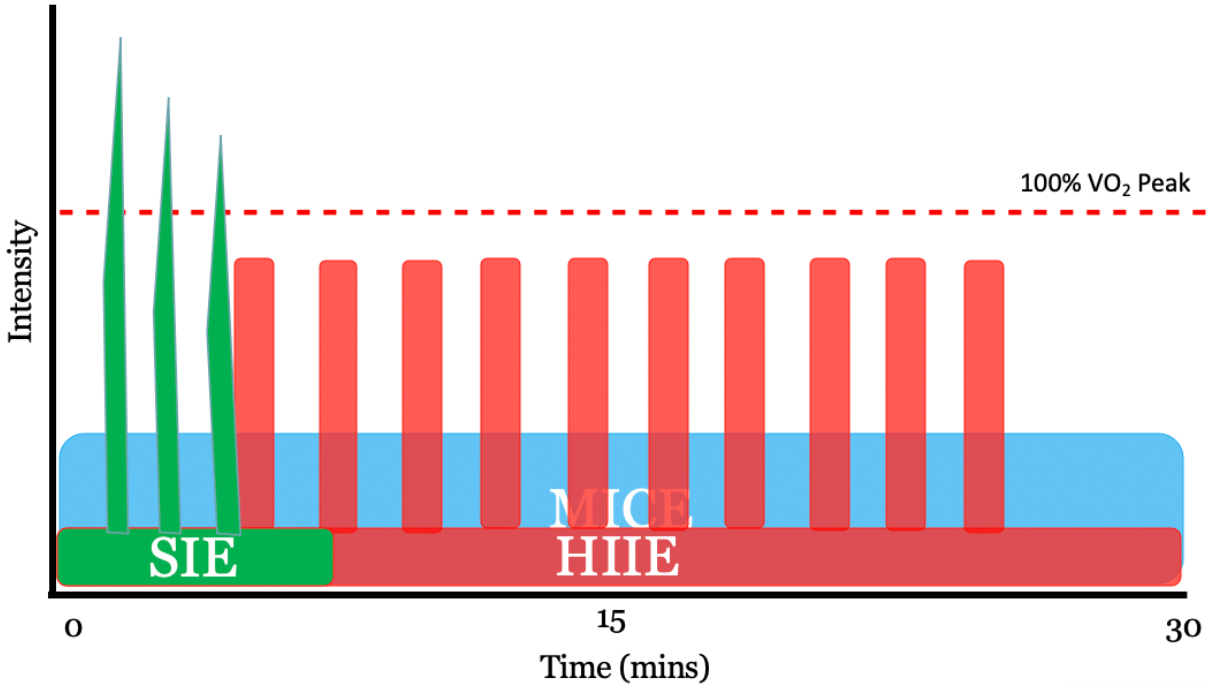

Figure 2.4: Differences between moderate intensity interval exercise, high intensity interval exercise and sprint interval exercise adapted from J. Gillen, CSEP 2019.

2.6.1.1 Moderate-intensity continuous exercise

MIC exercise is the most traditional version of AE and as the name suggests consists of moderate intensity endurance exercise performed continuously for longer durations. Traditionally, MIC exercise protocols use exercise intensities that correspond to approximately 65-70% of $\dot{V}O_{2\ peak}$ for durations of 30-60 mins (306). The effect of MIC exercise on glycemic control and IS has been well studied in the literature (301, 303). Acutely, MIC exercise has been well documented to transiently increase IS for several hours following a single bout of exercise in a variety of populations (307-310). IS has been shown to increase more than 50% for up to 72 hours following an acute bout of aerobic exercise (59). This transient increase in IS is in part due to the activation of AMPK following acute MIC exercise (311, 312). Additionally, TBC1D4 (a downstream target of AMPK) has increased phosphorylation at Thr^{649} and Ser^{711} and enhanced

glucose uptake in response to insulin following exercise in animals (311). Brestoff et al, (308) investigated the response to an acute bout of MIC exercise in healthy males and females and did not see any effect of sex on indices of IS. However, researchers did not control for menstrual cycle phase, oral contraceptive use or aerobic fitness based on LBM, all factors that could influence the post-exercise IS.

Chronic MIC exercise is a commonly advocated exercise prescription for adults with T2D and has been demonstrated to be effective in reducing markers of IR (313, 314). Walking three times per week for 30-60mins was stimulus enough to elicit improvements in HOMA-IR, fasting plasma insulin and fasting glucose in participants with pre-diabetes and T2D (313, 314), even though this exercise prescription does not meet minimum exercise recommendations. The effectiveness of MIC training at improving glucose handling has been demonstrated in both males (298, 315, 316) and females (317-319). However, there are also numerous studies that have shown that MIC exercise has not elicited beneficial changes in IS in females (298, 320, 321). Potteiger et al, (306) demonstrated that 16 months of MIC exercise at 60-75% HR reserve for 20-45min, 3-5 times per week improved markers of IS in males and not females. Similarly, exercise training for 9 months at a moderate intensity of 65% $\dot{V}O_{2peak}$ did not elicit any changes in glucose utilization in inactive older females (321). Therefore, it seems that there could be a sex-specific adaptation to chronic MIC exercise that could impact its effectiveness in females. This suggests that females may not respond in the same manner as males when it comes to the insulin sensitizing effects of MIC exercise and therefore females may benefit more from another exercise modality in terms of glycemic control and IS. However, the mechanisms behind this sex-specific response are currently not well understood.

2.6.1.2 High–intensity interval exercise and sprint-interval exercise

HIIE protocols typically involve repeated intervals of approximately 1-4 min in duration at high intensity (ie. 80-90% HR_{max}) interspersed with low-intensity intervals. Alternatively, SIE protocols involve repeated intervals of approximately 20-30s in duration at supramaximal (i.e., above $\dot{V}O_{2max}$)/'all-out' intensities interspersed with low-intensity intervals of longer duration (i.e., 1 – 4 minutes). HIIE and SIE have gained notoriety in recent years due their ability to elicit the same beneficial physiological effects as traditional endurance training (MIC exercise) while reducing total exercise volume and exercise time (302). Evidence also suggests that HIIE is perceived to be more enjoyable than MIC exercise and therefore has a vast importance in the clinical setting and its implications to increase exercise adherence (322). Acutely, a single bout of HIIE has been previously shown to enhance glycemic control and increase IS (323-325). While the mechanisms for the increased IS and glycemic control have yet to be fully elucidated, authors speculate that it could be due to muscle fibre recruitment and/or glycogen utilization following acute exercise, all which contribute to increased GLUT4 translocation (323). Parker et al, (307) have demonstrated that an acute bout of HIIE elicited greater insulin stimulation of JNK, p38 MAPK, and NK-KB signaling which coincided with whole body IS. The aforementioned study was conducted in obese middle-aged males, therefore whether this effect is also seen in females is currently lacking. Therefore, enhanced redox signaling following acute HIIE could also be contributing to the insulin sensitizing effects seen.

Chronic bouts of HIIE have also been shown to have a beneficial effect on IS and glycemic control. A recent study by Dela et al, (326) found that after a one-legged HIIE training

protocol involving 10x1minute intervals at >80% HR max for 8 sessions over 2 weeks, insulin stimulated glucose clearance was ~30% higher in the trained leg compared to the untrained leg. Similarly, a study by Sogaard et al (327) found improvements in IS after 6 weeks of HIIE training involving 5x1min intervals at >90% HRmax in both males and females. However, it is worth noting that the reported improvement was an 11% increase in IS in males and 1% increase in females (327). In healthy older adults, HIIE (4x4min at 90% peak HR) for 8 weeks reduced HOMA-IR values by 26% (328). HIIE training has also been shown to be an efficacious exercise mode to reduce IR in T2D (329). Therefore, HIIE is a viable exercise modality to increase IS and reduce IR.

Several studies have also found sex-specific physiological adaptations to both HIIE and SIE. A study by Gillen et al, (17) found that 6-weeks of SIE in overweight individuals lowered 24-h average glucose concentrations in males, but not females. There was also an increase in GLUT4 protein content in males compared to females, which may be indicative of improved insulin sensitizing capacity in males (17). However, it is important to note that the overall protein content increase in GLUT4 may not be as important as the quantity of GLUT4 that is being translocated to the plasma membrane during exercise. Additionally, a study by Metcalfe et al, (296) demonstrated improvements in IS in males after 6-weeks of SIE with no improvements seen in females undergoing the same training protocol (296). Studies that have looked at HIIE and its effects on IS have found that 6-weeks of supervised interval training improved IS in obese males and females (327); however, the response was found to be greater in males than females with glycosylated hemoglobin (HbA1C) levels being reduced after training in males only (327). Taken together, the findings of these studies suggest that interval training

may not be as effective a strategy to improve glycemic control in females as compared to males.

2.6.2 Resistance exercise

2.6.2.1 High-load resistance exercise

Resistance exercise (RE) is the primary mode of exercise used to elicit positive changes in muscle mass (330). RE increases muscle mass, muscle function, strength, and has a positive impact on cardiovascular health and bone mineral density (330, 331). Although RE has been shown to improve IS and glycemic control, it has been far less studied than MIC exercise (301, 332). Similar to aerobic exercise, trials using a single bout of RE have shown improvements in IS (333-335). While the mechanisms behind acute RE and the influence on IS are not yet fully elucidated, acute RE has been shown to increase AMPK activation and TBC1D4 Ser and Thr phosphorylation in skeletal muscle (123, 336). What is currently lacking however is if the mechanisms behind the acute response to RE are similar between the sexes.

RE may improve glycemic control via several mechanisms including through its ability to 1) increase muscle mass, which in turn will increase glucose storage capacity (training adaptation), 2) upregulate insulin signalling proteins (acute and training adaptation) and 3) induce GLUT4 translocation to the cell membrane to facilitate glucose clearance from circulation during and immediately after exercise (acute adaptation) (317). Furthermore, RE two to three times per week can increase GLUT4 concentrations and translocation by 30-70% and enhance IS by 10-48% (4, 59, 337). These beneficial adaptations on glycemic control may be

directly related to the extent of fibre recruitment during exercise (338). Recent meta-analyses have also suggested that the effects of MIC exercise and RE on glycemic control in individuals with T2D may be comparable and individuals may choose one or the other based on personal preference and feasibility while achieving the same beneficial effect through either exercise modality (301). RE has been shown to improve glycemic control in a variety of populations including older overweight individuals with prediabetes (339) and postmenopausal females (340) and is therefore an efficacious exercise modality for IS and IR.

2.6.3 Comparison between the exercise modes

While MIC exercise has been shown to be effective, it may not be as efficacious when compared to higher-intensity exercise bouts such as HIIE (341, 342) and RE (343). When comparing MIC exercise to HIIE, HIIE has been shown to induce similar (316, 344-346) or greater (347) improvements in IS in males, despite having a much lower exercise volume and time commitments (344). It has also been demonstrated that participants tend to find exercising at higher intensities more enjoyable, which could therefore increase participant compliance and program adherence (348). When comparing MIC exercise (30 mins at 60-65% $\dot{V}O_{2peak}$) to HIIE (4x1m in at 80% $\dot{V}O_{2peak}$) in a 12-week exercise training program fasting blood glucose concentrations decreased in both groups, but HbA1C levels decreased only in the HIIE group (341). Likewise, Karstoft et al (342) demonstrated that alternating walking sessions with 3-min repetitions at a low and then high intensity increased glycemic control and decreased glucose concentrations in individuals with T2D with no change in those who walked at a continuous intensity (342). Acutely, MIC exercise has been shown to increase IS relative to a

non-exercise control condition in healthy males and females with no change following an acute bout of SIE (308).

Similar to HIIE, RE may also be more efficacious at improving IS compared to MIC exercise. When comparing an acute bout of MIC exercise (1hour of cycling at 75% W_{max}) or RE (3 sets of 10 repetitions at 50-60% of 10RM), Venables et al (343), found that RE reduced plasma glucose AUC during a post-exercise oral glucose tolerance test (OGTT), whereas there was no change after MIC exercise. However, in sedentary, obese older adults who underwent a 6-month training protocol it has been found that only those in MIC exercise alone or combined MIC exercise and RE groups had improved IS compared to the RE group alone (349). Thus, there is conflicting evidence as to whether RE or MIC exercise training is best for inducing improvements in IS.

While some studies have found that high-load RE is beneficial to improve IS and markers of glycemic control (339, 340, 350), when RE is compared to MIC exercise, several studies have shown that there is a greater improvements in glycemic control and glucose handling with MIC exercise (301, 349). A study by Bacchi et al, (351) demonstrated that after 4 months of exercise intervention in patients with T2D who completed RE (3 sets of 10 reps at 70-80% 1RM) and MIC exercise (60 mins at 60-65% HR reserve), both exercise modalities decreased HbA_{1c} levels (-0.35% vs -0.40%, respectively) to a similar extent. There are studies, however that show that RE can improve glucose handling and markers of IS greater than training MIC exercise alone. After a 4-month training program that compared strength training to endurance training in males and females with T2D it was found that HbA_{1c} levels decreased in the strength training group alone (352). Additionally, there was a significant decrease in fasting blood glucose concentrations and

HOMA-IR levels both demonstrating an increase in IS in the RE group compared to the endurance exercise group (352). These findings are confirmed by Dipietro et al, (321) that demonstrated in healthy inactive older females who performed either MIC exercise or lower-intensity RE for 9 months, there were significant improvements in 2-hour glucose concentrations at 3, 6 and 9 months in the RE group but not the MIC exercise group. These findings are particularly interesting considering that in response to MIC exercise and HIIE improvements in IS have been reported to be absent or blunted in females (17, 296). As previously stated above, RE has been shown to have a positive influence on IS in both males and females, although there seems to be an influence of sex on the extent of the improvements seen with RE (10, 301). The mechanisms that underpin improvements in IS with RE alone warrant further research; however, taken together the research suggests that it may be a combination of RE and MIC exercise that will elucidate the most beneficial effects on IS and IR, however the increased time commitment of performing a combination of these exercises may present a barrier to exercise.

2.6.4 Low-load resistance exercise

The most studied mode of resistance training is high-load, low-repetition where an individual lifts a heavy load (>65% 1RM) for a low-moderate number of repetitions (6-12) for 1-3 sets (343, 351). However, recent studies have shown that low-loads can induce similar increases in muscle mass and strength as heavy loads as long as the loads are lifted to volitional failure (353-355). The current definitions for high and low load is defined as any loads >65% 1RM and any loads <60% 1RM, respectively (353). A typical low load RE bout is composed of 25-

35 repetitions per set, with 3 sets at approximately 30-50% 1RM with at least the last set completed to failure (330). An important component of low-load RE is to ensure that the participant is performing at least one of the sets of repetitions until they hit failure (dictated by being unable to perform the exercise at that given weight with proper form) in order to induce the same adaptations as high-load, low repetition RE (330, 356).

To the best of the authors knowledge LLHR RE has scarcely been studied in any population in relation to its effects on glucose handling and IS. Although traditional RE with loads of >65% 1RM are the typically prescribed resistance training program, this may not be practical for older adult populations due to an increase in adverse events such as musculoskeletal injury to cardiovascular events when compared to LLHR RE (331, 357). This is especially important for older and elderly adults who are at the greatest risk for developing T2D. A LLHR RE program may induce the same benefits of a high-load, low-repetition (HLLR) RE program on bone mineral density and muscular adaptations such as strength and hypertrophy with significantly lower adverse events (331). Furthermore, since each set of LLHR RE takes longer to complete than HLLR, LLHR may be more aerobic in nature and may induce greater cardiovascular adaptations than HLLR (358). Furthermore, as noted above, combined ET and RE may be the most effective exercise modality to improve IS, however, requires a greater time commitment to complete, which may decrease adherence. However, since LLHR RE is more aerobic in nature than high-load, low-repetition RE, yet induces similar muscular adaptations, LLHR RE may be able to induce the beneficial effects of both MIC exercise and RE on insulin sensitivity without requiring a greater time commitment.

Chapter 3: Overarching purpose and objectives

3.1 Thesis purpose

The overarching purpose of the research conducted in this thesis was to investigate the impact of different exercise modalities (MIC exercise, HIIE and a novel LLHR RE) on post-exercise glucose handling, IS and the metabolic response to exercise in recreationally active males and females without pathology. Additionally, I sought to examine whether biological sex inherently influences resting glucose metabolism and the metabolic responses at rest and following different bouts of acute exercise and whether these differences may influence post-exercise glucose uptake and IS. Lastly, I also wanted to examine whether anthropometric differences between males and females influence the glycemic response to an oral glucose tolerance test.

3.2 Objectives and hypotheses for thesis study 1

The main objectives for Study 1 were:

1. To compare the effects of an acute bout of HIIE on post-exercise glycemic control and insulin sensitivity between the sexes.
2. To examine how normalizing the relative dose of glucose consumed influences post-exercise glycemic control during an OGTT between the sexes.
3. To examine whether sex influences the content of insulin-dependent signaling proteins and/or the activation of insulin-independent signaling proteins.

4. To examine whether underlying differences in muscle glycogen utilization and/or AMPK activation during exercise differed between males and females and whether they are related to post-exercise glycemic control and/or insulin sensitivity.

The specific hypotheses for Study 1 were:

1. That the mean glucose concentration during the post-exercise OGTT would be higher and the improvement in insulin sensitivity would be blunted in females as compared with males
2. That when the dose of glucose consumed was normalized to body anthropometrics females would then have a lower glucose concentration than males.
3. That the content of insulin-dependent signaling proteins would be higher in females than males.
4. That activation of the insulin-independent signaling cascade would be blunted in females as compared with males following exercise.
5. That males would have a greater AMPK activation and muscle glycogen utilization during HIIE than females and that the extent of AMPK activation and glycogen utilization would relate to post-exercise glycemic control and insulin sensitivity.

3.3 Objectives and hypotheses for thesis study 2

The main objectives for Study 2 were:

1. To determine if there are basal differences in markers of inflammation, antioxidant status and oxidative stress between males and females.
2. To determine the effect of an acute bout of HIIE on markers of inflammation and oxidative stress.
3. To determine whether sex influences the effect of HIIE on markers of inflammation and oxidative stress

The specific hypotheses for Study 2 were:

1. That males would have greater resting levels of inflammation and oxidative stress and lower antioxidant status compared to females.
2. That HIIE would acutely increase circulating levels of IL-6 and TNFα and increase markers of oxidative damage.
3. That there would be a greater increase in markers of inflammation and oxidative stress following HIIE in males as compared with females.

3.4 Objectives and hypotheses for thesis study 3

The main objectives for Study 3 were:

1. To compare the effects of an acute bout of LLHR RE on post-exercise glycemic control and insulin sensitivity to that of an acute bout of MIC exercise.
2. To determine whether the post-exercise glycemic response and insulin sensitivity differs between males and females.

3. To compare the effects of LLHR RE and MIC exercise on insulin-independent signaling pathways.
4. To examine the effect of sex on insulin dependent and -independent signaling pathways.

The specific hypotheses for Study 3 were:

4. That an acute bout of LLHR RE would result in greater post-exercise glycemic control and insulin sensitivity than an acute bout of MIC exercise in both males and females.
5. That post-exercise glycemic control and insulin sensitivity would be blunted in females as compared with males following MIC, but not LLHR, exercise.
6. That there would be a greater activation of insulin independent signaling proteins (AMPK, Akt, TBC1D1, TBC1D4, p38 MAPK and PTEN) after an acute bout of LLHR as compared to MIC exercise.
7. That total content of insulin-signaling proteins would be greater in females, but that activation of these proteins during exercise would be blunted in females compared with males following both MIC exercise and LLHR RE.

Chapter 4: TBC1D1 Ser^{237} phosphorylation, but not insulin sensitivity, is higher following a bout of high-intensity interval exercise in healthy males as compared with females

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4.1 Abstract:

Interval training has been found to lower glucose concentrations and increase insulin sensitivity in males but not females, which may be due to inherent sex-based differences in metabolism. Twenty-four (12/sex) participants completed a bout of high-intensity interval exercise (HIIE, 10x1min at 90% HR_{max}) to evaluate whether sex influenced the physiological effects of HIIE on post-exercise glycemic control during an oral glucose tolerance test (OGTT). Given that body anthropometrics influence postprandial glucose, data were also expressed as a function of the normalized glucose dose. Additionally, we examined whether sex differences in post-exercise glycemic control were related to sex differences in muscle metabolism and/or insulin signaling proteins. HIIE increased insulin sensitivity in both sexes as characterized by the Matsuda ($p=0.03$, $\eta_p^2=0.20$) and HOMA-IR ($p=0.047$, $\eta_p^2=0.17$) indices. HIIE also lowered insulin

concentration during the OGTT ($p=0.04$, $\eta_p^2=0.18$) as compared with control. When normalized for glucose dose relative to lean body mass, glucose AUC was lower in females than males ($p=0.001$, $\eta_p^2=0.42$). TBC1D1 Ser^{237} phosphorylation increased in males, but not females, post-exercise ($p=0.02$, $\eta_p^2=0.22$). There was no difference in total insulin signaling protein content, muscle glycogen utilization or AMPK activation during exercise between the sexes. These findings indicate that when the glucose dose is normalized for differences in body composition glycemic handling is better in females and that an acute bout of HIIE improves insulin sensitivity equally in healthy males and females.

Keywords: sex-based differences, high intensity interval exercise, TBC1D1, insulin sensitivity, muscle metabolism

4.2 Introduction:

Biological sex is known to influence fuel utilization and muscle metabolism during exercise (16-18), which may ultimately lead to differential adaptations induced by training. Indeed, previous research has found that while insulin sensitivity improves following interval training in males, this effect is blunted or absent in females (323, 359, 360). Several studies have been conducted examining sex differences in fuel metabolism during interval exercise in young, healthy individuals (16, 18, 129), but have not examined whether these differences are related to differences in post-exercise glucose handling. Given that the effect of exercise on insulin sensitivity is thought to be the result of repeated acute exercise bouts (361), understanding the inherent physiological sex-based differences in muscle metabolism during interval exercise in relation to post-exercise glucose handling may provide important mechanistic insight as to why the insulin-sensitizing effect of interval training is blunted in females.

Differences in fuel metabolism during exercise can lead to differences in energy status within the myocyte, leading to differential activation of AMPK. In fact, Roepstorff et al (258) found that both AMPK Thr^{172} phosphorylation and α_2AMPK activity were increased in males, but not females following an acute bout of aerobic exercise. Furthermore, other studies have found that AMPK phosphorylation, mRNA expression and enzyme activity are higher in males than females at rest (362, 363). AMPK is thought to be critical to enhance post-exercise insulin-independent and -dependent glucose uptake and insulin sensitivity (76, 116, 311). Exercise-induced increases in AMPK activity increase TBC1D1 phosphorylation, promoting glucose uptake by inhibiting Rab GTPase activity, promoting GTP binding to Rabs therefore allowing

GLUT4 translocation to the plasma membrane (46, 76). Furthermore, increased AMPK activity during exercise also promotes insulin-stimulated phosphorylation of TBC1D4 following exercise, leading to enhanced insulin sensitivity by also allowing GLUT4 translocation to the plasma membrane (124, 311). Thus, the finding that AMPK activity increases in response to exercise in males only could be a potential reason why insulin sensitivity does not increase in response to interval training in females; however, whether differences in AMPK activation following an acute bout of HIIE are related to differences in post-exercise glucose handling in males and females has not been examined.

It is widely reported that males rely to a greater extent on carbohydrate stores during exercise (19, 20, 364). Low muscle glycogen content is an important regulator of glucose uptake and insulin sensitivity (365), thus differences in muscle glycogen utilization during exercise may also contribute to differences in post-exercise and training-induced insulin sensitivity. Whether muscle glycogen utilization is greater in males during interval exercise is controversial with one study finding that glycogen utilization was 50% greater in type I muscle fibres of males during sprint interval exercise (18), whereas another study found no difference in muscle glycogen utilization between the sexes (16). Importantly, in both trials the interval exercise was of short duration (maximum 7 minutes) and may have not been long enough to detect differences in fuel metabolism. To the best of our knowledge, no trial has examined sex differences in muscle glycogen utilization during interval exercise of longer duration, which is more representative of interval exercise training protocols that have been found to improve insulin sensitivity (329). Furthermore, whether sex differences in muscle glycogen utilization during interval exercise relates to post-exercise glucose uptake and insulin sensitivity has not been examined.

Individuals are classified for risk and presence of type 2 diabetes based on fasting plasma glucose and 2-hour glucose concentration following an oral glucose tolerance test (OGTT) (239). It has been repeatedly found that males are more frequently classified as having impaired fasting glucose, whereas females are classified as having impaired glucose tolerance due to elevated glucose concentrations at the end of the OGTT (239-241). Recent trials have shown that 2-hour glucose concentration, but not fasting glucose concentration, is negatively associated with body surface area and height (240, 241) and thus the greater prevalence of impaired glucose tolerance in females is likely due to the fact that the relative dose of glucose consumed during the OGTT is greater. Thus, the purpose of the present study was to examine whether sex inherently influences glycemic control during an oral glucose tolerance test conducted after an acute bout of HIIE in young, healthy males and females while taking into consideration the relative dose of glucose consumed. Additionally, we sought to examine whether metabolic differences in AMPK activation and glycogen utilization were related to post-exercise glycemic control and insulin sensitivity and whether there were sex-based differences in the content of proteins related to insulin signaling and exercise-induced activation of insulin-independent signaling. We chose to study young, healthy males and females in order to determine whether there are inherent sex differences in insulin-signaling proteins at rest or in metabolism during exercise that are related to post-exercise glycemic control in the absence of pathology.

4.3 Methods:

4.3.1 Participants

Twenty-four recreationally active young males (*n*=12) and females (*n*=12) took part in the study (Table 4.1). Participants were deemed recreationally active based on self-reported habitual physical activity indicating that they participated in no more than 3 sessions of cardiovascular exercise or 2 sessions of resistance exercise per week. Additionally, participants were excluded if they had any chronic health conditions, were unable to complete a single exercise session, had an allergy to local anaesthetic, were taking prescription anti-coagulant or anti-platelet medications, were unable to exercise as suggested by the Get Active Questionnaire (GAQ) or had a BMI >27 kg/m^2. Females were excluded if they were taking any form of monophasic birth control. Participants were instructed to maintain their habitual diet and physical activity throughout the trial. The study protocol was reviewed and received ethics clearance from the University of Waterloo Research Ethics Committee (ORE #22477). Prior to commencing the trial all participants provided written informed consent. The study conformed with all standards outlined by the Tri-Council Policy Statements for Ethical Conduct for Research Involving Humans (TCPS 2).

4.3.2 Experimental protocol

The experimental protocol consisted of 4 preliminary visits and an acute exercise session (Figure 4.1). The first preliminary visit included consent and anthropometric measurements (height, weight, BMI). At the end of this visit participants were instructed on how to complete a 3-day food log to determine habitual dietary intake and were given a pedometer to track habitual

physical activity to allow for comparison of habitual diet and physical activity between the sexes. The second preliminary visit included a DXA scan (non-fasted, DXA, Hologic Discovery W with QDR APEX software version 4.5.3, Mississauga, ON, Canada) for determination of body fat and lean body mass (LBM) and an assessment of aerobic fitness (VO_2 peak test). LBM measured from the DXA scan includes mass of muscle, vital organs, extracellular fluid and lipids in cellular membranes (366). The third and fourth visits included a familiarization session to the HIIE bout, and a control OGTT (75 g glucose, Trutol™, Thermo Scientific, Waltham, Massachusetts, United States), respectively. On the 5th visit participants completed the acute HIIE exercise bout with a blood draw taken prior to exercise and muscle biopsies taken immediately pre- and post-exercise. Ninety minutes after the end of the acute exercise bout participants underwent a 120 min OGTT. We conducted the post-exercise OGTT 90 minutes after the cessation of exercise in order to examine the effects of exercise on insulin-stimulated glucose uptake as previous research has found that exercise-induced GLUT4 at the sarcolemma reinternalizes ~85 minutes after the cessation of exercise (53). On the day before visits 4 and 5 participants consumed the same diet to minimize differences in fuel storage between assessments. Given that menstrual phase can influence fuel metabolism during exercise (20) and insulin sensitivity (367), females were tested in the midfollicular phase of the menstrual cycle (day 5-9 after the beginning of menses). Pre-exercise blood samples were assessed for estradiol and progesterone using commercially available kits (DKO003 and DKO006, Diametra, Spello PG, Italy). The overall study schematic is found in Figure 4.1.

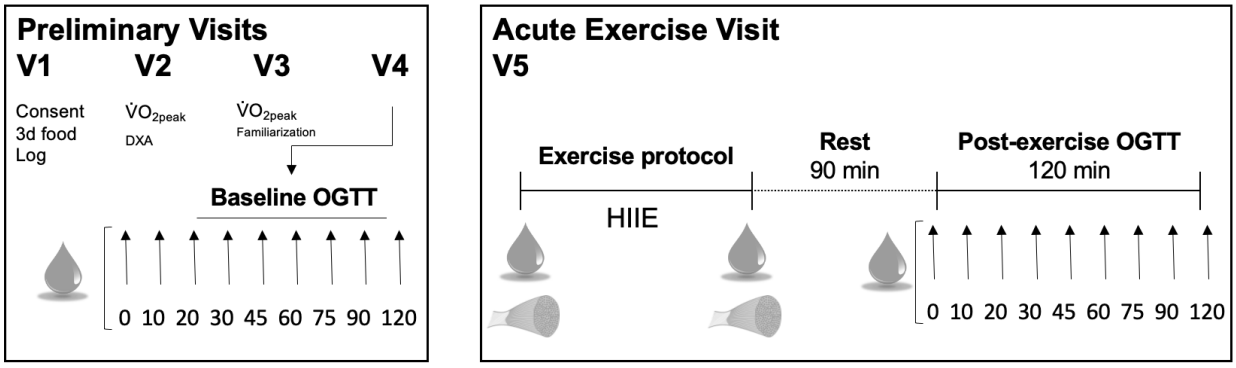

Figure 4.1: Protocol overview for preliminary visits (V1-V4), as well as the acute exercise visit (V5). HIIE, high-intensity interval exercise; OGTT, oral glucose tolerance test; $\dot{V}O_2$peak, peak oxygen consumption; DXA, dual-energy X-ray absorptiometry.

4.3.3 $\dot{V}O_2$ peak testing

To appropriately match males and females for aerobic fitness and to determine the work rate corresponding to 90% HR_{max} for the acute HIIE trial, participants underwent an incremental cycling test to volitional fatigue on an electronically braked cycle ergometer (Ergoselect 100, ergoline GmbH, Germany) using an online gas collection system (Vmax encore CPET Systems, Vyaire medical, Chicago). Participants began with a warmup at 50W for 2mins and thereafter the intensity was increased by 1W every 2s until volitional fatigue or the point at which pedal cadence fell below 50 rpm. A metabolic cart with an online gas collection system measured oxygen consumption and carbon dioxide production. The $\dot{V}O_{2peak}$ test was considered valid if the participant achieved at least 3 of the following criteria: a RER >1.15, blood lactate >8mmol/L, RPE >17, a failure of HR to increase with increased exercise intensity, and a $\dot{V}O_2$ increase of <2.2ml/kg/min with increased exercise intensity. In order to ensure that any sex-based differences are not the result of differences in aerobic fitness, males and females must be matched for aerobic fitness ($\dot{V}O_{2peak}$) relative to LBM (220). Heart rate was measured every

minute during the $\dot{V}O_{2peak}$ test using a chest strap HR monitor (H9 heart rate sensor, Polar, Kempele, Findland). The intensity for the acute HIIE bout was calculated from the highest HR reached during the $\dot{V}O_{2peak}$ test. Participants returned to the lab at least 24-hours after the $\dot{V}O_{2peak}$ testing and cycled at the prescribed HIIE wattage for 3-4 repetitions to confirm that the work rate was appropriate to achieve the target HR of 90% HR_{max}.

4.3.4 Oral glucose tolerance test

For the control OGTT, participants reported to the laboratory between 730-900am after a 12-hour overnight fast. Prior to the OGTT participants refrained from exercise for 72 hours and alcohol for 24 hours. Furthermore, on the day prior to the OGTT participants recorded everything they ate and repeated this diet prior to the acute exercise session to ensure similar nutrient availability. Upon arrival in the laboratory participants rested quietly and an indwelling catheter was inserted into a prominent forearm vein and a fasted blood sample was taken. Participants then consumed 75 grams of glucose and blood samples were taken at 10, 20, 30, 45, 60, 75, 90 and 120 minutes after drink consumption. Heparinized plasma samples were analysed for glucose and insulin concentrations. Blood glucose concentration was analysed using a spectrophotometric glucose assay using PGO enzyme preparation (Sigma-Aldrich, St. Louis, M0). Insulin concentrations were analysed using radioimmunoassay (RIA) kits (#HI-14K) according to manufacturer's instruction (Millipore Sigma, Burlington, Massachusetts, United States). Glucose and insulin area under the curve (AUC) values were calculated using the trapezoidal rule. Maximal glucose concentration (Cmax) represents the maximum glucose concentration that occurred during the OGTT. Time of maximal glucose concentration (Tmax) is

the time during the OGTT when the Cmax occured. Glucose concentrations and AUC values are also reported normalized to the glucose dose relative to body weight, LBM and height. For each normalization the relative dose was determined by dividing the glucose dose (75g) by body weight (kg), height (cm) or LBM (kg). HOMA-IR was calculated according to the formula: fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5. The Matsuda index was calculated according to the formula: [10,000 / √glucose minute 0 x insulin minute 0) (mean glucose (OGTT) x mean insulin OGTT)](368).

4.3.5 Acute high intensity exercise session

On the morning of the acute exercise session participants arrived at the laboratory following a 12-hour overnight fast. Additionally, participants refrained from alcohol for 24 hours and physical activity for 72 hours prior to the acute exercise bout. Upon arrival in the laboratory participants rested quietly and an indwelling catheter was inserted into a prominent forearm vein and a fasted blood sample was taken. A muscle biopsy (~100-150 mg) was then taken from the *vastus lateralis* muscle approximately 20cm proximal to the knee using a custom suction-modified Bergstrom needle (5 mm diameter) as previously described (369). The muscle sample was dissected free of fat and connective tissue and was snap frozen in liquid nitrogen and stored at -80˚C for analysis of muscle glycogen and protein content of enzymes/signaling proteins related to muscle metabolism and insulin-dependent and -independent signaling. Participants then commenced the acute exercise bout starting with a 5-min warm up at 50W, followed by 10 intervals of 60 sec at 90% HR_{max} interspersed with 60 sec at low intensity (50W) and ending with a 5 min cool down at 50W. Heart rate (HR) was measured at the end of each

minute in the warmup, cool down and at the end of each high and low interval. This specific protocol where the low intensity work rate was the same for males and females was chosen to represent a typical "low volume" interval exercise session that constitutes <10 mins of intense exercise and has been previously shown to induce favourable changes in IS (326, 327, 370). A second muscle biopsy was taken from the same leg immediately post exercise. Participants then sat quietly for 90 minutes and then completed a post-exercise OGTT identical to that performed for control. Average exercise heart rate (HR) was determined by averaging the HR at the end of each high and low intensity interval. Maximal work rate is the average of the wattage of the high-intensity bouts for males and females. Estimated energy expenditure during the HIIE bout was calculated using metabolic equations where the cycling work rates were used to determine the oxygen cost of the low and high intervals and then converted to calories burned (1 L O_2 consumed = 5 kcal) (371).

4.3.6 Muscle analysis

Muscle samples for Western Blot analyses were homogenized in ice cold 25mM Tris buffer [25mM Tris, 0.5% (v/v) Triton X-100 with protease/phosphatase inhibitor tablets (Roche Diagnostics, Laval, QC, Canada)] at a ratio of 10μL buffer to 1mg of muscle in a prechilled homogenization Biopur Eppendorf (Eppendorf, Mississauga, ON, Canada) using a bead homogenizer (TissueLyser II, Qiagen, Toronto, ON, Canada) run at 20 cycles/second for 40 seconds. Once the samples were sufficiently homogenized, they were spun at 10,000G for 10 minutes at 4^{o}C. The supernatant was separated and allocated into a prechilled Eppendorf while the pellet was frozen for potential use in additional analyses. Western blot analysis was

conducted using previously described techniques (372). Briefly, protein concentration of homogenates was determined using the BCA protein assay technique (Thermo Fischer Scientific, Waltham, MA) and equal amounts of protein were prepared in 4x Laemmli's buffer and then separated using 10% SDS-PAGE on 4%-15% Criterion TGX Stain-free protein gels (Bio-Rad, Hercules, CA) at 200V for 40mins and electro transferred to PVDF membranes. A protein ladder (Precision Plus Protein Standard, Bio-Rad, Hercules, CA, USA) and a standard curve (pooled from all samples) were run on every gel. Total protein and visual confirmation of protein transfer was done pre- and post-membrane transfer, respectively, using a Chemidoc MP (Bio-Rad, Hercules, CA, USA). Following 1h block in 5% bovine serum albumin (BSA) in 1X Tris-buffered saline and Tween 20 (TBST), membranes were incubated in primary antibody overnight at 4°C in 5% BSA/TBST based on previously optimized conditions. The primary antibodies used for western blotting were phosphorylated AMPKα Thr^{172} (2531S, 1:1000, Cell Signaling, Danvers, MA, USA), total AMPKα (2532S, 1:1000, Cell Signaling, Danvers, MA, USA), total Akt (9272S, 1:1000, Cell Signaling, Danvers, MA, USA), phosphorylated TBC1D4 Thr^{642} (4288S, 1:1000, Cell Signaling, Danvers, MA, USA), total TBC1D4 (2447S, 1:1000, Cell Signaling, Danvers, MA, USA), phosphorylated TBC1D1 Ser^{237} (07-2268, 1:1000, Millipore Sigma, Burlington, MA, USA), phospho-Acetyl-CoA Carboxylase Ser^{79} (3661S, 1:1000, Cell Signaling, Danvers, MA, USA), total PTEN (9552S, 1:1000, Cell Signaling, Danvers, MA, USA) and total GLUT4 (ab654, 1:1000, Abcam, Toronto, ON, Canada). After 3x5 min washes in TBST, membranes were incubated in horseradish peroxidase-conjugated goat anti-rabbit secondary (170-6515, Bio-Rad, Hercules, CA, USA) diluted to 1:20,000 in 5% BSA/TBST for 1h at room temperature and washed in TBST for 3x5 min. Membranes were then incubated for 1min with enhanced chemiluminescence (ECL) (Clarity

Western ECL Substrate, Bio-rad, Hercules, CA, USA) and imaged on the Chemidoc imaging system (Bio-Rad, Hercules, CA, USA). Bands were quantified using Image Lab software (Version 3.0, Bio-Rad, Hercules, CA, USA) and protein content was normalized within and between blots using total protein and the standard curve obtained from the gel, as previously described (372, 373).

4.3.7 Glycogen determination

For determination of muscle glycogen concentration frozen muscle tissue was first freeze-dried and processed using previously published methods (374-376). Briefly, pre-cooled perchloric acid (0.5M $HClO_4$) was added to an Eppendorf containing 3-5 mg of freeze-dried muscle sample. Samples were left for 10mins to allow for sufficient breakdown of muscle tissue and extraction of metabolites and then the supernatant was removed. The pellet was used for determination of muscle glycogen content as previously described (374). Briefly, hydrochloric acid (2M HCl) was added to the glycogen pellet (0.650mL/5 mg dry tissue) and heated at 100°C for 2 hours. Heated Eppendorf tubes were re-weighed and brought back to pre-heated weight with water. Sodium hydroxide (0.650mL/5 mg dry tissue; 2M NaOH) was subsequently added to the heated Eppendorf tube to neutralize the solution. The sample was further diluted (1µl sample: 4µl water) for use in the fluorometric determination of glycogen concentration.

Glycogen was measured using an NADP-linked assay as previously described (377). Briefly, a reaction mixture was created with 50mM tris (pH 8.1; T1503-100G, Sigma-Aldrich, St. Louis, Massachusetts, USA), 1mM $MgCl_2$ (10128-478, VWR, Mississauga, Ontario, Canada), 0.5mM dithiothreitol (646563, Sigma-Aldrich, St. Louis, Massachusetts, USA), 300µM ATP (ATP007.1,

BioShop, Burlington, Ontario, Canada), 50µM NADP (05-408-129, Fisher Scientific, Waltham, Massachusetts, USA), and 0.02U/mL glucose-6-phosphate dehydrogenase (10127655001, Sigma-Aldrich, St. Louis, Missouri, USA). In a black walled, black bottomed 96-welled plate, 200µl of the reaction mixture and 10µl of sample were added to the plate in triplicate. The plate was read fluorometrically [excitation= 365nm, emission= 455nm, Cytation5 (BioTek Instruments, Winooski, Vermont, USA)] after which 0.14U/mL of hexokinase (11426362001, Sigma-Aldrich, St. Louis, Missouri, USA) was added to each well and the plate was incubated in the dark at room temperature for 60 minutes. After incubation, the plate was once again read fluorometrically [excitation= 365nm, emission= 455nm, Cytation 5 (BioTek Instruments, Winooski, Vermont, USA)].

4.3.8 Statistical analysis:

All statistical analyses were conducted using SPSS (version 25, IBM, Armonk, NY, USA). Baseline differences between groups and total protein content of insulin-dependent and -independent signaling were assessed using a non-paired t-test. 2-way mixed model ANOVA with sex (2 levels, male/female) as the between variable and time (2 levels, pre/post exercise) as the within variable was used to determine the effects of sex and exercise on all other muscle variables. 2-way mixed model ANOVA with sex as the between variable and trial (Control/Exercise) as the within variable was used to determine the effects of sex and trial on all measures during the OGTT with the exception of glucose and insulin concentrations during the OGTT. 3-way mixed model ANOVA with sex as the between variable and trial and OGTT timepoint (6 or 9 levels) as the within variables was used to determine the effects of sex and trial on glucose

and insulin concentrations during the OGTT. Data sets were assessed for normality using the Shapiro-Wilk test and were found to be not normally distributed. Thus, values were log transformed prior to undergoing statistical analyses using ANOVA. ANOVA results using transformed data sets were consistent with using nontransformed data sets. For consistency, the ANOVA analyses reported are based on nontransformed data sets for all results, with the exception of insulin and insulin sensitivity data from the OGTT. Post-hoc analyses were conducted using a Tukey's HSD test where appropriate. Significance was set at $p \leq 0.05$. Partial eta-squared (η_p^2) values were calculated to estimate the effect sizes (small 0.04, medium 0.25, large 0.64) for main effects and interactions where necessary. Cohen's *d* values were calculated to estimate effect sizes (small 0.2, medium 0.5, large 0.8) for t-tests. In order to examine whether there was a relationship between activation of AMPK and/or glycogen utilization during exercise and post-exercise insulin sensitivity we performed correlational analyses. We correlated HOMA-IR and Matsuda Index with the change (pre – post) in AMPK phosphorylation, change in glycogen and post-exercise glycogen content. Additionally, we correlated the post-exercise glucose AUC during the OGTT with the change in glycogen content, post-exercise glycogen content and the change in AMPK phosphorylation. All data are presented as means ± SEM for *n*= 12 in each group. All graphs were created using GraphPad Prism (GraphPad Software Inc., CA).

4.4 Results:

4.4.1 Subject characteristics

Subject characteristics are found in Table 4.1. As to be expected there were significant differences in weight, %BF, LBM, and $\dot{V}O_{2peak}$ between males and females. Importantly, once

$\dot{V}O_{2peak}$ was adjusted based on LBM, rather than body weight, there was no significant difference between males and females, indicating that our males and females had similar aerobic fitness. Additionally, males had a significantly greater maximal workrate and estimated energy expenditure during the HIIE bout compared to females. When estimated energy expenditure was normalized to body weight males still expended more energy during the acute session compared to females (Table 4.1, p=0.02). Interestingly, when we normalized energy expenditure to LBM, females expended more energy compared to males (Table 4.1; p<0.001).

4.4.2 Plasma hormone concentrations

Serum estradiol (pg/mL) and progesterone (ng/mL) were not significantly different between males and females (p=0.21 and p=0.78, respectively) (Table 4.1).

Table 4.1: Participant characteristics

	Males	**Females**	**p value**	**Cohen's d**
Age (years)	22 ± 1	21 ± 1	p=0.45	*d*=0.46
Weight (kg)	76.6 ± 2.4	62.6 ± 3.1	p<0.01*	*d*=1.46
Height (cm)	177.9 ± 2.0	162.7 ± 2.6	p<0.01*	*d*=1.95
Body Mass Index (kg/m^2)	23.7 ± 0.8	22.9 ± 0.6	p=0.45	*d*=0.32
Body Fat (%)	20.9 ± 1.6	32.7 ± 1.3	p<0.001*	*d*=2.39
Fat Mass (kg)	15.4 ± 1.4	19.5 ± 1.2	p<0.05*	*d*=0.92
Lean Body Mass (kg)	55.1 ± 2.2	37.6 ± 1.8	p<0.001*	*d*=2.53
Fasting Blood Glucose (mmol/L)	5.1 ± 0.2	5.4 ± 0.2	p=0.43	*d*=0.33
Estradiol (pg/mL)	25.5 ± 5.7	38.9 ± 8.8	p=0.21	*d*=0.52
Progesterone (ng/mL)	3.6 ± 0.6	3.4 ± 0.6	p=0.78	*d*=0.10

$\dot{V}O_{2peak}$ (ml/kgBW/min)	43.1 ± 1.6	34.2 ± 1.4	p<0.001*	*d*=1.67
$\dot{V}O_{2peak}$ (ml/kgLBM/min)	59.5 ± 1.4	56.5 ± 1.7	p=0.21	*d*=0.52
Daily Average Steps	8487 ± 485	8603 ± 1043	p=0.92	*d*=0.05
Maximal Workrate (W)	199.2 ± 10.3	118.5 ± 8.5	p<0.001*	*d*=2.42
Average Heartrate (bpm)	151 ± 3	157 ± 2	p=0.12	*d*=0.51
Estimated Energy Expenditure (kcals)	240.0 ± 7.6	181.9 ± 7.5	p<0.001*	*d*=2.22
Estimated Energy Expenditure normalized to Body Weight (kcals/kgBW)	3.2 ± 0.1	3.0 ± 0.0	p=0.02	*d=1.00*
Estimated Energy Expenditure normalized to Lean Body Mass (kcals/kgLBM)	4.4 ± 0.3	4.9 ± 0.2	p<0.01	*d=1.96*

All results are shown as mean ± SE, n = 12 males, and n = 12 females.
* Significance from independent samples t-test, significantly different with p value <0.05

4.4.3 Habitual dietary intake

Table 4.2 shows the average dietary intake of participants from the 3-day food log. Males consumed more calories per day than females (p<0.01). Males also consumed more protein (p<0.01), fat (p<0.01) and carbohydrates (p<0.01) compared to females. However, when expressed as percent of total daily caloric intake, males and females ate similar percentages of protein, fat and carbohydrates. Males and females also consumed similar g/kg/day of protein, carbohydrates, and fat. The diet consumed the day before the CON and HIIE trials did not differ (Table 4.2).

Table 4.2: Dietary intake data

	Habitual Dietary Intake				Day Before CON/HIIE Diet			
	Males	**Females**	**p value**	**Cohen's d**	**Males**	**Females**	**p value**	**Cohen's d**
Kcal	2008.0 ± 119.9	1476.8 ± 60.7	**p<0.01**	*d*=0.93	2137.2 ± 232.4	1502.1 ± 133.3	**p=0.03**	*d*=0.97
PRO								
g	96.4 ± 7.8	65.0 ± 3.4	**p<0.01**	*d*=0.87	99.7 ± 14.4	64.7 ± 7.5	**p=0.04**	*d*=0.88
g/kgBW/d	1.3 ± 0.1	1.1 ± 0.1	p=0.10	*d*=0.39	1.4 ± 0.2	1.0 ± 0.1	p=0.10	*d*=0.70
% Of Kcals	18.8 ± 1.2	17.4 ± 0.8	p=0.35	*d*=0.23	18.5 ± 1.9	17.1 ± 1.8	p=0.61	*d*=0.21
FAT								
g	79.1 ± 5.6	56.5 ± 3.6	**p<0.01**	*d*=0.80	83.1 ± 11.2	61.9 ± 7.3	p=0.13	*d*=0.65
g/kgBW/d	1.0 ± 0.1	0.9 ± 0.1	p=0.26	*d*=0.25	1.1 ± 0.2	0.9 ± 0.1	p=0.19	*d*=0.49
% Of Kcals	34.8 ± 1.4	33.1 ± 1.4	p=0.38	*d*=0.20	34.8 ± 3.7	35.6 ± 2.8	p=0.87	*d*=0.07
CHO								
g	234.0 ± 15.8	185.6 ± 9.2	**p=0.01**	*d*=0.63	254.0 ± 32.5	180.7 ± 20.1	**p=0.07**	*d*=0.78
g/kgBW/d	3.1 ± 0.2	3.1 ± 0.2	p=0.86	*d*>0.01	3.5 ± 0.5	2.7 ± 0.3	p=0.12	*d*=0.62
% Of Kcals	46.3 ± 1.7	49.5 ± 1.5	p=0.16	*d*=0.34	46.7 ± 3.2	47.2 ± 2.8	p=0.89	*d*=0.05

All results are shown as mean ± SEM, n = 12 males and n = 12 females. PRO; protein, FAT; total fat, CHO; carbohydrates

* Significance from independent samples t-test, significantly different with p value <0.05

4.4.4 Plasma Glucose

Absolute blood glucose concentrations during the control and post-HIIE OGTT in males and females are shown in Figure 4.2A. As expected, there was a main effect of time ($p<0.001$, $\eta_p^2=0.51$) showing that blood glucose concentration increased and was significantly greater than baseline by 10 minutes after consumption, remained elevated throughout the OGTT, and declined but remained elevated above baseline at the end of the OGTT ($p<0.05$ for all comparisons). There was no effect of sex ($p=0.28$, $\eta_p^2=0.05$) and no effect of trial (control vs. HIIE, $p=0.64$, $\eta_p^2=0.01$) for blood glucose concentrations during the OGTT. There was no sex x trial ($p=0.87$, $\eta_p^2=0.001$) or sex x time interaction ($p=0.39$, $\eta_p^2=0.04$); however, there was a significant trial x time interaction ($p=0.01$, $\eta_p^2=0.14$) with the post-hoc indicating that glucose concentration was greater post-HIIE than control at 10mins, 20mins and 75mins into the OGTT. There was no sex x trial x time interaction ($p=0.65$, $\eta_p^2=0.03$). Additionally, there was no effect of sex ($p\geq0.26$, $\eta_p^2\geq0.00$), trial ($p\geq0.39$, $\eta_p^2\geq0.00$) or sex x trial interaction ($p\geq0.27$, $\eta_p^2\geq0.00$) for glucose AUC, glucose Cmax or glucose Tmax (Table 4.3).

Normalizing glucose concentrations during the OGTT for the glucose dose relative to body weight ($p=0.03$, $\eta_p^2=0.21$) and LBM ($p<0.001$, $\eta_p^2=0.47$) indicated that glucose concentrations were higher in males than females (Figure 4.2B, C) during the OGTT. There was no difference however when glucose values were normalized for the glucose dose relative to height ($p=0.53$, $\eta_p^2=0.02$; Figure 4.2D). Furthermore, there was a main effect of sex indicating that glucose Cmax was lower in females when the glucose dose was normalized relative to LBM ($p=0.001$, $\eta_p^2=0.38$; Table 4.3), but not total body weight ($p=0.07$, $\eta_p^2=0.14$) or height ($p=0.73$, $\eta_p^2=0.01$). Lastly, glucose AUC was lower in females when normalized for the glucose dose

relative to LBM (p=0.001, η_p^2=0.42), but not when normalized for body weight (p=0.05, η_p^2=0.16) or height (p=0.71, η_p^2=0.01).

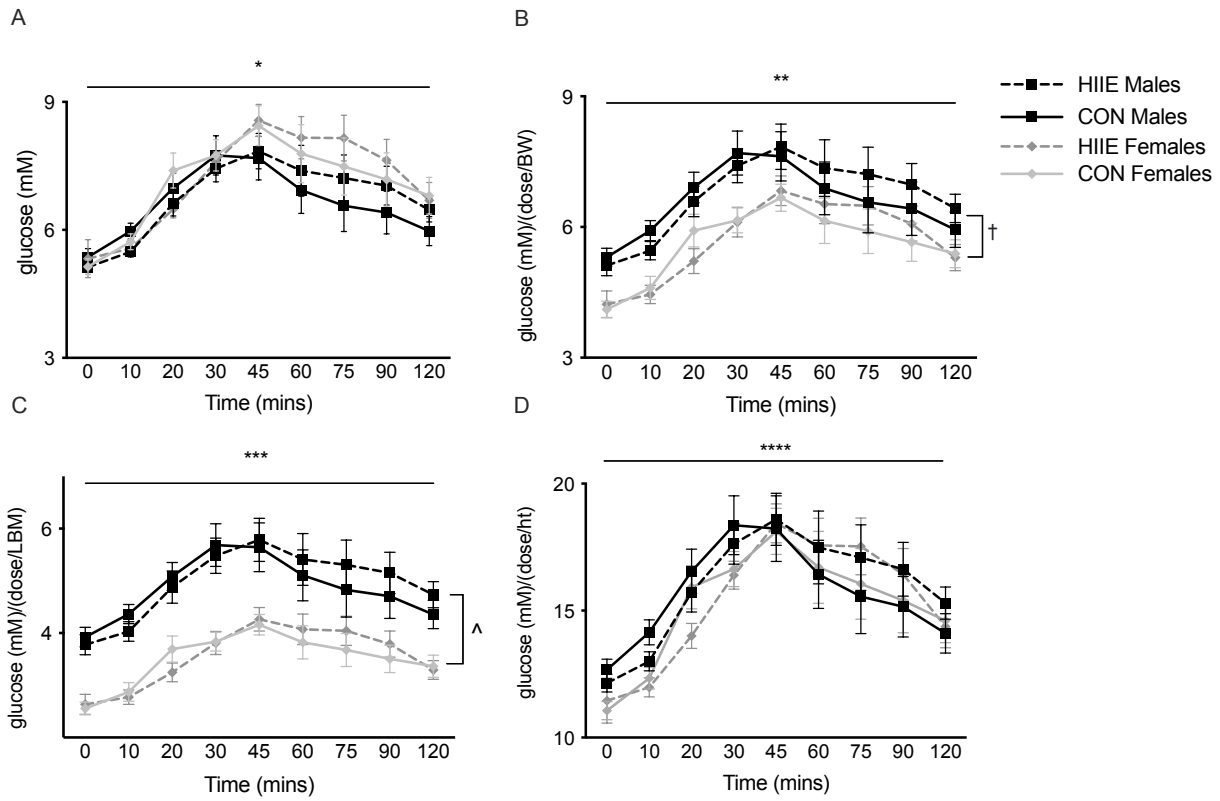

Figure 4.2**:** Control and post-HIIE oral glucose tolerance test glucose curves in males (n=12) and females (n=12). A) glucose (mmol/L) concentrations * represents significant main effect of time (p<0.001, η_p^2=0.51), B) Glucose (mmol/L) relative to dose normalized to body weight (kg) ** represents significant main effect of time (p<0.001, η_p^2=0.93), † represents significant main effect of sex (p=0.03, η_p^2=0.21), C) glucose (mmol/L) relative to dose normalized to LBM (kg) *** represents significant main effect of time (p<0.001, η_p^2=0.91), ^ represents significant main effect of sex (p<0.001, η_p^2=0.47) D) glucose (mmol/L) relative to dose normalized to height (cm) ****represents significant main effect of time (p<0.001, η_p^2=0.93)

4.4.5 Plasma insulin

Absolute plasma insulin concentrations during the control and post-HIIE OGTT in males and females are shown in Figure 4.3. As expected, blood insulin concentration increased after glucose consumption and was significantly higher by 10 mins and remained elevated at the 120min mark ($p<0.001$, $\eta_p^2=0.85$). There was no effect of sex ($p=0.33$, $\eta_p^2=0.04$); however, there was a main effect of trial on insulin concentration ($p=0.04$, $\eta_p^2=0.18$) indicating that insulin was lower following HIIE than control. While it did not reach statistical significance, the sex x trial interaction ($p=0.056$, $\eta_p^2=0.16$) has a medium effect size and therefore suggests that insulin concentration was lower in males following HIIE compared with control, but not females. There were no sex x time ($p=0.16$, $\eta_p^2=0.08$) or trial x time ($p=0.93$, $\eta_p^2=0.01$) interactions. Additionally, there was no sex x trial x time interaction ($p=0.57$, $\eta_p^2=0.03$). There was no effect of sex ($p\geq0.19$, $\eta_p^2\geq0.05$), trial ($p\geq0.29$, $\eta_p^2\geq0.00$), or sex x trial interaction ($p\geq0.09$, $\eta_p^2\geq0.02$) for insulin Cmax, insulin Tmax or insulin AUC (Table 4.3).

Figure 4.4 shows indices of insulin sensitivity during the control and post-HIIE OGTT in males and females. There was no effect of sex ($p=0.76$, $\eta_p^2<0.001$) and no sex x trial interaction ($p=0.58$, $\eta_p^2=0.01$) for the Matsuda Index; however, there was a significant main effect of trial ($p=0.03$, $\eta_p^2=0.20$), indicating that HIIE increased insulin sensitivity characterized by the Matsuda index. There was no main effect of sex ($p=0.39$, $\eta_p^2=0.34$) on HOMA-IR and no sex x trial interaction ($p=0.72$, $\eta_p^2=0.01$), however there was a significant effect of trial ($p=0.047$, $\eta_p^2=0.17$) indicating that males and females were less insulin resistant following HIIE characterized by HOMA-IR.

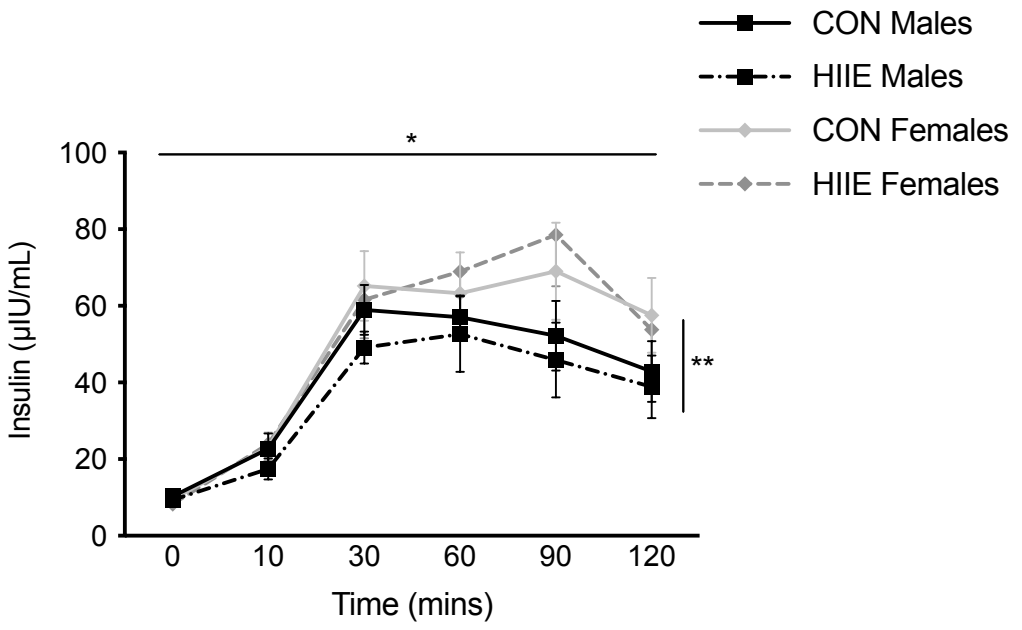

Figure 4.3: Control and post-HIIE oral glucose tolerance test insulin curves in males (n=12) and females (n=12). * Represents significant main effect of time ($p<0.001$, $\eta_p^2=0.85$) ** Represents significant main effect of trial ($p=0.04$, $\eta_p^2=0.18$).

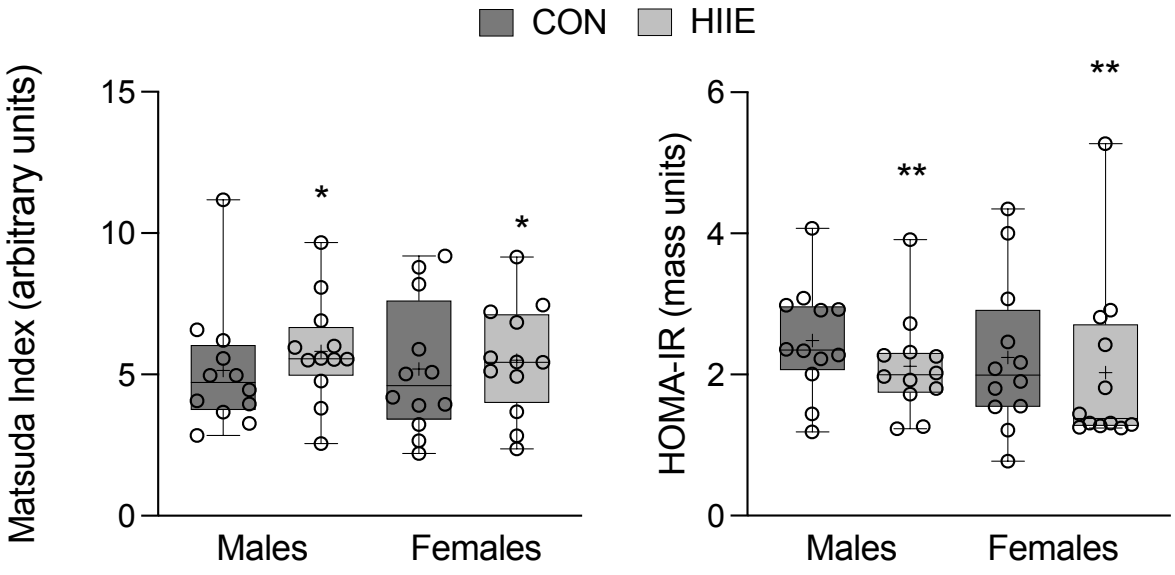

Figure 4.4**:** Indices of insulin sensitivity during control and post-exercise OGTT. A) Matsuda index, *denotes main effect of trial ($p=0.03$, $\eta_p^2=0.20$), B) HOMA-IR index, ** denotes significant main effect of trial ($p=0.047$, $\eta_p^2=0.17$).

Table 4.3. Glucose and insulin measurements during the control and post-HIIE OGTT in males and females

Variable	Males		Females		p values		
	CON	HIIE	CON	HIIE	S	T	SxT
Glucose AUC (mmol/L · 120min)	804.6 ± 40.2	831.8 ± 38.5	873.2 ± 50.9	887.4 ± 35.9	0.26	0.39	0.79
Glucose AUC (mmol/L · 120min) by dose/BW	800.6 ± 49.7	828.1 ± 47.1	691.4 ± 37.0	708.1 ± 33.7	0.052	0.33	0.81
Glucose AUC (mmol/L · 120min) by dose/LBM	590.1 ± 38.1	611.1 ± 38.1	430.6 ± 23.0	441.3 ± 21.9	**0.001**	0.32	0.74
Glucose AUC (mmol/L · 120min) by dose/ht	1905.5 ± 99.6	1969.4 ± 93.5	1874.5 ± 100.2	1910.4 ± 72.7	0.71	0.37	0.80
Glucose Cmax (mmol/L)	8.4 ± 0.4	8.4 ± 0.4	9.1 ± 0.5	9.1 ± 0.4	0.28	0.99	0.94
Glucose Cmax by dose/BW	8.4 ± 0.5	8.4 ± 0.5	7.2 ± 0.4	7.2 ± 0.4	0.07	0.89	0.96
Glucose Cmax by dose/LBM	6.2 ± 0.4	6.2 ± 0.4	4.5 ± 0.3	4.5 ± 0.3	**0.001**	0.87	0.93
Glucose Cmax by dose/ht	20.0 ± 1.1	20.0 ± 1.0	19.5 ± 1.0	19.5 ± 0.9	0.73	0.97	0.96
Glucose Tmax (mmol/L)	54.6 ± 9.5	51.3 ± 6.0	46.7 ± 7.7	53.8 ± 5.0	0.77	0.69	0.27
Insulin AUC (mmol/L · 120min)	5784.3 5761.2	5076.6 ± 720.3	6860.2 ± 947.9	7172.3 ± 1035.3	0.20	0.29	0.09
Insulin Cmax (µIU/mL)	70.8 ± 7.8	66.7 ± 8.1	86.2 ± 11.9	91.0 ± 12.8	0.19	0.90	0.53
Insulin Tmax (µIU/mL)	60.0 ± 7.4	60.0 ± 8.3	65.0 ± 8.9	80.0 ± 9.3	0.29	0.44	0.40

All results are shown as mean ± SEM, males (n=12) and females (n=12). P values are listed in order as sex (S), trial (T) and sex x trial (SxT) from RM ANOVA. AUC – area under the curve, BW – body weight in kg, Cmax – maximum concentration, LBM – fat-free mass in kg, ht – height in cm, Tmax – time of maximum concentration.

4.4.6 Muscle glycogen content

Muscle glycogen decreased pre (443.4mmol/mg ± 207.2, 387.8mmol/mg ± 139.3) to post-HIIE exercise (271.4mmol/mg ± 146.7, 193.9mmol/mg ± 123.0) in males and females, respectively (main effect of time, $p<0.001$, $\eta_p^2=0.67$). There was no main effect of sex ($p=0.26$, $\eta_p^2=0.06$) or a sex x time interaction ($p=0.69$, $\eta_p^2=0.01$). Additionally, there was no difference in the change ($p=0.69$, $d=0.16$) or percent change ($p=0.10$, $d=0.70$) in muscle glycogen between the sexes.

4.4.7 Mixed muscle protein content

Mixed muscle content for proteins related to the insulin-dependent signaling cascade are shown in Figure 4.5. There was no difference in total GLUT4 protein content ($p=0.33$, $d=0.41$), total TBC1D4 protein content ($p=0.27$, $d=0.46$), total AMPK protein content ($p=0.97$, $d=0.01$), total Akt protein content ($p=0.62$, $d=0.21$) or total PTEN protein content ($p=0.98$, $d=0.01$) between males and females.

Phosphorylation status of proteins related to muscle energetics, insulin-independent and -dependent signaling before and after HIIE are shown in 4.6. TBC1D1 Ser^{237} did not differ between the sexes ($p=0.22$, $\eta_p^2=0.07$); however, TBC1D1 Ser^{237} phosphorylation increased with exercise ($p=0.01$, $\eta_p^2=0.24$), which the sex x time interaction indicated was due to a significant increase in males, not females ($p=0.03$, $\eta_p^2=0.19$; Figure 6A). There was no effect of sex ($p\geq 0.30$, $\eta_p^2 \leq 0.05$), no effect of time ($p\geq 0.45$, $\eta_p^2\leq 0.03$) and no sex x time interaction ($p\geq 0.78$, $\eta_p^2\leq 0.01$) for TBC1D4 Thr^{642}, AMPK Thr^{172} or pACC Ser^{79} phosphorylation (Figures 6C-G).

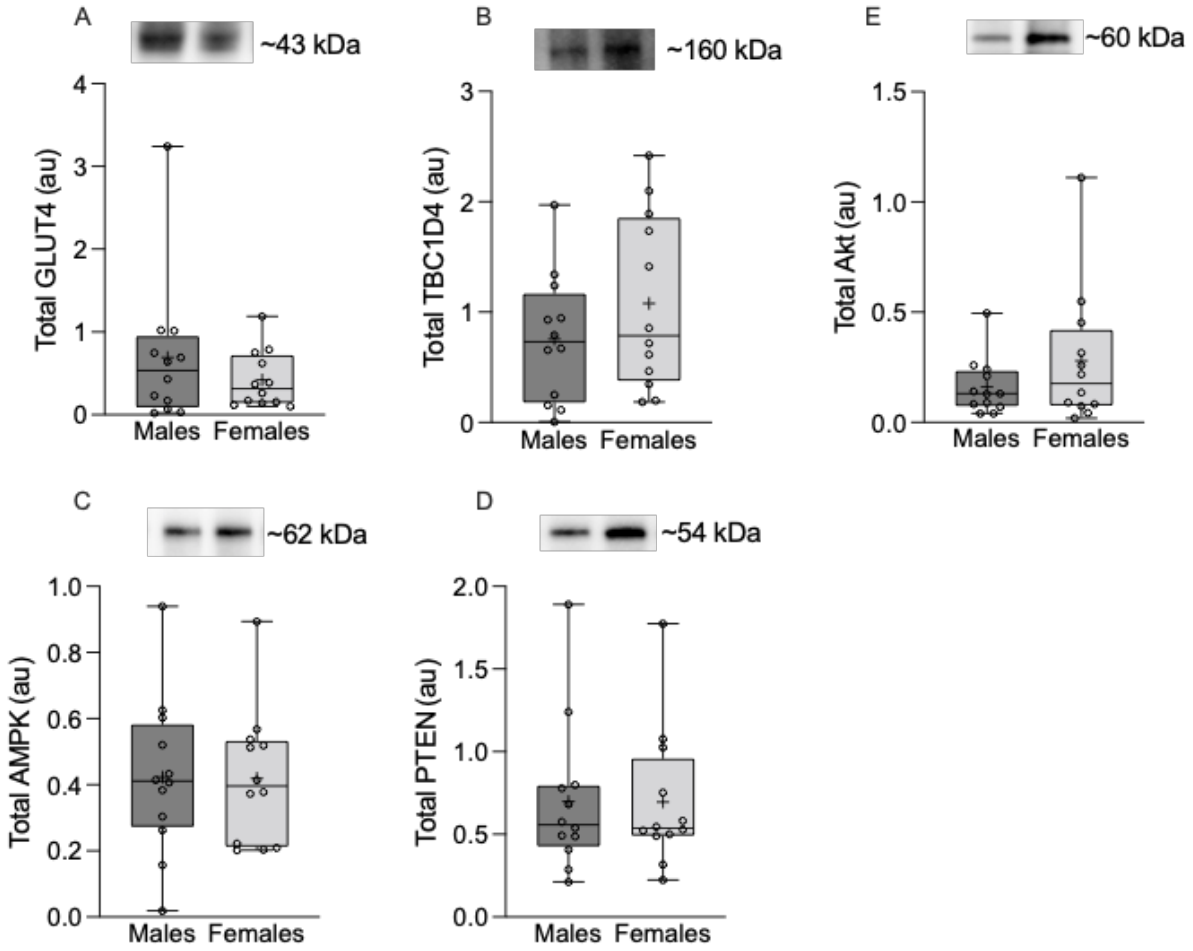

Figure 4.5**:** Content of proteins relating to insulin independent and dependent signaling. A) total GLUT4; B) total TBC1D4; C) total AMPK; D) total P5TEN; E) total Akt

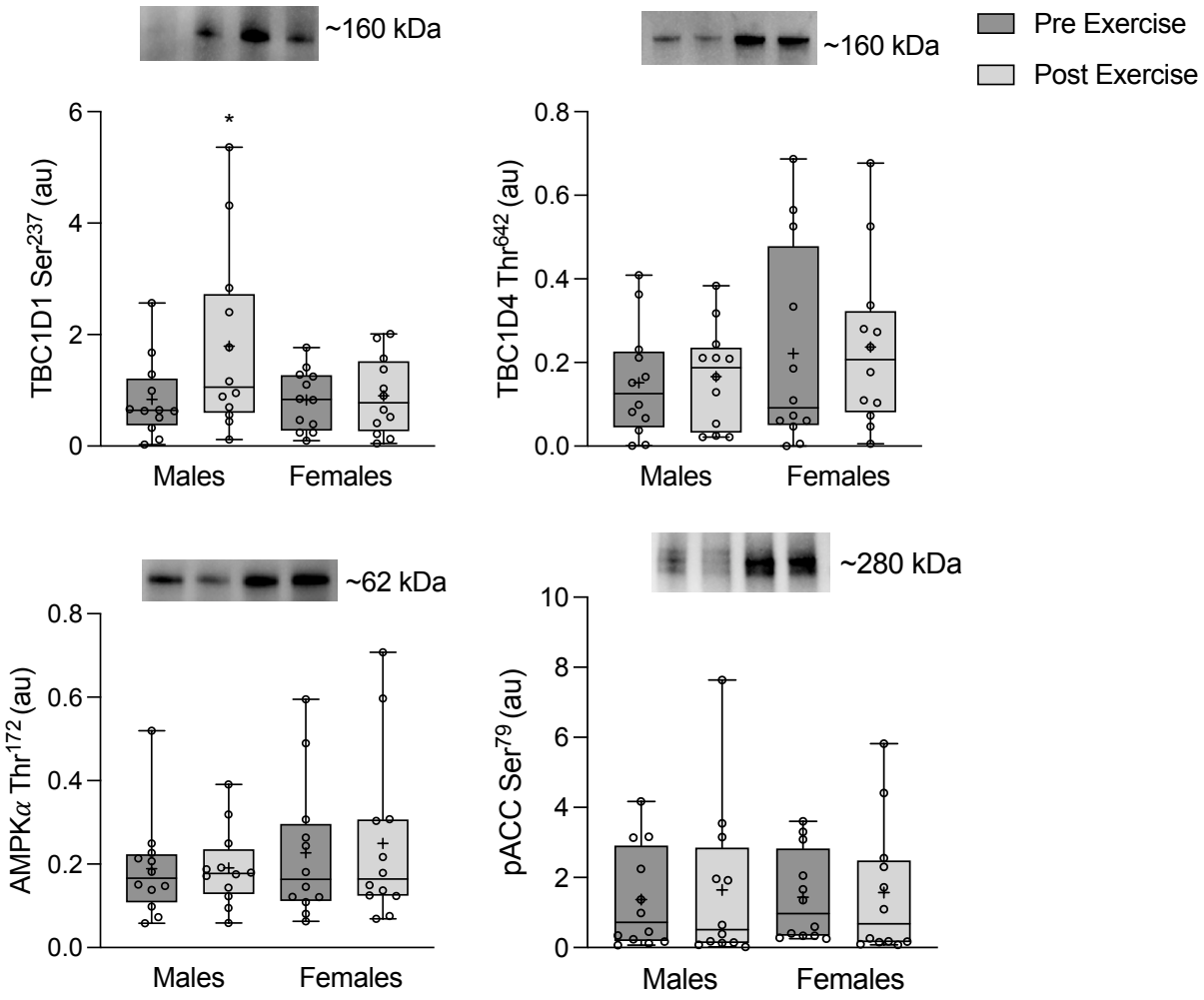

Figure 4.6**:** Phosphorylation status of proteins involved in insulin dependent and independent signaling before and after exercise in males (n=12) and females (n=12). A) TBC1D1 Ser237 phosphorylation, *denotes tukey's post hoc p value (p=0.002) from a significant sex x trial interaction effect from RM ANOVA. higher in males post-exercise (p<0.01); B) TBC1D4 Thr642 phosphorylation; C) AMPKThr172 phosphorylation; D) pACC Ser79 phosphorylation.

4.4.8 Correlations

Correlations are shown in Figure 4.7. The Matsuda index was negatively correlated with post-exercise glycogen content (r=-0.497, p=0.014), but was not correlated with the change in glycogen content (r=0.218, p=0.306) or the change in AMPK phosphorylation (r=-0.017, p=0.939). Similarly, post-exercise HOMA-IR was correlated with post-exercise glycogen content

(r=0.453, p=0.026), but not the change in glycogen content (r=-0.322, p=0.125) or change in AMPK phosphorylation (r=0.057, p=0.791).

There was no correlation between post-exercise glucose AUC and the change in glycogen (r=0.098, p=0.649), post-exercise glycogen content (r=-0.109, p=0.612) or change in AMPK phosphorylation (r=-0.020, p=0.925).

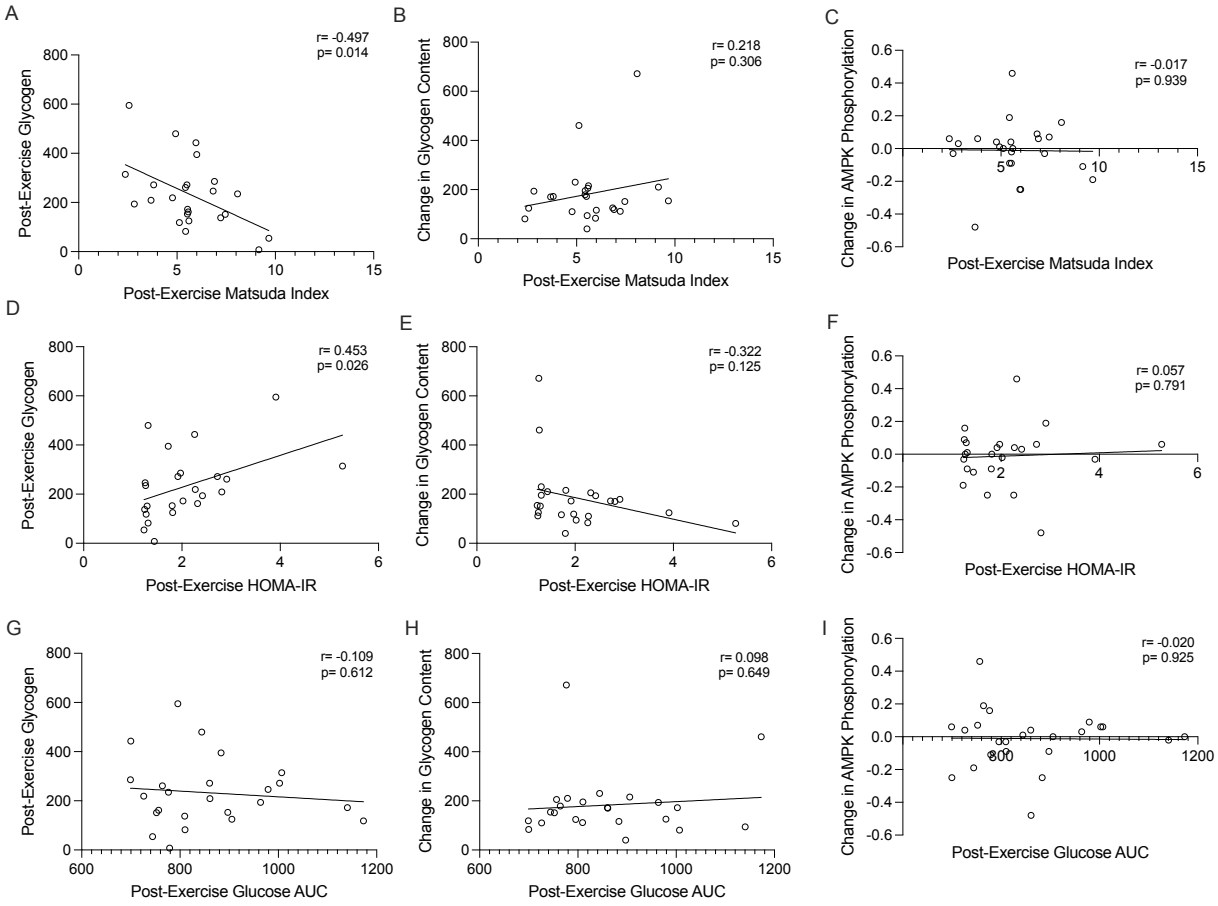

Figure 4.7**:** Correlation graphs (n=24) between A) post-exercise Matsuda index and post-exercise glycogen content (r=-0.497, p=0.014), B) post-exercise Matsuda index and change in glycogen content (r=0.218, p=0.306), C) post-exercise Matsuda index and change in AMPK phosphorylation (r=-0.017, p=0.939), D) post-exercise HOMA-IR and post-exercise glycogen content (r=-0.322, p=0.125), E) post-exercise HOMA-IR and change in glycogen content (r=0.453, p=0.026), F) post-exercise HOMA-IR and change in AMPK phosphorylation (r=0.057, p=0.791), G) post-exercise glucose AUC and post-exercise glycogen content (r=-0.109, p=0.612), H) post-exercise glucose AUC and change in glycogen content

(r=0.098, p=0.649), I) post-exercise glucose AUC and change in AMPK phosphorylation (r=-0.020, p=0.925).

4.5 Discussion:

The overarching goal of the present study was to characterize the effects of an acute bout of HIIE on post-exercise glycemic control during an OGTT in young, healthy males and females in the absence of pathology to further our understanding of whether there are inherent sex differences in exercise-induced glycemic control. Additionally, we sought to examine whether sex differences in muscle metabolism, content of insulin-dependent signaling proteins or activation of the insulin-independent signaling cascade could explain any sex differences in post-exercise glucose handling and insulin sensitivity. The main findings of this study were that an acute bout of HIIE increased insulin sensitivity similarly in both males and females as characterized by both the Matsuda and HOMA-IR indices. While there was no difference in glucose concentration during the OGTT or glucose AUC between males and females during either the control or post-HIIE OGTT, when the relative dose of glucose was considered in relation to body weight and fat-free mass, glucose concentration and glucose AUC were lower in females than males. Furthermore, we found that HIIE lowered insulin concentrations during the post-HIIE OGTT, which was driven by the effect in males as insulin concentration, while not statistically significant, was higher during the post-HIIE OGTT vs CON trial in females. At the metabolic level we found no differences in the total protein content of insulin-signaling proteins between males and females. However, we did find that TBC1D1 Ser^{237} phosphorylation increased to a greater extent in males than females during HIIE. Lastly, we found that glycogen content decreased during exercise with no differences between the sexes

and there was no effect of HIIE or sex on phosphorylation of AMPK or its downstream target ACC.

The main finding of this study was that an acute bout of HIIE resulted in similar improvements in insulin sensitivity, as determined by the Matsuda and HOMA-IR indices, in males and females. Our findings are in line with those of Metcalfe et al, (378) who found no sex difference in any OGTT-derived variable following an acute bout of sprint interval exercise in healthy males and females, despite having previously found that this mode of interval training improved insulin sensitivity in males, but not females (296). Importantly, unlike in our trial where insulin sensitivity improved following HIIE, Metcalfe at al (296), did not find that an acute bout of sprint interval exercise improved insulin sensitivity. Differences in interval exercise modality including fewer and shorter intervals (2 x 20s Wingate sprints vs 10 x 1 min at 90% HRmax) and overall shorter exercise duration (10 min vs 30 min) may be why differences in the acute effect of interval exercise on insulin sensitivity were observed. While no sex difference in post-exercise insulin sensitivity was found in the current trial, we did find that HIIE decreased insulin concentration, which while not statistically significant, was due to an effect in males, not females as insulin concentrations were higher in females during the post-HIIE OGTT. A lower insulin concentration in response to a given glucose load and glucose concentration is indicative of greater sensitivity of tissues to insulin signaling and suggests that there may be subtle differences in the effects of HIIE on insulin sensitivity in males and females, which may over time lead to different training induced adaptations. However, taken together, the findings of these two trials suggest that inherent sex differences in the insulin sensitizing effects of acute HIIE do not underpin sex differences in the effects of HIIE training on insulin sensitivity. This

hypothesis is further supported by findings in obese males and females where an acute bout of HIIE was found to increase insulin sensitivity in females, but decrease it is males (379), which is the opposite of what has been observed with interval training (17, 296, 380).

Height and body composition influence the glycemic response to an OGTT (240, 241). Furthermore, skeletal muscle is the largest storage depot for glucose in the body (24). As such, it is critical when examining differences in glycemic control between males and females, who inherently differ in body anthropometrics, that we consider the relative dose of glucose being consumed. Females are inherently shorter, weigh less and have less fat-free mass then males, thus are inherently disadvantaged to have a higher blood glucose concentration during a standard 75 g OGTT. In fact, while females have been found to have a higher 2h glucose concentration than males, when height and body surface area are accounted for these differences disappear (240, 241). In the current trial we found no difference in blood glucose concentration during the control or post-HIIE OGTT between males and females when no adjustments for anthropometrics were made. Given the differences in body anthropometrics between males and females, the lack of difference in glucose concentration during the OGTT suggests that females are better able to handle a glucose load than males. Indeed, when glucose data were normalized to both body weight and LBM, glucose concentrations during the OGTT were higher in males compared to females, indicating that for a given mass females are better able to clear glucose. Interestingly, we did not find any difference in blood glucose concentration when we normalized glucose values for dose relative to height. Sicree et al, (240) found that differences in height between the sexes was responsible for higher post-load glucose in females; however, they speculated that the association of height with post-load

glucose may ultimately be due to differences in muscle mass, as a taller person would have more muscle mass allowing for greater glucose uptake (240). We would be remiss if we did not also consider other inherent sex differences related to glucose metabolism that could influence glucose concentration during an OGTT. Anderwald et al, (381) found that gut glucose half-life was higher in females compared to males and was negatively related to body height. The higher gut glucose half-life is supported by findings from another study that found an elevated glucose concentration during the latter part of an OGTT in females. Together our results suggest that when differences in body weight and muscle mass are considered, females are inherently better able to clear glucose from the blood and highlight the importance of considering the relative dose when comparing glucose response to an OGTT between males and females. This however is a first step in understanding sex-based differences in glycemic control and future research should focus on administering a relative dose of glucose to individuals based on BW and/or LBM.

Our finding that blood glucose concentration is lower during an OGTT in females when the glucose dose is normalized for body weight is different from findings from Bartholomae et al (382). Important differences in how the data were normalized need to be addressed to explain these discrepancies. In the current trial we normalized glucose concentrations during the OGTT by dividing the observed blood glucose concentration by the glucose dose administered relative to each anthropometric variable as we wanted to look at whether the greater relative dose of glucose consumed by females was preventing us from detecting differences in the effects of sex on glycemic control. In contrast, Bartholomae normalized blood glucose concentrations by dividing the observed blood glucose concentration by the

participants body weight. Unsurprisingly, given that females weigh less than males, they found that when normalized for body weight glucose concentrations were higher in females than males. We would argue that this is not the appropriate manner in which to normalize blood glucose data to compare between males and females as it does not control for the dose of glucose administered. Our manner of adjusting glucose concentrations during the OGTT takes into consideration the fact that 75g of glucose is a higher dose in females than males since females are shorter, weigh less and have less fat-free mass and adjusts the glucose values by the glucose dose relative to body anthropometrics. As such, our method of adjustment allows us to normalize blood glucose concentrations for differences in body anthropometrics that have been shown to influence glucose uptake.

Despite no acute differences in post-exercise insulin sensitivity between males and females, differences in exercise metabolism may result in differences in signaling that over time could lead to different insulin sensitizing adaptations in response to HIIE training in males and females. Both muscle glycogen utilization and increased AMPK activity are important regulators of post-exercise glucose uptake and insulin sensitivity. As expected, we found a significant decrease in glycogen concentration following HIIE, and post-exercise glycogen content was correlated with the increase in post-exercise insulin sensitivity characterized by the Matsuda and HOMA-IR indices. Given the relationship between post-exercise glycogen content and glucose uptake and insulin sensitivity (365), the lack of difference in glycogen utilization and post-exercise glycogen content between males and females is supportive of the finding that post-exercise insulin sensitivity did not differ between males and females.

Unlike previous work that found that AMPK Thr^{172} phosphorylation and α_2AMPK activity increased in males, but not females during an acute bout of aerobic exercise (258), we did not find a sex difference in the effect of HIIE on phosphorylation of AMPK or its downstream target ACC. The lack of sex difference in AMPK phosphorylation with HIIE exercise is in line with our finding that sex did not influence the effect of HIIE on insulin sensitivity. AMPK plays a critical role in enhancing post-exercise insulin-independent and -dependent glucose uptake via phosphorylation of TBC1D1 and TBC1D4 (76, 311). Thus, the finding that TBC1D1 phosphorylation increased in males, not females, during HIIE despite no differences in AMPK phosphorylation is perplexing. However, it is important to note that AMPK activity is regulated both allosterically and covalently (383), thus perhaps there were differences in AMPK activity between males and females during HIIE that led to differences in TBC1D1 phosphorylation that we did not detect because we did not directly measure AMPK activity. Future studies should include an assessment of AMPK activity in order examine whether differences in AMPK activity are related to differences in insulin-dependent and -independent signaling between males and females.

One of the major strengths of this trial is that we ensured that our males and females were properly matched for training status and controlled for variations in sex hormones that may affect insulin sensitivity and glucose handling. It is imperative that males and females are matched for fitness relative to LBM ($\dot{V}O_{2peak}$/kgLBM/min), not body weight, as females naturally have a greater body fat percentage compared to males (220). Failure to properly match males and females in this manner could lead to inappropriate conclusions regarding the effects of sex or exercise on glycemic control. Three previous trials have compared the effects of acute bouts

of exercise on post-exercise glycemia and insulin sensitivity in healthy (382) and overweight/obese (384) males and females; however, differences in fitness between male and female participants may have impacted their findings. In the study by Bhammar et al (384), though aerobic fitness was not reported relative to LBM, using the available body composition data it is estimated that $VO2_{max}$ relative to LBM was ~25% higher in males compared with females (~50.9 vs. ~38.3 ml/kgLBM/min) and thus the greater effect of an acute bout of exercise on postprandial glycemia in females may have been due to baseline differences in fitness and/or insulin sensitivity (not reported) between the sexes. In the study by Delaney et al (385), participants were considered inactive; however, fitness was not assessed, thus whether differences in fitness may have impacted their results cannot be determined. Finally in the study by Bartholomae et al (382) fitness was determined but was not expressed relative to LBM so it is unclear whether males and females were equally trained. Given that aerobic fitness impacts insulin sensitivity (386) it is inappropriate to make comparisons between groups without properly matching participants for aerobic fitness.

Other strengths of the current trial are that both menstrual phase and diet were controlled for given that both can influence insulin sensitivity. Fluctuations of hormones across the menstrual cycle can influence insulin sensitivity, namely through fluctuations in insulin, resulting in lower insulin sensitivity in the luteal phase (265). Failing to control for menstrual cycle may lead to erroneous conclusions related to the effect of exercise on glycemic control within females and could introduce greater variability leading to an inability to detect differences between groups. Furthermore, dietary intake 24-hours before each trial was controlled and remained consistent between the control OGTT and the HIIE acute exercise

bout, thereby limiting any differences seen in insulin sensitivity and glucose control due to acute changes in dietary intake. Furthermore, comparison of dietary intake between males and females indicated no differences in habitual dietary intake, meaning that any differences between the sexes, or lack thereof, were not due to differences in dietary intake. Additionally, the baseline muscle glycogen concentration did not differ between the sexes which indicates that substrate availability was similar prior to the HIIE bout.

While this study has its strengths, there are also some limitations to discuss. First, there is evidence to support that several proteins involved in glucose uptake and insulin sensitivity may have a delayed response to exercise and may be more prominent during recovery (129). We may have missed potential differences in the acute effect of HIIE on ACC, AMPK and TBC1D4 by examining the changes immediately post-exercise and not during recovery. Additionally, we may have missed some changes in the activation of some proteins (i.e. TBC1D4, PTEN) by not assessing them during the OGTT in the insulin stimulated state. However, the main objective of the current study was to investigate whether sex influenced the acute effects of exercise on muscle metabolism in relation to post-exercise glucose control. Future trials should determine whether exercise influences activation of the insulin-signaling cascade differently in males and females in the insulin-stimulated state. Second, another limitation is the use of the OGTT rather than the gold-standard use of the hyperinsulinemic-euglycemic clamp. The use of the hyperinsulinemic-euglycemic clamp could potentially be more sensitive to slight differences in insulin sensitivity changes between the sexes; however, this procedure was not feasible to conduct in the current trial and would prevent insulin secretion induced by incretin hormones, which is known to be higher in females and thus may be mechanistically one

of the reasons females typically have higher insulin sensitivity than males (387). It is also worth mentioning that controlling for menstrual cycle phase, while a strength in sex comparative research, may also be considered a limitation as the findings are only generalizable to the phase in which the data were obtained. Because data was collected in the follicular phase of the menstrual cycle, findings from the current trial may not be generalizable to the luteal phase where females typically oxidize less carbohydrates and more lipids (263) and are reported to be less insulin sensitive (367). Importantly, differences in metabolism during exercise are found between males and follicular phase females and the differences between the sexes are greater than those observed between phases of the menstrual cycle (20). However, future trials should examine whether sex differences in post-exercise glycemic handling and insulin sensitivity occur when males are compared with females during the luteal phase of the menstrual cycle.

In conclusion, the present study reports that though there were no differences in glucose parameters when absolute glucose concentrations were reported, when data were normalized to the relative dose of glucose consumed, blood glucose concentrations were lower in females than males. Furthermore, we also report that an acute bout of HIIE increased insulin sensitivity equally in healthy males and females as characterized by the Matsuda index and HOMA-IR. In support of the finding that exercise-induced improvements in insulin sensitivity did not differ between the sexes, we did not find an effect of sex on muscle glycogen utilization or AMPK activation during exercise; however, we did find that exercise increased TBC1D1 Ser^{237} phosphorylation in males only. Overall, these findings confirm that acute HIIE is an effective mode of exercise to improve IS in healthy males and females and suggests that inherent differences in metabolism are not related to differences in the acute effects of exercise on

glycemic control in the absence of pathology. However, our finding that TBC1D1 phosphorylation increased in males only identifies a potential mechanistic target to examine in clinical populations in relation to the blunted effect of interval training on insulin sensitivity. Our findings add to our understanding of how sex influences muscle metabolism in relation to post-exercise glycemic control in the absence of pathology. Now that the normal physiological response to HIIE has been characterized in males and females, future studies should examine whether the response is altered in insulin resistant males and females following acute and chronic exercise, as well as in the insulin stimulated state.

Chapter 5: Acute high intensity interval exercise increases plasma markers of inflammation with no differences between recreationally active males and females

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5.1 Abstract:

High intensity interval exercise (HIIE) is less effective at improving insulin sensitivity in females. Transient increases in reactive oxygen species and inflammatory markers are known to mediate exercise-induced adaptations in skeletal muscle. Sex influences oxidative stress and inflammation with males having higher levels compared to females. Thus, the purpose of the current trial was to investigate the effect of sex on markers of oxidative stress and inflammation following an acute bout of HIIE and during an oral glucose tolerance test (OGTT) in young, healthy males and females. Twenty-four (12/sex) males and females matched for aerobic fitness ($\dot{V}O_{2peak}$ relative to LBM) had muscle biopsies taken at rest and following an acute bout of high-intensity interval exercise (HIIE, 10x1min at 90% HR_{max}) and blood taken at rest, post exercise, 90 minutes post exercise (immediately prior to the OGTT) and at 60 min during the OGTT. Muscle samples were analyzed for oxidative stress (NOX2, 4HNE, p38 MAPK) and antioxidant status (TRX1, glutathione reductase, GPX1). Blood samples were analyzed for inflammatory markers (IL-6 and TNFα). Following exercise there was an increase in plasma concentrations of IL-6 ($p>0.001$, $\eta_p^2=0.27$) and TNFα ($p=0.04$, $\eta_p^2=0.13$), with IL-6 continuing to

increase during the OGTT. There was no difference between the sexes at rest or following exercise ($p>0.11$, $\eta_p^2>0.12$). We did not see any effect of sex on antioxidant ($p>0.54$, $d=0.09$) or oxidative stress ($p>0.62$, $d>0.30$) status, however females had higher p38 MAPK phosphorylation at rest compared to males ($p=0.04$, $\eta_p^2=0.18$). Overall, these findings suggest that antioxidant status, ROS production and inflammation do not differ between males and females at rest, following an acute bout of HIIE or during a post-exercise glucose challenge.

Key Words: oxidative stress, inflammation, sex differences, high-intensity, interval exercise

5.2 Introduction

Chronic inflammation and oxidative stress have been implicated in the pathogenesis of a myriad of diseases such as cardiovascular disease, cancer, metabolic syndrome, and type 2 diabetes (175). Oxidative stress is caused by an imbalance between free radical production and antioxidant capacity, which leads to molecular damage within the cell and has recently been termed oxidative distress (388). However, acute increases in reactive oxygen species (ROS) and cytokines also act physiologically as secondary messengers and are critical to mediate metabolic stress-adaptations (389). The physiological role of ROS to induce metabolic adaptations is termed oxidative eustress (388). Interestingly, biological sex is known to influence levels of oxidative stress and inflammation, with males having higher markers of oxidative stress (271-277) and inflammation (IL-6 and TNFα) compared with females (278, 279). The lower levels of oxidative stress and inflammation in females is thought to be due to estrogen. Estrogen has antioxidant properties due to its ability to up-regulate the expression of antioxidant enzymes via intracellular signaling pathways (276). Additionally, estrogen acts in an anti-inflammatory manner through its inhibitory effect on the expression of inflammatory marker genes (278, 279). Lower levels of oxidative distress and inflammation in females are related to the lower risk of various chronic health conditions including cardiovascular disease and type II diabetes in females. However, if estrogen acts in an antioxidant and anti-inflammatory manner, acute increases in ROS and inflammatory markers in response to stressors may be blunted in females leading to reduced or absent signaling and subsequent adaptation (283).

Acute exercise increases ROS production in an intensity and duration dependent manner and these acute increases in ROS are critical to inducing training adaptations over time (390). In fact, numerous studies have found that ROS are required for exercise-induced adaptations in skeletal muscle (391-394) and that administration of antioxidants prevents exercise-induced adaptations, specifically preventing increases in peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) content, antioxidant enzymes and mitochondrial biogenesis (395-397). Given the importance of ROS in mediating exercise-induced adaptations, sex-differences in ROS production and/or antioxidant status may result in different adaptations within skeletal muscle in males vs. females. For example, interval training has been found to increase muscle protein synthesis, mitochondrial biogenesis, and insulin sensitivity to a greater extent in males than females (17, 296, 398). Acutely, a single session of interval exercise induced a significant increase in markers of lipid peroxidation and protein carbonylation, as well as an increase in the antioxidant defence mechanism in healthy young males (399). However, whether this response is similar in females is largely unexamined.

Acute exercise also results in cytokine/myokine production that can also induce metabolic effects (400). Specifically, acute exercise is known to induce acute elevations in IL-6 and IL-10, which in turn to inhibit TNFα (401). TNFα is a pro-inflammatory cytokine and is implicated in the development of insulin resistance and type II diabetes (402). However, sex differences in the effect of exercise on TNFα and IL-10 production and how it may relate to the differential effects of interval training on mitochondrial biogenesis and insulin sensitivity, is largely unexamined. Therefore, the purpose of this study was to investigate sex differences in markers of inflammation, oxidative stress and antioxidant status at rest, after an acute bout of

high intensity interval exercise (HIIE) and during a post-exercise glucose challenge in young, recreationally active males and females. We hypothesized that males would have greater resting levels of inflammation and oxidative stress and lower antioxidant status compared to females. Additionally, we hypothesized that HIIE would acutely increase circulating levels of IL-6, TNFα and markers of oxidative damage (4HNE, p38 MAPK), but that these changes would be greater in males than females.

5.3 Methods

5.3.1 Subjects

Twenty-four recreationally active young males (*n*=12) and females (*n*=12) took part in the study (Table 5.1, taken from Chapter 4). The participants in this study were the same as in Chapter 4. Participants were deemed recreationally active based on self-reported habitual physical activity indicating that they participated in no more than 3 sessions of cardiovascular exercise per week. Participants were excluded if they had any chronic health conditions, were unable to complete an acute exercise session, had an allergy to local anaesthetic, were taking prescription anti-coagulant or anti-platelet medications, were unable to exercise as suggested by the Get Active Questionnaire (GAQ) or had a BMI>27 kg/m^2. Females were excluded if they were taking any form of monophasic birth control. Participants were told to maintain habitual dietary intake and physical activity throughout the trial. Furthermore, to ensure that any sex-based differences were not due to differences in habitual diet and physical activity, all participants completed a 3-day diet record and 7-day physical activity log. Prior to commencing the trial all participants provided written informed consent. The study protocol was reviewed and received ethics

clearance from the University of Waterloo Research Ethics Committee (ORE #22477). The study conformed with all standards outlined by the Tri-Council Policy Statements for Ethical Conduct for Research Involving Humans (TCPS 2) (403).

5.3.2 Experimental Protocol

The experimental protocol consisted of three preliminary visits and one acute exercise session. The first preliminary visit included consent and anthropometric measurements (height, weight, BMI). The second preliminary visit included a DXA scan (DXA, Hologic Discovery W with QDR APEX software version 4.5.3, Mississauga, ON, Canada) for determination of body composition and an assessment of aerobic fitness ($\dot{V}O_2$ peak test). The third visit included a familiarization session to the HIIE bout. On the 4th visit participants completed the acute HIIE exercise bout with blood and muscle samples taken prior to and following exercise. Participants then underwent a 75g oral glucose tolerance test (OGTT) 90 min after exercise cessation with blood samples taken immediately before (90min) the OGTT and at 60 (150min) minutes during the OGTT to also assess sex differences in cytokine/myokines in the fed state. Females were tested in the mid-follicular phase of the menstrual cycle (day 5-9).

5.3.3 $\dot{V}O_2$ peak testing

To appropriately match males and females for aerobic fitness level and to determine the work rate corresponding to 90% HR_{max} for the acute exercise bout, participants underwent an incremental cycling test to volitional fatigue on an electronically braked cycle ergometer (Ergoselect 100, ergoline GmbH, Germany) using an online gas collection system (Vmax encore

CPET Systems, Vyaire medical, Chicago). Participants began with a warmup at 50W for 2mins after which the intensity was increased by 1W every 2s until volitional fatigue or the point at which pedal cadence fell below 50 rpm. A metabolic cart with an online gas collection system (Vmax encore CPET Systems, Vyaire medical, Chicago) measured oxygen consumption and carbon dioxide production. In order to ensure that males and females were appropriately matched for aerobic fitness (220) $\dot{V}O_{2peak}$ was expressed relative to fat-free mass. Participants returned to the lab at least 24-hours after the $\dot{V}O_{2peak}$ testing and cycled at the approximate wattage of the HIIE bout for 3-4 repetitions to ensure that the work rate was appropriate to achieve the target HR of 90% HR_{max}.

5.3.4 Acute high intensity interval exercise session

On the morning of the acute exercise session participants arrived at the laboratory following a 12-hour overnight fast. Additionally, participants refrained from alcohol for 24 hours and physical activity for 72 hours prior to the acute exercise bout. Upon arrival in the laboratory participants rested quietly and an indwelling catheter was inserted into a prominent forearm vein and a fasted blood sample was taken. A muscle biopsy (~100-150 mg) was then taken from the *vastus lateralis* muscle approximately 20cm proximal to the knee using a custom suction-modified Bergstrom needle (5 mm diameter) as previously described (369). The muscle sample was dissected free of fat and connective tissue and was snap frozen in liquid nitrogen and stored at -80˚C for analysis of oxidative stress and antioxidant status. Blood samples were assessed for plasma concentrations of IL-6 (HS600C, Bio-Techne, Minneapolis, Minnesota, USA) and TNFα (HSTA00E, Bio-Techne, Minneapolis, Minnesota, USA) in accordance with

manufacturer's instructions. Participants then commenced the acute exercise bout starting with a 5-min warm up at 50W, followed by 10 intervals of 60 sec at 90% HR_{max} interspersed with 60 sec at low intensity (50W) and ending with a 5 min cool down at 50W. Immediately following cessation of exercise a second muscle biopsy was taken from the same leg and a post-exercise blood sample was obtained. Participants than sat quietly for 90mins and then underwent a 120min oral glucose tolerance test (OGTT). Participants consumed 75 grams of glucose (Trutol™, Thermo Scientific, Waltham, Massachusetts, United States) and had blood draws taken immediately prior to (90mins post-exercise) and at 60 minutes (150min post-exercise) during the OGTT. Heart rate was measured every minute during the HIIE bout using a chest strap HR monitor (H9 heart rate sensor, Polar, Kempele, Findland). Maximal workload is the average of the wattage of the high-intensity bouts for each participant. Estimated energy expenditure from the HIIE bout was calculated from the oxygen cost of both the low and high intervals and then converting to calories burned.

5.3.5 Muscle analysis

Muscle samples for Western Blot analyses were homogenized in ice cold 25mM Tris buffer [25mM Tris, 0.5% (v/v) Triton X-100 with protease/phosphatase inhibitor tablets (Roche Diagnostics, Laval, QC, Canada)] at a ratio of 10μL buffer to 1mg of muscle in a prechilled homogenization Biopur Eppendorf (Eppendorf, Mississauga, ON, Canada) using a bead homogenizer (TissueLyser II, Qiagen, Toronto, ON, Canada) run at 20 cycles/second for 40 seconds. Once the samples were sufficiently homogenized, they were spun at 10,000G for 10 minutes at 4^{o}C. The supernatant was separated and allocated into a prechilled Eppendorf while

the pellet was frozen for potential use in additional analyses. Western blot analysis was conducted using previously described techniques (372). Briefly, protein concentration of homogenates was determined using the BCA protein assay technique (Thermo Fischer Scientific, Waltham, MA) and equal amounts of protein were prepared in 4x Laemmli's buffer and then separated using 10% SDS-PAGE on 4%-15% Criterion TGX Stain-free protein gels (Bio-Rad, Hercules, CA) at 200V for 40mins and electro transferred to PVDF membranes. A protein ladder (Precision Plus Protein Standard, Bio-Rad, Hercules, CA, USA) and a standard curve (pooled from all samples) were run on every gel. Total protein and visual confirmation of protein transfer was done pre- and post-membrane transfer, respectively, using a Chemidoc MP (Bio-Rad, Hercules, CA, USA). Following 1h block in 5% bovine serum albumin (BSA) in 1X Tris-buffered saline and Tween 20 (TBST), membranes were incubated in primary antibody overnight at 4°C in 5% BSA/TBST based on previously optimized conditions. The primary antibodies used for western blotting were phosphorylated p38 MAPK Thr^{180}/Tyr^{182} (92152, 1:1000, Cell Signaling, Danvers, MA, USA), total p38 MAPK (cell signaling, #9212S, 1:1000), 4 Hydroxynonenal (ab46545, 1:1000, Abcam, Cambridge, UK), glutathione peroxidase 1 (ab108427, 1/5000, Abcam, Cambridge, UK), thioredoxin 1 (MA5-14941, 1/1000, ThermoFischer, Waltham Massachusetts, USA), NOX2 (ab129068, 1/5000, Abcam, Cambridge, UK), glutathione reductase (sc-133245, 1/1000, Dallas, Texas, USA). After 3x5 min washes in TBST, membranes were incubated in horseradish peroxidase-conjugated goat anti-rabbit secondary (170-6515, Bio-Rad, Hercules, CA, USA) diluted to 1:20,000 in 5% BSA/TBST for 1h at room temperature and washed in TBST for 3x5 min. Membranes were then incubated for 1min with enhanced chemiluminescence (ECL) (Clarity Western ECL Substrate, Bio-rad, Hercules, CA, USA) and imaged on the Chemidoc imaging system

(Bio-Rad, Hercules, CA, USA). Bands were quantified using Image Lab software (Version 3.0, Bio-Rad, Hercules, CA, USA) and protein content was normalized within and between blots using total protein and the standard curve obtained from the gel, as previously described (372).

5.3.6 Statistical analysis:

All statistical analyses were conducted using SPSS (version 25, IBM, Armonk, NY, USA). Baseline differences between groups and change values for muscle analyses were assessed using a non-paired t-test. 2-way mixed model ANOVA with sex (2 levels, males/females) as the between variable and time (4 levels, pre/post exercise/90, 150min) as the within variable was used to determine the effects of sex and exercise on all blood variables. 2-way mixed model ANOVA with sex (2 levels, males /females) as the between variable and time (2 levels, pre/post exercise) as the within variable was used to determine the effects of sex and exercise on all muscle variables. Post-hoc analyses were conducted using a Tukey's HSD test where appropriate. Data sets were assessed for normality using the Shapiro-Wilk test and were found to be not normally distributed. Thus, values were log transformed prior to undergoing statistical analyses using ANOVA. Statistical analyses completed on log transformed and non-log transformed data sets were similar, therefore non log transformed results are displayed, with the exception of proteins related to antioxidant and oxidant status which shows log transformed ANOVA results. Significance was set at $p \leq 0.05$. Partial eta-squared (η_p^2) values were calculated to estimate the effect sizes (small 0.04, medium 0.25, large 0.64) for main effects and interactions where necessary. Cohen's *d* values were calculated to estimate effect

sizes (small 0.2, medium 0.5, large 0.8) for t-test and post hoc comparisons where necessary. All data are presented as means ± SEM for *n*= 12 in each group, based off non log transformed data sets. All graphs were created using GraphPad Prism (GraphPad Software Inc., CA).

5.4 Results

5.4.1 Participant characteristics

Participant characteristics have previously been reported (Beaudry et al, 2022- in review) and are shown in Table 5.1. As anticipated, there were significant differences in weight, percent body fat, lean body mass and $\dot{V}O_{2peak}$ between males and females. Once $\dot{V}O_{2peak}$ was adjusted based on lean body mass rather than body weight, there was no significant difference between males and females, indicating that our males and females were matched for aerobic fitness. Furthermore, during the acute HIIE bout males had significantly greater maximal workload and estimated energy expenditure compared to females.

Table 5.1: Subject characteristics from male and female participants.

	Males	Females	p value	Cohen's d
Age (years)	22 ± 1	21 ± 1	p=0.45	*d*=0.46
Weight (kg)	76.6 ± 2.4	62.6 ± 3.1	p<0.01*	*d*=1.46
Height (cm)	177.9 ± 2.0	162.7 ± 2.6	p<0.01*	*d*=1.95
Body Mass Index (kg/m^2)	23.7 ± 0.8	22.9 ± 0.6	p=0.45	*d*=0.32
Body Fat (%)	20.9 ± 1.6	32.68 ± 1.3	p<0.001*	*d*=2.39
Fat mass (kg)	15.4 ± 1.4	19.5 ± 1.2	p<0.05*	*d*=0.92
Lean Body mass (kg)	55.1 ± 2.2	37.6 ± 1.8	p<0.001*	*d*=2.53
$\dot{V}O_{2peak}$ (ml/kgBW/min)	43.1 ± 1.6	34.2 ± 1.4	p<0.001*	*d*=1.67
$\dot{V}O_{2peak}$ (ml/kgLBM/min)	59.5 ± 1.4	56.5 ± 1.7	p=0.21	*d*=0.52
Maximal Workload (W)	199 ± 10	118 ± 8	p<0.001*	*d*=2.42
Average heartrate (bpm)	151 ± 3	157 ± 2	p=0.12	*d*=0.51
Estimated Energy Expenditure (kcals)	240 ± 8	182 ± 7	p<0.001*	*d*=2.22

All results are shown as mean ± SE, *n*=12 males, and *n*=12 females.
* Significance from independent samples t-test, significantly different with P value <0.05

5.4.2 Basal Sex differences in plasma inflammatory markers and indices of Oxidative Stress

Basal sex differences in plasma inflammatory markers are reported in Table 5.2. There were no sex differences for IL-6 or TNFα (p>0.16, *d*=0.06; Table 5.2). Figure 5.1 shows total protein content of pro (NOX2, total p38 MAPK) and antioxidant (TRX1, GPX1, GR, GPX1/GR) enzymes in males and females. There was no difference in NOX2 (p=0.62, *d*=0.30), TRX1 (p=0.74, *d*=0.24), GPX1 (p=0.83, *d*=0.09), GR (p=0.54, *d*=0.25), or total p38 MAPK (p=0.45, *d*=0.36) content between the sexes. There was also no sex difference for the GPX1/GR ratio (p=0.72, *d*=0.25).

Table 5.2: Basal differences in plasma inflammatory markers

	Males	Females	p value
IL-6 (pg-mL)	1.52 ± 0.38	0.94 ± 0.12	p=0.16
TNFα (pg-mL)	0.88 ± 0.15	0.69 ± 0.06	p=0.23

All results are shown as mean ± SE, *n*=12 males, and *n*=12 females.

* Significance from independent samples t-test, significantly different with P value <0.05

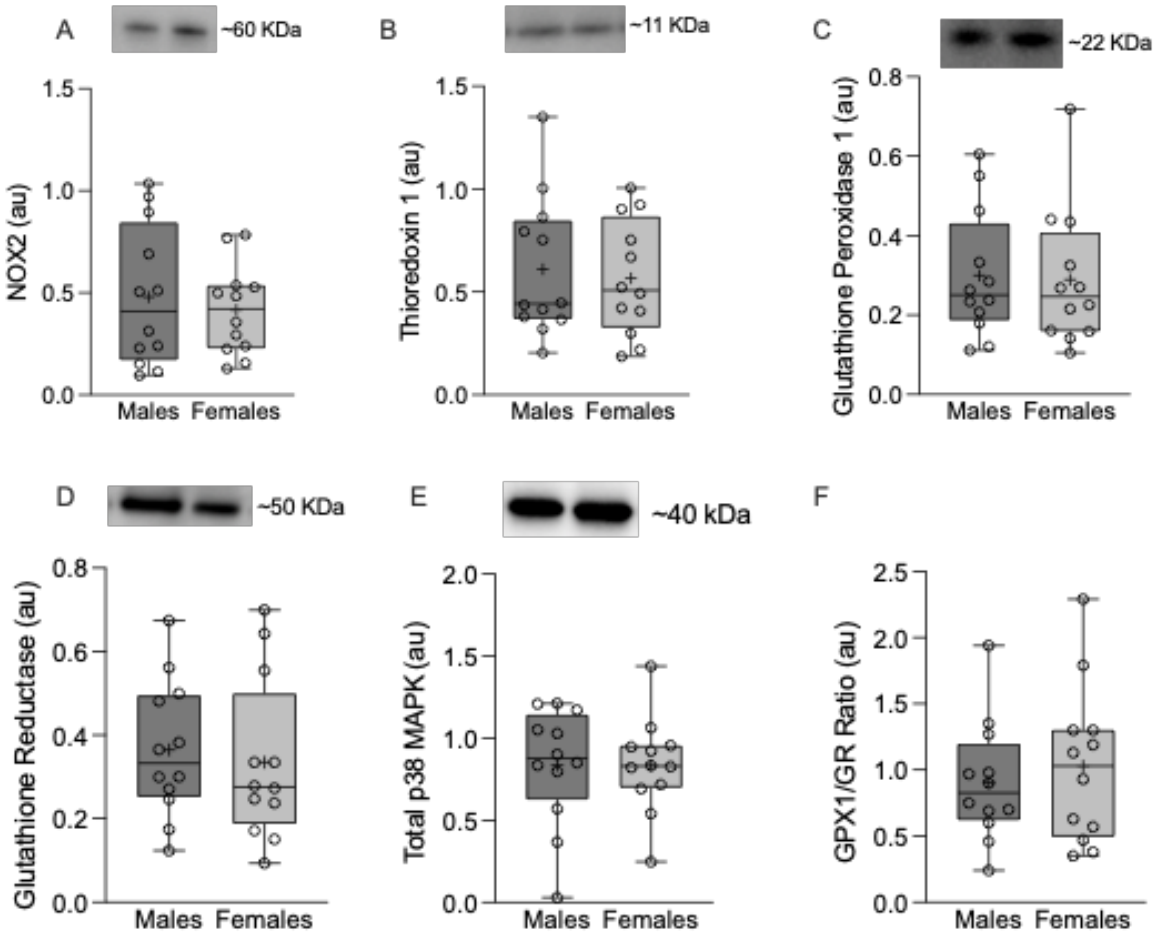

Figure 5.1: Total proteins relating to oxidative stress and antioxidant status in males (n=12) and females (n=12). A) NOX2, B) Thioredoxin 1, C) Glutathione Reductase, D) Glutathione Peroxidase 1, E) Total p38 MAPK and F) GPX1/GR Ratio

5.4.3 Effects of exercise and sex on markers of inflammation and oxidative stress

5.4.3.1 Plasma inflammatory markers

Plasma inflammatory markers are shown in Figure 5.2. There was no main effect of sex ($p=0.71$, $\eta_p^2=0.01$) or sex x time interaction ($p=0.41$, $\eta_p^2=0.03$) for IL-6 concentration (Figure 5.1A). There was a main effect of time ($p>0.001$, $\eta_p^2=0.27$) indicating IL-6 increased over time. Plasma TNFα concentrations are shown in Figure 5.2B. There was no main effect of sex ($p=0.11$, $\eta_p^2=0.12$) or sex x time interaction ($p=0.27$, $\eta_p^2=0.06$); however, there was a significant main effect of time ($p=0.04$, $\eta_p^2=0.13$) with post-hoc analysis showing that TNFα was higher following exercise but returned to baseline by 90 minutes after exercise and did not increase during the OGTT.

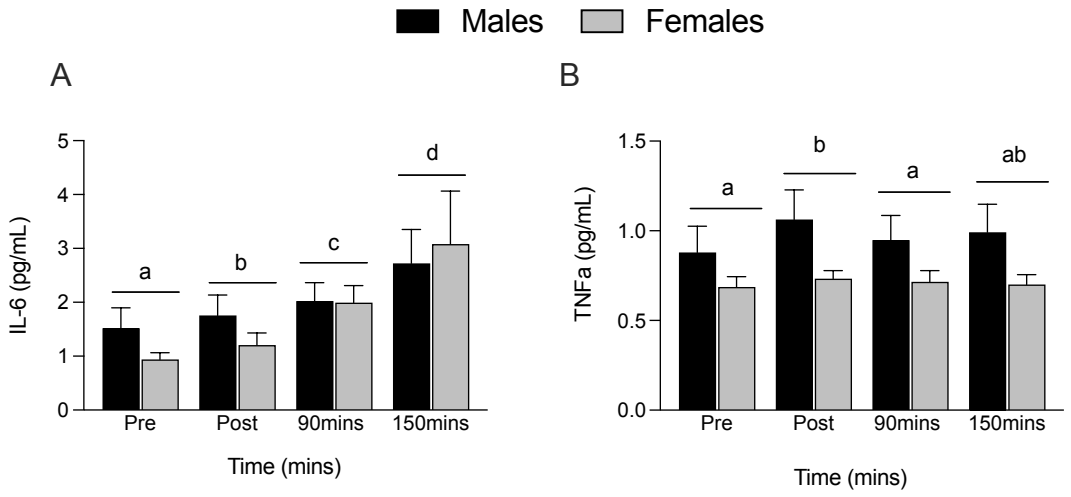

Figure 5.2: Plasma inflammatory markers immediately pre-exercise, immediately post-exercise, 90mins post-exercise and 150mins post-exercise in A) IL-6 and B) TNFα.

5.4.3.2 Proteins related to oxidative stress

Indices of oxidative stress are shown in Figure 5.3. 4HNE (Figure 6.3A), a marker of lipid peroxidation, did not show any effect of sex (p=0.86, η_p^2=0.001), time (p=0.42, η_p^2=0.03), or sex x time interaction (p=0.27, η_p^2=0.05). Furthermore, the change in 4HNE following exercise did not differ between males and females (males: -0.12 ± 0.08, females: -0.04 ± 0.05, p=0.39, *d*=0.36). There was no difference overall in p38 MAPK phosphorylation between males and females (p=0.26, η_p^2=0.06) and no main effect of time (p=0.62, η_p^2=0.01); however, there was a significant sex x interaction (p=0.04, η_p^2=0.18), which indicated that basal p38 MAPK phosphorylation was higher in females compared to males (Figure 5.3B).

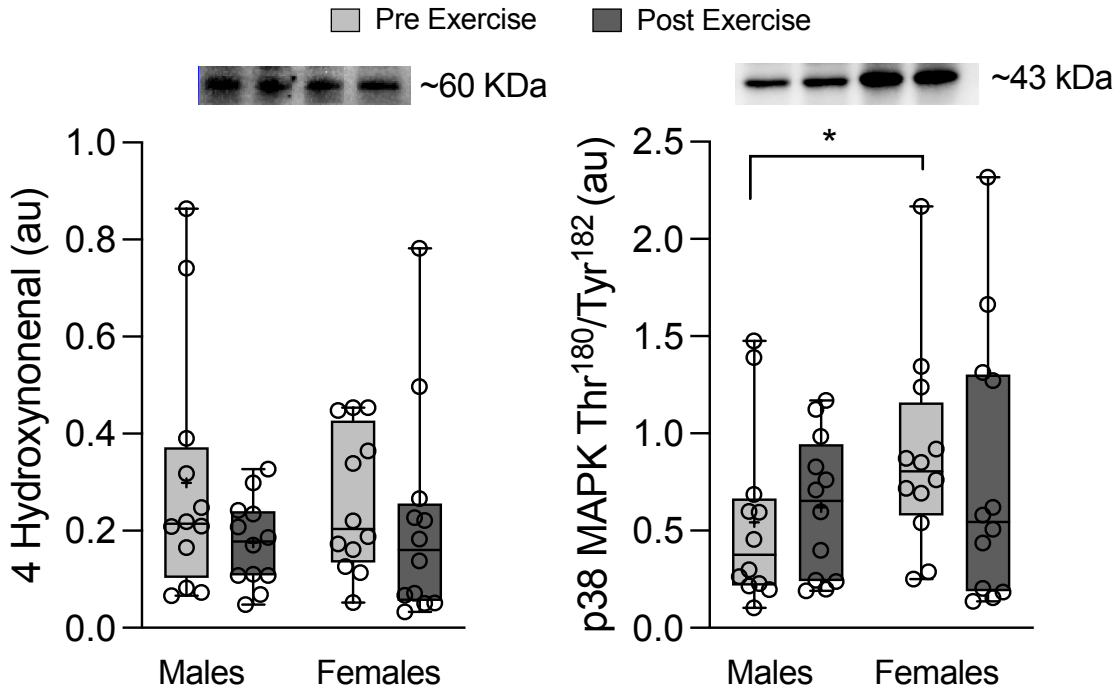

Figure 5.3: Markers of lipid peroxidation and activation during exercise for A) 4-Hydroxynonenal and B) p38 MAPK Thr^{180}/Tyr^{182} phosphorylation, *higher in females pre-exercise (p=0.04)

5.5 Discussion

The purpose of the current study was to investigate sex differences in inflammation, oxidative stress, and antioxidant status at rest, following exercise and during an OGTT in young, healthy males and females matched for training status. The main finding of this study was that in response to an acute bout of HIIE, plasma IL-6 concentrations increased immediately post-exercise, continued to increase during 90 min of rest and increased further during an OGTT with no differences between the sexes. We also found that plasma TNFα concentrations were raised acutely immediately following HIIE exercise in both sexes and then decreased by 90mins post-exercise and did not change during the OGTT. Additionally, we did not see any differences between the sexes in basal levels of NADPH oxidase (NOX2), thioredoxin 1 (TRX1), glutathione peroxidase 1 (GPX1) and glutathione reductase (GR). Furthermore, there was no effect of exercise or sex on 4HNE; however, we did find that p38 MAPK Thr^{180}/Tyr^{182} phosphorylation was higher in females compared to males at rest.

Plasma IL-6 concentration was increased immediately following exercise and continued to increase 90- and 150-minutes post-exercise. IL-6 has been classified as both a pro-inflammatory cytokine and anti-inflammatory myokine (404, 405). When acting as a pro-inflammatory cytokine, IL-6 results in an inhibitory response on the insulin signaling cascade through serine phosphorylation of IRS-1 (406). In response to exercise, IL-6 is secreted from the muscle in an exponential fashion (407), is related to the intensity of the exercise bout (405) and acts in an anti-inflammatory manner. IL-6 increases following exercise induces an increase in the production of IL-1ra and IL-10 by blood mononuclear cells which lead to the stimulation of anti-inflammatory cytokines (408). IL-6 also stimulates β-cell proliferation, regulates β-cell

mass and stimulates glucagon-like peptide 1 thereby improving insulin secretion (409, 410). The increases in IL-6 that were seen following the acute HIIE protocol are in agreeance with other studies that have reported increases in plasma IL-6 concentration following acute HIIE (411-415). Furthermore, we found no difference in IL-6 concentrations between males and females at rest, following exercise or during the OGTT. The lack of difference in basal IL-6 concentration between the sexes is in agreeance with previous research that found no sex difference in basal IL-6 concentrations (416, 417). However, our finding that there was no difference in post-exercise IL-6 concentration between the sexes is in disagreement with a previous study that reported that IL-6 concentration at 60mins following maximal exercise was greater in females than males (418). The study did not control for training status nor menstrual cycle/oral contraceptive use, which may explain why a sex difference was observed. Additionally, this study did not see any effect of sex following an acute submaximal aerobic exercise bout, therefore sex differences in IL-6 response following exercise could be intensity dependent, which warrants further examination.

We also investigated the effects of acute HIIE on plasma concentrations of TNFα, a major modulator of inflammation. We found that TNFα concentration was elevated immediately post-exercise, but that it returned to baseline by 90 mins post-exercise. This finding agrees with other studies that found a significant increase in TNFα following acute HIIE. (419-421). We did not however see any significant differences in TNFα concentrations between the sexes. Our finding is in agreeance with a study in multiple sclerosis patients that also reported no sex differences in proinflammatory cytokine profiles (422). Conversely it has been reported that in healthy adults TNFα is higher in males compared to females (423). This study

however included a small sample size of male subjects and did not control for oral contraception, menstrual cycle, or habitual training status all of which may affect TNFα concentration. Future studies should focus on basal sex differences of TNFα throughout both the luteal phase and follicular phase of the menstrual cycle where there may be differences in circulating levels of TNFα.

Numerous studies have found that males have higher levels of oxidative stress compared to females (271-277, 424). Specifically, previous research has found that NADPH oxidase (NOX) activity is higher in males compared to females (271, 273, 280, 281, 425). NOX2, an isoform of NADPH oxidase, is a key producer of ROS in many cells (282) and can lead to skeletal muscle insulin resistance (426); however, increased NOX2 activity during exercise has been found to be necessary to promote adaptations to high-intensity interval training (393). Thus, differences in NOX2 content between the sexes may explain why males respond more readily to high intensity interval training than females. The sex difference in NOX2 activity is thought to be due to estrogen. Miller et al, 2007 (427), found that in ovariectomized rats NOX2 stimulated O_2^- production was 3-fold higher compared to intact females. Additionally, hormone replacement therapy with 17β-estradiol prevented the increase in O_2^- production in the ovariectomized rats (280). In the current trial we did not find a difference in the content of NOX2 between males and females. The lack of sex difference could be because we tested our female participants during the mid-follicular phase of the menstrual cycle, when estrogen levels are low and not different compared to males (data not shown). However, it has been previously reported that males and females still have differences in metabolism when tested in the mid-follicular phase of the menstrual cycle (19, 20, 22, 246, 247, 428, 429). This difference could be

due to higher estrogen receptor mRNA content and percent estrogen-positive nuclei compared with males (430), rather than the amount of circulating estrogen. Furthermore, we assessed differences in NOX2 content, not activity therefore future trials should examine whether NOX2 activity differs in males and females either at rest or following exercise to see if differences in NOX2 activity could explain the differences in adaptations seen with HIIE training.

In the current trial we found higher basal phosphorylation status of p38 MAPK in females compared to males. This is in accordance with a 2019 study that reported that females had significantly higher phosphorylated p38 MAPK in resting skeletal muscle compared to males (431). The same authors also found a trend for total p38 MAPK content to be higher in females compared to males at rest (431), where our study found no significant differences in total p38 MAPK content between the sexes. However, our findings disagree with a previous study that found no sex difference in p38 MAPK phosphorylation at rest or following an acute bout of sprint interval exercise (432). Neither study however controlled for menstrual cycle or oral contraceptive use, which could have had an effect as estrogen has been previously shown to increase p38 MAPK phosphorylation in endometrial cells (433). Oxidative stress has been found to induce the activation of p38 MAPK (434). Interestingly, the higher basal p38 MAPK phosphorylation in our females was not related to an increased content of oxidative stress markers. Additionally, we did not find that markers of oxidant generation, oxidative stress or antioxidant status differed between the sexes. The lack of sex differences observed in the current trial could be reflective of the methodology used as we only measured the content of oxidant and antioxidant enzymes, where the activity could differ between the sexes since females typically have up-regulated activity of antioxidants and have been shown to have lower

levels of lipid peroxidation compared to males (424, 435). Thus, future work should examine the production of reactive oxygen species and antioxidant activity to see if this differs between the sexes at rest or following exercise.

We also looked at differences in 4HNE, a well-known by product of lipid peroxidation (436). In the current trial we did not find an effect of sex or exercise on 4HNE in skeletal muscle. A study investigating the effects of HIIE in obese middle-aged males found that 4HNE was increased in skeletal muscle 1-hour post-exercise, therefore the timing of our biopsy (immediately post-exercise) may not have accurately captured changes in 4HNE following exercise (325). Our findings disagree with previous work that found that lipid peroxidation was higher in males compared to females (437). However, this previous study did not control for menstrual phase, habitual training status, or overall health status, therefore it is difficult to ascertain whether the higher lipid peroxidation in males in that study was due to inherent sex differences or differences in health and/or training status of male and female participants. Importantly, the lack of sex difference in 4HNE at rest and following exercise is not necessarily indicative of no difference in oxidative stress between the sexes as this is just one indicator of oxidative stress. Future work should investigate other markers of oxidative stress such as total protein oxidation and total protein carbonylation, as well as measuring ROS production at rest and following exercise to ascertain whether there are differences in oxidative stress between the sexes.

We found no difference in antioxidant status between males and females in the current trial. The glutathione (GSH) system is critical to maintaining the redox status of the cell (438). GPX uses GSH to reduce hydrogen peroxide to water and oxygen and creating glutathione

disulfide (GSSG) while glutathione reductase reduces GSSG to reform GSH (439). Previous work has shown that GPX activity in erythrocytes is regulated by sex hormones (440-442), with higher activity in premenopausal females compared to postmenopausal females (442). In skeletal muscle however, it has been shown that GPX activity does not differ between males and females across three different age groups (424). Thus, our finding that GPX1 content did not differ between males and females is in line with previous work. We also did not find a sex difference in GR content. To the best of the authors knowledge, basal sex differences in GR has not yet been investigated in human skeletal muscle. In animal models, liver GR activity is higher in females than males (443, 444), however, as is expected if the greater activity was mediated by estrogen, no sex difference in GR activity is observed in aged rats (444). TRX1 is a key antioxidant system that regulates oxidative stress through providing electrons to thiol-dependent peroxidases to remove reactive oxygen and nitrogen species (445). It has been previously reported that plasma TRX concentrations tended to be higher in females than in males (446); however, in the current trial we did not find differences in skeletal muscle TRX1 content in young recreationally active males and females. Overall, the findings of our study suggest that males and females who are matched for aerobic fitness do not differ in the content of endogenous antioxidants; however, our results cannot conclude whether there are differences in antioxidant activity between males and females.

Overall, the current study had some strengths. We ensured that our males and females were properly matched for habitual training status and controlled for variations in sex hormones that may affect oxidative stress levels in females (447). In physiologically research, it is essential that males and females are matched for fitness relative to fat-free mass

($\dot{V}O_{2peak}$/kgLBM/min), not body weight, as females naturally have a greater body fat percentage compared to males (220). Failure to not properly match males and females in this manner could lead to inappropriate conclusions on the effectiveness of exercise on inflammation and oxidative stress due to baseline differences in fitness as both inflammation (448) and oxidative stress are lower in the trained state (449, 450). There are also some limitations of the current study to discuss. By only assessing the oxidative stress response immediately pre- and post-exercise we could have missed some activation of oxidative and antioxidant response to exercise during the recovery period. We also only measured oxidant and antioxidant status in a select number of proteins and one marker of lipid peroxidation. This unfortunately will not tell us a complete story of the effects of exercise and sex on oxidative stress and future studies should investigate HIIE on robust markers of oxidative stress and ROS generation between the sexes. Additionally, we choose to only look at the response of IL-6 and TNFα following exercise and therefore could have missed significant changes in other cytokines profiles following acute HIIE.

In conclusion, the current study reports that in response to an acute bout of HIIE, plasma concentrations of IL-6 and TNFα increased, and IL-6 continued to increase while TNFα returned to baseline by 90 minutes post exercise, with no differences in response between the sexes. While we did not see any effect of sex on antioxidant status, we did find that females have higher levels of p38 MAPK phosphorylation at rest compared to males. Overall, these findings suggest that differences in antioxidant status, ROS signaling, and inflammation do not contribute to differences in the adaptations induced by high intensity interval exercise in males

and females. However, much more work in this area is required, particularly examining sex-based differences in ROS production and antioxidant activity.

Chapter 6: No effect of sex, but greater post-exercise glucose handling following low-load, high-repetition resistance exercise compared with moderate-intensity continuous exercise in young, recreationally active males and females

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6.1 Abstract:

Exercise has beneficial effects on insulin sensitivity (IS) and glucose handling with both moderate-intensity continuous (MIC) exercise and resistance exercise (RE) inducing beneficial effects. In recent years, low-load high repetition (LLHR) RE has emerged as a strategy to increase muscle mass and strength to levels similar to heavy-load RE, however the effects of LLHR RE on glucose handling and whether the response is similar in males and females is largely unexamined. The purpose of this trial was to compare the acute effects of LLHR RE to MIC exercise on post-exercise glycemic control and insulin sensitivity and determine whether sex inherently influenced these effects. Twenty-four, young healthy (n=12/sex) participants completed acute bouts of MIC exercise (30mins at 65% $\dot{V}O_{2peak}$) and LLHR (3 circuits, 6 exercises/circuit, 25-35 repetitions/exercise/circuit) matched for time with an oral glucose

tolerance test (OGTT) taking place 90 minutes post-exercise. During the post-exercise OGTT blood glucose concentrations (males: p=0.047, η_p^2=0.31; females: p=0.02, η_p^2=0.42) and glucose AUC (males: p=0.04, *d*=0.67, females: p=0.03, *d*=0.74) were lower in both sexes and max glucose concentration was lower in females only (p=0.02, *d*=0.76) following LLHR RE vs MIC exercise. However, phosphorylated ACC Ser^{79} increased following MIC exercise (males: p=0.02, *d*=0.8; females: p=0.003, *d*=1.1). These findings suggest that LLHR RE is a feasible exercise modality to improve post-exercise glycemic control in males and females with no differences between sexes.

Key Words: low-load high repetition resistance exercise, moderate intensity continuous exercise, sex, glucose, insulin sensitivity

6.2 Introduction

A single acute exercise bout can markedly increase post-exercise glucose handling up to 20-fold for 2-72 hours, depending on exercise type, intensity, and duration (9-14). However, when looking at the effects of exercise on post-exercise glucose handling there is conflicting evidence in regards to the best type and duration of exercise to elicit the most beneficial impact on blood glucose concentration (15). There is some evidence that exercise elicits a dose-response effect such that higher energy expenditures and exercise intensities will elicit a greater response on glucose handling and IS, whereas others state that a combined effect of different exercise modalities (aerobic and resistance exercise) is the best approach (15).

In recent years, a more novel form of RE, low-load high repetition (LLHR) RE in which the load lifted is lower but the number of repetitions is higher, has emerged as a strategy to increase muscle mass and strength to levels similar to that of high-load RE (356, 451). The more aerobic nature of LLHR RE may also induce favourable changes in blood glucose handling, acting akin to the combination of aerobic and RE; however, the effects of an acute bout of LLHR RE on post-exercise blood glucose handling and IS is largely unexamined, particularly in females.

Biological sex is known to influence exercise-induced improvements in glycemic control, such that exercise training has been found to induce more favourable changes in glycemic control and insulin sensitivity in males compared to females (16-18, 306). Fuel utilization and muscle metabolism during exercise also differs by sex, such that females tend to oxidize more fat and less carbohydrates than males during a bout of exercise (19-22), which can lead to differences in muscle energetics and activation of signaling pathways (452). Over time these differences in metabolism may result in differential training adaptations, thus understanding

how sex inherently influences the response to acute bouts of exercise is important as it may further our understanding as to why males and females do not necessarily respond in the same manner to a training intervention.

Biological sex also influences the prevalence of impaired fasting glucose and impaired glucose tolerance, with males more frequently classified as having impaired fasting glucose and females more frequently classified as having impaired glucose tolerance (239-241). Importantly, both increased height and body weight, which are generally greater in males, are related to greater glucose tolerance (240). Thus, the observed sex-based differences in glucose tolerance may be related to inherent differences in height and body weight between males and females and thus, the dose of glucose consumed should be considered when comparing the effects of an intervention on glucose handling between males and females. Recently, work from our laboratory found no difference in blood glucose concentrations between males and females in response to an OGTT performed after an acute bout of high intensity interval training (Beaudry 2022, under review). However, when glucose concentrations were normalized to the dose of glucose consumed relative to body weight or lean body mass, glucose concentrations and glucose AUC were lower in females than males (Beaudry 2022- under review). This finding suggests that when differences in body weight and muscle mass are considered, females are inherently better able to clear a given amount of glucose from the blood during a post-exercise glucose challenge, at least following high intensity interval exercise.

Therefore, the purpose of the current study was to investigate the effects of an acute bout of LLHR RE and MIC exercise on post-exercise glycemic control in young, recreationally active males and females while taking into consideration the relative dose of glucose

consumed. Additionally, we sought to examine if there were any sex-based differences in the content of proteins related to insulin signaling and exercise-induced activation of insulin-independent signaling. We chose to study young healthy individuals so that we could determine the inherent effects of sex on post-exercise glucose handling, insulin sensitivity and insulin dependent and independent signaling in response to these exercise modes in the absence of pathology. We hypothesized that an acute bout of LLHR RE would result in a greater post-exercise glycemic control and insulin sensitivity compared to MIC exercise and that the effects of exercise on glycemic control and insulin sensitivity would be blunted in females compared with males. Additionally, we hypothesized that the activation of proteins relating to insulin-independent signaling would be greater following LLHR RE compared to MIC exercise. Lastly, we hypothesized that females would have greater total content of insulin-signaling proteins, but that activation of signaling proteins that lead to GLUT4 translocation would be blunted in females compared with males post-exercise.

6.3 Methods

6.3.1 Study participants

Twenty-four (12/sex) recreationally active young males and females took part in the study. Participants were the same as Chapter 4 and Chapter 5. Participants were deemed recreationally active based on self-reported habitual physical activity and participated in no more than 3 sessions of cardiovascular exercise or 2 sessions of RE per week. Additionally, participants were excluded if they had any chronic health conditions including type 1 or type 2 diabetes, cardiovascular, respiratory, or digestive disorders; an inability to complete a single

exercise session or perform exercise as suggested by the Get Active Questionnaire (GAQ); an allergy to local aesthetic; were taking prescription anti-coagulant or anti-platelet medications; or a BMI >27 kg/m^2. Females were excluded if they were taking any form of monophasic birth control. Habitual dietary intake and physical activity were maintained throughout the trial. Prior to commencing the trial all participants provided written informed consent. The study conformed with all standards outlined by the Tri-Council Policy Statements for Ethical Conduct for Research Involving Humans (TCPS 2) (403). The study protocol was reviewed and received ethics clearance from the University of Waterloo Research Ethics Committee (ORE #22477).

6.3.2 Experimental overview

The experimental protocol consisted of 3 preliminary visits and 2 acute exercise sessions completed in randomized order (Figure 6.1). The first preliminary visit included consent and anthropometric measurements such as height and weight. To ensure that differences in habitual diet and physical activity did not influence the results, participants were given a 3-day food log and a 7-day physical activity log and instructed on how to accurately complete these logs. The second preliminary visit included a DXA scan for determination of body composition, an assessment of aerobic fitness ($\dot{V}O_{2peak}$ test) and the first of two maximal strength assessments [one repetition max (1RM)]. The third visit included a familiarization session for the MIC exercise protocol and the second 1RM test. Visits 4 and 5 included the two acute exercise bouts completed in random order. The MIC exercise bout consisted of a 30-minute bike ride on a cycle ergometer at 65% $\dot{V}O_{2peak}$. The LLHR RE bout consisted of a whole-body circuit including chest press hamstring curl, lat pulldown, knee extension, shoulder press and leg press, repeated 3 times with

30s rest between exercises and 2 minutes rest between sets. For visits 4 and 5 participants reported to the laboratory after a 12-hour fast and refrained from exercise for 72 hours prior to and alcohol for 24 hours prior to each session. Additionally, to ensure similar nutrient availability, participants consumed the same diet for the 24h leading up to the acute exercise visits. Females were tested in the mid-follicular phase (day 5-9) of the menstrual cycle, as menstrual cycle phase can influence fuel metabolism during exercise (20) and insulin sensitivity (367). Participants completed the acute MIC exercise and LLHR bouts in a randomized order with blood draws and muscle biopsies immediately pre- and post-exercise. Ninety minutes after the end of each acute exercise bout participants underwent a 120 min post-exercise OGTT (75g glucose, Trutol™, Thermo Scientific, Waltham, Massachusetts, United States). Pre blood samples were assessed for estradiol and progesterone using commercially available kits (DKO003 and DKO006, Diametra, Spello PG, Italy). The overall study schematic is found in Figure 6.1.

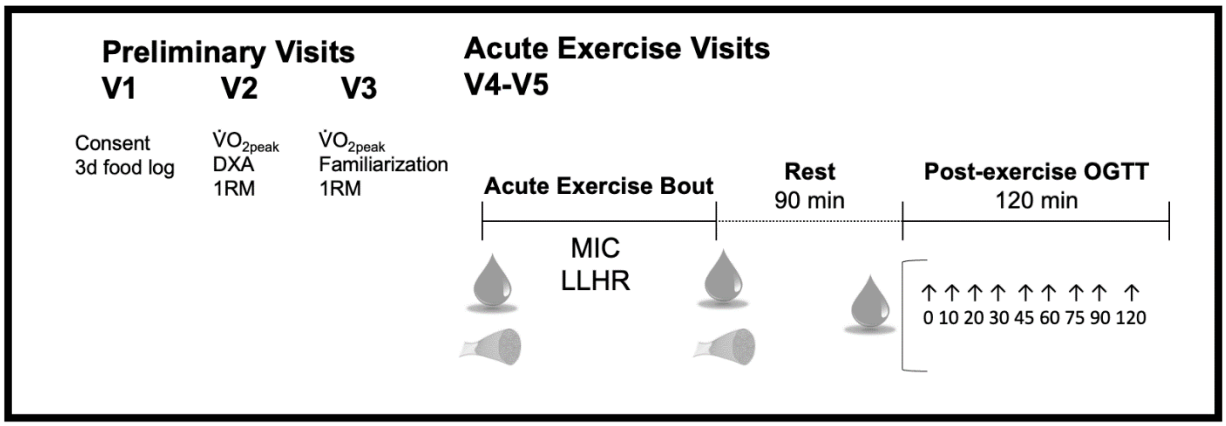


Figure 6.1: Protocol overview for preliminary visits (V1-V3), as well as the acute exercise visits (V4-V5). MIC, moderate intensity continuous exercise; LLHR, low-load high-repetition resistance exercise; OGTT, oral glucose tolerance test; $\dot{V}O_{2peak}$, peak oxygen consumption; DXA, dual-energy X-ray absorptiometry; 1RM, 1 repetition max strength testing

6.3.3 $\dot{V}O_2$ peak testing

To appropriately match males and females for fitness level and to determine the work rate corresponding to 65% $\dot{V}O_{2peak}$ for the MIC exercise bout, participants underwent an incremental cycling test to volitional fatigue on an electronically braked cycle ergometer (Ergoselect 100, ergoline GmbH, Germany) using an online gas collection system (Vmax encore CPET Systems, Vyaire medical, Chicago). Participants began with a warmup at 50W for 2 mins and thereafter the intensity was increased by 1W every 2s until volitional fatigue or the point at which pedal cadence fell below 50 rpm. A metabolic cart with an online gas collection system (Vmax encore CPET Systems, Vyaire medical, Chicago) measured oxygen consumption and carbon dioxide production. In order to ensure that males and females were matched for aerobic fitness (220), $\dot{V}O_{2peak}$ was expressed relative to fat-free mass. Participants returned to the lab at least 24-hours after the $\dot{V}O_{2peak}$ testing and cycled at the approximate wattage of the MIC exercise bout until steady state was reached to ensure that participants exercised at appropriate loads to achieve the target of 65% $\dot{V}O_{2peak}$.

6.3.4 1RM testing

To determine the workload that corresponded to 30% 1RM for each exercise completed during the LLHR RE trial participants completed two separate 1RM (strength) testing sessions. Participants completed 1-RM testing on chest press (Technogym, Cesena, Italy), shoulder press (Technogym, Cesena, Italy), lat pulldown (Technogym, Cesena, Italy), leg press (Technogym, Cesena, Italy), knee extension (Technogym, Cesena, Italy) and hamstring curl (Technogym, Cesena, Italy). The same investigators administered all strength testing. After a brief general

warm-up (5-minute, cycling), a specific warmup of the given exercise was performed at approximately 50% of the participants estimated 1RM based on habitual 10-RM. Load was progressively increased by ~10-20% for each attempt until a true 1RM was reached. Three to five minutes of rest was given between each attempt. A successful attempt required the participant to move the load throughout the full range of motion for the exercise with correct form. The higher load of the two separate 1RM tests was used to determine 30% of a participants 1RM for the LLHR RE bout.

6.3.5 Moderate-intensity continuous exercise bout

The MIC exercise bout consisted of participants performing a 30-minute bike ride on a cycle ergometer (Ergoselect 100, ergoline GmbH, Germany) at 65% of their predetermined $\dot{V}O_{2peak}$. This work rate was chosen to allow for a significant contribution from both carbohydrates and fat from both muscle stores and plasma (249).

6.3.6 Low-load high repetition resistance exercise bout

The LLHR RE bout consisted of lifting a load corresponding to 30% 1RM for 20-25 repetitions for each exercise in a circuit format. This workload was chosen because training to failure at 30% 1RM has been found to produce a similar acute muscle protein synthetic response to a 90% 1RM protocol (356, 451). 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 was performed to

volitional failure. The LLHR RE exercise session was designed to be completed in ~30 minutes so that the LLHR RE and MIC exercise bouts were matched for time.

6.3.7 Oral glucose tolerance test

In order to allow for reinternalization of GLUT4 that translocated to the membrane during exercise (53), an OGTT was performed following 90 minutes of rest after exercise cessation. Prior to consuming the 75g glucose beverage (Trutol™, Thermo Scientific, Waltham, Massachusetts, United States) participants had a blood sample taken (t=0 min) and blood samples were then taken 10, 20, 30, 45, 60, 75, 90 and 120 minutes after drink consumption. Plasma samples were analysed for glucose and insulin concentrations. Plasma glucose concentration was analysed using a spectrophotometric glucose assay using PGO enzyme preparation (Sigma-Aldrich, St. Louis, M0). Insulin concentrations were analysed using radioimmunoassay (RIA) kits (#HI-14K) according to manufacturer's instruction (Millipore Sigma, Burlington, Massachusetts, United States). Glucose and insulin area under the curve (AUC) values were calculated using the trapezoidal rule. Glucose max concentration (Cmax) is the maximum glucose concentration that occurred during the OGTT. Time of max glucose concentration (Tmax) is the time during the OGTT when the Cmax occurred. Glucose concentrations and AUC values are also reported normalized to the glucose dose relative to body weight, lean body mass and height. For each normalization the relative dose was determined by dividing the glucose dose (75g) by body weight (kg), height (cm) or lean body mass (kg). HOMA-IR was calculated according to the formula: fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5. HOMA-IR was chosen as a measure of fasting IR. The Matsuda index was

calculated according to the formula: [10,000 /√glucose minute 0 x insulin minute 0) (mean glucose (OGTT) x mean insulin OGTT)](368). The Matsuda index was chosen as an indicator of fed state IS.

6.3.8 Muscle analysis

Muscle samples for Western Blot analyses were homogenized in ice cold 25mM Tris buffer [25mM Tris, 0.5% (v/v) Triton X-100 with protease/phosphatase inhibitor Tablets (Roche Diagnostics, Laval, QC, Canada)] at a ratio of 10μL buffer to 1mg of muscle in a prechilled homogenization Biopur Eppendorf (Eppendorf, Mississauga, ON, Canada) using a bead homogenizer (TissueLyser II, Qiagen, Toronto, ON, Canada) run at 20 cycles/second for 40 seconds. Once the samples were sufficiently homogenized, they were spun at 10,000G for 10 minutes at 4^{o}C. The supernatant was separated and allocated into a prechilled Eppendorf while the pellet was frozen for potential use in additional analyses. Western blot analysis was conducted using previously described techniques (372). Briefly, protein concentration of homogenates was determined using the BCA protein assay technique (Thermo Fischer Scientific, Waltham, MA) and equal amounts of protein were prepared in 4x Laemmli's buffer and then separated using 10% SDS-PAGE on 4%-15% Criterion TGX Stain-free protein gels (Bio-Rad, Hercules, CA) at 200V for 40mins and electro transferred to PVDF membranes. A protein ladder (Precision Plus Protein Standard, Bio-Rad, Hercules, CA, USA) and a standard curve (pooled from all samples) were run on every gel. Total protein and visual confirmation of protein transfer was done pre- and post-membrane transfer, respectively, using a Chemidoc MP (Bio-Rad, Hercules, CA, USA). Following 1h block in 5% bovine serum albumin (BSA) in 1X Tris-buffered saline and

Tween 20 (TBST), membranes were incubated in primary antibody overnight at 4°C in 5% BSA/TBST based on previously optimized conditions. The primary antibodies used for western blotting were phosphorylated p38 MAPK Thr^{180}/Tyr^{182} (92152, 1:1000, Cell Signaling, Danvers, MA, USA), total p38 MAPK (9212s, 1:1000, Cell Signaling, Danvers, MA, USA), phosphorylated AMPKα Thr^{172} (2531S, 1:1000, Cell Signaling, Danvers, MA, USA), total AMPKα (2532S, 1:1000, Cell Signaling, Danvers, MA, USA), phosphorylated Akt Ser^{437} (9271T, 1:1000, Cell Signaling, Danvers, MA, USA), total Akt (9272S, 1:1000, Cell Signaling, Danvers, MA, USA), phosphorylated TBC1D4 Thr^{642} (4288S, 1:1000, Cell Signaling, Danvers, MA, USA), total TBC1D4 (2447S, 1:1000, Cell Signaling, Danvers, MA, USA), phosphorylated TBC1D1 Ser^{237} (07-2268, 1:1000, Millipore Sigma, Burlington, MA, USA) and total GLUT4 (ab654, 1:1000, Abcam, Toronto, ON, Canada). After 3x5 min washes in TBST, membranes were incubated in horseradish peroxidase-conjugated goat anti-rabbit secondary (170-6515, Bio-Rad, Hercules, CA, USA) diluted to 1:20,000 in 5% BSA/TBST for 1h at room temperature and washed in TBST for 3x5 min. Membranes were then incubated for 1min with enhanced chemiluminescence (ECL) (Clarity Western ECL Substrate, Bio-rad, Hercules, CA, USA) and imaged on the Chemidoc imaging system (Bio-Rad, Hercules, CA, USA). Bands were quantified using Image Lab software (Version 3.0, Bio-Rad, Hercules, CA, USA) and protein content was normalized within and between blots using total protein and the standard curve obtained from the gel, as previously described (372, 373).

6.3.9 Statistical analysis:

All statistical analyses were conducted using SPSS (version 25, IBM, Armonk, NY, USA). To compare the effects of LLHR RE and MIC exercise on post-exercise glycemic handling and phosphorylation of proteins relating to insulin independent and dependent signaling we performed a mixed model ANOVA with trial (MIC/LLHR RE) as the within variable in males and females separately. A repeated measures ANOVA with trial (MIC/LLHR RE) and OGTT timepoint (6 or 9 levels) as the within variables was used to determine the effects of exercise mode on glucose and insulin concentrations during the OGTT on males and females separately. A paired samples t-test was used to assess differences between exercise modes for all other measures in males and females separately. Secondly, to assess if the response to MIC and LLHR RE differed between the sexes we performed the same analyses as detailed above, but with sex as the between variable (2 levels, male/female), with MIC exercise and LLHR assessed separately. We decided to perform the statistical analyses separately so that we could ascertain the effects of exercise and sex separately. Data sets were assessed for normality using the Shapiro-Wilk test and were found to be not normally distributed. Thus, values were log transformed prior to undergoing statistical analyses using ANOVA. ANOVA results using transformed data sets were consistent with using nontransformed data sets. Post-hoc analyses were conducted using a Tukey's HSD test where appropriate. Significance was set at $p \leq 0.05$. Partial eta-squared (η_p^2) values were calculated to estimate the effect sizes (small 0.04, medium 0.25, large 0.64) for main effects and interactions where necessary. Cohen's *d* values were calculated to estimate effect sizes (small 0.2, medium 0.5, large 0.8) for t-test and post hoc comparisons where

necessary. All data are presented as means ± SEM for *n*= 12 in each group. All graphs were created using GraphPad Prism (GraphPad Software Inc., CA).

6.4 Results

6.4.1 Participant characteristics

Participant characteristics are found in Table 6.1 (same as Chapter 4) and have been reported previously (Beaudry 2022, under review). As to be expected there were significant differences in weight, %BF, lean body mass and $\dot{V}O_{2peak}$ between males and females. Importantly, once $\dot{V}O_{2peak}$ was adjusted based on lean body mass rather than body weight, there was no significant difference between males and females, indicating that our males and females were matched for aerobic fitness.

Table 6.1: Participant characteristics from male and female participants

	Males	Females	p value	Cohen's d
Age (years)	22 ± 1	21 ± 1	p=0.45	*d*=0.46
Weight (kg)	76.6 ± 2.4	62.6 ± 3.1	p<0.01*	*d*=1.46
Height (cm)	177.9 ± 2.0	162.7 ± 2.6	p<0.01*	*d*=1.95
Body Mass Index (kg/m^2)	23.7 ± 0.8	22.9 ± 0.6	p=0.45	*d*=0.32
Body Fat (%)	20.9 ± 1.6	32.7 ± 1.3	p<0.001*	*d*=2.39
Fat mass (kg)	15.4 ± 1.4	19.5 ± 1.2	p<0.05*	*d*=0.92
Lean Body mass (kg)	55.1 ± 2.2	37.6 ± 1.8	p<0.001*	*d*=2.53
$\dot{V}O_{2peak}$ (ml/kgBW/min)	43.1 ± 1.6	34.2 ± 1.4	p<0.001*	*d*=1.67
$\dot{V}O_{2peak}$ (ml/kgLBM/min)	59.5 ± 1.4	56.5 ± 1.7	p=0.21	*d*=0.52

All results are shown as mean ± SE, n = 12 males, and n = 12 females.
* Significance from independent samples t-test, significantly different with P value <0.05

6.4.2 Plasma hormone concentrations

Plasma hormone concentrations are shown in Table 6.2. Plasma estradiol (pg/mL) and progesterone (ng/mL) were not significantly different between females prior to MIC exercise or LLHR RE (p=0.99 and p=0.84, respectively). Furthermore, plasma estradiol concentrations did not differ between males and females prior to the MIC (p=0.16) or LLHR RE (p=0.13) exercise bouts. Similarly, plasma progesterone concentrations did not differ between males and females prior to the MIC (p=0.96) or LLHR RE (p=0.369 exercise bouts (Table 6.2).

Table 6.2: Plasma estradiol and progesterone concentrations

	Males	Females		p values		
		MIC	LLHR	Trial	Sex M	Sex R
Estradiol (pg/mL)	21.6 ± 4.1	31.3 ± 7.2	38.1 ± 6.0	p=0.99	p=0.16	p=0.13
Progesterone (ng/mL)	3.4 ± 0.5	3.4 ± 0.4	3.0 ± 0.4	p=0.84	p=0.96	p=0.69

All results are shown as mean ± SEM, n=12 males and n=12 females.
p values from non-paired t-test. Trial is the t-test within female subjects. Sex M is the t-test between females (MIC) and males. Sex R is the t-test between females (LLHR RE) and males.

6.4.3 Habitual dietary intake

Table 6.3 shows the average dietary intake of participants from the 3-day food log and has been reported previously (Beaudry 2022, under review). Males consumed more calories per day than females (p<0.01). Males also consumed more protein (p<0.01), fat (p<0.01) and carbohydrates (p<0.01) compared to females. When expressed as percent of total daily caloric intake, males and females ate comparable percentages of protein, fat, and carbohydrates.

Males and females also consumed similar g/kg/day of protein. Diets did not differ between males and females or between trials for the day before the acute exercise sessions (data not shown).

Table 6.3: Diet data for males and females

	Habitual Dietary Intake			
	Males	**Females**	**p value**	**Cohen's d**
Kcal	2008.0 ± 119.9	1476.8 ± 60.7	**p<0.01**	*d*=0.93
PRO				
g	96.4 ± 7.8	65.0 ± 3.4	**p<0.01**	*d*=0.87
g/kgBW/d	1.3 ± 0.1	1.1 ± 0.1	p=0.10	*d*=0.39
% Of Kcals	18.8 ± 1.2	17.4 ± 0.8	p=0.35	*d*=0.23
FAT				
g	79.1 ± 5.6	56.5 ± 3.6	**p<0.01**	*d*=0.80
g/kgBW/d	1.0 ± 0.1	0.9 ± 0.1	p=0.26	*d*=0.25
% Of Kcals	34.8 ± 1.4	33.1 ± 1.4	p=0.38	*d*=0.20
CHO				
g	234.0 ± 15.8	185.6 ± 9.2	**p=0.01**	*d*=0.63
g/kgBW/d	3.1 ± 0.2	3.1 ± 0.2	p=0.86	*d*>0.01
% Of Kcals	46.3 ± 1.7	49.5 ± 1.5	p=0.16	*d*=0.34

All results are shown as mean ± SEM, n= 12 males and n= 12 females.
* Significance from independent samples t-test, significantly different with P value <0.05

6.4.4 MIC exercise and LLHR average heart rates

Average heart rate (HR) during the acute exercise bouts is shown in Figure 6.2. There was a main effect of trial on HR (p=0.004, η_p^2=0.77) whereby average HR during LLHR was lower

than the MIC exercise bouts. There was no main effect of sex (p=0.83, η_p^2=0.01) and no trial x sex interaction (p=0.23, η_p^2=0.23).

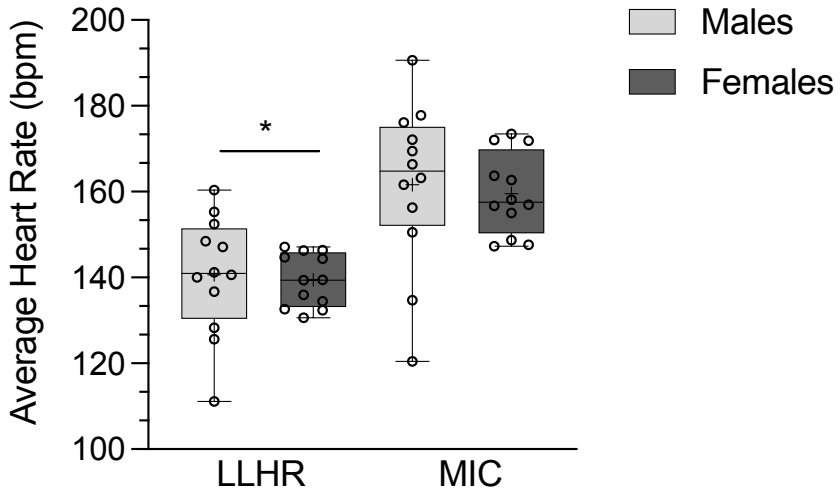


Figure 6. 2: Average heart rate during the acute exercise bouts in males (n=12) and females (n=12). *Denotes significantly different compared to MIC exercise (p<0.001, *d*=1.6) from repeated measures ANOVA.

6.4.5 Plasma glucose concentrations

Plasma glucose concentrations during the OGTT are shown in Figure 6.3. To determine the effect of exercise mode on post-exercise glycemic handling we assessed the effect of trial in males and female separately. In males, there was a main effect of time indicating that blood glucose concentration increased and was significantly greater than baseline by 10 mins post-consumption, remained elevated throughout the OGTT and declined but remained above baseline concentrations at the end of the OGTT (p<0.001, η_p^2=0.61). There was a main effect of trial (p=0.047, η_p^2=0.31) indicating that glucose concentrations during the OGTT were significantly lower following LLHR RE compared to MIC exercise. There was a significant trial x time interaction indicating specifically that blood glucose concentrations at 45mins, 60mins and

75mins were lower following LLHR RE compared to MIC exercise (p=0.01, η_p^2=0.25). In males, glucose AUC was lower following LLHR RE compared with MIC exercise (p=0.04, *d*=0.67). There was no significant difference in maximal glucose concentration (p=0.072, *d*=0.60) or time of maximal glucose concentration (p=0.68, *d*=0.09; Table 6.3) in males between LLHR RE and MIC exercise. Similar trends were found in females, with a main effect of time showing that blood glucose concentration increased and was significantly greater than baseline by 10 mins post-consumption, remained elevated throughout the OGTT and declined but remained above baseline concentrations at the end of the OGTT (p<0.001, η_p^2=0.66). There was also a main effect of trial in females (p=0.02, η_p^2=0.42) indicating that glucose during the OGTT was lower following LLHR RE compared to MIC exercise. There was no significant trial x time interaction in females (p=0.27, η_p^2=0.11). Maximal glucose concentration (p=0.02, *d*=0.76) and glucose AUC (p=0.03, *d*=0.74) were lower following LLHR RE compared with MIC exercise in females. There was no difference in time of maximal glucose concentration in females between LLHR RE and MIC exercise (p=0.61, *d*=0.15).

Next, we assessed if the response to either of the exercise bouts differed between the sexes. There was no effect of sex (p>0.11, η_p^2>0.06) and no sex x time interaction (p>0.26, η_p^2>0.06) during either post-exercise OGTT indicating that post exercise glucose concentrations did not differ between males and females following either bout of exercise. There were no significant differences between the sexes for maximal glucose concentration (p>0.07, *d*>0.33), time of maximal glucose concentration (p>0.11, *d*>0.51), and glucose AUC (p>0.07, *d*>0.33) during the post-MIC or LLHR exercise OGTTs.

When glucose concentrations during the post MIC exercise OGTT were normalized to both body weight ($p=0.03$, $\eta_p^2=0.19$) and lean body mass ($p<0.001$, $\eta_p^2=0.49$) glucose concentrations were higher in males than females (Figure 6.3B, C). Similarly, during the post LLHR RE OGTT glucose concentrations normalized to lean body mass ($p=0.001$, $\eta_p^2=0.40$), but not body weight ($p=0.057$, $\eta_p^2=0.16$), were higher in males than females. There was no effect of sex when adjusting glucose concentrations to height during the post-MIC exercise ($p=0.82$, $\eta_p^2=0.002$) or LLHR RE OGTTs ($p=0.70$, $\eta_p^2=0.01$). Additionally, when glucose values were normalized for lean body mass glucose AUC following both MIC exercise ($p<0.001$, $d=0.86$) and LLHR RE ($p<0.002$, $d=1.46$) was higher in males compared to females. This was also found when glucose values were normalized for body weight following MIC exercise ($p=0.046$, $d=0.86$), but not LLHR RE ($p=0.09$, $d=0.73$). Similarly, we found that when glucose values were normalized to lean body mass maximal glucose concentration was higher in males than females following both MIC exercise ($p=0.001$, $d=1.65$) and LLHR RE ($p=0.001$, $d=1.51$).

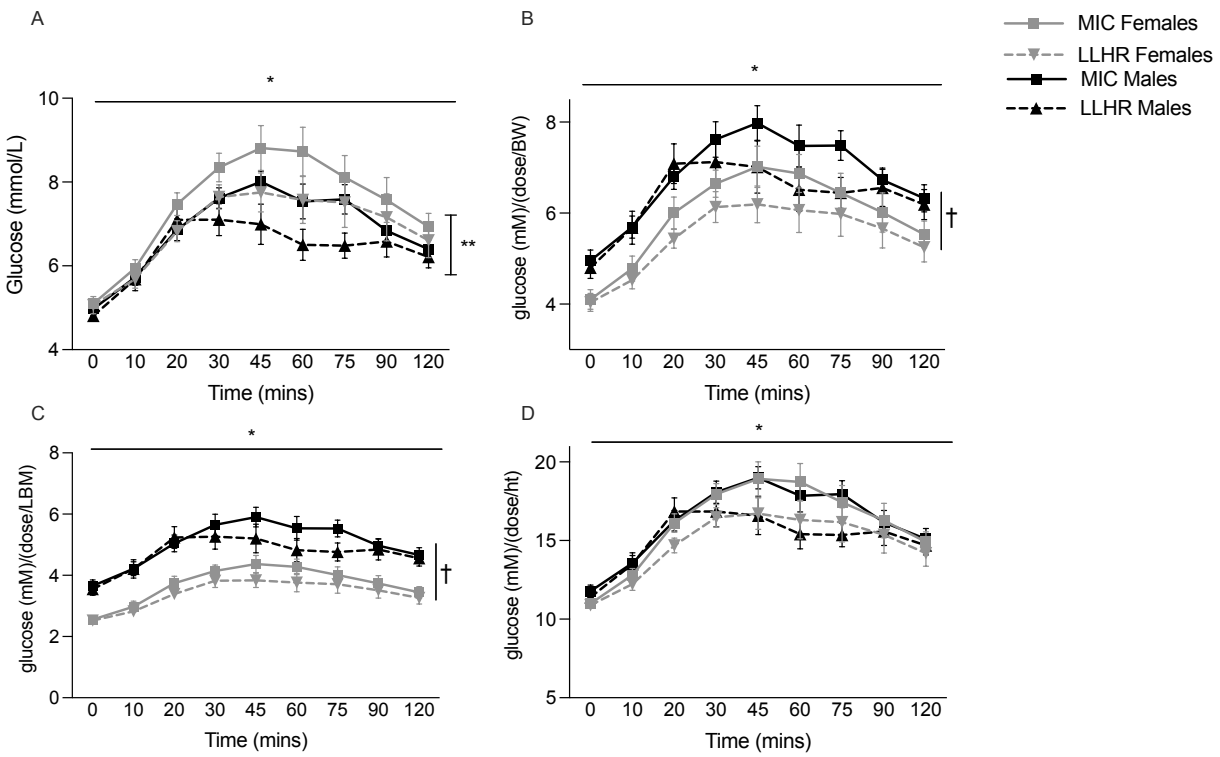


Figure 6.3: Control and post-exercise oral glucose tolerance test glucose curves in males and females. A) glucose (mmol/L) concentrations *represents main effect of time (p<0.05) ** represents main effect of trial (p=0.05), B) Glucose (mmol/L) relative to dose normalized to body weight (kg), *represents main effect of time (p<0.05), † represents main effect of sex (p<0.05), C) glucose (mmol/L) relative to dose normalized to lean body mass (kg), * represents main effect of time (p<0.05), † represents main effect of sex (p<0.05), D) glucose (mmol/L) relative to dose normalized to height (cm) * represents main effect of time (p<0.05)

6.4.6 Plasma insulin concentrations

Plasma insulin curves during the post-exercise OGTTs are shown in Figure 6.4. In males, blood insulin concentration increased after glucose consumption and was significantly higher by 10 mins and remained elevated at the 120min mark ($p<0.001$, $\eta_p^2=0.67$). There was no effect of trial ($p=0.65$, $\eta_p^2=0.02$) and no trial x time interaction ($p=0.15$, $\eta_p^2=0.13$). There were no differences in maximal insulin concentration ($p=0.88$, $d=0.05$), time of maximal insulin concentration ($p=0.28$, $d=0.04$), or insulin AUC ($p=0.59$, $d=0.16$) in males between exercise bouts. We found similar results in females, with a significant main effect of time ($p<0.001$,

η_p^2=0.68) also indicating that blood insulin concentration increased after glucose consumption and was significantly higher by 10 mins and remained elevated at the 120min mark. There was no main effect of trial (p=0.27, η_p^2=0.11) and no trial x time interaction (p=0.44, η_p^2=0.08). Additionally, we did not see any effect of trial on maximal insulin concentration (p=0.08, *d*=0.55), time of maximal insulin concentration (p=0.11, *d*=0.51) or insulin AUC (p=0.24, *d*=0.38) in females. When assessing differences between the sexes we found a significant sex x time interaction (p=0.01, η_p^2=0.15) during the post-LLHR RE OGTT indicating that males had significantly lower blood insulin concentration at the end of the OGTT compared to females. However, there was no main effect of sex (p<0.23, η_p^2<0.03) for insulin concentration during either the post MIC exercise or post LLHR RE OGTT and no sex x time interaction (p=0.73, η_p^2=0.02) during the post-MIC exercise OGTT. We found a sex difference in the time of maximal insulin concentration during the post-LLHR RE OGTT (p=0.02, *d*=1.1) indicating that peak insulin concentration occurred earlier in males compared to females. There were no sex differences in maximal insulin concentration (p<0.21, *d*>0.08) or insulin AUC (p>0.25, *d*>0.29) following either exercise bout and no sex difference for time of maximal insulin concentration during the post MIC exercise OGTT (p=0.86, d=0.07).

Insulin sensitivity measures are shown in Table 6.4. There was no difference in HOMA-IR values between MIC exercise and LLHR RE in males (p=0.38, *d*=0.27) or females (p=0.20, *d*=0.40). Additionally, there was no significant sex difference in HOMA-IR following either acute exercise bout (p>0.33, *d*<0.27). Similarly, there was no difference in Matsuda index between MIC exercise and LLHR RE in males (p=0.27, *d*=0.34) or females (p=0.94, *d*=0.21) and no sex difference in Matsuda index following either acute exercise bout (p>0.38, *d*>0.06).

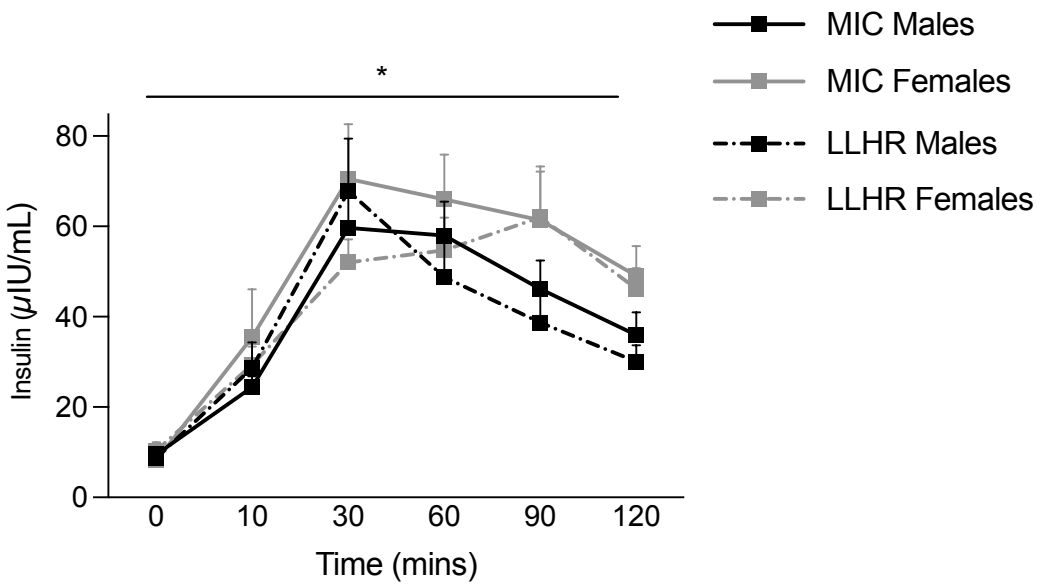


Figure 6.4. Control and post-exercise oral glucose tolerance test insulin curves in males (n=12) and females (n=12). * Represents main effect of time from RM ANOVA in males ($p<0.001$, $\eta_p^2=0.67$) and females ($p<0.001$, $\eta_p^2=0.68$).

Table 6.4: Glucose and insulin measurements during the post-exercise OGTTs in males and females

Variable	Males		Females		p values			
	MIC	LLHR	MIC	LLHR	Sex MIC	Sex LLHR	Trial Males	Trial Females
HOMA-IR (AU)	2.1 ± 0.3	1.9 ± 0.2	1.9 ± 0.2	2.5 ± 0.6	0.52	0.33	0.38	0.20
Matsuda Index (mass units)	5.7 ± 0.7	6.4 ± 0.7	5.5 ± 0.7	5.5 ± 0.7	0.90	0.38	0.27	0.94
Glucose AUC (mmol/L · 120min)	843 ± 25	782 ± 32	923 ± 44	849 ± 46	0.13	0.24	**0.04**	**0.03**
Glucose AUC (mmol/L · 120min) by BW	835 ± 30	780 ± 44	735 ± 37	676 ± 38	**0.05**	0.09	0.053	**0.03**
Glucose AUC (mmol/L · 120min) by LBM	618 ± 28	577 ± 37	457 ± 23	420 ± 23	**0.000**	**0.002**	0.054	**0.03**
Glucose AUC (mmol/L · 120min) by ht	1995 ± 63	1852 ± 83	1985 ± 87	1826 ± 93	0.93	0.84	**0.045**	**0.03**
Glucose Cmax(mmol/L)	8.4 ± 0.2	8.0 ± 0.3	9.4 ± 0.4	8.4 ± 0.5	0.07	0.42	0.06	**0.02**
Glucose Cmax by BW	8.4 ± 0.3	7.9 ± 0.4	7.5 ± 0.4	6.7 ± 0.5	0.09	**0.07**	0.09	**0.03**
Glucose Cmax by LBM	6.2 ± 0.3	5.9 ± 0.4	4.7 ± 0.3	4.2 ± 0.3	**0.001**	**0.001**	0.09	**0.03**
Glucose Cmax by ht	20.0 ± 0.6	18.9 ± 0.7	20.2 ± 0.9	18.1 ± 1.0	0.87	0.53	0.07	**0.03**
Glucose Tmax(mmol/L)	41.7 ± 5.1	39.2 ± 6.0	50.4 ± 4.7	54.2 ± 6.6	0.22	0.11	0.77	0.61
Insulin AUC (mmol/L · 120min)	5569 ± 571	5246 ± 780	6893 ± 968	5990 ± 678	0.25	0.48	0.59	0.24
Insulin Cmax (µIU/mL)	69.9 ± 7.4	71.2 ± 11.5	89.9 ± 13.6	68.3 ± 8.5	0.21	0.84	0.88	0.08
Insulin Tmax (µIU/mL)	50.0 ± 5.6	42.5 ± 4.5	48.3 ± 7.4	67.5 ± 8.4	0.86	**0.02**	0.28	0.11

All results are shown as mean ± SEM, males (n= 12) and females (n= 12). P values are listed in order effect of sex with MIC exercise, effect of sex with LLHR RE, effect of trial in males, effect of trial in females

6.4.7 Mixed muscle protein content

6.4.7.1 Effect of exercise bout

The effects of each acute exercise bout on the change in phosphorylation status of insulin dependent and independent signaling proteins in males and females are shown in Figure 6.5. In males, the change in phosphorylated ACC Ser^{79} was significantly greater following MIC exercise compared to LLHR RE (p=0.02, *d*=0.8). Neither exercise bout induced a significant change in phosphorylated TBC1D1 Ser^{237} (p=0.78, *d*=0.08), TBC1D4 Thr^{642} (p=0.86, *d*=0.05), AMPK Thr^{172} (p=0.60, *d*=0.16), p38 MAPK Thr^{180}/Tyr^{182} (p=0.77, *d*=0.09), Akt Thr^{308} (p=0.27, *d*=0.33) or PTEN Ser^{380} (p=0.59, *d*=0.16). In females, the change in phosphorylated ACC Ser^{79} was significantly greater following MIC exercise compared to LLHR RE (p=0.003, *d*=1.1). Neither exercise bout induced a significant change in phosphorylated TBC1D1 Ser^{237} (p=0.14, *d*=0.45), TBC1D4 Thr^{642} (p=0.06, *d*=0.61), AMPK Thr^{172} (p=0.76, *d*=0.09), p38 MAPK Thr^{180}/Tyr^{182} (p=0.47, *d*=0.21), Akt Thr^{308} (p=0.32, *d*=0.30), and PTEN Ser^{380} (p=0.12, *d*=0.49).

6.4.7.2 Effect of exercise sex

Total content of proteins related to insulin independent and dependent signalling are shown in Figure 6.6. There were no differences between the sexes for total content of TBC1D4 (p=0.94, *d*=0.03), AMPK (p=0.75, *d*=0.13), Akt (p=0.15, *d*=0.61), p38 MAPK (p=0.23, *d*=0.51), PTEN (p=0.87, *d*=0.07), and GLUT4 (p=0.99, *d*=0.004).

The effects of sex on the change in phosphorylation status of proteins related to insulin independent and dependent signaling following MIC exercise and LLHR RE are shown in Figure

6.5. There was no difference in the change in TBC1D1 Ser^{237} (p=0.25, *d*=0.48), TBC1D4 Thr^{642} (p=0.61, *d*=0.21), ACC Ser^{79} (p=0.68, *d*=0.17), AMPK Thr^{172}(p=0.74, *d*=0.14), p38 MAPK Thr^{180}/Tyr^{182} (p=0.66, *d*=0.18), Akt Thr^{308} (p=0.29, *d*=0.44), or PTEN Ser^{380} (p=0.68, *d*=0.17) between males and females following MIC exercise. Similarly, following LLHR RE there was no difference in the change in TBC1D1 Ser^{237} (p=0.95, *d*=0.03), TBC1D4 Thr^{642} (p=0.11, *d*=0.68), ACC Ser^{79} (p=0.13, *d*=0.64), AMPK Thr^{172}(p=0.98, *d*=0.01), p38 MAPK Thr^{180}/Tyr^{182} (p=0.92, *d*=0.04), Akt Thr^{308} (p=0.73, *d*=0.14), or PTEN Ser^{380} (p=0.15, *d*=0.60) between males and females.

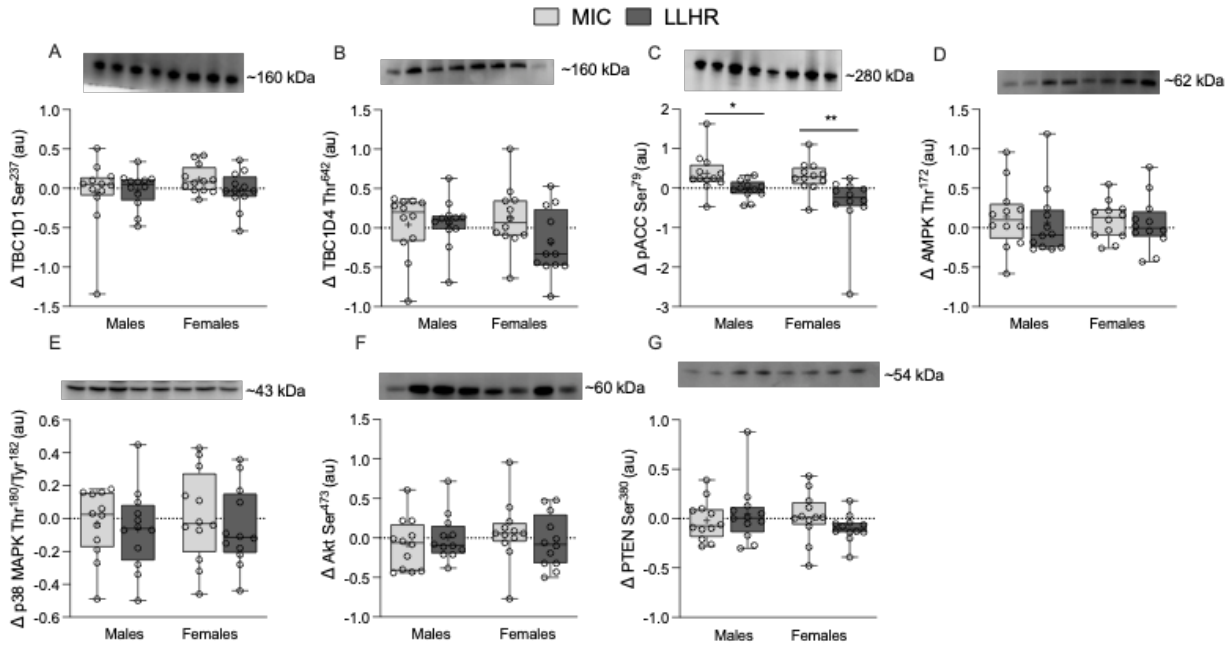


Figure 6.5: Phosphorylation status of proteins involved in insulin dependent and independent signaling before and after exercise in males (n=12) and females (n=12). A) change in TBC1D1 Ser^{237} phosphorylation, B) change in TBC1D4 Thr^{642} phosphorylation, C) change in pACC Ser^{79} phosphorylation, *represents significant difference between MIC exercise and LLHR in males (p=0.02, *d*=0.8), ** represents significant difference between MIC exercise and LLHR in females (p=0.003, *d*=1.1), D) change in AMPK Thr^{172} phosphorylation, E) change in p38 MAPK Thr^{180}/Tyr^{182} phosphorylation, F) change in Akt Ser^{437} phosphorylation, G) change in PTEN Ser^{380} phosphorylation.

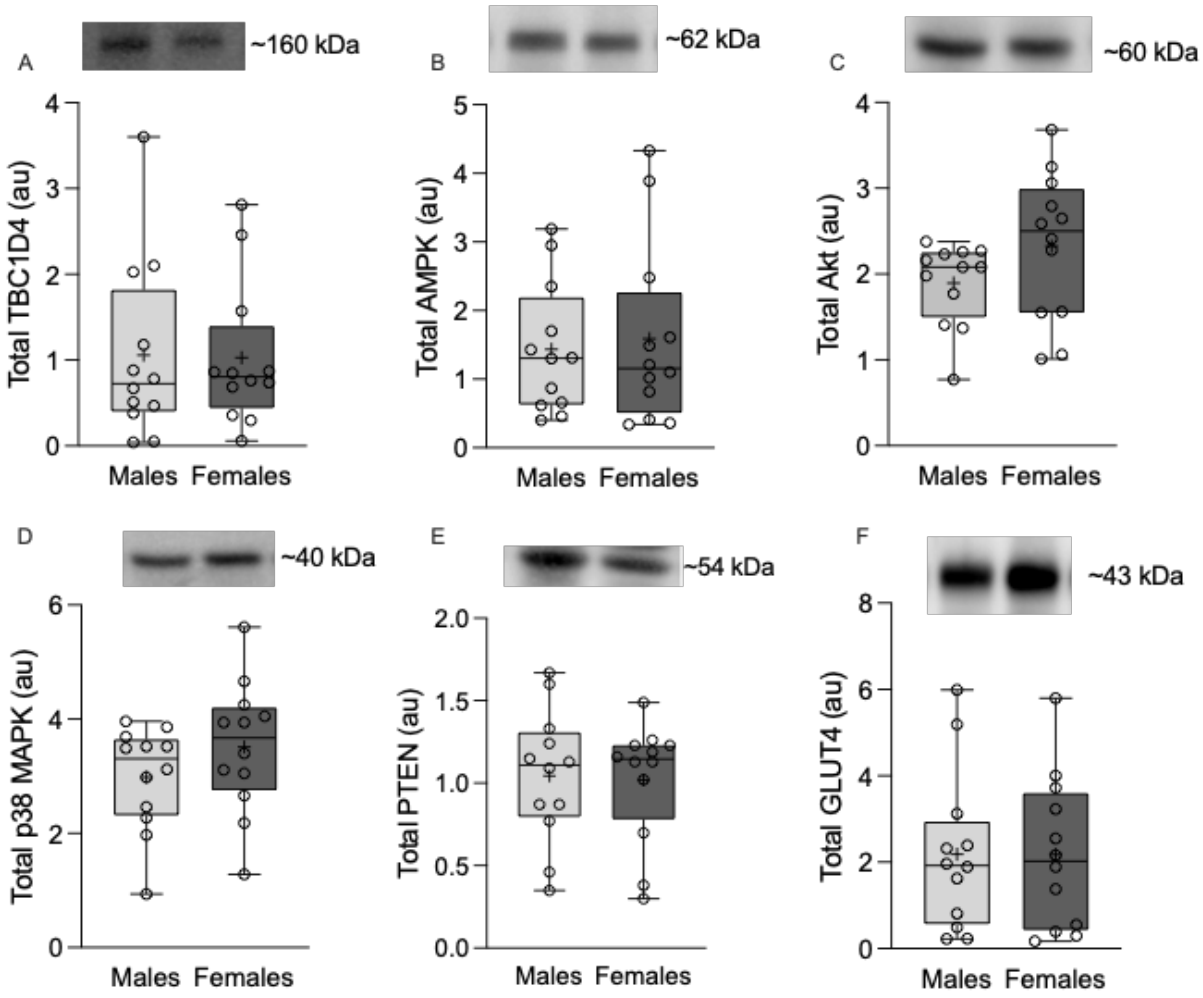


Figure 6.6: Content of proteins relating to insulin independent and dependent signaling. A) Total TBC1D4, B) Total AMPK, C) Total Akt, D) Total p38 MAPK, E) Total PTEN, F) Total GLUT4

6.5 Discussion

The purpose of the present study was to compare the effects of an acute bout of LLHR RE and MIC exercise on post-exercise glycemic control in young, recreationally active males and females. Additionally, we sought to examine if sex inherently influenced post-exercise glycemic control following either exercise bout while taking into consideration the relative dose of glucose consumed. Finally, we also sought to examine if sex influenced the content of insulin

signaling proteins or if exercise mode or sex inherently influenced the activation of insulin-independent signaling in the absence of pathology. The main finding of this study was that blood glucose concentrations during an OGTT were lower following LLHR RE compared to MIC exercise, with no difference between the sexes. The lower glucose concentration following LLHR RE resulted in a significantly lower glucose AUC during the OGTT following LLHR RE compared to MIC exercise in both sexes, but maximal glucose concentration was lower following LLHR RE in females only. While we found no sex differences when looking at absolute glucose concentrations following either exercise bout, once glucose was normalized to body weight and lean body mass, but not height, we found that males had higher glucose concentrations during the post-MIC exercise OGTT compared to females. This was also found following LLHR RE when glucose was normalized to lean body mass, but not body weight or height. There was no difference in post-exercise plasma insulin concentrations between the exercise bouts, however we did find that insulin peaked earlier in males than females following LLHR RE, which resulted in males having a lower insulin concentration at the end of the OGTT following LLHR RE compared to females. We did not find an effect of sex on the total content of insulin-independent or -dependent signaling proteins. With respect to exercise mode, we found that phosphorylated ACC Ser^{79} increased following MIC exercise, but not LLHR RE in both males and females.

The main finding of this study was that blood glucose concentration during a post-exercise OGTT was lower following LLHR RE compared to MIC exercise with no difference between the sexes. This finding is supported by the lower glucose AUC following LLHR RE compared to MIC exercise in males and females, and the lower max glucose concentration

following LLHR RE in females only. Our findings are in line with a previous trial that found that an acute bout of low intensity RE (40% of 1RM) with 16 repetitions for each set decreased glucose AUC and hyperglycaemic prevalence in females with type II diabetes (453). Furthermore, glucose AUC during a post-exercise OGTT was reported to be lower following high-load, low-repetition acute RE, but not MIC exercise (343). Together, the findings of our trial and others support the role of LLHR RE to acutely improve post-exercise glycemic control in healthy individuals and those with type II diabetes. It is worth noting however that it is unknown whether LLHR RE or MIC exercise improve glucose tolerance relative to no-exercise as we did not perform an OGTT in the non-exercised state. Future trials should examine the effects of LLHR resistance training on insulin sensitivity in comparison with aerobic exercise and 'traditional' heavy load, low repetition resistance training to determine its effectiveness long-term.

It is worth noting however, that the beneficial effect of acute exercise on glycemic control may be directly related to the extent of muscle fibre recruitment during exercise (338). This is of note because in the current trial the LLHR RE bout involved whole body exercise whereas the MIC exercise bout involved lower body exercise. However, it is important to recognize that the amount of time for each exercise bout was similar (i.e., 30 minutes) and thus while the LLHR RE bout included whole body exercise, since the time spent exercising was the same this implies that for a given amount of exercise time LLHR RE promotes better post-exercise glucose handling than MIC exercise. That said, we did not see any significant differences in indices of fasted (HOMA-IR) or fed (Matsuda index) insulin sensitivity between

exercise modes. This lack of difference between the exercise bouts could be reflective of the lack of effect of exercise on insulin concentrations prior to or during the OGTT.

While we did not find a sex difference in absolute glucose concentration during the OGTT between the sexes, when we normalized glucose concentration during the post-MIC exercise OGTT to the glucose dose relative to body weight and lean body mass, we found that glucose concentrations were higher in males compared to females. Similarly, following LLHR RE when glucose concentration was normalized to lean body mass, glucose concentrations were higher in males compared to females. These findings are in line with findings from a previous trial (Beaudry et al, 2022- in review) that also found higher glucose concentration in males compared to females once normalized to body composition. As discussed more fully in our previous paper, height and body composition are known to influence the glycemic response to an OGTT therefore it is imperative to normalize glucose concentrations to body composition especially when comparing between the sexes who inherently differ in body anthropometrics and composition. Our results ultimately suggest that when differences in body weight and muscle mass are considered, females have a greater ability to clear glucose at rest (Beaudry et al, 2022- in review) and following an acute bout of exercise. This highlights the importance of considering the relative dose when comparing glucose response to an OGTT between the sexes and/or between people of different body masses and composition.

We did not find any differences in the total content of proteins related to insulin signaling between males and females. Females have a lower risk of developing type II diabetes (454) and are more insulin sensitive than males, even after adjustment for age and BMI (387). In the current trial we did not find any differences in the total protein content of proteins

relating to glucose handling and insulin sensitivity between males and females who are matched for aerobic fitness. This could indicate that differences in the content of proteins related to insulin signaling may not be an inherent sex difference. However, it will be imperative in future studies to examine whether there are inherent sex differences in insulin signaling in the insulin-stimulated state since that was not assessed in this study.

The current trial found no change in TBC1D1 Ser^{237} following MIC exercise or LLHR RE. This is in contradiction with another study that reported that an acute bout of MIC exercise increased TBC1D1 Ser^{660} phosphorylation in obese participants (117). Previous work from our lab has also found that acute high intensity interval exercise increased TBC1D1 Ser^{237} phosphorylation in males, but not females (Beaudry et al, 2022- in review) immediately after exercise. Therefore, the lack of activation of TBC1D1 Ser^{237} phosphorylation following MIC exercise is not entirely clear. To the best of the authors knowledge this is the first time that the effects of RE on TBC1D1 Ser^{237} phosphorylation status has been investigated in human participants. However, previous work done in rats found that TBC1D1 Ser^{237} phosphorylation was significantly increased immediately following RE and returned to baseline by 3h post-exercise following maximum isometric contractions via electrical stimulation (333). More work in this area to clarify the time course and exercise-mode effect of exercise on TBC1D1 Ser^{237} phosphorylation is warranted, particularly in the insulin resistant state.

While the current study did not find any changes in AMPK phosphorylation following either of the acute exercise sessions, we did find that ACC Ser^{79} phosphorylation, which is phosphorylated by AMPK (455), increased following MIC exercise with no significant changes following LLHR RE. The lack of an increase in AMPK, ACC and TBC1D1 phosphorylation following

LLHR RE could be due to the activation of mTORC1 as activation of mTORC1 is thought to lead to deactivation of AMPK signaling (456-458). However, there is research indicative of simultaneous increases in AMPK and mTORC1 signaling following both aerobic (459-461) and resistance exercise (336, 461). Therefore, the lack of activation of AMPK, ACC and TBC1D1 following LLHR RE in the current study are not clear.

We also did not see any significant changes in phosphorylation status of Akt Ser^{473} following either MIC exercise or LLHR RE. Our findings agree with a similar study that used a 30-min MIC exercise protocol at 70% $\dot{V}O_{2peak}$ and did not find any activation of Akt (117). Their finding is not surprising as previous data suggests that exercising for 60 mins is needed to see increases Akt and TBC1D4 phosphorylation in human skeletal muscle (110, 119). Furthermore, while an acute cycling and RE (8 reps x 5 sets of leg extension at 80% 1RM) session induced comparable increases in Akt Thr^{308}/Ser^{473} phosphorylation, phosphorylation peaked 30-60mins following exercise (462) and thus we may have missed an effect of exercise on Akt by taking our biopsies immediately post-exercise. Furthermore, another study found that Akt Thr^{308}/Ser^{473} phosphorylation was decreased immediately following RE (10 sets of 10 reps of leg extension at 80% 1RM) and continued to be depressed at 24-hours post-exercise (112). These results could indicate that high intensity RE could inhibit Akt phosphorylation in the fasted state. More work examining the effects of different modes of exercise on Akt phosphorylation, considering the possible time-course of activation and including measurements in the insulin-stimulated state are needed.

We also saw a modest effect of sex on insulin concentration during the post-LLHR RE, but not MIC exercise, OGTT such that males had an earlier peak insulin concentration and

significantly lower insulin concentrations at the end of the OGTT compared to females. Following the LLHR RE bout insulin peaked at approximately ~42mins into the OGTT for males and at ~68mins into the OGTT for females. Of note, while not statistically different, glucose Tmax occurred at 39 min in males and 54 minutes in females during the post-LLHR RE OGTT. These findings are interesting as a delay in insulin and glucose Tmax has been found to be related to a decrease in insulin sensitivity and secretion and lower β-cell function in non-diabetic and type II diabetic individuals (463, 464). Again, while not statistically significant, HOMA-IR was higher (2.5 vs 1.9 AU), and Matsuda index was lower (5.5 vs 6.4 mass units) in females compared with males following LLHR RE. This could be related to gut glucose half-life being higher in females compared to males (381), which could be driving a higher glucose concentration during the latter part of an OGTT. Importantly, the delayed Tmax for insulin and glucose in females was only observed following LLHR RE indicating that this difference was related to exercise mode and not overall differences between the sexes. Indeed, we have previously found in these participants that insulin Tmax did not differ between males and females during an OGTT performed in the rested state (males: 65 min, females: 60 min, Beaudry et al, 2022 under review). Furthermore, despite the delayed insulin Tmax, glucose AUC was lower following LLHR RE vs MIC exercise in females, suggesting a greater effect of LLHR RE on post-exercise glycemic control. Thus, these findings suggest that despite similar health status and aerobic fitness, there may be a potential differential effect of LLHR RE on glycemic control in males vs females, which warrants further examination.

The current trial had several strengths including that our male and female participants were properly matched for habitual training status, that we controlled for variations in sex

hormones that may affect IS and glucose handling, that we performed dietary analyses to ensure that any sex differences observed were not due to differences in habitual dietary intake, and that we controlled diet in the day leading up to the two exercise bouts to ensure that there were no differences in nutrient availability between exercise bouts. Our trial also had several limitations including that we used an OGTT instead of the gold standard euglycemic, hyperinsulinemic clamp to determine insulin sensitivity, the lack of additional biopsies taken over time during recovery and the lack of a biopsy taken during the insulin-stimulated state. A more comprehensive discussion of these strengths and limitations is provided in the discussion of a previous paper that used the same study design and compared the effects of an acute bout of high intensity interval exercise on post-exercise glucose handling between males and females (Beaudry et al, 2022- in review). One final note, while the use of the OGTT is a limitation, it is also a strength as since it involves consumption, not infusion, of glucose, it allows incretin-induced insulin secretion to occur, which is known to be greater in females (387).

In summary, we conclude that blood glucose concentrations during an OGTT conducted after an acute bout of LLHR RE were lower than when the OGTT was conducted after an acute MIC exercise bout, with no difference between the sexes. We also found that once glucose concentrations were normalized to body weight and lean body mass, males had higher glucose concentrations during both post-exercise OGTTs compared to females, suggesting that for a given amount of (muscle) mass females are better able to clear glucose. We did not find any significant differences in the content of proteins related to insulin-independent or dependent signaling between males and females. Interestingly, we found that the phosphorylation status of ACC Ser^{79} was greater following MIC exercise compared to LLHR RE. The findings from this

study provide the necessary proof of concept that LLHR RE is a feasible exercise modality to improve post-exercise glycemic control in both males and females. These findings are significant as LLHR RE may be more feasible for older adults with IR and type II diabetes who are at an increased risk of adverse events such as musculoskeletal injury, while simultaneously promoting muscle hypertrophy and improved insulin sensitivity. Future trials should examine the effectiveness of LLHR resistance training on insulin sensitivity in insulin resistant populations. While we purposely chose to study young healthy individuals so that we could determine the inherent effects of sex on post-exercise glucose handling, insulin sensitivity and insulin dependent and independent signaling in the absence of pathology, future studies should build upon our findings to examine whether sex differences are maintained/become apparent in males and females with insulin resistance.

Chapter 7: Integrated Discussion

7.1 Thesis summary

The purpose of the research conducted in this thesis was to investigate the impact of different modes of exercise (MIC exercise, HIIE and LLHR RE) on post-exercise glucose handling and IS in young, recreationally active males and females without pathology. We additionally sought to examine whether biological sex influences the metabolic response to different bouts of acute exercise and how it may influence post-exercise glucose uptake and IS.

In Chapter 4, we examined whether sex influences glycemic control during an OGTT conducted after an acute bout of HIIE in young healthy males and females. Additionally, we sought to examine whether metabolic differences in AMPK activation and glycogen utilization were related to post-exercise glycemic control and IS and whether there were sex-based differences in the content of proteins related to insulin signalling and exercise-induced activation of insulin-independent signalling. The main findings of this study were that an acute bout of HIIE increased IS similarly in both males and females as characterized by both the Matsuda and HOMA-IR indices. When looking at absolute glucose concentrations, there was no difference between the sexes during either the control or post-HIIE OGTT; however, when considering the relative dose of glucose in relation to fat-free mass, glucose concentrations and glucose AUC were lower in females than males. Furthermore, HIIE lowered insulin concentration during the post-HIIE OGTT, an effect mediated by the effect of HIIE in males. We found no differences in total protein content of insulin-signalling proteins between males and females. However, TBC1D1 Ser^{237} phosphorylation increased to a greater extent in males than females during HIIE.

In Chapter 5, we investigated sex differences in markers of inflammation, oxidative stress, and antioxidant status at rest, after an acute bout of HIIE and during a post-exercise OGTT in young, recreationally active males and females. We found that in response to an acute bout of HIIE, plasma concentrations of IL-6 and TNFα increased, and IL-6 continued to increase while TNFα returned to baseline by 90 minutes post exercise, with no differences in response between the sexes. While we did not see any effect of sex on antioxidant status or oxidative stress status, we did see that females had higher p38 MAPK phosphorylation at rest compared to males.

In Chapter 6, we investigated the effects of an acute bout of LLHR RE and MIC exercise on post-exercise glycemic control in young, recreationally active males and females while taking into consideration the relative dose of glucose consumed. We found that LLHR RE was able to significantly lower blood glucose concentrations during a post-exercise OGTT compared to MIC exercise, with no difference between the sexes. Once glucose concentration was normalized to body weight and lean body mass, but not height we found that males had higher glucose concentrations during both post-exercise OGTTs compared to females. This was also seen following LLHR once glucose concentrations were normalized to lean body mass. Additionally, we found that phosphorylated ACC Ser^{79} increased following MIC exercise in males and females, with no changes following LLHR RE.

7.2 Effects of exercise and sex on post-exercise glucose concentrations

One of the main contributions of this thesis is providing the necessary proof-of-concept that LLHR RE was able to decrease post-exercise glucose concentration during an OGTT to a greater extent than MIC exercise in both males and females. This is the first study to directly compare LLHR RE to MIC exercise, which is the most commonly prescribed form of exercise upon T2D diagnosis (292). Previous research has already reported that LLHR RE can stimulate muscle protein synthesis and muscle growth to similar levels as traditional low-load high repetition RE (354, 451). These findings suggest an exciting area of research in exercise and diabetes, such that it may be possible to increase skeletal muscle mass while simultaneously incurring beneficial effects on blood glucose concentrations above what is seen with MIC exercise alone. Additionally, while we did not find that acute HIIE decreased post-exercise glucose concentrations during an OGTT we did find that HIIE increased IS, with no differences between the sexes. This indicates that acutely HIIE is a beneficial exercise modality to improve IS in males and females.

Furthermore, we found that while absolute glucose concentration following acute exercise (HIIE, MIC exercise, and LLHR RE) may not differ between males and females, normalizing glucose concentrations to anthropometric measures reveals that glucose concentrations were higher in males compared to females. This is noteworthy as previous work has demonstrated that height and body composition influence the glycemic response to an OGTT (240, 241). Since females are typically shorter, weigh less and have less fat-free mass compared to males, they are inherently disadvantaged to have a higher blood glucose concentration during a standard 75g OGTT. Our work demonstrates that once glucose concentration during the post-exercise OGTT following HIIE and MIC exercise were normalized to body weight and lean body mass, glucose concentrations during the OGTT were higher in males compared to females. This was also seen

when blood glucose concentration was normalized to lean body mass following LLHR RE. Overall these findings indicate that for a given mass females are better able to clear a given amount of glucose. Interestingly, we did not find any difference in blood glucose concentration when we normalized glucose values for dose relative to height. The lack of relationship between height and glucose concentration suggests that it is mainly differences in muscle mass, which is responsible for >80% of glucose disposal (465), that is related to differences in blood glucose handling. Our findings highlight the importance of normalizing glucose load to anthropometric measurements or using a relative glucose dose when conducting sex comparative research. Furthermore, our work suggests that the reason females may have a greater risk of impaired glucose tolerance than males could be related to the fact that the same dose of glucose is used for males and females despite differences in body weight and lean body mass.

7.3 Effect of sex and exercise on proteins related to oxidative stress, insulin-dependent and insulin-independent signaling

Biological sex appears to influence the effectiveness of exercise regimes to improve IS (17, 296, 297, 306) and the current mechanism(s) underlying this sexual dichotomy is/are not well understood. Training trials involving interval exercise have found that improvements in IS and glucose control is greater in males compared to females (17, 296). While we did not see any effect of sex on absolute glucose concentrations during a post-HIIE acute exercise bout, we did find that TBC1D1 Ser^{237} phosphorylation was increased following HIIE in males only. Interestingly, however we did not find any effect of sex on TBC1D1 Ser^{237} phosphorylation following MIC exercise or LLHR RE. Together these findings suggest that TBC1D1 may be

differentially activated based on exercise mode and/or intensity, a finding which warrants further investigation. However, the finding that TBC1D1 phosphorylation increased in males only following an acute bout of HIIE may explain a possible mechanism by which interval exercise has been found to be more efficacious in males compared to females.

TBC1D1 is an important regulator of GLUT4 translocation, promoting glucose uptake by inhibiting Rab GTPase activity, promoting GTP binding to Rabs therefore allowing GLUT4 translocation to the plasma membrane (46, 76). In our trial we found that glucose concentration and glucose AUC during a post-exercise OGTT was lower following LLHR RE than MIC exercise. Interestingly, we found no increase in TBC1D1 phosphorylation following LLHR RE. Mammalian target of rapamyocin (mTOR) has been previously demonstrated to be elevated following LLHR RE (356), and can stimulate glucose uptake through modulation of the transcription factor hypoxia-inducible factor (HIF1α) (466). Therefore, the effects of LLHR RE on post-exercise glucose handling could be due to a delayed effect on TBC1D1 phosphorylation that may be more evident in recovery or perhaps through a separate pathway not involving TBC1D1.

We did not find any effect of sex on total proteins relating to insulin-dependent, insulin-independent, or proteins relating to antioxidant or oxidative stress in our participants. This could be due to the young, healthy population that we investigated. A possible explanation is that sex differences in total protein could become more apparent in states of pathology, where estrogen has an added beneficial effect. Interestingly however, previous work by Tobias et al, (129) found that females expressed higher ACC than males in type IIa fibers and higher AMPK and TBC1D4 in both fiber types. The aforementioned study also studied young, recreationally

males and females and conducted the experiments in females during the mid-to-late follicular phase of the menstrual cycle, therefore the lack of sex difference in our studies is not clear. However, it is important to note that in the Tobias et al (129) studies the males and females were not matched for aerobic fitness, which could have influenced the findings. Conversely, Roepstorff et al, (452) found that AMPKα2 activity increased (~200%) in males, but not females following 90-mins of MIC exercise. The reasons for the discrepancies in both the aforementioned papers is not clear, and more research is warranted to fully elucidate potential sex differences in the activation of proteins related to metabolism.

7.4 Overarching Limitations

One of the overarching limitations to this thesis is the lack of data on the response to exercise during the luteal phase of the menstrual cycle. While controlling for menstrual phase when conducting sex-comparative research is a strength to ensure that menstrual cycle phase is not impacting insulin sensitivity and glucose handling independent of exercise modality. It is worth mentioning that these may only be generalizable to the mid-follicular phase of the menstrual cycle. Secondly, a limitation to this work is the timing of the muscle biopsies. Since we performed muscle biopsies immediately pre- and post- exercise, we may have missed activation of proteins related to both insulin independent and dependent signaling that are activated further into recovery. We also did not measure any of these proteins in the stimulated state, which would allow for determination of the acute effects of exercise on insulin-dependent signaling in males and females. Thirdly, a limitation to this work is the use of the OGTT and not the gold standard use of the hyperinsulinemic-euglycemic clamp. While the

use of the hyperinsulinemic-euglycemic clamp could potentially be more sensitive to slight differences in insulin sensitivity changes between the sexes, this procedure was not feasible to conduct in the current trial and would prevent insulin secretion induced by incretin hormones, which is known to be higher in females and thus may be mechanistically one of the reasons females typically have higher insulin sensitivity than males (387). Lastly, due to supply chain issues resulting from COVID-19, the markers of inflammation, ROS production and overall oxidative damage included in study 2 do not provide a complete representation of the effects of HIIE on oxidative stress and inflammation. With only IL-6 and TNFα included as markers of inflammation we cannot present a robust story of what is happening in the plasma both basally and post-exercise between the sexes. Additionally, we are lacking a measurement of inflammatory markers and additional indicators of ROS production (DCF ROS production assay) and oxidative damage (Oxyblot) in skeletal muscle following exercise. As such, much more work in this area is needed to fully understand whether sex influences the inflammatory or oxidative stress response to exercise.

7.5 Future Directions

The purpose of the work conducted in this thesis was to characterize the inherent effects of sex on muscle metabolism, inflammation, and oxidative stress in relation to insulin sensitivity in response to different modes of exercise the absence of pathology. It is important to examine the effects of sex without pathologies present to determine if mechanistically there are inherent sex differences in the response of proteins relating to glycemic control and the

oxidative/inflammatory response that could be influencing post-exercise glucose handling. Future research directions stemming from this thesis would be to investigate the efficacy of HIIE, MIC exercise and LLHR RE in a state of pathology such as IR and T2D and determine whether there are differences in the response in males vs. females. Specifically, investigating the effects of LLHR RE on post-exercise insulin sensitivity and glucose handling in IR is of specific interest. Given the efficacy of LLHR RE on glucose handling during a post-exercise OGTT in young, recreationally active males and females combined with the ability for LLHR RE to increase muscle mass (467) and strength (468) in older adults, LLHR RE may be particularly efficacious at improving IS in older adults with and without T2D. Training studies investigating the efficacy of LLHR RE on muscle mass and glycemic control should be conducted. Additionally, future studies should investigate if protein content and phosphorylation status of proteins relating to insulin-dependent and independent signaling, oxidative stress and inflammation differ between the sexes in both the insulin stimulates state and in older adults with IR and T2D. Further mechanistic insight behind why the efficacy of different exercise modes differ between the sexes will allow exercise recommendations to be tailored to the sexes and prevent IR and T2D in both sexes. Additionally, future research should focus on the inflammatory and oxidative stress response both across the entire menstrual cycle and additionally in states of pathology to see if they differ between the sexes.

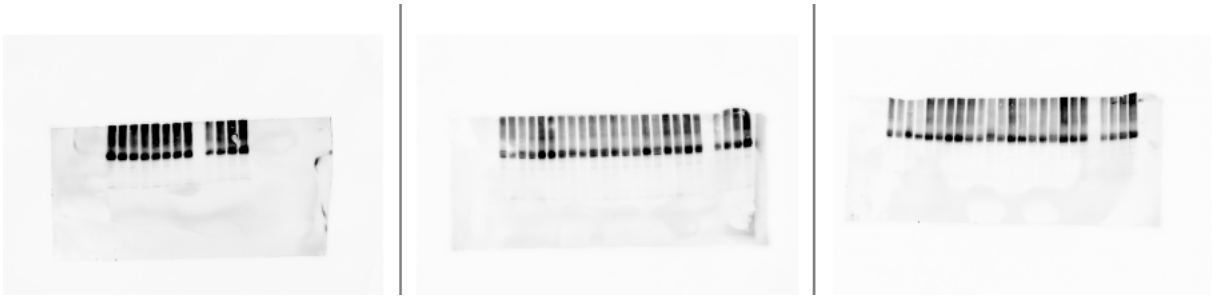
7.6 Conclusions

An understanding of the insulin sensitizing effects of acute exercise and whether they differ between the sexes is critical to provide the most efficacious exercise recommendations to prevent T2D in both males and females. In conclusion we found that LLHR RE was able to decrease blood glucose concentrations to a greater extent than MIC exercise. We also found that acute HIIE was able to improve post-exercise IS in both males and females. While absolute glucose concentrations did not differ between the sexes, we report that once glucose concentrations were normalized to body weight and lean body mass, females had lower glucose concentrations compared to males. We also report some slight differences between sexes in the mechanistic response to acute exercise that may indicate differences in pathways responsible for glucose uptake. Overall, this thesis provides necessary proof of concept for future sex comparative research done in the area of exercise and glucose handling.

Chapter 8: Supplementary Figures

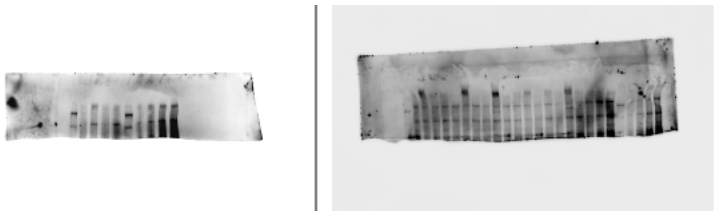
8.1 Supplementary Figures Chapter 4

Supplementary Figure 8.1.1: representative western blot images total AKT

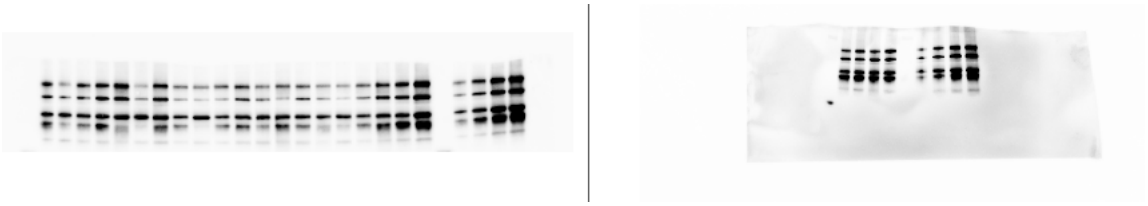


Supplementary Figure 8.1.2: representative western blot images GLUT4

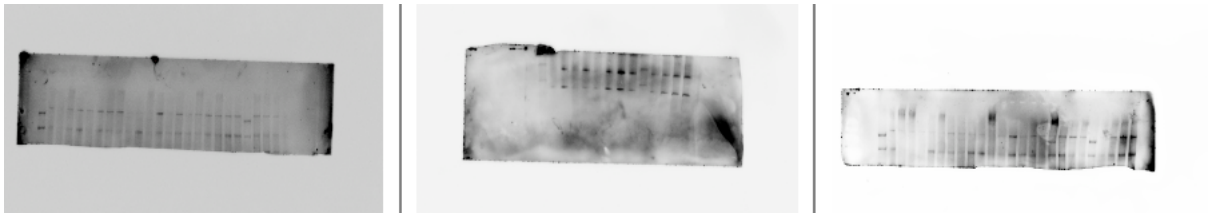
Supplementary Figure 8.1.3: representative western blot images total PTEN



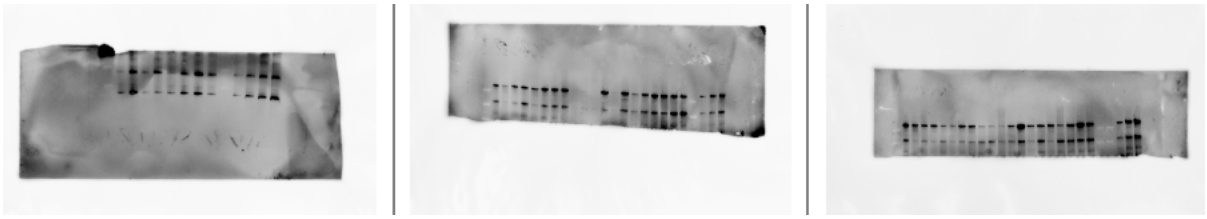
Supplementary Figure 8.1.4: representative western blot images total TBC1D4



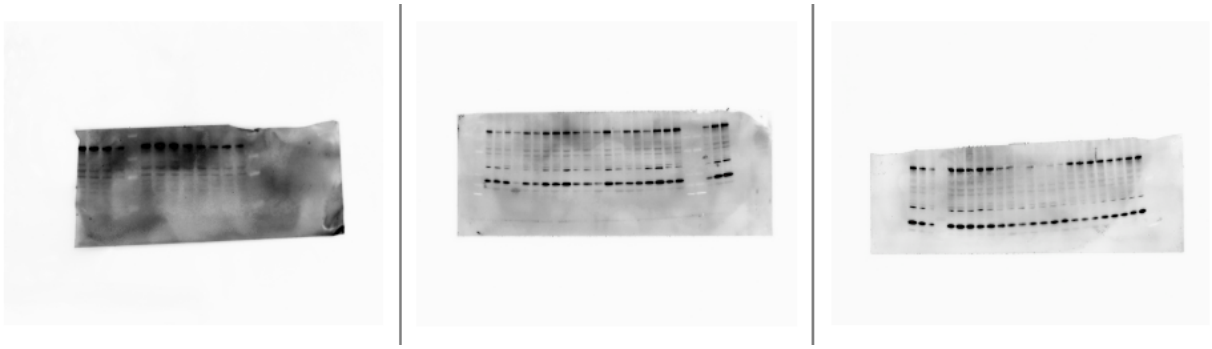
Supplementary Figure 8.1.5: representative western blot images total AMPK



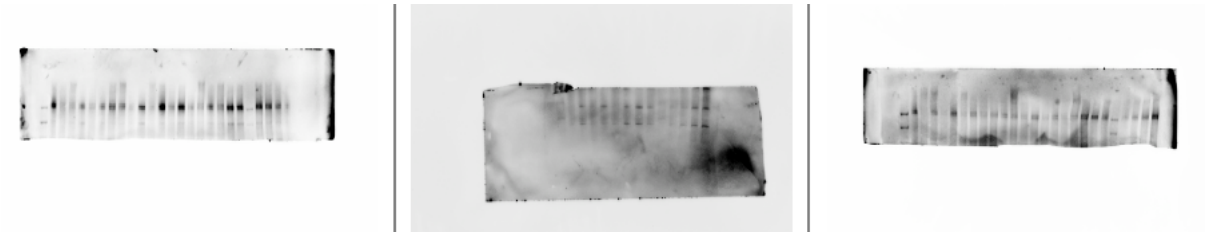
Supplementary Figure 8.1.6: Representative western blot images phosphorylated TBC1D1 Ser^{237}



Supplementary Figure 8.1.7: Representative western blot images phosphorylated TBC1D4 Thr^{642}

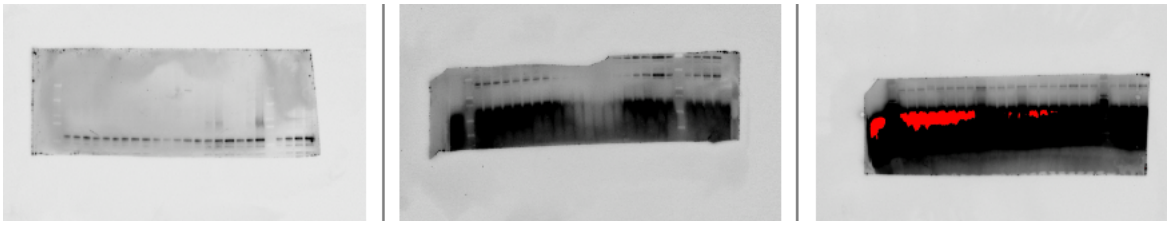


Supplementary Figure 8.1.8: representative western blot images phosphorylated AMPK Thr^{172}

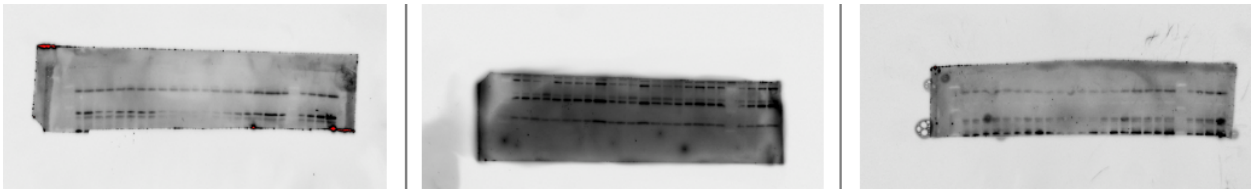


Supplementary Figure 8.1.9: Representative western blot images phosphorylated ACC Ser^{79}

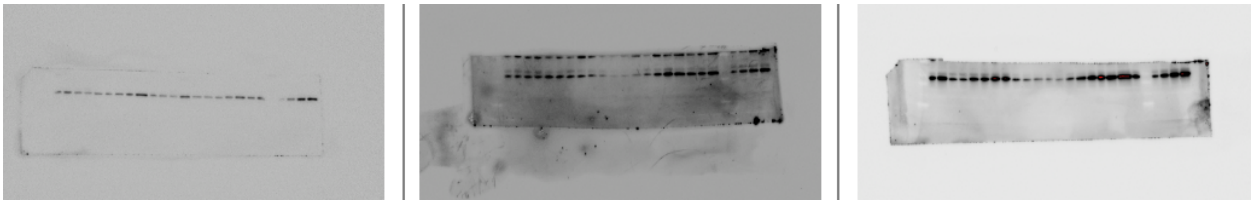
8.2 Supplementary Figures Chapter 5



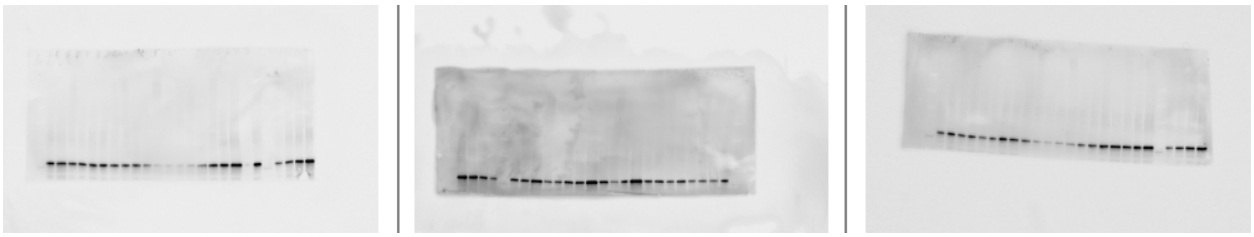
Supplementary Figure 8.2.1: Representative western blot images NOX2



Supplementary Figure 8.2.2: Representative western blot images TRX1

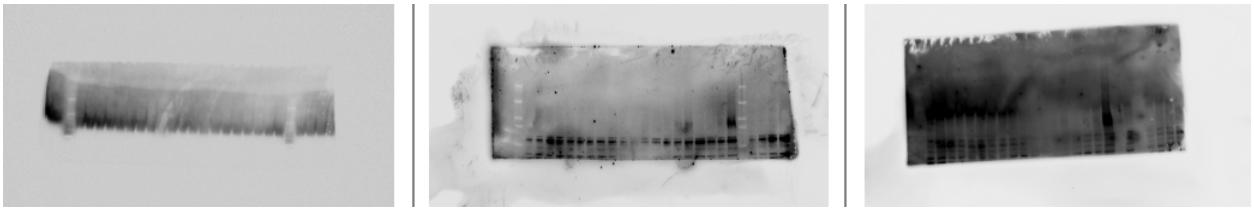


Supplementary Figure 8.2.3: Representative western blot image GPX1

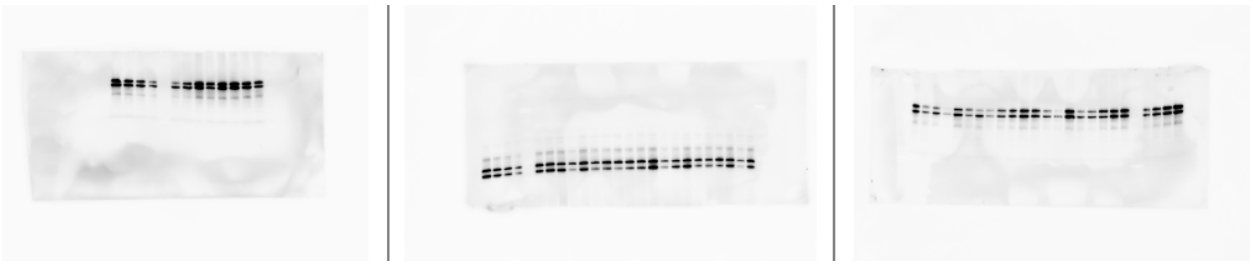


Supplementary Figure 8.2.4: Representative western blot image GR

Supplementary Figure 8.2.5: Representative western blot image total p38 MAPK

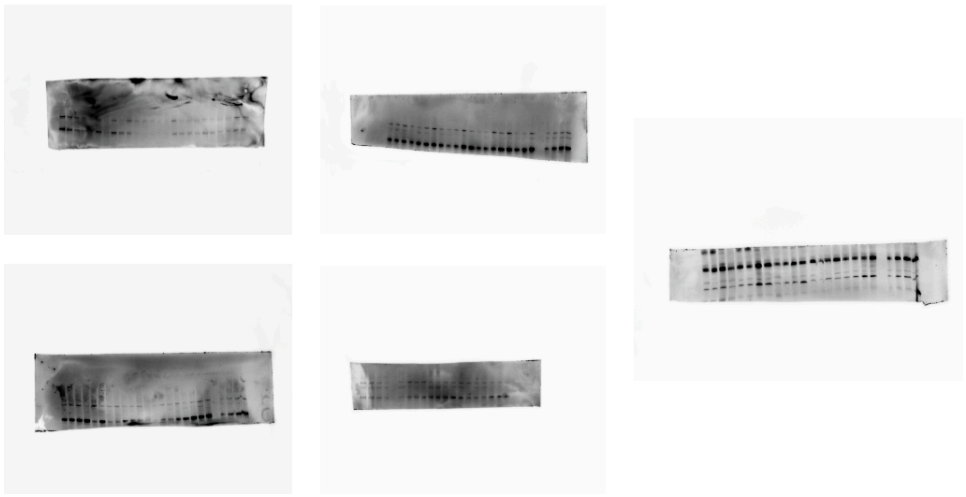


Supplementary Figure 8.2.6: Representative western blot image 4HNE

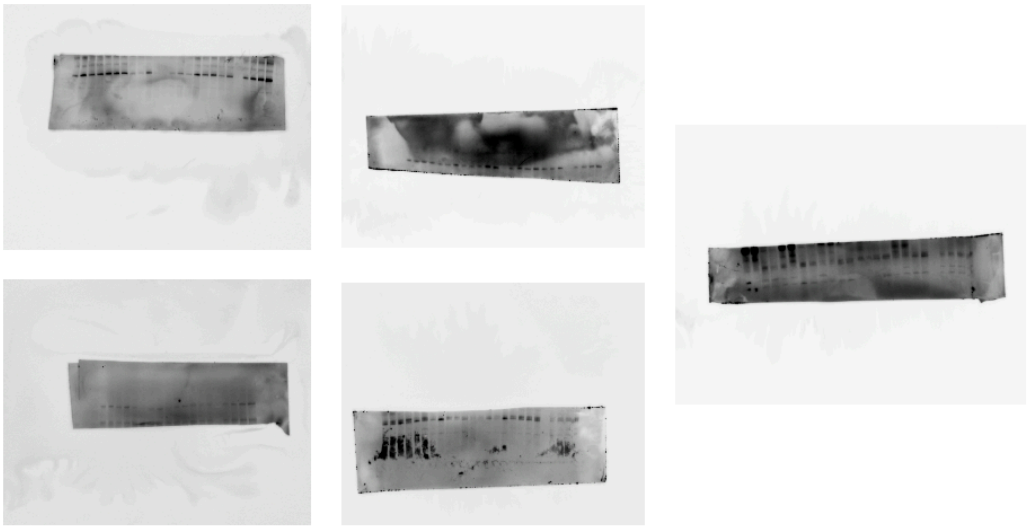


Supplementary Figure 8.4.7: Representative western blot image p38 MAPK $Thr^{180/}Tyr^{182}$

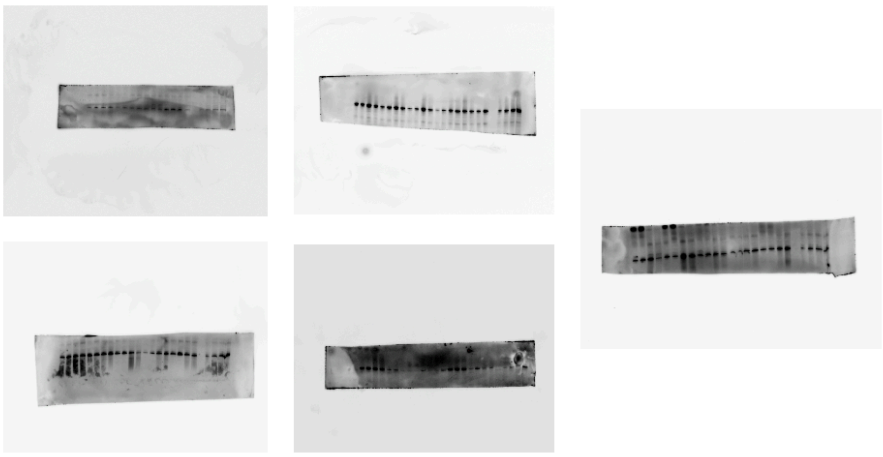
8.3 Supplementary Figures Chapter 6



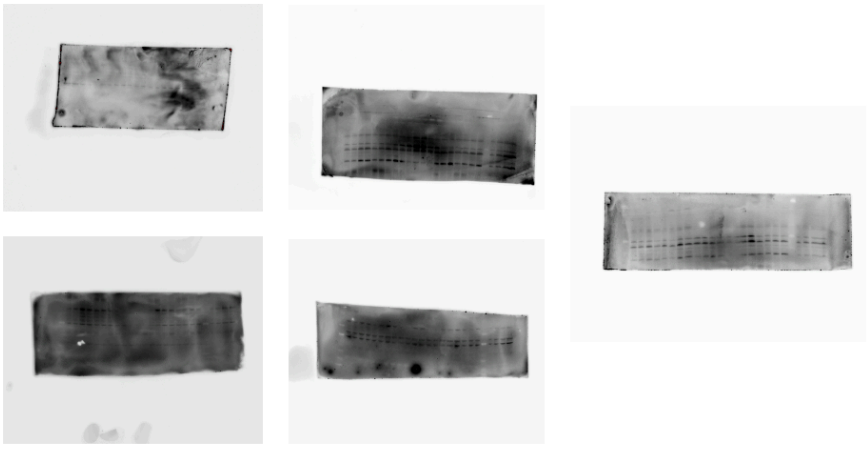
Supplementary Figure 8.3.1: Representative western blot images phosphorylated TBC1D1 Ser^{237}



Supplementary Figure 8.3.2: Representative western blot images phosphorylated TBC1D4 Thr^{642}

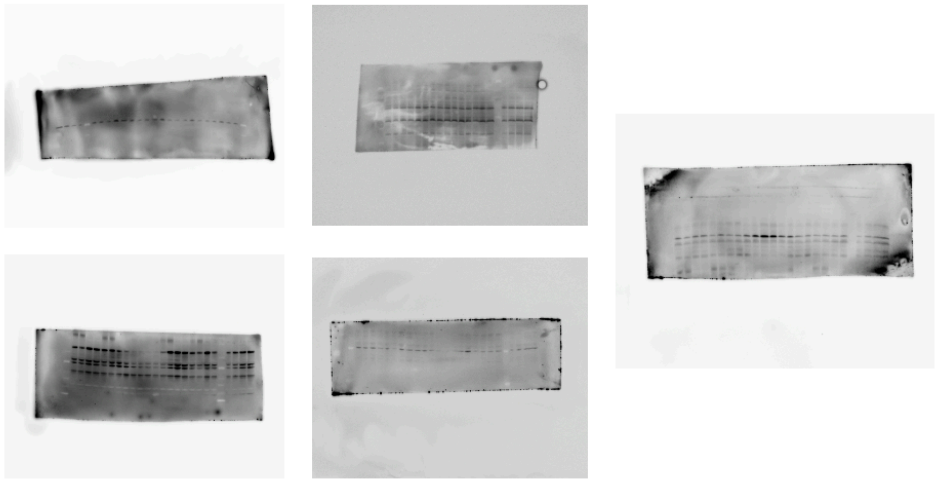


Supplementary Figure 8.3.3: Representative western blot images phosphorylated ACC Ser[79]

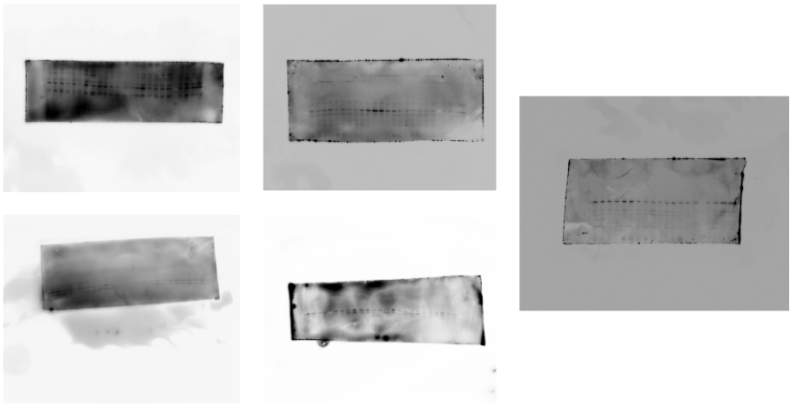


Supplementary Figure 8.3.4 Representative western blot images phosphorylated AMPK Thr[172]

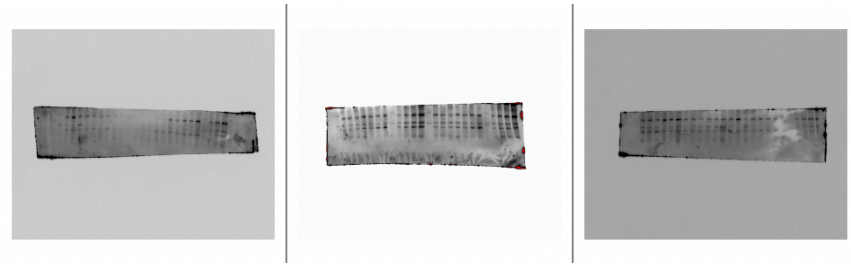
Supplementary Figure 8.3.5: Representative western blot images phosphorylated p38 MAPK Thr^{180}/Tyr^{182}



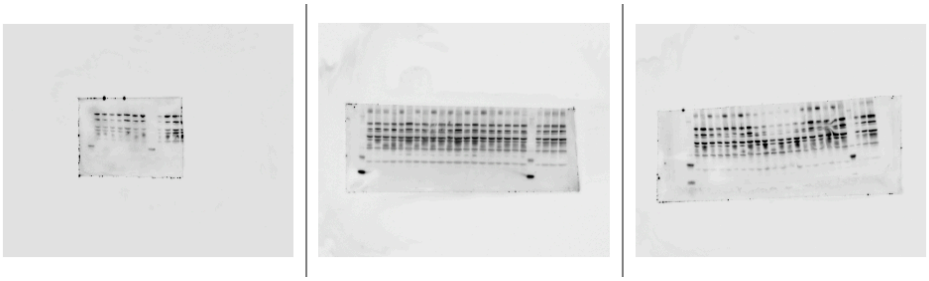
Supplementary Figure 8.3.6: Representative western blot images phosphorylated Akt Ser^{473}



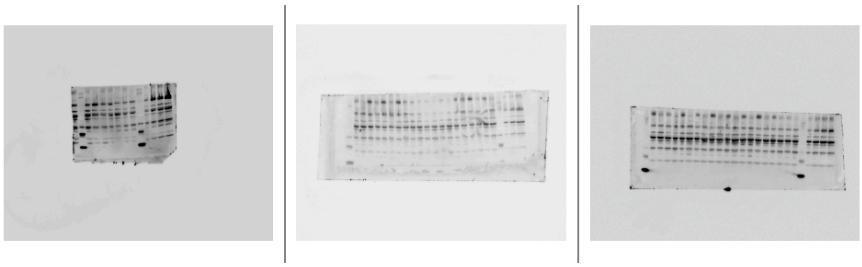
Supplementary Figure 8.3.7: Representative western blot images phosphorylated PTEN Ser^{380}



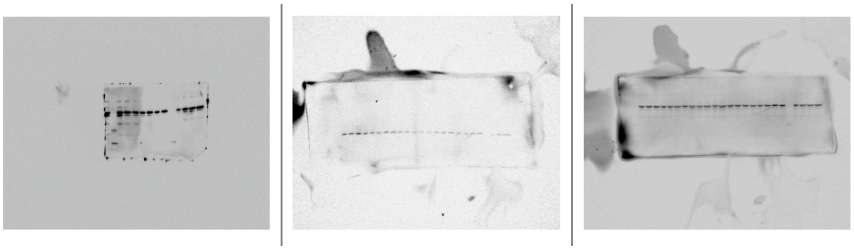
Supplementary Figure 8.3.8: Representative western blot images total TBC1D4



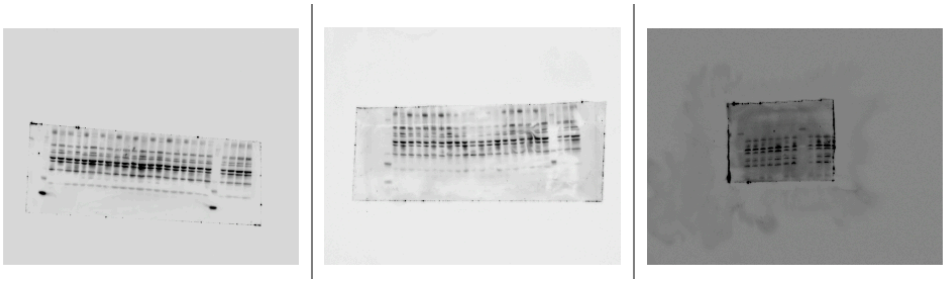
Supplementary Figure 8.3.9: Representative western blot images total AMPK



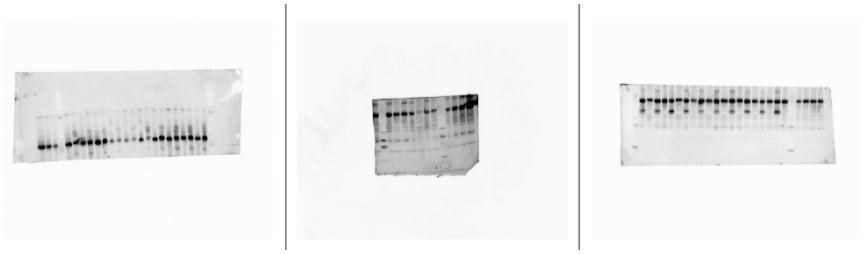
Supplementary Figure 8.3.10: Representative western blot images total Akt



Supplementary Figure 8.3.11: Representative western blot images total p38 MAPK



Supplementary Figure 8.3.12: Representative western blot images total PTEN



Supplementary Figure 8.3.13: Representative western blot images total GLUT4

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