EFFECT OF ACUTE EXERCISE ON WHOLE BODY FAT OXIDATION: CONTRIBUTIONS OF ABDOMINAL SUBCUTANEOUS ADIPOSE TISSUE

by

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A thesis submitted to the School of Kinesiology and Health Studies
In conformity with the requirements for
the degree of Master of Science

Queen’s University
Kingston, Ontario, Canada
(September, 2013)

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Lay Abstract

In consideration of the rising prevalence of obesity and its effect on metabolic health and disease, this study was conducted to examine mechanisms involved in adipose tissue function following an acute bout of exercise in abdominal subcutaneous adipose tissue. Sedentary, overweight/obese women (n=10, BMI=30.6±6.0 kg·m$^{-2}$, VO$_2$ peak=30.3±5.4 mL·kg$^{-1}$·min$^{-1}$) completed 2 visits to the lab in which they either exercised for 1 hour or rested in bed for the equivalent time (control). Experiments were executed randomly using a randomized cross-over study design. Gas exchange measures were collected at three time points before biopsies and subcutaneous adipose biopsies were obtained pre-condition, immediately after condition (0hr), two hours post (2hr), and four hours post (4hr). Acute exercise had significant effects whole body fat oxidation and phosphorylation of insulin signalling proteins, but had no effect on the phosphorylation of proteins regulating the expression of glyceroneogenic genes. In combination, these results suggest that acute exercise can transiently decrease insulin signalling although the mechanism by which this occurs is unclear. Additionally, acute exercise had no effect on the phosphorylation of proteins that are thought to regulate glyceroneogenic gene expression, suggesting that there are either alternative mechanisms involved or that time since the consumption of a meal is a greater stimulus for the activation/upregulation of glyceroneogenesis. Our findings suggest that acute exercise may acutely alter function of adipose tissue such that it contributes to elevations in whole body fatty acid metabolism, however, whether or not chronic adaptations are induced remains an important area for future study.
Co-Authorship

This thesis presents the work of Marysa Smith in collaboration with Dr. Brendon Gurd and Dr. David Wright.

*Effect of acute exercise on whole body fat oxidation: contributions of abdominal subcutaneous adipose tissue* is presented according to the guidelines for the Journal of Physiology. Marysa Smith was responsible for reviewing relevant literature, conducting the study, performing data analyses, and drafting the manuscript. All aspects were a collaborative effort between Marysa Smith and Dr. Brendon Gurd. Additionally, Dr. David Wright provided valuable insight and collaboration on developing the research question and methodological design. In addition, Dr. James Landine and Dr. Craig Simpson provided medical supervision. Finally, Trish Scribbans, and Cameron Williams were instrumental in aiding in the collection of data and analysis of adipose tissue.
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMPK</td>
<td>AMP-activated protein kinase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ATGL</td>
<td>adipose triglyceride lipase</td>
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<tr>
<td>ATP</td>
<td>adenosine-5'-triphosphate</td>
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<tr>
<td>β-AR</td>
<td>beta-adrenergic</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
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<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>CHO</td>
<td>carbohydrate</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<tr>
<td>DAG</td>
<td>diacylglycerol</td>
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<tr>
<td>EDTA</td>
<td>ethylene diamine tetra-acetic acid</td>
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<tr>
<td>EGTA</td>
<td>ethylene glycol tera-acetic acid</td>
</tr>
<tr>
<td>END</td>
<td>endurance exercise</td>
</tr>
<tr>
<td>G3P</td>
<td>glycerol-3-phosphate</td>
</tr>
<tr>
<td>GAPDH</td>
<td>glyceraldehyde 3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GLUT4</td>
<td>glucose transporter type 4</td>
</tr>
<tr>
<td>H₂O</td>
<td>water</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
</tr>
<tr>
<td>HIT</td>
<td>high intensity interval training</td>
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<tr>
<td>hr</td>
<td>hour</td>
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<tr>
<td>HSL</td>
<td>hormone sensitive lipase</td>
</tr>
<tr>
<td>IL-6</td>
<td>interlukin-6</td>
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<tr>
<td>LPL</td>
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<tr>
<td>NEFA</td>
<td>non-esterified fatty acid</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>OA</td>
<td>oxaloacetate</td>
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<tr>
<td>p38 MAPK</td>
<td>p38 mitogen-activated kinase</td>
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<tr>
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<td>pyruvate dehydrogenase complex</td>
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<td>phosphodiesterase-3B</td>
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<td>pyruvate dehydrogenase kinase 4</td>
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<tr>
<td>PEPCK</td>
<td>phosphoenolpyruvate</td>
</tr>
<tr>
<td>PKA</td>
<td>protein kinase A</td>
</tr>
<tr>
<td>PKB/Akt</td>
<td>protein kinase B (also known as Akt)</td>
</tr>
<tr>
<td>PPARγ</td>
<td>peroxisome proliferator-activated receptor gamma</td>
</tr>
<tr>
<td>RER</td>
<td>respiratory exchange ratio</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<td>SDS-PAGE</td>
<td>sodium dodecyl sulfate polyacrylamide gel electrophoresis</td>
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<tr>
<td>T2D</td>
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</tr>
<tr>
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<td>triglyceride</td>
</tr>
<tr>
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<td>tumor necrosis factor alpha</td>
</tr>
<tr>
<td>μL</td>
<td>microliter</td>
</tr>
<tr>
<td>VO₂</td>
<td>volume of oxygen</td>
</tr>
<tr>
<td>WAT</td>
<td>white adipose tissue</td>
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Chapter 1

Introduction

1.1 General Introduction

The prevalence of obesity in Canada has risen steadily over the last 30 years. Current reports indicate that approximately 34% of the Canadian population are classified as overweight and an additional 26% are obese (10, 12, 16). This has significant healthcare implications as obesity is associated with risk factors that are linked to the development of multiple chronic illnesses including: insulin resistance, diabetes, dyslipidemia, metabolic syndrome, hypertension, cardiovascular disease and certain types of cancer (16). Furthermore, obesity represents a considerable economic burden with approximately 3.9 billion dollars of Canadian health care costs directly attributed to the management and treatment of obesity, and an additional 3.2 billion in indirect costs (10, 11). Many of the related risk factors accompanied by obesity are due to chronic impairment of adipose tissue function, which directly and indirectly regulates systemic metabolism (21). Therefore, proper investigation and understanding of the mechanisms involved in adipose tissue function are important in the development of interventions to combat metabolic dysfunction.

1.2 Adipose Tissue: More than Storage

Healthy adipose tissue has many important functions in the regulation of fatty acid metabolism and systemic energy homeostasis (19, 22). Primarily, adipose tissue stores triglycerides during periods of substrate excess when energy demand is low, and releases fatty acids when energy demand is high (22). This process is tightly regulated by β- and α- receptor
stimulation which controls the metabolism of the cell (see Chapter 2 for details) (13). Additionally, adipose tissue is an endocrine organ that is capable of releasing a wide range of hormones and metabolites that contribute to whole body fuel metabolism (14). Specifically, the release of these hormones has been associated with local and systemic regulation of metabolic processes in both adipose tissue and other tissues such as the brain, skeletal muscle, pancreas and the liver (22). Therefore, examination of the mechanisms involved in the proper function of adipose tissue may aid in the design of interventions directed at improving metabolic health.

Obesity modifies both the energetic and endocrine functions of adipose tissue which is associated with a range of illnesses such as metabolic syndrome, insulin resistance, hyperlipidemia, and chronic low-grade systemic inflammation (5, 22, 27). Adipose tissue energetic dysfunction consists of attenuated β- and α- receptor stimulation which inhibits the ability of the cell to store triglycerides via insulin signalling pathways, and may also reduce its ability to stimulate the pathway responsible for the production of key glyceroneogenic proteins that are needed to produce triglycerides (8, 9, 26). The inability of adipose tissue to store triglycerides results in increased basal plasma fatty acid concentrations (8, 24). Additionally, obesity affects the ability of adipose tissue to function as an endocrine organ and changes the concentration of key hormones and cytokines that control local and systemic metabolism (27). Changes in these hormones increases fat storage in both adipose tissue and peripheral tissue, while decreasing fat oxidation in skeletal muscle and liver (22). This ectopic storage of lipids can lead to peripheral insulin resistance and metabolic syndrome (2, 15, 20). These hormonal changes are also followed by low-grade inflammation produced from proinflammatory cytokines (27). Cytokines are produced as a protective mechanism to aid in adipose tissue function, but by consequence, effect systemic function by further increasing basal plasma fatty acid concentrations.
and contributing to ectopic lipid distribution (27). This evidence of adipose tissue dysfunction and its consequences on systemic metabolic function justifies the investigation into the pathways involved in β- and α- receptor stimulation and the factors by which they are influenced.

1.3 Exercise: A Potential Stimulator of Adipose Tissue Health

It is well recognized that exercise is capable of inducing a wide array of central and peripheral adaptations which has many positive influences on overall health (1, 7). More recently, it has also been implicated in the treatment of metabolic syndrome (18). These benefits may in part be influenced by exercise-mediated changes in adipose tissue metabolic pathways. Exercise-mediated increases in plasma epinephrine are well known to influence a variety of tissues through β-adrenergic stimulation (3, 4, 23). Evidence with rodents has revealed acute and chronic changes in adipose tissue lipolysis, and glyceroneogenic and mitochondrial gene expression following acute exercise (17, 23, 25). Additional studies with humans have shown increased lipolysis, fatty acid mobilization and plasma fat oxidation, and that the increase in available plasma fatty acids was associated with greater total lipid oxidation up to 3 hours post-exercise (6). In combination, these findings demonstrate that adipose tissue is plastic in nature and may be changed in response to physiological and environmental stresses. Currently, the factors that influence healthy adipose tissue function and the understanding of the mechanisms involved are limited. Accordingly, investigation into the time-course stimulation of pathways responsible for proper adipose tissue function, and its response to acute environmental stresses such as exercise are required, as they may provide important clinical value.
1.4 Thesis Purpose and Objectives

The purpose of this study was to examine time-course changes in the phosphorylation status of pathways conducive to adipose tissue lipid storage. Specifically, the purpose of this study was to: 1) investigate the phosphorylation status of proteins within the insulin signalling pathway following acute exercise and control conditions, and 2) investigate the phosphorylation status of proteins known to be involved in the pathway regulating the expression of glyceroneogenic genes following acute exercise and control conditions. To accomplish this, a randomized cross-over design was implemented. Sedentary, overweight female participants completed 2 visits to the lab in which they either completed 1 hour of exercise or a time-matched bed rest control. Gas exchange measures were collected at three time points before biopsies and subcutaneous adipose biopsies were obtained pre-condition, immediately after condition (0hr), two hours post (2hr), and four hours post (4hr) in order to examine the effect of acute exercise on whole body fatty acid oxidation and phosphorylation of intracellular signalling proteins in adipose tissue.

1.5 Thesis Organization

Chapter 2 is a detailed literature review outlining metabolic syndrome and the function of adipose tissue along with its roles as both a metabolic tissue and an endocrine organ. It will also look at proposed exercise-induced mechanisms responsible for adipose tissue and systemic health. Chapter 3 contains the manuscript detailing the study of the impact of acute exercise on mechanisms of action in human adipose tissue in a time-course approach. Chapter 4 provides a discussion on the key findings of this research, the limitations, and future directions. Finally,
several appendices are attached to the end of this document to provide more complete examples of items referred to throughout this thesis.
1.6 References


Chapter 2
Literature Review

2.1 Overview

This literature review will begin with a discussion of metabolic syndrome and its causes. The function of adipose tissue and its roles as both a metabolic tissue and an endocrine organ, and the progression of adipose tissue dysfunction caused by obesity will then be highlighted. Finally, this review will cover the effects of exercise on metabolic health along with proposed exercise-induced mechanisms responsible for proper adipose tissue health and function.

2.2 The Metabolic Syndrome

The steady rise in prevalence of obesity in Canada over the last 30 years has impacted the risk of developing chronic illnesses such as cardiovascular disease (CVD) and type II diabetes (T2D) (75). The treatment of such illness has caused a tremendous financial burden on Canadian health care costs (42). The development of this trend has brought about the need to classify and characterize potential risk factors that contribute to the development of both obesity itself, and obesity related illnesses.

The metabolic syndrome refers to a combination of risk factors and conditions that are interrelated in the progression of CVD and T2D. These factors include high blood pressure, elevated triglyceride levels, low high-density lipoprotein (HDL) cholesterol concentrations, elevated fasting plasma glucose levels, and obesity (1). Although the precise criteria that define the metabolic syndrome have been debated, a collaborative effort by multiple health organizations has produced a unified set of criteria (1, 66). According to these criteria, metabolic
syndrome is present if three or more of the following are present: abdominal obesity (waist circumference ≥ 94cm for men and ≥ 80cm for women); elevated plasma triglyceride levels (≥ 1.7mmol/L); decreased HDL cholesterol levels (<1.03 mmol/L for men and < 1.30 mmol/L for women); elevated blood pressure (≥ 130/85mm Hg); or elevated fasting glucose levels (≥ 5.6 mmol/L) (1). The estimated prevalence of metabolic syndrome in Canada in 2009 was 19.1%, which corresponds to approximately 1 out of 5 Canadians (66). Individuals afflicted by metabolic syndrome have a 2-fold increase in the risk of developing CVD and a 5-fold increase in the risk of developing T2D (1). The primary cause underlying the development of metabolic syndrome appears to be the overconsumption of calories in combination with a decrease in physical activity (1, 85). This chronic positive energy balance leads to greater adiposity and obesity which can have many negative effects on both adipose tissue function and systemic health (6, 86). Available evidence suggests that adipose tissue dysfunction may be a primary determinant in the development of metabolic syndrome. If this is true, then a better understanding of the mechanisms of adipose function are important to our overall understanding of the maintenance of both adipose tissue and overall metabolic health.

2.3 Adipose Tissue Function

White adipose tissue (WAT) was once thought to only function as an energy storage depot for lipids. More recently, it has also been found to be a complex metabolic tissue capable of releasing a wide range of hormones and metabolites that contribute to whole body fuel metabolism (67). More specifically, WAT has three important functions in maintaining proper fatty acid metabolism: first, to control the storage of non-esterified fatty acids (NEFAs) as triglycerides (TGs); second, to hydrolyzes TGs and release glycerol and NEFAs that are used as fuel in working tissues allowing for fat oxidation in muscle, liver and other tissues while sparing
glucose for other processes (39, 67); and third, WAT is an endocrine organ that is capable of regulating energy metabolism through endocrine, paracrine and autocrine signals. Regarding the latter, adipose tissue derived hormones have the ability to regulate both local and systemic metabolism (83, 86). There is emerging evidence that all three of these processes are tightly regulated and increasing adiposity and obesity can cause multiple levels of dysfunction (44). This dysfunction appears to contribute to the development of chronic illnesses such as metabolic syndrome and progressive chronic illnesses such as CVD and T2D (85, 86, 91). Thus, understanding the proper function of adipose tissue will allow greater insight into the progression of dysfunction and the development of metabolic disease.

2.3.1 Adipose Tissue and Metabolism

Adipose tissue plays a key role in basal metabolism via its influence on substrate utilization and maintenance of energy homeostasis (39, 67). To this end, adipocyte (individual adipose tissue cells) metabolism is tightly regulated by β- and α- receptor stimulation (see Figure 1) (62). In the postprandial state (i.e. after a meal), plasma insulin is elevated promoting α-receptor stimulation of adipocytes which in turn promotes the storage of NEFAs, and glycerol as TGs via a process termed esterification (62). The Randle Cycle describes this action as the “glucose-fatty acid cycle” which explains that in the post absorptive state, when insulin and glucose are elevated, lipid and carbohydrate storage are promoted in both adipose tissue, muscle and other tissues (39). This allows for the clearance of excess substrate from the blood when energy demand is low (see Figure 1). In the fasted state, when substrate availability is low, low insulin and high glucagon and catecholamine levels stimulate adipose tissue lipolysis via β-adrenergic (β-AR) receptors. This results in the liberation of NEFAs from TGs to supply muscle, liver and other tissues with substrate for fatty acid oxidation to spare glucose (39, 67). In addition,
high glucagon to insulin ratios also promote the production of glucose in the liver to supply tissues such as the brain and nervous system, which are solely dependent on glucose for substrate in energy production (39). This becomes an important factor when discussing disturbances to the system later on in this review.

**Fasted/Glucagon/Exercise**

![Diagram of Adipose tissue and its pathways](image)

**Figure 1. Action of β- and α- receptor stimulated pathways during the fed/insulin and fasted/glucagon/exercise states.**

Therefore, it can be suggested that substrate selection and energy homeostasis during basal conditions are primarily controlled by β- and α- receptor stimulation of adipose tissue, as it is the primary storage site of lipids that ultimately dictates plasma NEFA concentrations (65). The proper functioning of this cycle helps in the understanding of how the system handles physiological stresses such as exercise. Similar to that of the fasted state, exercise promotes
lipolysis through the activation of β-AR signalling (37, 62), increasing plasma NEFA concentrations and allowing for increased fat oxidation in working muscle (37, 67). This tight regulation of NEFA release and storage seems to be a key mechanism in the overall maintenance of adipose tissue and metabolic health.

2.3.2 Adipose Tissue as an Endocrine Organ

In addition to its role in energy homeostasis, adipose tissue is also an endocrine organ that is capable of secreting a number of adipokines and proinflammatory cytokines that are implicated in systemic metabolic control (86). Although the list of hormones and adipokines are numerous, the two that have received the most attention over the past years are leptin and adiponectin. Additionally, proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) have received abundant consideration as adipose tissue both produces these cytokines and expresses receptors for them (43, 92).

Although expressed by other tissues, adipose tissue is the largest producer of leptin and accounts for the majority of all leptin secreted into the circulation (44, 86). Additionally, plasma leptin concentrations are directly proportional to the number of adipocytes present (86). Among many other functions, leptin regulates energy metabolism by increasing energy expenditure and decreasing energy consumption (83, 86), by both stimulating receptors on the hypothalamus that control the feeling of satiety and reducing appetite (44, 83) and by targeting receptors on tissues such as the liver, pancreas, and skeletal muscle that stimulate greater fatty acid uptake and oxidation (Figure 2) (83, 86). In addition, adipose tissue also expresses leptin receptors indicating an autocrine and paracrine function is also present (86). In rodents, leptin stimulates the oxidation of stored lipids within adipocytes therefore reducing their size (86). This acts as protective mechanism against over accumulation of lipids within the cell.
Adipose tissue also secretes adiponectin which has multiple effects on systemic energy metabolism. Adiponectin increases insulin sensitivity in peripheral tissues in two ways: 1) it decreases NEFA uptake and TG storage, and increases fat oxidation in liver, and 2) increases NEFA uptake and oxidation in muscle (12). Additionally, adiponectin activates AMP-activated protein kinase (AMPK) in both muscle and liver, which stimulates fat oxidation and glucose uptake in muscle, and reduces the expression of proteins involved in gluconeogenesis in liver (41, 93). In combination, these actions lower basal plasma glucose and NEFA concentrations, and decrease TG storage within peripheral tissues (Figure 2) (12, 41). Adiponectin also inhibits the production and action of inflammatory cytokines such as TNF-α (12, 44) positioning it as a protective adipokine against insulin resistance and metabolic syndrome. More specifically, adiponectin prevents accumulation of fat depots within tissues such as muscle and liver by facilitating fatty acid oxidation, an effect that is especially important given the increases in ectopic fat storage seen in obesity (85). Thus, the secretion of leptin and adiponectin by normally functioning adipose tissue seems to have multiple important roles in the maintenance of proper metabolic health.

In contrast, chronic secretion of proinflammatory cytokines, such as TNF-α and IL-6 by adipose tissue have been associated with obesity and the development of insulin resistance (44) (69, 73, 81). However, acute expression of TNF-α and IL-6 have been suggested to have beneficial physiological effects on systemic metabolism, and healing processes (58, 79). Expression of TNF-α acutely regulates many important factors in the progression of wound healing (30, 58). TNF-α is produced by macrophages in the acute inflammatory phase, and in combination with other cytokines is important for cell proliferation, angiogenesis, and migration of mesenchymal stem cells to injured areas (30). Conversely, chronic TNF-α expression by
adipose tissue interferes with adipocyte metabolism and function (69, 73). In addition to TNF-α, IL-6 is another pro-inflammatory cytokine secreted by adipose tissue and its suggested functions are two-fold: 1) circulating IL-6 increases whole body fat oxidation (64), and 2) increases lipolysis in adipose tissue therefore increasing plasma NEFA concentrations (43, 64). In response to acute exercise, interstitial concentrations of IL-6 in adipose tissue have been shown to remain elevated for several hours post exercise (32, 43). It is hypothesized that this transient elevation of IL-6 post-exercise may be related with maintaining elevated NEFA for post exercise fat oxidation (79). Although acute elevation of these proinflammatory cytokines may have physiological benefits, overexpression and chronic elevated concentrations are detrimental to proper adipose tissue function (Figure 2). The role of pro-inflammatory cytokines in obesity and metabolic syndrome are discussed in further detail below.

2.3.3 Obesity and Adipose Tissue

Adipose tissue is a complex tissue that is capable of many important functions that enable proper systemic energy homeostasis, however, when adipose tissue function is impaired its contributions to the maintenance of energy homeostasis becomes compromised (86). This dysfunction leads to hyperlipidemia, ectopic fatty acid storage, insulin resistance and low-grade chronic systemic inflammation (28, 86, 91).

There are many factors involved in the progression of adipose tissue dysfunction. Obesity is primarily associated with a positive energy balance that is characterized by excess caloric intake in the absence of caloric expenditure (28, 74). Healthy adipose tissue is capable of storing excess TGs and maintaining near normal basal lipolytic rates during fasting (28), through hyperplasia and hypertrophy of adipocytes, and fat oxidation in insulin sensitive skeletal muscle and peripheral tissues (86). Obese individuals display blunted adipose tissue lipolytic activity
which is responsible for the hydrolysis of TGs and release of NEFAs (28). This inability to release NEFAs may contribute to the augmented adipose cell hypertrophy that is associated with obesity (27). Although this may seem counter intuitive to maintaining proper adipose tissue function, this adaptation is positive and is most likely an attempt to keep lipid stores localized to adipose tissue as opposed to ectopic deposition. However, as a chronic positive energy balance is maintained and adiposity increases, the ability of adipocytes to function properly is eventually impaired (28).

Obesity modifies the endocrine function of adipose tissue by altering the production of key metabolic hormones such as leptin and adiponectin, and proinflammatory proteins such as TNF-α and IL-6 (91). The concentration and expression of leptin is both positively and directly associated with adiposity, more specifically cell hypertrophy (19). Hyperleptinemia (elevated leptin concentrations) accompanying obesity is also associated with leptin resistance which increases both adipose tissue and peripheral fat storage and decreases systemic fat oxidation (86). Ultimately, hyperleptinemia leads to ectopic storage of lipids in liver, skeletal muscle, pancreas and other peripheral tissues (85), which then contributes to systemic insulin resistance (4, 72). In addition to leptin, adiponectin is also a stimulator of fat oxidation in skeletal muscle and liver (86). Adiponectin is inversely correlated with adiposity, insulin resistance, and metabolic syndrome (44, 86). Plasma adiponectin is decreased during obesity (44), and may contribute to reduced fat oxidation in both skeletal muscle and liver.

Also accompanying excessive adiposity, potentially in an attempt to reduce adipocyte size, hypertrophied adipose tissue releases many proinflammatory proteins including TNF-α and IL-6, which contribute to chronic inflammation (91). TNF-α expression is positively correlated with adiposity and directly regulates insulin sensitivity and lipolysis in adipocytes (19, 91).
through reductions in the expression of genes that are responsible for the uptake and storage of NEFAs and glucose, while increasing adipose tissue lipolysis to reduce TG stores and therefore adipocyte size (19, 44, 91). As a consequence, plasma NEFAs are increased, which leads to ectopic NEFA storage in peripheral tissues and ultimately systemic insulin resistance (27, 44, 72). In addition to TNF-α regulating adipose tissue metabolism, it also modifies concentrations of adiponectin and IL-6 (69). TNF-α mediates reductions in adiponectin, which may contribute to the reduction in plasma adiponectin that accompanies obesity (69) while TNF-α mediated increases in IL-6 may trigger negative adaptation in both adipose tissue and peripheral tissues (91). Although IL-6 has many functions, chronic elevated plasma concentrations are linked with obesity and insulin resistance (44) via increases in adipocyte lipolysis, plasma NEFA concentrations, ectopic NEFA storage, and decreasing adiponectin concentrations (44). In combination with many other factors, these proinflammatory cytokines contribute to the progression of adipose tissue dysfunction, systemic insulin resistance, and the development of metabolic syndrome.
2.3.4 Obesity and Adipose Tissue in Women

It is now well established that individuals that are overweight or obese have increased basal plasma NEFA concentrations (35, 36, 40, 68). In comparison to lean women, obese women have less preadipocytes that are available for differentiation (15) decreasing the ability of adipose tissue to store lipids and resulting in elevated levels of circulating NEFAs. These chronic excess basal circulating NEFAs are then deposited in peripheral tissues such as liver and skeletal muscle where it effects the insulin sensitivity and functioning of those tissues (4, 5, 72). Obese women also exhibit a greater basal lipolytic rate when compared to lean women (35). This greater basal
lipolytic rate may be related to adipose tissue insulin resistance (35, 36) and TNF-α concentrations (44). As adipose tissue becomes insulin resistant its ability to store lipids is impaired which increases plasma NEFA concentrations. In addition to adipose tissue insulin resistance, obese women also exhibit attenuated lipolytic sensitivity to catecholamines in subcutaneous adipose tissue compared to lean women (36). Higher adipose tissue basal lipolytic rates observed in obese individuals may be induced by means of proinflammatory proteins rather than catecholamine-induced lipolysis (49). This is an important difference as epinephrine is an important catecholamine that contributes to the control of both the proper release and storage of NEFAs (37). Epinephrine is also responsible for the activation and regulation of important lipolytic and glyceroneogenic proteins in adipose tissue (26, 88, 89). These proteins are important in the proper balance between storage and release of NEFAs that contribute to proper systemic functioning. Therefore attenuated adipose tissue sensitivity to catecholamines may also contribute to the development of adipose tissue dysfunction.

Chronic exposure to excess circulating NEFAs is an important factor in the progression of insulin resistance and metabolic syndrome (5, 86) as ectopic storage of NEFAs leads to peripheral tissue insulin resistance (44, 72, 91). From the evidence provided it can be suggested that adipose tissue health and its proper function play key roles in general overall metabolic health and function. This becomes even more apparent as adipose tissue dysfunction directly and indirectly contributes to: increased adiposity; elevated plasma triglycerides; and elevated fasting glucose via insulin resistance, which are 3 of the criteria of metabolic syndrome. At present however, the mechanism(s) by which proper adipose tissue function is maintained are not well understood. With increasing obesity rates and subsequent illnesses caused by adiposity, there is an increasing need for a better understanding of the mechanism(s) controlling adipose tissue
function. As stated above, the progression of metabolic syndrome is caused by inhibition of adipose tissue function, therefore, investigation into factors known to benefit overall health, such as exercise, and their effect on adipose tissue mechanisms, need to be examined.

2.4 Exercise

It is well recognized that exercise induces many positive benefits to overall health. It is capable of increasing both central (3) and peripheral adaptations (33) that result in increases in aerobic fitness (22, 52) and metabolic health (8, 23, 80). Centrally, exercise increases factors related to cardiovascular function such as greater cardiac output, which allows for greater oxygen delivery to working muscles to produce energy (3). Peripheral adaptations, although numerous, include greater skeletal muscle oxidative capacity (8), and adipose tissue function (88, 89), influencing mitochondrial content and substrate utilization respectively (8, 21). Specifically, exercise is associated with acute and chronic adaptations in adipose tissue (76, 89) allowing for acute increases in lipolysis and chronic changes in the storage of NEFAs (29, 89). For example, 3hrs following a single bout of exercise lipolysis and NEFA mobilization from adipose tissue, and the systemic oxidation of plasma NEFA were all increased (29). In addition, studies with rodents have shown that exercise training also stimulates chronic changes in the storage and composition of adipose tissue, which aids in proper basal metabolic functioning (76, 89). These findings demonstrate that adipose tissue is plastic in nature, and at least in rodents, is able to change in response to physiological stresses such as exercise. The ability of exercise to influence both central and peripheral adaptation, improve overall aerobic fitness, exercise tolerance, and metabolic health is not questioned (8, 52, 80), but the mechanisms behind these acute and chronic adaptations and the factors by which adipose tissue may be effected in humans have yet to be elucidated.
Over the past couple of decades, cardio exercise utilized in experimental research has been characterized into two categories. The first comprises of traditional steady state endurance exercise (END). END is categorized as exercise that is maintained over a fixed distance or duration for a given submaximal workload (22). This type of training influences both central and peripheral adaptations and typically results in weight loss and improved body composition (78, 84). The second type of exercise includes high-intensity interval training (HIT), which is characterized by brief repeated intermittent exercise at near maximal or supramaximal intensity (22). HIT primarily effects peripheral adaptations (52), and also positively enhances body composition (82). Both END and HIT have positive effects on reducing obesity and improving metabolic health (80, 82).

2.4.1 Exercise and Weight loss

A well-established effective tool for reducing obesity is training with traditional END (59). More recently, HIT has also been associated with substantial or in some cases greater fat loss than END (80, 82, 84). HIT is also effective in improving aerobic fitness (52), skeletal muscle oxidative capacity (8, 22), and metabolic health (80). Weight loss from exercise is characterized as a negative energy balance caused by a greater energy expenditure than intake (84). Interestingly, energy expenditure with HIT is often markedly lower than END (8, 22, 52, 84), however, END and HIT have been demonstrated to result in similar gains in aerobic capacity, fatty acid oxidation and skeletal muscle mitochondrial biogenesis (8, 22). More recent evidence suggests that HIT is capable of inducing significant decreases in body fat (7, 82); however, the mechanisms by which this occurs, and the effects of HIT on adipose tissue function remains unclear.
2.4.2 Exercise and Plasma Epinephrine

Exercise mediated increases in plasma epinephrine have long been known to be associated with exercise intensity (20, 46, 54) and duration (38, 48). Based on this relationship, it seems reasonable to suggest that some of the systemic and intramuscular adaptations induced by HIT result from high levels of epinephrine accompanying the high relative intensities of exercise associated with low volume HIT protocols. Central to this hypothesis is the assumption that the intensity dependent increases in epinephrine associated with constant load submaximal exercise extend to maximal and supramaximal HIT protocols. Pilot data from our lab show significant increases in plasma epinephrine following increasing intensities of acute exercise with protocols being matched for exercise duration (Figure 3). This significant increase in plasma epinephrine is also seen despite greater total external work in the 60% VO$_{2}$peak and 80% VO$_{2}$peak steady-state endurance protocols (155±22kJ and 187±34kJ respectively) compared to the 100% VO$_{2}$peak and Wingate HIT protocols (125±15kJ and 49±8kJ respectively).

Exercise-mediated increases in epinephrine may present a plausible stimulus for both acute and chronic adaptations seen in adipose tissue. Epinephrine is a stimulator of β-AR receptors on adipose tissue (63), which can acutely increase lipolysis, and in rodents, can influence chronic changes in adipose tissue lipid storage (88). However, exercise-mediated changes in the signalling pathways of β- AR agonism in human adipose tissue have yet to be identified and have yet to be associated with the acute and chronic adaptations mentioned above. The changes observed in plasma epinephrine over increasing intensities of exercise supplies us with a model to observe potential mechanisms stimulated by β-AR agonism in adipose tissue.
2.5 Mechanisms of Adipose Tissue Adaptation to Exercise

Healthy adipose tissue function requires the stimulation of β- and α-receptors (62). The stimulation of these receptors dictates the activity of the cell by activating proteins and pathways that are responsible in either storing or releasing NEFAs (37, 62). These signalling cascades are fairly well understood in the basal state but have not been fully elucidated in human adipose tissue in response to stressors such as exercise. This becomes an issue with the rise in prevalence of obesity and metabolic syndrome (66, 75), as it is important that the mechanisms of adipose tissue function are better understood to combat such illnesses.

2.5.1 β-AR Stimulated Lipolysis in Adipose Tissue

It is well established that during and following traditional steady-state endurance exercise that there is an increase in skeletal muscle fatty acid oxidation (29, 53). Catecholamines
(epinephrine and norepinephrine) released during exercise activate β-AR receptors on adipocytes which result in the initiation of lipolysis. Following β-AR agonism, TGs within the lipid droplet are broken down by adipose triglyceride lipase (ATGL) to form diacylglycerols (DAGs). DAGs are then broken down to monoacylglycerols (MAGs) by phosphorylated hormone sensitive lipase (HSL; Figure 4). The final step of lipolysis requires monoacylglycerol lipase to break down MAGs into NEFA and glycerol, which are released into the circulation for the uptake of skeletal muscle for fatty acid oxidation (63). During each step of lipolysis a NEFA is released into the circulation for use as fuel in working tissues such as skeletal muscle.

In order for lipolysis to be initiated, activation of certain key proteins must first take place. Activation of β-AR receptors on adipose tissue results in the conversion of adenosine-5'-triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), which in turn activates protein kinase A (PKA) (34). PKA has two major targets in the initiation of lipolysis. The first target of PKA is the phosphorylation and activation perilipin A (47), which is a protein that is bound to lipid droplet and acts as a barrier to inhibit lipolysis by lipases located in the cytosol (25). The activation of perilipin A allows lipases such as ATGL and phosphorylated HSL to interact with the lipid droplet and breakdown TGs and DAGs (56, 57, 77). The second target of PKA is the phosphorylation and activation of HSL which allows for the translocation of HSL from the cytosol to the lipid droplet where it can hydrolyze TGs (25). Stimulation of the β-AR receptor on adipose tissue is important during fasting and both during and following exercise when NEFAs are required for fuel. However, altered activity of PKA in human adipose tissue in response to acute exercise has yet to be measured. Further investigation is required to establish whether or not PKA is influenced by potential exercise-mediated increases in β-AR agonism.
Figure 4. Epinephrine mediated pathways in adipose tissue.

Abbreviations: Adenosine-5'-triphosphate (ATP); phosphorylated hormone sensitive lipase (p-HSL); adipose triglyceride lipase (ATGL); p-38 mitogen-activated protein kinase (p38 MAPK); uncoupling protein 1 (UCP1).

2.5.2 α-Receptor Stimulated Storage of TGs by Insulin

In contrast to β-AR agonism, α- receptor stimulation by insulin attenuates the β-AR signalling pathway through activation of phosphodiesterase-3B (PDE-3B) (45, 63). PDE-3B reduces intracellular cAMP concentrations therefore decreasing its availability to activate PKA, and subsequently reducing the rate of lipolysis. PDE-3B is activated through phosphorylation of protein kinase B (PKB/Akt) which is regulated by the insulin signaling cascade (45, 63). Insulin
signals the storage of NEFAs within adipocytes and by consequence slows the release of NEFAs from the cell.

In addition to blunting lipolysis, α-receptor stimulation by insulin also stimulates the storage of plasma glucose and triglycerides into adipose tissue derived TGs (94). Insulin action increases glucose transport and lipoprotein lipase (LPL) activity, both of which supply substrate for the storage of lipids (18, 71). During the postprandial state, insulin stimulates the translocation of glucose transporter GLUT4 to the plasma membrane, which facilitates the transport of glucose into the adipocyte (24, 55). Glucose is utilized in multiple pathways in adipose tissue but the two fates associated with storage of TGs are: 1) de novo synthesis of NEFAs via lipogenesis (31) and, 2) production of glycerol-3-phosphate (G3P) to be utilized in the esterification of NEFAs, from preexisting and de novo pools, to adipose tissue TGs (13). Additionally, insulin stimulates LPL activity in adipose tissue which is responsible for hydrolyzing plasma TGs into NEFAs so that they can be taken up and esterified in adipose tissue as TGs (94). Therefore, insulin sensitivity is important for the storage of substrate as TGs in adipose tissue (94).

In addition, it had been established that insulin sensitivity is a positive mechanism which is increased in tissues such as muscle post exercise, and that these increases in sensitivity are accompanied by overall improvements in glucose homeostasis (51), and metabolic health (80). However, acutely increasing insulin sensitivity in adipose tissue post exercise may prove to be counterproductive in maintaining proper adipose tissue function. Although seemingly counterintuitive as chronic adipose tissue insulin resistance is detrimental to systemic metabolism (70, 86), acute insulin insensitivity post exercise may be beneficial in maintaining proper adipose tissue function. Although speculative, it may be proposed that post exercise, transient decreases in insulin signalling in adipose tissue may provide a mechanism by which increases in NEFA
release is sustained in adipose tissue. This plausible mechanism may explain the increases in NEFA release and subsequent oxidation that have been observed in humans up to 3hrs post exercise (29). Uncertainty remains around the mechanisms responsible for these increases in NEFA release observed, and it is unknown whether or not insulin signalling in adipose tissue may be involved. Additionally, altered insulin signalling in human adipose tissue in response to acute exercise has yet to be studied. Future investigation into the time-course changes of insulin signalling in human adipose tissue in response to acute exercise is required to fully understand the mechanisms involved post exercise.

2.5.3 Glyceroneogenesis

In contrast to the fed state, when insulin is high and glucose is the primary substrate for G3P production, fasting and exercise provoke an alternative pathway of G3P production from non-glucose derived pyruvate termed glyceroneogenesis (13, 61). Glyceroneogenesis is the process by which G3P, an important substrate to form TGs via its re-esterification with NEFAs, is produced during periods of β-AR agonism accompanying fasting and exercise (13, 88). The proteins primarily responsible for controlling the production of G3P are pyruvate dehydrogenase kinase 4 (PDK4) and phosphoenolpyruvate carboxykinase (PEPCK) (Figure 5) (17, 89). PDK4 is a potent inhibitor of the pyruvate dehydrogenase complex (PDC), the enzyme complex that catalyzes the irreversible formation of acetyl-CoA and CO₂ from pyruvate (90). Inhibition of PDC results in provision of pyruvate to the glyceroneogenic pathway, which catalyzes the conversion of pyruvate to oxaloacetate which is subsequently converted to phosphoenolpyruvate, a precursor for G3P by PEPCK. G3P is then used as substrate in the re-esterification process via its interaction with NEFAs to form TGs (Figure 5) (9, 17, 87, 89). This process allows for adipocytes to reduce and regulate the concentration of NEFA that leave the adipocyte, therefore,
regulating the amount of circulating NEFA. This is important as metabolic syndrome is associated with elevated plasma NEFA concentrations (1), and examination into the factors which influence this regulation of this pathway in adipose tissue is needed. Specifically, investigation into the regulation of this pathway in response to exercise in adipose tissue of humans is still required.

Figure 5. Insulin and re-esterification processes in adipose tissue.

Abbreviations: Phosphatidylinositol 3-kinase (PI3-K); phosphorylated protein kinase B (p-PKB/Akt); phosphodiesterases (PDE); cyclic adenosine monophosphate (cAMP); pyruvate dehydrogenase kinase 4 (PDK4); pyruvate dehydrogenase (PDH); pyruvate carboxylase (PC); oxaloacetate (OA); phosphoenolpyruvate carboxykinase (PEPCK); glycerol-3-phosphate (G3P); triglyceride (TG); diacylglycerol (DAG); monoacylglycerol (MAG).
Recent investigation with rodents has revealed both epinephrine induced and exercise-mediated changes in adipose tissue in regards to the expression of glyceroneogenic genes. These studies have revealed epinephrine-mediated increases in PDK4 mRNA following acute exercise and in-vivo epinephrine administration (87–89). Exposure of WAT to epinephrine and β-AR agonists increased the activation of p38 mitogen-activated protein kinase (MAPK) via PKA activation (11). This pathway was also further elucidated by the use of a potent p38 MAPK inhibitor SB202190. Use of this inhibitor on WAT attenuated the epinephrine-mediated increases in PDK4 gene expression (89). Therefore suggesting that p38 MAPK is a key upstream regulator of PDK4 expression. Although exercise-mediated increases in PDK4 have been illustrated in rodents, investigation into the phosphorylation status of p38 MAPK and pathways regulating PDK4 expression in response to exercise in human adipose tissue have yet to be performed.

While PEPCK has been explored widely in tissues such as muscle and liver, there is little research on its mechanism of expression in human adipose tissue in response to exercise. PEPCK acts in a different manner in adipose tissue than in liver and muscle as it is recognized as a key enzyme in the glyceroneogenic pathway of adipose tissue (2, 14, 17). In rodents, PEPCK expression in adipose tissue has shown to increase in response to acute exercise and β-AR agonists that increase cAMP availability (16, 88). Additionally, PEPCK expression has been shown to be inhibited by insulin (13). Whether acute exercise may be involved in increasing the pathways regulating PEPCK expression in human adipose tissue has yet to be investigated.

Glyceroneogenesis is important for regulating and reducing the amount of NEFA which leave the adipocyte and enter the circulation (17, 60). Evidence in rodents suggests that increased glyceroneogenic gene expression is a chronic adaptation to exercise (88), and aids in the regulation of NEFA release and storage (13). Additionally, abundance of glyceroneogenic genes
and proteins has shown to be beneficial in reducing plasma NEFA concentrations in humans (10, 50), however, to the best of our knowledge, there have been no investigations into the activation of the pathway regulating the expression of these genes in response to acute exercise in human adipose tissue. Whether this pathway can be regulated in response to acute exercise needs to be examined before a full understanding of the mechanisms involved in the chronic maintenance of proper adipose tissue lipid storage in humans is achieved.

2.6 Summary

In summary, available evidence suggests that adipose tissue function is a key component in the regulation of metabolic function and health, and there is some evidence demonstrating exercise-induced adaptations in adipocyte function (88). More specifically, the epinephrine responses following acute exercise provide us with a model to examine the impact of acute exercise on the mechanisms of adipocyte function. As such, there is a need for research examining the existence of exercise-induced adipocyte responses, the mechanisms by which these responses occur, and their implications on metabolic health. This information may allow for more appropriate and effective exercise prescription, and a decreased need for pharmacological interventions for individuals inflicted by adipocyte dysfunction and associated metabolic disorders including metabolic disease.

2.7 Thesis Objectives

Given the evidence provided, it is clear that our current understanding of acute exercise and its influence on acute and chronic changes in human adipose tissue is deficient. Therefore, the purpose of this thesis is two-fold: 1) to determine the effect of exercise on insulin signaling in human subcutaneous adipose tissue and to examine whether changes in insulin signaling are
related to changes in whole body fatty acid oxidation, and 2) to determine the effect of exercise on signaling pathways responsible for upregulation of glyceroneogenic genes, specifically the activation/phosphorylation of PKA and p38 MAPK.

2.8 Thesis Hypotheses

Based on evidence gained from rodents, we hypothesized that: 1) acutely following exercise, there would be decreased phosphorylation of insulin-signalling proteins, relative to control, and increased whole body fat oxidation and, 2) chronically following exercise, there would be an increase in the activity/phosphorylation of the signaling proteins involved in the upregulation of glyceroneogenic genes.
2.9 References


Chapter 3

Effect of acute exercise on whole body fat oxidation: contributions of abdominal subcutaneous adipose tissue
3.1 Introduction

Adipose tissue is a complex tissue capable of secreting a wide range of hormones and metabolites that contribute to whole body metabolism (24). Specifically, adipose tissue stores triglycerides (TGs) during periods of substrate excess and/or when energy demand is low (via glyceroneogenesis and esterification) and releases non-esterified fatty acids (NEFA) via lipolysis when substrate availability is low and/or energy demand is high (28). The regulation and maintenance of these pathways is important as excess basal plasma NEFA concentrations are associated with ectopic accumulation of TGs that contribute to systemic metabolic dysfunction (1, 28). The regulation of these processes in adipose tissue is tightly controlled by insulin and β-adrenergic (β-AR) receptor signaling, both of which contribute to activation/inhibition of pathways involved in the control of TG synthesis and lipolysis, ultimately dictating cellular and systemic metabolism (21).

The stimulation of β-AR receptors on adipose tissue initiates lipolysis by increasing the production of cyclic adenosine monophosphate (cAMP) which in turn activates protein kinase A (PKA) (10). Subsequent activation by PKA allows lipases to attach to the lipid droplet and results in the release of NEFAs and glycerol from adipocytes (17, 18, 27). In contrast, insulin signalling reduces the rate of lipolysis in two ways: first, insulin phosphorylates and activates protein kinase B (PKB/Akt) and phosphodiesterase-3B (PDE-3B) reducing intracellular cAMP, and the activity of PKA and PKA mediated activation of lipolysis (13, 22). Second, insulin action increases glucose transport and lipoprotein lipase activity (LPL), both of which supply substrate such as NEFAs and glycerol for the storage of lipids (6, 25). In addition, insulin stimulates the expression of lipogenic genes that contribute to the conversion of glycerol and NEFA to TGs (33). Interestingly, a recent investigation reported elevated NEFA release from adipose tissue in
women up to 3hrs post exercise (8). However, it remains unclear which, if any, of the mechanisms discussed above, contribute to the elevated lipolysis observed post exercise. In fact, the contributions of both insulin and β-AR signalling in human subcutaneous adipose tissue to exercise mediated increases in lipolysis and whole body fatty acid oxidation remain unclear.

Adipose tissue’s ability to re-esterify NEFAs back into TGs is partly dependent on the combination of NEFAs with glycerol-3-phosphate (G3P) from non-glucose derived pyruvate through a process termed glyceroneogenesis (5, 30). This process allows adipose tissue to reduce and regulate the concentration of NEFA that leave the adipocyte, therefore, regulating the amount of circulating NEFA during states of low plasma insulin availability (5, 30). In addition to stimulating lipolysis, β-AR agonism can increase the expression of glyceroneogenic genes in rodent adipose tissue (30, 31), through the phosphorylation and activation p38 mitogen-activated protein kinase (MAPK) (31). Phosphorylation of PKA appears to be required for the stimulation of p38 MAPK in rodent adipose tissue (4) suggesting that the activation of PKA and p38 MAPK contributes to the regulation of glyceroneogenic gene expression (31). In addition, acute exercise can mediated increases in β-AR activity which are associated with increased expression of glyceroneogenic genes in rodents (30). While studies examining the expression of glyceroneogenic gene expression in human adipose tissue are limited, a positive association between glyceroneogenic gene expression and basal plasma NEFA concentrations has been observed (3). With studies from rodents suggesting a role for exercise and β-AR signaling in the control of glyceroneogenic gene expression the activation of this pathway, specifically the activation of PKA and p38 MAPK, by exercise in human subcutaneous adipose tissue represents an important area of study.
The purpose of this study was therefore twofold: 1) to determine the effect of exercise on insulin signaling in human subcutaneous adipose tissue and to examine whether changes in insulin signaling are related to changes in whole body fatty acid oxidation, and 2) to determine the effect of exercise on signaling pathways responsible for upregulation of glyceroneogenic genes, specifically the activation/phosphorylation of PKA and p38 MAPK. We hypothesized that: 1) acutely following exercise, there would be decreased phosphorylation of insulin-signalling proteins, relative to control, and increased whole body fat oxidation and, 2) chronically following exercise, there would be an increase in the activity/phosphorylation of the signaling protein involved in the upregulation of glyceroneogenic genes.

3.2 Methods

3.2.1 Participants

Ten overweight/obese, sedentary female participants volunteered to take part in this study (participant characteristics are presented in Table 1). All participants reported less than 150 minutes per week of moderate to intense physical activity on the Physical Activity Readiness Questionnaire (PAR-Q) prior to enrollment and were overweight (waist circumference greater than 80 cm) (15). All participants were instructed to maintain exercise and nutritional habits throughout the study, but were asked to refrain from exercise and the consumption of caffeine and alcohol for a minimum of 24hrs prior to each visit. The week within each participant’s typical 28 day menstrual cycle was recorded at each visit, and no significant differences were found between conditions and the timing of menstrual cycles. Ethical approval for this study was granted by the Health Sciences Human Research Ethics board at Queen’s University and all
participants provided written informed consent prior to participation in the study (Appendix A and B).

Table 1. Summary of participant characteristics. Values are means (±SD)

<table>
<thead>
<tr>
<th>Participants</th>
<th>Female (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>22 (3.9)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65 (.10)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.9 (12.4)</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>102.0 (9.6)</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>30.6 (6.0)</td>
</tr>
<tr>
<td>VO₂ peak (mL·kg⁻¹·min⁻¹)</td>
<td>30.3 (5.4)</td>
</tr>
<tr>
<td>PAR-Q (minutes of moderate/vigorous activity)</td>
<td>84 (52)</td>
</tr>
</tbody>
</table>

Note: kg, kilograms; m, meters; BMI, body mass index; VO₂peak (mL·kg⁻¹·min⁻¹), peak oxygen uptake per kilogram body weight.

3.2.2 Experimental Design

The experimental design consisted of (i) baseline testing and (ii) a control and exercise intervention day where the impact of acute exercise on substrate utilization and adipocyte protein phosphorylation were assessed. The order of intervention was a randomized cross over design, with each condition separated by a minimum 14 day recovery period.

3.2.3 Baseline Testing

Participants reported to the laboratory for the first visit a minimum of 72 hours prior to any intervention visit. During their first visit, anthropometric measures were obtained and participants were instructed to complete a PAR-Q. Additionally, participants completed an incremental ramp exercise test to the participants’ limit of tolerance which was performed on a treadmill (Sport Art Fitness 6300) to determine peak O₂ uptake (VO₂peak). The VO₂peak ramp
protocol consisted of a 3 minute warm-up at a speed of 2mph and an incline of 2, followed by a step increase in speed to 4.5mph for 2 minutes and subsequent increases in incline at a rate of 1 every 2 minutes to volitional exhaustion (determined by the participant and their ability to maintain a safe position on the treadmill). Gas exchange measures were collected continuously using a metabolic cart (Moxus AEI Technologies, Pittsburgh, PA). Relative VO₂peak and peak heart rate were calculated as the average of their respective values measured in the final 30 sec of the protocol.

3.2.4 Intervention Protocol

Participants reported to the laboratory at the same time of day on two subsequent occasions to participate in a control intervention and an acute exercise intervention in randomized order. On the evening prior to each intervention visit, participants were provided a standardized dinner which included a Stouffer Sauté Sensation Mediterranean chicken dinner (260 calories; 37 g carbohydrate (CHO), 5 g fat, 16 g protein), and 500 mL of 1% chocolate milk (340 calories; 56 g CHO, 5 g fat, 18 g protein) before reporting to the lab after an overnight fast (~12 hours). Participants were then given a standardized breakfast which included a whole wheat bagel (240 calories; 45 g CHO, 2.5 g fat, 9 g protein), 2 tbsp peanut butter (180 calories; 8 g CHO, 16 g fat, 6 g protein) and 200ml apple juice (90 calories; 26 g CHO, 0 g fat, 0 g protein). After having rested for 15 minutes, resting gas exchange measures were collected (Moxus AEI Technologies, Pittsburgh, PA) for 15 minutes while participants rested in seated position. Immediately following resting gas exchange, a baseline adipose biopsy (Pre) was obtained (for details please see Physiological Measurements). Following the first biopsy, the participants completed a 65 minute acute exercise bout on a treadmill or a time matched bed rest control. The acute exercise protocol consisted of a 5 minute warm-up at a speed of 2 mph and incline of 2 which was followed by 10
intervals of 4 minutes at 90% heart rate max with 2 minutes at a speed of 2 mph and an incline of 2 between intervals (See Figure 2 for acute exercise protocol). Immediately following the acute exercise bout or time-matched bed rest a second adipose biopsy (0hr) was obtained. Additional adipose biopsies were taken 2 hours (2hr) and 4 hours (4hr) post-exercise or bed rest with 15 minutes of resting gas exchange collected beforehand as described above (See Figure 7 for experimental visit timing). Intervention visits were spaced a minimum of 14 days apart to allow for proper healing of adipose tissue and incision sites.

Figure 6. Acute exercise protocol.

Figure 7. Intervention visit timing.
3.2.5 Physiological Measurements

Adipose tissue biopsies were obtained using a Bergstrom needle with the addition of manual suction following local anaesthetization (2% lidocaine). Biopsies were obtained from the abdominal region with the incision made approximately 5 cm lateral to the umbilicus and the needle inserted laterally through this incision. All biopsies were taken from 2 incision points with the first two biopsies taken from the right side lateral to the umbilicus and the last two taken from the left side lateral to the umbilicus (see Figure 8). In the subsequent visit, the same two incision locations were used to reduce scarring while the starting side of the biopsies was switched to minimize the influence of adipocyte location. Adipose tissue biopsies were immediately blotted, snap-frozen in liquid nitrogen, and stored at -80°C for further analysis.

Figure 8. Location of incisions and direction of biopsy needle for adipose tissue biopsies.
3.2.6 Substrate Utilization

Respiratory exchange ratio (RER) was determined from recorded gas exchange values at fasted and post-absorptive time points. A 10 min average was taken from 2.5 to 12.5 min of recorded gas exchange values to minimize the impact of any adjustment period following putting on the mask and/or participant anticipation of the mask being removed. RER and VO₂ were used to estimate relative substrate contribution to total substrate oxidation assuming a linear relationship between an RER of 0.7 (100% fat, 0% CHO), and 1.0 (0% fat, 100% CHO) at rest. Specifically, the fat derived component for energy expenditure at each time point was calculated according to the following formula (9):

\[ \text{fat (g·min}^{-1} = 1.695 \cdot \text{O}_2 \text{production (l·min}^{-1} \) - 1.701 \cdot \text{CO}_2 \text{production (l·min}^{-1} \) \].

To convert fatty acid oxidation rates to kcal·min\(^{-1}\) resulting values were multiplied by 9 kcal·g\(^{-1}\).

3.2.7 Western Blot Analysis

Frozen adipose tissue (~80-100mg) was homogenized in 300 μL of pre-chilled (4°C) lysis buffer (210 mM sucrose, 2 mM EGTA, 40 mM NaCl, 30 mM Hepes, 20 mM EDTA). Samples were homogenized for 3-5 seconds at 15 000RPM (Polytron PT10/35 GT Benchtop Homogenizer, Kinematic, Luzern, Switzerland).

Protein concentrations were determined for all homogenates using a commercially available protein assay kit (Pierce, Rockford, IL). Samples were diluted to equivalent concentrations with a mixture of 4x Laemmli sample buffer and H₂O and then denatured by heating to 95°C for 5 min. Proteins were separated by SDS-PAGE using 10% (phospho-AMPK, phospho-AS160, phospho-PKA, GAPDH), and 15% (phospho-p38 MAPK, phospho-Akt Ser473) polyacrylamide gels and were subsequently transferred to a polyvinylidene difluoride membrane. For the detection of proteins, commercially available antibodies were used for GAPDH
(Millipore, Billerica, MA) and phospho-p38 MAPK Thr180/Tyr182, phospho-AMPK Thr172, phospho-Akt Ser473, and phosphor-PKA substrate (Cell Signaling Technologies, Danvers, MA). Membranes were blocked with 5% BSA in TBS-T (0.1%) and immunoblotted with primary antibody. Proteins were visualized by chemiluminescence detection according to the manufacturer’s instructions (Millipore, Billerica, MA). Blots were imaged using the FluorChem Cell Biosciences imaging system (ProteinSimple, Santa Clara, CA) and quantified using AlphaView technology (ProteinSimple, Santa Clara, CA). Equal protein loading for all western blots were confirmed using GAPDH. No significant time or interaction effects were detected from GAPDH protein content analysis.

3.2.8 Statistical Analysis

A two-way, repeated measures analysis of variance (ANOVA) was used to compare the effect of intervention (control and exercise) and time (pre, 0hrs, 2hrs, 4hrs) for adipose biopsy data. A Bonferroni correction was used for post hoc pairwise comparison of means for main effects and significant interactions. Data analysis was completed with GraphPad Prism v 5.01 (GraphPad Software Inc., La Jolla, CA). All values are represented as mean ± standard deviation and statistical significance was accepted at p < 0.05.

3.3 Results

3.3.1 Fatty Acid Oxidation

RER showed a significant interaction effect (p=0.02). Post hoc analysis revealed that RER values were significantly lower (p<0.05) 2 hours post exercise (0.78 ± 0.02) compared to control (0.84 ± 0.03) (Figure 10A). In addition, a significant main effect of time was observed for whole body fat oxidation (p<0.01). Post hoc analysis revealed a significant increase (p<0.05)
in whole body fat oxidation 2hs post exercise (0.955 ± 0.240 kcal/min) compared to control (0.106 ± 0.027 kcal/min) (Figure 10B).

### 3.3.2 Phosphorylation of Insulin Signalling Proteins

A significant main effect of condition was observed for phosphorylation of Akt (p=0.01). Post hoc analysis demonstrates a significant decrease (p < 0.05) in phosphorylation of Akt 2hr post exercise (5719 ± 2369 A.U.) compared to control (8117 ± 4188 A.U.) (Figure 11A), but that there was no significant interaction effect (p=0.21). There were no significant effects of time (p=0.09) on phosphorylation of Akt. In addition, there was no significant effect of condition (p=0.07) or interaction effect (p=0.79) observed for the phosphorylation of AS160. There was a significant main effect of time (p=0.01) on the phosphorylation of AS160 (Figure 11B). Lastly there was a no observed interaction effect (p=0.95) but a significant main effect of time on the phosphorylation status of AMPK (p=0.003).

### 3.3.3 Phosphorylation of β-receptor stimulated Signalling Proteins

There was no significant effect of condition (p=0.65) or interaction effect observed (p=0.25) for phosphorylation of p38 MAPK. There was a significant main effect of time (p=0.03) on phosphorylation of p38 MAPK (Figure 12). There were no significant effects of condition (p=0.87) or time (p=0.11) on the phosphorylation of PKA substrate. However, when broken down into 5 bands by molecular weight, there was a significant effect of time on the phosphorylation of band 1 (p=0.02) (Figure 13B).
Figure 9. RER and whole body fat oxidation are altered in response to acute exercise.

Resting respiratory exchange ratio (RER) (A) and fatty acid oxidation rate (B) were measured before intervention (Pre), 2 hours post intervention (2hr) and 4 hours post intervention (4hr). ‡Significantly different (p<0.05) from control condition.
Figure 10. Representative blots for all protein both phosphorylated and total.

The representative blots for (A) phosphorylated protein and protein content of Akt, AS160, AMPK, p-38 MAPK and (B) phosphorylated PKA substrate before intervention (Pre), immediately after intervention (0hrs), 2 hours post intervention (2hr) and 4 hours post intervention (4hr).
Figure 11. Acute exercise alters phosphorylation of Akt but does not alter other antilipolytic signalling proteins.

The change in phosphorylated protein content of Akt (A), AS160 (B), and AMPK (C), before intervention (Pre), immediately after intervention (0hrs), 2 hours post intervention (2hr) and 4 hours post intervention (4hr).

* Significant main effect (p<0.05) of time
† Significant main effect of (p<0.05) condition
‡ Significantly different (p<0.05) from control condition.
Figure 12. Acute exercise has no effect on the phosphorylation status of p38 MAPK.

The change in phosphorylated protein content of p38 MAPK before intervention (Pre), immediately after intervention (0hrs), 2 hours post intervention (2hr) and 4 hours post intervention (4hr).

* Significant main effect (p<0.05) of time
Figure 13. Phosphorylation of PKA substrate during control and exercise conditions.

The change in phosphorylated protein content including all bands (A), and as individual bands (B), (C), (D), (E), (F), before intervention (Pre), immediately after intervention (0hrs), 2 hours post intervention (2hr) and 4 hours post intervention (4hr).

* Significant main effect (p<0.05) of time
3.4 Discussion

The current study was conducted to evaluate the effect of acute exercise on changes in whole body fat oxidation and intracellular pathways involved in insulin signalling and regulation of glyceroneogenic gene expression in adipose tissue of overweight/obese females. Our results indicated that acute exercise: 1) increases whole body fatty acid oxidation 2 hours post exercise but does not alter PKA substrate phosphorylation immediately after exercise; 2) decreases the activation of Akt (an insulin signalling protein) 2 hours post exercise; and 3) does not appear to impact the activation of proteins (PKA, p38MAPK) associated with the regulation of glyceroneogenic gene expression, but rather suggests that time after consumption of a caloric load may be a primary stimulus for altered glyceroneogenic function.

3.4.1 Whole Body Fat Oxidation

In agreement with our hypothesis, acute exercise significantly increased whole body fat oxidation 2 hours post exercise. Our findings are in agreement with previous findings that acute exercise is capable of stimulating prolonged increases in whole body fat oxidation post-exercise (2, 8, 23, 26). Previous reports have identified that post-exercise, plasma NEFA and glycerol levels remain elevated for 2-3 hours in parallel with elevated fatty acid oxidation (2, 8). The availability of NEFA and glycerol have been suggested to determine substrate utilization in tissues such as muscle in order to spare glucose for other processes such as glycogenolysis (12, 24). Furthermore, research observing changes in skeletal muscle metabolism post exercise suggests that the substrate malonyl-CoA, a potent inhibitor of fatty acid transport, is depressed post-exercise for prolonged periods of up to 2 hours (23). These findings suggest that in combination with greater plasma concentrations of fatty acids, that fatty acid flux into muscle
mitochondria for oxidation may be increased as well, allowing for greater fatty acid oxidation and diversion of glucose for the glycogen synthesis pathway (2, 23).

Our demonstration of elevated fatty acid oxidation 2hr post exercise also support an earlier study (8), which found that women show elevated NEFA mobilization and subsequent oxidation following an acute bout of exercise. Our findings suggest that the intensity and duration of exercise used in this study is capable of prolonged increases in fat oxidation post-exercise which may be substrate derived from adipose tissue fatty acid release.

3.4.2 Acute Exercise and Insulin Signalling

A novel finding of this study is that acute exercise is associated with transient decreases in the phosphorylation status of Akt 2 hours post exercise (Figure 11). Akt is an important intermediate in the insulin signalling cascade (14), and regulates the storage of lipids in adipose tissue (22). Additionally, Akt is an upstream regulator of PDE-3B, which reduces cAMP concentration and decreases its availability to stimulate lipolysis (13, 22). While the exact mechanism explaining the reduced Akt phosphorylation is unclear, previous studies have observed lower plasma insulin post endurance exercise (8), a response that would be expected to be accompanied by reduced insulin signaling (and decreased Akt phosphorylation). Alternatively, it is possible that the observed decrease in phosphorylation of Akt may result from altered signaling, intrinsic to the adipocyte, which causes a transient phase of adipose tissue insulin insensitivity. Although the promotion of adipose tissue insulin insensitivity by exercise may seem contradictory, as excess basal plasma NEFA are associated with metabolic syndrome (1), transient periods of insulin insensitivity would support lipolysis and may help in reducing the size and/or number of adipocytes. In the context of obesity, where adipocyte hypertrophy is associated with metabolic dysfunction (7) and impaired adipose tissue endocrine and energetic
function (28), an acute increase in lipolysis following exercise, combined with increased fat oxidation in tissues such as muscle (8) may contribute to the improved adipose tissue health observed following exercise.

Regardless of the underlying mechanism(s), this is the first study to demonstrate an effect of high-intensity exercise on the phosphorylation of Akt in humans. Our findings suggest that transient decreases in the phosphorylation of Akt reduce the ability of adipose tissue to store lipids during this acute phase. If this is true, then plasma NEFAs would be expected to increase resulting in an elevated availability, and subsequent utilization of fatty acids for fuel post-exercise in tissues such as muscle, sparing glucose for other processes such as glycogenolysis (12, 24). Thus, our results suggest a novel mechanism linking acute alterations of subcutaneous adipose tissue function, either through altered plasma insulin or altered adipocyte insulin sensitivity, to changes in whole body fatty acid oxidation typically observed after high-intensity exercise. These results, obtained in human adipose tissue, broaden our understanding of mechanisms involved in healthy adipocyte function.

While the phosphorylation status of AS160 was not statistically significant between conditions, there was a trend for lower phosphorylation following exercise (Figure 11B; p=0.07). AS160 is a downstream target of the insulin signalling pathway and its phosphorylation triggers the translocation of glucose transporters to the cell membrane (16). These observations suggest that decreased phosphorylation of AS160 may reduce the amount of glucose transporters that are translocated to the plasma membrane (16), and by consequence, reduce the ability of glucose to be transported into the cell. Similar to the changes observed with the phosphorylation status of Akt, this would also push the balance of adipocyte function away from storage of CHO and lipid towards elevated lipolytic activity. A hypothesis for this interaction has been proposed, which
suggests there are two separate types of adipocytes: 1) adipocytes which do not store TGs (known as preadipocytes) but instead are glycolytic and release lactate into the interstitium, and 2) adipocytes which store TGs (mature adipocytes) and use lactate as the primary substrate for glyceroneogenesis (19, 20). If this is true, then reduced glucose transport would consequently reduce the ability of substrate for glyceroneogenesis, and ultimate impede re-esterification of NEFAs, therefore increasing the amount NEFA that are available to leave the cell. In combination with the action of Akt, altered AS160 phosphorylation may contribute to transient elevated plasma NEFA concentrations and greater fat oxidation post-exercise (8). These novel results in combination provide insight into the mechanisms of function within adipose tissue in response to time and exercise, and are worthy of further investigation.

To the best of our knowledge this is the first study to measure PKA activity in human adipose tissue in response to acute exercise. PKA is known to be activated by β-AR signalling and its regulation in adipose tissue is described as two-tiered: 1) it phosphorylates lipases to initiate lipolysis (13, 22), and 2) phosphorylates p38 MAPK which may contribute to the expression of glyceroneogenic genes (4, 32). The inability of acute exercise to increase the activity of PKA, in comparison to the time-matched control condition, indicates that epinephrine mediated activation of β-AR signaling in abdominal, subcutaneous adipose tissue, is minimal during and following an acute bout of exercise. These results suggest that changes in adipose tissue lipolysis and TG synthesis/glyceroneogenesis may be impacted to a greater extent by changes in insulin/glucagon (5, 11, 20) than by epinephrine.

### 3.4.3 Acute Exercise and Glyceroneogenic Gene Signalling

Also in contrast to our hypotheses, the results from the current study demonstrate no effect of acute exercise on the phosphorylation status of p38 MAPK or PKA substrates. There
are limited studies that have examined the effect of exercise on the signalling pathways associated with glyceroneogenic gene expression (30, 31) and no study, to the best of our knowledge, has previously examined this pathway in human adipose tissue in response to acute exercise. Interestingly, in agreement with our findings a recent study found that the phosphorylation of p38 MAPK was unchanged following 90 minutes of treadmill running in epididymal adipose tissue of rodents (30). Other studies have found that epinephrine and its subsequent β-AR stimulation are capable of increasing the phosphorylation of p38 MAPK in rodent epididymal adipose tissue (29, 31). In rodents, both exercise (in the absence of p38 phosphorylation) and β-AR stimulation (in the presence of p38 MAPK phosphorylation) result in an upregulation of glyceroneogenic genes (29–31). It is currently unclear whether the acute exercise bout utilized in the current study altered glyceroneogenic gene expression independent of p38 MAPK phosphorylation, and this remains an important area of future study.

3.5 Limitations

While considerable attention was given to study design and execution, there are some limitations of the present work. First, due to the significant number and discomfort of multiple biopsies requested of our participants, it was decided that blood would not be collected; consequently, we were unable to measure plasma epinephrine, insulin, glucagon, and NEFA or glycerol concentrations. Furthermore, due to the limited amount of tissue obtained from adipose biopsies, western blot and additional analysis was restricted. Lastly, while our findings suggest implications to the pathway governing the expression of glyceroneogenic genes, we were unable to directly measure changes in genes associated with the glyceroneogenic pathway.
3.6 Summary

The purpose of this current study was to examine the effect of acute exercise on whole body fat oxidation and its subsequent effects on intracellular signalling in human subcutaneous adipose tissue. Our results suggest that acute exercise has significant effects on the phosphorylation of Akt, potentially due to lower circulating insulin levels or changes to intrinsic signalling, and may help to explain the increased whole body fat oxidation observed post exercise. Interestingly, this was not accompanied by altered PKA substrate phosphorylation suggesting that elevated NEFA availability post exercise results from decreased insulin mediated TG synthesis rather than PKA stimulated lipolysis. Acute exercise also had no effect on the phosphorylation of proteins thought to regulate glyceroneogenic gene expression in subcutaneous adipose tissue. Limitations associated with measuring glyceroneogenic genes directly requires further research to determine whether or not exercise increases glyceroneogenic genes independent of PKA and p38 MAPK phosphorylation in human adipose tissue.
3.7 References


Chapter 4

Discussion

4.1 Summary of Key Findings

Our results suggest that acute exercise can significantly increase whole body fat oxidation, but not PKA-stimulated lipolysis. Additionally, acute exercise can transiently alter insulin signalling in subcutaneous adipose tissue in humans. The decrease in insulin signalling may be attributed to decreases in plasma insulin that have been previously reported post exercise (1) or may be due to changes to the insulin sensitivity of the adipose tissue itself (David Wright, University of Guelph, Personal Communication). While speculative, the transient decrease in insulin signalling may offer a plausible mechanism which could account for the increased release of NEFA into the plasma and subsequent oxidation that have been observed post exercise (1), as perhaps re-esterification of NEFAs is reduced acutely post-exercise. Lastly, acute exercise had no effect on the phosphorylation of the p38 MAPK or PKA which are believed to be part of the pathway thought to regulate glyceroneogenic gene expression in adipose tissue (6, 7). These findings suggest that the pathway regulating glyceroneogenic gene expression in human subcutaneous adipose tissue may not respond to acute exercise.

4.2 Study Strengths

To the best of our knowledge, this the first study to investigate the acute effects of exercise, over a time course of 4+ hours, on the phosphorylation of both insulin signalling proteins and proteins involved in the regulation of glyceroneogenic genes in human adipose tissue. Further, notable results from studies using pharmaceuticals to induce glyceroneogenic
genes to treat metabolic dysfunction have disregarded the acute mechanisms involved, and the potential side effects of these drugs (4). Our study has taken the initiative to investigate both alternative methods, and potential acute mechanisms that may contribute to maintaining adipose tissue health, as it is proving to be an increasingly important factor in treating obesity and metabolic syndrome. Additionally, the use of a cross-over randomized design strengthens our observations, as we were able to compare individuals to their own control, therefore better compensating for individual variability. Lastly, in an effort to control for depot specific heterogeneity between adipose tissue samples, biopsies were taken from 4 areas (Chapter 3, Figure 8) and then on the subsequent visit were taken in reverse order.

4.3 Study Limitations

The limitations to our study have been previously reported in Chapter 3. To summarize, due to the significant number of biopsies taken, and the limited amount of tissue we obtained, we decided not to collect additional blood samples, and the analysis of tissue was restricted. The ability to collect blood would have allowed us to quantify plasma epinephrine, insulin, NEFA and glycerol. The ability to quantify plasma epinephrine would have given us insight into whether or not plasma epinephrine levels in this current study achieved concentrations associated with our pilot data (See Figure 3 in Chapter 2). Plasma insulin measurement would have given us evidence of whether plasma insulin was depressed post-exercise mediating the observed changes in insulin signalling. Additionally, the ability to collect plasma NEFA and glycerol concentrations would have also given us a better indicator of whether or not plasma NEFA or intramuscular TG stores were being used for whole body fat oxidation. In addition, limited adipose tissue yields prevented us from running adipose tissue explant experiments, and further analysis with western blots. The adipose tissue explant experiments would have allowed us to establish whether adipose tissue
becomes insulin insensitive after acute exercise or whether decreased plasma insulin is the cause of the attenuated insulin signalling. Further to the low adipose yields, there was also lower protein yield compare to tissues such as muscle, which restricted our ability to perform additional western blots. Additional western blots would have potentially permitted us to look at lipolytic proteins downstream of PKA, and additional insulin signalling proteins. Lastly, due the combined yield and protein content of the adipose tissue obtained, expression of glyceroneogenic genes were unable to be observed. The ability to measure the expression of these genes would have allowed us to verify whether or not PKA and p38 MAPK stimulation is required or if there are other stimulating pathways that exist in human adipose tissue in response to exercise.

4.4 Future Research

Based on the study limitations discussed above, there are several areas that would benefit from future research. Primarily, a continued investigation of acute exercise and its effects on the mechanisms of adipose tissue insulin signalling is needed. While the current study suggests there are transient decreases in the phosphorylation of Akt post exercise, there is little insight into whether or not this is caused by decreased plasma insulin concentrations or if the tissue itself has altered insulin sensitivity. Adipose tissue explants would allow for a greater understanding of the acute mechanisms involved in insulin sensitivity post exercise in human subcutaneous adipose tissue. Furthermore, studies in rodents have suggested that exhaustive exercise promotes a pro-inflammatory response in adipose tissue which may be associated with increased lipolysis (2). Due to the influence of cytokines on adipose tissue function (see Chapter 2), it would be interesting to observe the acute mechanisms of these pro-inflammatory cytokines post exercise on insulin signalling, as they may provide additional mechanisms of action in human adipose tissue. Additionally, future investigation is warranted examining the effect of acute exercise on the
actual expression of glyceroneogenic genes, as further information is required to determine if these genes are indeed expressed by acute exercise independent of PKA and p38 MAPK phosphorylation. Furthermore, if it were possible, exploration into the acute effects of exercise on the mechanisms of function in different adipose tissue depots (visceral vs. subcutaneous or leg vs. abdominal), would give further insight about potential differences in mechanisms involved post exercise and if they are depot specific. Additionally, comparisons of acute exercise in adipose tissue from lean vs. obese individuals would give valuable information into potential differences in intracellular signalling and gene expression post exercise, as differences in gene expression in human adipose tissue have already been observed in response to a caloric load (3). Lastly, future research should also be conducted on chronic adaptations of adipose tissue function and the mechanisms involved in response to exercise training as there may be additive effects of exercise that we were unable measure acutely. These training studies may give valuable insight into the possible mechanisms involved in altering the function of adipose tissue as perhaps a single bout of exercise is not an effective method to promote chronic changes to adipose tissue function. Additionally, training studies may reveal that there are differences in the mechanisms of action involved in chronic adipose tissue function that are secondary to weight loss. Furthermore, if future training studies do unveil mechanisms involved in chronic adipose tissue functioning, it would be interesting to see how these mechanisms are affected by varying doses and intensities of exercise. These studies may provide clinically relevant information on how to treat adipose tissue dysfunction caused by obesity without the need of pharmaceutical intervention.
4.5 MSc. Research Experience

My research experience in graduate school over the last two years has provided me with several new skills and experiences. My pilot and thesis work, along with aiding labmates in the muscle biochemistry lab has provided me the opportunity to develop a wide array of laboratory techniques. I have learned how to successfully schedule and interact with participants, carry out fitness tests and exercise training on both cycle ergometers and treadmills, with additional skills in exercise training using full body exercises, use and troubleshoot metabolic carts, assist in blood draws, muscle and adipose tissue biopsies, and perform the analysis that follows these techniques. Along with my work in the muscle biochemistry lab, I have had the opportunity to work in the exercise physiology lab. This experience also aided in the development of my skills in regard to successfully interacting with participants, especially those from a broader age range in Kingston and the surrounding area, and how to acquire and analyze data obtained from training sessions and accelerometers. In addition to laboratory skills, I have also learned how to think critically, ask questions and search for answers within the available literature. Furthermore, I have had the opportunity to have daily exchanges with my lab mates, Dr. Gurd, faculty members, and SKHS staff that have contributed to my broader understanding of research, and have aided in my ability to work as a team and provide and receive constructive criticism. Lastly, I have been fortunate enough to have had the experience of working in collaboration with other labs both within the faculty of SHKS and from other educational institutes. These experiences have enhanced my ability to view research questions from many angles and have given me a new perspective on the value of collaborative research.
4.6 Conclusions

An acute bout of exercise can increase whole body fat oxidation and transiently decrease insulin signalling in adipose tissue, but has no effect on the phosphorylation of proteins involved in regulating glyceroneogenic gene expression in subcutaneous adipose tissue of human. The lack of effect on the phosphorylation of proteins regulating glyceroneogenic genes, suggests that exercise does not acutely increase expression of glyceroneogenic genes in human subcutaneous adipose tissue.
4.7 References


Appendix A
Research Ethics Boards - Letter of Informed Consent

Consent to Volunteer for Participation in a Study

TITLE: Changes in adipose tissue glyceroneogenic gene expression after acute exercise in women.

PRINCIPAL INVESTIGATOR: Brendon J. Gurd, PhD
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You are being invited to participate in a study examining the influence of different exercise protocols that vary in intensity (difficulty), duration (length) and mode (type) on adipose tissue function (the response of fat tissue to exercise). This study will compare the effects of these different exercise protocols in young women who are overweight/obese. You have been invited to participate in this study because you are a healthy adult (18-40 years) who is overweight (waist circumference >80 cm). The following brief is intended to provide you with the details you should be aware of prior to your consent as a participant in this study. Please read the following information carefully and feel free to ask any question that you may have.

BACKGROUND INFORMATION

Exercise capacity (fitness) is an important predictor of long term health. More specifically, the ability of adipose cells (fat tissue) to release fuel are all impaired with obesity and are predictors of both weight gain, the development of type II diabetes and cardiovascular disease. In healthy active populations interval training (repeated bouts of exercise separated by periods of recovery) is a potent, time effective stimuli for increasing exercise capacity. This study will examine the ability of different exercise protocols to activate processes that lead to increases in adipose tissue function.

You will not be able to participate in the study if you have been diagnosed previously with any respiratory, cardiovascular (eg. High blood pressure,
EXPLANATION OF PROCEDURES

Participation
Participation in the study is voluntary. You may refuse to participate or withdraw from the study at any time with no effect on your academic or employment status.

Should you choose to participate you will take part in experimental procedures outlined below. These include 4 visits to the lab for familiarization (approximately 1.5 hour visit) and 3 experimental days (each experimental visit will take approximately 6 hours). On one experimental visit you will be asked to lie in a bed for your entire visit, while the other two experimental visits will involve performing either moderate, or high intensity exercise followed by a period of bed rest. All of these visits will occur within approximately 1 month of your enrolment in this study. The investigator will explain to you, in detail how each of these procedures will be conducted in the study in which you have agreed to participate.

Exercise tests:
During each of the exercise tests you will be required to wear a nose-clip (to prevent you from breathing through your nose) and a rubber mouthpiece (similar to breathing through a snorkel or diving mask). This will enable us to measure the volume of air that you breathe in and out, and measure the gas concentration in that air. You may experience some initial discomfort from wearing the nose-clip and mouthpiece. You will also be required to wear a heart rate monitor around your chest during all tests. You will be

heart conditions), metabolic (eg. Diabetes), neurological or musculoskeletal disease; or you are currently on medication; or you are a smoker; or you respond to the exercise protocol in an irregular manner (i.e. chest pains, dizziness, shortness of breath, excessive awareness of breathing)
asked to perform each of these tests on one occasion before and once occasion following exercise training.

**Incremental exercise test:** This test is performed on either a cycle ergometer (a stationary bike) or a treadmill and is designed to measure your fitness level. During this test the intensity of exercise increases gradually until you are physically unable to continue exercising because the intensity is either too high or too uncomfortable. The test will begin with the exercise intensity being very light and easy. After a few minutes the exercise intensity will gradually and continuously increase until you are unable to continue because of fatigue, or until you wish to stop.

**Exercise Training Protocols:**
Any exercise carries a slight risk of heart attack or may be uncomfortable if you are unfit or not used to exercise. The risk of a cardiac event (heart attack, dysrhythmias etc.) in a mixed subject population (healthy low risk and unhealthy high risk patients together) is approximately 6:10 000, however this risk decreases in a previously healthy (i.e. young, moderately active) population. There may be some minor discomfort during the exercise testing. You may experience increased awareness of breathing, muscle pain and/or fatigue, increased sweating, or a general feeling of fatigue or nausea, all of which are not unexpected consequences of exercise. You are being asked to participate in the following exercise training programs. The investigator will explain to you exactly what is involved in each specific protocol you are being asked to complete.

You will be asked to complete each of the following protocols on 2 separate occasions. On the each visit for each protocol you will have adipose tissue samples taken. These procedures are explained below.

**Low-intensity exercise training:** This protocol will involve riding a stationary bike or jogging on a treadmill for a period of up to 90 minutes at a low intensity similar to a leisurely bike ride.
**High-intensity exercise training:** This protocol involves riding a bike or running on a treadmill at a high-intensity, like an all out sprint, for 30 seconds at a time (called an interval) followed by 4.5 minutes of rest. This interval will be repeated up to 6 times.

**Physiological tests:**

**Adipose Tissue Biopsy:** On each of the 3 experimental visits you will have 4 biopsies adipose tissue (fat) biopsies taken (before, immediately after (0hrs), 2 and 4 hours after either exercise or bed rest). **A total of 12 biopsies will be taken during this study.** For each biopsy you will also be asked to have small amounts of adipose tissue removed from your abdomen by means of a needle biopsy. The biopsy procedure is detailed on the biopsy information sheet, briefly the procedure is as follows: while you are resting on a bed, an anesthetic will be applied locally to anesthetize the skin over your abdominal at the 2 sites where the biopsies will be taken (there is an extremely low risk of allergic reaction to the local injection; see biopsy information sheet). A small incision (approximately 1 cm each) will be made through your skin approximately 5 cm from the belly button. A needle will then be inserted into your fat layer and a small piece of tissue will be removed. This procedure is referred to as a biopsy.

On each experimental visit, you will have 2 incisions made (approximately 1 cm each) through your skin, approximately 5 cm on either side of your belly button. On each visit 2 biopsies will be taken through each incision for a total of 4 biopsies. A minimum of 7 days will be given between biopsy visits to allow for proper healing. The adipose biopsies will be taken either by a medical doctor or a professor trained in this technique.

During fat biopsies you may feel slight pressure and/or discomfort in your fat layer but this discomfort will pass very quickly. Following your biopsies there may be light bruising in the area where the biopsies were taken but this will generally fade within a couple of
days. There is also a slight risk of infection following a biopsy but proper sterilization of equipment and cleaning of the sampled area minimizes this risk. If the site of the fat biopsy becomes more tender and redness and/or swelling develops in the area over the next five to seven days, or if you have any concerns whatsoever you should contact the research person supervising your study immediately and seek medical attention as needed.

Please refer to the Biopsy Information Sheet for more information regarding this procedure.

RISK OF INJURY

All exercise also carries a small risk of personal injury. Should any such injury occur during your participation in this study you will be initially cared for by the study administrators, all of whom are certified in first aid. Should further assistance be required you will be taken to the university health centre/hospital or emergency as required.

POTENTIAL BENEFITS OF PARTICIPATION

You will gain no direct benefit through participation in this study.

CONFIDENTIALITY

During the course of your participation in this study you will not be required to provide any personal information beyond your name and phone number (for study purposes only). All information obtained during the course of this study, including your name and fitness results, is strictly confidential and your anonymity will be protected at all times. All samples obtained will be stored in a freezer, no personal information will be attached to your sample
(all samples will be labeled with subject ID only). Samples will be destroyed following completion of analysis. Your information will be kept in locked files and will be available only to Dr. Brendon Gurd and those working within his laboratory. Your identity will not be revealed in any description or publication.

By signing this consent form, you do not waive your legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities.

**VOLUNTARY CONSENT**

I have been given an opportunity to ask any questions concerning the procedures. All of my questions regarding the research project have been satisfactorily answered. I understand that my test results are considered confidential and will never be released in a form that is traceable to me. I do understand that I am free to deny consent if I so desire, and may withdraw from the study at any time without any effect on my academic or employment status.

Should I have any questions about the study, I know that I can contact Dr. Brendon Gurd (613 533-9023), Dr. Jean Coté, Head, School of Kinesiology and Health Studies (613 533-6601). Questions concerning your rights as a research participant may be directed to Dr. Albert Clark, Chair, Queen’s Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (613 533-6081). A copy of this consent form will be provided me for my records. My signature below means that I freely agreed to participate in this study.

__________________________________________________________________________

Volunteer’s Signature

Date
STATEMENT OF INVESTIGATOR
I, or one of my colleagues, have carefully explained to the subject the nature of the above research study. I certify that, to the best of my knowledge, the subject understands clearly the nature of the study and demands, benefits, and risks involved to participants in this study.

________________________________________  ____________________________
Principal Investigator’s Signature                  Date
Appendix B
Adipose Tissue Biopsy Sheet

Biopsy Information Sheet

You have volunteered to take part in a research study that requires you to undergo 12 fat biopsies. These are commonly performed procedures in research studies and for the medical diagnosis of disease. The procedure will be performed by a medical doctor trained to perform muscle biopsies or a specially trained researcher directly supervised by a medical doctor.

The fat biopsies involve the removal of a small piece of tissue (fat) from your abdomen using a sterile hollow needle. The area over one of your abdominal muscles (5 cm lateral to the umbilicus) will be carefully cleaned. A small amount of local freezing (anesthetic) will be injected into and under the skin. You will likely experience a burning sensation while the freezing is injected. Then a small, 4 – 5 mm incision will be made in your skin in order to create an opening for the biopsy needle. There is often a small amount of bleeding from the incision, but this is usually minimal.

For each fat biopsy, the biopsy needle will then be inserted through the incision into the layer of fat between your skin and your muscle (100 – 200 mg), about the size of a pencil eraser, will be quickly removed and the needle taken out. During the time that the sample is being taken (about 5 seconds), you may feel the sensation of pressure in your abdomen and on some occasions this is moderately painful. However, the discomfort very quickly passes and you are quite capable of performing exercise and daily activities. There may be some minimal bleeding when the needle is removed which may require application of pressure for a few minutes.
Following biopsies, the incision will be closed with sterile tape (steri-strips), and wrapped with a tensor bandage. You should refrain from excessive muscle use for the remainder of the day. Once the freezing wears off, your abdomen may feel tight and often there is the sensation of a deep bruise. Pain killers such as Acetaminophen (e.g. Tylenol) or Ibuprofen (e.g. Advil) are acceptable if you experience pain associated with the biopsy. It is also beneficial to periodically apply an ice pack to the biopsy site the following day, as this will help to reduce any swelling and any residual soreness. The following day your abdomen may feel uncomfortable. The tightness in the abdomen usually disappears within 2 days and subjects routinely begin exercising at normal capacity within 2 days. In order to allow the incisions to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 4 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided for at least 4 days following the biopsy procedure.

Potential Risks

- The local freezing will likely result in a burning feeling in the abdomen at the time of the injection. This will last only 5 – 10 seconds. There is an extremely low risk of allergic reaction to the local injection (1 in 1 million).

- The chance of a local skin infection in less than 1 in 1000. Carefully cleaning the skin and keeping the area clean and dry until the skin heals will minimize this.

- Most subjects experience local soreness in the abdomen for two or three days after the fat biopsy similar to a deep bruise. There is a very low risk of internal bleeding at the biopsy site which can result in more prolonged pain and stiffness in the leg.

- On occasion, a small lump of scar tissue may form under the site of the incision, but this normally disappears within 2-3 months, or within a few weeks if massaged. A small visible scar often remains from the biopsy incision.
There is the possibility of a small area of numbness (about the size of a one dollar coin) around either biopsy site. This usually resolves over 5 – 6 months. There is a very low risk (estimated at less than 1/5000) of damage to a small nerve branch to the muscle. This would result in partial weakness of abdominal muscle and would likely have no impact on day-to-day activities. Nerve injuries like this usually resolve in 8 – 12 months, but there is a theoretical risk of mild leg weakness.

**Concerns or Problems**

Infection can be serious, if you experience excessive redness, swelling or infection around the biopsy site or pain or stiffness in your abdominals you must contact Dr. Simpson right away. Dr. Simpson will be available 24 hours a day to answer any of your concerns or questions about the biopsy.

**Dr. Craig Simpson:** Cell Phone (XXX) XXX-XXXX

However, if for some reason, you are not able to contact Dr. Simpson then you should contact your family doctor or go to the Emergency Department.

Please keep this Information Sheet until such time as your biopsy site has fully healed.
FAT BIOPSY SUBJECT SCREENING FORM

To help us ensure your safety and wellbeing please answer the following questions.

1. Have you ever had a negative or allergic reaction to local freezing (e.g. during dental procedures)?
   
   No □  Yes □

2. Do you have any tendency toward easy bleeding or bruising (e.g. with minor cuts or shaving)?
   
   No □  Yes □

3. Are you currently taking any medications that may increase the chance of bleeding or bruising (e.g. Aspirin, Coumadin, Anti-inflammatories, Plavix)?
   
   No □  Yes □

4. Have you ever fainted or do you have a tendency to faint when undergoing or watching medical procedures?
   
   No □  Yes □

5. Will you contact Dr. Craig Simpson directly if you have any concerns about the biopsy site including: excessive redness, swelling, infection, pain or stiffness of the leg?
   
   No □  Yes □
Subject Name (print) :_____________________________________
Subject Signature :________________________________________
Date :_________________
Signature of Person Conducting Assessment: ___________________
Appendix C
Research Ethics Board Approval

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD

March 04, 2013

Dr. Brendon Gurd
School of Kinesiology & Health Studies
Queen’s University

Dear Dr. Gurd,

Study Title: Changes in adipose tissue glyceroneogenic gene expression after acute exercise in women.
Co-Investigators: Dr. R. Ross, Ms. M. Smith

Full Board Meeting Date: December 12, 2012

The members of the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board have examined the revised protocol, signed directive, revised fat biopsy information sheet, revised letter of information/consent form for your project (as stated above) and consider it to be ethically acceptable. This approval is valid for one year from the date of this letter. Please attend carefully to the following list of ethics requirements you must fulfill over the course of your study:

Reporting of Amendments: If there are any changes to your study (e.g. consent, protocol, study procedures, etc.), you must submit an amendment to the Research Ethics Board for approval.

Reporting of Serious Adverse Events: Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other serious adverse events must be reported within 15 days after becoming aware of the information.
**Reporting of Complaints:** Any complaints made by participants or persons acting on behalf of participants must be reported to the Research Ethics Board within 7 days of becoming aware of the complaint. Note: All documents supplied to participants must have the contact information for the Research Ethics Board.

**Annual Renewal:** Prior to the expiration of your approval (which is one year from the date of the Chair's signature below), you will be reminded to submit your renewal form along with any new changes or amendments you wish to make to your study. If there have been no major changes to your protocol, your approval may be renewed for another year.

Yours sincerely,

Chair, Research Ethics Board

**Study Code:** PHE-130-12Romeo #6007600

Investigators please note that if your trial is registered by the sponsor, you must take responsibility to ensure that the registration information is accurate and complete. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards and operates in compliance with the Tri-Council Policy Statement; Part C Division 5 of the Food and Drug Regulations, OHRP, and U.S DHHS Code of Federal Regulations Title 45, Part 46 and carries out its functions in a manner consistent with Good Clinical Practices.

Federalwide Assurance Number: #FWA00004184, #IRB00001173

**Current 2012 membership of the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board:**

- Dr. A.F. Clark, Emeritus Professor, Department of Biomedical and Molecular Sciences, Queen's University (Chair)
- Dr. H. Abdollah, Professor, Department of Medicine, Queen's University
- Dr. R. Brison, Professor, Department of Emergency Medicine, Queen's University
- Dr. C. Cline, Assistant Professor, Department of Medicine, Director, Office of Bioethics, Queen's University, Clinical Ethicist, Kingston General Hospital
- Dr. M. Evans, Community Member
- Ms. J. Hudacin, Community Member
- Dr. J. MacKenzie, Pediatric Geneticist, Department of Paediatrics, Queen's University
Mr. D. McNaughton, Community Member

Ms. P. Newman, Pharmacist, Clinical Care Specialist and Clinical Lead, Quality and Safety, Pharmacy Services, Kingston General Hospital

Ms. S. Rohland, Privacy Officer, ICES-Queen's Health Services Research Facility, Research Associate, Division of Cancer Care and Epidemiology, Queen's Cancer Research Institute

Dr. B. Simchison, Assistant Professor, Department of Anesthesiology and Perioperative Medicine, Queen's University

Dr. A. Singh, Professor, Department of Psychiatry, Queen's University

Dr. J. Tang, Medical Resident, Department of Emergency Medicine, Queen's University
Ms. W. Weisbaum, LL.B. and Adjunct Instructor, Department of Family Medicine (Bioethics)