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The effects of chronic and acute exercise modalities on substrate utilization and plasma adiponectin concentration

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THE EFFECTS OF CHRONIC AND ACUTE EXERCISE MODALITIES ON SUBSTRATE UTILIZATION AND PLASMA ADIPONECTIN CONCENTRATION

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Impaired lipid metabolism at rest has been linked to the development of insulin resistance and an attenuation of plasma concentration of the hormone adiponectin. However, it has been suggested that increasing fatty acid oxidation at rest, via exercise or otherwise, may increase plasma adiponectin. The current literature addressing changes substrate utilization and possible direct effects on adiponectin during and following exercise is not well established. Therefore, the purpose of this dissertation was to examine concomitant changes in adiponectin and substrate utilization both at rest and during various exercise modalities, as this may provide implications for improving metabolic responses and in turn, affect insulin sensitivity. Pre-, mid-, and post-exercise measurements were obtained for five separate studies. Each study incorporated a single, unique exercise modality, which included: acute aerobic, chronic aerobic, acute resistance, chronic resistance, or acute stretching exercise. Chronic resistance training significantly increased adiponectin levels, although resting substrate utilization did not significantly change pre- to post-exercise. All acute exercise modalities significantly increased glucose utilization during exercise. However, none of the acute exercise modalities nor the chronic aerobic exercise modality elicited significant changes in adiponectin. First degree family history of type 2 diabetes mellitus had a significant inverse correlation with adiponectin concentration, whereas regular aerobic exercise had a significant direct correlation with adiponectin. Body fat percentage and body mass index did not have significant inverse correlations with adiponectin. These results suggest that substrate utilization does not directly affect adiponectin concentration and that the regulation of this hormone is likely dependent on other factors. The results do, however, highlight the importance of implementing a chronic exercise training program to limit the development of insulin resistance, especially in populations genetically predisposed to type 2 diabetes mellitus.

CHAPTER 1. INTRODUCTION

1.1 Overview

Regardless of the type, intensity, or duration of exercise, chronic has irrefutably been shown to help to prevent, or at least limit several health disparities including, but not limited to cardiovascular disease, cancer, obesity, and diabetes (1). The underlying biological mechanisms responsible for the improvements in health parameters as a result of exercise are not fully understood. Some of the possible metabolic mechanisms associated with lipid-induced insulin resistance and the reversal of insulin resistance following exercise are discussed in depth in Appendix 1. Determining the role of specific mechanisms associated with improvements in insulin sensitivity, fitness, and overall health is critical to strengthen the evidence for implementing specific exercise prescriptions among healthy and diseased populations.

Acute exercise, defined as a single bout of exercise, can elicit changes in the molecular biology, biochemistry, and physiology of the human body. Acute exercise studies have demonstrated changes in the expression of several genes (2, 3), the expression of lipid and glucose metabolites (4), increased expression of some inflammatory markers, (5) and decreased the expression of other inflammatory markers (6). Acute exercise also causes a variety of vascular, neural, metabolic, and endocrine responses (7).

Chronic exercise, defined as consistently repeated acute exercise bouts over a given period of time, yields adaptations which are much different to those seen following an acute exercise bout. The adaptations which occur at the molecular, biochemical, and physiological level following chronic exercise are primarily due to the cumulative effects of repeated acute exercise responses (7), and therefore, distinctions must be made between adaptations to chronic exercise and responses to acute exercise. While each response and adaptation to exercise is

important and evidently interdependent, the focus of this dissertation is centered on the physiological aspects, specifically the metabolic and endocrine responses and adaptations to various exercise modalities.

Metabolic activity in the human body is highly regulated and dependent on several feed-forward and feedback mechanisms which are activated by numerous external and internal environmental cues. The external cue of physical inactivity can lead to impaired substrate (lipid, carbohydrate, or amino acid) metabolism and negatively affect exercise performance as well as energy homeostasis and insulin sensitivity (8-10). Much like exercise, acute and chronic inactivity results in numerous responses and adaptations via changes in molecular biology, biochemistry, and physiology. The external cue of exercise, on the other hand, has been shown to improve energy homeostasis and reverse several metabolic impairments in glucose metabolism (11-13) and lipid metabolism (14-16) as seen in insulin resistant (17) and diabetic patients (18, 19).

Changes in substrate metabolism have been demonstrated during and following acute aerobic exercise studies (16, 20-22), chronic aerobic exercise studies (23, 24), acute resistance exercise studies (25, 26), chronic resistance exercise studies (12, 13, 27), and combined exercise studies (28, 29). Some studies have shown increases in glucose oxidation (30), while others have shown decreases in glucose oxidation (31). Some studies have shown increases in fat oxidation (32), some have shown no increases in fat oxidation (33), and others have shown increases in both glucose and fat oxidation (4). These equivocal results have made it difficult to identify the exact underlying mechanisms responsible for the improvements in insulin sensitivity observed following exercise.

2

Similarly, the variability in the adipose tissue-derived hormone, adiponectin, responses have been highly variable following exercise. Adiponectin has been shown to play an important role in insulin sensitivity as well as glucose and lipid metabolism (2), thus changes in adiponectin should theoretically be seen as a result of changes in substrate utilization. Currently however, there are few studies that have examined the role of substrate utilization on plasma adiponectin during and following exercise. Several studies have examined changes in glucose kinetics (12, 30) and free fatty acid (FFA) oxidation (14, 34) following exercise; though, none have measured these changes concomitantly with changes in plasma adiponectin nor compared the differences in plasma adiponectin concentration following aerobic versus resistance training exercise. The effects of any type of exercise on the expression of adiponectin have shown variable results. Some suggest exercise yields increased adiponectin levels following acute (35) or chronic training (36), while other studies suggest no change following acute (37) or chronic training (38).

Due to the variability in methodology and analysis between these studies, it has been difficult to establish which variables play the most significant roles in altering substrate utilization and improving insulin sensitivity. Not only can the measurement technique (gas analysis, tracers, biopsy) and experimental model (cell cultures, animal models, humans) affect the results of a study, but gender, training status, diet, muscle fiber type, muscle glycogen and lipid content, exercise intensity, exercise duration, exercise type, all affect substrate metabolism differently during and following exercise, making it difficult to compare the effects between studies. Exercise intensity and intramuscular glycogen and fat stores have been studied extensively and have been shown to greatly affect substrate utilization and exercise performance (4); however, further research is needed to determine the role of various exercise modalities on

substrate utilization. It is currently unknown if a specific form of exercise, or exercise modality, generates superior changes compared to other modalities. Therefore the focus of this dissertation was on exercise modality, which included acute aerobic, acute resistance, chronic aerobic, chronic resistance, and acute stretching exercise.

Purpose:

The purpose of this dissertation was to:

1.)   Examine changes in substrate metabolism during and following various exercise training modalities to determine how specific exercise modalities affect fuel utilization during and following exercise.

2.)   Examine changes in adiponectin levels following various exercise training modalities to determine if adiponectin is directly linked to changes in substrate metabolism and determine if altered substrate utilization (via supplementation) directly affects plasma adiponectin concentration.

3.)   Provide implications for which modality, if any, produces the greatest changes and how these changes may lead to improved exercise performance and insulin sensitivity.

1.2 Substrate Metabolism

Acute Exercise

A single bout of exercise is known to affect systemic metabolism and consequently cause a shift in energy homeostasis in an intensity-dependent fashion (30, 39). Adiponectin has been implicated in the regulation of energy homeostasis, which suggests that substrate metabolism

may be tightly linked with adiponectin concentration. Numerous factors contribute to the shift in energy homeostasis following acute exercise; however, the likely initial factor is a result of skeletal muscle contraction. Muscle contraction, whether through resistance or aerobic exercise mitigates the adenosine triphosphate / adenosine monophosphate (ATP/AMP) ratio, which in turn activates 5' AMP activated protein kinase (AMPK) (40). The extent of AMPK activation appears to be directly related to exercise intensity (40, 41). Higher exercise intensity yields a lower ATP/AMP ratio, greater AMPK activation, and thus, greater substrate oxidation. Numerous studies have suggested AMPK phosphorylates and inhibits acetyl-CoA carboxylase (ACC), which inhibits the production of malonyl-CoA and relieves the inhibition of carnitine palmitoyltransferase 1 (CPT1) to transport long-chain fatty acids into the mitochondria to allow increased FA oxidation (40). AMPK also increases skeletal muscle glucose uptake, increases citrate levels, and increases levels of nicotinamide adenine dinucleotide ($NAD^{+)}$ and Sirtuin-1 (SIRT-1), both of which increase peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) expression. Some studies have suggested that SIRT-1 may regulate the expression of adiponectin (42) via NAD^{+} (43) further supporting the notion that exercise-induced changes in substrate metabolism can alter plasma adiponectin. Chronically, this signaling cascade acts to induce mitochondrial biogenesis, improve glucose and lipid transport, and in turn, alter substrate utilization and protect against insulin resistance (40, 41). Thus, the intensity-dependent shift in energy balance appears to be dependent on the activation of AMPK and its subsequent signaling cascade.

Muscle contraction also induces calcium release from the sarcoplasmic reticulum, which in turn, activates calcineurin (40). Calcineurin has been shown to up-regulate the expression of insulin receptor, protein kinase B (Akt), glucose transporter type 4 (GLUT-4), and PGC-1α

expression in the glycolytic extensor digtorum longus (EDL) muscle of transgenic mice given a high fat diet (44), indicating increased substrate oxidation in skeletal muscle. In normal functioning muscle cells, calcineurin is activated by increased levels of cytoplasmic calcium, thus, muscle contraction alone does indeed improve insulin sensitivity and glucose uptake via an insulin-independent mechanism. Adiponectin appears to up-regulate the expression of several of these signaling molecules involved in acute muscle contraction and improved insulin sensitivity (45); however, the current literature examining acute muscle contraction and changes in plasma adiponectin are equivocal and will be discussed further in the next section.

Exercise is the product of continually repeated muscle contraction and as a result of this repeated contraction, the associated signaling cascade and the replenishment of ATP is amplified to meet the necessary energy demands. ATP replenishment is achieved via the creatine phosphate pathway, anaerobic glycolysis, glucose oxidation, amino acid deamination, or FFA oxidation (7). Untrained individuals participating in less than one hour of moderate- to high-intensity aerobic exercise are highly dependent on blood glucose to provide fuel for the increased energy demand (46). In fact, it has been shown that glucose oxidation may account for 30-50% of substrate metabolism during exercise in untrained populations (47). During short-duration exercise, blood glucose is replenished 3-5 fold by liver glycogenolysis, and glucose uptake is increased 20-35 fold. Exercise intensity and duration appear to have a direct linear relationship with the extent of glucose uptake as a result of the muscle contraction signaling cascade described above. A greater percentage of glucose uptake has been observed with shorter duration, higher intensity exercise (46). Thus, performance during short-duration, high-intensity aerobic exercise in untrained individuals is highly dependent on glucose oxidation (11). Not

surprisingly, this type of exercise has been shown to be effective in improving insulin sensitivity (48).

The role of fat metabolism during and following short-duration acute aerobic exercise; however, is quite complex and should not be overlooked. Coggan et al. (16) demonstrated a small, but significant percentage of fat is oxidized during 30 minutes of high-intensity aerobic exercise (75-80% $VO_{2\ max}$) in recreationally active individuals. This finding suggests that if the ability to utilize fat becomes impaired, then exercise performance may be compromised for both trained and untrained individuals, even for short-duration, high-intensity aerobic exercise. Similarly, 30 minutes of resistance training has been shown to activate lipolysis, the degree of which is dependent on the availability of intramyocellular lipids (IMCL) (4). The authors suggested that if IMCL stores are available, they will be utilized regardless of exercise intensity in order to spare muscle glycogen. More recently, Koopman et al. (26) found that IMCL content, as well as muscle glycogen content, significantly decreased following 45 minutes of high-intensity resistance training. The authors suggested that both lipid and glucose oxidation are important for exercise performance and improved insulin sensitivity but that substrate utilization is dependent upon muscle fiber type.

Thus substrate oxidation may be more dependent on availability than intensity. This notion is supported by the idea that although the body primarily oxidizes substrates during acute exercise, a single aerobic exercise session has been shown to augment IMCL synthesis, particularly when fat availability is high (22), and consequently prevents the accumulation of deleterious metabolites such as diacylglycerol (DAG) and ceramides, which are known to activate pro-inflammatory pathways associated with insulin resistance. Similar results have been seen in rats. When given the drug Thiazolidinedione, IMCL synthesis increased while

metabolite accumulation decreased and subsequent pro-inflammatory pathways were inactivated (49), suggesting that an acute bout of aerobic exercise can act to down-regulate pro-inflammatory pathways (21). Given that the pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF-α) has antagonistic effects to adiponectin, these findings further suggest that adiponectin expression is up-regulated following acute aerobic exercise, which is supported by some studies (35), but not others (37), thus further research is still necessary.

It is currently unknown if acute stretching has similar effects on lipid metabolism to those seen with resistance training. Recent studies have demonstrated that stretching exercise elicits many of the same benefits of resistance training including increased flexibility and maintained strength (50), improved exercise performance (51), and improved glucose transport (52). Acute resistance training exercise has been shown to increase lipid oxidation for up to 24 hours post-exercise, but it is unknown if stretching yields similar results. Acute resistance training has also been shown to improve insulin sensitivity via improvements in glucoregulatory function (44) and lipid metabolism (40). It is likely that the mechanisms responsible for these changes following acute resistance training and the changes following acute stretching exercise are due to different mechanisms than those seen following acute aerobic exercise since resistance training is known to activate, rather than repress, pro-inflammatory pathways (53). Thus the expression of adiponectin may not be the same with this type of training and deserves further investigation. Currently, no studies have examined changes in substrate utilization or adiponectin levels following acute stretching exercise.

Chronic Exercise

It has been clearly demonstrated that chronic aerobic exercise training leads to increases in the size and number of mitochondria, in addition to increases in mitochondrial enzymes involved in fatty acid oxidation, the citrate cycle, respiratory chain, ATP synthesis, and insulin sensitivity (54). It has been suggested that these changes are due to muscle fibers switching to more oxidative type, increasing mitochondrial density, GLUT-4 concentration, glycogen synthase activity (23) and increased reliance on more on IMCL stores for fat oxidation during exercise (16). Even independent of weight loss, exercise seems to elicit improvements in insulin sensitivity. One study involving obese girls showed that twelve weeks of aerobic exercise improved insulin sensitivity without any changes in body weight, percent body fat, or markers of inflammation (23). This further suggests that changes such as increased oxidative muscle fiber type, increased mitochondrial density, GLUT-4 concentration, and glycogen synthase activity, and decreased IMCL are more responsible for changes in insulin signaling as a result of exercise rather than changes in hormone and inflammatory signaling seen with acute aerobic exercise studies. These results also imply that adiponectin expression may not change following chronic aerobic exercise, which has been demonstrated in some studies (38), but not others (36), thus further examination is necessary.

A lack of physical activity or excess caloric intake leads to excess lipid storage in adipose tissue and within skeletal muscle. This excess lipid storage has been linked to an increased reliance on glucose oxidation (55) and increased insulin resistance as reviewed by Samuel et. al (56). Impaired lipid metabolism not only limits aerobic performance during exercise (57), but has also been linked to the development of insulin resistance (17). Impaired FFA oxidation during exercise leads to several changes in metabolism including increased reliance on other

9

substrates via muscle and liver glycogenolysis, increased blood glucose utilization, increased amino acid deamination, or increased ketogenesis, all of which hasten fatigue and negatively affect exercise performance. Conversely, improved lipid metabolism, as seen following aerobic exercise (58), leads to improved exercise performance and improved insulin sensitivity. Substrate utilization in working skeletal muscle is highly regulated by several feed-forward and feedback mechanisms, many of which are affected as a result of chronic exercise training or lack thereof. Aerobically trained individuals are able to oxidize a high percentage of fat, even during high-intensity exercise (58). This is because aerobic exercise leads to up-regulation in IMCL storage, lipolysis, lipid oxidation, improvements in exercise performance (58) and improvements in insulin sensitivity, (23).

Much like chronic aerobic exercise, chronic resistance training augments GLUT-4 protein, insulin receptor protein, and glycogen synthase protein and activity, thereby improving skeletal muscle glucose uptake and glycemic control (12). This may be partially due to changes in muscle fiber type characteristics. A study on chronically resistance trained powerlifters and recreationally active controls observed significant differences in the composition of skeletal muscle fibers, with trained lifters demonstrating a greater percentage of type II fibers, suggesting that muscle fiber type shifting as a result of chronic resistance training may contribute to changes in substrate metabolism during exercise (59). This hypothesis is supported by another study which observed elevated post-prandial carbohydrate oxidation in resistance trained males compared to sedentary controls (60). Area under the curve for both blood glucose and insulin (over time) was lower in resistance trained males compared to sedentary males, suggesting better insulin sensitivity in the resistance trained group and as previously suggested, the increase in type II fiber expression in resistance trained individuals could explain the difference in post-

prandial carbohydrate metabolism. Interestingly, resistance training has also been shown to attenuate mitochondrial content following chronic high-volume, resistance training (61), due to an augmentation in type II fiber area, suggesting a decreased ability to oxidize lipids. Therefore, it can be suggested that the mechanisms responsible for improved insulin sensitivity following chronic resistance training are significantly different than those seen following chronic aerobic training.

Chronic resistance training has also shown improvements in glycelated hemoglobin (HbA1C levels), (12) insulin sensitivity, and increased levels of adiponectin in diabetic men (62) without changes in plasma glucose, insulin, or lipid levels. This may suggest adiponectin does not play a role in changes in substrate utilization following resistance training. However, a recent study showed that augmented levels of adiponectin are associated with improvements in fat oxidation and insulin sensitivity and that plasma adiponectin concentration is up-regulated with aerobic exercise (35). Further research is needed to confirm these findings following resistance training and to determine if aerobic training is superior to resistance training in terms of adiponectin expression.

1.3 Adiponectin

Adiponectin is an adipocyte-derived hormone that plays an important role in insulin sensitivity as well as glucose and lipid metabolism (2). There are three different isoforms of adiponectin, including low molecular weight (LMW), medium molecular weight (MMW) and high molecular weight (HMW). HMW appears to be the more active form of adiponectin compared to MMW or LMW (63) and greater expression of this isoform has been implicated in greater insulin sensitivity (64). However, the HMW form has not been shown to change

following exercise in lean, healthy individuals (65) and is not associated with acute improvements in insulin-sensitivity (66) whereas total adiponectin has been shown to change following exercise in some studies in lean, healthy individuals (67). All participants for the current dissertation were lean and insulin-sensitive and therefore, total, rather than HMW adiponectin was measured. There are two types of adiponectin receptors, AdipoR1 receptors are expressed in skeletal muscle cells, AdipoR2 receptors are expressed in skeletal muscle and liver cells, and both receptor types are expressed in adipose tissue (68).

Adiponectin functions to regulate glucose and lipid storage as well as transport and oxidation, which in turn, affects and insulin sensitivity (69). Some research suggests that adiponectin mitigates hepatic gluconeogenesis while hepatic glycolysis and glucose uptake are unaffected (70). Other research suggests that adiponectin may act to increase FFA oxidation via activation of AMPK, and in turn, reduce the concentration of FFAs in the blood and the concentration of lipid metabolites in skeletal muscle and liver (71). Further research suggests that in addition to increased fat oxidation, adiponectin may act to increase lipogenesis and lipid transport, and to increase glycolysis. The liver adiponectin knock-out (KO) mice have demonstrated down-regulated mRNA expression of: glycolytic enzymes, TCA cycle enzymes, lipogenic enzymes, fatty acid activation enzymes, and beta-oxidation enzymes (69). Expectedly, the KO mice exhibited less fat accumulation in white adipose tissue (WAT) and lower total body weight compared to wild-type (WT) after 16 weeks of high-fat diet (HFD) feeding. After only 30 days of HFD, WT and KO mice did not exhibit differences in WAT or skeletal muscle, but liver had significantly less lipid droplets due to decreased lipogenesis. Thus adiponectin appears to function as a mediator to clear lipids from plasma via increased β-oxidation through up-regulation of the AMPK pathway as well as increased expression of rate-limiting enzymes

involved in lipogenesis. The down-regulation of lipogenesis, lipid transport, and oxidation will result in delayed clearance of lipids from plasma, which may contribute to the development of insulin resistance and lipotoxicitiy associated with low expression of adiponectin.

Currently, the mechanisms responsible for the regulation of adiponectin are a matter of debate. There are several genetic factors which likely influence adiponectin regulation and adipocyte mRNA expression including differences in PGC-1α, nuclear respiratory factor-1 (NRF1) (72), SIRT-1 (42), and peroxisome proliferator-activated receptor- gamma (PPARγ) (73) gene expression, among others. First degree family history of type 2 diabetes, defined as a first-degree relative (mother or father) having type 2 diabetes is a major risk factor for the development of insulin resistance and type 2 diabetes in the offspring (74). This risk factor may be partially due to differences in several key metabolic genes as mentioned above, but is also likely due to lifestyle factors including habitual physical inactivity and poor diet (75). Further investigation is necessary to determine if first degree family of type 2 diabetes is correlated with plasma adiponectin concentration. If so, this correlation could highlight the importance of regular physical activity for this population to maintain insulin sensitivity.

Aside from genetic factors, there are several other proposed mechanisms responsible for the regulation of adiponectin. Despite the apparent role of adiponectin in lipid clearance from the blood, postprandial and post-absorptive states do not appear to affect plasma adiponectin concentration. Studies in human models have demonstrated that HMW adiponectin concentration fluctuates in healthy, lean males by approximately 40 percent from late night to early morning, whereas the fasted versus fed state appears to have no significant effect on adiponectin (76). Therefore, circadian rhythm appears to be involved, at least to some extent, in the regulation of adiponectin. In rats, adiponectin messenger ribonucleic acid (mRNA) was

down-regulated following fasting or stimulation of beta-adrenergic receptors in WAT and brown adipose tissue (BAT), likely due to a combination of an increased rate of lipolysis, lipid transport, and less lipid storage; however, serum adiponectin was unaffected. Refeeding normalized mRNA expression (77), which suggests that insulin may be an agonist to adiponectin mRNA expression in the short-term, but insulin does not appear to have an immediate effect on plasma protein expression. Conversely, chronic overfeeding has been shown to down-regulate the secretion of adiponectin from the adipocyte (78). Combined, these results suggest that the secretion of adiponectin may be regulated by chronic, rather than acute changes, namely the development of insulin resistance in adipocytes.

Insulin appears to regulate adiponectin mRNA expression in adipocyte cell culture as well (79). In addition, Motoshima et. al (80) suggested that adiponectin secretion from visceral, but not subcutaneous, fat in humans is regulated by insulin and correlates with obesity, in vitro. Degawa-Yamauchi et al. (78); however, did not find any correlation between serum adiponectin and serum insulin, suggesting insulin does not regulate adiponectin expression. Combined, these results may indicate that insulin initiates alterations in mRNA expression; however, protein expression of adiponectin may be regulated by unknown secondary mechanisms or as previously suggested, the secretion may become impaired in an insulin-resistant state.

Motoshima et al. (76) also noted that adiponectin secretion from visceral fat was higher than from subcutaneous fat, and the secretion of adiponectin from visceral fat had a stronger negative correlation with body mass index (BMI) compared to the secretion of adiponectin from subcutaneous fat. This is an apparent paradox given that numerous studies have found negative correlations between plasma adiponectin and waist-to-hip ratio (WHR), body fat percentage, and BMI (81). In contrast to these ideas, Degawa-Yamauchi et. al (82) found that subcutaneous

14

adiponectin mRNA negatively correlated with BMI and positively correlated with serum adiponectin, while there was no correlation between omental adiponectin mRNA and BMI or serum adiponectin. In addition, the secretion of adiponectin from subcutaneous versus omental adipose was not different over a 24 hour period. These conflicting results demand further research.

Gender differences could potentially explain some of these discrepancies; though, the aforementioned studies did not separate results based on gender. Men generally have more omental fat compared to women and tend to have lower levels of adiponectin, although some researchers suggest these differences are due to testosterone levels (83). Another study found that growth hormone may act to regulate the expression of adiponectin (84), and still others suggest glucocorticoids significantly decrease adiponectin release from subcutaneous, but not omental adipose (82). This suggests that adiponectin is directly regulated by changes in glucose metabolism. Most studies; however, have suggested that hormones, such as leptin, testosterone, growth hormone, and cortisol have no correlation with adiponectin (77, 81). It may be that the metabolic state has a greater influence on plasma adiponectin, but this too deserves further investigation. Furthermore, additional research is needed to determine if a first degree family history of type 2 diabetes, which is a major risk factor in the development of insulin resistance, (74) is linked to lower plasma adiponectin concentration.

Exercise

Despite the clear improvements in insulin sensitivity following exercise, study results examining the effects of exercise on adiponectin have shown equivocal results. Most studies have demonstrated that HMW form of adiponectin tends to increase with higher intensities or in

the presence of weight loss, while no change or a decrease occurs following low- to moderate-exercise intensities (85); however, the reasoning for this remains unclear. It may be partially due to the exercise intensity-dependent activation of AMPK and an increased accumulation of NAD^+ (41), as this has been shown to increase SIRT-1 expression and in turn, regulate the expression of adiponectin (42).

Exercise appears to elicit changes in the expression of adiponectin receptors, though this regulation is a matter of debate. When a wide range of normal weight, overweight, and obese individuals with normal glucose tolerance, impaired glucose tolerance, and type II diabetes were placed on a four-week physical training regimen, (2) exercise increased the expression of adiponectin and its receptors (AdipoR1 & AdipoR2). This increased expression may act to mediate improvements in insulin sensitivity, glucose metabolism, and lipid metabolism seen as a result of exercise. However, the authors also found that the more insulin resistance that a given participant had, the higher the expression of the AdipoR2, regardless of body fat percentage. In an earlier study, this research lab also found that an increased expression of AdipoR1 and AdipoR2 mRNA positively associated with obesity, plasma glucose, plasma lipid levels, and insulin resistance. The same four week exercise regimen increased serum adiponectin, and AdipoR1 & AdipoR2 mRNA in skeletal muscle. They also found that three hours of exercise was sufficient to increase mRNA expression of AdipoR1 & AdipoR2, phosphorylation of AMPK, but did not have an effect on serum adiponectin. (36). These results suggest that both insulin resistance and exercise mediate up-regulation in adiponectin receptor expression, thus further research is needed to clarify these findings.

It is possible that exercise may cause an initial decrease in plasma adiponectin due to increased free fatty acid concentration in the plasma, thereby increasing the expression of

adiponectin receptor mRNA. The increased clearance of lipid metabolites from the plasma as a result of chronic exercise may be responsible for higher levels of plasma adiponectin and lower expression of adiponectin receptors. This hypothesis is supported by (67) who found that 20 minutes of rowing in elite male rowers decreased adiponectin immediately post-exercise, but adiponectin levels increased above resting levels 30 minutes after exercise. Conversely, adiponectin significantly decreased 30 minutes after exercise in non-elite rowers. Growth hormone, leptin, insulin, and glucose were not different from resting levels, further supporting the previously mentioned notion that adiponectin changes independently of these hormones. The initial decrease in adiponectin seen in the elite rowers may have been due to increased lipolysis and lipid transport into plasma, while an increase in FFA oxidation and lipid clearance from plasma 30 min post-exercise may explain increase in adiponectin, given that trained individuals are able to oxidize lipids post-exercise more quickly than untrained individuals. This study, along with a more recent acute aerobic exercise study have shown an increase in adiponectin (35), but others (38, 86), have shown no change. Similarly, chronic aerobic exercise has been shown to increase adiponectin (36) or cause no change in adiponectin (38). Chronic resistance training has been shown to increase adiponectin (87) or cause on change in adiponectin (88). The acute effects of acute resistance training or stretching on adiponectin are relatively unknown. It appears that the regulation of substrate utilization and adiponectin share similar signaling molecules and thus, may be dependent on one another. The combination of these equivocal results and the unknown regulation of adiponectin warrant further investigation.

1.4 Effects of Supplementation

Several supplements are known to affect exercise performance, substrate utilization, plasma glucose concentration, plasma lipid levels, and plasma adiponectin concentration.

17

Therefore, two exercise intervention modalities in the current study incorporated supplementation to determine if the combination of a supplement plus exercise would amplify the effects of the supplement on exercise performance, substrate utilization, and adiponectin concentration.

Caffeine

In several studies, caffeine has been shown to enhance fatty acid mobilization from adipose tissue, and in turn lower the respiratory exchange ratio (RER) and spare muscle glycogen in submaximal acute exercise lasing 30-60 minutes (7, 89). This mechanism is likely due to an increase in catecholamine concentration, which blocks glucose uptake, thereby increasing FFA oxidation. Increased catecholamine release also enhances glucose release from the liver and FFA release from adipose tissue (7). If plasma adiponectin is regulated by plasma lipid levels as hypothesized, this supplement should mitigate plasma adiponectin expression.

Despite the proposed mechanism of increased FFA oxidation with caffeine ingestion, some studies have seen no effect of caffeine on aerobic function, RER or FFA uptake into skeletal muscle (90-92). Untrained individuals tested at 75% VO_{2max} elicited no benefit from caffeine supplementation (92). Overall however, caffeine ingestion appears to be effective for improving low- to moderate-intensity aerobic exercise performance regardless of training status, (93, 94) whereas caffeine ingestion prior to high-intensity exercise may only be beneficial to trained athletes (95); however, more studies are needed to verify these outcomes and further research is needed to determine whether a caffeine-induced substrate shift is associated with changes in plasma adiponectin expression.

Niacin

Nicotinic acid, also known as niacin, is pharmacologically used to improve cholesterol levels by blocking fat breakdown in adipose and liver tissue. As a consequence of this action, very low density lipoprotein (VLDL) formation decreases in the liver, thus less low density lipoprotein (LDL) and more high density lipoprotein (HDL) are formed, thereby lowering blood lipids (162). Consequently, this supplement should increase plasma adiponectin concentration, and indeed that has been demonstrated (96).

Recently, Plaisance et al. (92) demonstrated that an acute dosage of niacin decreased plasma non-esterified fatty acid (NEFA) levels by decreasing lipolysis in adipocytes. Niacin administration also increased adiponectin, primarily HMW adiponectin, but not other adipokines such as leptin or resistin in lean mice. The authors suggested that NEFA may inhibit adiponectin secretion by acting on adipocyte receptors. It was also suggested that beta-hydroxybuterate, which is up-regulated during starvation, acts on one of the adiponectin receptors (AdipoR1 or AdipoR2) to decrease serum FFA and decrease ketogenesis while increasing adiponectin concentration. Thus, it appears that adiponectin is inhibited by high concentrations of NEFA and is stimulated by low concentrations of NEFA. Based on this evidence, it can be hypothesized that adiponectin is a catabolic hormone, which may partially explain why it is inhibited in diabetic and insulin resistant individuals, which demonstrate high levels of the anabolic hormone, insulin (63); however, insulin regulation of adiponectin remains a matter of debate. Other studies, such as the recent study by Kelley et al. (35) in which exercise training increased HMW adiponectin while increasing fat oxidation, support the findings that the adiponectin hormone is up-regulated by low levels of NEFA, which activates PPARγ, and in turn, stimulates

mitochondrial biogenesis and increases fat oxidation; although, this theory has not been directly measured therefore further investigation is needed.

Given that HMW adiponectin may be directly affected by NEFA concentration, it is possible that there may be a direct link between substrate utilization and adiponectin concentration in the plasma. Following fasting, NEFA concentration will rise; however, the fasted state appears to have no effect on adiponectin. Time of day does have a significant effect on adiponectin and therefore, participants were not fasted for the current research studies examining adiponectin, but measurements were taken at the same time of day.

Melatonin

Also of interest, melatonin supplementation has been shown to increase glucose transport (97), increase liver and muscle glycogen content, maintain homeostatic blood glucose concentrations, and increase lipid utilization immediately following aerobic exercise (98). Whether or not melatonin affects substrate utilization following resistance or stretching exercise and what effect melatonin has on plasma adiponectin concentration following resistance or stretching exercise is currently unknown. A recent study by Nelson et al. (103) demonstrated that acute melatonin supplementation increases glucose oxidation during aerobic exercise, which suggests the supplement acts to improve insulin sensitivity.

Melatonin supplementation has been shown to attenuate body weight gain in rats given a high fat diet, decrease plasma glucose, insulin and triglycerides, while reducing adiponectin concentration (99). These results suggest that the regulation of adiponectin may not be regulated by blood glucose or lipid concentration and are supported by earlier studies which demonstrated that melatonin leads to an inhibition in the accumulation of lipid droplets in pre-adipocytes

20

(100). However, if melatonin does indeed increase lipid utilization, it could encourage free fatty acid availability and oxidation, in other words, high NEFA concentration in plasma. As previously mentioned, this has been proposed to cause a reduction in adiponectin expression, as was seen in high-fat fed rats given melatonin. The extent of change in substrate utilization and plasma adiponectin concentration following melatonin supplementation in humans is largely unexplored and deserves further investigation.

Rationale

Considering exercise modality may have a large effect on substrate utilization, plasma adiponectin concentration and exercise performance, various exercise modalities were examined in the current study. Higher intensity exercise, whether acute, chronic, aerobic, resistance, or stretching, should theoretically augment plasma adiponectin and alter substrate utilization. Therefore all modalities were performed at moderate-to-high intensities. Acute aerobic exercise was performed to complete exhaustion, chronic aerobic exercise was performed at an average high rating of perceived exertion, acute resistance training was performed at 75% one-repetition maximum (1RM), chronic resistance training was performed at an average of > 75% 1RM, and acute stretching exercise was performed by stretching each muscle to its maximal threshold. Furthermore, limiting or enhancing lipid or glucose metabolism via supplementation during high-intensity chronic or acute aerobic exercise in untrained individuals should diminish or improve plasma adiponectin concentration, respectively. Finally, first degree family history of type 2 diabetes, high body fat percentage, high BMI, high waist-to-hip ratio, and lifestyle habits including physical inactivity, caffeine consumption, and high-fat diet are all major risk factors in the development of insulin resistance (74, 75, 101). Each of these variables will be correlated

21

with plasma adiponectin concentration to determine if any relationships exist between lower

levels of adiponectin and variables known to decrease insulin sensitivity.

CHAPTER 2. METHODS

2.1 Acute Aerobic Exercise

Subjects

Untrained males (n = 17) and females (n = 8) between the ages of 18-26 were recruited to participate in the study. All participants were non-smokers, were not taking any medication, with the exception of oral contraceptives, and did not regularly perform any type of cycling exercise more than one time per week. Subject characteristics are shown in table 1. All participants were healthy as indicated by the Physical Activity Readiness Questionnaire (PAR-Q). Informed consent was obtained from all participants prior to the onset of the first exercise trial and the study protocol was approved by the Institutional Review Board of Louisiana State University.

Table 1. Subject Characteristics for the acute aerobic exercise modality.

Subject Characteristics	Men n = 17	Women n = 8
Age (yr)	21.3 ± 2.2	20.8 ± 1.7
Weight (kg)	78.0 ± 13.9	60.1 ± 5.1 *
Height (cm)	177.9 ± 6.7	167.9 ± 4.6 *
BMI	24.5 ± 2.2	21.5 ± 2.3 *
Body Fat %	19.3 ± 6.1	22.4 ± 2.6
Fat Mass (kg)	15.1 ± 0.9	13.5 ± 0.3
Fat Free Mass (kg)	63.0 ± 13.0	46.7 ± 4.7 *
Reg. Caffeine Consumption (%)	17.6	50.0
Reg. Aerobic Exercise (%)	35.3	75.0
Reg. High Fat Diet (%)	41.8	37.5

* Denotes $p < 0.05$ between groups. BMI Body Mass Index.

Experimental Protocol

Participants were asked to abstain from caffeine and alcohol consumption 48 hours prior to each trial and were asked to abstain from exercise for 24 hours prior to each trial. In addition, participants entered the lab three hours postprandial and were asked to consume a standard meal of approximately 270 kilocalories (60% CHO, 15% PRO, 25% Fat) prior to each exercise trial to ensure consistent results across all participants and between each trial. It should be noted that studies have demonstrated that an overnight fast does provide valid and reliable responses to exercise; however, by examining participants three hours postprandial, the current study provided responses that are more applicable to real-world settings. Presumably, most of the population does not exercise on a regular basis following an overnight fast, rather exercise takes place after some form of nutrients have been consumed. Additionally, several studies have shown that the ingestion of carbohydrates prior to exercise enhances endurance performance (102). Thus, by examining lipid metabolism three hours postprandial, we were able to provide much more useful information as it applies to the general public, and as previously mentioned, the fasting state has no effect on plasma adiponectin concentration (76).

Upon arrival to the testing site, all baseline measures were obtained (table 1). Each participant sat quietly for five minutes, after which resting heart rate was obtained. Heart rate data was obtained through a Polar heart rate monitor (Polar Electro, Kempele, Finland). Height and weight were measured using a Detecto medical scale (Cardinal Scale Manufacturing Co., Webb City, MO), body fat was measured via the seven-site skinfold technique using Lange skinfold calipers (Beta Technology, Inc., Cambridge, MD), and a questionnaire regarding exercise and dietary habits was completed and analyzed using My Diet Analysis software

(Pearson Education Inc., Upper Saddle River, NJ). A sample questionnaire is provided in Appendix 2. Regular caffeine consumption was considered a combination of any of the following equating to ≥ five per week: eight ounces of coffee/tea, twelve ounces of caffeinated soda, and one energy shot/drink. Regular aerobic exercise was considered ≥ 150 minutes of total aerobic exercise per week. Regular high fat diet was considered ≥ an average of 35% of total daily caloric intake from fat.

Exercise Protocol

Following the completion of baseline testing, resting respiratory exchange ratio (RER) data was obtained through indirect calorimetry (Moxus Modular VO_2 System, Pittsburgh, PA). Heart rate, volume of oxygen consumed (VO_2), and respiratory exchange ratio (RER) were monitored for the duration of the study. All exercise protocols were performed on a mechanically braked cycle ergometer (Monark Exercise AB, Norrbotten, Sweden). Before each exercise session, male participants completed a 10 minute warm- up at a 50 watt (W) power output maintaining 50 revolutions per minute (rpm). Female participants completed a 10 minute warm-up at a 25 W power output, then began the initial workload at 50W, increasing the workload by 25 W every 6 minutes until exhaustion. This was done to compensate for the reduced muscle mass and aerobic capacity seen in the female group. A pedal frequency of 50 rpm was maintained for the entirety of the warm-up and exercise phases for both males and females. In addition, rating of perceived exertion (RPE) and signs and symptoms of possible cardiopulmonary dysfunction was monitored every 2-3 minutes throughout the test. Participants then began the initial workload at 100W. The workload then increased by 50W every 6 minutes until the participant could no longer maintain his or her pedal frequency. This time point was deemed exhaustion and time to exhaustion for the exercise trial was recorded.

25

Supplement Dosage

Supplements were administered using a balanced, randomized, double-blind, crossover design. Upon arrival to the research site, each participant was randomly assigned to a placebo, caffeine, or nicotinic acid supplement. For each subsequent visit, the participant was administered a different supplement, ensuring that each of the three supplements were ingested for three different exercise trials. A one-week washout period separated each exercise trial to eliminate any carry-over effect. For the niacin trial, each participant was administered 1000 milligrams (mg) of niacin, for the caffeine trial, each participant was administered five mg/kilogram (kg) of body weight, and for the placebo trial, each participant was administered a methylcellulose placebo supplement capsule. Each supplement was ingested at least 30 minutes prior to the onset of the exercise protocol.

Plasma Analysis

For each trial, the participant sat quietly for 30 minutes after ingesting the supplement, after which ten milliliters (ml) of blood was drawn from the cubital vein to determine initial plasma adiponectin values. Immediately following the completion of each exercise trial, an additional ten ml of blood was drawn from the same location to determine post-exercise plasma adiponectin levels. Blood was collected into sodium heparin vacutainers and immediately centrifuged. Plasma was then separated and stored at -80 degrees Fahrenheit until further analysis. All plasma samples were analyzed to determine the concentration of total plasma adiponectin via western blot. Protein was quantified for each sample, as determined by bicinchoninic acid (BCA) assay (Sigma-Aldrich, St. Louis, MO), and equal amounts of protein from each sample were subjected to electrophoresis. Proteins were separated in 7.5%

polyacrylamide (acrylamide from National Diagnostics) gels containing sodium dodecyl sulfate (SDS) and transferred to nitrocellulose membrane in 25 mM Tris, 192 mM glycine, and 20% methanol. Following transfer, the membrane was blocked overnight in 4% milk at 4°C then probed with human-anti-adiponectin (1:1000 in 1% BSA in 1X Tris-buffered saline-tween). Results were visualized with horseradish peroxidase (HRP)-conjugated mouse-anti-human secondary antibody (1:1000 in 1% nonfat milk in 1X Tris-buffered saline-tween) and enhanced chemiluminescence. Each membrane was probed with anti- α Tubulin as a loading control (1:1000 in 1% BSA in 1X Tris-buffered saline-tween) and densitometric analysis of blots was performed using Image J software.

Statistical Analysis

All data were analyzed using a repeated-measures analysis of variance (ANOVA) to compare differences in RER, time to exhaustion, and plasma adiponectin and alpha-tubulin concentration between and within subjects as well as between and within trials. Correlations of co-variables were tested using simple linear regression and significant differences were identified using Tukey post-hoc tests. $p < 0.05$ was considered significant. All values are presented as mean ± SD. All statistical analysis was completed using SAS v9.2 (SAS Institute, Inc. Cary, NC).

2.2 Chronic Aerobic Exercise

Subjects

Recreationally active females (n = 38) ages 18-24 were recruited to participate in the study. All participants were non-smokers and were not taking any medication, with the exception of oral contraceptives. Subject characteristics are shown in table 2. All participants

27

were healthy as indicated by the Physical Activity Readiness Questionnaire (PAR-Q). Informed

consent was obtained from all participants prior to the onset of the first exercise trial and the

study protocol was approved by the Institutional Review Board of Louisiana State University.

Table 2. Subject Characteristics for the chronic aerobic exercise modality.

Baseline Subject Characteristics	Exercise n = 18	Control n = 20
Age (yr)	21 ± 0.8	21 ± 1.3
Weight (kg)	62.9 ± 6.3	61.5 ± 9.2
Height (cm)	166.0 ± 5.1	164.9 ± 4.2
BMI	22.79 ± 2.1	21.87 ± 3.1
Body Fat %	32.4 ± 5.1	29.7 ± 6.1
Fat Mass (kg)	20.5 ± 3.8	18.6 ± 6.3
Fat Free Mass (kg)	42.8 ± 5.7	42.8 ± 4.3

* No statistical differences between groups at baseline. BMI Body Mass Index.

Experimental Protocol

At the onset of the study, participants were randomly assigned to an aerobic exercise

group (E) or a control group (C). In order to minimize subject variability, participants were

asked to abstain from alcohol and caffeine consumption as well as strenuous physical activity for

48 hours prior to baseline testing.   Baseline measurements were established for height and body

weight using a Detecto medical scale (Cardinal Scale Manufacturing Co., Webb City, MO), body

fat percentage was determined by duel energy x-ray absorptiometry (DXA), and estimated VO_{2max} was determined by a 1.5 mile run.

Participants then completed the 15-week exercise intervention. A week-by-week

description for the exercise intervention is described in table 3. Participants were required to

participate in a minimum of 80% of the exercise sessions to be included in the analysis.

Following training, and prior to running a full marathon, all baseline measurements were re-

tested. This was done to minimize confounding inflammatory responses that are associated with completing a full marathon (26.2 mile) race.

Table 3. Training Protocol for the chronic aerobic exercise modality.

Week	Mon	Tues	Wed	Thur	Fri	Sat	Sun	Total (miles/wk)
1	Rest	3	3	3	Rest	6	Cross	15
2	Rest	3	4	3	Rest	7	Cross	17
3	Rest	3	4	3	Rest	8	Cross	18
4	Rest	3	4	3	Rest	7	Cross	17
5	Rest	3	5	3	Rest	10	Cross	21
6	Rest	3	6	3	Rest	12	Cross	24
7	Rest	3	5	4	Rest	11	Cross	23
8	Rest	4	7	4	Rest	14	Cross	29
9	Rest	4	8	5	Rest	16	Cross	33
10	Rest	4	9	5	Rest	18	Cross	36
11	Rest	5	9	5	Rest	20-22	Cross	39-41
12	Rest	5	10	5	Rest	20	Cross	40
13	Rest	5	10	5	Rest	12	Cross	32
14	Rest	5	6	5	Rest	8	Cross	24
15	Rest	3	4	2	Rest	RACE		

Numbers= number of miles run; cross= cross training.

Plasma Analysis

During initial baseline testing at the onset of the study, an overnight fasting blood sample was drawn from the cubital vein of each participant. Prior to the blood draw, participants were asked to abstain from caffeine and alcohol consumption as well as exercise for 48 hours. This procedure was repeated at the conclusion of the study and a second fasting blood sample was obtained 48 hours after the final exercise testing; this was done to minimize any carry-over effects from an acute exercise bout. Blood was collected into sodium heparin vacutainers and immediately centrifuged. Plasma was then separated and stored at -80 degrees Fahrenheit until further analysis. All plasma samples were analyzed to determine the concentration of total

plasma adiponectin via western blot. Protein was quantified for each sample, as determined by

bicinchoninic acid (BCA) assay (Sigma-Aldrich, St. Louis, MO), and equal amounts of protein

from each sample were subjected to electrophoresis. Proteins were separated in 7.5%

polyacrylamide (acrylamide from National Diagnostics) gels containing sodium dodecyl sulfate

(SDS) and transferred to nitrocellulose membrane in 25 mM Tris, 192 mM glycine, and 20%

methanol. Following transfer, the membrane was blocked overnight in 4% milk at 4°C then

probed with human-anti-adiponectin (1:1000 in 1% BSA in 1X Tris-buffered saline-tween).

Results were visualized with horseradish peroxidase (HRP)-conjugated mouse-anti-human

secondary antibody (1:1000 in 1% nonfat milk in 1X Tris-buffered saline-tween) and enhanced

chemiluminescence. Each membrane was probed with anti- α Tubulin as a loading control

(1:1000 in 1% BSA in 1X Tris-buffered saline-tween) and densitometric analysis of blots was

performed using Image J software.

Statistical Analysis

All data were analyzed using a repeated-measure ANOVA and paired t-tests where

appropriate. Correlations of co-variables were tested using simple linear regression and

significant differences were identified using Tukey post-hoc tests. $p < 0.05$ was considered

significant. All values are presented as mean ± SD. All statistical analysis was completed using

SAS v9.2 (SAS Institute, Inc. Cary, NC).

2.3 Acute Resistance Training

Subjects

Untrained healthy males (n = 17) and females (n = 12) age 18-35 with a body mass index

(BMI) of less than 30 were recruited to participate in the study. All participants were non-

smokers, were not taking any medication, with the exception of oral contraceptives, and did not

regularly perform any type of resistance training exercise more than one time per week. Subject

characteristics are shown in table 4. All participants were healthy as indicated by the Physical

Activity Readiness Questionnaire (PAR-Q). Informed consent was obtained from all participants

prior to the onset of the first exercise trial and the study protocol was approved by the

Institutional Review Board of Louisiana State University.

Table 4. Subject Characteristics for the acute resistance exercise modality.

Subject Characteristics	Men n = 17	Women n = 12
Age (yr)	23.1 ± 4.1	20.6 ± 1.36
Weight (kg)	78 ± 10.3	62.0 ± 8.8 *
Height (cm)	176.5 ± 6.4	163.7 ± 4.7 *
BMI	25.0 ± 2.8	23.1 ± 2.9 *
Body Fat %	17.9 ± 5.9	24.3 ± 5.7 *
Fat Mass (kg)	14.3 ± 6.5	15.4 ± 5.2
Fat Free Mass (kg)	63.7 ± 6.1	46.6 ± 4.7 *
Reg. Caffeine Consumption (%)	36.4	35.0
Reg. Resistance Exercise (%)	27.3	60.0 *

* Denotes $p < 0.05$ between groups. BMI Body Mass Index. BMI Body Mass Index.

Experimental Protocol

Participants were asked to abstain from vigorous physical activity, caffeine and alcohol

consumption 48 hours prior to each trial. In addition, participants came in a minimum of three

hours postprandial and were asked to consume the same meal (60% CHO, 15% protein, 25% fat)

prior to each trail to ensure consistent results across all participants and between each trial.

Upon arrival to the research site, height and weight were measured using a Detecto medical scale

(Cardinal Scale Manufacturing Co., Webb City, MO), body fat was measured via the 7-site

skinfold technique using Lange skinfold calipers (Beta Technology, Inc., Cambridge, MD), and a

questionnaire regarding exercise and dietary habits was completed and analyzed using My Diet

Analysis software (Pearson Education Inc., Upper Saddle River, NJ). A sample questionnaire is

provided in Appendix 2. Regular caffeine consumption was considered a combination of any of

the following equating to $\geq$ 5 per week: cups of coffee/tea, 12 oz caffeinated sodas, and energy

shots/drinks. Regular aerobic exercise was considered $\geq$ 150 minutes of total aerobic exercise

per week. Regular high fat diet was considered $\geq$ an average of 35% of total daily caloric intake

from fat.

Each participant was then randomly assigned to the control or exercise trial. On the

subsequent visit, the participant was assigned to whichever trial was not administered during the

first visit. Each exercise trial was separated by a minimum of one week. Each participant

completed a total of three trials. The first trial was used to determine a one repetition maximum

(1RM) for box squat and bench press exercises. The next trial consisted of either a high-

intensity resistance training protocol lasting approximately 30 minutes or a control trial, in which

the participants sat quietly for 30 minutes.

Venous blood samples were collected immediately before and after each protocol and

dermal punctures were administered pre- and post-exercise to determine changes in blood

glucose concentration.

Exercise Protocol

The exercise intervention set, rep, and rest scheme was based off of previous acute

resistance training studies that have demonstrated increases in lipid oxidation, decreases in

intramyocellular lipid (IMCL) stores, decreases in glycogen stores (26), and increases in plasma

32

glycerol (4). Therefore it can be assumed that lipid utilization was increasing with this type of exercise.

A 1RM for the box squat and bench press exercises were determined during the first trial. On one of the two subsequent trials, participants completed 6 sets of 12 repetitions on squats and bench press at 75% 1RM following a pre-exercise blood draw. 60 seconds of rest were allotted between sets. For the box squat exercise, participants lowered themselves down onto the box in a slow, controlled manner to prevent injury. Boxes were used as a depth cue so participants know when they have reached the proper depth (approximately 90 degree angle at the knee). The boxes also ensured that there were separate eccentric and concentric phases of the squat. Once the proper depth had been reached, participants raised themselves to an upright, standing position, and then repeated this process for the allotted number of repetitions. For the bench press exercise, participants lowered the bar down onto their chest in a slow, controlled manner to prevent injury. Once the bar had touched the chest, participants pressed the bar upward until their arms were fully extended, and then repeat this process for the allotted number of repetitions. If necessary, the weight lifted was decreased to ensure all sets and reps were completed and that the allotted 60 second rest period was maintained for the entirety of the exercise protocol.

Plasma Analysis

Blood glucose concentration measurements were taken to via dermal puncture immediately pre- and post-exercise to determine the extent of glucose utilization and uptake. Prior to the exercise intervention, 10 ml of blood was drawn from the cubital vein to determine initial plasma adiponectin values. Immediately following the completion of each exercise trial,

33

an additional 10 ml of blood was drawn from the same location to determine post-exercise

plasma adiponectin levels. Blood was collected into sodium heparin vacutainers and

immediately centrifuged. Plasma was then separated and stored at -80 degrees Fahrenheit until

further analysis. All plasma samples were analyzed to determine the concentration of total

plasma adiponectin via western blot. Protein was quantified for each sample, as determined by

bicinchoninic acid (BCA) assay (Sigma-Aldrich, St. Louis, MO), and equal amounts of protein

from each sample were subjected to electrophoresis. Proteins were separated in 7.5%

polyacrylamide (acrylamide from National Diagnostics) gels containing sodium dodecyl sulfate

(SDS) and transferred to nitrocellulose membrane in 25 mM Tris, 192 mM glycine, and 20%

methanol. Following transfer, the membrane was blocked overnight in 4% milk at 4°C then

probed with human-anti-adiponectin (1:1000 in 1% BSA in 1X Tris-buffered saline-tween).

Results were visualized with horseradish peroxidase (HRP)-conjugated mouse-anti-human

secondary antibody (1:1000 in 1% nonfat milk in 1X Tris-buffered saline-tween) and enhanced

chemiluminescence. Each membrane was probed with anti- α Tubulin as a loading control

(1:1000 in 1% BSA in 1X Tris-buffered saline-tween) and densitometric analysis of blots was

performed using Image J software.

Statistical Analysis

All data were analyzed using repeated-measures ANOVA and paired t-tests where

appropriate. Correlations of co-variables were tested using simple linear regression and

significant differences were identified using Tukey post-hoc tests. $p < 0.05$ was considered

significant. All values are presented as mean $\pm$ SD. All statistical analysis was completed using

SAS v9.2 (SAS Institute, Inc. Cary, NC).

2.4 Chronic Resistance Exercise

Subjects

Untrained, healthy men (n=40) age 18-35 with a body mass index (BMI) of less than 30 were recruited to participate in the study. All participants were non-smokers, were not taking any medication, and did not regularly perform any type of resistance training exercise more than one time per week. Subject characteristics are shown in table 5. All participants were healthy as indicated by the Physical Activity Readiness Questionnaire (PAR-Q). Informed consent was obtained from all participants prior to the onset of the first exercise trial and the study protocol was approved by the Institutional Review Board of Louisiana State University.

Table 5. Subject characteristics for the chronic resistance exercise modality.

Subject Characteristics	Exercise n = 18	Control n = 22
Age (yr)	20.4 ± 2.1	22.9 ± 4.7 *
Weight (kg)	82.1 ± 11.3	82.1 ± 13.9
Height (cm)	177.2 ± 7.7	176.2 ± 5.6
BMI	26.1 ± 3.4	26.8 ± 5.5
Body Fat %	20.4 ± 6.4	24.4 ± 8.8
Fat Mass (kg)	17.3 ± 7.4	20.3 ± 11.6
Fat Free Mass (kg)	64.8 ± 6.1	61.9 ± 5.9
Reg. Caffeine Consumption (%)	55.0	45.5
Reg. Resistance Exercise (%)	25.0	41.0

* Denotes statistically significant differences between groups, $p < 0.05$. BMI Body Mass Index.

Experimental Protocol

Students currently enrolled in a beginning weight training course and a lecture course at Louisiana State University were recruited to participate as the exercise (E) and control (C) groups, respectively. In order to minimize subject variability, at the onset of the study, participants were asked to abstain from alcohol and caffeine consumption as well as strenuous

physical activity for 48 hours. Following this time period, participants completed an overnight fast and 10 ml of blood was drawn from the cubital vein, height and weight were measured using a Detecto medical scale (Cardinal Scale Manufacturing Co., Webb City, MO), body fat was measured via the 7-site skinfold technique using Lange skinfold calipers (Beta Technology, Inc., Cambridge, MD), and a questionnaire regarding exercise and dietary habits was completed and analyzed using My Diet Analysis software (Pearson Education Inc., Upper Saddle River, NJ). A sample questionnaire is provided in Appendix 2. Regular caffeine consumption was considered a combination of any of the following equating to ≥ 5 per week: cups of coffee/tea, 12 oz caffeinated sodas, and energy shots/drinks. Regular aerobic exercise was considered ≥ 150 minutes of total aerobic exercise per week. Regular high fat diet was considered $\geq$ an average of 35% of total daily caloric intake from fat.

Exercise Protocol

As outlined in table 6, following a familiarization phase, the initial strength of each participant was determined by one-repetition maximum (1RM) for free weight squat, free weight bench press, and free weight deadlift. Following the exercise intervention, all baseline measurements and strength measurements were re-tested.

Participants completed the twelve-week exercise intervention. A week-by-week description for the exercise intervention is described in table 6. Resistance training was performed three times per week for 40-50 minutes per exercise session. Each exercise session was separated by a minimum of 48 hours. Participants were required to participate in a minimum of 80% of the exercise sessions to be included in the analysis.

Table 6 Week-to-week periodization training protocol for the chronic resistance training protocol.

Week	Sets	Reps	Intensity (%1RM)
1	Familiarization/Baseline Testing		N/A
2	4	3	60
3	4	6	60
4	12	2	80
5	1 RM Testing	1	100
6	3	8	60
7	3	6	70
8	4	2	85
9	5	5	80
10	4	4	85
11	3	12	55
12	3	6	70
13	1 RM Testing	1	100
14	Repeat Baseline Testing	N/A	N/A

1RM one-repetition maximum.

Total work volume varied from week to week, each day consisted of three-to-five exercises depending on the number of sets and reps. Three exercises were used for higher set and rep schemes whereas four or exercises were used for lower set and rep schemes. Specific exercises varied from week-to-week and included free weight box squats, back squats, flat bench press, incline bench press, conventional deadlifts, Romanian deadlifts, sumo deadlifts, bent over rows, upright rows, good mornings, glut/ham raises, pull ups, lat pull downs, and push press. To minimize the risk of overtraining and injury, no single muscle group was targeted on back-to-back exercise sessions. Each of the baseline variables were re-assessed at week twelve following the baseline testing procedures discussed.

Plasma Analysis

At the onset of the study, ten ml of blood was drawn from the cubital vein to determine initial plasma adiponectin values. Following a 48-hour rest period, in which participants

abstained from vigorous exercise, caffeine, and alcohol for 48 hours, an additional ten ml of blood was drawn from the same location to determine post-exercise intervention plasma adiponectin levels. Blood was collected into sodium heparin vacutainers and immediately centrifuged. Plasma was then separated and stored at -80 degrees Fahrenheit until further analysis. All plasma samples were analyzed to determine the concentration of total plasma adiponectin via western blot. Protein was quantified for each sample, as determined by bicinchoninic acid (BCA) assay (Sigma-Aldrich, St. Louis, MO), and equal amounts of protein from each sample were subjected to electrophoresis. Proteins were separated in 7.5% polyacrylamide (acrylamide from National Diagnostics) gels containing sodium dodecyl sulfate (SDS) and transferred to nitrocellulose membrane in 25 mM Tris, 192 mM glycine, and 20% methanol. Following transfer, the membrane was blocked overnight in 4% milk at 4°C then probed with human-anti-adiponectin (1:1000 in 1% BSA in 1X Tris-buffered saline-tween). Results were visualized with horseradish peroxidase (HRP)-conjugated mouse-anti-human secondary antibody (1:1000 in 1% nonfat milk in 1X Tris-buffered saline-tween) and enhanced chemiluminescence. Each membrane was probed with anti- α Tubulin as a loading control (1:1000 in 1% BSA in 1X Tris-buffered saline-tween) and densitometric analysis of blots was performed using Image J software.

Statistical Analysis

All data were analyzed using repeated-measures ANOVA and paired t-tests where appropriate. Correlations of co-variables were tested using simple linear regression and significant differences were identified using Tukey post-hoc tests. $p < 0.05$ was considered significant. All values are presented as mean ± SD. All statistical analysis was completed using SAS v9.2 (SAS Institute, Inc. Cary, NC).

2.5 Acute Stretching Exercise

Subjects

Recreationally active, healthy men age 18-35 with a body mass index (BMI) of less than 25 were recruited to participate in the study. All participants were non-smokers, were not taking any medication, and did not regularly participate in vigorous exercise more than one time per week. Subject characteristics are shown in table 7. All participants were healthy as indicated by the Physical Activity Readiness Questionnaire (PAR-Q). Informed consent was obtained from all participants prior to the onset of the first exercise trial and the study protocol was approved by the Institutional Review Board of Louisiana State University.

Table 7. Subject characteristics for the acute stretching exercise protocol.

Subject Characteristics	Men n = 23
Age (yr)	22.5 ± 4.3
Weight (kg)	78.8 ± 11.4
Height (cm)	177.9 ± 5.7
BMI	25.0 ± 3.9
Body Fat %	16.2 ± 5.9
Fat Mass (kg)	13.4 ± 6.5
Fat Free Mass (kg)	65.5 ± 7.1
Reg. Caffeine Consumption (%)	30.0
Reg. Resistance Exercise (%)	48.0
Reg. Aerobic Exercise (%)	65.0

BMI Body Mass Index.

Experimental Protocol

Each participant completed a total of four trials. Participants were asked to abstain from caffeine and alcohol consumption 48 hours prior to each trial. In addition, participants came into the lab a minimum of three hours postprandial and were asked to consume the same meal (60% CHO, 15% protein, 25% fat) prior to each trail to ensure consistent results across all participants

and between each trial. Upon arrival to the research site, height and weight were measured using a Detecto medical scale (Cardinal Scale Manufacturing Co., Webb City, MO), body fat was measured via the 7-site skinfold technique using Lange skinfold calipers (Beta Technology, Inc., Cambridge, MD), and a questionnaire regarding exercise and dietary habits was completed and analyzed using My Diet Analysis software (Pearson Education Inc., Upper Saddle River, NJ). A sample questionnaire is provided in Appendix 2. Regular caffeine consumption was considered a combination of any of the following equating to $\geq$ 5 per week: cups of coffee/tea, 12 oz caffeinated sodas, and energy shots/drinks. Regular aerobic exercise was considered $\geq$ 150 minutes of total aerobic exercise per week. Regular high fat diet was considered $\geq$ an average of 35% of total daily caloric intake from fat.

Supplement dosage

Following baseline measurements, participants were administered a methylcellulose placebo or melatonin (6mg) supplement, utilizing a counterbalanced randomized double blind, crossover design. This ensured that each participant was administered each supplement twice over the course of the four exercise trials, one placebo and one melatonin supplement for each of the exercise trials, and one placebo and one melatonin supplement for each control trial. At the onset of each trail and for the duration of each trial, respiratory gases were monitored using indirect calorimetry. This was done to determine changes in substrate utilization during the exercise protocol.

Exercise Protocol

Two exercise trials consisted of a 30 minute passive resistance stretching protocol, the other two trials consisted of a 30 minute mock stretching protocol, meaning no tension was

applied to the muscle, but participants went through the same motions as the passive resistance protocol. The order of the trials was randomized and balanced for each participant. Each exercise trial was separated by a minimum of one week.

The passive resistance stretching exercise protocol was done in the following order: Hamstring stretch, quadriceps stretch, shoulder stretch, triceps stretch, hip adductor stretch, and hip flexor stretch. Each stretch was held for 30 seconds followed by 20 seconds rest, then repeated 2 more times (three sets of 30 seconds for each exercise per leg/arm). 6 exercises X 2 limbs X 2.5 minutes/exercise = a total time of 30 minutes. The details of each exercise are outlined and diagramed below, however some of these stretches were modified to have the participants lying in the supine position. This allowed for more accurate measurements of the respiratory gases. For each exercise, the range of motion was increased until the participant acknowledged a stretch-induced mild tightness in the muscle similar to that normally felt during a stretching activity. For the control trials, participants completed these same motions for the same period of time, but no tension will be applied to the muscle.

Stretching Illustrations and Descriptions

Hamstring stretch: Participants were in a supine position on a table with one leg fully extended. The other leg was flexed at the hip (45 degrees), with the knee fully extended. From this position, the extended leg was raised (hip flexion) to the vertical position or beyond to the point of stretching. During the stretch, the buttocks remained in complete contact with the ground, and the knee of the stretched leg was fully extended.
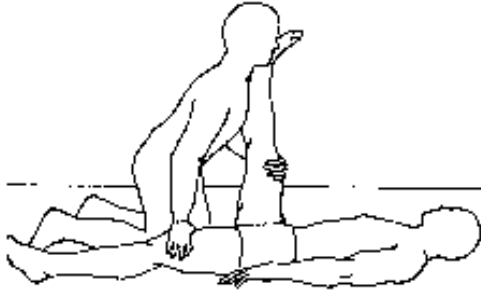
Quadriceps stretch: Participants were in a supine position with one leg hanging off the edge of the table and knee flexed at approximately 90 degrees. The hip was then hyperextended by pushing down on the thigh and the foot was pushed upward to increase tension on the quadriceps muscle. This was done to the point of stretching.
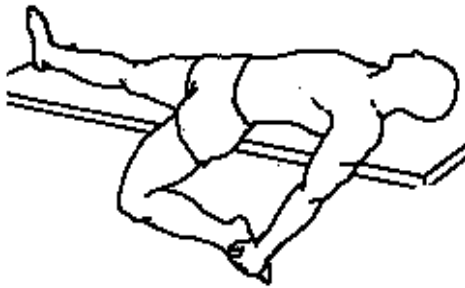
Shoulder stretch: Participants were in a supine, anatomical position on a table. The shoulder was then adducted; the arm was rotated to a pronated position. The arm was then hyperextended to the point of stretching, keeping the opposite shoulder flat on the table.

Chest stretch: Participants were in a supine, anatomical position on a table. The shoulder was then adducted and the arm remained in a supinated position. The arm was then pressed down toward the floor to the point of stretching, keeping the opposite shoulder flat on the table.

Glutes stretch: Participants were in a supine position on a table with one leg flexed at the knee (90 degrees) and the hip (45 degrees), with the foot placed firmly on the table. The ankle of the opposite leg was placed on the knee of the flexed leg. From this position, the hip of the first leg was flexed back to the point of stretching in the gluteus maximus muscle.

Low back stretch: Participants were in a supine position on a table with one leg fully extended. The other leg was flexed at the knee (90 degrees) and the hip (45 degrees), with the hip placed firmly on the table. From this position, the flexed leg was adducted and brought across the body. The leg was then pushed upward toward the head until the point of stretching in the low back.

Plasma Sample Analysis

For each trial, the participant sat quietly for 30 minutes after ingesting the supplement, after which ten ml of blood was drawn from the cubital vein to determine initial plasma adiponectin values. Immediately following the completion of each exercise trial, an additional ten ml of blood was drawn from the same location to determine post-exercise plasma adiponectin levels. Blood was collected into sodium heparin vacutainers and immediately centrifuged. Plasma was then separated and stored at -80 degrees Fahrenheit until further analysis. All plasma samples were analyzed to determine the concentration of total plasma adiponectin via western blot. Protein was quantified for each sample, as determined by bicinchoninic acid (BCA) assay (Sigma-Aldrich, St. Louis, MO), and equal amounts of protein from each sample were subjected to electrophoresis. Proteins were separated in 7.5% polyacrylamide (acrylamide from National Diagnostics) gels containing sodium dodecyl sulfate (SDS) and transferred to nitrocellulose membrane in 25 mM Tris, 192 mM glycine, and 20% methanol. Following

transfer, the membrane was blocked overnight in 4% milk at 4°C then probed with human-anti-adiponectin (1:1000 in 1% BSA in 1X Tris-buffered saline-tween). Results were visualized with horseradish peroxidase (HRP)-conjugated mouse-anti-human secondary antibody (1:1000 in 1% nonfat milk in 1X Tris-buffered saline-tween) and enhanced chemiluminescence. Each membrane was probed with anti- α Tubulin as a loading control (1:1000 in 1% BSA in 1X Tris-buffered saline-tween) and densitometric analysis of blots was performed using Image J software.

Statistical Analysis

All data were analyzed using repeated-measures ANOVA and paired t-tests where appropriate. Correlations of co-variables were tested using simple linear regression and significant differences were identified using Tukey post-hoc tests. $p < 0.05$ was considered significant. All values are presented as mean $\pm$ SD. All statistical analysis was completed using SAS v9.2 (SAS Institute, Inc. Cary, NC).

CHAPTER 3. RESULTS

3.1 Acute Aerobic Exercise

In males, the niacin treatment had significantly higher ($p < 0.05$) respiratory exchange ratio (RER) compared to the placebo and caffeine treatments (N = 0.95 ± 0.05, P = 0.88 ± 0.05, C = 0.88 ± 0.06) (mean $\pm$ SD.) (Fig. 1.1a). Females, however, did not demonstrate significant differences in RER when given the niacin treatment compared to the placebo treatment, while the caffeine treatment yielded a significantly lower RER compared to the placebo treatment (C = 0.84 ± 0.09, P = 0.88 ± 0.04) (Fig. 1.1b). Males demonstrated a significantly higher RER when given niacin compared to females (males = 0.95 ± 0.05, females = 0.85 ± 0.08). When all exercise trails were combined, fat utilization did not significantly differ between males and females for stages one and two of exercise; however, the utilization of fat at the third stage of exercise was significantly lower in males (males = 0.97 ± 0.06, females = 0.90 ± 0.08).

In males, the percentage of carbohydrate (CHO) utilization was higher for the niacin treatment compared to placebo and caffeine treatments (N = 80% $\pm$ 13, P = 61% $\pm$ 17, C = 62% $\pm$ 20) (Fig. 1.2a) while there were no significant differences in CHO utilization in females (Fig. 1.2b). Similar to absolute RER, males demonstrated a significantly higher %CHO utilization when given niacin compared to females (males = 80% $\pm$ 13, females = 51% $\pm$ 26) and when all exercise trails were combined, %CHO during the third stage of exercise was significantly higher in males (males = 85% $\pm$ 14, females = 60% $\pm$ 27), while there were no significant differences in %CHO utilized during stages one and two.

Time to exhaustion was significantly different for all three trials for both males (N = 24.4 ± 3.8, P = 25.5 ± 3.1, C = 27 ± 3.4) (Fig. 1.3a) and females (N = 29.7 ± 5.6, P = 31.3 ± 5.0, C = 32.8 ± 5.1) (Fig.1.3b).

The caffeine and niacin supplements did not significantly alter plasma adiponectin pre-exercise compared to the placebo trial. (Fig. 1.4) Exercise did not significantly alter plasma adiponectin in the placebo or caffeine trials, however, plasma adiponectin significantly decreased following the niacin trial (Pre = 1.00 AU, Post = 0.74 AU). (Fig. 1.5).

Simple linear regression was used to determine correlations between average plasma adiponectin concentration (Adpn) and: first degree family history of type 2 diabetes mellitus (FH+), regular aerobic exercise (Reg. Aex), regular resistance training exercise (Reg. Rex), regular caffeine consumption (Reg. Caff), body fat percentage (BF%), BMI, and average RER during exercise for each subject (Table 8). Regular aerobic exercise (r = 0.28) and regular caffeine consumption (r = .32) were both statistically significant correlations (p < 0.05), no other correlates were significant. No subjects for this intervention were FH+ and therefore no values are given for this variable.
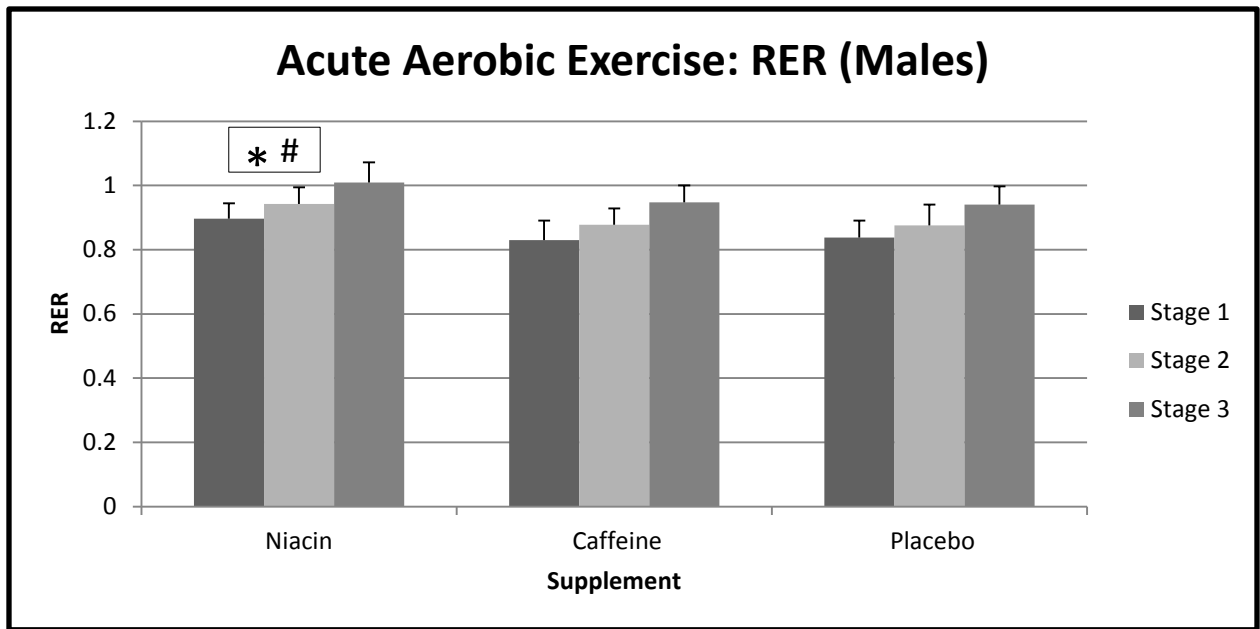
A.
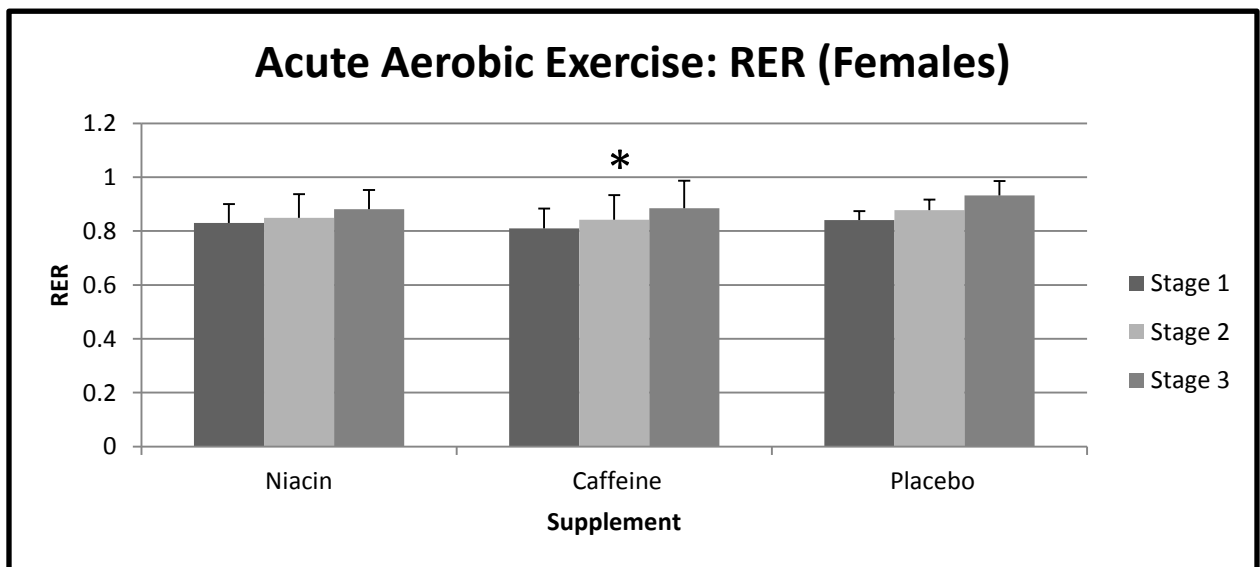
B.

Figure 1.1. Comparison of respiratory exchange ratio (RER) during three stages of exercise for niacin, placebo, and caffeine exercise trials (means ± SD) for males (A) and females (B). * $p < 0.05$ compared to placebo treatment. # $p < 0.05$ compared to caffeine treatment. (Males: N = 0.95 ± 0.05, P = 0.88 ± 0.05, C = 0.88 ± 0.06; Females: N = 0.87 ± 0.07, C = 0.84 ± 0.09, P = 0.88 ± 0.04).
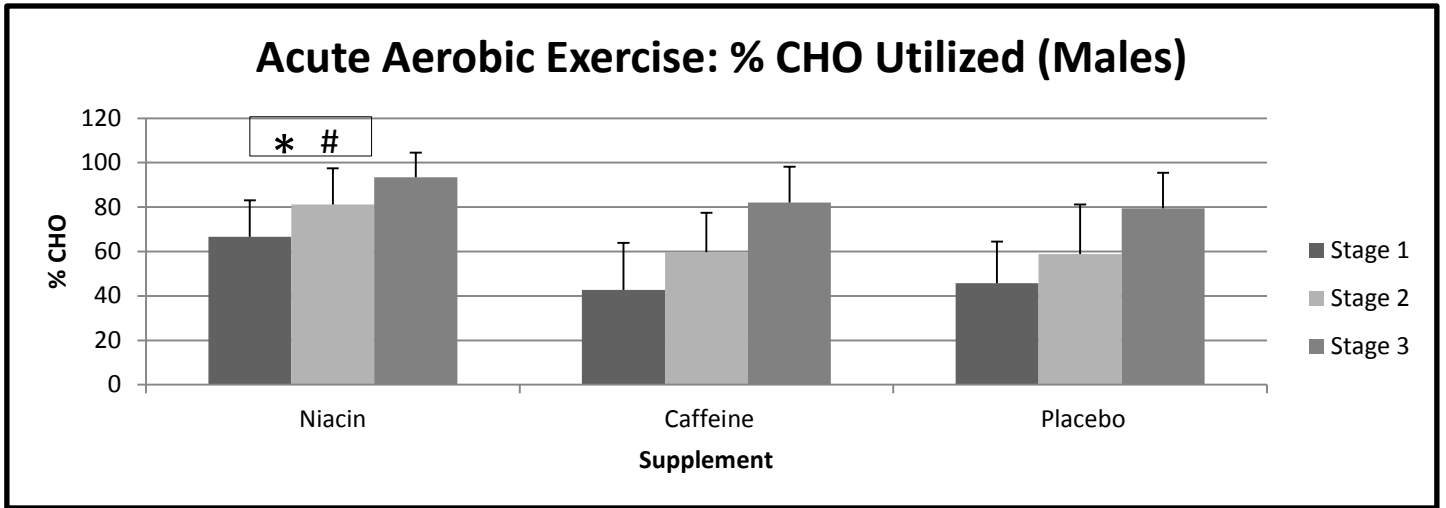
A.
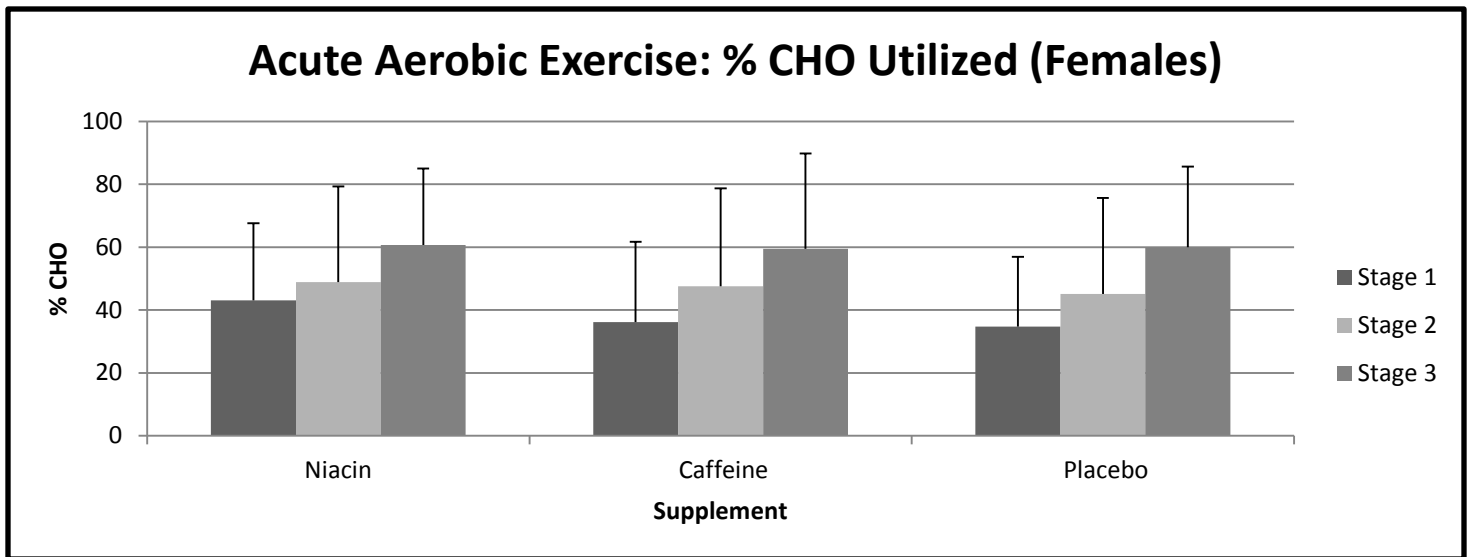
B.

Figure 1.2. Comparison of %CHO utilization during three stages of exercise for niacin, placebo, and caffeine exercise trials (means ± SD) for males (A) and females (B). *p > 0.05 compared to placebo treatment. # p > 0.05 compared to caffeine treatment. (Males: N = 80 ± 13%, C = 62 ± 20%, P = 61 ± 17%; Females: N = 51 ± 26%, C = 48 ± 29%, P = 47 ± 26%).
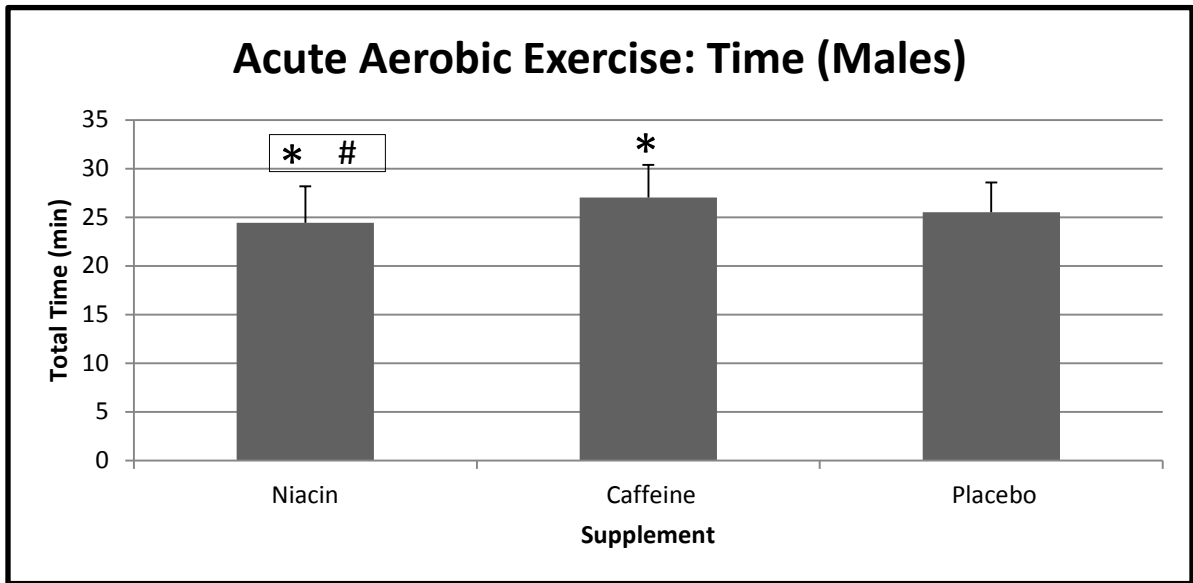
A.
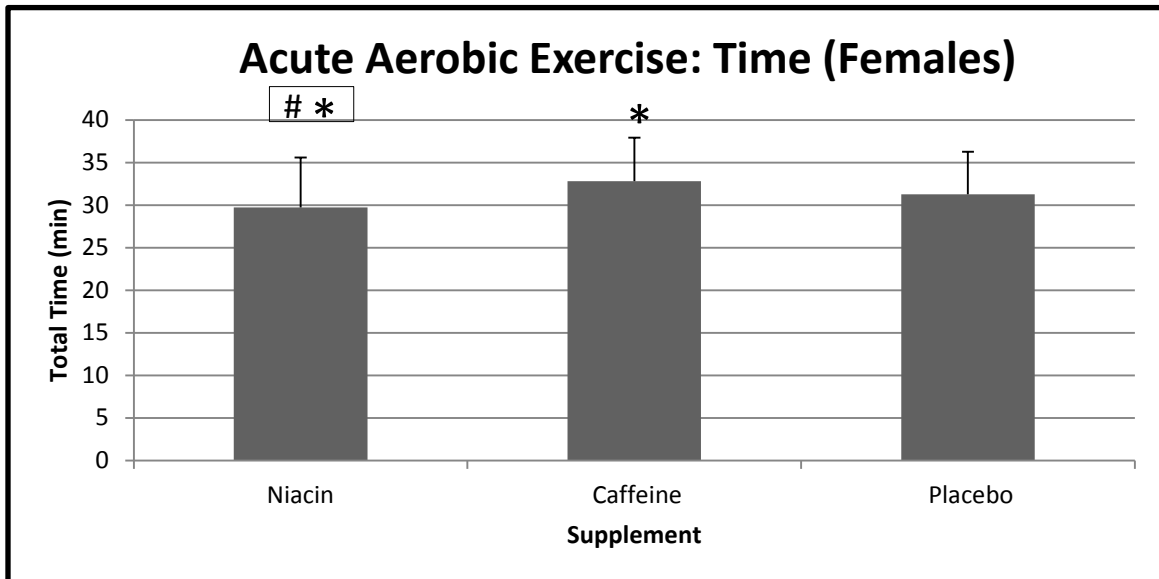
B.

Figure 1.3. Comparison of time to exhaustion for niacin, placebo, and caffeine exercise trails in minutes (min) (mean ± SD) for males (A) and females (B). *p < 0.05 compared to placebo treatment. # p < 0.05 compared to caffeine treatment. (Males: N = 24.4 ± 3.8 vs. P = 25.5 ± 3.1 vs. C = 27 ± 3.4; Females: (N = 29.7 ± 5.6 vs. P = 31.3 ± 5.0 vs. C = 32.8 ± 5.1).

Placebo   Niacin   Caffeine

Subject 2   -   +   -   +   -   +

Adiponectin
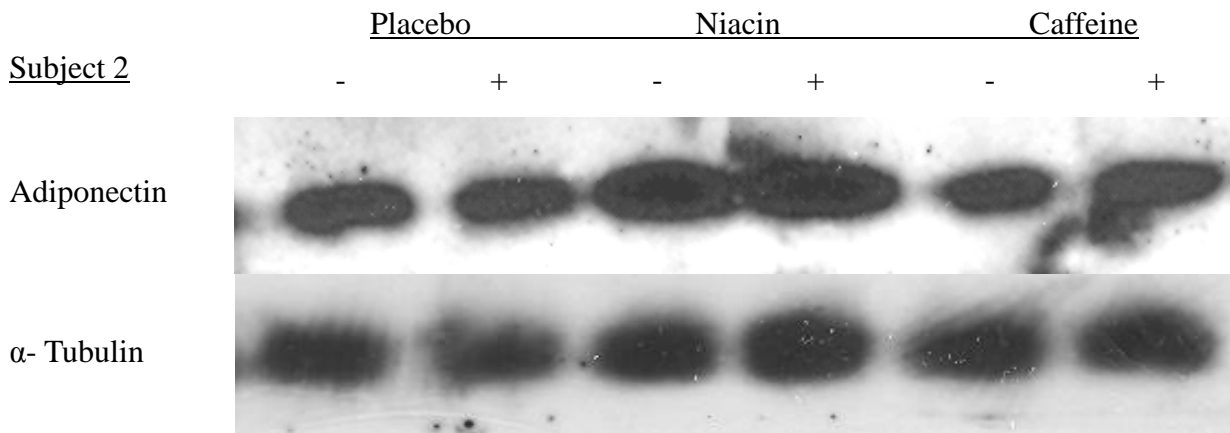
α- Tubulin

Figure 1.4. Acute Aerobic Exercise. Western blot comparison of plasma adiponectin (~ 31 kda) for placebo, niacin, and caffeine exercise trials pre- and post- exercise for subject 2. α-Tubulin (~ 55 kda) served as a loading control. - Pre-Exercise Intervention; + Post-Exercise Intervention.
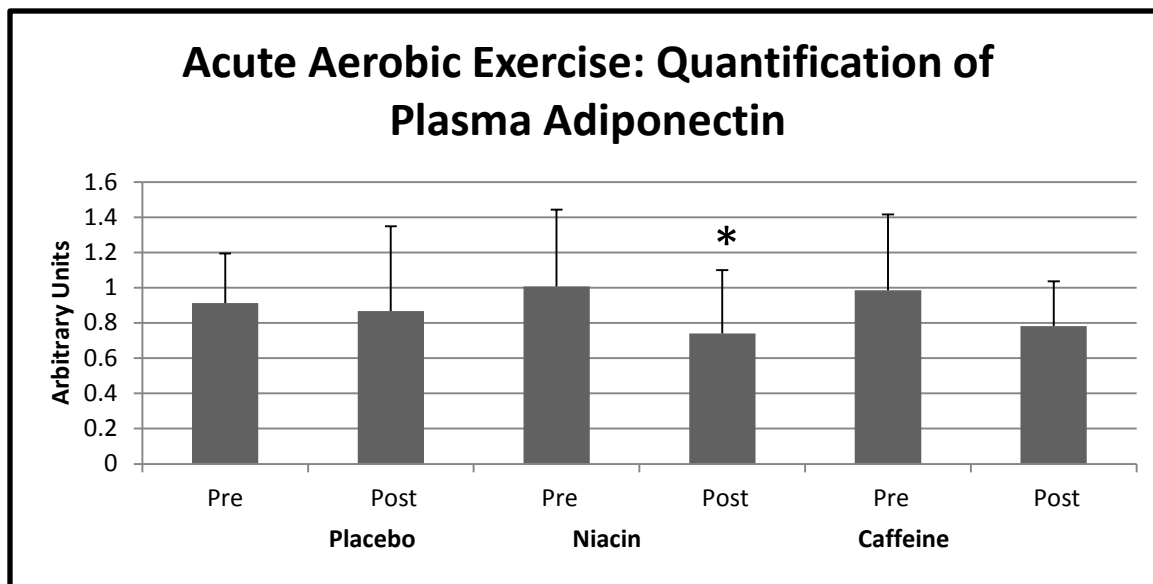
Figure 1.5. Quantitative comparison of plasma adiponectin for placebo, niacin, and caffeine exercise trials. Area under the curve for each plasma sample was calculated and normalized against the loading control. Results are given in arbitrary units (mean ± SD) for all male subjects (n = 17). (P Trial: Pre = 0.91 ± 0.28, Post = 0.87 ± 0.48; N Trial: Pre = 1.01 ± 0.44, 0.74 ± 0.36, C Trial: Pre = 0.99 ± 0.43, Post = 0.78 ± 0.25).

Table 8. Acute Aerobic Exercise.

Adiponectin Correlations

FH+	-	-
Reg. AEX.	**r = .28**	**p = 0 .05**
Reg. Rex	r = -.08	NS
Reg. Caff.	**r = .32**	**p = 0.03**
BF%	r = -.14	NS
BMI	r = -.06	NS
RER	r = -.14	NS

Average plasma adiponectin concentration compared to various correlates. Reg AEX. Regular Aerobic Exercise; BMI Body Mass Index; RER Respiratory Exchange Ratio; Reg. Caff. Regular Caffeine Consumption; NS non-significant; statistics represent data from all subjects (n = 17).

3.2 Chronic Aerobic Exercise

There were no significant differences among the groups for any of the measured parameters at baseline. Significant improvements were observed for estimated VO_{2max} in the E group (37.38 ± 2.39 ml/kg/min pre-intervention to 42.32 ± 7.67 ml/kg/min post-intervention) (mean ± SD) p = 0.02, but not the C group (36.85 ± 2.98 ml/kg/min pre to 39.79 ± 4.46 ml/kg/min) (Fig 2.1). The 1.5 mile run time changed 8.5% from 13.57 ± 1.3 to 12.41 ± 2.7 minutes (mean ± SD), and the C group changed from 14.24 ± 1.7 to 13.31 ± 2.0 minutes (mean ± SD) neither of which reached significance (p > 0.05).

The E group decreased body fat significantly from 29.4% ± 8.3 to 27.7% ± 8.7 (mean ± SD) p = 0.04 (Fig. 2.2). The percent change for this reduction in body fat was 1.7%; however, the reduction in total body weight was not significant. There was no significant change in body fat for the C group. The C group and E group had no significant changes in lean tissue mass pre- to post- exercise.

Plasma adiponectin was not significantly different pre- to post-intervention in the E group

or the C group (Fig 2.3), however, plasma adiponectin was significantly lower in the C group for

both pre- and post-intervention time points p = 0.05 (Fig 2.4).

Simple linear regression was used to determine correlations between average plasma

adiponectin concentration (Adpn) and: VO_{2max}, waist-to-hip ratio (WHR), body fat percentage

(BF %), BMI, android percent fat (And.), and gynoid percent fat (Gyn.) for each subject (Table

9). No correlates were found to be significant (p > 0.05).
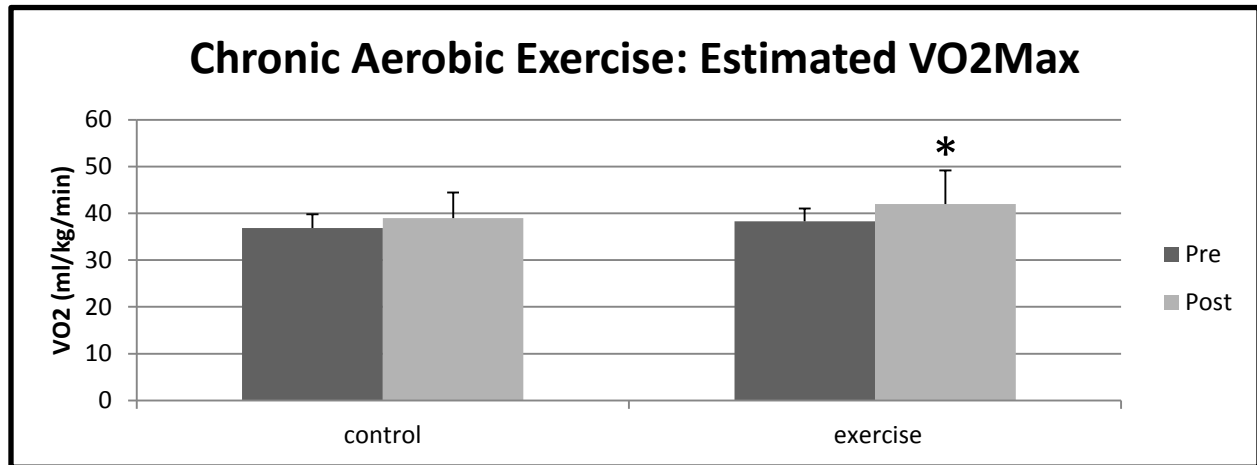
Figure 2.1. Comparison of estimated VO_{2max} pre- to post-exercise between C and E groups
(mean ± SD). * p = 0.02 compared to pre-exercise intervention. (E Group: pre = 37.38 ± 2.39
ml/kg/min, post = 42.32 ± 7.67 ml/kg/min; C Group: pre = 36.85 ± 2.98 ml/kg/min, post = 39.79
± 4.46 ml/kg/min).
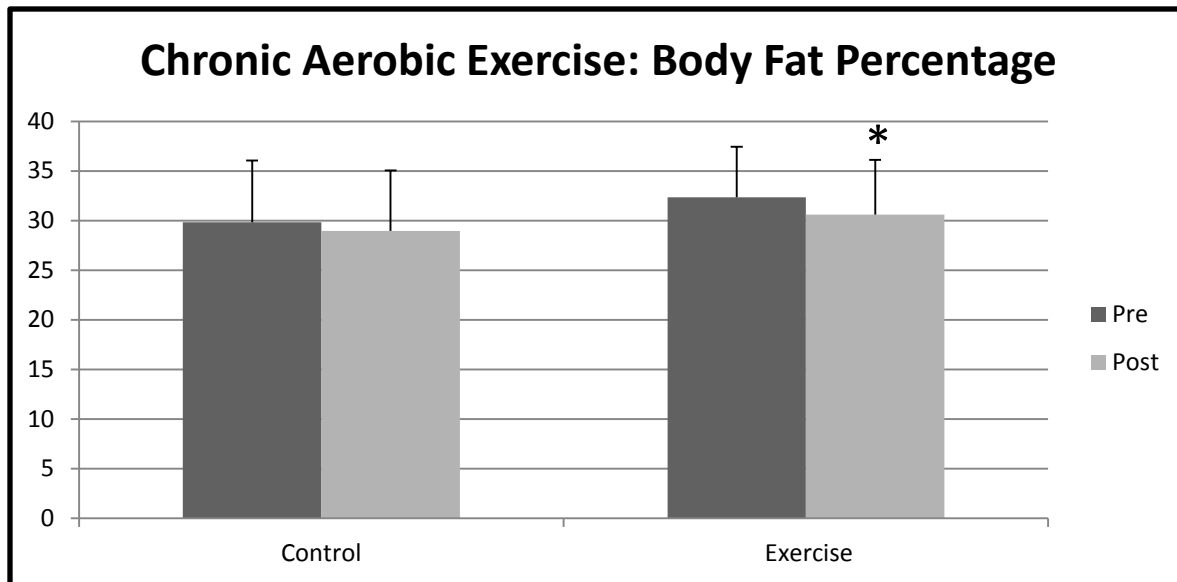
Figure 2.2. Comparison of body fat percentage pre- to post-exercise between C and E groups (mean ± SD). * p = 0.04 compared to pre-exercise intervention. (E Group: pre = 32.36 ± 5.09, post = 30.62 ± 5.5%; C Group: pre = 30.17 ± 5.9, post = 28.9 ± 6.0%).
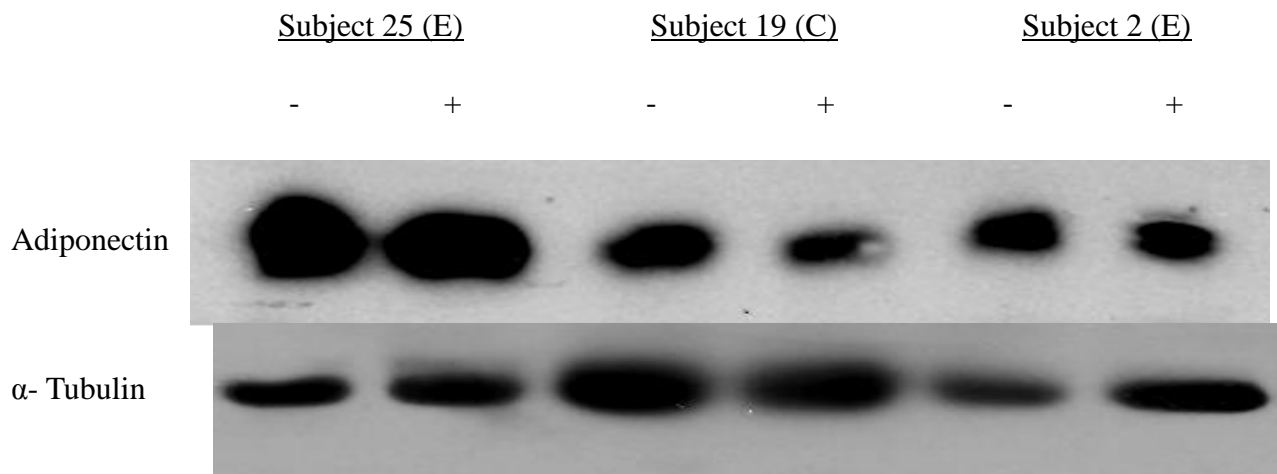
Figure 2.3. Chronic Aerobic Exercise. Western blot comparison of plasma adiponectin (~ 31 kda) for pre- and post- exercise time points for subject 25, 19, and 2. α-Tubulin (~ 55 kda) served as a loading control. - Pre-Exercise Intervention; + Post-Exercise Intervention.

Figure 2.4. Quantitative comparison of plasma adiponectin for C and E group pre- and post-intervention. Area under the curve for each plasma sample was calculated and normalized against the loading control. Results are given in arbitrary units (mean ± SD) for all subjects (n = 11 for both groups). * p = 0.04 compared to control group. (E Group: pre = 1.41 ± 0.66, post = 1.51 ± 0.69; C Group: pre = 1.06 ± 1.06, post = 0.88 ± 0.95).

Table 9. Chronic Aerobic Exercise.

Adiponectin Correlations

VO_{2max}	r = .17	NS
WHR	r = -.21	NS
BMI	r = -.12	NS
BF %	r = .09	NS
And.	r = -.15	NS
Gyn.	r = -.15	NS

Average plasma adiponectin concentration compared to various correlates. WHR Waist-to-hip ratio; BMI Body Mass Index; BF% Body Fat Percentage; And. Android percent fat; Gyn. Gynoid percent fat; NS non-significant; statistics represent data from all subjects (n = 22).

3.3 Acute Resistance Exercise

Blood glucose significantly increased pre-to post-exercise (pre = 4.72 mmol/l ± 0.45, post = 5.21 mmol/l ± 0.49). The increase in blood glucose immediately following exercise was

10.4 %. There was no significant difference in blood glucose 24-hours post-exercise compared to pre-exercise levels (24 = 4.71 mmol/l $\pm$ 0.43) (Fig. 3.1). The control trial did not yield any significant differences in blood glucose from pre- to immediately post- or 24 hours post-exercise (pre = 4.70 mmol/l $\pm$ 0.41, post = 4.70 mmol/l $\pm$ 0.44 vs. 24 = 4.72 mmol/l $\pm$ 0.45) (mean $\pm$ SD).

Despite apparent changes in glucose utilization, plasma adiponectin was not significantly different pre- to post-intervention for the exercise trial nor was adiponectin significantly different 24 hours post-exercise or between pre- post- and 24 hours post-control trial (Fig 3.2).

Simple linear regression was used to determine correlations between average plasma adiponectin concentration (Adpn) and: resting blood glucose, relative strength (RS), body mass index (BMI), body fat percentage (BF%), regular caffeine consumption (Reg. Caff), regular aerobic exercise (Reg. Aex), regular resistance exercise (Reg. Rex), and first degree family history of type 2 diabetes mellitus (FH+). Reg. Aex (r = 0.44) and RS (r = 0.43) were significantly correlated with average plasma adiponectin (p < 0.01) and FH+ (r = -0.56) was significantly inversely correlated with average plasma adiponectin (p < 0.01). No other correlates were significant (Table 10).
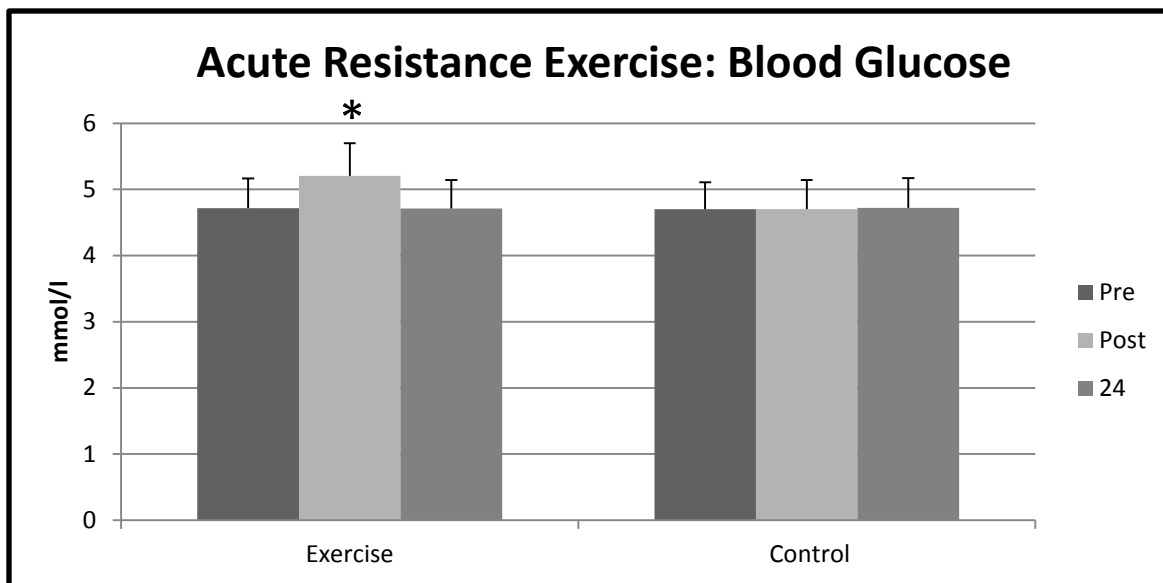
Figure 3.1 Blood glucose concentration pre-exercise, immediately post-exercise, 24 hours post intervention. * $p < 0.01$ compared to the pre-exercise time point. (E Trial: pre = 4.72 ± 0.45, post = 5.21 ± 0.49, 24 = 4.71 ± 0.43; C Trial: pre = 4.70 ± 0.41, post = 4.70 ± 0.44, 24 = 4.72 ± 0.45 mmol/l).
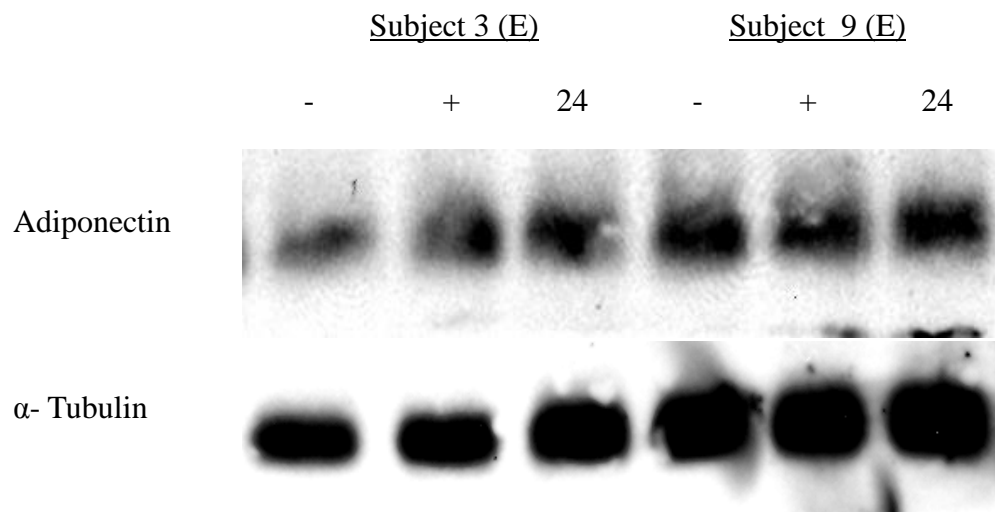
Figure 3.2. Acute Resistance Exercise. Western blot comparison of plasma adiponectin (~ 31 kda) for pre-, post-, and 24 hours post- exercise time points for subject 3 and 9. α-Tubulin (~ 55 kda) served as a loading control. - Pre-Exercise Intervention; + Post-Exercise Intervention; 24 24 hours-post intervention.
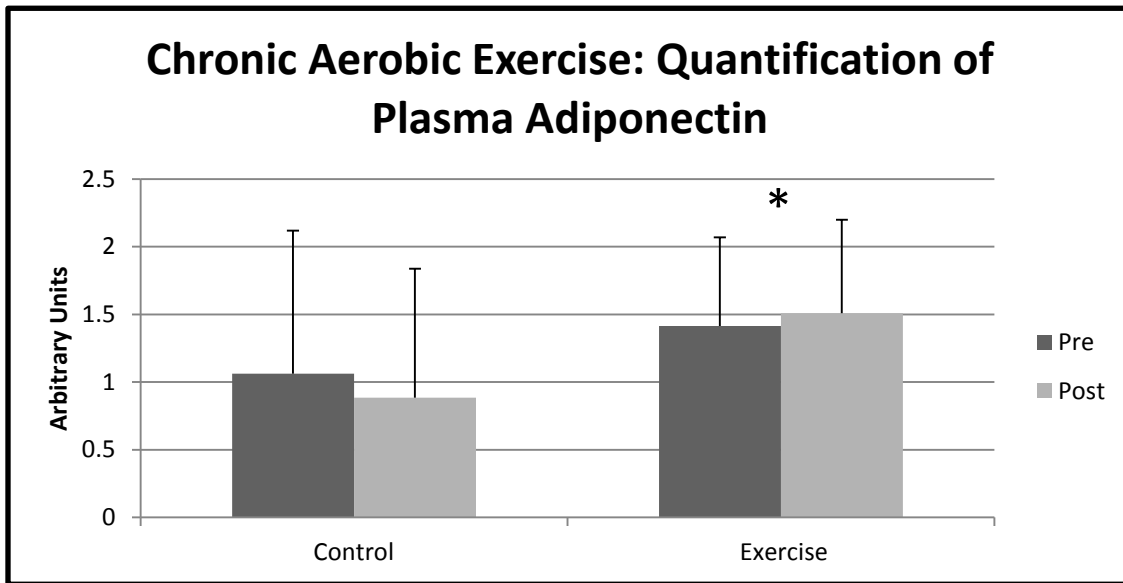
Acute Resistance Exercise: Quantification of Plasma Adiponectin
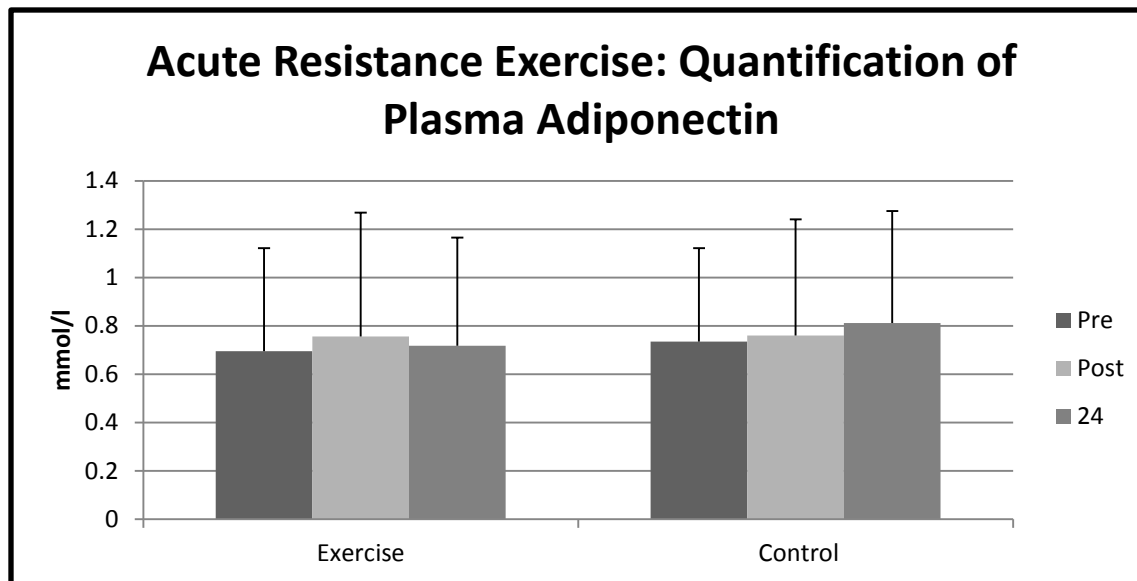
Figure 3.3. Quantitative comparison of plasma adiponectin for pre- post- and 24 hours post-intervention. Area under the curve for each plasma sample was calculated and normalized against the loading control. Results are given in arbitrary units (mean $\pm$ SD) for all subjects (n = 17). (E Trial: pre = 0.69 $\pm$ 0.43, post = 0.75 $\pm$ 0.51, 24 = 0.72 $\pm$ 0.45; C Trial: pre = 0.74 $\pm$ 0.39, post = 0.76 $\pm$ 0.48, 24 = 0.81 $\pm$ 0.46 AU).

Table 10. Acute Resistance Exercise.

Adiponectin Correlations		
RBG	r = -.03	NS
RS	**r = .42**	**p < 0.01**
BMI	r = -.12	NS
BF%	r = -.20	NS
Reg. Caff.	**r = .45**	**p < 0.01**
Reg. Aex.	r = .22	NS
Reg. Rex	r = .18	NS
FH+	**r = -.56**	**p < 0.01**

Average plasma adiponectin concentration compared to various correlates. RBG Resting Blood Glucose; RS Relative Strength; BMI Body Mass Index; BF% Body Fat Percentage; Reg. Caff. Regular Caffeine Consumption; Reg Aex. Regular Aerobic Exercise; Reg. Rex Regular Resistance Exercise; FH+ First Degree Family History of Type 2 Diabetes Mellitus; NS non-significant; statistics represent data from all subjects (n = 17).

3.4 Chronic Resistance Exercise

Strength, as measured by 1RM increased significantly following training (Fig 4.1). Bench press increased from 97.7±18.4 kilograms (kg) to 101.8±16.2 kg, an 8.2% increase. Squat increased from 129.6±30.3 kg to 143± 28.3 kg, a 13.4% increase. Deadlift increased from 126.3±35.7 kg to 137.2±33.2 kg, a 12.2% increase.

Resting blood glucose did not change significantly in the E group (pre = 4.87 ± 0.34 mmol/l, post = 4.84 ± 0.36 mmol/l) or the C group (pre = 4.94 ± 0.43 mmol/l, post = 4.83 ± 0.42 mmol/l) (Fig 4.2).

Body fat percentage did not change significantly in the E group (pre = 18.38 ± 4.94%, post = 17.73 ± 5.42%) or the C group (pre = 21.49 ± 2.02, post = 21.19 ± 3.04) and neither group had significantly different body fat percentage at the pre- nor post-intervention time points (Fig. 4.3).

Plasma adiponectin did change significantly in the E group (pre = 0.90 ± 0.48 AU, post = 1.00 ± 0.59 AU) but not the C group (pre = 1.04 ± 0.37, post = 1.15 ± 0.53 AU) and neither group had significantly different concentrations of plasma adiponectin at the pre- or post-intervention time points (Fig. 4.4).

Simple linear regression was used to determine correlations between average plasma adiponectin concentration (Adpn) and: resting blood glucose (RBG), relative strength (RS), body mass index (BMI), body fat percentage (BF%), regular caffeine consumption (Reg. Caff), regular aerobic exercise (Reg. Aex), regular resistance exercise (Reg. Rex), and first degree family history of type 2 diabetes mellitus (FH+). Reg. Aex (r = 0.32) was significantly correlated with

average plasma adiponectin ($p < 0.05$) and FH+ ($r = -0.58$) was significantly inversely correlated with average plasma adiponectin ($p < 0.01$). No other correlates were significant (Table 11).

Chronic Resistance Exercise: 1RM
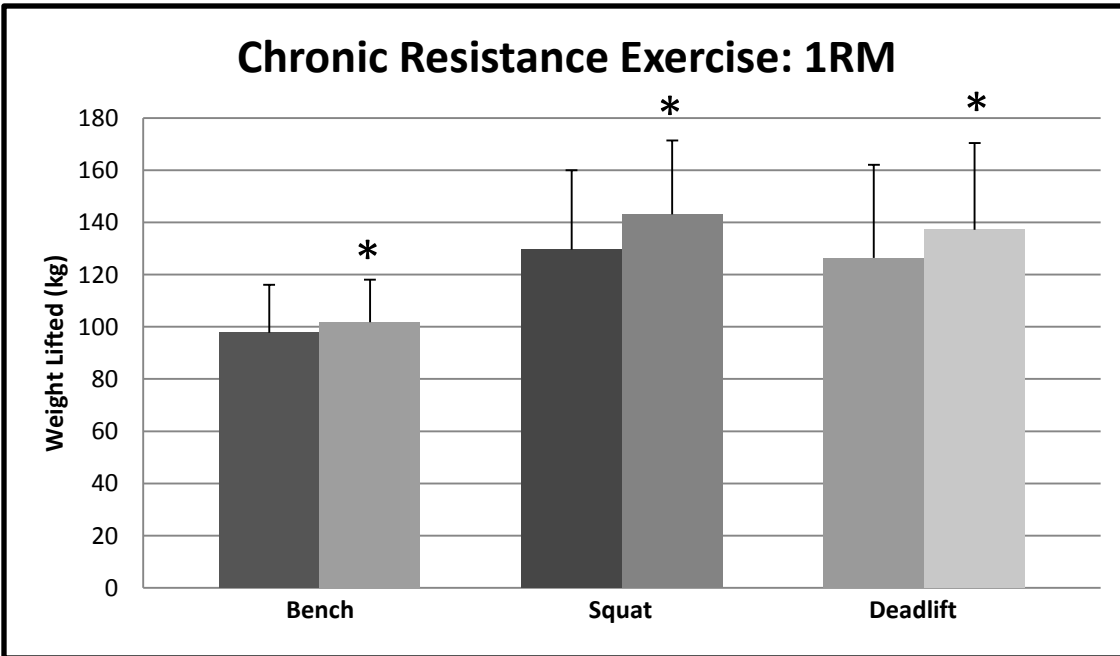
Figure 4.1. Absolute weight lifted in kilograms (kg) for a one-repetition maximum lift effort (1RM) (means ± SD). * P < 0.01 vs. baseline measurements. Dark bars, pre-intervention; Light bars, post-intervention (n = 18).
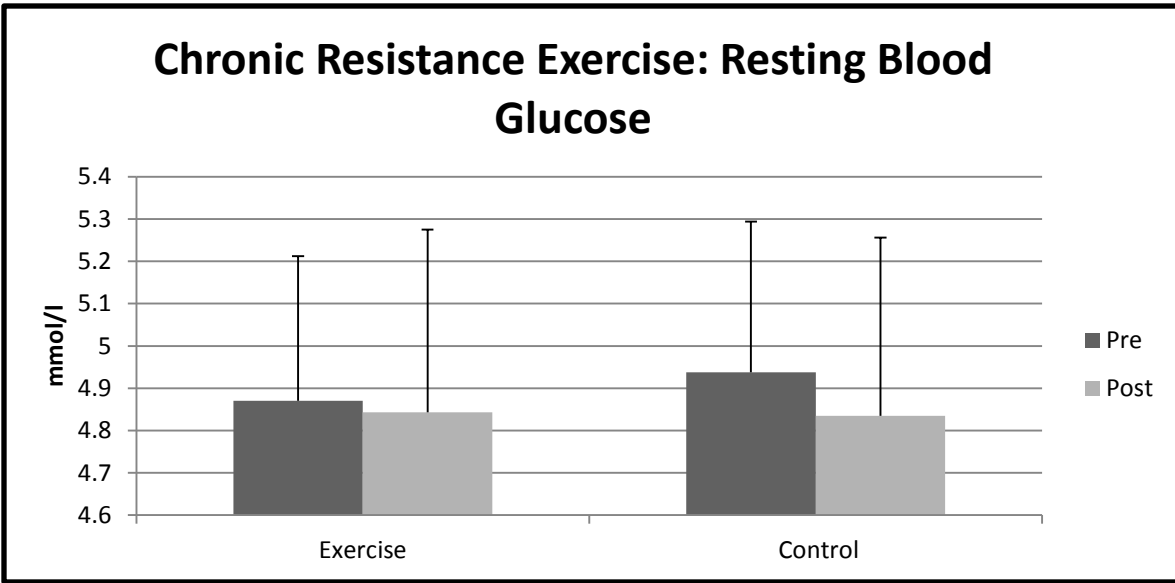
Figure 4.2. Comparison of resting blood glucose between E and C groups pre- and post-intervention. There were no significant differences pre- to post-exercise for the E or C group and there were no significant differences between groups.
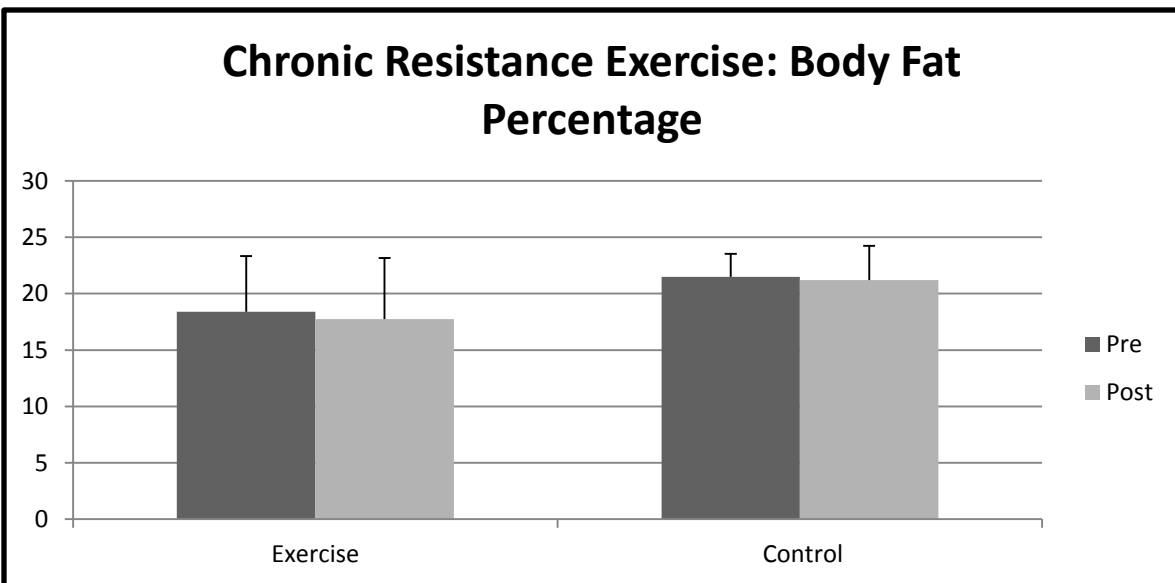
Figure 4.3. Comparison of body fat percentage between E and C groups pre- and post-intervention. There were no significant differences pre- to post-exercise for the E or C group and there were no significant differences between groups.
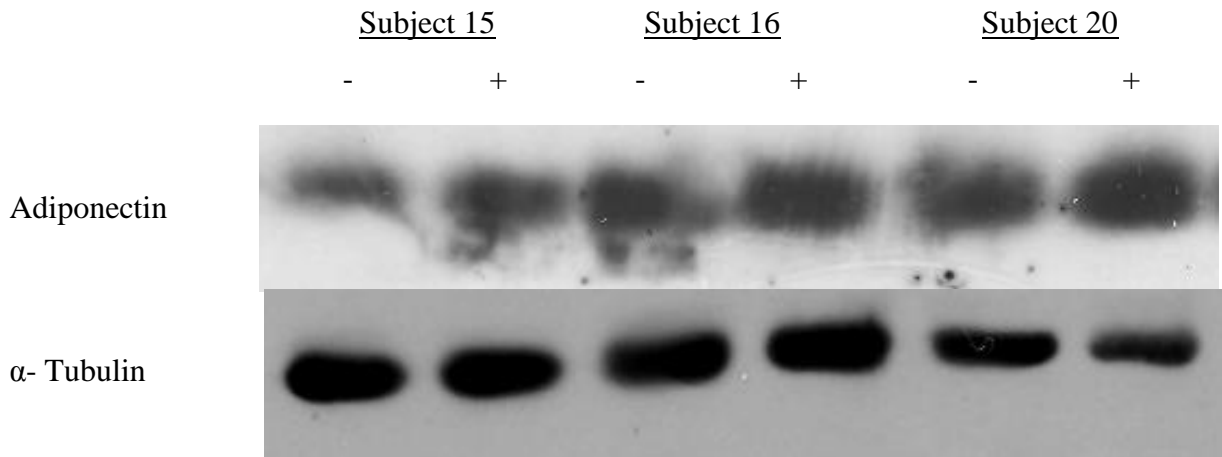
Figure 4.4. Chronic Resistance Exercise. Western blot comparison of plasma adiponectin (~ 31 kda) for pre- and post-exercise time points for subjects 15, 16, and 20. α-Tubulin (~ 55 kda) served as a loading control. - Pre-Exercise Intervention; + Post-Exercise Intervention.

Figure 4.5. Quantitative comparison of plasma adiponectin for pre- and post-intervention. Area under the curve for each plasma sample was calculated and normalized against the loading control. Results are given in arbitrary units (mean ± SD) for all subjects (n = 36). *p < 0.05 compared to control.

Table 11. Chronic Resistance Exercise.

Adiponectin Correlations

RBG	r = .14	NS
RS	r = .27	NS
BMI	r = -.08	NS
BF %	r = .08	NS
Reg. AEX.	r = .36	p = 0.02
Reg. Caff.	r = .25	NS
FH+	r = -.58	p < 0.01

Average plasma adiponectin concentration compared to various correlates. RBG Resting Blood Glucose; RS Relative Strength; BMI Body Mass Index; BF% Body Fat Percentage; Reg. Caff. Regular Caffeine Consumption; Reg Aex. Regular Aerobic Exercise; Reg. Rex Regular Resistance Exercise; FH+ First Degree Family History of Type 2 Diabetes Mellitus; NS non-significant; statistics represent data from all subjects (n = 18).

3.5 Acute Stretching Exercise

Stretching did significantly increase RER. Post-hoc analysis revealed that supplementation did not significantly affect RER, regardless of whether subjects were stretching or not. The average RER for the placebo stretching trial was 0.72, average RER for the melatonin stretch trial was 0.70, average RER for the placebo control trial was 0.66, and average RER for the melatonin control trail was 0.64 (Fig 5.1).

Plasma adiponectin did not change significantly as a result of supplementation pre- or post-exercise, (M Trial: 0.99 ± 0.37; P Trial: 1.01 ± 0.38 AU) (Fig. 5.2 & 5.3a) nor as a result of exercise (pre = 0.99 ± 0.40; post = 1.02 ± 0.36 AU) (Fig 5.3b) and there was no interaction effect for supplement and exercise on plasma adiponectin concentration (p = 0.25). (PC Trial: Pre = 0.91 ± 0.47, Post = 1.18 ± 0.34; PS Trial: Pre = 0.90 ± 0.76, Post = 0.74 ± 0.59; MC Trial: Pre = 1.00 ± 0.49, Post = 1.02 ± 0.56; MS Trial: Pre = 1.18 ± 0.30, Post = 1.22 ± 0.47) (Fig 5.3c).

Simple linear regression was used to determine correlations between average plasma adiponectin concentration (Adpn) and: respiratory exchange ratio (RER), body mass index (BMI), body fat percentage (BF%), regular caffeine consumption (Reg. Caff), regular aerobic exercise (Reg. Aex), regular resistance exercise (Reg. Rex), and first degree family history of type 2 diabetes mellitus (FH+). FH+ (r = -0.14) was significantly inversely correlated with average plasma adiponectin (p = 0.04). No other correlates were significant (Table 12). No participants regularly consumed caffeine and therefore, no values are provided for the variable.
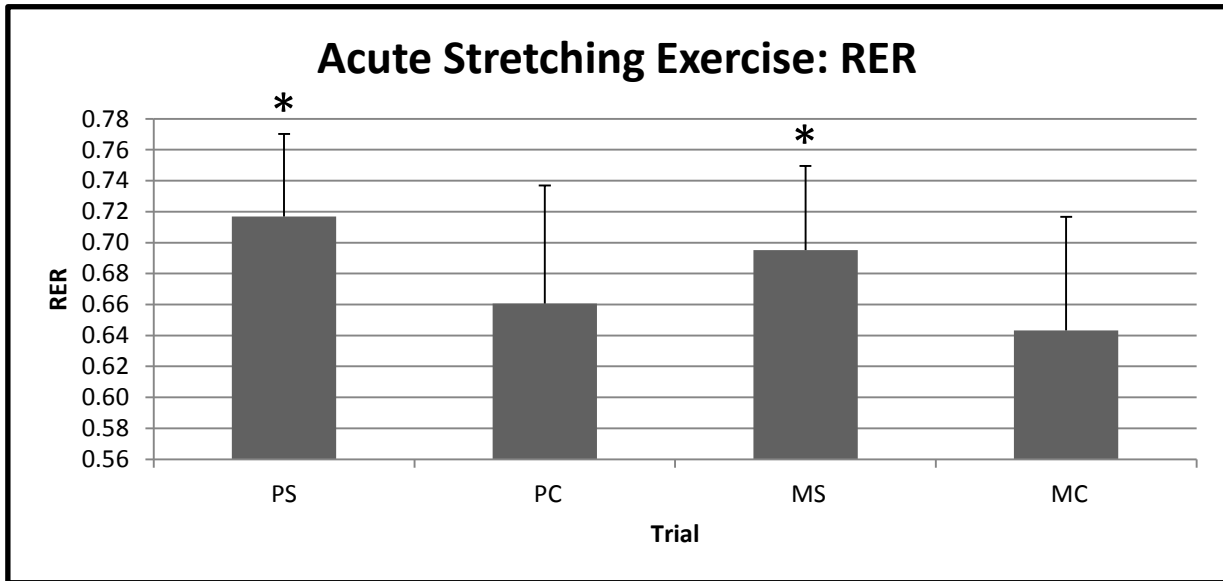
Figure 5.1. Respiratory exchange ratio (RER) measured via indirect calorimetry (means ± SD) for placebo and melatonin trials (n = 21). *$p < 0.05$ vs. control trial. PS, placebo stretch trial; PC, placebo control trial; MS, melatonin stretch trial; MC, melatonin control trial.

|	Placebo (S)		Placebo (C)		Mel (S)		Mel (C)	
	-	+	-	+	-	+	-	+

Adiponectin
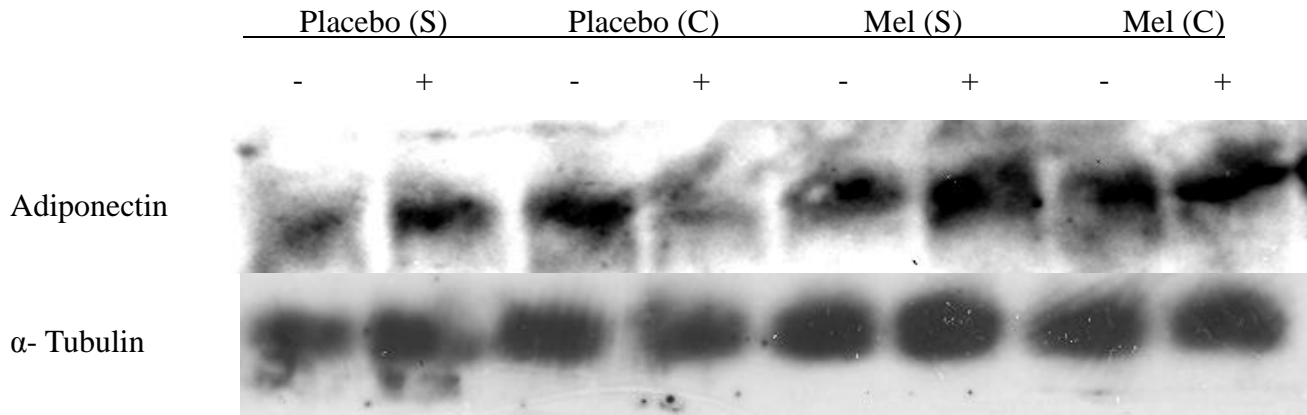
α- Tubulin

Figure 5.2. Acute Stretching Exercise. Western blot comparison of plasma adiponectin (~ 31 kda) for pre- and post-exercise time points for subjects for PS, PC, MS, and MC trials. α-Tubulin (~ 55 kda) served as a loading control. - Pre-Exercise Intervention; + Post-Exercise Intervention.
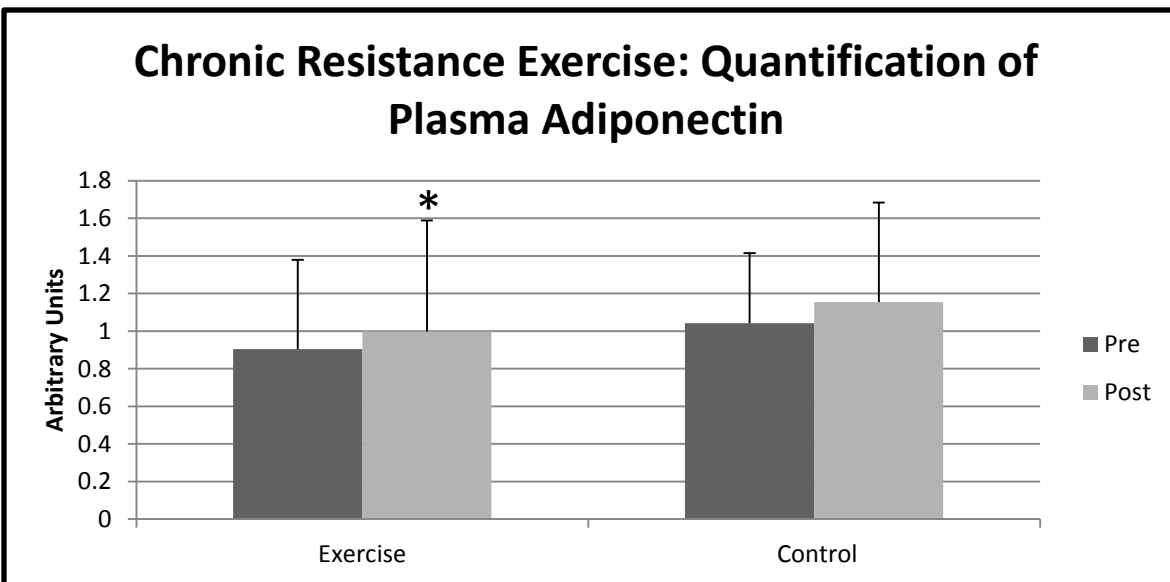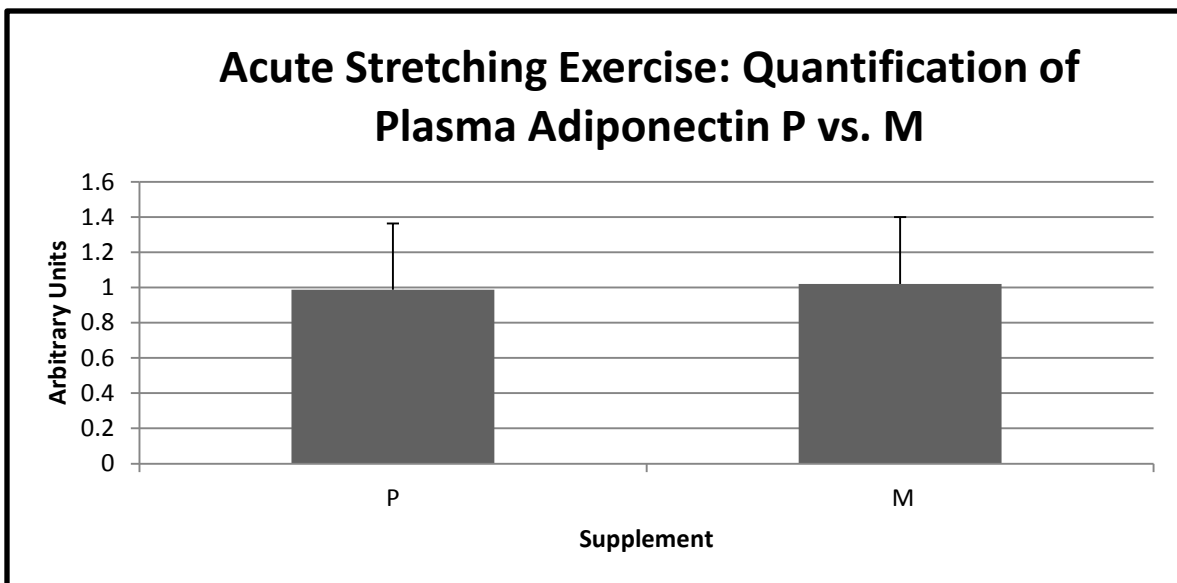

A. Figure 5.3. Comparison of plasma adiponectin between supplements (A). No significant differences were found for supplement. M Melatonin; P placebo; statistics represent data from all subjects (n = 21).
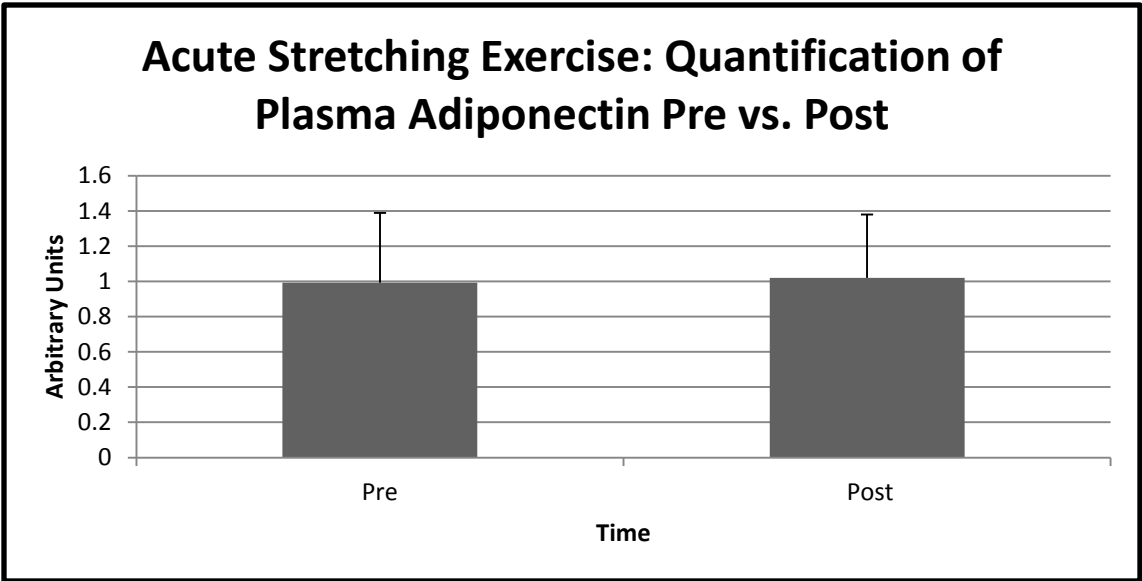
B.
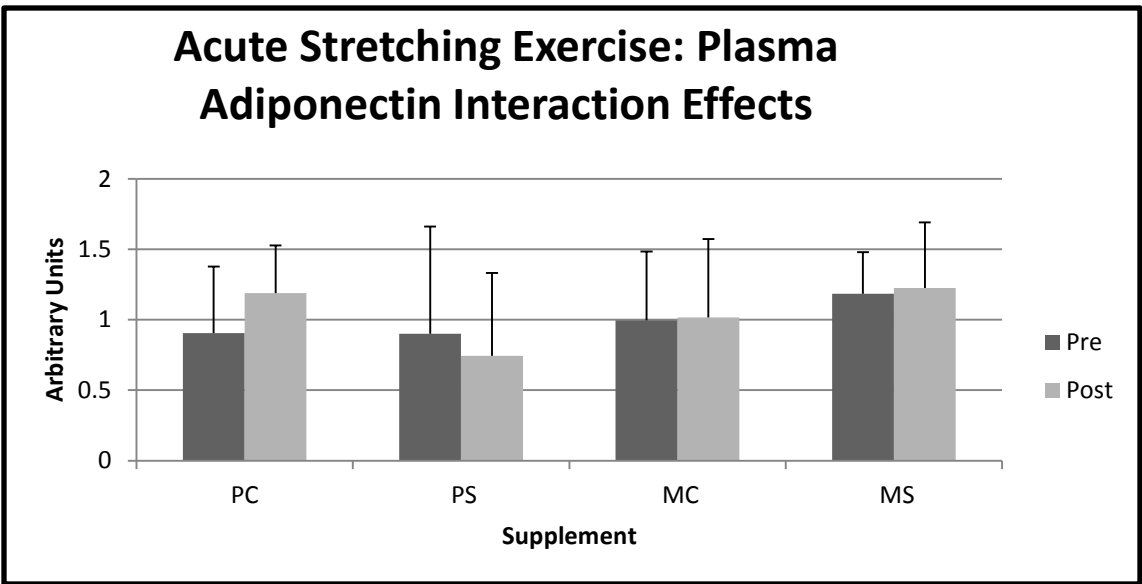
C.

Figure 5.3. Comparison of plasma adiponectin between supplements (A), pre- to post-exercise (B), and the interaction effect of supplement and exercise (C). No significant differences were found for supplement, exercise, or interaction. - Pre-Exercise Intervention; + Post-Exercise Intervention; S Stretch; C Control; M Melatonin; P placebo; statistics represent data from all subjects (n = 21).

Table 12. Acute Stretching Exercise.
Adiponectin Correlations

RER	-0.19	NS
BMI	-0.11	NS
BF %	-0.15	NS
Reg. Caff	-	-
Reg. Aex	0.24	NS
Reg. Rex	-0.20	NS
FH+	-0.14	$p = 0.04$

Average plasma adiponectin concentration compared to various correlates. RER Respiratory Exchange Ratio; BMI Body Mass Index; BF% Body Fat Percentage; Reg. Caff. Regular Caffeine Consumption; Reg Aex. Regular Aerobic Exercise; Reg. Rex Regular Resistance Exercise; FH+ First Degree Family History of Type 2 Diabetes Mellitus; NS non-significant; statistics represent data from all subjects (n = 21).

CHAPTER 4. DISCUSSION

4.1 Substrate Metabolism and Adiponectin

Changes in substrate utilization during exercise do not appear to directly regulate plasma adiponectin concentration. Despite significant changes in substrate metabolism, measured via RER and BG, during and immediately following exercise, plasma adiponectin concentration was largely unaltered. It is worth noting that changes in metabolism may still alter plasma adiponectin but not immediately post-exercise. Previous research has demonstrated that niacin supplementation suppresses lipolysis in adipocytes (103), mitigates plasma FA, and increases plasma adiponectin (96). The current study did not yield any significant increases in adiponectin following niacin supplementation; in fact, the combination of niacin and aerobic exercise significantly attenuated plasma adiponectin post-exercise. The aforementioned study was performed on mice and the dosage of niacin was 30 mg/kg of body weight, which was approximately twice the dosage administered in the current study. It is likely if a higher dose of niacin had been administered, adiponectin would have significantly increased.

Niacin supplementation negatively affected acute aerobic exercise performance in the current study, which may be partially attributed to changes in substrate utilization, as indicated by changes in RER, though there are clearly other factors, such as catecholamine release involved as well. Carlson et al. (103) suggested that niacin supplementation does not directly affect free fatty acid (FFA) uptake or oxidation, rather it inhibits norepinephrine from binding at its receptor site, which in turn, inhibits FFA mobilization. Therefore, niacin has directly opposing mechanisms to those of caffeine to alter substrate utilization, as discussed below.

Taken together, these results suggest that even for high-intensity aerobic exercise lasting less than 30 minutes, impaired lipid metabolism negatively affects exercise performance.

The reasons for the significant attenuation in plasma adiponectin following niacin supplementation post-exercise in the current study are not readily apparent, but may have been due to decreased lipid oxidation or increased plasma glucose concentration. During the niacin trial, RER was significantly higher compared to the placebo trial, which suggests decreased lipid utilization and increased glucose utilization. Increased plasma lipids are a likely candidate for decreasing the expression of adiponectin. In addition, increased plasma glucose is a major characteristic of insulin resistant and diabetic populations expressing low levels of plasma adiponectin (69). Thus, a combination of these two factors may have driven down the expression of adiponectin. Although high-intensity exercise elicits significant increases in growth hormone and glucocorticoids, the placebo and caffeine trials did not yield significant changes in plasma adiponectin. These findings support previous research which suggests adiponectin is not directly regulated by endocrine responses (77, 81).

In contrast to the effects of niacin, previous research has indicated that caffeine augments lipolysis and possibly lipid oxidation as well (89, 93). Caffeine supplementation has been shown to elicit a greater benefit for aerobic performance compared to placebo in the current and previous studies (94, 104), as measured by time to exhaustion. A study by Graham & Spriet (89), suggested that 6 mg/kg of body weight of a caffeine supplement improved exercise performance and increased plasma epinephrine concentration. Epinephrine acts to increase both lipid and glucose substrate availability; although, epinephrine seems to have a greater effect on lipolysis than glycogenolysis (105). Previous studies have also demonstrated that caffeine supplementation increased FFA mobilization from adipose tissue, lowered RER, and spared

muscle glycogen during submaximal acute exercise lasing 30-60 minutes (89). Since plasma lipid metabolites were not elevated in the study by Grahm & Spriet (89), it is likely that FFA mobilization as well as uptake and oxidation were up-regulated. Compared to the placebo trial, the caffeine trial of the current study did not demonstrate significantly lower RER values during exercise, which suggests that caffeine increases lipolysis, but not necessarily lipid oxidation. These results may also explain why plasma adiponectin did not significantly change post-exercise for the caffeine trial. These conflicting results are likely due to differences in exercise intensity. The current study incorporated higher intensity exercise compared to previous studies. Untrained individuals primarily oxidize glucose at high exercise intensities and therefore, the effects of increased catecholamines may have been overridden by other factors such as lactate accumulation and decreased blood flow to adipose tissue, thereby limiting lipid oxidation.

The current study revealed that regular caffeine consumption was significantly correlated with adiponectin. This is in agreement with a previous research study involving both diabetic and non-diabetic women which found habitual coffee consumption correlated with higher levels of adiponectin (106). This is an interesting finding given that caffeine increases lipolysis but not necessarily lipid oxidation (91). Although the cellular mechanisms responsible for increased expression of adiponectin with caffeine consumption are currently unknown, it has been suggested that regular consumption of caffeine may act to down-regulate inflammatory markers associated with low levels of adiponectin (107). It is also possible that habitual caffeine consumption may down-regulate β-adrenergic receptor sensitivity, and consequently attenuate the lipolytic effects of caffeine; however, more research needed to fully investigate these hypotheses.

69

Melatonin supplementation has been shown to decrease plasma glucose, increase glucose transport, and increase glucose oxidation during and post-exercise (98, 108). Melatonin has also been shown to decrease plasma adiponectin in rats given a high fat diet (99). Therefore it seems feasible that melatonin may decrease lipid uptake and oxidation, thereby decreasing plasma adiponectin. The current study; however, did not demonstrate any significant changes in plasma adiponectin following melatonin supplementation nor did the combination of exercise and melatonin have any significant effect on plasma adiponectin. The discrepancies in these findings may be explained by the lack of a high fat diet in the current study, which may have eliminated any detectible changes in plasma adiponectin. Interestingly, melatonin supplementation did not significantly affect RER in the current study, suggesting that substrate utilization was not altered following the administration of this supplement. Since previous melatonin supplement research has revealed changes in substrate utilization during and following exercise, it seems likely that the stretching exercise modality was not metabolically challenging enough to elicit any significant changes in substrate utilization. Whether or not other exercise modalities, in combination with melatonin supplementation, are sufficient to alter plasma adiponectin concentrations remains to be determined.

4.2 Effects of Chronic Exercise Modalities

During exercise, substrate metabolism is primarily dependent upon training status, exercise intensity, and duration (39); however, the current study suggests that substrate metabolism following chronic training is affected, at least to some extent, by exercise modality as well. Plasma adiponectin concentration following chronic training also appears to be somewhat dependent on exercise modality, although the link between substrate metabolism and adiponectin remains unclear.

70

The current study revealed that chronic resistance training significantly increased plasma adiponectin concentration whereas chronic aerobic training did not significantly increase adiponectin. Combined, these results further suggest that substrate metabolism does not directly regulate plasma adiponectin concentration. Chronic exercise modality; however, does have a significant effect on substrate utilization. The present study did not reveal any significant changes in resting blood glucose; however, the participant population for both the E and C groups were young, active, and insulin sensitive, therefore detecting significant changes in this variable was difficult. Although not directly measured for this exercise modality, it is likely that glucose utilization during exercise was up-regulated following chronic training. Previous studies have demonstrated that resistance-trained individuals appear to have a high percentage of type II, glycolytic fibers, and thus few mitochondria and a low capacity to oxidize fatty acids (61), but increased glycolytic enzyme activity (12) and capacity to oxidize carbohydrates (60).

Conversely, endurance-trained individuals demonstrate a high percentage of type I, oxidative fibers, and thus, an increased size and number of mitochondria and an elevated capacity to oxidize fatty acids (14). However, unlike resistance training, which exclusively increases the body's ability to utilize carbohydrate sources for energy, endurance training increases the body's ability to use all substrates. Following endurance training, GLUT-4 (109) and hexokinase (110) activity increase, gluconeogenic capacity has been shown to increase in rat liver (111), and glycogen is spared to a greater extent compared to sedentary controls (39). It has been suggested that this glycogen sparing in endurance-trained individuals is due to increased blood glucose availability and uptake, not increased lipid utilization and that catecholamines and glucagon raise less while insulin falls less following training, thereby sparing glycogen by increasing blood glucose utilization at low exercise intensities (39).

Despite the increased blood glucose utilization, lipid oxidation has been shown to significantly increase following chronic aerobic training (14). Lipid oxidation has also been shown to increase during long duration (> 1 hour) aerobic exercise (30). Participants for the current study's chronic aerobic training modality were training for a marathon and therefore, one-to-two sessions per week were greater than an hour in duration, on average. Although duration is a confounding variable for the present study, several studies have shown that chronic aerobic training of less than one hour elicits an up-regulation in lipid metabolism (16), thus, a chronic aerobic exercise training modality will increase the body's dependence on lipid metabolism during exercise. It is likely that this shift in lipid metabolism may partially account for the observed significant reduction in body fat following the chronic aerobic exercise modality, despite the lack of overall body weight reduction in the present study. While a shift in energy balance will result in total body weight reduction, body fat reduction in the absence of weight loss may be dependent on increased lipid oxidation.

In the present study, regular aerobic exercise weakly, but significantly correlated with adiponectin, which may suggest that this type of exercise limits plasma lipid concentration via increased lipid transport and storage, as previous research has suggested (69). Specifically, there may be increased lipid synthesis in the form of IMCL, given that this form of lipid storage is increased following endurance exercise (22). Further research examining lipogenic enzymes and transporters concomitantly with plasma adiponectin is needed.

Despite the correlation with regular aerobic exercise and plasma adiponectin, the chronic aerobic exercise intervention did not significantly increase adiponectin. Conversely, regular resistance training exercise did not significantly correlate with plasma adiponectin, whereas the chronic resistance training intervention did significantly increase adiponectin. The reasoning for

72

this apparent disconnect is likely due to both training status as well as exercise intensity and is consistent with the literature which shows an augmentation in adiponectin following some chronic exercise studies (87), but no changes in adiponectin following other chronic exercise studies (88). The less trained an individual and the higher the exercise intensity, the more likely it is that adiponectin will significantly increase as a result of training. In the present study, the chronic aerobic training modality was high-intensity marathon training; however, all participants were previously engaged in habitual, recreational aerobic exercise, and therefore these participants exhibited significantly higher levels of plasma adiponectin compared to the control group both at the onset of the study and following the exercise intervention, limiting the degree of change in this hormone as a result of exercise. Conversely, participants for the chronic resistance training modality did not engage in habitual physical activity prior to the exercise intervention. The chronic resistance training modality was high-intensity, well above the typical intensity for individuals that regularly engage in this type of exercise. Combined, these variables readily explain why plasma adiponectin increased following chronic resistance, but not aerobic training. The mechanisms for this change remain unknown. Further research examining lipogenic or glycogenic enzymes and plasma adiponectin in sedentary individuals may reveal specific mechanisms responsible for the changes in adiponectin concentration.

4.3 Effects of Acute Exercise Modalities

It should be noted that most studies examining complete substrate utilization during and following acute exercise consist of protocols that are upwards of two hours in duration (30, 47, 112) yet most individuals that participate in regular exercise for health purposes are not likely to engage in any type of exercise activity exceeding an hour in length in a given day. Thus, it was important to examine substrate utilization during and following less than one hour of activity.

73

Similar to chronic exercise modalities, the present study demonstrated that substrate metabolism during and following acute exercise modalities was primarily dependent upon intensity and habitual training modality. In contrast to chronic exercise modalities, acute exercise modality did not appear to have any effect on substrate utilization. The acute aerobic, acute resistance, and acute stretching modalities for the current study were similar in duration (approximately thirty minutes each) and the intensity for each modality was considered high-intensity, according to the 2011 American College of Sports Medicine (ACSM) position stand for exercise guidelines (113). This approach minimized potential confounding variables.

As expected, the acute aerobic exercise modality revealed that as exercise intensity increases, the body's dependence on glucose oxidation increases while the body's dependence on lipid oxidation decreases. Independent of the addition of supplements, this change in substrate metabolism as a result of exercise did not significantly change plasma adiponectin concentration. Previous acute aerobic exercise studies have shown decreases in plasma adiponectin (67), increases in HMW adiponectin (35), and no change in plasma adiponectin (86). However, decreases in total adiponectin were seen in elite athletes immediately post-exercise, increases in HMW adiponectin were seen in obese individuals, and no changes in total adiponectin were seen in healthy, untrained individuals. Therefore, the current results support the existing research on plasma adiponectin responses to acute aerobic exercise and further suggest that acute changes in adiponectin may be dependent on plasma lipid content, which is elevated in elite endurance athletes immediately post-exercise and is suppressed in sedentary, obese individuals immediately post-exercise.

The incorporation of supplements in concert with acute aerobic exercise revealed that caffeine enhances aerobic performance while niacin negatively affects aerobic of performance.

The underlying cause for this response is partially attributed to changes in substrate utilization as previously suggested, though the current study suggests that these changes are different between males and females. Previous studies have also demonstrated greater fat oxidation and higher β oxidative enzyme content in female skeletal muscle compared to male counterparts (114). However, the study also revealed that there were no significant differences between males and females for the transcription factor PPAR, which plays a major role in several genes involved in lipid metabolism and the regulation of adiponectin. Interestingly, females have exhibited higher plasma HMW adiponectin concentration (53) as well as total plasma adiponectin concentration (115) compared to males matched for BMI. It is likely that increased lipid oxidation in females may be partially responsible for the gender differences in adiponectin; therefore further research is warranted to investigate these issues.

The acute stretching exercise modality of similar duration and intensity (30 minutes at high-intensity) significantly increases RER compared to the control trials; however, plasma adiponectin did not significantly change post-exercise. This appears to be the first study to examine the effects of passive resistance stretching on adiponectin. Similar to acute aerobic exercise, it seems that even in the presence of substrate shift, plasma adiponectin is unaffected in young, healthy populations. The increase in RER and subsequent increase in glucose oxidation is likely due to increased muscle contraction. Increased muscle contraction alone does improve insulin sensitivity (40), although this improvement appears to be independent of adiponectin. Therefore, it may be suggested that while improvements in insulin sensitivity as a result of chronic exercise training are partially due to increased plasma adiponectin, improvements in insulin sensitivity as a result of acute exercise appear to be independent of plasma adiponectin. Acute high-intensity, passive-resistance stretching exercise will result in only minimal increases

in overall energy expenditure and elevation in metabolism as seen in the present study; therefore; further research investigating the effects of chronic stretching exercise on plasma adiponectin in sedentary populations may be useful given that habitual exercise is correlated with increased insulin sensitivity and increased adiponectin. This exercise modality could be an attractive alternative form of exercise for individuals unwilling or unable to engage in aerobic exercise modalities.

The acute resistance exercise modality of the present study was similar in duration and intensity to both the acute aerobic and acute stretching modalities (30 minutes, high-intensity) and resulted in significantly increased blood glucose immediately post-exercise. Not surprisingly, blood glucose levels 24-hours post-exercise were similar to those seen at baseline. An augmentation in hepatic glycogenolysis, and to a lesser extent, gluconeogenesis, are responsible for the observed increases in blood glucose immediately post-exercise (39), indicating that glucose utilization is up-regulated following an acute resistance exercise regimen. The increased utilization of glucose; however, was not associated with any changes in plasma adiponectin immediately post-exercise. Previous research has indicated that acute resistance training leads to an up-regulation of lipolysis from adipose tissue post-exercise (116) and an increase in lipid oxidation for up to 24 hours post-exercise (26). Importantly, immediately following exercise, the activation of lipolysis is delayed in obese populations compared to lean populations and fat oxidation during acute resistance exercise is lower. This may help to clarify why sedentary or obese populations continually demonstrate improvements in plasma adiponectin following acute exercise while lean populations do not. Limited lipolysis and consequently, limited plasma lipids may up-regulate plasma adiponectin immediately following exercise. To date, no studies have examined plasma adiponectin responses to acute resistance

76

exercise. It seems likely that, similar to other acute exercise modalities, changes in adiponectin are more likely to occur in sedentary or obese populations. Further research is needed to examine plasma adiponectin responses to acute resistance exercise in these populations.

It should be noted that plasma adiponectin concentration is lower in patients with coronary artery disease as well as patients with type 2 diabetes, and is inversely correlated with a poor lipid profile (117) and elevated inflammatory markers such as TNF-α (118). Acute aerobic exercise has been shown to increase TNF-α in healthy populations without any concomitant increase in plasma adiponectin (86). Combined, these results suggest that inflammatory markers do not regulate adiponectin, but rather they are a likely byproduct of chronic elevated plasma lipids, which as suggested by the current study, acts to drive down the expression of adiponectin. Also, while plasma lipids are controlled in large part by diet and exercise, genetic predisposition to high plasma TAG levels, high LDL, or low HDL may contribute to lower levels of plasma adiponectin (119) and may help explain why some individuals are more susceptible to the development of insulin resistance and type 2 diabetes.

The most significant and striking correlation of the current study was the strong inverse correlation between plasma adiponectin and first degree family history of type 2 diabetes. This finding further suggests genetic predisposition to the development of insulin resistance may be a direct result of low levels of adiponectin. Elevated plasma lipids likely drive down the expression of plasma adiponectin via down-regulated expression of mRNA in the adipocyte (82). It has also been suggested that adiponectin mRNA is directly regulated by insulin (79). The specific regulation of various gene transcription factor activation is beyond the scope of this study, but this area of investigation can potentially provide more in-depth explanations for the

link between first degree family history of type 2 diabetes and low expression of plasma adiponectin.

Previous findings combined with the current findings on adiponectin and exercise indicate that exercise-mediated improvements in insulin sensitivity following chronic training may be partially due to increased plasma adiponectin; however, short-term improvements in insulin sensitivity are likely due to other factors. It is worth investigating substrate utilization and plasma adiponectin several hours post-exercise as lipid oxidation and lipid storage both increase following exercise (22) while plasma lipids decrease, potentially leading to an up-regulation in adiponectin.

4.4 Limitations

The participant population for the current study was young, active, and insulin sensitive. It is likely that this population had high levels of adiponectin at the onset of the study; therefore, eliciting significant changes in this hormone may have been compromised in this population. If sample sizes for both of the chronic exercise modalities had been larger, it would have been useful to stratify participants based on percent decrease in body fat pre- to post-exercise to determine potential correlations with changes in adiponectin and total body fat percentage. Studies have shown that plasma adiponectin concentration significantly increases following weight loss (118), however the number of participants in the current studies that demonstrated significant decreases in percent body fat following exercise intervention were too few to determine statistical significance. Additionally, relative concentration of plasma adiponectin was measured using a western blot technique, as opposed to the measurement of absolute concentration of adiponectin via enzyme-linked immunosorbent assay (ELISA) or

radioimmunoassay (RIA). While specific concentration cannot be determined from a western blot technique, it does account for plasma volume shift which is not accounted for in numerous exercise studies involving plasma adiponectin. Also, plasma adiponectin concentration in humans varies considerably in healthy populations. Some studies report concentrations as low as 6 µg/ml (88) while other studies report concentrations as high as 20 µg/ml (86). Thus the current study eliminated this issue by determining relative change.

The current study did not incorporate muscle or adipose tissue biopsies. While muscle biopsies would have provided useful information in regards to changes in adiponectin mRNA, AdipoR1, and AdipoR2 receptor expression, the use of biopsies were beyond the scope of the current study and likely would have compromised the number of study participants. In addition, skeletal muscle fiber composition, and likely receptor expression, at the origin may be different than at the insertion (120), making it difficult to determine which changes are truly due to the training adaptation.

Lastly, the HMW isoform of adiponectin, which has been correlated more strongly with insulin sensitivity than total serum adiponectin (64), was not measured in the current study. However, all subjects were insulin sensitive and the HMW form has not been shown to change following exercise in lean, healthy individuals (65). Furthermore, this isoform is not associated with acute improvements in insulin-sensitivity (66) whereas total adiponectin has been shown to change following exercise in some studies involving lean, healthy individuals (67). Thus, the focus of this dissertation was to determine if changes in substrate utilization directly affected total plasma adiponectin concentration rather than a specific isoform of adiponectin.

CHAPTER 5. CONCLUSIONS

The current study demonstrates that changes in substrate utilization during and following exercise are not directly related to changes in plasma adiponectin. Furthermore, while both chronic and acute exercise modalities affect substrate utilization during exercise, only high-intensity chronic exercise appears to increase plasma adiponectin concentration. Substrate oxidation may not be responsible for the shift in plasma adiponectin concentration following chronic exercise training; rather the increase seen in the present and previous studies may be due to increased lipid clearance from the plasma, as was evident following niacin supplementation.

Inducing significant changes in adiponectin via exercise appears to be dependent on habitual activity as indicated by the lack of prior physical activity in the participants in the chronic resistance training modality, which demonstrated significant augmentation in plasma adiponectin post-exercise. On the other hand, the higher levels of prior physical activity in the participants in the chronic aerobic training modality minimized improvements in plasma adiponectin post-exercise, but demonstrated significantly higher plasma adiponectin compared to the less active control group at both time points.

The results of this study provide important implications for exercise prescription to both improve insulin sensitivity and increase plasma adiponectin. While high-intensity exercise increases the body's dependence on glucose oxidation acutely, lipid clearance appears to improve following this type of training both acutely and chronically. Thus, engaging in high-intensity exercise may provide greater benefits in improving insulin sensitivity compared to low- or moderate-intensity exercise.

Whether or not aerobic or resistance training exercise elicits greater improvements in plasma adiponectin remains to be determined. While aerobic exercise clearly has its benefits, if patients do not regularly adhere to this type of exercise throughout their lives, plasma adiponectin and overall health status is not likely to improve. With a limited aerobic capacity in these patients, especially in the early stages of training, engaging in resistance training appears to offer an attractive alternative to possibly help combat obesity and the development of insulin resistance and type 2 diabetes; however, much more research is needed to establish specific changes that occur from this type of exercise.

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APPENDIX 1. GENERAL EXAMINATION

"Effects of exercise on lipid synthesis and degradation in healthy vs. insulin resistant skeletal muscle"

A Review of the Literature for the General Examination for the degree of Doctor of Philosophy in the department of Kinesiology

Louisiana State University, Agricultural & Mechanical College
Department of Kinesiology

Gregg Davis

B.S. Elon University, 2007

CONTENTS

*Note: All citations for Appendix 1 are included in the references list of the main text.

CHAPTER 1: The problem with excess energy intake/insufficient energy expenditure

Introduction

Diabetes is a chronic disease that is characterized by elevated blood glucose levels. In 2005, the centers for disease control (CDC) estimated that approximately 20.8 million people were suffering with diabetes, and of those, 90-95% had non-insulin dependent Diabetes Mellitus (NIDDM or Type 2 Diabetes). The CDC also states that this disease leads to a variety of secondary health problems including heart disease, stroke, hypertension, blindness, kidney disease, nervous system damage, amputations, and dental disease.

Although the etiology of diabetes is far from being fully understood, there are several known factors that contribute to the development of diabetes including genetics, lifestyle factors, and levels of adiposity (18, 121), all of which must be taken into consideration when examining this disease. Substantial evidence over the years has indicated that the one of the major predictive causes of type 2 diabetes is obesity, the prevalence of which has increased dramatically over the past 20 years (122) and is likely to continue to increase primarily due modern lifestyle factors including an overconsumption of calories and lack of physical activity. Therefore, gaining further insight into the development and prevention of diabetes is of high importance to help minimize the prevalence of this disease. Research has overwhelmingly indicated that type 2 diabetes is preceded by several years of insulin resistance, hyperglycemia, and hyperlipidemia both in skeletal muscle and in the liver. In this review, the main focus for the development of type 2 diabetes will revolve around this concept. The main focus for the prevention of type 2 diabetes will be centered on various forms of acute and chronic exercise given that numerous studies have shown that insulin resistance can be reversed with the addition of acute or chronic exercise (15, 22).

Insulin, which is one of the hormones responsible for maintaining blood glucose homeostasis, is released at an elevated rate following feeding. Insulin signals skeletal muscle and adipose tissue to uptake the circulating glucose, which is either stored in the form of glycogen or it can be oxidized and used for energy (7). In either case, blood glucose levels are maintained following feeding via insulin signaling. When an individual becomes insulin resistant, the tissue's ability to uptake glucose from the blood becomes impaired, resulting in chronic elevated blood glucose levels. From a mechanistic standpoint, hepatic, adipose, and skeletal muscle tissue insulin resistance do not develop following identical signaling pathways nor is this insulin resistance reversed following the same pathways (34). This notion is extremely important must be considered when examining the various mechanisms involved in insulin resistance.

Although the liver and adipose tissue play critical roles in energy homeostasis and whole-body insulin sensitivity, the focus of this review will be on the role of skeletal muscle. There are two critical reasons for this. The first is that skeletal muscle has a large relative mass which requires a high amount of energy to maintain. In addition, skeletal muscle is responsible for all mechanical work, and is thus metabolically active tissue with high energy demands. In fact, when the body is at a relatively stable balance, the majority of ingested carbohydrates are either stored in skeletal muscle or oxidized in skeletal muscle to provide immediate energy (123). The second reason for investigating skeletal muscle in greater detail is that skeletal muscle plays a critical role in fatty acid oxidation. At rest, approximately 60-80% of muscle energy production is provided by fat oxidation (124). During exercise, there is a shift of substrate utilization and the body becomes more dependent on glucose oxidation. Clearly, energy homeostasis is largely dependent on the metabolic state of skeletal muscle. When the metabolism of skeletal muscle

becomes impaired, fat oxidation is compromised and insulin resistance can result. Indeed, the development of insulin resistance has been linked to impaired lipid metabolism in skeletal muscle, although the mechanisms remain somewhat unclear.

Therefore, the purpose of this review is to address lipid metabolism in skeletal muscle, its links to insulin resistance, and how this insulin resistance can be reversed with the addition of exercise training or calorie restriction. Since there are numerous interacting factors that could possibly contribute to insulin resistance, the first chapter will briefly address some of the current major focus areas relating to lipid metabolism and cellular signaling. The second and third and chapters will address lipogenesis and lipolysis, respectively, in healthy and insulin resistant individuals. This will identify possible cellular mechanisms that may be responsible for impaired insulin signaling as a result of lipid accumulation and lipid intermediate accumulation. The fourth chapter will discuss the effects of exercise on lipid synthesis and degradation to identify mechanisms that may be responsible for improved insulin signaling following exercise. Based on these findings, the fifth and final chapter will offer future directions for this area of research.

Energy Imbalance

During periods of starvation, the human body will readily break down energy stores, mainly energy stores in the form of lipids, in order to sustain normal function. Similarly, during periods of food abundance, the human body has an astounding ability store excess energy, mostly in the form of triacylglycerols (TAG) in white adipose tissue (WAT) (125), which once again can be used as an energy source during periods of starvation. This ability has been critical to the survival of our species during times of famine. However, in more developed countries in modern times, a combination of technology and societal norms has encouraged sedentary

behavior and overconsumption (126). Thus, the body's efficient ability to store excess energy has recently lead to a wide range of health disparities. Over an extended period of time, excessive physical inactivity in combination with overconsumption of food can result in an array of chronic diseases including obesity, cardiovascular disease, and diabetes (127). As will be discussed in detail in chapter four, many of these abnormalities can be reversed with the addition of exercise although the mechanisms for these adaptations remain unclear.

It will become evident throughout this review that although many of the cellular mechanisms responsible for changes in insulin sensitivity are currently unknown, the human body will adapt regardless of the stress placed on the body in order to maintain a homeostatic state. Much like the body becomes trained to utilize fats as a primary fuel following chronic endurance training, in the case of chronic energy abundance, the body becomes trained to store rather than oxidize excess fat. With a decreased ability to oxidize fats, the body must primarily rely on glucose oxidization, even in a fasted state, to provide energy. This is what has been observed in obese human subjects. A study by Kelley et. Al (55) revealed that when the respiratory quotient (RQ) was measured across the leg muscles of obese subjects, RQ averaged 0.90 in a fasted state, indicating glucose was the primary fuel source. On the other hand, fasted lean subjects had an RQ of 0.82 indicating a greater reliance on fatty acids for fuel (55). This increased reliance on glucose for fuel has been linked to hyperlipidemia and metabolic dysfunction. This makes sense given that if fat is not utilized for fuel, it will remain in the circulation, raising lipid levels, or will be stored in adipose tissue which seemingly has no maximal storage capacity. Conversely, although glucose can be stored to some extent in skeletal muscle and liver in the form of glycogen, there is a limit to how much of this can be stored.

Thus, the body may preferentially oxidize excess glucose first, while only minimally oxidizing lipids, storing the rest in adipose, skeletal, and hepatic tissue.

It is likely that excess lipid storage, not only in adipose tissue, but also in skeletal muscle and around organs such as the heart, liver, and kidneys can lead to metabolic dysfunction (8). However, the exact mechanism(s) responsible for this abnormal metabolic regulation is not fully understood. There are several current hypotheses to explain the development of insulin resistance, many of which are interconnected as seen in figure A1.
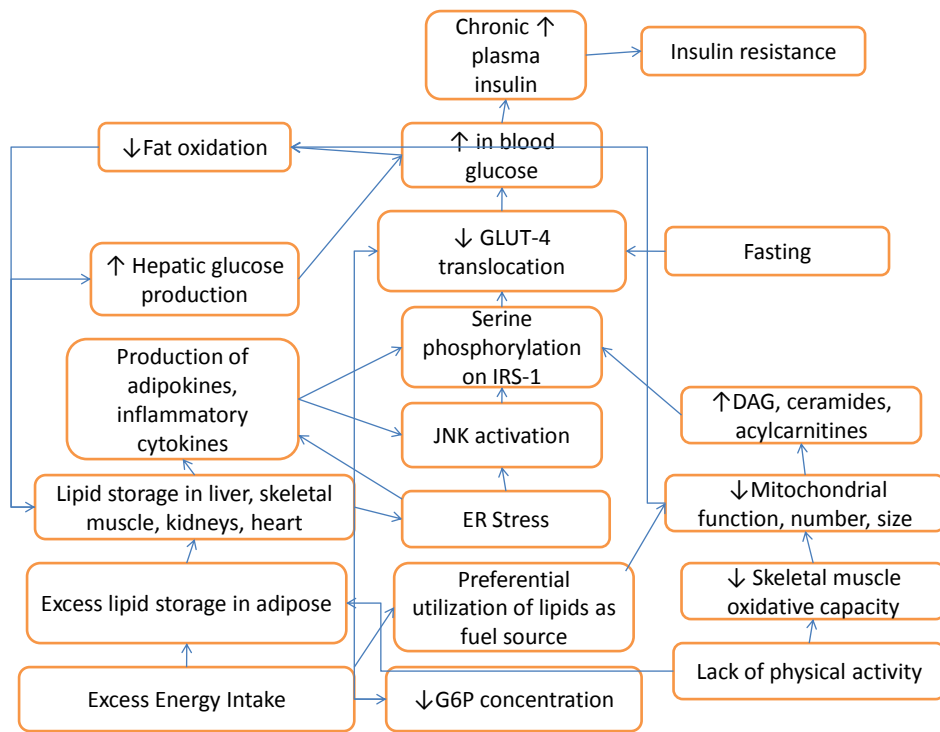
Figure A1. A general overview of some of the proposed mechanisms linked to the development of diabetes. G6P= glucose-6 phosphate; ER= endoplasmic reticulum; IRS-1= insulin receptor substrate-1; DAG= diacylglycerol.

Recent research has suggested that specific peptide hormones and inflammatory cytokines, specifically adipokines, which control the communication between various metabolically active tissues and organs, including the liver, skeletal muscle, and fat (128) may

play a role in regulating insulin sensitivity. Adipocyte signaling can greatly affect the development of skeletal muscle insulin resistance and will be addressed briefly here.

Adipocyte-derived hormones

Peptide hormones including leptin, adiponectin (ACRP30), retinol-binding protein (RBP4), and resistin have all been implicated in the development of insulin resistance (18). Each of these hormones are secreted by adipocytes and play an active role in lipid storage and mobilization. In healthy, normal-weight individuals, adiponectin and leptin act to regulate energy storage and energy expenditure. As leptin levels increase, metabolism increases and appetite decreases (129). However, as a result of obesity, individuals become leptin resistant, resulting in higher levels of circulating leptin. This mechanism is similar to that seen in insulin resistance, in which hormone signaling is impaired despite increased circulating levels. Conversely, adiponectin, which plays a critical role in glucose and lipid metabolism, has been shown to decrease with obesity (71). Since these two hormones appear to have complementary actions and it has been suggested that both act to decrease TAG synthesis, increase β-oxidation, and activate 5'-AMP-activated protein kinase (AMPK). Thus, they are said to be insulin-sensitizing hormones, which are secreted preferentially from small adipocytes. RBP4 and resistin on the other hand, have been deemed insulin-desensitizing and are secreted preferentially from large adipocytes (8). RBP4 and resistin act to decrease glucose uptake and encourage gluconeogenesis, which during fasting, helps maintain normal glycemic levels in the blood. However, in a state of chronic overfeeding, the protective action of these hormones becomes a plausible contributor to the development of insulin resistance and type 2 diabetes (128).

It has been demonstrated that fasting can lead to a down-regulation of adipose GLUT-4 mRNA expression in rats, (130) and several studies have shown that lower GLUT-4 levels are

linked to the development of insulin resistance (131). Although there are several tissue-specific

glucose transporters, GLUT-4 is the primary transporter in skeletal muscle and adipose tissue

and appears to be the only one which is insulin-dependent (132). Thus, it is possible that chronic

depressed levels of the GLUT-4 protein in adipose tissue as a result of fasting can lead to

hyperinsulinaemia and eventual insulin resistance, although further research is still needed to

support these ideas. This may seem counterintuitive considering that overwhelming evidence

points to overconsumption leading to insulin resistance. However, consider that in a fasted state

plasma insulin levels are low. In turn, to prevent hypoglycemia, GLUT-4 expression will be

down-regulated. This concept has been supported by adipose GLUT-4 knockout (KO) mouse

models, which demonstrate decreased glucose uptake into the cell and increased insulin

resistance in skeletal muscle and liver despite normal GLUT-4 function in these tissues (131).

It should be noted that excess body fat does not cause insulin resistance per se. Though

adipose tissue appears to have a limitless capacity, the formation of adipocytes is highly

regulated and it has been suggested by some that adipose tissue storage capacity is limited via

TNF-α or other inflammatory cytokine signaling molecules (133). Some researchers have

suggested that once this storage capacity is reached, excess lipids are forced to be stored in and

around organs that do not normally accumulate high lipid levels, this in turn, makes the organs

more susceptible to lipotoxicity and local insulin resistance (128). Therefore in theory, a

therapeutic strategy to combat insulin resistance could involve the expansion in the storage of

lipids in subcutaneous adipose tissue, blunting the metabolic deficiencies that are normally

associated with obesity, as long as lipid overflow into other tissues and organs is limited. The

problem with this idea, however, is that adipose tissue, as previously mentioned, is an endocrine

organ which secretes hormones and adipokines. In the presence of expanding adipose tissue,

110

specific signaling molecules are likely to lead to systemic insulin resistance, similar to what has

been seen in mouse models (131).

Based off these findings, it is apparent that adipose tissue is a metabolically active

endocrine organ that responds to changes in energy demands. Excess energy intake results in

adipogenesis, however, this is not a passive process, rather it requires energy, is highly regulated,

and involves a complex array of transcriptional activation as well as inter- and intracellular

signaling (128) through hormones, as previously discussed, as well as adipokines.

Inflammatory Cytokines

While there are several pro-inflammatory cytokines that may be linked to obesity and

insulin resistance, tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6) are two of the

most studied and will be addressed briefly here. TNFα is involved in the regulation of immune

cells, it can induce apoptosis, inhibit tumor growth, and induce inflammation (134). However,

this cytokine has also been implicated in the development of insulin resistance. Over-expression

of this cytokine acts to inhibit insulin action and was demonstrated in obese mouse and human

models (135). TNFα also activates the c-Jun amino terminal kinases (JNKs), which promotes

the phosphorylation of the serine residue on insulin receptor supstrate-1 (IRS-1), which has been

shown to induce insulin resistance (136). Interestingly, TNFα also acts to promote lipolysis as

well as inhibit adipogenesis and adipocyte differentiation (133). Although this may seem

contradictive given that insulin resistance is highly correlated with excess lipid storage, as will

be discussed in chapters two and three, incomplete synthesis and degradation of lipids via TNF-α

or otherwise, is a plausible underlying mechanism which contributes to insulin resistance.

Increased lipids in the circulation due to increased lipolysis and decreased adipogenesis may also

111

be partially responsible for impaired insulin signaling. Furthermore, inhibition of adipogenesis also suggests that TNF-α may act to limit the storage capacity of adipose tissue. Thus as an oversupply of calories continues, this creates a need for excess lipids to be stored in other organs such as skeletal muscle and liver. As a result, lipotoxic intermediates such as diacylglycerol (DAG) and ceramides are formed. The deleterious effects of these metabolites on the insulin signaling cascade is well established (56) and will be discussed further in the next section of this chapter.

IL-6, which much like TNFα, acts to stimulate immune response to injury, is another pro-inflammatory cytokine that has also been shown to induce insulin resistance. The accumulation of fat deposits around organs appears to be responsible for this outcome through activation of the transcription factor NF-κB, which is responsible for the production of several cytokines, including IL-6 and TNFα (137). It is likely that these and other cytokines have additive effects; however, the relative contribution of each of these cytokines is not readily apparent. What is apparent is that adipokines play a significant role in the development of insulin resistance and that there is a link between these adipokines and lipid accumulation in organs such as skeletal muscle and the liver. The question becomes whether these cytokines are a cause or an effect of abnormal lipid storage. The nature of cytokines would suggest the latter; however, further research is still needed to support this notion.

The treatment of chronic inflammation has been targeted as a potentially novel strategy in the prevention and treatment of type 2 diabetes. Non-steroidal anti-inflammatory drugs (NSAID), particularly aspirin, which is an acetylated salicylate drug, has been shown to reduce inflammation by irreversibly modifying the active site on cyclooxygenase (COX) 1 and 2 and in turn, inhibiting prostaglandin formation. High doses of salicylates have also been shown

directly lower blood glucose levels and improve insulin sensitivity in diabetic patients by direct inactivation of IKKβ (138). When activated, IkB kinase-β (IKKβ) will activate NF-kB, which produces pro-inflammatory markers such as IL-6, TNF-α, and resistin, which as mentioned in chapter one, have been linked to insulin resistance. Also, macrophages may amplify this signal, which demonstrates that the development of IR may have similar/parallel mechanisms to other inflammatory diseases such as atherosclerosis. However, it is important to note that it is assumed that adipocytes, not skeletal muscle cells, initiate inflammation-mediated IR since accumulated lipid stores are predominantly in these types of cells (139). The inflammatory cascades seen in adipose and liver cells from increased adiposity are not seen in skeletal muscle, there is no activation of IKKβ/NF-kB or increases in macrophages. Inflammation in skeletal muscle is only seen in lipid infusion models, which indicates again that skeletal muscle IR does not cause inflammatory responses, rather skeletal muscle IR occurs as a result of inflammatory responses.

Though a link appears to be established between insulin resistance and lipid accumulation around organs, rather than in subcutaneous adipose tissue, the exact mechanism(s) responsible for this dysregulation is currently a matter of debate and clearly requires further investigation. There are two opposing theories, one suggesting decreased β oxidation, and the other suggesting increased, but incomplete β oxidation, both of which will be addressed here.

Decreased β oxidation

The rationale for the popular theory which suggests reduced β oxidation leads to insulin resistance follows a seemingly intuitive and logical line of reasoning. Feeding stimulates the conversion of acetyl CoA to malonyl CoA by acetyl-CoA carboxylase (ACC). Malonyl CoA is responsible for the inhibition of carnitine palmitoyltransferase 1 (CPT1), which controls entry of

113

fatty acids into the mitochondria for β oxidation (18). Immediately following feeding, the oxidation of fat stores is no longer necessary to provide energy. Thus, long chain acyl CoAs (LC-CoAs) which are partitioned toward mitochondrial oxidation in a fasted state, are redirected toward the synthesis of TAG, diacylglycerol (DAG), and ceramides in a fed state (140). Chronic overfeeding, therefore, leads to decreased β oxidation and an accumulation of lipid intermediates, particularly DAG, which phosphorylates several kinases, including several protein kinase C (PKC) isoforms (54). This in turn, phosphorylates serine kinases on insulin receptor substrate-1 and -2 (IRS-1 and IRS-2), which again, have been shown to negatively influence insulin-induced glucose uptake (136). This is likely due to a decrease in GLUT-4 translocation via a signaling cascade described elsewhere (54).

In addition, it has been demonstrated that patients with type 2 diabetes have smaller and fewer number skeletal muscle mitochondrial as well as decreased mitochondrial enzyme activity compared to healthy controls (141), thus the rate of oxidative phosphorylation is significantly reduced, causing lipid accumulation within the muscle. However, it should be noted that in this study, the diabetic subjects were approximately ten years older than the control subjects. It has been shown that mitochondrial density and mitochondrial adenosine triphosphate (ATP) synthesis are reduced with age (142), therefore it is possible that age may have influenced these results and must be taken into consideration. However, other studies have revealed similar results which point toward overall reduced fat oxidation in skeletal muscle of fasted insulin resistant subjects (143). The activity of citrate synthase (CS) and cytochrome c oxidase, which are thought to be the rate-limiting enzymes involved in the tricarboxcylic acid (TCA) cycle and Electron Transport system (ETS), respectively were also down-regulated in insulin resistant, obese subjects (144). It has also been shown that the expression of hormone sensitive lipase

114

(HSL) and adipose triglyceride lipase (ATGL), both of which hydrolyze triglyceride stores, are down-regulated in insulin resistance (145). Together, these results suggest that not only is β oxidation decreased, but fatty acid hydrolysis may be down regulated as well. The extent of lipid breakdown in insulin resistant individuals is yet to be determined and warrants further investigation as will be discussed further in chapter three.

Another reason that decreased fatty acid oxidation is a popular theory to explain the connection between lipid accumulation in skeletal muscle and insulin resistance is because of changes in fatty acid transport and translocation. Initially, it may seem logical that decreases in oxidative enzymatic activity as well as decreased expression of the fatty acid translocation protein (FAT/CD36) could explain the lack of fat utilization as seen in some studies (9) (146). However, given that insulin resistant individuals have elevated levels of intramyocellular lipids (IMCL), more recent studies have shown that CD36 expression is actually increased in type-2 diabetics, yet the translocation of CD36 to the plasma membrane is impaired. This results in increased fatty acid uptake, but decreased oxidation due to the decreased transport of fatty acids to the mitochondria (147). Indeed, CD 36 KO mice have shown improvements in insulin sensitivity in skeletal muscle (148). This is likely due to the lack of lipid accumulation in the muscle; however, these KO mice had decreased insulin sensitivity in the liver. This was likely due to increased lipid accumulation in the liver and suggests tissue-specific mechanisms involved in the development of insulin resistance. It also suggests that there are significant differences between the regulation of glucose versus lipid transporters. While increased lipid transport is associated with insulin resistance, decreased glucose transport is associated with insulin resistance.

Incomplete β oxidation

In contrast to the theory of decreased β oxidation, recent studies have suggested that insulin resistance leads to increased, but incomplete, β oxidation. No matter what type of stress placed on the body, under normal conditions, the human body will adapt to better handle the stress and maintain a homeostatic state, beginning with changes at the molecular level. Thus, the theory of incomplete β oxidation is as follows:

Much like the changes seen in gene expression following chronic exercise training, chronic exposure to elevated lipids in skeletal muscle up-regulates various genes involved in β oxidation to compensate for this constant stress. However, unlike the changes seen with exercise, exposure to high levels of lipids appears to have no effect on the TCA cycle or ETS (140). This results in an accumulation of acetyl CoA, which is the end product of β oxidation. When energy supply is greater than energy demand, these accumulated acetyl CoAs are converted to acylcarnitines (8). A recent study demonstrated that high-fat feeding in mice induces similar increases in even-chain acylcarnitines in skeletal muscle to those seen in fasted standard chow-fed mice, however, acylcarnitine levels significantly decreased following feeding in standard chow mice whereas they remained elevated in the high-fat fed mice (149). This gives rise to two important outcomes. The first is that the mitochondria in high-fat fed mice are unable to adapt to changes in fuel sources, this has recently been deemed "metabolic inflexibility". Studies have shown that there are direct links to metabolic inefficiency, particularly in fat utilization in insulin resistant individuals (150, 151). Essentially what these studies indicate is that insulin resistant individuals may not be able to switch from fat utilization to glucose or vice versa under stress conditions, which leads back to mitochondrial dysfunction or mitochondrial overload, which will be discussed further in chapter four.

The second outcome from the acylcarnitine study indicates that both high fat feeding and prolonged starvation can lead to incomplete β oxidation. These results were further supported by examining malonyl-CoA decarboxylase (mcd) KO mice. As previously mentioned, malonyl-CoA inhibits CPT1 to prevent the import of fatty acids into the mitochondria. Mcd degrades malonyl-CoA, thus reliving the inhibition of CPT1, allowing fatty acid entry into the mitochondria for oxidation. Mcd KO mice have partial inhibition of CPT1 and were in turn, protected from glucose intolerance and skeletal muscle remained insulin sensitive when fed a high-fat diet (140). Thus, by limiting β oxidation, the build-up of metabolic byproducts such as DAGs, ceramides, acylcarnitine, and non-esterified fatty acids (NEFAs) (121), all of which are associated with the development of insulin resistance, are limited. This is an apparent paradox given that these same intermediates accumulate in studies examining incomplete β oxidation and decreased β oxidation.

Experimental Variation

There may be a wide range of explanations for the conflicting data between decreased β oxidation and incomplete β oxidation. The most likely of these is tissue specificity. A recent study showed that this incomplete β oxidation was more pronounced in white muscle compared to red muscle in high-fat fed mice (140). This is likely due to the intrinsic properties of white muscle, which has a much lower oxidative capacity than red muscle, and therefore fewer mitochondria, leading to higher levels of incomplete oxidation. It should be noted here that many insulin resistant individuals have a higher percentage of white, glycolytic fibers compared to their insulin-sensitive counterparts (152) which may be attributed to a combination of genetics and physical activity level. Therefore, it seems likely that this is a contributing factor to the

development of insulin resistance. For that reason, when examining various mechanisms of insulin resistance, muscle fiber type must be taken into consideration.

The examination of other tissues must also be considered. The previously mentioned study which elicited changes in the skeletal muscle of mcd KO mice had no changes in β oxidation in the liver. Likewise, CD36 KO mice were insulin sensitive in skeletal muscle, but insulin resistant in the liver. Thus, insulin resistance appears to be tissue specific. Interestingly, some of the currently investigated mechanisms involving LC-CoA's, DAG's, endoplasmic reticulum (ER) stress, and inflammation have shown changes in hepatocytes while they have shown little or no changes in myocytes. For example, acetyl-CoA carboxylase (ACC2) null mice have lower levels of malonyl-CoA and are in turn, resistant to diet-induced diabetes. However, these changes occur only in liver while adjustments in muscle lipid metabolism were minor (153). This may suggest that the liver is the site of initiation of insulin resistance, whereas skeletal muscle is a secondary target, or responder to insulin resistance (139).

Other factors, such as cell cultures versus animal models versus human models must also be taken into consideration as cells behave differently *in vitro* compared to *in vivo*; likewise, mouse models do not always respond in the same manner as human models. In the study by Koves et al. (133), cell cultured myotubes exposed to insulin increased glycogen synthesis, but only minor increases were seen in glucose oxidation. On the other hand, fatty acid exposure decreased both glycogen synthesis and glucose oxidation by 40-50%. Insulin resistance increased with higher exposure to carnitine, indicating fatty acids must enter mitochondria to induce insulin resistance. This leads to the possibility that elevated carnatine levels may contribute to insulin resistance. However, it is also possible that when cultured cells are exposed to carnitine, they will preferentially utilize fatty acids rather than glucose due to the absence of

systemic conditions and input, and in turn, these cells appear to be more insulin resistant. *In situ*, body will preferentially oxidize glucose and store fat following feeding, thus it cannot be concluded that elevated levels of carnitine alone subsequently increase β oxidation and lead to insulin resistance. More studies done with animal or human models are needed to support these outcomes seen in cell cultures.

Individual diet, total adiposity, and gender are also important considerations and can greatly affect the outcome of a study. These variables, as well as others, will be examined in greater detail in the remaining chapters of this review.

Athlete's Paradox

Regardless of what the mechanism may be, it is evident that excess lipid storage, especially within skeletal muscle, leads to insulin resistance, and therefore limiting this accumulation may improve insulin sensitivity. Interestingly, insulin sensitive, endurance-trained athletes have demonstrated IMCL stores comparable to, or even higher than sedentary, insulin resistant individuals (16), which has been deemed the "athlete's paradox". One possible explanation may be that these particular insulin sensitive individuals have a high rate of lipid turnover as a result of endurance training (22) and store TAGs which consist mostly unsaturated fatty acids. On the other hand, insulin resistant individuals tend to store IMCL as a result of an energy surplus, have a low rate of lipid turnover, and store TAGs which consist of a higher percentage of saturated fatty acids. In addition, unlike insulin sensitive individuals, those with insulin resistance may not be able to completely synthesize triglycerides within the muscle or within adipocytes, thus these lipids may not be fully synthesized TAG, rather they may be partial lipid intermediates such as DAG or monoacylglycerol (MAG), which have been linked to impaired insulin signaling and glucose transport (22); however, further research is needed to

support these hypotheses. The cause for this dysfunction is still not fully understood and

warrants further investigation. Therefore, the remainder of this review will focus on differences

in lipid synthesis and degradation in healthy and insulin resistant individuals to determine

plausible mechanistic pathways linking incomplete lipid metabolism to insulin resistance and

how this metabolism changes with the addition of exercise in these two populations.

CHAPTER 2: Lipid synthesis in healthy vs. insulin resistant skeletal muscle

Since lipid intermediates such as DAG, ceramides, and acylcarnitine appear to have deleterious effects on insulin signaling pathways, it is necessary to examine both TAG breakdown into FFA, also known as lipolysis, and TAG synthesis from FFA, also known as lipogenesis, to determine exactly how these metabolites accumulate. Currently, both lipolysis and lipogenesis within skeletal muscle are poorly understood. By gaining a better understanding of the mechanisms involved in these processes and by understanding differences in lipolysis and lipogenesis in healthy skeletal muscle compared to insulin resistant muscle, it may be possible to determine more definitive causes that lead to a build-up of these lipid intermediates and contribute to insulin resistance.

As mentioned in chapter one, insulin is a hormone which acts to control blood glucose levels. It is also an anabolic hormone and therefore promotes the synthesis and storage of various substrates, including lipids. Thus, following feeding, insulin is released activating various enzymes involved in lipogenesis. It should be noted that most of the current knowledge regarding lipogenesis comes from *E.Coli* or hepatic cell culture studies though it is assumed that similar mechanisms occur in skeletal muscle. Briefly, the synthesis of fatty acids begins with acetyl CoA, which is formed from the enzyme pyruvate dehydrogenase (PDH) (154). When pyruvate is decarboxylated by PDH, the newly formed acetyl CoA can be converted to malonyl CoA by acetyl CoA carboxylase (ACC). The carbon units from this malonyl CoA can then be used to synthesize part of a fatty acid chain via the action of an enzyme complex called fatty acid synthase (154). Both PDH and ACC are activated by insulin and as will be discussed, alterations to these enzymes or variations in the level of substrate can greatly affect lipid metabolism and in turn, insulin sensitivity.

Once a fatty acid is formed, it, along with two other fatty acids must attach to a glycerol molecule, which is derived from carbohydrate sources, to form TAG (154). Complete lipogenesis involves both the formation of fatty acids and the partitioning of fatty acids into TAG molecules. It should be noted that TAGs can be any permutation of various fatty acid chain lengths (14-24 carbon atoms) and saturation level for each of the three fatty acids, thus the rate and extent of degradation and esterification of TAGs have a large degree of variation.

TAG synthesis can result from one of two biochemical pathways, as seen in figure 2. The MAG pathway is the predominant pathway following feeding and is likely responsible for the storage of TAG in adipose tissue whereas the glycerol-3 phosphate (G3P) pathway is responsible for *de Novo* synthesis of TAG in most tissues. In both pathways, monoacylglycerol acyltransferase (MGAT) and glycerol phosphate acyltransferase (GPAT) act to acetylate the substrate with fatty acyl-CoA. The G3P pathway requires an additional acetylation followed by dephosphorylation. Each pathway produces DAG and thus the final step of converting DAG to TAG by diacylglycerol acyltransferase (DGAT) is shared by both pathways and is considered to be the rate-limiting step in TAG synthesis (155, 156).

Monoacylglycerol Pathway

Glycerol Phosphate Pathway

G-3-P

GPAT

LPA

LPAAT

Monoacylglycerol

PA

MGAT

PAP

Diacylglycerol

DGAT

PC or PE   TAG
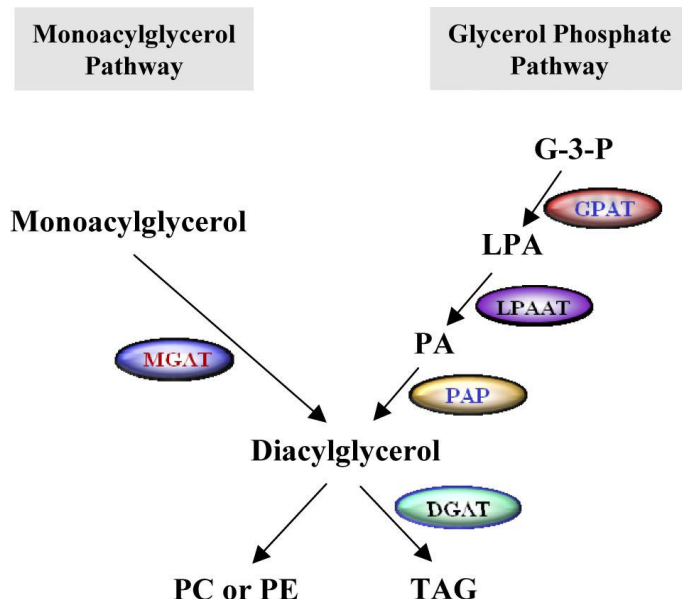
Figure A2. Lipid Synthesis Pathway. *(155).* MGAT=monoacylglycerol acyltransferase; GPAT= glycerol-3-phosphate acyltransferase; LPAAT= lysopophosphatidic acidacyltransferase; PAP= phosphatidic acid phosphorylase; DGAT=diacylglycerol acyltransferase; PC= phosphatidic choline; PE= phosphatidic ethanolamine.

Variation in Lipid Storage

Multiple factors such as gender, training status, measurement used, muscle analyzed, and most importantly, energy status all affect the synthesis and partitioning of lipids to various storage sites. The storage of lipids in non-adipose tissues may contribute to insulin resistance, but an overconsumption of calories will not result in the same gains in adiposity in all individuals. In fact, a study done with overfeeding in twins revealed that there was a large variation between pairs of twins in energy expenditure, weight gain, and regional adiposity (157), all of which were thought to be due to genetic variation. These results highlight the importance of acknowledging the differences in the expression of genes responsible for the partitioning of lipids to adipose versus skeletal muscle. Further insight is still needed to explain how lipids are synthesized within skeletal muscle and how insulin resistance develops as a result of excess lipid storage. More visceral adiposity and lipid storage in and around organs and

within skeletal muscle contribute to increased insulin resistance, yet endurance athletes remain insulin sensitive despite high IMCL stores (16).

With that in mind, the question becomes, "what are the primary mechanistic differences in lipogenesis between healthy and insulin resistant individuals"? It is likely that endurance athletes store lipids in muscle for quick access to a lipid energy source as a result of training and thus, have a high turnover of lipid storage and oxidation within the muscle. A high mitochondrial density and increased oxidative fiber characteristics likely contribute to the lack of intermediate metabolites, which may be the reason these athletes remain insulin sensitive(16). Another major reason endurance athletes remain insulin sensitive despite high levels of IMCL may be due to complete synthesis of triglyceride stores. NEFAs must be esterified to MAG, DAG, and finally TAG by multiple enzymes including glycerophosphate, mitochondrial glycerol-3 phosphate, sterol CoA desaturase 1 (SCD1), and DGAT. If any of these enzymes are down-regulated, lipids may not be completely converted into TAG, causing intermediates to accumulate and interfere with insulin signaling. Aside from genetic disturbances, numerous dietary and supplemental factors can influence this lipogenesis. Therefore, some of the major factors will be addressed here.

Following Feeding

Lipids within the body are stored in the body primarily as TAGs. Therefore having a firm understanding of how TAGs are formed is critically important. Once food is ingested, there are many enzymatic processes involved in the digestion of various food stuffs and eventual transport into the circulation, the details of which are described elsewhere (158). Once in the circulation via very low density lipoproteins (VLDLs) and chylomicrons, TAGs are hydrolyzed into free fatty acids (FFAs) via lipoprotein lipase (LPL) (125), which is discussed in further

detail in chapter three. The FFAs are transported into skeletal muscle, adipose, and liver cells via fatty acid transport proteins (159). Once inside the cell, the fate of that fatty acid is highly dependent on the cell type. In adipose tissue, fatty acyl-CoA's are re-esterified to TAGs, in the liver, fatty acids are re-esterified to TAGs or are packaged into VLDLs for export into the circulation and eventual storage in adipose tissue as TAGs (8). In skeletal muscle, which is the primary focus of this review, FFA are primarily oxidized to provide energy; however, some FFA are stored as TAGs within the muscle cell, which is likely due to an increase in the expression and activity of various lipogenic enzymes. The extent of this activation and expression may be partially due to the type of macronutrient consumed.

Carbohydrate ingestion

In healthy and insulin resistant individuals, there are numerous factors that will ultimately determine whether ingested carbohydrates will immediately be utilized for energy or stored in various tissues. Both the amount and the type of carbohydrate consumed will affect the rise in blood glucose concentrations. Simple carbohydrates as well as high amounts of carbohydrates consumed at one time will increase blood glucose more rapidly, and in turn increase the rate of insulin release. In insulin resistant individuals, glucose stimulated insulin secretion (GSIS) is impaired, leading to chronic elevated levels of blood glucose (160). In healthy individuals, insulin stimulates uptake of glucose and transport into numerous cell types including, skeletal muscle, liver, and adipose (7), but again, this action is impaired in insulin resistant individuals (161). This has major implications for the development of diabetes given that up to 90% of insulin-stimulated glucose disposal is accounted for by skeletal muscle (162) which will either immediately use the glucose to meet its energy needs, or it will store the glucose in the form of glycogen. In healthy individuals, if skeletal muscle and the liver already have large amounts of

glycogen stored, it is likely that this excess glucose will be partitioned toward adipose cells, the presence of which stimulates lipogenesis. However, insulin resistant individuals have an impaired ability to synthesize glycogen (161), which means even more lipogenesis is likely to occur, further exacerbating signaling impairments associated with excess lipid storage and increased reliance on glucose oxidation.

This is why the RQ is high in a fasted state in insulin resistant individuals and remains that way following feeding (55). The authors from this study suggest that obese, insulin resistant individuals oxidize proportionally more fat than lean individuals following insulin stimulation. These conclusions cause a fair amount of confusion, as seen in chapter 1 (increased versus decreased β oxidation) and deserve clarification. It is important to understand that while the RQ of obese was approximately 0.91 and the RQ of lean was approximately 0.99 following insulin stimulation, the RQ of obese in a fasted state was 0.90 compared to 0.82 in lean. Clearly the obese subjects were metabolically inflexible (0.90 fasted versus 0.91 following insulin stimulation), but they do not necessarily metabolize more fat than lean individuals as previously suggested, rather they are unable to adapt or respond to insulin stimulation and are in a chronic state of elevated glucose oxidation.

Fat ingestion

Similar to the ingestion of carbohydrates, the ingestion of fat, in the presence of an energy surplus, can lead to increased fat synthesis within skeletal muscle, liver, and adipose tissue. Some studies suggest increased fat oxidation following high fat feeding in insulin resistant individuals compensate for the influx of fat while others suggest decreased fat oxidation or an inability to adjust with fat overfeeding (163) (164). Differences are likely attributed to skeletal muscle composition as well as dietary fat composition (saturated versus unsaturated) and

total energy expenditure. Some of these discrepancies may also be due to reporting relative changes seen in substrate utilization in insulin resistant individuals compared to lean counterparts, not absolute changes in fat oxidation.

One particular study has suggested that fat over-feeding increases adiposity more so than CHO over feeding (164). The authors showed that isocaloric carbohydrate overfeeding for 14 days in lean and obese humans led to an increased oxidation of carbohydrates with 75-85% excess energy being stored. Fat overfeeding did not increase fat oxidation and 90-95% excess energy was stored, indicating that excess dietary fat increased fat accumulation more so than excess dietary carbohydrate. This seems logical given that glucose is the body's immediate preferred energy source there is a limitation to how much glycogen can be stored in skeletal muscle and the liver. Contrasting results were seen in another study which demonstrated that RQ was not significantly different in lean subjects given a high-fat diet compared to a high carbohydrate diet or mixed (all isocaloric) on consecutive days, indicating that the metabolism of these subjects were unable to immediately respond to changes in macronutrient intake (163). Taken together, these results suggest that regardless of macronutrient consumption, continual overfeeding will result in increased fat storage; however, unlike excess carbohydrate intake, excess fat intake is not compensated for by an increased rate of fat oxidation, thus, lipids accumulate and levels of adiposity become elevated.

Also of interest, in the study by Horton et al, (158), the obese subjects oxidized proportionally more carbohydrates and less fat than the lean subjects, regardless of diet composition, elucidating to the likelihood of metabolic inflexibility in obese individuals as seen in previous studies (55). In addition, plasma insulin levels increased in carbohydrate overfeeding in both lean and obese subjects, but insulin concentration decreased in fat overfeeding in lean

127

subjects while it increased in obese subjects, which could explain why obese individuals often exhibit hyperinsulimia. In contrast to these findings, a recent study demonstrated that muscle mitochondrial number increases from a high fat diet in rats, which increases the capacity for fat oxidation through post-transcriptional modifications for Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), but insulin resistance increases as well (165). This indirectly suggests that decreased mitochondrial content in muscle may not be directly responsible for insulin resistance as previously suggested, rather it may be a result of an energy imbalance (lack of physical activity) as suggested in chapter one combined with impaired lipid transport and increased lipid accumulation. Chapter four will describe in greater detail how even a single bout of physical activity can reverse some of the insulin desensitizing effects of physical inactivity.

Lipid Storage

 Ingesting a mixed diet, which consists of a percentage of both carbohydrates and fats is much more applicable to the general public and does not suggest the preferred storage of one substrate over another, especially in an isocaloric diet. It is likely that lipid storage in skeletal muscle for this type of diet is largely dependent on the metabolic state of the muscle (energy consumption versus energy expenditure). However, a recent study also demonstrated that dietary fat composition can affect insulin sensitivity. Male Sprague-Dawly rats given a high-saturated fat diet for eight weeks exhibited greater levels of saturated fatty acids incorporated into IMCL compared to rats given a high-polyunsaturated diet, which incorporated more unsaturated fatty acids into IMCL. However, IMCL content increased in both high-fat diets compared to the control group. The high saturated fatty acid group also exhibited increased homeostasis model assessment of insulin resistance (HOMA-IR) and glucose intolerance (166). In addition, L6

myotubes treated with saturated fatty acids had decreased insulin-stimulated glucose uptake and increased DAG and ceramide accumulation. On the other hand, myotubes treated with polyunsaturated fatty acids had no changes in insulin-stimulated glucose uptake or DAG/ceramide content, but TAG levels did increase significantly (166). Together, these results indicate that high levels of dietary saturated fatty acids may directly contribute to the development of insulin resistance independent of systemic hormone regulation or tissue cross-talk. Rather, saturated fatty acid-induced insulin resistance may occur via incomplete degradation of TAG's (DAG, MAG, or ceramides). The results may also partially explain the athlete's paradox given that obese individuals likely have a much higher intake and storage of saturated fatty acids compared to lean endurance athletes.

Higher levels of unsaturated fatty acids incorporated into TAG stores may improve insulin sensitivity by limiting lipid metabolite accumulation. This may be due to the fact that one of the enzymes involved in lipogenesis (DGAT, discussed below) preferentially utilizes unsaturated fatty acids rather than saturated fatty acids (167). Thus, not only is total TAG storage important, but also the content of the TAG stores. Unfortunately, as previously mentioned, this content can be quite variable given that fatty acids can range from 14-24 carbons and have a variety of locations which may be unsaturated and each TAG can contain any combination of these fatty acids (7).

Niacin

To gain a more complete understanding of lipid formation, it is helpful to examine models in which this process is either accelerated or inhibited. Aside from exercise intervention, which will be thoroughly examined in chapter four, one such way to accomplish this task in human models is through pharmacological intervention. Nicotinic acid, also known as niacin or

vitamin B3, is pharmacologically used to improve cholesterol levels by blocking fat breakdown in adipose and liver tissue, but it also appears to play a role in lipogenesis. As a consequence of this supplement's actions, VLDL formation decreases in the liver, and in turn, less low-density lipoprotein (LDL) is formed, and more high-density lipoprotein (HDL) is formed, thereby lowering blood lipids (168). Niacin is also a precursor to the coenzymes NAD+/NADH as well as NADPH. In addition to being involved in DNA repair and steroid hormone production, these coenzymes are involved in fatty acid synthesis via NADPH, tissue respiration, and utilization of carbohydrates via NADH in skeletal muscle (7).

Interestingly, the effect of niacin appears to have different effects in hepatocytes, myocytes, and adipocytes, suggesting tissue-specific effects which must be taken into consideration when examining the whole body effects of this supplement. One study investigating the cellular mechanisms of niacin demonstrated an up-regulation in the degradation of ApoB, which is a protein involved in LDL transport, in human HepG2 (hepatic) cell cultures, thus lowering plasma LDL concentrations possibly by decreasing the synthesis and secretion of TAG and VLDL into the blood stream. Supplementation also inhibited fatty acid synthesis and esterification (169). Niacin also appears to limit the degradation of ApoA (a protein component of HDL) in hepatic cells, thus increasing plasma HDL concentration. Niacin appears to elicit its effects by acting on hormone sensitive lipase (HSL) to inhibit lipolysis in adipocytes and it acting on diacylglycerol acetyltransferase (DGAT) to inhibit TAG synthesis (170). Thus it appears that in skeletal muscle, niacin can contribute to fatty acid synthesis while inhibiting lipolysis. On the other hand, niacin can also block fatty acid synthesis in adipose and liver cells. This apparent disconnect may be explained by niacin's effects on the various tissue-specific enzyme isoforms involved in TAG synthesis, specifically DGAT.

DGAT

Diacylglycerol acetyltransferase (DGAT) catalyzes the only committed step in TAG

synthesis using DAG and fatty acyl CoA as substrates (156). Thus, without proper expression

and activity of this enzyme, lipid intermediates will not be incorporated into TAG, rather they

will accumulate. Accumulation of these intermediates, as previously discussed, play an integral

role in the development of insulin resistance. There are two basic isoforms of DGAT, both of

which appear to be necessary for proper functioning in mammals (171). DGAT1, which is

considered "overt" and is involved in formation of TAG in cytoplasm of ER in liver cells and

DGAT2, which is "latent" and is involved in formation of TAG in lumen of ER in liver cells,

which are then involved in the formation of VLDL (172).

The DGAT reaction can come from multiple pathways (1) de novo synthesis catalyzed by

mitochondrial glycerol-3 phosphate acetyltransferase (mGPAT), (2) esterification of MAG

catalyzed by monoglyceride acetyltransferase (MGAT), or (3) hydrolysis of TAG or

phospholipids.

Given its critical role in TAG synthesis, it has been suggested that inhibiting DGAT1

may decrease the expression of genes involved in fatty acid oxidation (i.e. PPAR genes) and

decrease adiposity and diet-induced obesity (173). Indeed DGAT1 KO mice exhibited these

traits and improved insulin sensitivity, however, mice deficient only in skeletal muscle DGAT1,

rather than whole body DGAT1 deficiency exhibited decreased insulin sensitivity, again

demonstrating the importance of tissue-specific isoenzymes and tissue-specific development of

insulin resistance (156). In fact, DGAT1 gene expression has been shown to be up-regulated

following a single bout of moderate-intensity aerobic exercise in humans (56). This increased

expression of DGAT1 increased IMCL stores, while decreasing DAG concentration. As

131

mentioned in chapter one, high levels of DAG are thought to activate pro-inflammatory

pathways as well as specific protein kinase C's (PKC's), which in turn phosphorylate serine

residues on insulin-receptor 1 (IRS-1), and decrease insulin sensitivity (22, 140). Thus, up-

regulating TAG synthesis is skeletal muscle may act to limit the development of insulin

resistance.

This hypothesis is supported by earlier findings in mice which showed that DGAT1 over-

expression increases small lipid droplet accumulation around the cell periphery, whereas DGAT2

over-expression increases large lipid droplet accumulation in the cytosol (171). As mentioned in

chapter one, small adipocytes have been shown to secrete insulin-sensitizing hormones such as

adiponectin and leptin, whereas large adipocytes have been shown to secrete insulin-

desensitizing hormones such as retinol-binding protein (RBP-4) and resistin (8). In addition to

forming large lipid droplets, which may be linked to insulin resistance, the over-expression of

DGAT2 in mice has been shown to decrease insulin sensitivity due to storage of TAG in

glycolytic fibers (174). Oxidative fibers have many mitochondria and rely primarily on fatty

acid oxidation for energy. These types of fibers are capable of storing and utilizing TAG's

within the muscle and are more predominant in aerobically trained individuals. Glycolytic

fibers, conversely, have few mitochondria and therefore cannot effectively oxidize stored TAG's

to produce energy. Therefore, it seems plausible that in obese, insulin resistant individuals,

DGAT2 expression is increased in skeletal muscle. Since these individuals are less active, it is

likely that they have fewer oxidative fibers and more glycolytic fibers. Thus, the paradox

between IMCL storage in insulin resistant individuals and IMCL storage endurance trained

athletes could also be due to a combination of the inability of the glycolytic fibers to break down

and oxidize stored TAG as well as an up-regulation in DGAT2, which furthers TAG storage in these glycolytic fibers.

With the given information about DGAT2, it may seem plausible that inhibition of DGAT2 may help limit the development of insulin resistance. Whether or not there is an up-regulation in DGAT1 and a down-regulation of DGAT2 following chronic exercise is currently unknown and warrants further investigation.

The over-expression of DGAT 1 and DGAT2 in mouse liver cells has been shown to cause an excessive accumulation of lipid stores within the liver without inducing insulin resistance or glucose intolerance (175). This may indicate that excess lipid storage in liver alone may not be a direct cause of insulin resistance; rather it may be an effect of the development of insulin resistance via inflammatory pathways.

Other Enzymes

Though DGAT is the rate-limiting enzyme and is the only committed step in lipogenesis, the role of other possible enzymes involved in lipid synthesis, such as mGPAT and SCD1, which desaturates fatty acids into monounsaturated fatty acids to be incorporated into TAG stores, must also be examined. Alterations in the expression of these enzymes may also lead to a build-up of fatty acid metabolites which have been linked to insulin resistance (155). Work on these enzymes, particularly in skeletal muscle is limited, but studies examining whole organisms and other tissues can provide possible future directions for study. For example, SCD1 KO mice are resistant to diet-induced obesity, likely due to the inability to store saturated fatty acids (176). At the other end of the spectrum, SCD1 protein expression and activity is up-regulated in rats following a high fat diet, causing the rats to became more insulin resistant (166). This does not, however, indicate that up-regulation of this enzyme induces insulin resistance per se; rather it

may act as a protective mechanism to limit the formation of DAG and ceramides associated with high levels of saturated fatty acids as seen in the study by Lee et al. (166). In contrast, GPAT over-expression in rats directly contributes to DAG storage in the liver, increased IMCL storage and increased hepatic, peripheral, and systemic insulin resistance (177).

With this in mind, it seems that increasing the expression of specific lipogenic enzymes through dietary manipulations or otherwise can contribute to insulin resistance while the increased expression of other enzymes may have no effect or may improve insulin sensitivity. The reasoning for this is not entirely clear although it seems likely that the over-expression of an enzyme such as GPAT, involved in the first step of TAG synthesis may take away from the FFA pool that may have otherwise been utilized for oxidation. On the other hand, depending on the tissue, increased expression of DGAT or SCD1 may limit the accumulation of DAG and ceramides.

While it is apparent that variations in lipid synthesis can greatly affect insulin sensitivity, variations in lipid mobilization, hydrolysis, and oxidation are just as critically important to examine in order to gain a more complete understanding of lipid metabolism and its links to insulin resistance. Though as seen with lipid synthesis, much remains unknown about lipid utilization.

CHAPTER 3: Lipid utilization in healthy vs. insulin resistant skeletal muscle

There are multiple enzymes involved in the complete process of degrading TAGs to FFA. The extent to which each of these enzymes are complimentary versus redundant is still not clear. Therefore it is important to examine some of the major enzymes involved in lipolysis and how alterations in the expression or activity of these enzymes may contribute to insulin resistance. Lipoprotein lipase (LPL), hormone sensitive lipase (HSL), and adipose triglyceride lipase (ATGL) are three of the major enzymes contributing to the initial breakdown of TAGs. As discussed in earlier chapters, numerous factors can affect the activity of these enzymes, and in turn, the extent of lipid breakdown. This chapter of the review will examine the fasting state, which activates these specific lipolytic enzymes, but to a different degree in healthy versus insulin resistant individuals. Another aspect that will be examined is tissue specificity given that lipolysis in liver, skeletal muscle, and different muscle fiber types are regulated by differing mechanisms. Finally, the mobilization of lipids will be examined since the amount of lipolytic enzymes and intermediates in the blood vary between insulin resistant and healthy, but also depend on where the TAG are mobilized from (skeletal muscle, liver, or adipose tissue).

Major Enzymes

As its name suggests, LPL is involved in the lipolysis of TAG in lipoproteins. Following feeding (high insulin levels), excess circulating TAG will be stored primarily in adipose tissue. However, TAG must first be hydrolyzed from VLDL or chylomicrons via LPL into FFA and then re-esterified into TAG. Adipose tissue and skeletal muscle have different isoenzymes of LPL which are regulated by differing mechanisms (178) which helps explain why the storage of TAG in adipose occurs readily while storage of TAG in skeletal muscle is more limited, at least in healthy, untrained individuals.

A second enzyme involved in lipolysis, which essentially has the opposite actions of LPL hydrolyzes TAG, DAG, and MAG to be oxidized for fuel (rather than stored) and is sensitive to the concentrations of insulin and glucagon (7). Therefore this lipase is often referred to as LPL-HS or hormone sensitive lipase (HSL). Much like LPL, HSL is expressed in both adipose and all types of skeletal muscle fibers, but the expression appears to be the highest in adipose, followed by oxidative, then glycolytic muscle fibers (159). LPL hydrolyzes TAG from lipoproteins for storage in adipose tissue and skeletal muscle whereas HSL hydrolyzes TAG from adipose or IMCL reserves for oxidation (125). In the presence of insulin, HSL activity decreases in both adipose and skeletal muscle, whereas LPL activity increases in adipose and skeletal muscle tissue. As a result, lipid degradation increases, but lipid storage increases as well. This is due to the anabolic action of insulin (179), which makes sense considering that the body always attempts to be as energy efficient as possible. If both enzymes were up-regulated, there would be a futile cycle between lipid storage and breakdown. Interestingly, skeletal muscle inactivity also appears to decrease LPL activity in all muscle fiber types (180), suggesting a role of physical activity in maximizing lipolysis, which will be discussed further in chapter four. However, more recent studies have indicated that these two enzymes alone are not solely responsible for the hydrolysis of TAG and that there may be numerous other complimentary or redundant enzymes or pathways involved.

ATGL

One such enzyme involved in lipolysis is adipose triglyceride lipase (ATGL). Once again, as its name suggests, it is involved in the hydrolysis and mobilization of TAG from adipose tissue, however this enzyme is also expressed in skeletal muscle (145). ATGL has been shown to be expressed exclusively in type I fibers, appears to play a role in converting TAG to

136

DAG and may play a role in fatty acid handling, lipid storage and breakdown (145). Given the deleterious effects of excessive plasma DAG, one school of thought is that if TAG degradation to DAG is limited, insulin sensitivity will improve. This has been shown to be the case in ATGL KO mice, which demonstrated increased glucose tolerance and insulin sensitivity despite TAG accumulation in multiple tissues. Insulin-stimulated PI3K, Akt activity also increased and phosphorylation of tyrosine residues on IRS-1 increased in skeletal muscle and adipose tissue in vivo (10). While this could potentially be an attractive pathway for the prevention of diabetes, it has been demonstrated that ATGL and HSL are needed for lipolysis in skeletal muscle and adipose to sustain normal substrate metabolism both at rest and during exercise (57). ATGL gene deletion in mice causes an increase in carbohydrate utilization, a decrease in circulating FFA at rest. Decreased circulating FFA leads to an increased dependence on glucose oxidation, thus, liver and muscle glycogen stores decrease. Interestingly, resting glycogen storage was not affected in HSL KO mice nor was fatty acid transport affected in ATGL or HSL knockout mice (57). However, the exercise-induced increase in FFA and glycerol mobilization was blunted in both KO mice, indicating a decrease in lipolysis, an increase in CHO oxidation, and a reduced endurance capacity. A similar study examining ATGL KO mice yielded similar results, again indicating that ATGL is required for the hydrolysis of TAG from skeletal muscle and adipose tissue, and thus, proper energy supply during exercise (181). Without this enzyme, the body cannot utilize lipid for energy and is in turn, forced to rely on glucose metabolism. This will lead to an up-regulation in glucose transport, which could help explain the improvement in insulin sensitivity seen in these KO mice.

Tri-di- and monoglycerides

Currently, the role of individual enzymes responsible for the conversion of TAG to DAG, DAG to MAG, and MAG to FFA and glycerol has not been examined in great detail. However, recent evidence suggests that these enzymes may play a key role in the accumulation of DAG and ceramides, and in turn, contribute to the development of insulin resistance. Triacylglycerol hydrolase, which converts TAG to DAG and diacylglycerol hydrolase, which converts DAG to MAG was recently examined in lean and healthy male and female subjects, all of which were sedentary. As seen in past studies, it was found that females exhibited higher levels of IMCL than males, obese subjects exhibited higher IMCL, DAG and ceramides than lean subjects (182). Expectedly, TAG hydrolase activity was higher in obese compared to lean, and higher in females compared to males. Interestingly, despite elevated DAG and ceramide levels in obese subjects, DAG hydrolase activity was actually lower for this population compared to lean subjects (182). This is critical as it suggests that while TAG hydrolase activity may be up-regulated in individuals with higher levels of IMTG storage, such as females or endurance trained athletes, obesity appears to induce changes in DAG hydrolase in skeletal muscle, though increased levels of adiposity are independent of this change in enzymatic activity. This incomplete lipolysis could contribute to the formation of DAG seen in insulin resistance; however, the mechanisms are not yet apparent.

It should be noted that ATGL acts as TAG hydrolase and HSL acts as both a TAG hydrolase and a DAG hydrolase, at least in the white adipose tissue of murine models (183) and therefore the activity of ATGL and HSL are often representative of these various hydrolases. MAG lipase appears to be its own unique enzyme and may also play a role in incomplete lipolysis although currently there are no studies examining the role of this enzyme in insulin

resistance. This may be because this particular enzyme is neither rate-limiting nor a committed step in lipolysis; however, examining this enzyme along with others could provide greater insight into differences between insulin resistant and healthy skeletal muscle metabolism. A fasting state (i.e. low insulin, high glucagon concentration) will clearly activate these enzymes in adipose tissue and to some extent in skeletal muscle; however, there is limited research on the extent that each of these enzymes truly function in healthy and insulin resistant human skeletal muscle. This will be an important area of future research with regards to possible links between incomplete lipolysis and insulin resistance.

The study by Schenk and Horowitz (22) is one of the few studies that have examined DAG levels following an acute aerobic exercise bout. In the aforementioned study, DAG levels were significantly reduced following exercise, but further study is clearly necessary to either support or oppose this research and to further determine if mono- and diacylglycerol levels are altered following chronic exercise. Even without the incorporation of exercise, few studies have explicitly examined MAG and DAG levels. This is due largely to the fact that standard enzymatic assays measure all partial acylglycerols as triacylglycerols. Fielding et al. (184) have suggested that these partial acylglycerols may account for less than 3% of total acylglycerols in fasted human subjects. Despite this low percentage, these partial acylglycerols may still play a critical role in the development of insulin resistance. However, at this point it is unknown if, and to what extent, those numbers may be altered as a result of acute aerobic exercise. It is possible that there may be a link between changes these lipid intermediates and insulin sensitivity. In both humans (16) and mice (57), increased fat oxidation in skeletal muscle has been shown to improve insulin sensitivity.

Fasting vs. Fed state

In a fasted state, the body will rely primarily on fat stores to supply energy and will conserve glycogen stores for essential brain, kidney, and red blood cell function or sudden increases in energy demand (7). However, the dependence on fat utilization in a semi-fed state will depend greatly on diet. A recent study demonstrated that in a fasted state, serum NEFA levels were not different in high fat (HF) and standard chow (SC) fed mice, indicating both were able to utilize fatty acids as a fuel source. However, NEFA concentration was significantly lower in fed SC mice compared to the fed HF mice, which suggests that SC mice were able to uptake and store fatty acids then subsequently utilize the immediately available glucose for fuel. On the other hand, the authors suggested that the mitochondria in HF fed mice apparently could not adjust to the feeding and continued to oxidize fatty acids despite the increased available glucose; indicating an impaired ability to switch which substrate is utilized for energy (140). This could provide implications for testing in post-prandial state as well as in a fasted state. As previously suggested, fasting lipid responses in healthy and insulin resistant individuals is drastically different (55). In addition, the study by Koves et al. suggests differences following feeding, therefore testing in a fed or semi-fed state may elicit more pronounced responses in lipid metabolism and should be examined in addition to the fasted state.

The differences seen in the fasted state may, however, be tissue specific. In a study done on diabetic and lean rats, fasting induced significant increases in IMCL storage in white tibialis anterior (wTA) and extensor digitorium longus (EDL) in both groups, though to a greater extent in the diabetic group (185). In addition, malonyl CoA concentrations decreased in these muscles of both groups, but to a greater extent in the lean group. The red soleus (SOL) muscle on the other hand did not exhibit changes in malonyl CoA concentrations in either group (185). This

suggests that starvation does not induce changes in lipid metabolism to oxidative muscles, such as the SOL, regardless of insulin sensitivity, whereas both lipid uptake and oxidation are activated in more glycolytic muscles like wTA and EDL when FFA availability is high due to starvation. However, since glycolytic fibers contain fewer mitochondria than oxidative fibers, even if malonyl CoA levels are suppressed, β oxidation will still be limited by the number of mitochondria and mitochondrial enzymes present. It may be that wTA and EDL muscles can re-esterify FFA and store them as IMCL primarily to cope with elevated FFA levels during fasting whereas the SOL muscles can cope with elevated FFA levels primarily via β oxidation. There also appear to be CPT1 enzymes that are insensitive to malonyl CoA, (186) which may also help partially explain why malonyl CoA levels were not altered in the SOL muscles for this study.

Gender Differences

Though lipolytic enzyme activity may be similar between men and women, lipid metabolism is clearly different in men and women and is worth briefly addressing here. In general, women tend to perform better at ultra-endurance events and there may be several reasons for this occurrence. Women naturally store more fat, in the form of IMCL or otherwise, and utilize a greater percentage of fat for energy compared to men. The role of estrogen likely has an effect on both of these. Variations in fiber type percentages could help partially explain why women tend to have higher gene expression for specific β-oxidative enzymes. In a recent study comparing differences in fatty acid handling in men and women, results indicated that there were no significant differences in mRNA content for β oxidative enzymes, yet there were significant differences in protein content of β oxidative enzymes (114). However, this study did not show any significant differences between men and women for the transcription factor PPAR. Thus, it seems very likely not only that various systems are interacting, but there are several

post-translational modifications taking place. If women do indeed preferentially utilize fat as a fuel source, it would be expected that genes responsible for β oxidation as well as lipolysis and the TCA cycle would be up-regulated. Further studies need to investigate additional transcription factors as well as other proteins involved in fatty acid transport, binding, and oxidation. It would also be useful to examine various key enzymes involved in the TCA cycle to help determine if all components of fatty acid oxidation are higher in women or if it is just specific enzymes, such as the ones involved in β oxidation. An interesting point, although not significant was that short-chain hydroxyacyl co-A dehydrogenase (SCHAD), responsible for short-chain oxidation, was slightly higher in men. This may indicate that men have the same or slightly higher capacity than women to break down short-chain fatty acids. It may be possible that there are gender differences in the percentage of short, medium, - and long-chain fatty acids, all of which have unique lipolytic enzymes. It is important to remember that this study examined only skeletal muscle and some if its respective β-oxidative enzymes. Adipocyte, skeletal muscle, and hepatic lipolysis are all different and therefore, future studies are needed to examine these aspects as well. From an observational perspective, it seems logical that if women naturally store more fat than men, they should be able to utilize fat more readily as a fuel source, thus the findings of this study seem plausible; however, the underlying mechanisms, particularly at a transcriptional and translational level are not yet clear.

Though it is apparent that much work is still needed to gain a better understanding of complete lipid hydrolysis, mobilization, and oxidation within skeletal muscle, even less is known about how this pathway and its respective enzymes are affected by exercise. Though exercise acts in a similar manner to fasting in order to provide energy to working muscles, it is evident that there are several differences that separate the effects of exercise and the effects of fasting.

Numerous studies have shown that exercise improves several health parameters, including insulin sensitivity. However, the mechanisms for this improvement, specifically with regards to lipid metabolism are still not fully understood. Chapter four will discuss these differences and shed some light on possible mechanisms linking exercise to improved insulin resistance.

CHAPTER 4: Effects of exercise on lipid synthesis/degradation in healthy vs. insulin resistant

Weight loss, specifically a decrease of abdominal adiposity, through caloric restriction, exercise, or a combination of the two, has been linked to improved insulin sensitivity (187) through various mechanisms. In addition, a reduction of abdominal adiposity has been shown to significantly increase insulin sensitivity. Interestingly, even without weight loss, exercise can improve insulin sensitivity (23) though the mechanisms responsible for the improvements in insulin sensitivity appear to be much different when weight loss is not present. Regardless of the type, intensity, or duration of exercise, chronic or acute, exercise has irrefutably been shown to help to prevent, or at least limit several health disparities including cardiovascular disease, obesity, cancer, and diabetes. However, the mechanisms are apparently variable and are dependent on numerous factors. This chapter will be broadly divided into acute aerobic, chronic aerobic, acute resistance, and chronic resistance training to examine how the modality of exercise affects changes in lipid metabolism and how a single bout of exercise can affect lipid metabolism compared to several weeks of training.

Aerobic Exercise

Aerobic exercise has been shown to up-regulate lipolysis and improve insulin sensitivity whereas excess lipid storage in adipose tissue as well as within skeletal muscle has been linked to insulin resistance (56). In general, any form of exercise appears to improve insulin sensitivity; however, the underlying mechanism is still largely unknown. It may be due to interconnections of improved mitochondrial function and enzymatic activity, decreased lipid metabolites, and decreased inflammatory cytokines. Due to the variability in methodology and analysis between studies, it has also been difficult to establish which variables play the most significant roles for increasing lipid breakdown and improving insulin sensitivity. Not only can the measurement

144

technique used (gas analysis, tracers, biopsy, plasma) and experimental model (cell cultures, animal models, humans) affect the results of a study, but gender, training status, diet, muscle fiber type, muscle glycogen content, exercise intensity, exercise duration, exercise type, all affect lipolysis and lipid synthesis differently during and following exercise, making it difficult to compare the effects between studies and thus, all of these variables need to be taken into consideration when conclusions are made regarding the effects of exercise on lipid metabolism.

Acute aerobic exercise

Before examining the literature, it should be noted that in general, most acute exercise studies are carried out when participants are in a fasted state. It has been demonstrated that an overnight fast does provide valid and reliable responses to exercise. However, examining participants in a postprandial state may provide a more "real world response". Exercising, especially at high intensities, immediately after consuming a meal may result in undesired consequences, specifically vomiting. Therefore, allowing food to digest for a few hours prior to exercise may be ideal. Presumably, most of the population does not exercise on a regular basis following an overnight fast, rather exercise takes place after some form of nutrients have been consumed. Additionally, several studies have shown that the ingestion of carbohydrates enhances endurance performance (102). In addition, as mentioned in chapter three, one study demonstrated that serum NEFA levels were not different in fasted high fat and standard chow mice; however, NEFA concentration was significantly lower in the fed standard chow mice whereas concentrations remained elevated in the high fat mice (140). This suggests an impaired ability in the high fat fed mice to switch from utilizing primarily fats in the fasted state to carbohydrates in the fed state. This provides clear implications for exercise testing in a fed or

145

semi-fed state to see more distinguishable results between populations and to provide much more useful information as it applies to the general public.

Timing

When examining the effects of an acute exercise bout, a very important consideration is the timeline for which the measurements are taken. The rate of fat oxidation changes during exercise compared to immediately, and several hours afterward. Changes in metabolite concentration, enzymatic activity, or gene expression may be rapid, but changes in protein expression or protein modification may be much more delayed.

Within skeletal muscle, an acute bout of aerobic exercise will increase cytosolic calcium levels and decrease the ATP/AMP ratio, both of which result in the activation and increased expression of PGC-1α (188). Several studies have demonstrated that increased levels of PCG-1α result in mitochondrial biogenesis by means of a complex signaling cascade described elsewhere (188, 189). However, previous studies have shown that exercise-induced mitochondrial biogenesis is altered or reduced in both humans and rodents with obesity and insulin resistance (54, 190). The question becomes whether the obesity caused the reduction of mitochondria or if low mitochondrial density caused the obesity. Warram et al. (19) suggested it can be the latter for some individuals. This predisposition to obesity can easily lead to insulin resistance and the development of type 2 diabetes, both of which have been linked to the down-regulation in the expression of several genes responsible for oxidative phosphorylation, several of which are under the control of PGC-1α (191). A likely contributor to this down-regulation is a dysfunction of the mitochondria (54). It may also be due to a loss of oxidative fibers and an increase in the percentage of glycolytic fibers, which in turn decreases PGC-1α expression (190). It is likely a

combination of several factors, each of which plays a role in the development of insulin resistance.

Some authors have suggested that an incomplete breakdown of fatty acids may lead to an accumulation of lipid intermediates such as DAG, ceramides, and acylcarnitine species, increasing the likelihood of developing insulin resistance (8, 22). However, no studies to date have examined specific enzyme activity of ATGL, HSL, and MAGL in a single study, all of which are involved in the complete breakdown of lipids. Furthermore, while a sufficient number of studies have looked a TAG levels and exercise, very few studies have reported information on DAG or MAG following exercise.

Complete Synthesis

A single aerobic exercise session has been shown to increase IMCL synthesis, particularly when fat availability is high (22). These increased levels of IMCL synthesis by increasing protein expression of DGAT1, mGPAT, and SCD1, which in turn, prevented the accumulation of metabolites like DAG and ceramides known to activate pro-inflammatory pathways. Similar results have been seen in rats. When given the drug Thiazolidinedione, IMCL synthesis increased while metabolite accumulation decreased and subsequent pro-inflammatory pathways were inactivated (49). Interestingly, the research shows that, at least in rats, it is an acute bout of aerobic exercise, rather than chronic training, which can act to down-regulate these pro-inflammatory pathways (21). As seen in the study by Nassis et al. (23), improvements in insulin sensitivity from chronic aerobic training may be more attributed to fiber type shifting, increased mitochondrial density, and improved glucose transport.

Intensity

Another variable that greatly influences the relative contribution of fat versus glycogen utilization is exercise intensity. High-intensity exercise results in, among other things, an accumulation of ammonia, which activates phosphofructokinase (PFK), and thus accelerates the rate of glycolysis (192). This results in a greater percentage of fuel utilization from anaerobic glucose metabolism and in turn, the production of lactate of and H+ ions which decrease blood pH. Lower pH levels have been shown to inhibit CPT I, thus limiting fat oxidation (193). This indicates that the onset of lactate accumulation inhibits FFA release from adipose tissue. It has been suggested that maximum fat oxidation in untrained individuals occurs between 35-48% VO_{2max}, which is considered to be low-intensity (188). This may be due to the fact that fast twitch fibers are recruited more heavily during high-intensity exercise, which is when the production of ammonia has been shown to be highest and product inhibition limits lipid metabolism (192).

Caffeine

One way to increase lipid utilization during acute aerobic exercise is to ingest a caffeine supplement. The extent of enhanced lipid utilization via caffeine can be altered by the dosage taken, intensity, duration, and type of exercise being performed, whether the exercise is chronic or acute, as well as the training status, and diet of the participants. Maximal fat oxidation in trained individuals appears to occur around 62.5% VO_{2max}, and minimal fat oxidation appears to be around 86% VO_{2max}. Untrained individuals tested at 75% VO_{2max} elicited no benefit from caffeine supplementation (92). It is likely that the intensity of this exercise necessitated a greater reliance on glucose oxidation for these untrained individuals, and thus, the caffeine was ineffective. In addition, the total duration of this study was only 30 minutes, subjects did not go

until volitional fatigue and thus, it is possible that metabolism and rating of perceived exertion (RPE) may have been different between trials. Prior to the exercise intervention, these individuals did not participate in any regular physical activity. During and immediately following exercise, participants demonstrated high levels of lactate, likely due to their untrained status. High lactate levels are known to inhibit lipolysis and thus, the effects of caffeine may be overridden by the action of lactate.

In several studies, caffeine has been shown to enhance FFA mobilization from adipose tissue, and in turn lowering the respiratory exchange ratio (RER) and spare muscle glycogen in submaximal acute exercise lasing 30-60 minutes (7, 89). This mechanism is likely due to an increase in catecholamine concentration, which enhances glucose release from the liver, FFA release from adipose tissue and FFA oxidation (7).

Despite this proposed mechanism, some studies have seen no effect of caffeine on aerobic function, RER or FFA uptake into skeletal muscle (90-92). Although in these studies, individual fatty acids were not examined nor was lipid breakdown examined. It is possible that while total fatty acid uptake did not change, there may have been changes in individual fatty acid uptake or breakdown. This was seen one a study where the authors showed increased unsaturated fatty acid mobilization (c18:1) and decreased saturated fatty acids (16:0 and 18:0) (194). This suggests that future studies involving changes in lipid synthesis and degradation following exercise should include not only overall changes, but also changes in individual fatty acid chain lengths and level of saturation. Another important consideration is whether the lipids being utilized during exercise are hydrolyzed and utilized from IMCL or from liver or adipose tissue.

IMCL Stores and Muscle Fiber Type

The extent to which IMCL contributes to fuel utilization during exercise is not well known though it seems likely that there is more contribution from these stores with longer duration, lower intensity type exercise. In healthy untrained individuals, glycogen stores will decrease with prolonged exercise and lipids will take over as main substrate. The extent of glycogen depletion will vary depending on the type of muscle an extent of muscle activity. Indeed, it has been shown that IMCL stores decrease with prolonged exercise and there is more use of IMCL in oxidative muscles compared to glycolytic muscles (195) further supporting the notion that lower intensity exercise maximizes fat utilization.

While low-intensity exercise will yield a greater percentage of fat utilization compared to high-intensity exercise, high-intensity aerobic exercise has still been shown to improve fat oxidation during exercise in recreationally active individuals (16, 24). An interesting note is that lipolysis apparently was not up-regulated. These subjects had increased expression of fatty acid binding protein (FABP), hydroxyacyl-CoA dehydrogenase, and citrate synthase, while fatty acid translocase CD36 and HSL were not up-regulated. Although these results cannot be readily explained, this may indicate that the oxidation of free fatty acids is up-regulated, as suggested in chapter one, while the mobilization of stored triglycerides is not up-regulated following this type of training. This may also indicate that lipid oxidation is more of a limiting factor than hydrolysis or transport during exercise and thus, there was no need for an up-regulation in HSL or CD36. During exercise, increased oxidation may be due to enhanced mitochondrial fatty acid uptake through the up-regulation of carnitine transport system, allowing long-chain fatty acids LCFA's into the mitochondria, which in turn, results in more CPT1 activity (16).

Clearly, the type of activity, or lack thereof, in addition to genetics can greatly affect muscle fiber type, and thus, influence the rate of lipid metabolism. In a comparison of ob/ob mice to lean littermates, the cross-sectional area of the EDL and sternomastoid were significantly reduced in the obese group. The obese mice also had smaller type II (glycolytic) fibers, and more "hybrid" fibers, suggesting fiber type shifting in obese mice (196). Similar fiber type shifting occurs following chronic aerobic training and will be discussed later in this chapter.

Altered Oxidative Gene Expression

Another change that occurs as a result of acute aerobic training is the up-regulation of various genes involved in fat metabolism. Uncoupling protein 2 and 3 (UCP-2 & UCP-3) gene expression has been examined in detail in both human and mouse models. UCP is responsible for transforming substrates to ATP via uncoupling mitochondrial respiration and therefore plays an important role in oxidative metabolism (197). UCP-3 in particular is expressed primarily in human skeletal muscle and may act to help regulate thermogenesis as well as utilize lipids as fuels by increasing fatty acid oxidation within the muscle (198). This FFA oxidation is seen with endurance exercise as well as fasting. Because of this, it would make sense that endurance exercise may act to increase UCP-3 mRNA. Noland et. al (197) discovered that an acute bout of endurance exercise does, in fact, up-regulate UCP-3 in untrained humans, however, this up-regulation was not seen in trained individuals. The exact reasoning for this is unclear. It may be attributable to a lower body fat percentage in trained individuals and therefore less need for the expression of UCP gene or it may be due to a higher initial expression of the UCP gene in trained individuals and thus, less of a need to up-regulate gene expression.

Specific factors that regulate UCP gene expression are not well understood, however it is evident that UCP-3 mRNA expression is dependent on the metabolic state of the skeletal muscle.

Cortright et. al (199) for example, identified that fasting increases UCP-3 mRNA up to eight fold and that re-feeding brings UCP-3 back down to basal levels or below. Part of the reasoning for why it has been so difficult to establish specific mechanisms behind changes in gene expression within skeletal muscle may be because skeletal muscle grown in culture lacks systemic input, lacks a motor nerve, and exclusively relies on glycolytic metabolism, consequently making it difficult to compare to fatty acid handling in intact, fully functioning adult myofibers within an organism.

However, several studies have been done *in vivo* to gain a better understanding of fatty acid metabolism. Hilderbrandt et. al (200) used a technique which involved isolating nuclei from skeletal muscle in combination with real-time polymerase chain reaction techniques (RT-PCR) to determine muscle fiber-specific and gene-specific changes in transcription rate of several genes including UCP-3, LPL, muscle CPT I, long-chain acetyl-CoA dehydrogenase (LCAD), and medium-chain acetyl-CoA dehydrogenase (MCAD), all of which are intricately involved in fatty acid metabolism. The authors found that fasting induces increased expression of the UCP-3 gene as well as several other genes associated with lipid metabolism, specifically in fast-twitch muscle fibers, which is intriguing given that fast twitch fibers are primarily glycolytic. However, considering that oxidative fibers likely already express high levels of the UCP-3 gene, these results suggest some level of fiber-type fuel shifting, reinforcing the notion that the body relies heavily on fatty acid metabolism during starvation.

Interestingly, the addition of acute aerobic exercise, which increases metabolic demand and glucose utilization, actually decreased the transcription of genes associated with lipid metabolism, specifically in slow-twitch muscle fibers. This showed that the physiological mechanisms responsible for these changes in gene expression due to fasting may oppose the

mechanisms responsible for changes in gene expression due to exercise. This may help explain why UCP-3 gene expression after acute bouts of exercise varies among trained and untrained individuals. Trained individuals may have a more repressed UCP-3 gene due to the chronic increased metabolic demand placed on the muscles. The important point to keep in mind is that although individuals that exercise regularly (trained) appear to have specific genes which are responsible for lipid metabolism to be repressed more than in those individuals that do not exercise regularly (untrained), this does not mean a lack of exercise leads to increased fat oxidation. It may simply indicate that trained individuals tend to carry a lower body fat percentage, and hence, are more metabolically efficient and do not need to transcribe those genes at such high rates. In addition, as mentioned in chapter one, increased β oxidation does not necessarily indicate improved lipid metabolism. Without up-regulation of complete lipolysis, the TCA cycle and ETS, increased fat oxidation may actually increase the concentration of deleterious lipid intermediates associated with insulin resistance.

Understanding how exercise can regulate the transcription of specific genes is an important first step in understanding the mechanisms of how exercise can help prevent metabolic diseases such as diabetes. As previously discussed, skeletal muscle plays a central role in metabolic homeostasis; however, other tissues such as liver and adipose tissue are involved as well. Hoene et al. (20) demonstrated that hepatic gene expression PCG-1α and PDK4, both of which are involved in lipid utilization hepatic gluconeogenesis, were up-regulated as a result of 60 minutes of aerobic exercise. This particular study also demonstrated that acute aerobic exercise induces increased IRS-2 protein expression while decreasing serine residue phosphorylation on the IRS residues known to interfere with proper insulin signaling (20). One important note is that the expression of these genes was higher in the liver than in slow-twitch or

fast-twitch skeletal muscle, indicating that some of the immediate benefits of exercise on insulin sensitivity take place in the liver. However, all tissues for this study were dissected and frozen immediately following the completion of the exercise session. It is possible that transcriptional changes in the liver may occur rapidly, whereas translational or protein expression changes in skeletal muscle may be more delayed. In addition, protein expression of PGC-1α and PDK4 were not examined in this study. Therefore before any conclusions can be made regarding differences in these genes in various tissues, further study examining protein expression at various time points following the completion of acute aerobic exercise is deemed necessary.

Chronic Aerobic

It has been clearly demonstrated that aerobic exercise training increases the size and number of mitochondria in addition to increases in mitochondrial enzymes involved in fatty acid oxidation, TCA cycle, ETS, and ATP synthesis, and thus, insulin sensitivity (54). Even independent of weight loss, exercise seems to elicit improvements in insulin sensitivity. One study involving obese girls showed that twelve weeks of aerobic exercise improved insulin sensitivity without any changes in body weight, percent body fat, or markers of inflammation (23). This may suggest that changes such as muscle fibers switching to more oxidative type, increasing mitochondrial density, GLUT-4 concentration, glycogen synthase activity, and decreased IMCL are more responsible for changes in insulin signaling as a result of exercise compared to changes in hormone and inflammatory signaling seen in acute aerobic studies.

In an untrained state, PGC-1α is expressed at a higher level in more oxidative muscles and thus, has a greater ability to oxidize free fatty acids (201). Previous studies have shown that following chronic aerobic exercise training, all fiber types increase their capacity to oxidize FFA by increasing activation, transport and β oxidation of, among several others, LCFAs and

154

components of the ETS (202). However, since fast-twitch muscle intrinsically has a lower initial oxidative capacity, these types of fibers may have a greater ability to adapt to the exercise stimulus (203), whereas a more oxidative muscle will not have to undergo any major biochemical changes and therefore will not adapt to the same extent.

One study has shown that plasma FFA decreased in trained individuals following cycling indicating that trained individuals rely more on intramuscular TAG stores for fat oxidation during exercise (16). It also demonstrated that trained individuals have higher rate of FFA uptake and oxidation during exercise compared to untrained, both of which were tested at 75% VO_{2max} (high-intensity). This indicates the important role of training status, as will be discussed later in this chapter.

Regardless of the mechanisms involved, clearly chronic aerobic exercise improves insulin resistance, although different exercise and measurement variables create different effects on lipid metabolism. Some of those variables will be addressed here.

Muscle Tissue Variation

Another variable that must be taken into consideration when examining changes within skeletal muscle following chronic exercise is muscle fiber type. The vast majority of studies involving human models have taken muscle samples from the vastus lateralis, mainly because this muscle is easily accessible. The vastus lateralis is more involved with short, quick contractions and less involved with long, slow contractions. Therefore it generally has a higher percentage of fast-twitch fibers and a lower percentage of slow-twitch fibers whereas the soleus for example, is involved with standing and posture and therefore has a higher percentage of slow-twitch fibers. If a researcher were to obtain muscle samples from the vastus lateralis and the soleus of a trained athlete, the percentage of fast-twitch fibers in the soleus would likely not

be the same as an untrained individual, making it difficult to infer that one individual may be more effective at metabolizing lipids than another. The "in between" muscle fiber types must be considered as well. Most, if not all muscles, are not purely fast-twitch or slow twitch, rather they are on a continuum somewhere in between and the percentage of a specific fiber type can vary within the same muscle. The percentage of fast-twitch fibers at the origin may be different than at the insertion, so unless multiple samples are taken from the same muscle, it will be challenging to assess how lipids are truly metabolized within a muscle, and even then, there are other factors to consider.

Training Status

Endurance trained individuals rely more on intramuscular IMCL stores for fat oxidation during exercise whereas untrained individuals will rely more heavily on muscle glycogen and circulating FFA. As previously mentioned, plasma FFA concentration decreases in trained individuals following cycling (16). In addition to utilizing a higher percentage of IMCL, this also shows that trained individuals have higher rate of FFA uptake and oxidation during exercise compared to untrained, even at 75% VO_{2Max} (high-intensity). Also, the rate of appearance of glycerol higher in trained men vs. untrained indicating that esterified fatty acids are being hydrolyzed in the muscle. Though endurance trained individuals will store higher levels of IMCL, skeletal muscle does not contain glycerol kinase, which is required to re-phosphorylate glycerol into glycerol-3 phosphate (G3P), thus once stored TAGs have been hydrolyzed, glycerol must be released from the muscle. Thus, although the FFA's released via lipolysis was equal in both groups, the trained individuals were clearly utilizing a greater percentage of lipid stores (16). Fat oxidation was also twice as high in trained, likely due to the fact that that training increases fat oxidation during exercise by enhancing mitochondrial density and thus, fatty acid

156

uptake through higher expression and activity of CPT1. Though not specifically measured in this study, it may be concluded that aerobic exercise training up-regulates not only the number of mitochondria which can be utilized for β oxidation, but the carnitine transport system for LC-FAs into the mitochondria may be up-regulated as well though further research is needed to confirm these findings.

Implications for Diabetes

Adiponectin, as mentioned in chapter one, is a cytokine secreted by adipose tissue, plays an important role in insulin sensitivity as well as glucose and lipid metabolism (2). When a wide range of normal weight, overweight, and obese individuals with normal glucose tolerance, impaired glucose tolerance, and type II diabetes were placed on a four-week physical training regimen, Bluher et al. (2) found that exercise increased the expression of adiponectin and its receptors (AdipoR1 & AdipoR2). AdipoR1 receptors are expressed in skeletal muscle cells and AdipoR2 receptors are expressed in skeletal muscle and liver cells. This increased expression may act to mediate improvements in insulin sensitivity, glucose metabolism, and lipid metabolism seen as a result of exercise. They also noted that the more insulin resistance that a given participant had, the lower the expression of the AdipoR2, regardless of body fat percentage, indicating that even if an individual is not obese, poor insulin sensitivity can drive down the expression of important proteins needed for glucose and lipid metabolism.

Non-insulin dependent diabetes also induces several impairments, specifically in mitochondrial function. This includes decreased lipid metabolism, decreased UCP-3, low and mitochondrial density, which in turn, limits oxidative capacity (204). As discussed above, exercise counteracts several of these imbalances although the physiological mechanisms remain unclear. It has been determined by Lumini et al. (199) that exercise, in addition to restoring

157

UCP-3 gene expression, may increase mitochondrial biogenesis by increasing the expression of PGC-1α and heat shock proteins (HSPs) ultimately resulting in helping to prevent the development of diabetes. Similar results were seen by Toledo, Watkins, and Kelly (205). They demonstrated that when sedentary obese humans without diabetes were placed on a calorie restricted diet in combination with an exercise regimen, there was a significant increase in both the size and total number of mitochondria in the skeletal muscle. This increased oxidative capacity, increased lipid metabolism, and improved insulin sensitivity. This demonstrated that improvement in mitochondrial size and density as a result of exercise leads to not only improved muscle metabolism, but improved insulin sensitivity as well, although changes in insulin sensitivity were likely partially due to caloric restriction. Again, this may be a result of more complete β oxidation as well as more complete breakdown of esterified fat stores.

Evidence in the literature clearly indicates the high correlation between obesity (lipid accumulation) and type 2 diabetes (insulin resistance). Kelly, Goodpaster, Wing, and Simoneau (55) made this correlation even more evident by showing that obese individuals express diminished CPT activity as discussed earlier and they have a reduced oxidative enzyme capacity. Improvements in insulin sensitivity and fat oxidation in these obese participants improved as a result of a four month weight loss intervention using caloric restriction. Once again, insulin sensitivity and metabolic flexibility improved following feeding, but no changes were observed in a fasted state. It seems plausible that an exercise intervention, which clearly improves insulin sensitivity, may act to limit lipid utilization following feeding while caloric restriction may act to increase lipid utilization following fasting, though more research is needed to confirm this theory.

More recently, Haskell-Luevano et al. (206) looked at a melanocortin-4 receptor (MCR4) knockout (KO) mouse model to see if exercise alone affected specific genes associated with obesity, insulin resistance, and other metabolic syndromes. Altered MCR4 gene phenotypes have been identified in obese human and mouse models and therefore mice without this gene will express obesity, insulin resistance, and hyperleptinemia. Indeed, the study showed that eight weeks of exercise improves insulin sensitivity and effects the expression of proopiomelanocortin (POMC), orexin, melanocortin-3 receptor (MCR3), and neuropeptide Y (NPY), all of which are important in maintaining energy homeostasis. Exercise intervention creates this homeostasis and can, in turn, prevent obesity and diabetes, even in genetically predisposed MCR4 KO mice. It can also help prevent hyperleptinemia, which is simply high levels of circulating leptin, a hormone derived from adipocytes that is necessary for controlling body weight. Björnholm et al. (207) demonstrated that mutations in leptin gene phenotype or leptin receptor expression results in the inability of leptin to bind to its receptor site in the hypothalamus. When leptin is unable to bind, this creates higher levels of circulating leptin and increases excess lipid storage. Interestingly, following chronic aerobic exercise intervention these perturbations are reversed only in the presence of weight loss (208). This demonstrates that levels of adiposity may be directly responsible for changes in circulating leptin levels and that improvements seen following exercise intervention independent of weight loss occur from mechanisms other than changes in leptin levels. Indeed this has been seen in previous studies (23).

Clearly, aerobic exercise, whether acute or chronic, improves insulin sensitivity and some of the cellular mechanisms are beginning to emerge. However, it is currently unknown if resistance training has similar effects on lipid metabolism to those seen with aerobic training. Since resistance training is an attractive alternative form of exercise for individuals that are

unable or unwilling to perform aerobic exercise, understanding the effects of resistance training on insulin sensitivity compared to those seen with aerobic training is critical.

Resistance Training

Much like aerobic exercise training, there are multiple variables that can affect lipid metabolism responses following resistance exercise training (training status, gender, tissue type, etc). However, there are far fewer studies that have examined this particular research area. This is probably attributed to the fact that this type of exercise is not continuous and that the variability between different types of exercises, number of exercises, muscle groups worked, type of resistance used, sets, reps, rest between sets, relative intensity, and number of days per week make this type of exercise much more difficult to control and measure compared to aerobic type exercise. In addition, until fairly recently, resistance type exercise was not given much consideration as to its effectiveness in improving insulin sensitivity, especially with regards to lipid metabolism. Given that much of the United States population is now overweight or obese, it is evident that most of these types of patients, especially those with type 2 diabetes, do not engage in regular physical activity. While aerobic exercise clearly has its benefits, if patients do not regularly adhere to this type of exercise throughout their lives, health status is not likely to improve. With a limited aerobic capacity in these patients, especially at first, engaging in resistance training offers and attractive alternative to possibly help combat obesity and the development of diabetes; however it should appear evident by the end of this chapter that much more research is needed in this area to establish specific changes that occur from this type of exercise.

Acute resistance

As previously mentioned, skeletal muscle accounts for the majority of glucose uptake, therefore, by increasing the size of the muscle via resistance training, one should be able to increase the capacity of whole-body glucose disposal and in turn, limit hyperglycemia. Muscle contraction alone, whether through resistance or aerobic exercise requires the utilization of ATP (energy expenditure) and induces calcium release. This alone may have some protective effects against insulin resistance. This has been demonstrated in numerous studies. Transgenic mice expressing activated calcineurin in skeletal muscle had improved insulin uptake in the glycolytic extensor digtorum longus (EDL) muscle when fed a high fat diet. This was associated with an up-regulated expression of insulin receptor, Akt, GLUT-4, and PGC-1α expression (44). In normally functioning muscle cells, calcineurin is activated by increased levels of cytoplasmic calcium, thus this study implies that muscle contraction alone does indeed improve insulin sensitivity and glucose uptake via an insulin-independent mechanism.

Numerous studies have also suggested that AMP, which is a product resulting from ATP hydrolysis, activates AMP activated protein kinase (AMPK), and in turn phosphorylates and inhibits acetyl-CoA carboxylase (ACC), which stops the production of malonyl-CoA and relieves the inhibition of CPT1 to transport long-chain fatty acids into the mitochondria (40). AMPK also increases skeletal muscle glucose uptake, increases citrate levels, and increases levels of NAD^+ and Sirt-1, both of which increase PGC-1α expression. The extent of AMPK activation appeared to be directly related to exercise intensity, and thus ACC inactivation appears to be dependent on exercise intensity (40, 41).

However, other studies suggest insulin sensitivity appears to be independent of exercise intensity with acute resistance training. One study examined glucose uptake and insulin sensitivity in resistance trained individuals following a control (no exercise), low-intensity, or

161

high-intensity workload and found no differences at the two intensities (209). All participants

for this study were trained individuals, which may have confounded the results. It seems likely

that if untrained or insulin resistant individuals were to complete this protocol, differences may

be observed.

Acute resistance training also improves post-exercise lipid oxidation for up to 24 hours,

although the mechanism is unknown. Some authors have attributed this improvement to

decreased IMCL stores (26), however, this alone is not likely responsible for these changes as

the literature points more toward the notion that changes in IMCL content and lipid turnover may

be altered.

In resistance trained men, caffeine supplementation increased 1RM but had no effect on

leg extension or muscle endurance. This may indicate again that lactate supersedes the effects of

caffeine. It could also suggest that caffeine elicits different effects in different muscle groups

(104). However, this study had no crossover and participants only consumed 200 mg of caffeine,

and habitual vs. non-habitual consumption was not taken into consideration. It is likely that if

more caffeine were ingested, there may have been more beneficial effects given that caffeine

dosages between 3-6 mg/kg of body weight have been identified as being optimal dosages for

improving performance (93).

Chronic Resistance Training

As seen with aerobic exercise, there are significant differences between the beneficial

effects of acute resistance training compared to chronic resistance training, however, there are

some similarities as well. A six-week study comparing untrained healthy men and type 2

diabetic men showed that 30 minutes of resistance training three days per week increased

strength and muscle mass, as expected (12). Since skeletal muscle is responsible for the majority

162

of glucose disposal, a larger muscle mass allows for greater glucose uptake, and thus better glycemic control. Although six weeks of resistance training will improve strength significantly in untrained individuals, is not an adequate enough time period to see any significant changes in muscle mass. Rather, the results of this study indicated that GLUT-4 protein, insulin receptor protein, and glycogen synthase protein and activity increased as a result of training. This suggests that although there may have been slight changes in muscle mass, changes in protein expression were primarily responsible for the improved glucose uptake and insulin sensitivity. This change in protein expression appears to be due to increased muscle contraction, as was seen in acute resistance training. This makes sense given that resistance training is predominantly and anaerobic activity and therefore the working muscles must predominantly rely on glucose metabolism. Thus, the question becomes whether the effects seen in this study were truly due to chronic adaptations or residual effects of an acute bout of exercise.

It is important to keep in mind that the time course in which measurements are taken can factor into the outcomes. Measurements for the previously mentioned study were carried out 16 hours after the final exercise session. In another study, diabetic men were placed on a resistance training plus weight loss protocol or just a weight loss protocol for three days per week for six months. Measurements were taken 48 hours after the last exercise training session and it was found that, similar to the study by Holten et al. (12) that resistance training increased strength and muscle mass and had greater improvements in glycated hemoglobin (HbA1c) levels (13). However, plasma insulin and glucose as well as blood lipid concentrations did not improve. Thus, this study indicates that whole-body glycemic control improves as a result of chronic resistance training whereas changes in plasma insulin glucose concentrations may only improve transiently as a result of acute resistance training.

A more recent study supports these findings but suggests that insulin sensitivity does improve as a result of chronic resistance training. In measurements taken 72 hours after the completion of a 16 week resistance training intervention in older type 2 diabetic Hispanic men and women, whole-body insulin sensitivity and HbA1c levels improved (62). This correlated with decreased inflammatory markers and increased adiponectin levels. Similar to Dunstan et al. (13), plasma glucose and insulin did not change significantly, however, HOMA, determined as [fasting glucose*fasting insulin/22.5] was significantly improved following training. This may be due to improvements in glucose translocation, possibly via insulin-independent mechanisms such as increased calcineurin activation as suggested by Ryder et al. (44).

While these, along with several other studies have shown clear improvements in insulin sensitivity and glycemic control with resistance training, few have truly examined changes in lipid handling. Some studies have examined parameters such as plasma TAG, HDL, LDL, VLDL, and total cholesterol and seen improvements (27) whereas others have not seen improvements in these parameters (25). The different results yielded in these studies and others can likely be attributed to the heterogeneity of exercise modality, duration, and intensity.

Given the mostly anaerobic metabolic demands placed on skeletal muscle during resistance training, chronic exposure will cause muscle fibers to shift to more fast-twitch, glycolytic (type II fibers). As mentioned previously, this will affect the ability of the muscle cell to oxidize fatty acids since this type of fiber contains fewer mitochondria compared to oxidative fibers. Initially, this may seem detrimental to lipid metabolism, but it is important to keep in mind that other factors such as complete synthesis and hydrolysis may have a larger impact on insulin sensitivity, and other factors, such as fatty acid and glucose translocation also play a role, which is why despite the fiber type shifting, resistance training can still improve insulin

sensitivity. Also, other changes, such as increases in resting metabolic rate (RMR), following resistance training results in greater daily energy expenditure, and thus there is less likelihood that energy consumption will exceed energy expenditure, leading to less lipid storage, less lipid intermediates, and improved insulin sensitivity.

Combined, these results suggest that exercise improves insulin sensitivity primarily by increasing metabolic demand on skeletal muscle. This increases lipid turnover, improves oxidative capacity, up-regulates GLUT-4 translocation, and increases glycogen storage capacity. In turn, skeletal muscle can store more glycogen and utilize more fat. This in turn is likely to lower fasting RQ and improve metabolic flexibility, however further research is needed to confirm this theory.

Further study following both acute and chronic resistance training is clearly necessary to examine parameters such as TAG, DAG, MAG, glycerol and their associated lipolytic and lipogenic enzymes to gain a better understanding of changes in lipid handling following this type of training. In addition, examining changes in the RQ in both the fasted and fed state of insulin resistant individuals following resistance training may provide a more mechanistic insight as to why exercise improves insulin sensitivity and metabolic flexibility.

CHAPTER 5: Conclusions and Future Directions

Though there are several aspects involved in the development of insulin resistance, including but not limited to lipid intermediate accumulation, inflammation, change in adipocyte-derived hormone expression, changes in oxidative gene expression, impaired glucose stimulated insulin secretion, impaired glucose uptake, impaired glucose and fatty acid transport and translocation, incomplete lipolysis, decreased or incomplete β oxidation, incomplete TAG synthesis, the literature irrefutably shows that all of these aspects are interconnected and result from an energy imbalance (8, 126, 155). Linking of all these aspects of insulin resistance has not fully been established, due in large part to the fact that insulin resistance can develop from numerous pathways. Furthermore, the improvement of insulin resistance through means of calorie restriction, exercise, or otherwise can result in various metabolic changes depending on the means utilized. Finally variation among populations (training status, age, gender, ethnicity, diet, level of insulin sensitivity) along with measurements taken (enzymatic assays, mRNA, protein expression, chromatography, glucose clamp, HOMA, biopsy, tracer) and the time course for these measurements can all yield different outcomes. Thus, broad, general statements regarding the development of insulin resistance as it relates to lipid metabolism cannot be made. However, evidence in the literature has allowed for some more specific conclusions to be made.

Physical activity, or lack thereof, calorie restriction, calorie overconsumption, weight loss and weight gain all lead to changes in gene expression, which in turn, affects the capacity to hydrolyze, mobilize, oxidize, and synthesize lipids. Research has indicated that lipid accumulation in general (i.e. obesity) is highly correlated with the development of insulin resistance, but since high levels of lipids do not directly cause insulin resistance, these changes in

gene expression may help partially explain how elevated lipid levels can lead to the development of insulin resistance.

Altered gene expression and enzymatic activity is tissue-specific. Changes in the liver do not necessarily represent changes in the muscle and vice versa. It seems likely that individual organs and tissues are specifically regulated, but inter-organ communication creates a global effect on lipid metabolism and insulin sensitivity, thus when examining possible mechanisms linking lipid metabolism and insulin resistance, the state of each of these tissues and organs must be considered. Also, the effects of exercise on lipid degradation and lipid synthesis is different in healthy versus insulin resistant individuals and therefore, it is important to keep in mind that simply because studies demonstrate improvements in lipid metabolism in healthy skeletal muscle does not necessarily mean the same results will be seen in insulin resistant skeletal muscle.

Given the "athlete's paradox" and the multiple studies directly linking excess IMCL stores to insulin resistance, the composition TAGs themselves need to be considered. Fatty acid chains can vary in length and level of saturation. A TAG consists of a glycerol molecule and three fatty acids, all of which can contain short, medium, or long chains, and can be saturated or unsaturated. TAGs consisting of more saturated fatty acids may be more difficult to metabolize and may lead to incomplete degradation. This could partially explain why obese individuals with high IMCL are more insulin resistant compared to their endurance-trained counterparts.

Future Directions

An important note for future studies is that the heterogeneity of exercise and measurement variables have not allowed for any consistent results to be established. While it is not possible to standardize one procedure given that each of these variables have their pros and cons depending on resources, subject availability, and outcome measurements, following

167

previously established effective exercise programs and measurement techniques will make studies more comparable in the future.

Currently, few studies have examined aerobic exercise-induced changes in lipid synthesis and degradation and its effects on insulin sensitivity and even fewer studies have examined changes in lipid metabolism following resistance training. Given the numerous metabolic changes that occur as a result of resistance training, it is likely that resistance training will elicit changes in lipid metabolism, leading to improved insulin sensitivity. Other types of training, such as yoga, pilates, or intensive stretching, which also alter skeletal muscle metabolism through contraction-mediated responses, may also lead to improvements in lipid metabolism; however, this has not been examined. If found to improve lipid handling, resistance and alternative types of training could prove to be an attractive alternative to endurance type training in insulin resistant or obese individuals who may not otherwise engage in regular physical activity.

Exercise does improve insulin sensitivity, regardless of training status, modality, duration, or intensity. In terms of "the big picture", for the general population this is the most important outcome. This general idea has been known for years, the question has now become "what are the primary mechanisms responsible for this improvement and is there a more ideal exercise in terms of modality, time, intensity, and frequency?" In order to gain a better understanding of the mechanisms behind this improvement, it will be necessary to develop more specific pathways linking exercise to improved insulin sensitivity, particularly with regards to lipid metabolism. This may be done by examining each step involved in lipid degradation and synthesis in various populations and how these steps are affected by different types of exercise training. The activity of LPL, ATGL, HSL, MAG lipase, G3P, and various enzymes β oxidative,

TCA, and ETS enzymes, in healthy and insulin resistant individuals may allow researchers to

more accurately pinpoint where decrements in lipid metabolism occur both at rest and following

exercise.

*Note: All citations for Appendix 1 are included in the references list of the main text.

APPENDIX 2. PARTICIPANT QUESTIONNAIRES

<u>All questions contained in this questionnaire are strictly confidential</u>

Questionnaire

Name_____

Gender_____

Age_____

Height_____

Weight_____

Date/Time_____

Resting Blood Glucose_____

Has Father_____   Mother_____ been diagnosed with type 2 diabetes? Mark all that apply

How many times/week do you drink coffee/tea_____ _   Energy drinks/shots_____
        Soda_____

How many times/week do you typically perform the following exercises?

Running_____   Walking_____   Cycling_____   Swimming_____

Weight Lifting_____   Other (specify) _____

% BF from 7-site skinfold

Chest_____   Ab_____   Thigh_____Tricep_____   Suprailliac_____

Subscapular_____   Midaxilary_____

1 mile run time_____

Estimated 1 RM bench_____1RM squat_____1RM deadlift_____

3 Day Dietary Recall (Please be as specific as possible. What did you eat? How much? Where was it from? What time? Also include all drinks except water.)

<u>3 Days Ago</u>

Morning

Afternoon

Evening

<u>2 Days Ago</u>

Morning

Afternoon

Evening

<u>1 Day Ago</u>

Morning

Afternoon

Evening

<u>Notes:</u>

List your prescribed drugs and over-the-counter drugs, such as vitamins and inhalers		
Name the Drug	Strength	Frequency Taken

Allergies to medications/foods	
Name the Drug/Food	Reaction You Had

	HEALTH HABITS AND PERSONAL SAFETY
	ALL QUESTIONS CONTAINED IN THIS QUESTIONNAIRE WILL BE KEPT STRICTLY CONFIDENTIAL.

Exercise	☐ Sedentary (No exercise)				
	☐ Mild exercise (i.e., climb stairs, walk 3 blocks, golf)				
	☐ Occasional vigorous exercise (i.e., work or recreation, less than 4x/week for 30 min.)				
	☐ Regular vigorous exercise (i.e., work or recreation 4x/week for 30 minutes)				
Diet	Are you dieting?			☐ Yes	☐ No
	If yes, are you on a physician prescribed medical diet?			☐ Yes	☐ No
	# of meals you eat in an average day?				
	Rank salt intake	☐ Hi	☐ Med	☐ Low	
	Rank fat intake	☐ Hi	☐ Med	☐ Low	
Caffeine	☐ None	☐ Coffee	☐ Tea	☐ Cola	[] Energy Shots/Drinks
	# of cups/cans per day?				
Alcohol	Do you drink alcohol?			☐ Yes	☐ No
	If yes, what kind?				
	How many drinks per week?				
Tobacco	Do you use tobacco?			☐ Yes	☐ No
	☐ Cigarettes – pks./day	☐ Chew - #/day	☐ Pipe - #/day	☐ Cigars - #/day	
	☐ # of years	☐ Or year quit			
Drugs	Do you currently use recreational or street drugs?			☐ Yes	☐ No

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
☐	☐	2.	Do you feel pain in your chest when you do physical activity?
☐	☐	3.	In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5.	Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
☐	☐	6.	Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7.	Do you know of <u>any other reason</u> why you should not do physical activity?

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to <u>all</u> PAR-Q questions, you can be reasonably sure that you can:
- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:
- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

<u>Informed Use of the PAR-Q:</u> The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____   DATE_____

SIGNATURE OF PARENT _____   WITNESS _____
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

© Canadian Society for Exercise Physiology   Supported by: Health Canada   Santé Canada

APPENDIX 3. IRB/CONSENT FORMS

ACTION ON PROTOCOL APPROVAL REQUEST

LSU

TO: Greg Davis
Kinesiology

FROM: Robert C. Mathews
Chair, Institutional Review Board

DATE: March 15, 2011
RE: IRB# 3150

TITLE: Effects of nicotinic acid and caffeine supplementation on lipid metabolism following acute high-intensity aerobic exercise training

New Protocol/Modification/Continuation: _New Protocol_

Review type: Full _X_ Expedited __   **Review date:** _3/15/2011_

Risk Factor: Minimal _____ Uncertain ___X___ Greater Than Minimal_____
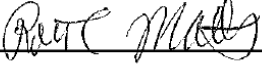
Approved_____X_____ **Disapproved**_____

Approval Date: _3/15/2011_   **Approval Expiration Date:** _3/14/2012_

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: _40_

Protocol Matches Scope of Work in Grant proposal: (if applicable)_____

By: Robert C. Mathews, Chairman _____

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. SPECIAL NOTE:
*All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb

Application for Approval of Projects Which Use Human Subjects

This application is used for projects/studies that cannot be reviewed through the exemption process.

LSU

– Applicant, Please fill out the application in its entirety and include two copies of the competed application as well as parts A-E, listed below. Once the application is completed, please submit to the IRB Office for review and please allow ample time for the application to be reviewed Expedited reviews usually takes 2 weeks. Carefully completed applications should be submitted 3 weeks before a meeting to ensure a prompt decision.

– A Complete Application Includes All of the Following:
  (A) Two copies of this completed form and two copies of part B thru E.
  (B) A brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts 1&2)
  (C) Copies of all instruments to be used.
        *If this proposal is part of a grant proposal, include a copy of the proposal and all recruitment material.
  (D) The consent form that you will use in the study (see part 3 for more information.)
  (E) Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project, including students who are involved with testing or handling data, unless already on file with the IRB. Training link: (http://phrp.nihtaining.com/users/login.php.)
  (F) IRB Security of Data Agreement: (http://www.lsu.edu/irb/IRB%20Security%20of%20Data.pdf)

1) Principal Investigator*: Arnold G. Nelson   **Rank** Professor
*PI **must be** an LSU Faculty Member

Dept: Kinesiology   **Ph:** 578-3114   **E-mail:** anelso@lsu.edu

2) Co Investigator(s): please include department, rank, phone and e-mail for each
Greggory R. Davis
Department of Kinesiology- Graduate Assistant
336-263-7101
gdavi14@lsu.edu

IRB# 350   LSU Proposal #_____

- ☒ Full
- ○ Expedited
- ☒ Human Subjects Training
- ☒ Complete Application

3) Project Title: Effects of nicotinic acid and caffeine supplementation on lipid metabolism following acute high-intensity aerobic exercise training

4) Proposal Start Date: January 18, 2011   **5) Proposed Duration Months:** 12

6) Number of Subjects Requested: 40   **7) LSU Proposal #:**

8) Funding Sought From: N/A

ASSURANCE OF PRINCIPAL INVESTIGATOR named above
   I accept personal responsibility for the conduct of this study (including ensuring compliance of co-investigators/co-workers) in accordance with the documents submitted herewith and the following guidelines for human subject protection: The Belmont Report, LSU's Assurance (FWA00003892) with OHRP and 45 CFR 46 (available from http://www.lsu.edu/irb). I also understand that copies of all consent forms **must be maintained at LSU for three years after the completion of the project.** If I leave LSU before that time, the consent forms should be preserved in the Departmental Office.

Signature of PI _____   Date _17 Nov 2010_

ASSURANCE OF STUDENT/PROJECT COORDINATOR named above. If multiple Co-Investigators, please create a "signature page" for all Co-Investigators to sign. Attach the "signature page" to the application.

Study Approved By:
Dr. Robert C. Mathews, Chairman
Institutional Review Board
Louisiana State University
203 B-1 David Boyd Hall
225-578-8692 | www.lsu.edu/irb
Approval Expires: 3-14-2012

I agree to adhere to the terms of this document and am familiar with the documents referenced above.

Signature of Co-PI (s) _____   Date _11/17/2010_

1. Study Title:

Effects of nicotinic acid and caffeine supplementation on lipid metabolism following acute high-intensity aerobic exercise training

2. Performance Sites:

Long Fieldhouse Room 54

3. Contacts:

Dr. Arnold Nelson

Wrk. 578-3114/ Hm. 766-4621

Gregg Davis

Cell. 336-263-7101

4. Purpose of the Study:

The study will investigate whether blood lipid breakdown immediately following an aerobic exercise bout is affected by caffeine or nicotinic acid supplementation.

5. Subjects:
A. Inclusion Criteria

Healthy, endurance trained (VO2 max> 48 ml/kg/min for men, VO2 max> 40 ml/kg/min for women) and untrained individuals age 18-35 with a BMI<30 will be recruited to participate in the study.

B. Exclusion Criteria

Anyone with a history or current cardiovascular disease and/or individuals that answer "yes" to any question on the PAR-Q will be excluded from the study.

C. Maximum number of subjects: 40

6. Study Procedures:

Experimental Overview

A. Design

A randomized crossover design will be used to test the acute effects of the three supplements. Each participant will be tested on three separate days, separated by a minimum of one week. At the onset of the study, each participant will be randomly assigned to the order in which they receive the caffeine, nicotinic acid, or placebo supplement. Supplementation will be followed by pre-exercise blood collection, an aerobic exercise protocol, and post-exercise blood collection.

B. Supplement Dosage

For caffeine- 5mg/kg/body weight will be consumed. For a 150 pound person, this would equate to approximately 340 mg of caffeine. There is approximately equivalent to a Strarbucks Grande Coffee (16 oz), which has 330 mg of caffeine. The amount of caffeine consumed would be slightly lower for a lower body weight individual, and slightly higher for a higher body weight individual.

For Nicotinic acid (Vitamin B3)- 1000 mg will be consumed. Niacin is commonly used for the treatment of high cholesterol, and dosages are usually around 1000 mg. The negative side effects seen with niacin supplements usually occur with dosages between 1500 mg-6000 mg per day. Thus, the 1000 mg dosage should have minimal ill effects, especially in a young, healthy population.

Exercise Protocol

All exercise protocols will be performed on a mechanically braked Monark cycle ergometer. Before each exercise session, participants will complete a 10 minute warm- up at a light intensity with low resistance, 1 kilogram (kg) for males, 0.5 kg for females. Following the warm-up, male participants will begin the initial workload with 2 kg resistance; females will begin the initial workload with 1 kg resistance. The workload will then increase every six minutes (1 kg for males, 0.5 kg for females) until the volume of oxygen consumed during exercise reaches a plateau. This will be determined by indirect calorimetry, which will measure gas analysis throughout the exercise protocol. A pedal frequency of 50 revolutions per minute (rpm) will be maintained for the entirety of the warm-up and exercise phases.

Blood Collection Protocol

Thirty minutes after the supplement has been ingested, a pre-exercise blood draw will be taken from the cubital vein to determine initial blood lipid values. The participant will complete the exercise protocol on a cycle ergometer. Immediately after exercise, a post-exercise blood draw will be taken from the cubital vein to determine lipid values following an acute aerobic exercise bout.

7. Benefits:

Personal benefits gained through participation in this study include knowledge about how an acute bout of high-intensity exercise affects lipid breakdown and how it may be enhanced and/or limited by caffeine or nicotinic acid ingestion prior to exercise.

8. Risks/Discomforts:

The major undesired effect from this project is the possibility of muscular injury, breathing difficulty, fainting, dizziness, cardiovascular abnormalities, or fatigue during the graded exercise. Nicotinic acid supplementation can lead to flushing, itching, and redness of the skin, as well as gastrointestinal issues such as nausea, vomiting, and diarrhea. Caffeine supplementation can lead to increased heart rate, flushing of the skin, as well as gastrointestinal issues such as nausea and diarrhea. Also, there is always a chance of infection when any invasive procedure (needle stick for blood draws) is used.

9. Measures Taken to Reduce Risk:

Injury to the muscles can be avoided be ensuring that the muscles are "warmed up" prior to activity, which is included in the exercise protocol. Chances of breathing difficulty, fainting, dizziness, and

177

cardiovascular abnormalities will be reduced by using only young healthy individuals and by monitoring heart rate, signs and symptoms of abnormal response to exercise, and checking how difficult the exercise is perceived to be by the individual throughout the exercise protocol. All exercises will be performed under careful supervision of these parameters. To prevent any infection from the needle stick, the site will be cleaned with an alcohol pad.

10. Right to Refuse:

Participation in research studies at LSU is completely voluntary and LSU is not responsible for any harm that may affect research participants. All volunteers may terminate their participation at any time and without warning. The individual just needs to stop the activity and leave the testing room. No attempt will be made by the research personnel to stop the volunteer.

11. Privacy:

The results of this study will be tabulated in a confidential manner. This means that the researchers will be unable to give you your results, as they will not be able to identify yours from the rest.

12. Financial Information:

The project will not affect the participants' finances in either a positive or negative manner.

13. Withdrawal:

Withdrawal from the study can occur at any time without any fear of punitive action.

14. Removal:

If the researcher has trouble scheduling the volunteer to come into the testing room, that volunteer will be dropped from the list of volunteers. The volunteer has the right to change their minds about participation at any time before or during the study.

---

15. Signatures:

The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have questions about subjects' rights or other concerns, I can contact Robert C. Mathews, Institutional Review Board, (225) 578-8692, irb@lsu.edu, www.lsu.edu/irb. I agree to participate in the study described above and acknowledge the investigator's obligation to provide me with a signed copy of the consent form.

Subject Signature:_____ Date:_____

The study subject has indicated to me that he/she is unable to read. I certify that I have read this consent form to the subject and explained that by completing the signature line above, the subject has agreed to participate.

Signature of Reader: _____ Date:_____

ACTION ON PROTOCOL APPROVAL REQUEST

Institutional Review Board
Dr. Robert Mathews, Chair
131 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.6792
irb@lsu.edu | lsu.edu/irb

LSU

TO: Arnold Nelson
          Kinesiology

FROM: Robert C. Mathews
          Chair, Institutional Review Board

DATE: August 22, 2011
RE: IRB# 3201

TITLE: Changes in Lipid Metabolism Following Aerobic Versus Resistance Exercise Training

New Protocol/Modification/Continuation: _New Protocol_

Review type: Full ___ Expedited _X_   **Review date:** _8/17/2011_

Risk Factor: Minimal __X__ Uncertain _____ Greater Than Minimal_____

Approved_____X_____ Disapproved_____

Approval Date: _8/23/2011_   **Approval Expiration Date:** _8/22/2012_

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: _120_

Protocol Matches Scope of Work in Grant proposal: (if applicable)_____

By: Robert C. Mathews, Chairman _[signature]_

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. SPECIAL NOTE:

All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb

Application for Approval of Projects Which Use Human Subjects

This application is used for projects/studies that cannot be reviewed through the exemption process.

LSU

-- Applicant, Please fill out the application in its entirety and include two copies of the competed application as well as parts A-E, listed below. Once the application is completed, please submit to the IRB Office for review and please allow ample time for the application to be reviewed Expedited reviews usually takes 2 weeks. Carefully completed applications should be submitted 3 weeks before a meeting to ensure a prompt decision.

Institutional Review Board
Dr. Robert Mathews, Chair
131 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.6792
irb@lsu.edu
lsu.edu/irb

-- A Complete Application Includes All of the Following:
   (A) Two copies of this completed form and two copies of part B thru E.
   (B) A brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts 1&2)
   (C) Copies of all instruments to be used.
      *If this proposal is part of a grant proposal, include a copy of the proposal and all recruitment material.
   (D) The consent form that you will use in the study (see part 3 for more information.)
   (E) Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project, including students who are involved with testing or handling data, unless already on file with the IRB. Training link: (http://phrp.nihtaining.com/users/login.php.)
   (F) IRB Security of Data Agreement: (http://www.lsu.edu/irb/IRB%20Security%20of%20Data.pdf)

1) Principal Investigator*: Arnold G. Nelson   **Rank** Professor
*PI **must be** an LSU Faculty Member

Dept: Kinesiology   **Ph:** 225-578-3114   **E-mail:** anelso@lsu.edu

2) Co Investigator(s): please include department, rank, phone and e-mail for each
Greggory R. Davis, Graduate Assistant, 336-263-7101, gdavi14@lsu.edu

IRB# ____ LSU Proposal # ____
◯ Full
⊗ Expedited
⊗ Human Subjects Training
⊘ Complete Application

3) Project Title: Changes in Lipid Metabolism Following Aerobic Versus Resistance Exercise Training

4) Proposal Start Date: 8/22/2011   **5) Proposed Duration Months:** 12

6) Number of Subjects Requested: 120   **7) LSU Proposal #:**

8) Funding Sought From: N/A

ASSURANCE OF PRINCIPAL INVESTIGATOR named above
   I accept personal responsibility for the conduct of this study (including ensuring compliance of co-investigators/co-workers) in accordance with the documents submitted herewith and the following guidelines for human subject protection: The Belmont Report, LSU's Assurance (FWA00003892) with OHRP and 45 CFR 46 (available from http://www.lsu.edu/irb). I also understand that copies of all consent forms **must be maintained at LSU for three years after the completion of the project.** If I leave LSU before that time, the consent forms should be preserved in the Departmental Office.

Signature of PI _____   Date _____

ASSURANCE OF STUDENT/PROJECT COORDINATOR named above. If multiple Co-Investigators, please create a "signature page" for all Co-Investigators to sign. Attach the "signature page" to the application.

I agree to adhere to the terms of this document and am familiar with the documents referenced above.

Signature of Co-PI (s) _____   Date 7/19/2011

Consent Form

1. Study Title:

Changes in Lipid Metabolism Following Aerobic Versus Resistance Exercise Training

2. Performance Sites:

Huey Long Fieldhouse Room 30, LSU Football Stadium Weight Room, Carl Maddox Fieldhouse

3. Contacts:

Dr. Arnold Nelson

 Wrk. 578-3114/ Hm. 766-4621

Gregg Davis

 Cell. 336-263-7101

Cardyl Trionfonte

 Cell. 216-637-7581

4. Purpose of the Study:

The study will investigate changes in various metabolites and enzymes before, during, and after an aerobic training activity course compared to a resistance training activity course.

5. Subjects:
A. Inclusion Criteria

Healthy, recreationally active individuals between ages 18-35 currently enrolled in a LSU Kinesiology activity course or lecture course will be recruited to participate in the study.

B. Exclusion Criteria

Anyone with a history or current cardiovascular disease and/or individuals that answer "yes" to any question on the PAR-Q will be excluded from the study.

C. Maximum number of subjects: 120

6. Study Procedures:

Experimental Overview

Students enrolled in the beginning jogging class will assigned to the aerobic (A) group, students enrolled in the beginning weight training class will be assigned to the resistance (R) group, and students enrolled in a lecture course will be assigned to the control (C) group. Participants will then participate normally in their respective activity or lecture courses.

At weeks 1, 6, and 12, participants will complete an overnight fast and 10 ml of blood will be drawn from the cubital vein to determine initial blood metabolite values.

7. Benefits:

Personal benefits gained through participation in this study include knowledge about how various forms of exercise affect lipid metabolism and enzymatic activity associated with improved fat utilization and insulin sensitivity.

8. Risks/Discomforts:

The major undesired effect from this project is the possibility of muscular injury, breathing difficulty, fainting, dizziness, cardiovascular abnormalities, or fatigue during physical activity. Also, there is always a chance of infection when any invasive procedure (needle stick for blood draws) is used.

9. Measures Taken to Reduce Risk:

Injury to the muscles can be avoided be ensuring that the muscles are "warmed up" prior to activity. The instructors for the courses will ensure that this is done each day. Chances of breathing difficulty, fainting, dizziness, and cardiovascular abnormalities will be reduced by using only young healthy individuals and by monitoring signs and symptoms of abnormal response to exercise, and checking how difficult the exercise is perceived to be by the individual throughout the exercise protocol. All exercises will be performed under careful supervision of these parameters by instructors certified in BLS CPR and First-Aid. To prevent any infection from the needle stick, the site will be cleaned with an alcohol pad.

10. Right to Refuse:

Participation in research studies at LSU is completely voluntary and LSU is not responsible for any harm that may affect research participants. All volunteers may terminate their participation at any time and without warning. The individual just needs to stop the activity and inform the researcher(s) that he/she no longer wishes to participate in the study. No attempt will be made by the research personnel to change the mind of the volunteer.

11. Privacy:

The results of this study will be tabulated in a confidential manner. This means that the researchers will be unable to give you your results, as they will not be able to identify yours from the rest.

12. Financial Information:

The project will not affect the participants' finances in either a positive or negative manner.

13. Withdrawal:

Withdrawal from the study can occur at any time without any fear of punitive action.

14. Removal:

If the researcher has trouble scheduling the volunteer to come into the testing room, that volunteer will be dropped from the list of volunteers. The volunteer has the right to change their minds about participation at any time before or during the study.

---

15. Signatures:

The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have questions about subjects' rights or other concerns, I can contact Robert C. Mathews, Institutional Review Board, (225) 578-8692, irb@lsu.edu, www.lsu.edu/irb. I agree to participate in the study described above and acknowledge the investigator's obligation to provide me with a signed copy of the consent form.

Subject Signature:_____ Date:_____

The study subject has indicated to me that he/she is unable to read. I certify that I have read this consent form to the subject and explained that by completing the signature line above, the subject has agreed to participate.

Signature of Reader: _____ Date:_____

Study Approved By:
Dr. Robert C. Mathews, Chairman
Institutional Review Board
Louisiana State University
203 B-1 David Boyd Hall
225-578-8692 | www.lsu.edu/irb
Approval Expires: 8-22-2012

ACTION ON PROTOCOL APPROVAL REQUEST

Institutional Review Board
Dr. Robert Mathews, Chair
131 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.6792
irb@lsu.edu | lsu.edu/irb

TO: Arnold Nelson
Kinesiology

FROM: Robert C. Mathews
Chair, Institutional Review Board

DATE: January 31, 2012
RE: IRB# 3231

TITLE: Effects of passive resistance (partner-assisted) stretching on lipid metabolism

New Protocol/Modification/Continuation: New Protocol

Review type: Full ___ Expedited _X_   **Review date:** 1/26/2012

Risk Factor: Minimal __X__ Uncertain _____ Greater Than Minimal_____

Approved_____X_____ Disapproved_____

Approval Date: 1/31/2012   **Approval Expiration Date:** 1/30/2013

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 40

Protocol Matches Scope of Work in Grant proposal: (if applicable)_____

By: Robert C. Mathews, Chairman _____

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING –
Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants, including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. SPECIAL NOTE:
All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb

Application for Approval of Projects Which Use Human Subjects

This application is used for projects/studies that cannot be reviewed through the exemption process.

LSU

-- Applicant, Please fill out the application in its entirety and include two copies of the competed application as well as parts A-E, listed below. Once the application is completed, please submit to the IRB Office for review and please allow ample time for the application to be reviewed Expedited reviews usually takes 2 weeks. Carefully completed applications should be submitted 3 weeks before a meeting to ensure a prompt decision.

Institutional Review Board
Dr. Robert Mathews, Chair
131 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.6792
irb@lsu.edu
lsu.edu/irb

-- A Complete Application Includes All of the Following:
(A) Two copies of this completed form and two copies of part B thru E.
(B) A brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts 1&2)
(C) Copies of all instruments to be used.
  *If this proposal is part of a grant proposal, include a copy of the proposal and all recruitment material.
(D) The consent form that you will use in the study (see part 3 for more information.)
(E) Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project, including students who are involved with testing or handling data, unless already on file with the IRB. Training link: (http://phrp.nihtaining.com/users/login.php.)
(F) IRB Security of Data Agreement: (http://www.lsu.edu/irb/IRB%20Security%20of%20Data.pdf)

1) Principal Investigator*: Arnold G. Nelson **Rank** Professor
*PI **must be** an LSU Faculty Member

Dept: Kinesiology **Ph:** 578-3114 **E-mail:** anelso@lsu.edu

2) Co Investigator(s): please include department, rank, phone and e-mail for each
Greggory R. Davis, Kinesiology, Graduate Assistant, 336-263-7101, gdavi14@lsu.edu

IRB# 3281 LSU Proposal #

○ Full
⊗ Expedited
⊗ Human Subjects Training
⊗ Complete Application

3) Project Title: Effects of passive resistance (partner-assisted) stretching on lipid metabolism

4) Proposal Start Date: 02/01/2012 **5) Proposed Duration Months:** 12

6) Number of Subjects Requested: 40 **7) LSU Proposal #:**

8) Funding Sought From: N/A

ASSURANCE OF PRINCIPAL INVESTIGATOR named above
  I accept personal responsibility for the conduct of this study (including ensuring compliance of co-investigators/co-workers) in accordance with the documents submitted herewith and the following guidelines for human subject protection: The Belmont Report, LSU's Assurance (FWA00003892) with OHRP and 45 CFR 46 (available from http://www.lsu.edu/irb). I also understand that copies of all consent forms **must be maintained at LSU for three years after the completion of the project.** If I leave LSU before that time, the consent forms should be preserved in the Departmental Office.

Signature of PI _____ Date _1/17/2012_

ASSURANCE OF STUDENT/PROJECT COORDINATOR named above. If multiple Co-Investigators, please create a "signature page" for all Co-Investigators to sign. Attach the "signature page" to the application.

I agree to adhere to the terms of this document and am familiar with the documents referenced above.

Signature of Co-PI (s) _____ Date _1/17/2012_

185

Consent Form

1. **Study Title:**

Effects of passive resistance (partner-assisted) stretching on lipid metabolism

2. **Performance Sites:**

Long Fieldhouse Room 54

3. **Contacts:**

Dr. Arnold Nelson

      Wrk. 578-3114/ Hm. 766-4621

Gregg Davis

      Cell. 336-263-7101

4. **Purpose of the Study:**

The study will investigate whether blood lipid metabolism is affected immediately following a passive resistance stretching exercise protocol.

5. **Subjects:**
   A. **Inclusion Criteria**

Healthy, men age 18-35 with a BMI<25 will be recruited to participate in the study.

   B. **Exclusion Criteria**

Anyone with a history or current cardiovascular disease and/or individuals that answer "yes" to any question on the PAR-Q will be excluded from the study.

   C. **Maximum number of subjects: 50**

6. **Study Procedures:**

Experimental Overview

   A. **Design**

A randomized balanced design will be used to test the acute effects of passive resistance stretching. Each participant will be tested on two separate days, separated by a minimum of one week. At the onset of the study, each participant will be randomly assigned to either the 36 minute passive stretching protocol or the 36 minute control protocol (mock stretching). Blood will be collected immediately before and after each protocol and respiratory gases will be analyzed for the duration of each protocol.

<u>Exercise Protocol</u>

The passive resistance stretching protocol will consist of the following stretches: Hamstring stretch, quadriceps stretch, shoulder stretch, triceps stretch, hip adductor stretch, and hip flexor stretch. Each stretch will be held for 30 seconds followed by 20 seconds rest, then repeated 3 more times (4 sets of 30 seconds for each exercise per leg/arm). 6 exercises X 2 limbs X 3 minutes/exercise= a total time of 36 minutes. Each participant will perform the exercises in the order listed above. The control trial will consist of the participant moving through the same motions for the same amount of time as the exercise trial, but no tension will be applied to the muscle.

<u>Blood Collection Protocol</u>

A pre-exercise blood draw will be taken from the cubital vein to determine initial blood lipid values. The participant will then complete the exercise or control protocol. Immediately following, a post-exercise blood draw will be taken from the cubital vein to determine lipid values following an acute stretching exercise bout.

7. Benefits:

Personal benefits gained through participation in this study include knowledge about how an acute bout of stretching affects lipid breakdown.

Risks/Discomforts:

There is minimal risk associated with this study, however, there may be some slight discomfort associated with stretching exercises. In addition, there is always a chance of infection when any invasive procedure (needle stick for blood draws) is used.

8. Measures Taken to Reduce Risk:

To avoid injury, all stretching exercises will be performed to the point of the participant feeling the muscle stretch, but not to the point of pain or extreme discomfort. To prevent any infection from the needle stick, the site will be cleaned with an alcohol pad.

9. Right to Refuse:

Participation in research studies at LSU is completely voluntary and LSU is not responsible for any harm that may affect research participants. All volunteers may terminate their participation at any time and without warning. The individual just needs to stop the activity and leave the testing room. No attempt will be made by the research personnel to stop the volunteer.

10. Privacy:

The results of this study will be tabulated in a confidential manner. This means that the researchers will be unable to give you your results, as they will not be able to identify yours from the rest.

11. Financial Information:

The project will not affect the participants' finances in either a positive or negative manner.

12. Withdrawal:

Withdrawal from the study can occur at any time without any fear of punitive action.

13. Removal:

If the researcher has trouble scheduling the volunteer to come into the testing room, that volunteer will be dropped from the list of volunteers. The volunteer has the right to change their minds about participation at any time before or during the study.

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14. Signatures:

The study has been discussed with me and all my questions have been answered. I may direct additional questions regarding study specifics to the investigators. If I have questions about subjects' rights or other concerns, I can contact Robert C. Mathews, Institutional Review Board, (225) 578-8692, irb@lsu.edu, www.lsu.edu/irb. I agree to participate in the study described above and acknowledge the investigator's obligation to provide me with a signed copy of the consent form.

Subject Signature:_____ Date:_____

The study subject has indicated to me that he/she is unable to read. I certify that I have read this consent form to the subject and explained that by completing the signature line above, the subject has agreed to participate.

Signature of Reader: _____ Date:_____

Study Approved By:
Dr. Robert C. Mathews, Chairman
Institutional Review Board
Louisiana State University
203 B-1 David Boyd Hall
225-578-8692 | www.lsu.edu/irb
Approval Expires: _1/30/2013_____

VITA

Greggory R. Davis spent most of his childhood and adolescence in Littleton, Colorado as well as in Salt Lake City, Utah. Gregg has always been highly involved in and has enjoyed sports and athletics, playing everything from soccer and baseball as a young kid, to varsity football in high school, to club to lacrosse and long-distance running in graduate school. He obtained his bachelor's degree in Exercise/Sport Science from Elon University in 2007. During his time at Elon, Gregg was an active member and scholarship chairman of Pi Kappa Phi Fraternity and was a varsity cheerleader and club swim team member.

Following graduation, Gregg obtained his Certified Strength and Conditioning Specialist (CSCS) certification from the National Strength and Conditioning Association (NSCA). He spent his college career and a year after graduation training a wide variety of clients, from high school athletes to diabetic, obese patients. It was during this year that he became more interested in the science behind metabolic adaptations to exercise, and with the support of his undergraduate professors, initially pursued an opportunity to obtain a Doctoral degree in exercise physiology at Louisiana State University (LSU).

Upon entry into the graduate program at LSU in the fall of 2008, Gregg received a recurring teaching assistantship in the College of Education through the Department of Kinesiology and has held the position for the past five years.

Gregg is currently a member of the American College of Sports Medicine (ACSM), National Strength and Conditioning Association (NSCA), American Heart Association (AHA), and the National Academy of Sports Medicine (NASM) and remains certified as a CSCS through the NSCA and as a CPT through NASM. He plans to graduate from LSU in May of 2013 with a Doctor of Philosophy in Kinesiology and a minor in Biological Sciences.

189