PPARs: POTENTIAL MECHANISMS REGULATING BLOOD LIPID AND LIPOPROTEIN CONCENTRATIONS AT REST AND FOLLOWING EXERCISE IN THE OBESE

A Dissertation

by

NICHOLAS PERRY GREENE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2010

Major Subject: Kinesiology

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ABSTRACT

PPARs: Potential Mechanisms Regulating Blood Lipid and Lipoprotein Concentrations at Rest and Following Exercise in the Obese. (August 2010)

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Obesity is associated with greater rates of cardiovascular disease, dyslipidemia and dysfunctional lipid metabolism. Exercise may provide an effective therapeutic tool to ameliorate dyslipidemia. However, how exercise attenuates dyslipidemia with obesity is not fully understood. Additionally, whether acute exercise or exercise training is the primary driver of such changes in this population is unknown. Furthermore, mechanisms mediating these exercise responses are not elucidated. The peroxisome proliferator-activated receptors (PPARs) provide a likely mechanism through enhanced expression of oxidative metabolism and cholesterol transport proteins augmenting fatty acid oxidation and cholesterol transport.

Study one describes blood lipid and lipoprotein responses to acute aerobic exercise and exercise training in obese men and women. The primary measured effects include: increased HDL-C in men following 12 wks exercise training, and a shift from HDL_3-C to HDL_2-C, with concomitantly reduced HDL-C mean density and LDL_3-C in women. Acute exercise of 400 kcal duration performed before and after training, yielded a

decreased TC: HDL-C ratio in men, which was unaffected by training. Thus, the primary exercise-based treatment for dyslipidemia with obesity appears to be exercise training.

In study two, PPARδ and PGC-1α content were significantly enhanced after acute exercise, whereas PPARα and AMPKα content were augmented only after training. These effects were seen with concomitantly increased content of target proteins involved in oxidative and lipoprotein metabolism including lipoprotein lipase, CPT-I, COX-IV, and FAT/CD36. PPARδ expression was correlated with total and LDL-cholesterol concentrations. AMPKα expression was correlated with the concentration of HDL-C and its subfractions, suggesting regulation of blood cholesterols by PPARδ and AMPKα.

Study three demonstrates comparative responses to high volume resistance exercise (RE) in lean and obese Zucker rats. RE enhanced PPARδ expression regardless of phenotype, but PGC-1α in obese only. Mitochondrial biogenesis was enhanced in lean animals only, indicating PPARδ and PGC-1α content is disconnected from mitochondrial biogenesis with obesity.

These studies enhance our understanding of exercise as a therapeutic tool in treating dyslipidemia and dysregulated lipid metabolism often associated with obesity. They further demonstrate the necessity for exercise training to attenuate dyslipidemia, while illustrating PPAR-mediated augmentations in oxidative and lipoprotein metabolism following exercise with obesity.

DEDICATION

To my lovely wife and our beautiful daughter, Liz and Amelia Greene.

Liz, you have been everything to me since the moment we met. I know it sounds cliché but I fall in love with you more by the day. Thank you for supporting me over these last few years. Without you I don't know if I could have done it! You have been the one dealing with me when I was most frustrated and have been and always will be the one by my side through thick and thin.

Amelia, you are much too young to understand now, but you and your mother have been the two best things to ever happen to me.

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Everywhere you look in science, the harder it becomes to understand the universe without God
-Robert Herrmann

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

This dissertation adheres to the journal article format method for dissertations. This document is organized into five chapters (Chapters II-IV are intended to serve as standalone manuscripts to be submitted for publication in peer-reviewed journals). In accordance with these guidelines, this chapter provides a review of pertinent literature and presents the questions to be addressed in Chapters II-IV. Chapter V presents general conclusions to the dissertation as a whole.

Rates of obesity and diabetes have risen to epidemic proportions in the United States (27) These disease states, along with other metabolic diseases, are characterized by impairments in energy metabolism including insulin resistance and debilitated lipid metabolism. Dysregulated oxidative metabolism is evident in reduced content and activity of oxidative enzymes within skeletal muscle. Due to impairments in cellular metabolic function, individuals suffering from these disease states often exhibit elevated blood glucose and dyslipidemia including elevated total cholesterol (TC), LDL-cholesterol (LDL-C), and triacylglycerols (TAG, also known as triglycerides), as well as depressed HDL-cholesterol (HDL-C).

This dissertation follows the style of the *American Journal of Physiology-Endocrinology and Metabolism*.

Dysregulation of Lipid Metabolism with Obesity

Obesity is strongly associated with incidences of diabetes (57) and dyslipidemia (6). In addition, obesity is an established risk factor for cardiovascular disease (CVD) (2) and has been associated with increased risk for several types of cancer (10). Obesity can induce metabolic disruptions by multiple mechanisms, potentially leading to development of disease. For example, Koves et al. (35) described increased serum nonesterified fatty acids (NEFA) and plasma oleate, linoleate, stearate and palmitate concentrations in high fat feeding-induced obesity in mice compared to standard chow fed controls. In that study high fat fed animals exhibited increased concentrations of several acylcarnitine species within the gastrocnemius muscle and an impaired ability to switch to carbohydrate fuel supplies, concomitant with increased rates of incomplete mitochondrial β-oxidation. In contrast, Holloway et al. (30) suggested that the primary cause of impaired energy metabolism in obesity is reduced mitochondrial content. Regardless, it appears a major problem with the development of obesity is the inability to properly utilize fuel stores which can lead to an accumulation of lipid and lipid intermediates both in the tissue and in the blood compartments which may cause further disease.

Classic Exercise Training, General Physiologic Adaptations

Exercise training has the potential to ameliorate many of these obesity-related issues, and is classically defined as either endurance or resistance, each with its own general training adaptations. Classic endurance training (ET; e.g., running, cycling) results in elevated maximal aerobic capacity (VO_2max), and decreased body weight and fat, with

little or no change in lean mass (5, 229). In comparison, classic resistance training (RT; e.g., weight training) is most commonly known for yielding increases in lean mass and muscular strength. However, similar to ET, RT can produce improvements in muscle oxidative capacity (203) and fat metabolism (161), particularly if the exercise mode is of relatively high volume (e.g., circuit training). This is reflected in the shift toward type IIA muscle fibers with RT (121). However, studies examining mitochondrial aspects of skeletal muscle following high volume RT observed either no change or even reduced mitochondrial density (volume mitochondria to volume total muscle). Thus, while mitochondrial proliferation after higher volume RT may be similar to that which occurs with ET, changes in mitochondrial content may be diluted by concomitant increases in myofibrillar growth.

In our laboratory, we have recently investigated the comparative effects of a classic ET model (land treadmill, LTM) to a novel form of ET, aquatic treadmill (ATM) (82). ATM training incorporates a motor-driven treadmill placed in the floor of a pool with frontal water resistance jets and water depths generally at the 4^{th} intercostal space. Data from these comparisons show that ATM training is equally capable of producing improved VO_2max and reduced body weight and fat as LTM training. However, we demonstrated that ATM training produces greater improvements in leg lean mass than LTM and potential improvements in total body lean mass ($P=0.059$). These data suggest that ATM training may act similarly to circuit training methods in which RT is performed at a high pace with little rest and produces both gains in lean mass and VO_2max (92).

Due to potential lean mass gains accompanying enhanced oxidative function with the ATM type exercise, for the study presented in Chapter IV of this dissertation the model of high volume (relatively low intensity with a higher number of repetitions) resistance exercise (RE) has been chosen because it may better mimic the concentric overload which is apparent during the ATM training utilized for the studies presented in Chapters II and III. Due to the potential gains in lean body mass with ATM training (82), a response not seen for LTM training; a treadmill analogue for the study presented in Chapter IV would not resemble adaptations seen with ATM training. However, the RE model presented here has been shown to elicit increases in muscle mass and protein synthesis in muscles of the hindlimb (64).

Guillet et al. (84) have demonstrated elevated mitochondrial protein synthesis rates (mtFSR) following high volume RE. Therefore, high volume RE may be similarly capable of eliciting mitochondrial biogenesis and enhanced oxidative function as some forms of aerobic exercise. We propose that mitochondrial biogenesis with RE has been masked in previous literature due to the limitations of assessing this phenomenon by mitochondrial density. If muscular hypertrophy is occurring, specifically of the myofibrillar compartment, mitochondrial growth may occur but be hidden by the growth of other cellular compartments when mitochondrial density is the chosen marker. Together, increases in lean mass and mitochondrial growth, which is associated with enhanced oxidative potential and aerobic capacity, suggest the viability of high volume RE as an analogue for the ATM training in Chapters II and III.

Cholesterol Transport and Metabolism

One of the hallmark conditions associated with obesity is dyslipidemia. This dissertation explores the use of exercise as a therapeutic agent for dyslipidemia with obesity. Furthermore, mechanisms by which skeletal muscle may influence lipid and lipoprotein concentrations are explored. To better understand the underlying mechanisms let us first review what is known about cholesterol and fatty acid (FA) transport and metabolism.

Lipoproteins

Lipoproteins are customarily classified based on hydrated density ($g \cdot cm^{-2}$), which is commonly determined by gradient ultracentrifugation and electrophoresis (103). Classes of lipoproteins have since come to be further described by the lipid and protein composition of the particle and the particle diameter. The largest, most buoyant lipoproteins contain a greater relative amount of lipid in their core with reduced protein mass. With decreasing hydrated density the particle is characterized by reduced lipid content and a relatively greater protein mass (85). In order of lowest to highest density the generally accepted lipoprotein fractions are: chylomicrons, very low density lipoprotein-cholesterol (VLDL-C), intermediate density lipoprotein-cholesterol (IDL-C), LDL-C, and HDL-C.

Apolipoproteins. Each class of lipoprotein is associated with a characteristic composition of apolipoproteins (Apo). The Apos both enable the solubilization and stabilization of the lipoprotein complex within the circulation, and serve a vital role in the lipoprotein complex's interaction with tissue receptors and lipolytic enzymes (141).

The accepted nomenclature for the different types of apolipoproteins was developed based on the lipoprotein fraction they were first identified with. Based on this nomenclature those designated ApoA are primarily associated with HDL-C, those designated ApoB are primarily associated with LDL-C, and ApoC associated with VLDL-C. However, some overlap in Apo constituents does occur where ApoB is also associated with VLDL-C and ApoC represents a minor portion of the HDL-C associated Apo (103). Other types of Apo include Apos D and E. ApoE is associated with chylomicrons and VLDL-C (141). ApoB represents two proteins from one gene, where ApoB48 is a smaller protein associated with chylomicron and ApoB100 is associated with VLDL-C and LDL-C.

Subfractions. Beyond these standard lipoprotein fractions HDL-C and LDL-C can be further divided into subfractions based on electrophoretic mobility. Using SDS-PAGE electrophoresis combined with Gaussian summation techniques, Verdery et al. (215) were able to identify six HDL-C class subfractions. The six subfractions were designated based on their mobility during gel electrophoresis relative to albumin and particle size (nm). The smallest HDL-C subfraction was HDL_{3c}-C with a radius of 3.9 nm and the largest was HDL_1-C with a radius of 6.0 nm. Of these subfractions the most commonly measured are HDL_3-C and $HDL_{2(a\ and\ b)}$-C.

Though less well described than HDL-C subfractions, LDL-C can also be divided into several subfractions. Using density gradient ultracentrifugation, Krauss and Burke (125) identified six total subclasses of LDL-C. Currently, four main subclasses of LDL-

C are readily accepted, denoted LDL_{1-4}, based on size and density, where the largest and most buoyant class is LDL_1-C and the smallest, most dense is LDL_4-C (124).

Transport

Cholesterol transport and metabolism has been previously reviewed by Ikonen (100). Cholesterol transport is broken into two main pathways: exogenous and endogenous, depicted in **Figure 1**. The exogenous pathway involves lipid and cholesterol absorption by the gut and packaging into chylomicrons for release into the lymphatics to be placed

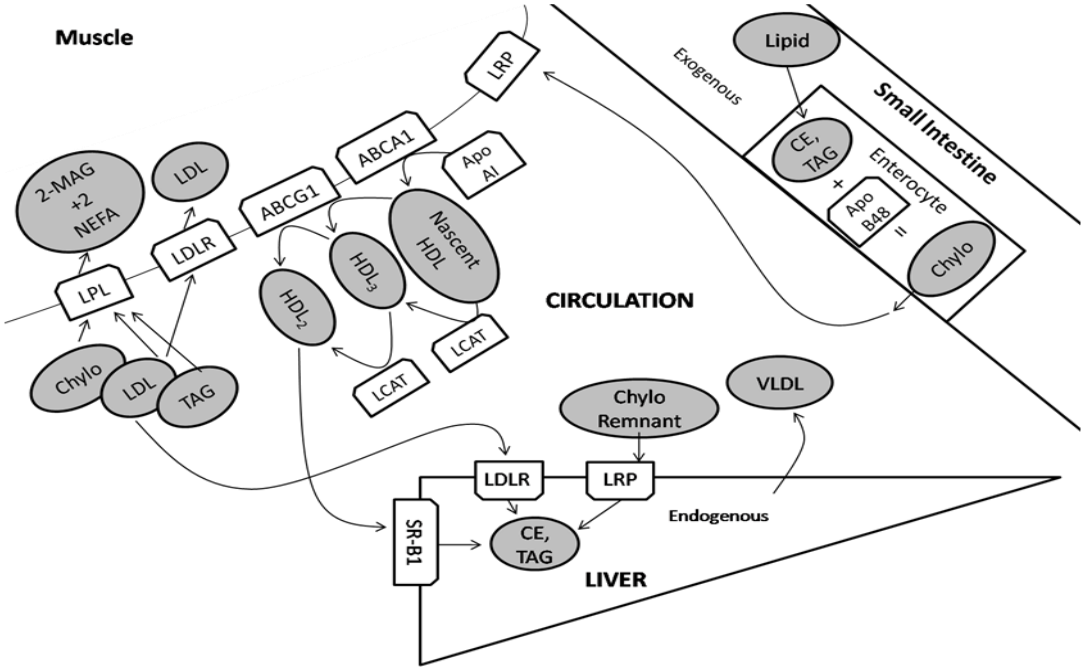


Figure 1. Vascular transfer of cholesterol and TAG. Transfer is depicted at cell membranes of the small intestine, liver, and skeletal muscle (peripheral tissue). CE, cholesterol ester; TAG, triacylglycerols; Chylo, chylomicron; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; 2-MAG, 2-monoacylglycerol; NEFA, nonesterified fatty acid; LPL, lipoprotein lipase; LDLR, LDL receptor; ABCA1/G1, ATP-binding cassette A1/G1; LRP, LDLR like protein; Apo, apolipoprotein; SR-B1, scavenger receptor-B1; LCAT, lecithin: cholesterol acyltransferase

into the circulation. The endogenous pathway includes hepatic packaging and release of very low density lipoprotein (VLDL) and the return of excess cholesterol ester (CE) by way of the reverse cholesterol transport system.

Exogenous pathway. The exogenous pathway begins with the uptake of dietary lipid and cholesterol via enterocytes by the NPC1L1 receptor into the brush border of the gut (100). Upon absorption by the enterocytes, cholesterol is esterified by Acyl CoA: Cholesterol Acyltransferase (ACAT) (100). Cholesterol and TAG can then either be packaged into chylomicrons or re-released into the gut via the ABCG5 and -G8 transporters (100). For incorporation into chylomicrons, lipid is packaged with ApoB48 and then released into the lymphatic system. From the lymphatics, the chylomicrons can be released into the circulation, generally within 40 minutes of a meal.

Chylomicrons are hydrolyzed in the circulation primarily by two mechanisms: lipoprotein lipase (LPL) and LDL-receptor like protein (LRP) (28). LPL is a lipase produced by most tissues including skeletal muscle and adipose tissue (85). The translated LPL is released to the vascular endothelium and located in the membrane of neighboring endothelial cells. From here LPL can hydrolyze TAG from chylomicrons and other cholesterol carrying species, including VLDL-C, IDL-C, LDL-C, and free TAG. This hydrolysis, like that of most lipases, yields 2-monoacylglycerol (2-MAG) and two nonesterified fatty acids (NEFA, also known as free fatty acids, FFA) (85). LRP is a specific cell receptor for chylomicron which recognizes ApoB48 on the surface of the chylomicron (214). As the chylomicron is degraded, the remnant is absorbed by the liver.

Endogenous pathway. The endogenous pathway begins with the packaging and release of VLDL-C from the liver. VLDL-C is packaged with a large portion of TAG in the core of the lipoprotein and contains ApoB100 and ApoE as primary Apo constituents

(85). Once released into circulation, the ApoB100 and ApoE are recognized as substrate for the LDL-receptor (LDLR) originally discovered by Brown and Goldstein (20, 76). VLDL-C is an ideal substrate for this receptor, as the receptor's highest affinity is for ApoE. As the CE and TAG are hydrolyzed from VLDL-C, the particle becomes more dense, and is eventually known as IDL-C (85). IDL-C is generally removed from the circulation within about 2-6 hours due to the high affinity of ApoE for the LDLR. During this process, however, about half of the ApoE dissociates from IDL-C in circulation. This dissociation carries two consequences: the half-life of IDL-C in the circulation extends to approximately 2 or 3 days, and IDL-C becomes known as LDL-C as CE and TAG are removed, creating an increasingly dense particle (85).

Reverse cholesterol transport. The process of reverse cholesterol transport involves the removal of excess cholesterol from peripheral tissues and blood and packaging into HDL-C for return to the liver, where cholesterol can either be repackaged into VLDL-C or used to make bile acids (100). HDL-C is originally formed as little more than ApoAI, which is synthesized in the liver and sent into the circulation. The peripheral tissues have a receptor known as ATP-binding cassette A1 (ABCA1) (100). This receptor packages CE with ApoAI, forming a nascent HDL-C. CE from the circulation can be added to this nascent HDL-C, producing HDL_3-C, a process mediated by the enzyme lecithin: cholesterol acyltransferase (LCAT) in addition to ABCA1. HDL_3-C incorporates additional CE either by recognition by the ABC transporters (A1 and G1) of peripheral tissues or by further incorporation by the LCAT mechanism. With this further addition of CE, the HDL_3-C particle becomes a larger and more buoyant form of

HDL-C known as HDL_2-C (177, 215). Due to the nature of the accumulation of CE, HDL_3 is generally the smaller, more dense species, whereas HDL_2 is the larger, less dense species of HDL (177, 201). HDL_2-C then directs the return of CE and TAG to the liver where scavenger receptor B1 (SR-B1) binds the HDL_2-C and facilitates the absorption of CE and any associated TAG or NEFA into the liver (100). The HDL-C itself then remains in the circulation where it can release from SR-B1 and repeat its cycle. HDL-C can also transfer its cholesterol to chylomicron and VLDL-C remnants, IDL-C and LDL-C by way of the cholesterol ester transfer protein (CETP).

Metabolism

Primary metabolism of cholesterol and FA in peripheral tissues (outside of the liver) in humans is the oxidation of FA and the use of cholesterol as a membrane constituent and substrate for hormone production. The liver is the principal site for cholesterol and FA synthesis. Generally, both cholesterol and FA synthesis begin with the substrate acetyl-CoA (85). Acetyl-CoA in the cytoplasm can be derived from citrate released by the tricarboxylic acid (TCA) cycle. When the $NADH+H^+$ concentration is high in the mitochondria, isocitrate dehydrogenase (ICDH) is inhibited. This inhibition causes an accumulation of mitochondrial citrate, which can be transported to the cytosol (85). Once in the cytosol, the citrate is split by the enzyme ATP-citrate lyase yielding oxaloacetate (OAA) and acetyl-CoA. OAA can be used to form malate, which will inhibit the entry of pyruvate into the mitochondria, thus slowing flux through the TCA cycle. The acetyl-CoA, however, can then be used as substrate for either cholesterol or FA synthesis (85).

Cholesterol synthesis. Cholesterol synthesis begins with the joining of two acetyl-CoAs by thiolase, yielding acetoacetyl-CoA (85). Acetoacetyl-CoA and a third acetyl-CoA now can form HMG-CoA via HMG-CoA synthase. HMG-CoA is a substrate for HMG-CoA reductase (HMG-CoAR), a rate limiting and committed step in the synthesis of cholesterol (85). Cholesterol can then be formed following a series of reactions which have not yet been completely elucidated. After synthesis the CE can be packaged with TAG, NEFA and Apos B and E to form VLDL-C and released to the circulation. Cholesterol homeostasis further depends on regulation by the sterol regulatory element–binding proteins (SREBPs), transcription factors which stimulate the transcription of HMG-CoAR and LDLR (100).

Fatty acid synthesis. *De novo* FA synthesis begins with the temporary fixation of CO_2 with acetyl-CoA by acetyl-CoA carboxylase (ACC) (85). This reaction forms malonyl-CoA, which is a primary substrate for fatty acid synthase (FAS). FAS can systematically add acetyl-CoA to malonyl-CoA one at a time to a growing FA chain. The affinity of FAS is reduced as the carbon chain becomes longer (85). In most tissues, FAS will release the newly synthesized FA when it reaches 16 carbons in length. Beyond 16C, the new FA can undergo further elongation to long chain and very long chain FAs using a series of enzymes known as elongases (85). The cell can also modify the FA by use of desaturases. In most animals, three desaturases are present including n-5, n-6, and n-9 (85). FAs must also be esterified to avoid cellular damage. This process is mediated by acyl-CoA synthase (ACS), which forms a fatty acyl-CoA using NEFA and CoASH as substrates.

Fatty acid oxidation. The first step of fatty acid oxidation (FAO) is the uptake of FA. Primary mechanisms for FA incorporation by the tissue include fatty acid transporters such as fatty acid transport protein (FATP) and fatty acid translocase (FAT/CD36), LPL, and LDLR mediated endocytosis of LDL (85). With the LDLR mechanism, LDL is taken to the lysosome, where LDLR can be re-sent to the membrane and LDL can be hydrolyzed, releasing TAG and CE (106). The TAG can then be hydrolyzed by hormone sensitive lipase (HSL) to yield 2-monoacylglycerol (2-MAG) and 2 NEFA (85). 2-MAG can be further hydrolyzed by a MAG lipase to yield NEFA plus glycerol. In this process the regulation of HSL is very important. As the name suggests, the lipase is susceptible to regulation by blood hormones such as insulin and epinephrine (EPI)/norepinephrine (NE). In the skeletal muscle, insulin will ultimately inhibit HSL, while NE/EPI will stimulate HSL (222). Adrenergic stimulation by EPI promotes the activation of adenylate cyclase and cAMP. cAMP then stimulates cAMP-dependent protein kinase A (PKA) which can then promote HSL activity by phosphorylation of HSL at three stimulatory sites (200). Previous evidence demonstrates that this EPI dependent stimulation of HSL is an important aspect to the enhanced activity of HSL at the onset of exercise (200). Insulin, however, stimulates phosphodiesterase (PDE) which causes the degradation of cAMP to AMP. This causes the deactivation of PKA, and thus removes PKA mediated stimulation of HSL (222).

As previously stated, to prevent cellular damage, NEFA must be esterified by ACS. This yields FA-CoA, which can be used as substrate for carnitine: palmitoyl acyltransferase-I (CPTI, also known as CATI) (85). This is necessary as long chain

NEFA and FA-CoA cannot cross the mitochondrial membrane without the aid of facilitated transport mechanisms. Of note, a mitochondrial pool of FAT/CD36 can also facilitate FA transport into the mitochondria (96). CPTI exchanges the CoA from the FA-CoA for carnitine, allowing the FA to traverse the mitochondrial membrane. Once at the inner membrane, CPTII can re-exchange the carnitine, reforming the FA-CoA and sending carnitine back to the outer membrane to pick up another FA (85). CPTI is considered the primary rate limiting step of FAO, and is inhibited by malonyl-CoA, the product of ACC. Thus when energy supply is high, $NADH+H^+$ inhibits isocitrate dehydrogenase (ICDH) increasing citrate which leaks out of the mitochondria and is split to form acetyl-CoA and OAA, providing substrate for ACC forming malonyl-CoA and inhibiting CPTI (85).

Once in the mitochondria, FA-CoA undergoes β-oxidation, which for every turn through the cycle will split off two carbons in the form of acetyl-CoA, $NADH+H^+$, and $FADH_2$. The acetyl-CoA can then be used as substrate for the TCA cycle yielding GTP, 3 $NADH+H^+$, and $FADH_2$ (85). NADH and $FADH_2$ then provide substrate for the electron transport system (ETS). ETS will use these molecules as reducing agents thereby pumping H^+ into the inner-membrane space. H^+ is the substrate for ATP synthase (85).

In times of energy surplus, the uncoupling proteins (UCPs) will uncouple ETS from ATP synthase allowing H+ to go back into the mitochondria. In excess this may cause the formation of H_2O_2 (a form of reactive oxygen species, ROS). Furthermore, during energy surplus, lipids will accumulate in the cell. Both the free radical formation and

lipid accumulation may activate a series of serine kinases which phosphorylate and thereby inhibit the insulin receptor substrate (IRS) (13, 120). This serves an important mechanism in maintaining the cell's energy homeostasis, but with chronic elevated lipid and ROS driving inhibition of IRS, glucose will not be taken up by the cell. The pancreatic β cells may then fatigue trying to overcome the cell's inhibition of IRS. This process will yield insulin resistance and eventually type 2 diabetes mellitus.

Dyslipidemia

Perhaps the most well known association of dyslipidemia with the risk of CVD and atherogenesis comes from the Framingham Heart Study. This study began in 1948 in an effort to identify characteristics which are associated with the onset and development of CVD and now includes data from multiple generations of the residents of the town of Framingham, Mass. The first report from the study to associate risk of coronary heart disease with elevated serum cholesterol was published in 1961 (108). Results from the study indicate the relationship of blood lipids and lipoproteins to general CVD risk (45), coronary heart disease risk (44, 230), and risk for intermittent claudication (153). In addition, Drexel et al. (54) have demonstrated that LDL-C, HDL_2-C, HDL_3-C, and TAG are each independently predictive of the extent of coronary atherosclerosis in a sample of patients undergoing angiography for evaluation of suspected or established coronary artery disease.

Due to these associations, abnormal blood lipid and lipoprotein concentrations are a recognized risk factor for the development of CVD by the American College of Sports Medicine (ACSM) and American Heart Association (AHA) (2, 156). Therefore, the

potential to relieve dyslipidemia using exercise-based therapy is of great interest. Dyslipidemia is characterized by elevated TC (>200 $mg \cdot dL^{-1}$), LDL-C (>130 $mg \cdot dL^{-1}$) and TAG (>200 $mg \cdot dL^{-1}$), and low HDL-C (< 40 $mg \cdot dL^{-1}$) (2, 156). In addition to total concentration of the lipoprotein fractions, subfractions of HDL-C and LDL-C represent differential atherogenic risk. Increased blood concentrations of HDL_2-C signals the acquisition of cholesterol by HDL, thus a shift from HDL_3-C to HDL_2-C and concomitantly enhanced reverse-cholesterol transport is consistent with reduced atherogenic risk (177, 201). Such a shift toward enhanced HDL_2-C concentration yields a decrease in HDL mean density, a factor also associated with reduced atherogenic risk (91). Smaller LDL-C subfractions (LDL_3-C and LDL_4-C) are considered to represent greater atherogenic risk because they are more susceptible to oxidation and greater binding to the arterial wall (124) and thus are correlated with coronary artery disease (37). Therefore, the understanding of cholesterol based therapies must be expanded beyond the assessment of total HDL and LDL related cholesterol and evaluate the influence of therapies on the lipoprotein subfractions.

Exercise as a Therapeutic Agent in the Treatment of Dyslipidemia

The potential for exercise to act as a therapeutic agent in the treatment of dyslipidemia has long been known. As far back as 1964, Holloszy et al. (94) reported a decrease in the blood concentration of TAG in subjects who performed exercise 3 $days \cdot wk^{-1}$ for 6 months. To properly understand the influence of exercise on blood lipids and lipoproteins, we must understand the factors which may influence the efficacy of the protocol including: 1) the comparative influence of type of exercise (endurance vs.

resistance), 2) exercise training compared to acute exercise, 3) differences among varying populations (i.e., men vs. women, hypercholesterolemic vs. exercise trained, lean vs. obese, etc.), and 4) the aspects of exercise itself which determine an effective exercise regimen (i.e., intensity, duration, and frequency). Research in the understanding of exercise-mediated alterations in blood lipids and lipoproteins has been carried out in both cross-sectional and longitudinal study designs. Here we provide a brief review of some of the more influential findings.

Cross-sectional studies.

Cross-sectional studies have contributed much of the basis for the concept that exercise training produces beneficial effects on blood lipids and lipoproteins. Two such studies by Williams et al. (226-227) have included data from more than 7,000 subjects. In one study, they examined the relationship of heart disease risk to exercise quantity and intensity and found that both quantity and intensity of habitual exercise were associated with reduced risk, including greater HDL-C and lower LDL-C and TAG concentrations (227). In the National Runners' Health Study (226), they reported that run distance per week was associated with reduced TAG and increased HDL-C. In addition, Halle et al. (86) have previously observed that increasing levels of habitual physical activity are correlated with reduced concentrations of TAG and small, dense LDL-C (presumably LDL_3-C and LDL_4-C) in hypercholesterolemic men.

Exercise training.

Exercise training can beneficially alter blood lipids and lipoproteins. In general, plasma TAG are reduced following exercise training, with the greatest effects often seen

in subjects who were previously inactive and with higher baseline TAG (115, 207). In contrast, TC is normally unchanged by exercise training (143, 233) unless accompanied by reductions in body fat (111). Similarly, total LDL-C is often unaffected by exercise training (115, 207). HDL-C appears to increase following exercise training in a dose dependent manner with energy expenditure. Changes in HDL-C are more likely with training protocols lasting greater than 12 weeks (179, 196). Absolute increases in HDL-C with training are generally seen to approximate 2-8 $mg \cdot dL^{-1}$. For example, Thompson et al. (206-207) have previously measured increases in HDL-C of 3 $mg \cdot dL^{-1}$ (206) and 8 $mg \cdot dL^{-1}$ (207) when subjects' diets were manipulated to maintain constant body weight and body fat for the duration of the study. Our laboratory (42) has demonstrated the potential of exercise training to reduce CVD risk by causing a shift in HDL-C subfractions including increased HDL_2-C and decreased HDL_3-C concentrations in hypercholesterolemic men.

To understand the effect of exercise training intensity, our laboratory (41) performed a study in which subjects were trained for an equal frequency and duration (assessed by caloric expenditure), but assigned into training groups for high (80% VO_2max) or low intensity (50% VO_2max). Decreased TC and HDL_3-C, and increased HDL_2-C concentrations were reported with no observed effect of training intensity. These findings indicate that intensity of exercise training does not appear to be a primary determining factor in exercise training induced changes in blood lipid and lipoprotein concentrations. However, a previous review of pertinent literature by Durstine et al. (59) suggests the importance of a caloric threshold, which is suggested to occur at

approximately 1200-1500 kcal·wk^{-1}, for exercise training mediated changes in lipids and lipoproteins. Thus it appears that training volume, rather than intensity, is the key to exercise training induced changes in blood lipids and lipoproteins. Based on these data, the exercise training protocol presented in Chapters II and III of this dissertation was designed to elicit 1500 kcal·wk^{-1} in exercise training-based caloric expenditure.

Acute exercise.

In addition to the adaptive influence of exercise training, acute exercise presents transient effects on blood lipids and lipoproteins which often peak 24 h after exercise and are no longer seen by 72 h (42-43, 65, 223, 234). Like exercise training, the primary effect of acute exercise on blood lipids and lipoproteins appears to involve a reduction in TAG and an increase in HDL-C. Though TC can change following acute exercise, it generally requires a very large caloric expenditure. Previous work from our laboratory (43) examining lipid and lipoprotein responses to high compared to low intensity acute exercise in hypercholesterolemia shows increased HDL-C and LDL-C, and decreased TAG 24 and 48 h post-exercise, following a single exercise session of 350 kcal expenditure, regardless of intensity (80% vs. 50% VO_2max). Similar to exercise training, these findings indicate that exercise intensity may not be a major determining factor in eliciting changes in blood lipids and lipoproteins.

However, findings regarding the influence of exercise duration, frequently defined by caloric expenditure, have provided information detailing population based caloric thresholds. Ferguson, et al. (65) investigated the effect of exercise duration on blood lipid responses in trained male runners performing exercise sessions of 800, 1100, 1300,

and 1500 kcal, showing that 1100 kcal was required to elicit changes in lipids and lipoproteins. Our laboratory demonstrated that an energy expenditure of approximately 350 kcal is sufficient to elicit changes in blood lipids and lipoproteins in previously untrained, hypercholesterolemic men (43, 81). In physically active postmenopausal women, Weise et al. (223) have shown that 400 kcal was sufficient to reduce TAG at 24 h post exercise, regardless of prior cholesterol concentrations. By contrast, Wooten et al. (234) demonstrated no significant change in blood lipids and lipoproteins following exercise of 500 kcal in duration in physically inactive postmenopausal women. These data indicate that they may have failed to reach caloric threshold for their sample of physically inactive women. Together previous data suggest that exercise duration (as defined by caloric expenditure) is the key to blood lipid and lipoproteins responses seen with acute exercise, and that the population studied influences the effective duration, whereby gender, cholesterol status, previous exercise training, and obesity may all have an effect.

Influence of exercise training on the transient response to acute exercise.

Only one known published study has compared and contrasted the influence of acute exercise and exercise training on blood lipids and lipoproteins (42), demonstrating changes in lipids and lipoproteins consistent with reduced atherogenic risk following both acute exercise and exercise training in hypercholesterolemic men. Interestingly, in the untrained state, acute exercise yielded an increase in LDL-C concentration which was no longer seen after 16 wks of training, suggesting that training may alter the acute response to exercise. However, the influence of training on the transient response to

acute exercise requires further examination to elucidate the nature of such an interaction. To better discern the nature of exercise-induced changes in lipids and lipoproteins, Chapter II of this dissertation examines the possibility of such an interaction in overweight and obese individuals.

Resistance exercise.

Relatively little is known about the effect of RE on blood lipids and lipoproteins. Many of the previous studies investigating RT have suffered design flaws, including low caloric expenditure, and inadequate quantification of the exercise training. However, the limited findings include no change in TAG and no change in TC when body weight, lean body mass, and percent body fat are unchanged (18, 187, 210). When lean body mass is increased and percent body fat decreased with RT, both TC and LDL-C have been seen to decrease (18, 210). These findings indicate the importance for a reduction in body fat to elicit changes in these cholesterol concentrations with RT. Results for changes in HDL-C have been mixed demonstrating either unchanged (116) or increased (210) HDL-C concentration following an RT regimen.

Acute RE has the potential to produce beneficial changes to the blood lipid and lipoprotein profile. Wallace et al. (218) have previously shown that a high volume, but not low volume, bout of RE can elicit increases in HDL-C and HDL_3-C concentration, with concomitant decreases in TAG content and LCAT activity. These findings indicate that RE may be able to improve blood lipid and lipoprotein concentrations, but only when a volume-based threshold, similar to aerobic exercise, is attained.

Obesity, exercise, and blood lipids and lipoproteins.

Despite known therapeutic benefits of exercise for the obese, the effects of exercise on blood lipids and lipoproteins in this population are incompletely described. The bulk of published studies examining interventions to reduce blood lipids and lipoproteins in this population have used methods designed to induce weight loss either by diet or by combination of diet and exercise (61, 180, 212, 232). Such weight loss interventions can be effective in producing beneficial changes in blood lipids and lipoproteins. In fact, Williams et al. (228) have previously demonstrated increased HDL_{2b}-C in men following a 12 month weight loss program by either diet (-7.2 kg) or exercise (-4.0 kg). However, weight loss may not be necessary to elicit the therapeutic benefits of exercise in the obese (33, 126). Studies examining the effects of exercise training alone on lipids and lipoproteins in the obese have often focused solely on men and included blood sampling within 24 h of the cessation of exercise training (157, 228). Such timing of blood sampling may be confounded by the transient response to the last exercise session which may last up to 72 h following exercise (42-43, 65, 223, 234); therefore, the interpretation of these studies should be taken with caution. Also, the influence of acute exercise has rarely been considered in this population.

Exercise training with obesity. Nicklas et al. (157) have previously demonstrated an increase in HDL-C and HDL_2-C in lean and overweight men following 9 months of exercise training, however, no such effect was observed in obese men. Slentz et al. (183) have demonstrated increased HDL-C and "large" HDL-C, presumably HDL_2-C, in overweight and mildly obese men in high volume exercisers (1700 $kcal \cdot wk^{-1}$), but not

with lower volume (approximately 1100 kcal·wk^{-1}). Their findings were seen 24 h following the last exercise session, and lasted through 15 days following the cessation of exercise training. They further showed that any exercise was sufficient to prevent increased LDL-C concentration and particle number and reduced LDL size observed in sedentary controls over the course of their study. Their findings suggest the efficacy of an exercise regimen alone to yield beneficial changes in HDL-C so long as a minimum duration is achieved. These data are suggestive of the benefits of exercise training on these cardiovascular risk markers but do not provide information regarding any influence of acute exercise or understanding of potential gender interactions in the overweight and obese population.

Skeletal Muscle in the Regulation of Lipids and Lipoproteins

Particularly with regards to physical exercise, skeletal muscle is the primary tissue involved in energy catabolism. Skeletal muscle may account for as much as 56-65% of total body weight (190) and as such contributes 20-30% of total energy expenditure at rest, a figure which increases greatly during exercise. Of total energy expenditure, lipid catabolism may account for 70% of skeletal muscle energy expenditure during rest (185), the rate of which only increases during prolonged exercise. Therefore, skeletal muscle must rely on mobilization of lipid stores from other tissues to supply its energetic demands. Considering that changes in serum lipids and lipoproteins during exercise appear dependent upon caloric expenditure, skeletal muscle must be considered as a primary source for the mediation of serum lipids and lipoproteins following exercise.

Previous studies have been conducted to directly understand the influence of skeletal muscle on exercise-derived changes in blood lipids and lipoproteins by assessing alterations in these factors across the vascular beds of skeletal muscle. Kiens and Lithell (113) reported the effects of one-leg exercise on the arterial-venous difference in blood lipids and lipoproteins. They demonstrated significantly greater HDL-C and HDL_2-C in the venous compared to arterial blood of the exercised leg. Furthermore, they demonstrated significant clearance of VLDL-TAG from arterial to venous compartments in the exercised leg, but not in the non-exercised leg. They additionally observed enhanced muscle LPL activity in the exercised leg. Together these data suggest a vital role of skeletal muscle in the regulation of blood lipids and lipoproteins, particularly during and following exercise. Therefore, the studies presented in this dissertation explore the role of skeletal muscle in the regulation of blood lipids and lipoproteins with and without exercise in overweight and obese individuals.

Peroxisome Proliferator Activated Receptors

To understand the ability of obese individuals to respond and adapt to the metabolic challenges of exercise and to exhibit normal metabolic function, it is necessary to understand the mechanisms by which metabolic capabilities are regulated and how these mechanisms may be altered in obesity. Furthermore, since little is understood about the mechanisms behind changes in blood lipids and lipoproteins with exercise, it is also necessary to elucidate such mechanisms. By doing so practitioners will better be able to treat both the obese and other populations to yield antiatherogenic changes in blood lipid and lipoprotein concentrations. The peroxisome proliferator-activated receptors

(PPARs) are transcription factors which may prove essential for these metabolic functions.

Issemann and Green (102) first discovered PPAR in 1990, when they identified PPARα, the first of three known PPAR isoforms (-α, -γ and -β/δ). The PPARs are a family of transcription factors with the ability to increase or decrease the rate of transcription of genes generally corresponding to metabolic enzymes and FA transport proteins. The PPARs have been found to have roles in glucose and lipid metabolism, endothelial function, and in dictating muscle fiber type. Previous work has related enhanced PPAR expression to improved insulin sensitivity (238) and beneficial changes in blood lipid and lipoprotein concentrations (160), providing a direct link to the treatment of metabolic diseases by enhancing PPAR expression and activity. Therefore, PPARs have been implicated as potential therapeutic targets for the treatment of several metabolic diseases including obesity, diabetes mellitus, dyslipidemia and the metabolic syndrome.

Tissue distribution.

The PPAR isomers show tissue specific expression. PPARs -α and -δ are expressed at the greatest levels in tissues with high metabolic activity, including liver and muscle. PPARα is primarily expressed in liver, kidneys, heart and skeletal muscle (15). However, PPARδ is ubiquitously expressed throughout the body (15). Though PPARδ expression is relatively low in the liver and kidneys, it is the most abundant isoform in skeletal muscle (152) and is believed to be the primary isoform in this tissue. Due to its importance in skeletal muscle, PPARδ will be the primary focal point for metabolic

regulation in this dissertation. PPARγ is primarily expressed in both the brown and white adipose tissue, and to a lesser degree in immune cells such as macrophages (15). Due to its lack of expression in skeletal muscle, PPARγ will not be discussed further in this review.

Structure.

The generic structure of PPAR is seen in **Figure 2**. The PPARs exhibit a domain structure that is similar to other nuclear receptors such as thyroid and steroid hormone receptors. The NH_2-terminal region of PPAR consists of a ligand-independent binding domain, followed by a DNA binding domain consisting of two zinc fingers, a ligand binding and dimerization domain and a ligand-dependent activation domain at the carboxy-terminus (49).

Mechanism of action.

The mechanism of action is depicted in **Figure 2**. PPAR can be activated by phosphorylation and/or ligand binding; upon activation PPAR forms a heterodimer with the retinoid X receptor (RXR) (49). Binding with the RXR in the cytoplasm allows

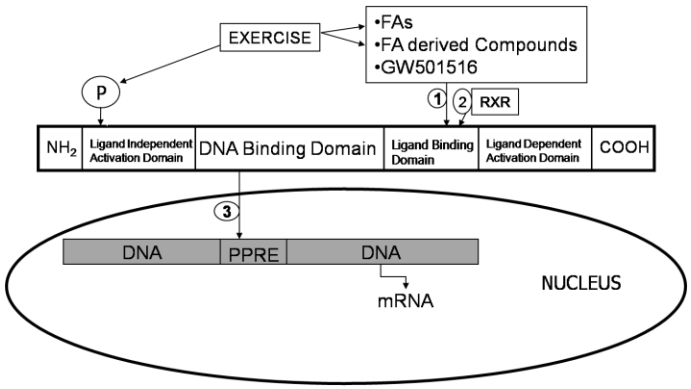


Figure 2: Basic structure and mechanism of action of the PPARs. RXR, Retinoid X Receptor; FA, Fatty Acids; GW501516, synthetic PPARδ specific pharmaceutical agonist; PPRE, Peroxisome Proliferator Response Element

PPAR to enter the nucleus where it can bind DNA. Once in the nucleus, PPAR recognizes and binds specific promoter regions of target genes, termed peroxisome proliferator response elements (PPRE) (49). This mechanism allows the PPARs to stimulate transcription of target genes, primarily those involved in lipid and glucose metabolism. The PPARs are also capable of repressing transcription of specific genes by a DNA binding independent manner, relying on protein-protein interactions and cofactor competition.

Regulation and activation.

PPAR activity can be regulated by a variety of methods. Primary means of activation include ligand binding and phosphorylation (23, 49).

Ligand-binding. PPARs are activated by a variety of natural and synthetic ligands. Primary natural ligands for the PPAR family are FA and their derivatives (8). These ligands are capable of recruiting specific cofactors which can either enhance or diminish the ligand's ability to bind PPAR (49).

All three isoforms of PPAR are activated by saturated FAs, such as palmitic acid, and polyunsaturated fatty acids (PUFAs) such as arachidonic and linoleic acids. PPARs appear to be more responsive to n-3 and n-6 PUFAs as ligands than to saturated FAs or monounsaturated FAs (49, 112). In addition, other natural substances, primarily FA derivatives from arachidonic acid metabolism, can selectively bind and activate each individual isoform. For example, PPARα is activated by 8S-HETE and leukotriene B_4, among others (49, 112).

Synthetic ligands have also been developed as a means of potential pharmacological treatments for metabolic disease. These synthetic ligands include the fibrates (e.g., fenofibrate) which stimulate PPARα, the thiazolidinediones (TZDs; e.g., rosiglitazone) which stimulate PPARγ, and experimental ligands including GW501516 which stimulate PPARδ (112, 160). Other synthetic ligands have also been developed, such as ragaglitazar, which act on all three isoforms (10).

Phosphorylation. In addition to ligand dependent activation, the PPARs are phosphoproteins, and are targets of several phosphorylation cascades involved in energy metabolism. PPAR was first recognized as a phosphoprotein in 1996, when it was discovered that PPARα was a target of insulin signaling via the ERK-MAPK pathway (181). In general, phosphorylation of PPARs -α and -δ yields enhanced basal and ligand mediated activity. Phosphorylation and activation of PPARα occurs via several kinase cascades, including the mitogen-activated protein kinases (MAPKs) ERK-MAPK and p38 MAPK, PKA, AMP-activated protein kinase (AMPK), and calcium-dependent protein kinase (PKC) (9, 24, 52, 181). Much less is known about the phosphorylation of PPARδ, but PKA, cyclic AMP dependent protein kinase (cAMP), and likely p38 MAPK are capable of phosphorylating PPARδ, thus increasing both basal and ligand-stimulated activity of PPARδ (15, 24, 52). Furthermore, AMPK appears essential for maximal activation of PPARδ (122); though a direct phosphorylation of PPARδ by AMPK has yet to be described.

PPARα.

The primary functions of PPARα involve the influx and efflux of cholesterol by the liver, delivery of FAs to peripheral cells and their metabolism therein. In fact, this isoform has proven to have a primary role in lipid metabolism, atherosclerosis and inflammation (238). Expression of PPARα is enhanced during fasting, a time in which FAs become more prevalent as an energy source, aiding in the switch from primary carbohydrate metabolism to lipid metabolism.

Lipid and lipoprotein metabolism. The role of PPARs -α and -δ in lipoprotein and cholesterol transport and metabolism is depicted in **Figure 3**. PPARα functions to aid in the delivery of FAs to peripheral tissues and their subsequent processing and metabolism within the cell. PPARα expression and activity thus yields increased transcription of genes for enzymes such as FAT/CD36 and FATP which mediate FA transport into the cell (144). This is followed by increased expression of ACS, which activates the FA by adding an acyl group, thereby trapping the FA within the cell, since activated FAs are incapable of transport out of the cell (144). Within the mitochondria, PPARα is capable of upregulating the expression of genes for enzymes such as carnitine-palmitoyl transferase-I (CPT-I), which transports FAs into the mitochondria for β-oxidation (145). Expression of PPARα also increases expression of acetyl-CoA synthase, a mitochondrial enzyme necessary for β-oxidation.

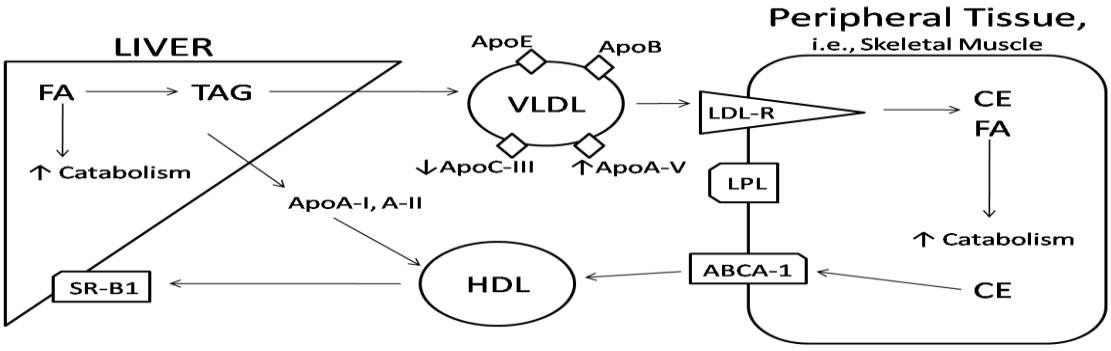


Figure 3: PPAR (-α and -δ) and cholesterol metabolism. Depiction of proteins whose transcription is stimulated by PPARs -α and -δ in liver and peripheral tissue. Proteins depicted are transcriptionally upregulated by PPAR unless otherwise indicated. LDL-R, LDL-Receptor; LPL, Lipoprotein Lipase; ABCA-1, ATP-Binding Cassette A-1; SR-B1, Scavenger Receptor-B1; FA, Fatty Acids; TAG, Triacylglycerol; CE, Cholesterol Ester; Apo, Apolipoprotein; VLDL, Very Low Density Lipoprotein; HDL, High Density Lipoprotein

The second role of PPARα in lipid and lipoprotein metabolism is the stimulation of lipolysis and cholesterol metabolism. PPARα directly increases LPL expression, which functions in lipolysis and serves to enhance clearance of TAG rich lipoproteins (178). PPARα also inhibits expression of apolipoprotein CIII (ApoCIII) (149), a protein which inhibits the TAG hydrolysis activity of LPL, contributing to the lipid lowering effects of fibrates.

Lipid metabolism involves not only the delivery of lipids and lipoproteins to the periphery and use within the peripheral cells, but also the ability to transport back to the liver. As discussed, the method by which this occurs is termed reverse cholesterol transport. Reverse cholesterol transport involves three major steps: (1) transport of lipids out of the peripheral cells, (2) delivery back to the liver via the blood, and (3) uptake by the liver.

ABCA1 is a primary mediator of the delivery of CE to HDL particles, whereas SR-B1 is a receptor protein on the liver which binds HDL and stimulates the absorption of CE from HDL. Both SR-B1 (29) and ABCA1 (30) transcription are enhanced by PPARα activators. In addition, PPARα expression has been shown to increase transcription of HDL-associated apolipoproteins including ApoAI and ApoAII (216). Interestingly, the opposite effect occurs in the rat model; HDL concentrations in the circulation are decreased corresponding to decreased ApoAI and ApoAII levels. This is likely due to PPARα inducing the expression of Rev-erbα, an orphan nuclear receptor which inhibits the transcription of ApoAI in the rat (216).

Integrative metabolism. A primary role of the PPARs is to facilitate a shift in energy metabolism from carbohydrate (CHO) to predominantly FAO, by promoting both enhanced FAO and reduced glucose metabolism. One pertinent observation regarding the role of PPARα in glucose metabolism is that PPARα-null mice exhibit extreme hypoglycemia, characterized by a 50% decrease in blood glucose following a 24 hour fast (114). The primary influences of PPARα on blood glucose likely occur in the liver. PPARα appears to modulate glycerol metabolism and gluconeogenesis in the liver (163). The likely cause of this shift is a PPARα transcriptional dependency for the production of glyceraldehyde 3 phosphate dehydrogenase, glycerol kinase, and glycerol transporters (aquaporins 3 and 9) (163). PPARα overexpression may impair glucose metabolism. Finck et al. (66) have reported reduced insulin-stimulated glucose uptake in transgenic mice overexpressing PPARα. In that study, they showed PPARα overexpression resulted in depressed mRNA content of the glucose transporter GLUT4, and

phosphofructokinase, a glycolytic rate-limiting enzyme, as well as enhanced expression of the glycolysis inhibitor pyruvate dehydrogenase kinase 4 (66).

One focus of research of the PPARs has been their relationship to insulin concentrations and sensitivity. Results of various studies have suggested seemingly contradictory roles of PPARα in insulin sensitivity. Knauf et al. (114) found that whole body insulin sensitivity is increased 15% in PPARα-null mice compared to wild type controls. However, several other published reports suggest that activation of PPARα enhances insulin sensitivity. In these studies, researchers have used nutritional, genetic (Zucker rat), and lipoatrophic models for insulin resistance and found improved insulin sensitivity following treatment with pharmaceutical agonists of PPARα (32, 83, 237).

PPARα may also have some influence on protein synthesis through the insulin/mTOR pathway. In addition to influencing insulin sensitivity, PPARα exhibits control over the expression of the protein TRB3. Koo et al. (118) demonstrated that PPARα knockout mice exhibit greatly reduced TRB3 mRNA content. TRB3 influences the insulin/mTOR pathway by inhibition of Akt, such that greater PPARα content and activity will yield increased TRB3 concentration and therefore potentially inhibit Akt.

PPARδ.

Perhaps the least studied of the three isoforms, PPARδ is the most abundantly expressed isoform. PPARδ's importance in FA metabolism was identified by the use of knockout animals. Animals lacking endogenous expression of PPARδ often die during embryogenesis, and those that do survive tend to present decreased fat mass (7, 155). Muoio et al. (152) performed the first known study documenting the function of PPARδ.

Their research showed evidence for what appeared to be compensatory regulation of lipid metabolism by PPARδ in skeletal muscle of PPARα knockout mice. Since this study it has become generally accepted that the primary isoform of skeletal muscle is PPARδ. With the use of synthetic agonists it was discovered that PPARδ increases FAO in both human and rodent myocytes (129, 139, 170).

With respect to its function in lipid metabolism, PPARδ activation in genetically obese mice enhances skeletal muscle β-oxidation and protects against diet-induced obesity (202, 219). Wang et al. (220) demonstrated the importance of PPARδ on oxidative phosphorylation by showing that transgenic mice over-expressing skeletal muscle PPARδ display a switch in muscle fiber type to favor type I (slow-oxidative) muscle fibers. PPARδ transgenic mice only gained a third as much weight after prolonged high fat feeding compared to wild type (220). PPARδ over-expression, independent of exercise, also facilitates an increase in muscle oxidative properties similar to that observed with endurance exercise training (199). Therefore, PPARδ is best known for regulating transcription of genes involved in FA transport, β-oxidation, and mitochondrial respiration (119).

Lipid and lipoprotein metabolism. Fatty acid transport inside of the cell is modulated by PPARδ through enhanced transcription of several key genes. With activation of PPARδ, LPL expression is increased, resulting in greater breakdown of VLDL cholesterol yielding NEFAs. This prepares the FAs for transport into the cell where FATP and FAT/CD36 are upregulated resulting in the accumulation of intracellular NEFA (192, 199). The trapping of the FA into the cell is then modulated by

increased expression of ACS. PPARδ upregulates the expression of CPT-I enhancing FA transport into the mitochondria. Within the mitochondria, PPARδ enhances the expression of genes for several enzymes involved in β-oxidation including long-chain acyl CoA dehydrogenase, and acetyl-CoA acyl transferase 2. This yields enhanced catabolism of FAs to acetyl-CoA for use in the TCA cycle (199). See **Figure 4** for influence of PPARδ on intracellular lipid metabolism.

The seminal study to show an effect of PPARδ activation on blood lipids and lipoproteins was conducted by Oliver, et al. (160). They observed that treating obese rhesus monkeys with the PPARδ agonist GW501516 resulted in decreased TAG, LDL-C and LDL particle number, and increased HDL-C concentration (160). In fact, genetic studies have linked PPARδ to cholesterol metabolism and transport in man (93, 182). Unfortunately, studies in humans using PPARδ agonists are currently few and restricted to hospitalization protocols. A bedrest study in humans has demonstrated that subjects receiving a PPARδ agonist maintained HDL-C concentration while those on placebo displayed reduced HDL-C. In addition, although not significant ($P = 0.08$), subjects receiving the PPARδ agonist showed decreased TAG (192). In moderately obese men, treatment with the PPARδ agonist reduces TAG, LDL-C and insulin, with a concomitant increase in expression of skeletal muscle CPT-I (170).

Treatment with pharmacological PPARδ agonists has been shown to promote reverse cholesterol transport (159, 211), an effect attributed to PPARδ dependent enhanced expression of ABCA1. This suggests that the role of PPARδ goes beyond that of increasing the cell's use of FA for an energy source, and indicates a major role for

PPARδ in lipoprotein metabolism. In fact, the findings of Oliver, et al. (160) compare favorably to the changes in blood lipids and lipoproteins seen with aerobic exercise (41, 81, 223).

Integrative metabolism. In addition to influencing lipid and lipoprotein transport and metabolism, PPARδ affects genes inhibiting glucose metabolism. By upregulating the expression of pyruvate dehydrogenase kinase 4 (PDK4), PPARδ is able to indirectly inhibit pyruvate dehydrogenase (**Figure 4**) (202). This prevents the conversion of pyruvate to acetyl-CoA, creating an abundance of pyruvate, and inhibiting

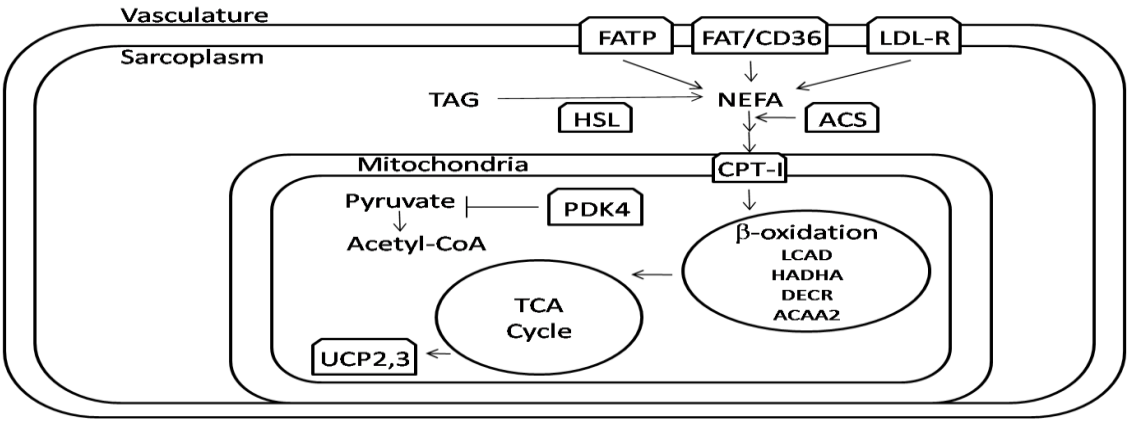


Figure 4: PPARδ and fatty acid oxidation in skeletal muscle. Skeletal muscle expressed proteins which are transcriptional targets of PPARδ. FATP, Fatty Acid Transport Protein; FAT/CD36, Fatty Acid Translocase; LDL-R, LDL-Receptor; HSL, Hormone Sensitive Lipase; ACS, Acyl CoA Synthase; CPT-I, Carnitine Palmitoyl Transferase; PDK4, Pyruvate Dehydrogenase Kinase; UCP2,3, Uncoupling Proteins 2 and 3.

glycolysis by product inhibition. This mechanism ultimately results in sparing glucose stores. However, though no effect is seen on lactate concentrations at rest, PPARδ agonists given at high doses (100 $mg \cdot kg^{-1}$ body mass) resulted in significant

accumulation of muscle lactate (35-36). By an apparent contrast, Burkart et al. (23) have reported increased mRNA content of GLUT4 and PFK in mouse cardiomyocytes in cardiac-specific PPARδ transgenic mice, which corresponded with increased glucose oxidation and glycogen stores in this tissue. Kramer et al. (123) demonstrated enhanced glucose uptake following treatment with GW501516 and insulin compared to an equal treatment of insulin alone. This effect occurred despite no effect on GLUT4 mRNA content; however, content and phosphorylation of p38 MAPK, AMPK, and ERK1/2 MAPK were enhanced in GW501516 treated cells (123). These findings further illustrate the importance of PPARδ in glucose uptake by the cell.

PPARδ also shows a potential effect on insulin sensitivity, similar to that seen with PPARα. Lee et al. (128) have reported improved insulin sensitivity following treatment with GW501516 in *db/db* mice (a model for type II diabetes). Tanaka et al. (202) reported similar findings in dietarily induced obese mice, with glucose tolerance and insulin sensitivity enhanced by treatment with GW501516. However, studies using both rat and human myocyte cultures have shown no effect of PPARδ on insulin sensitivity (47, 51, 205). Therefore, the role of PPARδ with regards to insulin sensitivity is not fully understood.

PGC-1α.

The actions of PPARs are heavily reliant upon PPARγ coactivator-1α (PGC-1α). Though first identified for its role in the coactivation of PPARγ (167), PGC-1α serves as a coactivator for all PPARs and a variety of other nuclear factors including nuclear respiratory factors 1 and 2 (NRF-1, and NRF-2) (151, 166). Through the coactivation of

various transcription factors, PGC-1α serves to regulate several biological functions including mitochondrial proliferation, fatty acid catabolism, angiogenesis, fiber type shifts, and antioxidant defense (134). The most well known of these functions of PGC-1α is to serve as a ”master regulator” of mitochondrial biogenesis (147). Perhaps due to its role in mitochondrial biogenesis, PGC-1α is believed to be tightly coupled with FAO; however, Holloway et al. (97) observed that though this is true in lean women, FAO appears uncoupled from PGC-1α expression in obese women. These data suggest a disconnect between PGC-1α and its normal functions with obesity.

PGC-1α may itself be a suitable target for treatment of metabolic diseases, especially type II diabetes, as PGC-1α has been linked to insulin sensitivity (147). However, the precise role of PGC-1α in insulin resistance remains controversial. Similar to findings linking PPARs -α and -δ to insulin sensitivity, attempts to link PGC-1α have produced mixed results. Studies have shown reduced PGC-1α mRNA content in human diabetics, suggesting a potential role in insulin resistance (188). At least one specific polymorphism of the PGC-1α gene, Gly482Ser, has been directly linked to reduced insulin sensitivity (62, 90). Such single nucleotide polymorphisms of PGC-1α are prevalent in obese and diabetic individuals (62, 90). Interestingly, transgenic mice overexpressing PGC-1α are insulin resistant (150), while PGC-1α-null mice show improved glucose tolerance and insulin sensitivity (133). Targeted skeletal muscle-specific overexpression of PGC-1α has been seen to induce muscle specific insulin resistance (31). Animals with whole body overexpression of PGC-1α show hepatic insulin resistance. However, contrary to findings with muscle specific overexpression,

these animals show enhanced muscle insulin sensitivity. Thus the role of PGC-1α in insulin sensitivity is unquestioned, but the nature of that role is complex and may vary based on the tissue studied.

PGC-1α activation is largely regulated by phosphorylation. Two primary proteins involved in the activation of PGC-1α by phosphorylation are AMPK (104) and p38γ MAPK (1, 164) (**Figure 5**). At least two AMPK phosphorylation sites have been identified on PGC-1α (137). Work by Irrcher and Hood (101) shows that AMPK not only activates PGC-1α, but appears necessary for the transcription of PGC-1α itself. Therefore, AMPK may control PGC-1α both as a regulator of PGC-1α content and activity. Akimoto et al. (1) first demonstrated the importance of p38 MAPK in the activation of PGC-1α. They further showed the importance of p38 MAPK activation in the exercise-induced increase in PGC-1α expression. This group has since demonstrated that this activity of p38 MAPK is specific to the -γ isoform (164), proposing that the -α and -β isoforms of p38 MAPK are primarily involved in autophagy (148).

AMP-activated protein kinase.

The importance of AMPK in the stimulation of PPAR and PGC-1α should be acknowledged. Kramer et al. (122) have shown that PPARδ-stimulated increases in FAO are abolished when AMPKα has been silenced. Further evidence also shows that phosphorylation of AMPKα is a potent stimulator of PGC-1α transcription (101). AMPK has been referred to as a "master metabolic switch," which is not only necessary for the immediate regulation of energy metabolism, but aids in the induction of exercise training adaptations. To examine the influence of AMPK the structure of this protein

must be considered. AMPK is a heterotrimer with subunits -α, -β, and -γ; of these the -α subunit is the catalytic and primary subunit (154). Primary means of activation of AMPK include an increase in AMP: ATP and creatine: phosphocreatine ratios, phosphorylation at Thr172 of the -α subunit, and allosteric modification (154).

AMPK is most known for its ability to stimulate ATP production pathways under conditions of energy depletion, however, AMPK is also capable of regulating long-term adaptive responses (154). For example, AMPK can induce expression of GLUT4 and mitochondrial genes, actions that may work through PGC-1α (137), and appears important in exercise training-mediated muscle fiber type shifts (172). AMPK is further capable of regulating FAO by influencing FA transport at the cell membrane, within the cytosol and at the mitochondrial membrane, and mediating content and activity of mitochondrial enzymes (208). To mediate these effects, AMPK has been demonstrated to induce enhanced content of FAT/CD36 at the cellular and mitochondrial membranes

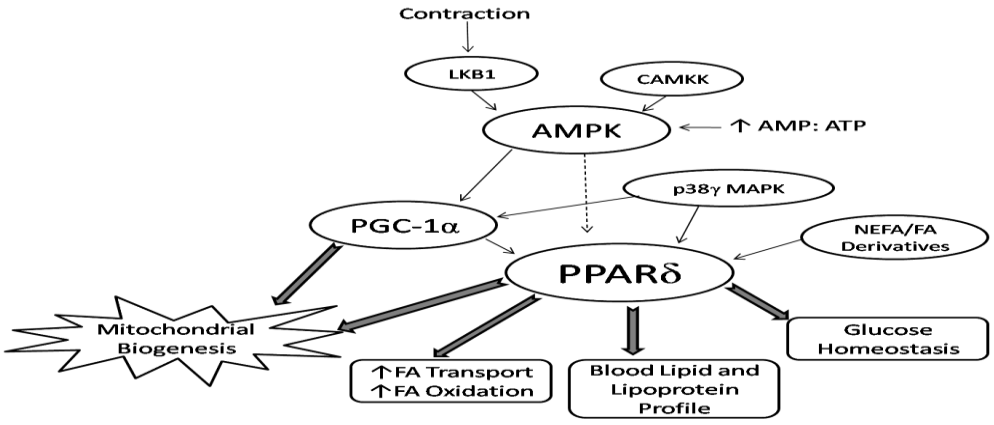


Figure 5: Activation of PPARδ, AMPK and PGC-1α with exercise and downstream effects. AMPK, AMP-activated protein kinase; p38 MAPK, p38 mitogen-activated protein kinase; NEFA, nonesterified fatty acids; FA, fatty acids; LKB1, serine/threonine kinase 11; CAMKK, calcium/calmodulin dependent protein kinase kinase.

(208), an effect also ascribed to PPARδ (151). Considering that PPARδ stimulated increases in FAO are not seen when AMPK is silenced, AMPK likely serves to direct the activity of PPARδ (122), even though a direct action of AMPK on PPARδ remains only postulated.

PPAR and Exercise

Exercise mediated activation of PPAR.

Exercise mediated mechanisms for activation of PPARs -α and -δ, PGC-1α, and AMPK can be seen in **Figure 5**. As previously stated, the major natural ligands for PPARs are FA and their derivatives (8). Both fasting and exercise states create an increase in serum FA concentration and FA uptake (81, 217). Furthermore, endurance exercise trained individuals display greater concentrations of intramuscular fat than untrained individuals (77). Therefore, an increase in FA availability created by exercise and exercise training provides ligand for PPAR, potentially increasing activity and signaling the need for an increase in PPAR expression.

Also, as mentioned, PPARs are phosphoproteins which can be acted upon by multiple kinases (24, 52). Among those kinases several have been shown to be activated by exercise including: ERK-MAPK, p38 MAPK, JNK-MAPK, and AMPK (16, 79, 174), creating a second mechanism for exercise based stimulation of PPAR. Increased PPAR activity may then mediate enhanced expression of genes involved in lipid and lipoprotein metabolism, yielding enhanced FAO, glucose sparing, mitochondrial biogenesis, and improved cholesterol metabolism and transport. Although this

dissertation does not test ligand binding or phosphorylation of PPAR, these processes provide a link by which exercise may stimulate enhanced PPAR expression and activity.

PPAR and acute exercise.

To the author's knowledge, two previous studies have examined the effect of acute endurance exercise on PPAR mRNA content in humans. Mahoney et al. (142) and Watt et al. (221) have each previously considered the effect of an acute bout of fatiguing cycle exercise on PPAR mRNA expression in humans. In both studies, PPARs -α and -δ exhibited increased mRNA expression at 3 h post exercise. Mahoney et al. (20) further demonstrated that at 48 h post exercise, mRNA levels had returned to baseline values. Watt et al. (221) also demonstrated enhanced PGC-1α mRNA and protein content 3 h post exercise.

PPAR and exercise training.

Several studies have investigated the effect of exercise training on the expression of PPARs -α and -δ. In 2000, Horowitz et al. (98) published one of the first studies evaluating the role of PPARα in the metabolic adaptations to exercise training. Their findings indicated an increase in PPARα content in the muscle, along with enhanced muscular oxidative capacity after exercise training. Kannisto et al. (109) revealed a differential response of the PPARs to exercise training. Their study demonstrated the greatest increase in expression of either PPAR isoform, compared to sedentary control condition, was with high fat feeding, but PPARδ expression was increased with exercise training regardless of diet. In addition, the exercise training effect on PPARδ was accompanied by a decrease in intramuscular fat content. Fritz et al. (24) explored the

effect of a low-intensity 4 month endurance exercise program on PPARδ in type 2 diabetics, and showed a significant increase in PPARδ protein expression and a tendency toward an increase in PPARδ mRNA expression. In contrast, following six weeks of endurance training, Russell et al. (175) measured elevated skeletal muscle mRNA content of PPARα and PGC-1α, with no change in PPARδ mRNA content.

PGC-1α and exercise.

In contrast with PPARδ, a relative wealth of data has been published to document the effect of exercise and exercise training on PGC-1α expression. From that work, a relative consensus exists that PGC-1α mRNA and protein expression are enhanced with both acute exercise and exercise training (4, 71, 146, 172). Sriwijitkamol, et al. (194) have previously shown that seven weeks of exercise training induced greater PGC-1α protein expression in obese Zucker rats. Given a potential uncoupling of PGC-1α from FAO in the obese (97) it is of extreme interest to understand how exercise in the obese affects the relationship of PGC-1α to responses such as enhanced FAO and mitochondrial proliferation. In addition to aerobic exercise effects, Deldicque et al. (48) showed that a single bout of RE yields an increase in PGC-1α mRNA content immediately post-exercise. However, the effects of acute exercise and exercise training on PPARδ and PGC-1α have not previously been compared and contrasted (Chapter III of this dissertation).

AMPK and exercise.

The response of AMPK to exercise must be considered on both the acute exercise and exercise training levels. The influence of acute exercise on AMPK appears to be

driven primarily by an increase in activity, as measured by phosphorylation at Thr172, seen immediately post-exercise, which returns to baseline by 2 h following exercise (131). Interestingly, an attenuation of AMPK phosphorylation at Thr172 has been observed following moderate intensity exercise in obese and type 2 diabetic humans compared to lean controls (193).

Exercise training is associated with an increase in expression of AMPKα. As previously stated, AMPK protein can be divided into several subunits; these subunits may be differentially affected by exercise training. Exercise trained individuals have been shown to express greater content of AMPKα1 but similar expression of the other AMPK subunits as compared to sedentary individuals (158). Sriwijitkamol et al. (194) have shown an increase in AMPKα1 with exercise training in the obese Zucker rat, which is consistent with exercise training in humans (34). In contrast, Leick et al. (131) demonstrate no influence of either acute exercise or exercise training on AMPKα protein content in PGC-1α knockout mice.

Summary

In summary, obesity is associated with increased rates of mortality and CVD concurrent with potentially severe metabolic perturbations. Among the risks involved in obesity is dyslipidemia, characterized by elevated blood concentrations of TC, LDL-C, and TAG and reduced HDL-C concentrations. Properly prescribed exercise can be a powerful therapeutic tool to combat such dyslipidemia; however, little is currently known about the mechanism by which this occurs. The PPARs are a family of proteins which mediate adaptive responses congruent with those seen with exercise training and

appear to mediate changes in blood lipids and lipoproteins consistent with reduced atherogenic risk. These studies were conducted to evaluate the efficacy of acute exercise and exercise training to elicit changes in blood lipids and lipoproteins consistent with reduced atherogenic risk and to explore the possibility that such responses are mediated by PPAR.

Aims of the Experiments

The primary **purpose** of these investigations is to explore the influence of exercise on lipids and lipoproteins and the regulation of lipid metabolism in obesity. These studies were designed to explore the response of blood lipids and lipoproteins to acute exercise *(Chapters II and IV)* and exercise training *(Chapter II)*, the influence of obesity *(Chapter IV)* and exercise *(Chapters III and IV)* on the regulation of lipid metabolism in skeletal muscle, and how skeletal muscle PPAR relates to blood lipid and lipoprotein concentrations *(Chapters III and IV)*. Therefore, this dissertation addresses the following aims:

Specific Aim 1 (Chapter II). Determine the response of traditional and nontraditional CHD risk markers in previously untrained, overweight and obese men and women to acute exercise and exercise training before and after exercise training implementing ATM or LTM exercise.

Specific Aim 2 (Chapter III). Determine if PPAR expression is altered following acute exercise and exercise training and if PPAR expression is associated with increases in

expression of PPAR target proteins including those of lipid metabolism and markers of mitochondrial growth, and blood lipids and lipoproteins in previously untrained overweight and obese men and women.

Specific Aim 3 (Chapter IV). Determine if PPAR protein expression is altered following a short term, high volume resistance exercise protocol in lean compared to obese Zucker rats, and if PPAR expression is associated with expression of PPAR target proteins, including those involved in lipid metabolism and markers of mitochondrial growth, and blood lipids and lipoproteins.

CHAPTER II

ACUTE EXERCISE AND AQUATIC TRAINING AFFECT BLOOD LIPID-LIPOPROTEIN CONCENTRATIONS, PARTICLE NUMBERS AND DENSITY, AND hsCRP IN OVERWEIGHT/OBESE MEN AND WOMEN

Introduction

Rates of obesity have steadily risen to epidemic proportions (27). Obesity is associated with greater incidence of diabetes (213) and dyslipidemia (6), and is an established independent risk factor for cardiovascular disease (CVD) (156). With regard to dyslipidemia, both acute exercise and exercise training have been demonstrated in other populations to elicit changes in blood lipid and lipoprotein concentrations consistent with reduced risk for CVD (40-43, 46, 58, 60, 80, 94). Furthermore, few have reported the influence of an exercise intervention on lipoprotein particle numbers in any population, though LDL particle number is considered a strong indicator of CVD (39), and reduced HDL particle numbers may facilitate development of atherosclerosis (117). In addition, inflammatory markers such as high sensitivity C-reactive protein (hsCRP) are related to obesity (162), and are independent predictors of CVD (169). Despite these findings, the response of blood lipid and lipoprotein concentrations and particle numbers to exercise and exercise training in the obese remains inadequately described. Additionally, few have compared and contrasted such responses between men and

women or described the influence of exercise training on the transient response to acute exercise.

Exercise training has been reported to induce changes in blood lipids and lipoproteins, including reductions in total cholesterol (TC), LDL-cholesterol (LDL-C) and triacylglycerols (TAG), and increased HDL-cholesterol (HDL-C) (59). Our laboratory (41) has reported decreased TC and HDL_3-C, and increased HDL_2-C concentrations in hypercholesterolemic men following 24 weeks of exercise training (1050 $kcal \cdot wk^{-1}$). Conversely, our laboratory demonstrated no influence of exercise training of similar frequency and duration in women (80). While increased HDL-C concentrations have been commonly reported in men, women often exhibit unchanged (136) or reduced HDL-C concentration following training (14). Furthermore, the influence of exercise on lipoprotein particle numbers has rarely been reported, primarily demonstrating reduced LDL particle number with training (183). In addition to the influence of exercise training on lipids and lipoproteins, cardiorespiratory fitness is inversely related to hsCRP (127).

Although the influence of exercise training on blood lipids and lipoproteins has been reported in a relatively large number of studies, results vary considerably, and often there was no control for the transient effects of the most recent session of exercise. Therefore, the interpretation of these studies must be taken with caution as the transient effects of acute exercise reportedly include increased HDL-C and decreased LDL-C and TAG, which last up to 72 h following exercise (43, 65, 223). However, the exercise duration required to elicit such transient changes varies with the population studied,

ranging from 350 kcal in hypercholesterolemic men (43) to 1100 kcal in endurance trained runners (65). By contrast, our laboratory has failed to measure such changes in women following exercise of 350 kcal (165, 223). Wooten et al. (234) explored the influence of a greater volume of exercise (500 kcal) in premenopausal, sedentary women, observing reductions in TC and HDL-C. Acute exercise has previously been reported to increase serum hsCRP 24 h after exercise (176). Given the varied nature of lipid and lipoprotein responses to both acute exercise and exercise training, understanding how a given population will respond to these stimuli is imperative. In addition, despite apparent differences in lipid and lipoprotein responses to exercise and exercise training, researchers have rarely compared and contrasted these responses in men and women in the same study.

Although of practical importance, only one study has been published to date to compare acute exercise and training effects on blood lipids and lipoprotein-lipids (42). In that study, changes in lipids and lipoproteins consistent with reduced atherogenic risk were demonstrated after both acute exercise and exercise training in hypercholesterolemic men. Interestingly, in the untrained state, acute exercise yielded an increase in LDL-C concentration which was no longer measured after 16 wks of training, suggesting that training may alter the acute response to exercise. To our knowledge, no single study has been published to characterize the independent and interactive effects of acute exercise and exercise training in obese men and women. Such information would be of benefit to practitioners in designing appropriate exercise intervention strategies to reduce CVD risk.

Despite known therapeutic benefits of exercise for the obese, the effects of exercise on blood lipids and lipoproteins in this population are incompletely described. The bulk of published studies have used interventions designed to induce weight loss either by diet or by combination of diet and exercise (61, 180, 212, 232). However, weight loss may not be necessary to elicit the therapeutic benefits of exercise in the obese (33, 126). Studies examining the effects of exercise training alone on lipids and lipoproteins in the obese have often focused solely on men and included blood sampling within 24 h of the cessation of exercise training (157, 228). As previously discussed, such timing of blood sampling may be confounded by the transient response to the last exercise session; therefore, the interpretation of these studies should be taken with caution. Also, the influence of acute exercise has rarely been considered in this population. Thus, while previous results appear promising, the blood lipid and lipoprotein responses to a single session of exercise and exercise training in overweight and obese individuals are not fully understood, particularly in women.

In addition, we have previously demonstrated the efficacy of aquatic treadmill (ATM) exercise to increase aerobic fitness and reduce weight and body fat percentage in the obese (82). In that previous report we further revealed the potential of ATM exercise to elicit enhanced lean body mass, providing a potential advantage to ATM training. However, the efficacy of ATM training to elicit reductions in cardiovascular risk has not yet been examined.

The purpose of our present study was to test the hypothesis that both acute exercise and exercise training will elicit changes in blood lipid and lipoprotein concentration and

particle numbers consistent with reduced atherogenic risk in overweight and obese men and women. We hypothesized that exercise training would not affect the acute response to exercise. In addition, we report the efficacy of ATM training compared with traditional land treadmill (LTM) training to induce changes in blood lipids and lipoproteins. Thus, our study sheds new light on the effects of exercise and exercise training on lipid and lipoprotein concentrations and particle numbers in overweight and obese men and women.

Methods

Physically inactive, overweight and obese men (n = 10) and women (n = 8) were recruited from the Texas A&M University and College Station, TX communities to participate in the study. The mean (SEM) age of subjects was 41 ± 2 years and 52 ± 2 years for men and women, respectively. Subjects were part of a larger cohort whose physiologic adaptations to the exercise training paradigm have previously been reported (82). Potential volunteers were recruited through informational flyers, email announcements, and by word of mouth. Volunteers were screened to ensure that they had not participated in regular aerobic activity for the previous 3 months (physically inactive), and were classified as overweight or obese initially by BMI and subsequently by percent body fat as measured by dual-energy x-ray absorptiometry (DEXA, Lunar Prodigy, GE Healthcare, Madison, WI). Subjects were stratified according to ACSM standards for risk of CVD, and those for whom it was required underwent a physical examination by a cardiologist before participation in the experiment (2). Preliminary physiological characteristics of the 18 subjects are presented in **Table 1**.

Table 1: Physical characteristics, and physical activity and dietary information before and after training in all subjects, women, and men.

	All Subjects (n = 18)		Women (n = 8)		Men (n = 10)	
	Pre-Tr	Post-Tr	Pre-Tr	Post-Tr	Pre-Tr	Post-Tr
Physical Characteristics						
Body Mass (kg)	101.50 ± 5.47	98.81 ± 5.08*	98.01 ± 8.16	94.53 ± 7.58	104.99 ± 7.30	103.09 ± 6.76
Body Mass Index ($kg \cdot m^{-2}$)	31.94 ± 1.41	31.18 ± 1.28*	32.03 ± 2.26	30.94 ± 2.06	31.86 ± 1.68	31.42 ± 1.54
Body Fat % †	41.14 ± 1.52	39.94 ± 1.60	44.26 ± 2.44	43.96 ± 2.57	38.02 ± 1.82	35.92 ± 1.91
Lean Body Mass (kg) †	52.70 ± 2.18	51.87 ± 2.08	45.53 ± 3.49	44.49 ± 3.34	59.88 ± 2.60	52.26 ± 2.49
Fat Mass (kg)	36.91 ± 3.15	35.73 ± 3.15	36.82 ± 5.05	35.37 ± 5.04	36.99 ± 3.77	36.09 ± 3.76
VO_2max ($L \cdot min^{-1}$) †	2.49 ± 0.08	2.74 ± 0.10*	2.04 ± 0.11	2.27 ± 0.15	2.95 ± 0.10	3.22 ± 0.12
VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$) †	25.15 ± 1.30	28.82 ± 1.44*	21.97 ± 1.94	25.35 ± 2.20	28.33 ± 1.73	32.29 ± 1.86
Activity and Nutritional Intake						
Caloric Expenditure ($kcal \cdot day^{-1}$) †	3005 ± 133	2971 ± 98	2595 ± 213	2582 ± 160	3414 ± 159	3360 ± 119
Caloric Intake ($kcal \cdot day^{-1}$) †	2362 ± 167	2137 ± 221	2019 ± 267	1780 ± 355	2705 ± 199	2495 ± 265
Carbohydrate ($g \cdot day^{-1}$) †	293 ± 21	261 ± 33	235 ± 34	207 ± 53	350 ± 26	316 ± 39
Total Fat ($g \cdot day^{-1}$)	91.4 ± 8.5	87.4 ± 10.6	84.0 ± 13.7	79.2 ± 17.0	98.9 ± 10.2	95.6 ± 12.7
Monounsaturated Fat ($g \cdot day^{-1}$)	19.2 ± 2.8	17.6 ± 3.5	13.0 ± 4.5	17.6 ± 5.6	25.3 ± 3.3	17.7 ± 4.2
Polyunsaturated Fat ($g \cdot day^{-1}$)	8.0 ± 1.1	10.1 ± 2.6	5.8 ± 1.7	11.0 ± 4.1	10.1 ± 1.3	9.2 ± 3.1
Saturated Fat ($g \cdot day^{-1}$)	31.9 ± 2.8	27.9 ± 3.1	29.8 ± 4.5	27.0 ± 4.9	34.0 ± 3.3	28.8 ± 3.7
Protein (g)	91.6 ± 8.0	76.1 ± 7.4	81.0 ± 12.8	67.8 ± 11.9	102.1 ± 9.5	84.4 ± 8.9

*P<0.05 significant main effect of exercise training. †P<0.05 significant main effect of gender. Data are means collapsed across mode of exercise training factor; error, SEM.

General protocol

All methods and procedures were approved by the Texas A&M University Institutional Review Board for Human Subjects in Research. On the first visit to the laboratory, subjects were informed of the study procedures and read and signed an institutionally approved informed consent. At this time, instructions were provided for completing physical activity and diet records. Physiological and demographic assessments were completed on the second visit to the laboratory (methods to follow). The following week, the first of two acute exercise sessions was performed on a standard motor-driven land treadmill (methods to follow). Fasting blood samples were obtained immediately prior to exercise and repeated 24 h following exercise. Within two weeks of physiological testing, subjects were matched for age, gender, and BMI, and then randomly assigned to 12-weeks of either LTM or ATM training (methods to follow). All physiological and demographic testing procedures were repeated 72-96 h after the final exercise training session. The acute exercise session was repeated approximately 7 days following post-training physiologic assessments, and blood sampling was again performed immediately prior to and 24 h following exercise.

Diet and activity logs

To study subjects in a free-living state, no attempt was made to modify diet or physical activity outside of the exercise training protocol. To this end, subjects were instructed to maintain their accustomed dietary and physical activity habits throughout the course of the study. To verify compliance with these instructions, dietary and activity habits were assessed on two occasions coinciding with the beginning and end of

exercise training. Subjects were instructed to complete dietary and physical activity records on days which would best represent their normal daily habits. On both occasions dietary logs were recorded for 3 consecutive days, including 1 weekend day. The 3-day dietary records were analyzed for total caloric intake and for carbohydrate, fat, and protein composition using commercially available computer software (Food Processor® 8.4, ESHA Research, Salem, OR). The physical activity records used were an adaptation of a previously described protocol for physical activity recall (12). Activity records were recorded for seven consecutive days and were analyzed for total energy expenditure. In addition, upon completion of the study all subjects were required to read and sign dietary and activity compliance forms.

Physiological assessments

These procedures have been previously published (82). In brief, body composition, including whole body percent fat, fat mass and lean body mass, were assessed using DEXA. An incremental maximal graded exercise test (GXT) was conducted on a motor-driven treadmill according to the Bruce protocol (21). Oxygen consumption during exercise was assessed using an automated metabolic gas-analysis system (CPX/D Exercise Stress Testing System, Medical Graphics, Minneapolis, MN or Oxycon Pro, Erich JAEGER, Hoechberg, Germany) calibrated with gasses of known concentration before and after each exercise test. VO_2max was taken as the highest 15 s average oxygen uptake achieved during the exercise test. Heart rate (HR) and rhythm were monitored continuously from a 12-lead electrocardiogram. Ratings of perceived exertion (RPE) using a Borg 15-point scale ranging from 6-20 (17) and manual blood pressures

(BP) were obtained during the last 30 s of each treadmill stage and at maximal exercise. At least two of the following criteria were required for the maximal exercise test to be considered valid: 1) achievement of maximum heart rate within 10 bpm of the age-predicted maximum; 2) rating of perceived exertion ≥ 18; 3) respiratory exchange ratio >1.1 at maximal exertion; or 4) O_2 uptake plateau despite further increases in workload. The same skilled laboratory personnel consistently performed all physiological measurements.

Acute exercise sessions

All non-study exercise was prohibited for 72 h prior to the experimental acute exercise sessions. The experimental acute exercise sessions were performed on a motorized land treadmill approximately seven days after completion of maximal exercise testing at pre- and post-training timepoints. The exercise protocol began with a 3 min warm-up period at 53.6 $m \cdot min^{-1}$ at a 0% grade. The duration of each acute exercise session was defined as the time required to expend 400 kcal at an intensity of 70% of VO_2max, based on the most recently acquired VO_2max. The necessary duration was estimated by dividing 400 kcal by the rate of energy expenditure at the required exercise intensity based on the respiratory exchange ratio energy-oxygen equivalent ($kcal \cdot LO_2^{-1}$). Initial treadmill velocity and grade were estimated using published metabolic equations for LTM exercise (2). Heart rate, RPE and VO_2 were measured every 5 min at the beginning of exercise to adjust treadmill velocity and grade until the required VO_2 was achieved; thereafter VO_2 measurements were taken every 10 min until the cessation of exercise, and minor adjustments were made as necessary to the treadmill

velocity and grade to maintain the required VO_2, as well as, exercise time to ensure the expenditure of 400 kcal. Subjects were asked to avoid any physical exertion outside of that required by their work until after all blood collection procedures were completed.

Exercise training

The exercise prescription and training progression for this study have previously been reported (82). ATM training was conducted using a HydroWorx 1000 series treadmill (HydroWorx International, Inc., Middletown, PA) as previously described (82). LTM training was conducted on a standard motor-driven treadmill. Subjects exercised three times per week for a 12-week period. Training sessions were performed by all subjects at an equivalent caloric expenditure and relative intensity progressively increased from 250 kcal·session^{-1}, 60% VO_2max during the first week to 500 kcal·session^{-1}, 85% VO_2max during weeks 6-12. The prescribed exercise progression was such that by week 6 subjects expended approximately 1500 kcal·wk^{-1} in exercise training. Exercise duration and intensity were calculated as described above for acute exercise sessions. For ATM training treadmill velocity and jet resistance were estimated using a previously developed metabolic equation for ATM (Greene et al., manuscript accepted for publication in Research Quarterly for Exercise and Sport). Treadmill velocity and grade/jet resistance were adjusted as necessary during the training session to attain the HR and RPE which matched the prescribed intensity. Each individual's exercise prescription was adjusted for increases in VO_2max during week 6 such that the prescribed intensity and duration were maintained throughout the study.

Blood sampling

Blood samples were obtained on four occasions. Before training, blood sample one was obtained with the subject at rest immediately preceding the acute exercise session and a second resting blood sample was drawn 24 h after a single aerobic exercise session. After training, blood samples three and four were obtained before and after an acute exercise session in an identical manner to the pre-training procedures. Resting samples were taken following at least 72 h without strenuous physical activity. For all blood sampling, each subject reported to the laboratory, time of day controlled, after a 12 h fast (water allowed *ad libitum*). Prior to each blood draw, the subjects completed a form reporting their physical activity (previous 72 h) and dietary (previous 24 h) adherence, and the time of their last meal. Blood samples were drawn without stasis from an antecubital vein with the subject seated at quiet rest into vacutainer tubes containing 10.5 mg Na-EDTA for plasma collection and a serum clotting factor (Becton Dickinson and Company, Rutherford, NJ) for serum collection. All blood sampling was performed by trained and experienced personnel according to well-accepted, sterile protocols. Serum and plasma were immediately isolated by centrifugation at 1500 x g for 30 minutes at 4°C. Aliquots of serum were stored at -80°C for later analysis. All blood variables (not including HDL and LDL mean densities) were adjusted for plasma volume shifts that occurred as a result of acute exercise using hematocrit and hemoglobin measurements obtained from each plasma sample (50).

Blood analysis

Frozen aliquots of serum were sent to SpectraCell Laboratories, Inc (Houston, TX) for complete LDL density and lipoprotein particle number analyses. A complete "Lipoprotein Particle Profile™" test was performed using the lipoprotein subgroup particle number analysis method. The lipoprotein particle separation utilized a patent pending (209) continuous gradient generated by analytical ultracentrifugation. The lipoprotein particles were stained on their surface with a fluorescent dye and then separated in the gradient over a range of d = 1.000 - 1.300 $g \cdot cm^{-3}$. After separation, the contents of the centrifuge tube were extracted and the fluorescence of the lipoprotein particles was measured in an HPLC-type flow system. For processing, the fluorescence response was normalized to a cholesterol scale with a proprietary algorithm. Values for each lipoprotein subgroup at their specific densities were determined using a multiple Gaussian fit/integration routine (209). The total number of VLDL (VLDL#), LDL (LDL#), remnant lipoprotein (RLP#), dense low-density lipoprotein$_3$ (LDL_3#), dense low-density lipoprotein$_4$ (LDL_4#), HDL (HDL#) and buoyant high-density lipoprotein$_{2b}$ (HDL_{2b}#) particles were determined in addition to the lipoprotein density. The coefficient of variation for this analysis using known standards has been reported as 2-3%. Analysis of serum nonesterified fatty acids (NEFA) was performed colorimetrically using a commercially available kit (Wako Diagnostics, Richmond, Va).

Statistical analysis

An acute exercise (Rest and + 24 h) by exercise training (pre-tr vs. post-tr) by exercise mode (ATM vs. LTM) by gender (men vs. women) ANOVA repeated across

acute exercise and exercise training was employed as the global analysis for each dependent variable of interest, except for VO_2max, body composition, diet and activity measures, in which case the acute exercise factor was not included. Dependent variables of interest included VO_2max , body composition, body weight, serum lipid and lipoprotein concentrations including TC, TAG, HDL-C, HDL_{2b}-C, HDL_{2a}-C, HDL_3-C, LDL-C, LDL_3-C, LDL_4-C, RLP, intermediate density lipoprotein (IDL-C), VLDL-C, lipoprotein (a) (Lp(a)), and NEFA, serum lipoprotein particle numbers for HDL#, HDL_{2b}#, LDL#, LDL_3#, LDL_4#, and RLP, TC: HDL-C ratio and mean densities of HDL and LDL, and blood concentrations of glucose, insulin and hsCRP. Mode of aerobic exercise training did not significantly affect the exercise response for any of the variables of interest in either gender ($P>0.05$); therefore, we elected to remove this factor for all subsequent analyses. The comparison-wise error rate, α, was set at 0.05 for all statistical tests. When significant F ratios were found, a Fisher's LSD post hoc analysis was used to distinguish differences among means. All data were analyzed using the Statistical Analysis System (SAS, version 9.2, Cary, NC) and expressed as mean ± SEM.

Results

Physical characteristics and nutritional intake before and after training.

Physiologic characteristics of these subjects have been previously reported as part of a larger cohort (82). For the subset of subjects included in this current study, body weight (-2.7 kg) and BMI (-0.8 $kg \cdot m^{-2}$) were significantly reduced following exercise training, and VO_2max (+3.7 $mL \cdot kg^{-1} \cdot min^{-1}$) was significantly increased (**Table 1**). No other body composition variables tested were significantly affected by exercise training

(**Table 1**). Body fat %, lean body mass, VO_2max, caloric expenditure, caloric intake, and carbohydrate intake were all different between men and women (**Table 1**). No dietary or non-study physical activity changes were observed throughout the study (**Table 1**).

Exercise training does not affect the lipid and lipoprotein response to acute exercise in overweight and obese men and women.

Exercise training did not influence the acute exercise response at + 24 hr of any measured blood lipid and lipoprotein variables. Lipid and lipoprotein concentrations at rest and + 24 h after a single session of aerobic exercise before and after exercise training are shown in **Table 2**, corresponding lipoprotein particle numbers and mean densities are reported in **Table 3**.

Exercise training increases HDL-C in overweight and obese men and induces a shift in HDL-C subfractions in women.

HDL-C concentration and particle number were significantly lower in men compared to women (-15 $mg \cdot dL^{-1}$ at baseline), but exercise training significantly enhanced HDL-C concentration (+4 $mg \cdot dL^{-1}$) and HDL particle number (+588 $nmol \cdot L^{-1}$) in men only (**Figure 6A and B**). Although mean HDL-C concentration was increased in women following exercise training (+2 $mg \cdot dL^{-1}$) this change was not significant. The HDL_{2b}-C concentration (+2.4 $mg \cdot dL^{-1}$) and particle number (+206 $nmol \cdot L^{-1}$) were enhanced by exercise training in both men and women (**Figure 6C and D**). In addition, despite already greater HDL_{2a}-C concentrations in women compared to men (+2.7 $mg \cdot dL^{-1}$), exercise training enhanced HDL_{2a}-C concentrations (+2.2 $mg \cdot dL^{-1}$) in women only

(**Figure 7A**). This finding was paralleled by reductions in HDL_3-C (-2.7 $mg \cdot dL^{-1}$) and HDL mean density (-0.0041 $g \cdot cm^{-2}$) in women following exercise training (**Figure 7B and C**). Conversely, HDL_3-C was significantly increased in men (+1.4 $mg \cdot dL^{-1}$) following exercise training (**Figure 7B**). We also found that LDL_3-C concentration (-1.6 $mg \cdot dL^{-1}$) and particle number (-16 $nmol \cdot L^{-1}$) were significantly reduced in women following exercise training, but not in men. This reduction led to significantly lower LDL_3-C concentration (-5.5 $mg \cdot dL^{-1}$) and particle number (-53 $nmol \cdot L^{-1}$) in women compared to men following exercise training (**Figure 8**). In addition to these effects on serum lipoprotein-cholesterol concentrations, exercise training proved equally efficacious at reducing serum NEFA concentrations (-0.26 $umol \cdot L^{-1}$) in overweight and obese men and women (**Figure 9A**). Exercise training did not affect serum concentrations of TC, TAG, LDL-C, LDL_4-C, IDL-C, RLP, VLDL-C, glucose, insulin, hsCRP and Lp(a), or corresponding lipoprotein particle numbers, densities, and ratios (e.g., the TC: HDL-C ratio) (**Tables 2 and 3**).

Table 2: Lipid and lipoprotein concentrations in overweight and obese men and women.

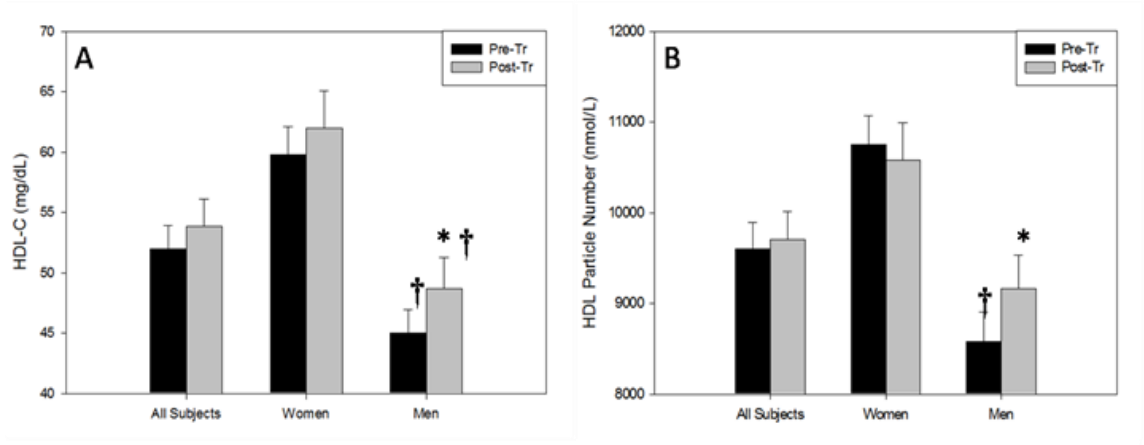
	All Subjects (n = 18)				Women (n = 8)				Men (n = 10)			
	Pre-Training		Post-Training		Pre-Training		Post-Training		Pre-Training		Post-Training	
	Rest	+ 24 h	Rest	+ 24 h	Rest	+ 24 h	Rest	+ 24 h	Rest	+ 24 h	Rest	+ 24 h
TC	190 ± 11	186 ± 10	186 ± 9	182 ± 8	201 ± 10	201 ± 9	201 ± 12	204 ± 10	180 ± 20	172 ± 16	173 ± 9	168 ± 9
TAG	93 ± 11	96 ± 12	102 ± 12	94 ± 8	82 ± 10	85 ± 10	108 ± 19	97 ± 12	103 ± 18	105 ± 22	99 ± 16	92 ± 11
LDL-C	120 ± 9	116 ± 7	112 ± 7	114 ± 9	126 ± 8	126 ± 8	124 ± 11	135 ± 14	115 ± 15	107 ± 12	105 ± 9	101 ± 10
LDL_3-C	20 ± 2	20 ± 2	19 ± 2	19 ± 3	17 ± 2	17 ± 2	16 ± 1	16 ± 1	22 ± 4	21 ±4	21 ± 4	22 ± 4
LDL_4-C	7.1 ± 0.7	6.7 ± 0.5	6.7 ± 0.5	6.8 ± 0.7	6.4 ± 0.5	6.3 ± 0.5	6.2 ± 0.4	6.2 ± 0.5	7.8 ± 1.2	7.0 ± 0.9	7.0 ± 0.8	7.2 ± 1.0
IDL-C†	29 ± 3	28 ± 3	27 ± 3	27 ± 3	32 ± 5	32 ± 4	36 ± 7	35 ± 6	26 ± 4	24 ± 4	22 ± 2	22 ± 2
RLP	33 ± 4	32 ± 3	32 ± 3	31 ± 3	36 ± 5	36 ± 4	40 ± 7	39 ± 6	30 ± 5	29 ± 4	26 ± 2	25 ± 2
VLDL-C	18 ± 2	18 ± 3	20 ± 2	18 ± 2	15 ± 2	16 ± 2	21 ± 4	19 ± 3	20 ± 4	21 ± 4	20 ± 3	18 ± 2
HDL-C†	52 ± 3	52 ± 3	54 ± 3	54 ± 3	60 ± 3	60 ± 4	62 ± 5	62 ± 4	45 ± 3	45 ± 3	49 ± 4	49 ± 4
HDL_{2b}-C†	19 ± 2	20 ± 2	22 ± 2	22 ± 2	24 ± 2	24± 3	27 ± 3	28 ± 3	15 ± 2	16 ± 2	18 ± 2	18 ± 2
HDL_{2a}-C†	7.1 ± 0.6	7.4 ± 0.8	8.0 ± 1.1	7.7 ± 1.1	8.8 ± 0.8	8.5 ± 1.4	11.0 ± 1.6	10.7 ± 1.9	5.6 ± 0.6	6.3 ± 0.8	6.1 ± 0.9	5.8 ± 0.8
HDL_3-C	25 ± 1	25 ± 1	24 ± 1	24 ± 1	27 ± 1	27 ± 1	24 ± 2	23 ± 1	24 ± 2	23 ± 1	24 ± 1	25 ± 1
TC: HDL	3.72 ± 0.22	3.65 ± 0.20	3.55 ± 0.20	3.46 ± 0.19	3.30 ± 0.21	3.38 ± 0.22	3.30 ± 0.30	3.31 ± 0.31	4.05 ± 0.33	3.87 ± 0.31	3.68 ± 0.27	3.54 ± 0.25
NEFA	0.60 ± 0.05	0.73 ± 0.08	0.40 ± 0.03	0.41 ± 0.03	0.51 ± 0.06	0.87 ± 0.10	0.36 ± 0.05	0.45 ± 0.07	0.69 ± 0.07	0.59 ± 0.09	0.42 ± 0.04	0.39 ± 0.04
Glucose	93 ± 2	93 ± 2	93 ± 2	95 ± 2	90 ± 3	93 ± 2	91 ± 1	90 ± 2	95 ± 3	93 ± 3	94 ± 2	98 ± 3
Insulin	13 ± 2	11 ± 2	11 ± 2	12 ± 2	11 ± 3	10 ± 2	11 ± 2	10 ± 3	15 ± 2	12 ± 2	14 ± 3	13 ± 3
hsCRP	0.22 ± 0.04	0.30 ± 0.07	0.20 ± 0.06	0.22 v 0.06	0.18 ± 0.06	0.22 ± 0.06	0.10 ± 0.02	0.14 ± 0.04	0.25 ± 0.06	0.36 ± 0.11	0.25 ± 0.08	0.27 ± 0.08
Lp(a)	23 ± 6	22 ± 5	23 ± 8	23 ± 8	30 ± 11	27 ± 10	31 ± 22	31 ± 22	18 ± 5	18 ± 5	19 ± 6	19 ± 7

†$P<0.05$ significant main effect of gender. All concentrations expressed as $mg \cdot dL^{-1}$ except for insulin ($uIU \cdot mL^{-1}$). Data are means collapsed across mode of exercise training factor; error, SEM.

Table 3: Lipid and lipoprotein particle numbers and densities in overweight and obese men and women.

	All Subjects (n = 18)				Women (n = 8)				Men (n = 10)			
	Pre-Training		Post-Training		Pre-Training		Post-Training		Pre-Training		Post-Training	
	Rest	+ 24 h	Rest	+ 24 h	Rest	+ 24 h	Rest	+ 24 h	Rest	+ 24 h	Rest	+ 24 h
LDL#	822 ± 60	793 ± 50	769 ±46	758 ± 50	838 ± 47	839 ± 49	815 ± 60	814 ± 52	808 ± 108	751 ± 85	739 ± 65	723 ± 75
LDL_3#	189 ± 22	185 ± 20	178 ± 21	185 ± 25	164 ± 15	166 ± 15	149 ± 11	149 ± 10	210 ± 39	203 ± 35	197 ± 33	207 ± 39
LDL_4#	87 ± 8	83 ± 7	81 ± 6	85 ± 8	78 ± 5	78 ± 6	76 ± 5	78 ± 6	95 ± 15	87 ± 12	84 ± 9	89 ± 13
LDL Density†	1.03 18 ± 0.00 03	1.03 20 ± 0.00 03	1.03 20 ± 0.00 03	1.03 20 ± 0.00 04	1.03 12 ± 0.00 04	1.03 13 ± 0.00 03	1.03 12 ± 0.00 04	1.03 12 ± 0.00 04	1.03 23 ± 0.00 04	1.03 24 ± 0.00 04	1.03 24 ± 0.00 04	1.03 26 ± 0.00 04
RLP#	140 ± 15	136 ± 12	134 ± 14	131 ± 14	154 ± 22	153 ± 17	171 ± 30	166 ± 27	128 ± 20	122 ± 17	112 ± 8	108 ± 8
VLDL #	68 ± 9	70 ± 10	78 ± 9	71 ± 7	58 ± 9	60 ± 10	80 ± 16	73 ± 11	77 ± 14	79 ± 16	76 ± 12	69 ± 9
HDL# †	9676 ± 450	9524 ± 395	9698 ± 469	9720 ± 400	1084 1 ± 457	1065 7 ± 474	1064 3 ± 703	1051 2 ± 523	8640 ± 563	8516 ± 381	9108 ± 553	9225 ± 507
HDL_{2b} # †	1733 ± 139	1795 ± 163	1949 ± 197	1944 ± 201	2131 ± 149	2193 ± 234	2451 ± 261	2499 ± 245	1378 ± 150	1442 ± 158	1635 ± 216	1598 ± 216
HDL Density	1.09 60 ± 0.00 01	1.09 46 ± 0.00 01	1.09 30 ± 0.00 01	1.09 40 ± 0.00 02	1.09 39 ± 0.00 01	1.09 33 ± 0.00 02	1.08 98 ± 0.00 02	1.08 92 ± 0.00 02	1.09 74 ± 0.00 02	1.09 58 ± 0.00 02	1.09 54 ± 0.00 02	1.09 65 ± 0.00 02

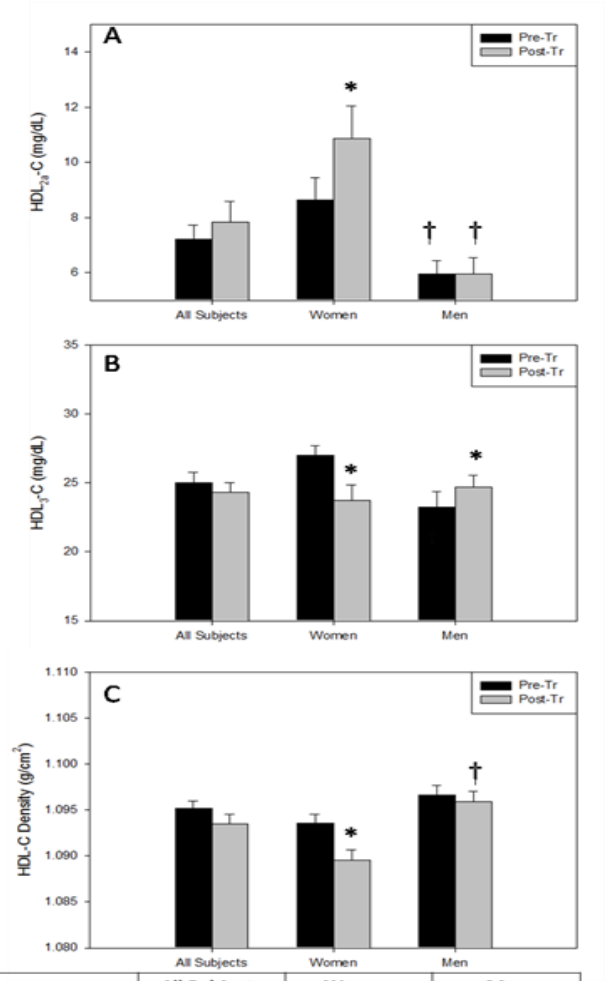
†$P<0.05$ significant main effect of gender. Particle numbers expressed as $nmol \cdot L^{-1}$, densities expressed as $g \cdot cm^{-2}$. Data are means collapsed across mode of exercise training factor; error, SEM.



Concentration		All Subjects	Women	Men
HDL-C	Pre-Tr	52 ± 2	60 ± 2	45 ± 2
	Post-Tr	54 ± 2	62 ± 3	49 ± 3
HDL_{2b}-C	Pre-Tr	20 ± 1	24 ± 1	16 ± 1
	Post-Tr	22 ± 2	28 ± 2	18 ± 2

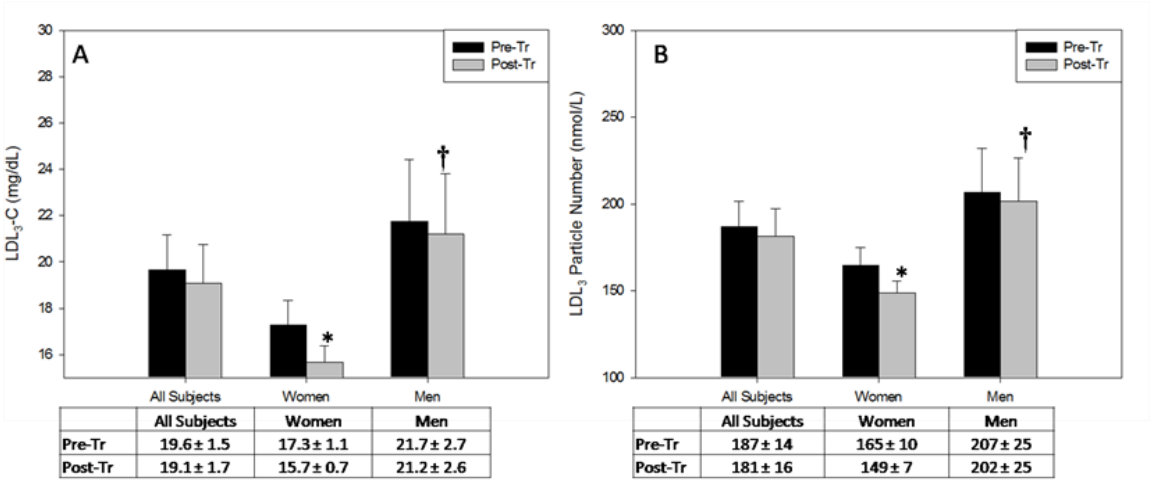
Particle Number		All Subjects	Women	Men
HDL-C	Pre-Tr	9600 ± 295	10749 ± 319	8578 ± 330
	Post-Tr	9709 ± 302	10577 ± 413	9166 ± 363
HDL_{2b}-C	Pre-Tr	1764 ± 106	2162 ± 134	1410 ± 106
	Post-Tr	1947 ± 138	2475 ± 169	1616 ± 148

Figure 6: Effect of exercise training on HDL-C and HDL_{2b}-C in overweight and obese men and women. Data presented are collapsed across mode of exercise training and presented for all subjects combined (n = 18), women (n = 8) and men (n = 10) before and after exercise training. A: HDL-C concentration. B: HDL particle number. C: HDL_{2b}-C concentration. D: HDL_{2b} particle number. *P<0.05 Compared to pre-training value. †P<0.05 Different between genders within exercise training timepoint. Bars, means collapsed across mode of exercise training factor; error bars, SEM.



		All Subjects	Women	Men
HDL_{2a}-C	Pre-Tr	7.2 ± 0.5	8.6 ± 0.8	5.9 ± 0.5
	Post-Tr	7.8 ± 0.7	10.9 ± 1.2	5.9 ± 0.6
HDL_3-C	Pre-Tr	25.0 ± 0.7	27.0 ± 0.7	23.2 ± 1.1
	Post-Tr	24.3 ± 0.7	23.7 ± 1.1	24.7 ± 0.9
HDL-C Density	Pre-Tr	1.0952 ± 0.0008	1.0936 ± 0.0009	1.0966 ± 0.0011
	Post-Tr	1.0935 ± 0.0010	1.0895 ± 0.0012	1.0959 ± 0.0011

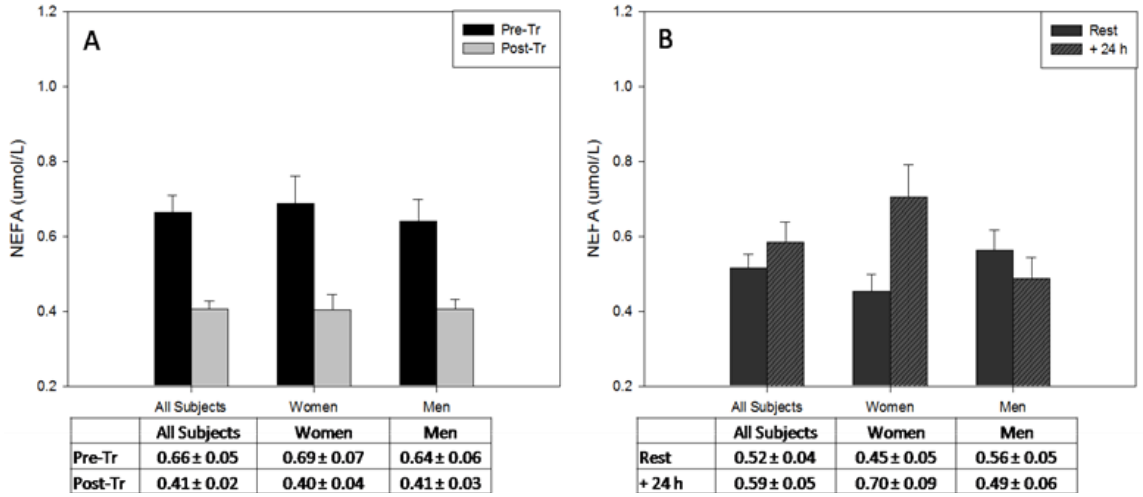
Figure 7: Effect of exercise training on HDL_{2a}-C, HDL_3-C and HDL-C mean density in overweight and obese men and women. Data presented are collapsed across mode of exercise training and presented for all subjects combined (n = 18), women (n = 8) and men (n = 10) before and after exercise training. A: HDL_{2a}-C concentration. B: HDL_3-C concentration. C: HDL-C mean density. *P<0.05 Compared to pre-training value. †P<0.05 Different between genders within exercise training timepoint. Bars, means collapsed across mode of exercise training factor; error bars, SEM.



	All Subjects	Women	Men
Pre-Tr	19.6 ± 1.5	17.3 ± 1.1	21.7 ± 2.7
Post-Tr	19.1 ± 1.7	15.7 ± 0.7	21.2 ± 2.6

	All Subjects	Women	Men
Pre-Tr	187 ± 14	165 ± 10	207 ± 25
Post-Tr	181 ± 16	149 ± 7	202 ± 25

Figure 8: Effect of exercise training on LDL_3-C in overweight and obese men and women. Data presented are collapsed across mode of exercise training and presented for all subjects combined (n = 18), women (n = 8) and men (n = 10) before and after exercise training. A: LDL_3-C concentration. B: LDL_3 particle number. *P<0.05 Compared to pre-training value. †P<0.05 Different between genders within exercise training timepoint. Bars, means collapsed across mode of exercise training factor; error bars, SEM.



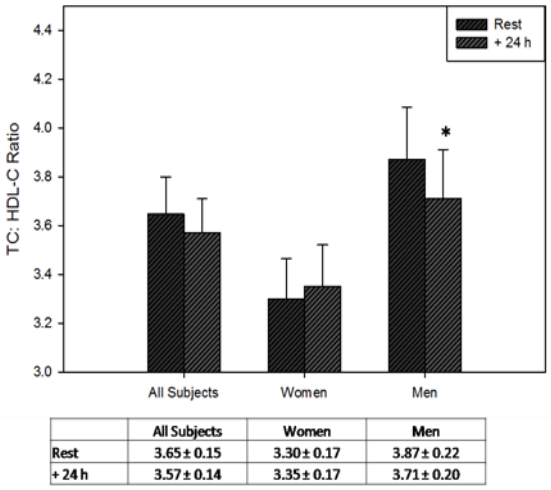
	All Subjects	Women	Men
Pre-Tr	0.66 ± 0.05	0.69 ± 0.07	0.64 ± 0.06
Post-Tr	0.41 ± 0.02	0.40 ± 0.04	0.41 ± 0.03

	All Subjects	Women	Men
Rest	0.52 ± 0.04	0.45 ± 0.05	0.56 ± 0.05
+ 24 h	0.59 ± 0.05	0.70 ± 0.09	0.49 ± 0.06

Figure 9: Effect of acute exercise and exercise training on serum NEFA concentrations in overweight and obese men and women. Data presented are collapsed across mode of exercise training and presented for all subjects combined (n = 18), women (n = 8) and men (n = 10). A: Serum NEFA concentration before and after exercise training. B: Serum NEFA concentration before and after acute exercise. *P<0.05 Compared to pre value. †P<0.05 Different between genders within exercise training timepoint. Bars, means collapsed across mode of exercise training factor; error bars, SEM.

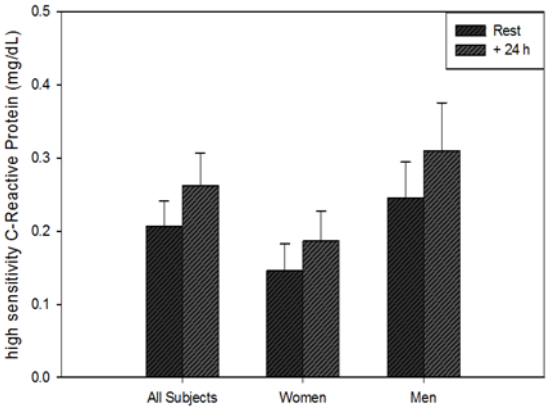
Acute exercise reduces the TC: HDL-C ratio in overweight and obese men, and raises serum hsCRP in both men and women.

Acute aerobic exercise reduced the ratio of TC: HDL-C (-0.16) in men but not women regardless of training status (**Figure 10**). Furthermore, whether our subjects were untrained or trained, serum concentrations of NEFA (+0.25 umol·L^{-1}) in women (**Figure 9B**), as well as hsCRP (+0.06 mg·dL^{-1}) in men and women (**Figure 11**) were significantly higher 24 h after a single session of 400 kcal of aerobic exercise. Acute exercise did not affect serum concentrations of TC, TAG, LDL-C, LDL_3-C, LDL_4-C, IDL-C, RLP, VLDL-C, HDL-C, HDL_{2b}-C, HDL_{2a}-C, HDL_3-C, glucose, insulin, and Lp(a), or corresponding lipoprotein particle numbers and densities (**Tables 2 and 3**).



	All Subjects	Women	Men
Rest	3.65 ± 0.15	3.30 ± 0.17	3.87 ± 0.22
+ 24 h	3.57 ± 0.14	3.35 ± 0.17	3.71 ± 0.20

Figure 10: Effect of acute exercise on the ratio of total cholesterol to HDL-C in overweight and obese men and women. Data presented are collapsed across mode of exercise training and presented for all subjects combined (n = 18), women (n = 8) and men (n = 10) before and after acute exercise. *P<0.05 Compared to pre-exercise value. Bars, means collapsed across mode of exercise training factor; error bars, SEM.



	All Subjects	Women	Men
Rest	0.21 ± 0.03	0.15 ± 0.04	0.25 ± 0.05
+ 24 h	0.26 ± 0.04	0.19 ± 0.04	0.31 ± 0.07

Figure 11: Effect of acute exercise on high sensitivity C-reactive protein in overweight and obese men and women. Data presented are collapsed across mode of exercise training and presented for all subjects combined (n = 18), women (n = 8) and men (n = 10) before and after acute exercise. *P<0.05 Compared to pre-exercise value. Bars, means collapsed across mode of exercise training factor; error bars, SEM.

Discussion

In the current study we have compared the effects of acute aerobic exercise and exercise training on blood lipids, lipoproteins, and hsCRP in overweight and obese men and women. We are among the first to report the influence of an exercise regimen on lipoprotein particle numbers and to compare and contrast acute exercise responses with the adaptations observed following exercise training. Our findings demonstrate that exercise training did not affect the lipid and lipoprotein-lipid response to a single session of aerobic exercise in our sample of subjects. Furthermore, we have demonstrated the efficacy of either ATM or LTM exercise training of 1500 kcal·wk^{-1} to elicit changes in blood lipid and lipoprotein concentrations consistent with reduced atherogenic risk in overweight and obese men and women, with only minimal weight loss. In addition, acute exercise of 400 kcal·session^{-1} was insufficient to elicit significant effects on blood cholesterol concentrations, however a significant reduction in the ratio of TC: HDL-C was observed in men.

The influence of exercise training on the acute response to exercise has been reported in only one previously published paper (42). In that study by our laboratory and using hypercholesterolemic men as subjects, exercise training nullified an acute exercise induced increase in LDL-C concentration. By contrast, in our current study we found no influence of exercise training on the acute response to exercise of blood lipids and lipoproteins. While the lack of such an interaction may be due to the fact that we observed only limited changes in lipids and lipoproteins following acute exercise, some differences between our current study and our previous study may at least partially

explain our divergent findings. First, we did not measure a transient increase in LDL-C concentrations after an acute exercise session regardless of training status in the men and women in our current study. As this was the primary effect of acute exercise influenced by training in our previous study (14) we are unlikely to detect a similar effect without this acute increase in LDL-C concentration. Second, our previous study was carried out across 24 wks with acute exercise sessions every 8 wks; the effect of exercise training on this transient response to acute exercise was not observed until 16 wks into exercise training, whereas our current study was included only 12 wks of training. Finally, in our current study we examined the influence of acute exercise and exercise training on blood lipids and lipoproteins in obese men and women, whereas hypercholesterolemic men served as subjects in our previous study.

An important finding of our current study is that exercise training elicited a reduction in atherogenic risk by HDL-C mediated mechanisms in both men and women; however, the nature of this effect was different between genders. More specifically, we have shown that exercise training induced a significant increase in both HDL-C concentration and HDL particle number in overweight and obese men, but not in women (**Figure 6**). Similar to previous findings (240), this effect was mediated in large part by a concomitant increase in HDL_{2b}-C concentration, and in our study increased HDL_{2b} particle number. Our finding that exercise training enhanced HDL-C concentrations in obese men is comparable to previously published reports in other populations including older and hypercholesterolemic individuals (43, 87, 207). We expand on previous studies by showing that the increase in HDL-C and HDL_{2b}-C concentrations in men is

driven by an increase in particle number of this lipoprotein fraction, rather than changes in particle size or density.

Nicklas et al. (157) have previously demonstrated an increase in HDL-C and HDL_2-C in lean and overweight men following 9 months of exercise training. In contrast to our findings, however, beneficial HDL-C changes did not occur in their obese men. Nicklas et al. (157) sampled blood lipids 24 hr after the last session of exercise. We (42-43, 223) and others (65, 234) have previously shown that the transient response to the last exercise session often peaks at 24 h and can last 48-72 h following exercise. Therefore, while training induced alterations in lipids and lipoproteins cannot be ruled out as a contributing factor for the observations by Nicklas et al. (157), it is likely that at least part of their findings reflect a transient response to the final exercise session. By contrast, in our present study we eliminated this possibility by restricting vigorous activity for 72 h prior to blood sampling. Williams et al. (228) have previously demonstrated increased HDL_{2b}-C in men following a 12 month weight loss program by either diet (-7.2 kg) or exercise (-4.0 kg), however, in their study blood samples were obtained after 12-16 hours without vigorous activity. In our study, we accomplished similar changes with less weight loss (-1.9 kg in men), and after controlling for the transient effects of the last exercise session. Our study may better compare to that by Slentz et al. (183) who showed similar increases in HDL-C and "large" HDL-C, presumably HDL_2-C, in overweight and mildly obese men in high volume exercisers (1700 $kcal \cdot wk^{-1}$), but not with lower volume (approximately 1100 $kcal \cdot wk^{-1}$). Their findings were seen 24 h following the last exercise session, and lasted through 15 days

following the cessation of exercise training. Combined with our results, this suggests the efficacy of exercise training of 1500 kcal·wk^{-1} to increase blood HDL-C in overweight and obese men.

By contrast, the mean HDL-C concentration was not significantly elevated following exercise training in the overweight and obese women in our study. These results in women corroborate those published by others after acute exercise (223) and exercise training (136). Conversely, in some published studies a decrease in HDL-C in women following exercise training has been reported (14, 80). We note that the mean HDL-C concentration of our women was 60 mg·dL^{-1} prior to training; this already elevated HDL-C concentration may reduce the likelihood of inducing an increase in the concentration of this fraction (132).

Despite the lack of a significant change in total HDL-C in our sample of women, we measured a shift in HDL-C subfractions from HDL_3-C to the HDL_2-C (both HDL_{2b}-C and HDL_{2a}-C, **Figures 6 and 7**) subfractions, similar to that previously observed in hypercholesterolemic men (41). This change in HDL subfractions could reduce atherogenic risk, since increased blood concentrations of HDL_2-C signals acquisition of cholesterol by HDL, and thus an enhanced reverse-cholesterol transport process (177, 201). Consistent with this increase in HDL_2-C at the expense of HDL_3-C concentration, a decrease in HDL mean density was measured, a finding also associated with reduced atherogenic risk (91). Thus, our results suggest that exercise training by overweight and obese women results in a reduction in atherogenic risk through a redistribution of cholesterol among the HDL subspecies, without a significant net change in total HDL-C

concentration. Our data support the need for a more detailed assessment of HDL-associated cholesterol and density beyond traditional approaches of simply measuring total HDL-C to assess the efficacy of an intervention aimed at improving the lipid profile and reducing atherogenic risk in women. Furthermore, we demonstrate that overweight and obese men and women each display a reduction in atherogenic risk by HDL mechanisms but that this effect differs between genders.

We show for the first time that exercise training induced a 9% decrease in LDL_3-C concentration and a 10% decrease in LDL_3 particle number in overweight and obese women, but not in men (**Figure 8**). LDL-C categories include subfractions 1 through 4, defined based on size and density; the largest and most buoyant class is LDL_1-C and the smallest, most dense is LDL_4-C (124). The smaller subfractions (LDL_3-C and LDL_4-C) are considered to represent greater risk because they are more susceptible to oxidation and greater binding to the arterial wall (124). Therefore, a reduction in either of these subfractions, as observed in LDL_3-C in our women subjects, is consistent with reduced atherogenic risk. Although not significant, men displayed greater mean concentrations of these subfractions than did women (+4.5 $mg \cdot dL^{-1}$ for LDL_3-C, and +1 $mg \cdot dL^{-1}$ for LDL_4-C) at the onset of training, and no significant decrease in either of these subfractions was measured in men after training. We note, however, that despite no significant effects on these subfractions men displayed a mean decrease in LDL_3 and LDL_4 particle numbers following exercise training.

We cannot conclude that the reduction in LDL_3-C in women was independent of the weight loss seen with our exercise training regimen, nevertheless, reduced LDL_3-C in

endurance trained compared with sedentary individuals has been reported (239). In addition, Halverstadt et al. (87) have previously shown exercise training induced reductions in "small" LDL, along with modest changes in body weight, BMI and VO_2max similar to those measured in our current study. Halverstadt et al. (87) also reported that these changes remained significant after adjusting for changes in body fat. We conclude that the reduction in LDL_3-C concentration was the result of reduced LDL_3 particle number. This finding is similar to our findings regarding HDL-C in men in that significantly altered lipoprotein-cholesterol concentrations appear to be mediated primarily by a change in lipoprotein particle number.

In both overweight and obese men and women we observed a 38% reduction in serum NEFA concentration following training (**Figure 9**). Our data corroborate previously published findings regarding exercise training in the obese (107). Elevated serum NEFA have previously been proposed to contribute to CVD by an oxidative stress mechanism (197). Therefore, the observed reduction in serum NEFA with exercise training by our subjects may also serve to reduce atherogenic risk. In contrast, we measured no significant effect of exercise training on hsCRP, confirming previous reports in overweight and obese men and women (99). Based on our data, exercise training consisting of 1500 $kcal \cdot wk^{-1}$ is sufficient to induce changes in blood lipids and lipoproteins in overweight and obese men and women consistent with reduced atherogenic risk, although the nature of this adaptation appears to differ between genders.

In addition to our examination of exercise training induced changes in lipids and lipoproteins, we have further described the influence of a single session of exercise on these factors in our sample of overweight and obese men and women. Considering that exercise duration, as measured by total caloric expenditure, appears to be the determining factor in eliciting transient changes in blood lipids and lipoproteins following acute exercise, it is likely that these transient responses are resultant of a differential ability to handle the energetic demands of the exercise. In this study we have examined these transient responses in both the untrained state and after 12 wks of exercise training. Based on previously published data from our laboratory (42-43, 81, 223) describing the effects of acute exercise on lipids and lipoproteins in other populations we elected to perform these exercise sessions at an energy expenditure of 400 kcal. We have shown that acute exercise reduced the ratio of TC: HDL-C in obese men but had no such effect in women (**Figure 10**). However, acute exercise also induced an increase in hsCRP in both men and women (27% increase, **Figure 11**) and NEFA (55% increase, **Figure 9B**) in women. Such increases in hsCRP (110) and NEFA (81) have been previously reported. Our finding that no cholesterol or TAG changes were significant in women corroborates previous findings in which 350 kcal of aerobic energy expenditure was insufficient to elicit such changes at 24 h post-exercise in both pre- and postmenopausal women (165).

That we were unable to elicit significant increases in HDL-C or reductions in TAG concentrations, as noted in other studies of acute exercise (43, 65) suggests that we may have been below the necessary caloric threshold for this overweight and obese

population to elicit such responses. That the necessary caloric threshold may be greater in the obese compared to other populations appears analogous to endurance trained individuals (65). A greater caloric threshold in obese and endurance trained individuals, compared to other populations, may be due to the fact these populations have been reported to exhibit greater stores of intramuscular fat than lean controls (77-78). If the transient blood lipid and lipoprotein response to exercise is driven by energetic need, as previously mentioned and evidenced by the apparent importance of a caloric threshold, an increase in intramuscular fat stores could blunt the need to mobilize fatty acids from other stores, therefore reducing the need for a change in blood lipids and lipoproteins. However, a greater caloric expenditure for acute exercise should be taken with caution as the acute exercise performed here induced increased serum hsCRP concentrations, and NEFA in women, and thus may be associated with a negative effect on these risk markers.

In the current study, we found no significant changes in serum insulin, glucose, or Lp(a) following either acute exercise or exercise training in our sample of overweight and obese men and women. Our findings regarding Lp(a) concentration corroborate previous work demonstrating no influence on this risk marker of acute exercise (57) or exercise training (140). We also measured no effect of the exercise protocol on fasting serum glucose or insulin. These findings are contrary to previous reports of exercise training in the overweight (184), in that we saw no significant change in fasting insulin. We should note, however, that our subjects, despite being overweight and obese, were not insulin resistant as defined by fasting glucose (mean fasting glucose at onset of study

93 mg·dL^{-1}) (73). Since fasting insulin and glucose levels were in normal range at the onset of the experiment, exercise and exercise training are less likely to cause significant reductions in these blood variables.

In summary, we have shown that both acute exercise and exercise training by obese men and women generally produce changes in blood lipids and lipoprotein-lipids congruent with reduced cardiovascular risk without the use of dietary measures to aid in weight loss. These data support the notion that exercise can have therapeutic benefits in the overweight and obese population without the need for dramatic weight loss. We have also shown that aerobic exercise training using either ATM or LTM is equally efficacious for the reduction of cardiovascular risk markers so long as frequency, intensity, and duration of exercise are matched. In addition, while acute exercise induced a reduction in the ratio of TC: HDL-C in overweight and obese men, acute exercise was also associated with an increase in serum hsCRP concentration in men and women, and NEFA in women. In addition, exercise training did not appear to affect the transient response to acute exercise in this population. Based on our present study, exercise training expending at least 1500 kcal·wk^{-1} is an effective course of action to reduce cardiovascular risk in the obese population.

CHAPTER III

PPARδ AND AMPKα PROTEIN CONTENT ARE ENHANCED FOLLOWING EXERCISE AND CORRELATED WITH BLOOD LIPID AND LIPOPROTEIN CONCENTRATIONS IN THE OBESE

Introduction

Obesity and diabetes have become a major public health concern in the United States with prevalence of these diseases rising to epidemic levels (27). In addition, obesity is an established independent risk factor for cardiovascular disease (156). These disease states are characterized by impairments in energy metabolism including insulin resistance and dysregulation of lipid metabolism. Dysregulated lipid metabolism is evident by reduced content and activity of oxidative enzymes within skeletal muscle. As a result, individuals suffering from these disease states often exhibit elevated blood glucose and dyslipidemia including elevated total cholesterol (TC), LDL-cholesterol (LDL-C), and triacylglycerols (TAG), as well as depressed HDL-cholesterol (HDL-C).

Our laboratory (41-42) and others (60, 94) have previously demonstrated that both acute exercise and exercise training are capable of eliciting beneficial changes in blood lipid and lipoprotein concentrations consistent with reduced risk for CVD, including reduced total cholesterol (TC), LDL-cholesterol (LDL-C) and triacylglycerol (TAG) concentrations, and elevated HDL-cholesterol (HDL-C) concentrations. However, the mechanisms responsible for alterations in blood lipids and lipoproteins with exercise are currently poorly understood. Both acute exercise and exercise training-induced changes

in blood lipids and lipoproteins appear to be dependent on the attainment of a necessary minimum caloric expenditure. In fact, a review by Durstine et al. (59) has previously examined necessary caloric thresholds to elicit changes in the various cholesterol fractions and TAGs. Considering that elevations in caloric expenditure with exercise are the result of enhanced skeletal muscle activity, we propose that skeletal muscle plays an essential function in mediating exercise-derived changes in blood lipids and lipoproteins, a concept which has recently been advanced by others (185). Previous evidence suggests arterial-venous differences across vascular beds of exercising skeletal muscle, whereby HDL-C concentration is elevated and VLDL-TAG reduced in venous compared to arterial blood (113).

Although there have been a plethora of therapeutic strategies for the treatment of metabolic dysregulation, pharmacological activation of the peroxisome proliferator-activated receptors (PPARs; a family of nuclear receptors involved in the regulation of lipid metabolism and glucose homeostasis) has shown promise for the treatment of several metabolic diseases. PPARδ, in particular, appears to play a critical role in metabolic adaptation in skeletal muscle. In fact, pharmacological evidence suggests that the activation of PPARδ may improve insulin sensitivity (238) and induce beneficial changes in blood lipids and lipoproteins (160), providing a direct link to the treatment of these disease states by enhancing PPAR expression.

The PPAR family members show tissue specific distribution. PPARγ is primarily expressed in adipose tissue; while, PPARs -α and -δ are present in tissues with high metabolic activity, including liver and muscle. The primary isoform in skeletal muscle

is PPARδ. Animals bred to overexpress PPARδ exhibit increased oxidative myofiber content (220) and a general increase in muscle oxidative capacities analogous to that seen following exercise training (199). In spite of such findings, the response to exercise of PPARδ remains inadequately described. Acute fatiguing exercise has previously been demonstrated to induce increased mRNA content of PPARδ and PPARα (142, 221). However, it remains uncertain as to how PPARδ might respond to non-fatiguing exercise or whether previously observed increases in mRNA content yield elevated levels of this protein. Exercise training has previously been demonstrated to enhance expression of PPARα protein (98), but less is known about PPARδ. Studies involving PPARδ protein content and exercise training are few and present mixed findings showing either unchanged (175) or increased (70) content. Further research is necessary to better understand how PPARδ responds to exercise and thereby mediates oxidative adaptations to exercise stress.

Stimulation of PPARδ in obese rhesus monkeys using a synthetic pharmaceutical agonist has been shown to alter blood lipids and lipoproteins, including increased HDL-C and decreased TAG and LDL-C concentrations, each congruent with reduced atherogenic risk (160). Furthermore, a bedrest study in humans demonstrated that subjects receiving a PPARδ agonist maintained HDL-C concentration while those on placebo displayed reduced HDL-C. In addition, although not significant ($P = 0.08$), subjects receiving the PPARδ agonist showed decreased TAG (192). However, it is not currently known whether the exercise induced alteration in blood lipids and lipoproteins may be mediated by PPARδ. In this study we specifically examined the role of skeletal

muscle PPARδ in the regulation of blood lipid and lipoprotein concentrations following exercise.

AMP-activated protein kinase (AMPK) and PPARγ coactivator-1α (PGC-1α) are vital to the effects of PPARδ on skeletal muscle and whole body metabolism. AMPK is considered to be a 'master metabolic switch' most commonly associated with regulating the use of metabolic fuel supplies, but also involved in the stimulation of the transcription of oxidative genes (208). PGC-1α, on the other hand, is believed to serve as a 'master regulator of mitochondrial biogenesis' (147). Both AMPK and PGC-1α appear necessary for the maximal activation of PPAR activity (122, 151). Therefore, the role of these proteins cannot be overlooked in assessing the influence of exercise on skeletal muscle, and whole muscle metabolism in the obese.

In addition, we have previously demonstrated the efficacy of aquatic treadmill (ATM) exercise to increase aerobic fitness and reduce weight and body fat percentage in the obese (82). In that previous report we further revealed the potential of ATM exercise to elicit enhanced lean body mass, providing a potential advantage to ATM training. However, mechanisms mediating enhanced oxidative function with this form of exercise have yet to be described.

Therefore, the purpose of this study was to test the hypotheses that: 1) both acute aerobic exercise and exercise training will elicit enhanced protein content of PPARδ and related proteins in the skeletal muscle of overweight and obese men and women, 2) enhanced PPARδ protein content will be associated with enhanced content of PPARδ target proteins involved in fatty acid and lipoprotein transport and metabolism, and 3)

PPARδ protein content will be correlated with the blood concentrations of lipids and lipoproteins at rest and following exercise in the obese. In addressing these hypothesis, this study provides: 1) new understanding of the comparative adaptations of skeletal muscle proteins regulating oxidative function following either ATM or land treadmill (LTM) training; 2) novel evidence regarding the regulation of metabolism with obesity, as well as how obesity affects the individual's ability to adapt and respond to exercise; and 3) additional insight into the mechanisms involved in the regulation of blood lipids and lipoproteins in response to exercise and exercise training.

Methods

Physically inactive, overweight and obese men (n = 9) and women (n = 7) were recruited from the Texas A&M University and College Station, TX communities to participate in the study. The mean (SEM) age of subjects was 41 ± 2 years and 52 ± 2 years for men and women, respectively. Subjects were part of a larger cohort whose physiologic adaptations to the exercise training paradigm have previously been reported (82). Potential volunteers were recruited through informational flyers, email announcements, and by word of mouth. Volunteers were screened to ensure that they had not participated in regular aerobic activity for the previous 3 months (physically inactive), and were classified as overweight or obese initially by BMI and subsequently by percent body fat as measured by dual-energy x-ray absorptiometry (DEXA, Lunar Prodigy, GE Healthcare, Madison, WI). Subjects were stratified according to ACSM standards for risk of cardiovascular disease, and those for whom it was required underwent a physical examination by a cardiologist before participation in the

experiment (2). Preliminary physiological characteristics of the 16 subjects who completed the study are presented in **Table 4**.

Table 4: Physiological variables, and activity and dietary habits before and after training in all subjects, women, and men.

	All Subjects (n = 16)		Women (n = 7)		Men (n = 9)	
	Pre-Tr	Post-Tr	Pre-Tr	Post-Tr	Pre-Tr	Post-Tr
Physical Characteristics						
Body Mass (kg)*	98.9 ± 5.7	96.4 ± 5.3	96.4 ± 8.6	93.1 ± 8.0	101.4 ± 7.6	99.8 ± 7.0
Body Mass Index ($kg \cdot m^{-2}$) *	31.9 ± 1.4	31.2 ± 1.3	32.0 ± 2.3	30.9 ± 2.1	31.9 ± 1.7	31.4 ± 1.5
Body Fat % †	41.1 ± 1.5	39.9 ± 1.6	44.3 ± 2.4	44.0 ± 2.6	38.0 ± 1.8	35.9 ± 1.9
Lean Body Mass (kg) †	52.7 ± 2.2	51.9 ± 2.1	45.5 ± 3.5	44.5 ± 3.3	59.9 ± 2.6	59.3 ± 2.5
Fat Mass (kg)	36.9 ± 3.2	35.7 ± 3.1	36.8 ± 5.1	35.4 ± 5.0	37.0 ± 3.8	36.1 ± 3.8
VO_2max ($L \bullet min^{-1}$) * †	2.52 ± 0.08	2.77 ± 0.10	2.05 ± 0.12	2.28 ± 0.15	2.99 ± 0.11	3.26 ± 0.13
VO_2max ($mL \bullet kg^{-1} \bullet min^{-1}$) * †	26.0 ± 1.3	29.6 ± 1.4	22.5 ± 1.9	25.8 ± 2.2	29.4 ± 1.7	33.3 ± 1.8
Activity and Nutritional Intake						
Caloric Expenditure ($kcal \bullet day^{-1}$) †	3005 ± 133	2971 ± 98	2595 ± 213	2582 ± 160	3414 ± 159	3360 ± 119
Caloric Intake ($kcal \bullet day^{-1}$) †	2362 ± 167	2137 ± 221	2019 ± 267	1780 ± 355	2705 ± 199	2495 ± 265
Carbohydrate ($g \bullet day^{-1}$) †	293 ± 21	261 ± 33	235 ± 34	207 ± 53	350 ± 26	316 ± 39
Total Fat ($g \bullet day^{-1}$)	91.4 ± 8.5	87.4 ± 10.6	84.0 ± 13.7	79.2 ± 17.0	98.9 ± 10.2	95.6 ± 12.7
Monounsaturated Fat ($g \bullet day^{-1}$)	19.2 ± 2.8	17.6 ± 3.5	13.0 ± 4.5	17.6 ± 5.6	25.3 ± 3.3	17.7 ± 4.2
Polyunsaturated Fat ($g \bullet day^{-1}$)	8.0 ± 1.1	10.1 ± 2.6	5.8 ± 1.7	11.0 ± 4.1	10.1 ± 1.3	9.2 ± 3.1
Saturated Fat ($g \bullet day^{-1}$)	31.9 ± 2.8	27.9 ± 3.1	29.8 ± 4.5	27.0 ± 4.9	34.0 ± 3.3	28.8 ± 3.7
Protein (g)	91.6 ± 8.0	76.1 ± 7.4	81.0 ± 12.8	67.8 ± 11.9	102.1 ± 9.5	84.4 ± 8.9

*P<0.05 significant main effect of exercise training. †P<0.05 significant main effect of gender. Data are means collapsed across mode of exercise training factor; error, SEM.

General protocol

All methods and procedures were approved by the Texas A&M University Institutional Review Board for Human Subjects in Research. On the first visit to the laboratory, subjects were informed of the study procedures, and read and signed an institutionally approved informed consent. At this time, instructions were provided for completing physical activity and diet records (methods to follow). Briefly, physiological and demographic assessments were completed on the second visit to the laboratory. Approximately four days following physiological assessments, resting muscle biopsies were obtained from the vastus lateralis (resting/untrained state, methods to follow). Approximately three days later (seven days following physiological testing) the first of two acute exercise bouts was performed on a standard motor-driven land treadmill with pre-exercise blood samples obtained immediately prior to exercise (resting/untrained state, methods to follow). Blood and muscle biopsy samples were obtained 24-h after the acute exercise session (exercised/untrained state). Within two weeks of physiological testing, subjects were matched for age, gender, and BMI, and then randomly assigned to 12-weeks of exercise training using either LTM or ATM (methods to follow). All physiological and demographic testing procedures were repeated within 72-96 h after the final exercise training session. Blood samples and muscle biopsies were again obtained 24 hours after a single exercise session performed within 7 days of the end-of-study physiological assessments (exercised/trained state).

Diet and activity logs

To study subjects in a free-living state, no attempt was made to modify diet or physical activity outside of the study protocol. To this end, subjects were instructed to maintain their accustomed dietary and physical activity habits throughout the course of the study. To verify compliance with these instructions, dietary and activity habits were assessed on two occasions coinciding with the beginning and end of exercise training. Subjects were instructed to complete dietary and physical activity records on days which would best represent their normal daily habits. On both occasions dietary logs were recorded for 3 consecutive days, including 1 weekend day. The 3-day dietary records were analyzed for total caloric intake and for carbohydrate, fat, and protein composition using commercially available computer software (Food Processor® 8.4, ESHA Research, Salem, OR). The physical activity records used were an adaptation of a previously described protocol for physical activity recall (6). Activity records were recorded for seven consecutive days and analyzed for total energy expenditure. In addition, upon completion of the study all subjects were required to read and sign dietary and activity compliance forms.

Physiological assessments

These procedures have been previously published (82). In brief, body composition, including whole body percent fat, fat mass and lean body mass, were assessed using DEXA. An incremental maximal graded exercise test (GXT) was conducted on a motor-driven treadmill according to the Bruce protocol (21). Oxygen consumption during exercise was assessed using an automated metabolic gas-analysis system (CPX/D

Exercise Stress Testing System, Medical Graphics, Minneapolis, MN or Oxycon Pro, Erich Jaeger, Hoechberg, Germany) calibrated with gasses of known concentration before and after each exercise test. VO_2max was taken as the highest 15 s average oxygen uptake achieved during the exercise test. Heart rate (HR) and rhythm were monitored continuously from a 12-lead electrocardiogram. Ratings of perceived exertion (RPE) using a Borg 15-point scale ranging from 6-20 (17) and manual blood pressures (BP) were obtained during the last 30 s of each treadmill stage and at maximal exercise. At least two of the following criteria were required for the maximal exercise test to be considered valid: 1) achievement of maximum heart rate within 10 bpm of the age-predicted maximum; 2) rating of perceived exertion $\geq$ 18; 3) respiratory exchange ratio >1.1 at maximal exertion; or 4) O_2 uptake plateau despite further increases in workload. The same skilled laboratory personnel consistently performed all physiological measurements.

Acute exercise sessions

All non-study exercise was prohibited for 72 h prior to the experimental acute exercise sessions. The experimental acute exercise sessions were performed on a motorized land treadmill approximately seven days after completion of maximal exercise testing at pre- and post-training timepoints. The exercise protocol began with a 3 min warm-up period at 53.6 $m \cdot min^{-1}$ at a 0% grade. The duration of each acute exercise session was defined as the time required to expend 400 kcal at an intensity of 70% of VO_2max, based on the most recently acquired VO_2max. The necessary duration was estimated by dividing 400 kcal by the rate of energy expenditure at the required

exercise intensity based on the respiratory exchange ratio energy-oxygen equivalent ($kcal \cdot LO_2^{-1}$). Initial treadmill velocity and grade were estimated using published metabolic equations for LTM exercise (2). Heart rate, RPE and VO_2 were measured every 5 min at the beginning of exercise to adjust treadmill velocity and grade until the required VO_2 was achieved; thereafter VO_2 measurements were taken every 10 min until the cessation of exercise, and minor adjustments were made as necessary to the treadmill velocity and grade to maintain the required VO_2, as well as, exercise time to ensure the expenditure of 400 kcal. Subjects were asked to avoid any physical exertion outside of that required by their work until after all blood and muscle collection procedures were completed.

Exercise training

The exercise prescription and training progression for this study have previously been reported (82). ATM training was conducted using a HydroWorx 1000 series treadmill (HydroWorx International, Inc., Middletown, PA) as previously described (82). LTM training was conducted on a standard motor-driven treadmill. Subjects exercised three times per week for a 12-week period. Training sessions were performed by all subjects at an equivalent caloric expenditure and relative intensity progressively increased from 250 $kcal \cdot session^{-1}$, 60% VO_2max during the first week to 500 $kcal \cdot session^{-1}$, 85% VO_2max during weeks 6-12. The prescribed exercise progression was such that by week 6 subjects expended approximately 1500 $kcal \cdot wk^{-1}$ in exercise training. Exercise duration and intensity were calculated as described above for acute exercise sessions. For ATM training treadmill velocity and jet resistance were estimated

using a previously developed metabolic equation for ATM (Greene et al., manuscript accepted for publication in Research Quarterly for Exercise and Sport). Treadmill velocity and grade/jet resistance were adjusted as necessary during the training session to attain the HR and RPE which matched the prescribed intensity. Each individual's exercise prescription was adjusted for increases in VO_2max during week 6 such that the prescribed intensity and duration were maintained throughout the study.

Blood and muscle sampling

Blood and muscle samples were obtained on three occasions: muscle sample one was obtained 3 days prior to acute exercise (resting/untrained state), blood sample one was obtained with the subject at rest immediately preceding the acute exercise session (resting/untrained state). Blood and muscle sample two was obtained 24 h after a single aerobic exercise session (exercised/untrained state). The third blood and muscle sample was obtained 24 h following acute exercise after 12 weeks of exercise training (exercised/trained state). Resting samples were taken following at least 72 hours without strenuous activity. For blood and muscle sampling each subject reported to the laboratory, time of day controlled, after a 12-hour fast (water allowed *ad libitum*). Prior to sample collection, subjects completed a form reporting their physical activity and dietary adherence over the previous 24 hours and the time of their last meal.

The same trained and experienced personnel consistently performed phlebotomy and biopsy procedures according to well-accepted, sterile protocols. Blood samples were drawn without stasis from an antecubital vein with the subject seated at quiet rest into vacutainer tubes containing 10.5 mg Na-EDTA for plasma collection and a serum

clotting factor (Becton Dickinson and Company, Rutherford, NJ) for serum collection. Serum and plasma were immediately isolated by centrifugation at 1500 x g for 30 minutes at 4°C. Aliquots of serum were stored at -80°C for later analysis. All blood variables (not including HDL and LDL mean densities) were adjusted for plasma volume shifts that occurred as a result of acute exercise using hematocrit and hemoglobin measurements obtained from each plasma sample (50).

The muscle biopsy procedure used is a modification of Bergstrom's technique (11), as described by Evans et al. (63). Muscle biopsies were taken from the vastus lateralis under local anesthetic (1% Xylocaine HCl) using a 5-mm needle. All muscle samples were cleaned of visible fat, connective tissue, and blood. Muscle samples were immediately frozen in liquid nitrogen (-190ºC), and then stored at -80ºC until analyzed.

Blood analysis

Frozen aliquots of serum were sent to SpectraCell Laboratories, Inc (Houston, TX) for complete LDL density and lipoprotein particle number analyses. A complete "Lipoprotein Particle Profile™" test was performed using the lipoprotein subgroup particle number analysis method. The lipoprotein particle separation utilized a patent pending (209) continuous gradient generated by analytical ultracentrifugation. The lipoprotein particles were stained on their surface with a fluorescent dye and then separated in the gradient over a range of d = 1.000 - 1.300 $g \cdot cm^{-3}$. After separation, the contents of the centrifuge tube were extracted and the fluorescence of the lipoprotein particles was measured in an HPLC-type flow system. For processing, the fluorescence response was normalized to a cholesterol scale with a proprietary algorithm. Values for

each lipoprotein subgroup at their specific densities were determined using a multiple Gaussian fit/integration routine (209). The total number of VLDL (VLDL#), LDL (LDL#), remnant lipoprotein (RLP#), dense low-density lipoprotein$_3$ (LDL_3#), dense low-density lipoprotein$_4$ (LDL_4#), HDL (HDL#) and buoyant high-density lipoprotein$_{2b}$ (HDL_{2b}#) particles were determined in addition to the lipoprotein density. The coefficient of variation for this analysis using known standards has been reported as 2-3%. Analysis of serum nonesterified fatty acids (NEFA) was performed colorimetrically using a commercially available kit (Wako Diagnostics, Richmond, Va).

Isolation of protein and western blot analysis

Western blot analysis of proteins was performed as previously described with minor modifications (56). Briefly, tissue was weighed, powdered at the temperature of liquid nitrogen, and then homogenized in cold buffer (25 mM HEPES, 4 mM EDTA, 25 mM benzamidine, 1 µM leupeptin, 1 µM pepstatin, 0.15 µM aprotinin, 2 mM phenylmethylsulfonyl fluoride, pH 7.4). The homogenate was then centrifuged (10,000 *g* for 30 min at 4°C). Protein concentration of the supernatant was determined according to the method described by Smith et al. (186). Samples were stored at -80°C until sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

Immediately prior to gel electrophoresis, an aliquot of the supernatant was diluted in an equal volume of buffer (125 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 200 mM DTT, and 0.002% bromophenol blue). Protein was then separated by electrophoresis across a polyacrylamide gel and transferred to nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ). Membranes were then incubated in blocking solution (containing 5%

nonfat dried milk in Tris-buffered saline) at room temperature. Following blocking, membranes were incubated with rabbit anti-PPARδ, PGC-1α, PPARα, carnitine palmitoyl transferase-Iβ (CPT-Iβ), fatty acid translocase (FAT/CD36), F_1 ATPase, ATP binding cassette A1 (ABCA1), LDL receptor (LDLR), and lipoprotein lipase (LPL) (Santa Cruz Biotechnology, Santa Cruz, CA), AMPKα, and cytochrome c oxidase-IV (COX-IV)(Cell Signaling Technology, Danvers, MA). The AMPKα antibody used detects total $AMPK\alpha_{1 \text{ and } 2}$ expression.

The membranes were then washed and incubated again with anti-rabbit IgG coupled to horseradish peroxidase (Cell Signaling) and developed using chemiluminescence (Alpha Innotech, FluorChem SP, San Leandro, CA). Absorbance was normalized to a protein standard (obtained from rat quadriceps) loaded on each gel and expressed as normalized absorbance units (AU).

Statistical analysis

A timepoint (resting/untrained, exercised/untrained, and exercised/trained) by exercise training mode (LTM vs. ATM) by gender ANOVA repeated across timepoint was employed as the global analysis for each dependent variable of interest. Dependent variables of interest included skeletal muscle protein expression of PPARδ, PPARα, PGC-1α, AMPKα, CD36/FAT, CPT-I, COX-IV, ABCA1, LDLR, and LPL. Neither mode of aerobic exercise training nor gender significantly affected the exercise response for any of the variables of interest ($P>0.05$); therefore, we elected to remove these factors for all subsequent analyses. The comparison-wise error rate, α, was set at 0.05 for all statistical tests. When significant F ratios were found, a Tukey-Kramer post hoc

analysis was used to distinguish differences among means. Correlation analyses were performed using Pearson's product-moment correlations for PPARδ, PPARα, PGC-1α, and AMPK to target proteins and serum lipids and lipoproteins at each of the sample collection timepoints. All data were analyzed using the Statistical Analysis System (SAS, version 9.13, Cary, NC) and expressed as mean ± SEM.

Results

Physical characteristics and nutritional intake before and after training.

Physiologic characteristics of these subjects have been previously reported as part of a larger cohort (82). For the subset of subjects included in the current study, body weight (-2.3 kg) and BMI (-0.7 $kg \cdot m^{-2}$) were significantly reduced following exercise training and VO_2max (+3.6 $mL \cdot kg^{-1} \cdot min^{-1}$) was significantly increased (**Table 4**). No other body composition variables tested were significantly affected by exercise training (**Table 4**). Body fat %, lean body mass, VO_2max, caloric expenditure, caloric intake, and carbohydrate intake were all different between men and women (**Table 4**). No dietary or non-study physical activity changes were observed following exercise training (**Table 4**).

Exercise enhances skeletal muscle protein content of PPARδ, PPARα, PGC-1α, and AMPKα in overweight and obese men and women.

PPARα content was significantly increased following 12 wks of exercise training (exercised/trained), but was not significantly affected by a single session of exercise compared to the resting/untrained state (**Figure 12A&B**). PPARδ content was elevated from baseline following both an acute session of aerobic exercise and was two-fold

greater in the exercised/trained state compared to the resting/untrained state; however, the difference in measured expression between the exercised/untrained and exercised/trained states was not significant (**Figure 12A&C**). AMPKα content was enhanced following exercise training but not after a single session of exercise (**Figure 13A&B**). PGC-1α protein content, on the other hand, was enhanced following a single session of aerobic exercise and remained elevated in the exercised/trained state (**Figure 13A&C**).

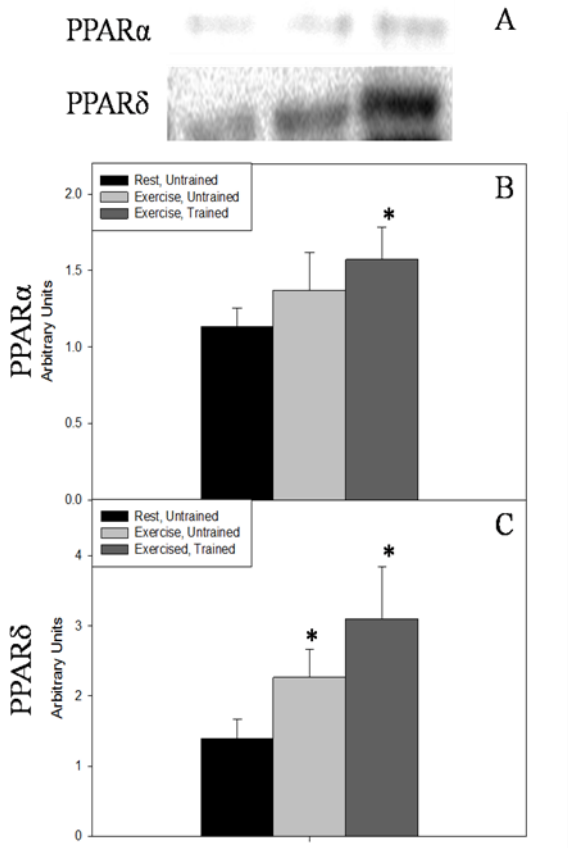


Figure 12: Influence of exercise and exercise training on PPARα and PPARδ expression in overweight and obese men and women. A: Sample immunoblot analysis of PPARα and PPARδ. B: Quantification of the relative abundance of PPARα. C: Quantification of the relative abundance of PPARδ. *P<0.05 Compared to resting, untrained. Data are mean ± SEM.

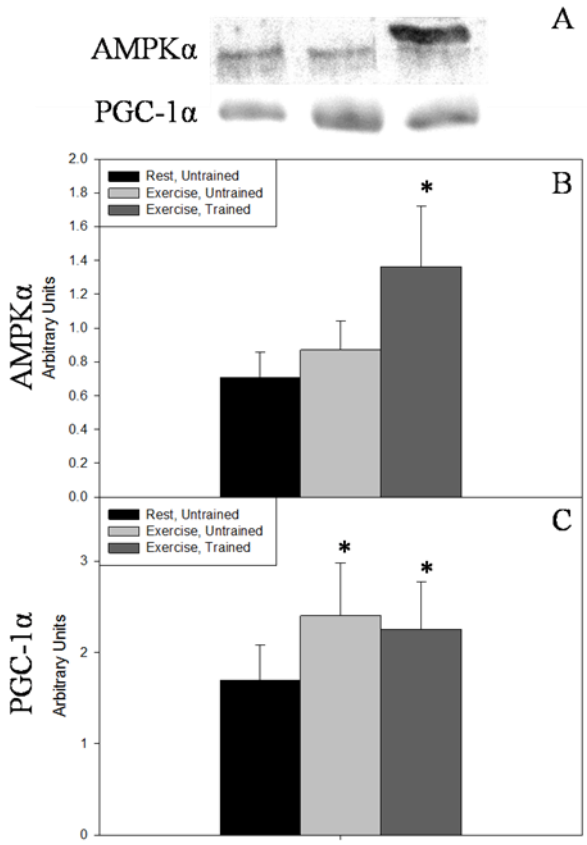


Figure 13: Influence of exercise and exercise training on AMPKα and PGC-1α expression in overweight and obese men and women. A: Sample immunoblot analysis of AMPKα and PGC-1α. B: Quantification of the relative abundance of AMPKα. C: Quantification of the relative abundance of PGC-1α. D: *P<0.05 Compared to resting, untrained. Data are mean ± SEM.

Exercise enhances expression of PPAR and PGC-1α target proteins involved in oxidative and lipoprotein metabolism.

Similar to PPARδ and PGC-1α, CD36/FAT protein content was enhanced following a single session of exercise and remained elevated in the exercised/trained state (**Figure 14A&B**). Expression of CPT-I (**Figure 14A&C**) and COX-IV (**Figure 14A&D**) proteins were significantly increased in the exercised/trained state compared to resting/untrained. In addition, LPL expression was significantly enhanced following a

single session of aerobic exercise (**Figure 15A&B**). Protein expression of F_1 ATPase (ATP synthase), ATP-binding cassette A1 (ABCA1) and LDL-receptor (LDLR) were not significantly affected by the exercise protocol.

PPARδ and AMPKα protein content are correlated with the content of blood lipids and lipoproteins in the obese at rest and following exercise training.

Table 5 shows the correlation of PPARα, PPARδ, PGC-1α, and AMPKα protein content to serum lipids and lipoproteins and target proteins. Of note in all three exercise conditions (resting/untrained, exercised/untrained, and exercised/trained) AMPKα protein content was significantly positively correlated with HDL-C, HDL_{2b}-C, HDL_{2a}-C, and HDL_{2b}-C particle number. In addition, PPARδ protein content was significantly negatively correlated with total cholesterol in the resting/untrained and exercise/trained states. PPARδ was also significantly negatively correlated with LDL-C, LDL_3-C, LDL_4-C, LDL-C particle number, and LDL_3-C particle number in the resting/untrained state. PPARδ content approached significant negative correlations with LDL_4-C particle number in the resting/untrained state, and with LDL-C in the exercised/trained state. In addition, in all three conditions PGC-1α protein content was significantly positively correlated with protein content of F_1 ATPase.

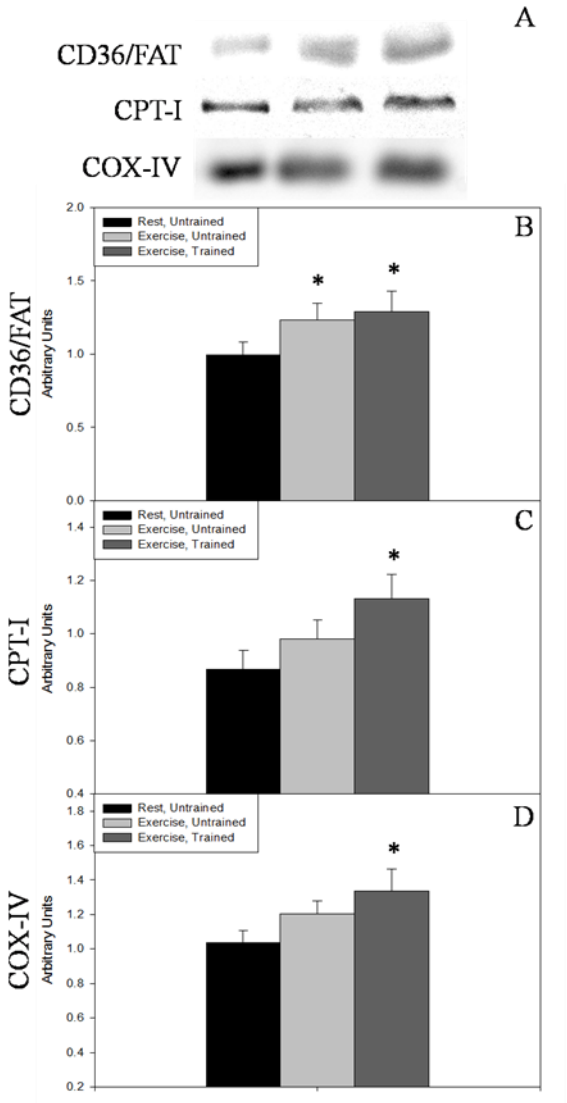


Figure 14: Influence of exercise and exercise training on CD36/FAT, CPT-I and COX-IV expression in overweight and obese men and women. A: Sample immunoblot analysis of CD36/FAT, CPT-I, and COX-IV. B: Quantification of the relative abundance of CD36/FAT. C: Quantification of the relative abundance of CPT-I. D: Quantification of the relative abundance of COX-IV. *P<0.05 Compared to resting, untrained. Data are mean ± SEM.

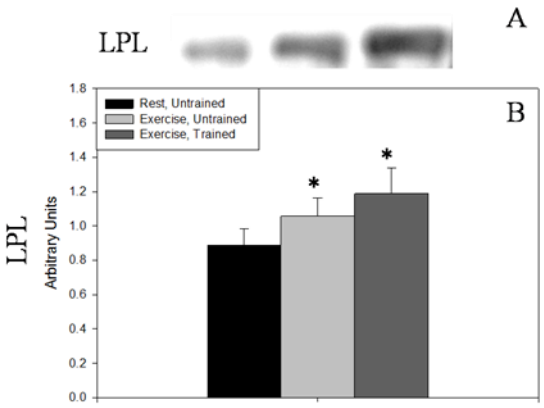


Figure 15: Influence of exercise and exercise training on LPL expression in overweight and obese men and women. A: Sample immunoblot analysis of LPL. B: Quantification of the relative abundance of LPL. *P<0.05 Compared to resting, untrained. Data are mean ± SEM.

Table 5: Correlation of PPARα , PPARδ, AMPKα, and PGC-1α to blood lipids and lipoproteins and target proteins.

	PPARα	PPARδ	AMPKα	PGC-1α
Resting, Untrained State				
F_1 ATPase	-0.3374	0.0014	-0.3523	0.8487*
CPT-I	0.6191	0.2810	-0.2765	0.2308
TC	0.1860	-0.6021*	0.0199	-0.223
LDL-C	0.1835	-0.5975*	0.066	-0.2545
LDL_3-C	-0.1104	-0.6307*	0.1834	-0.2342
LDL_4-C	-0.0642	-0.504†	0.3289	-0.056
HDL-C	0.1469	-0.2082	0.5543*	-0.0301
HDL_{2B}-C	0.2120	-0.1417	0.7234*	-0.0341
HDL_{2A}-C	0.0387	-0.138	0.7376*	-0.1404
LDL PRT	0.1170	-0.6813*	0.0022	-0.2616
LDL_3 PRT	-0.1031	-0.6354*	0.1994	-0.2278
LDL_4 PRT	-0.0501	-0.4946†	0.3021	-0.0691
HDL_{2B} PRT	0.1977	-0.1552	0.7151*	-0.045
CRP	-0.1869	0.0772	-0.6124*	-0.0109
Exercised, Untrained State				
F_1 ATPase	-0.2089	0.0897	-0.4765	0.7082*
Glucose	0.5321*	0.0376	-0.2685	0.5071*
HDL-C	-0.1527	-0.2788	0.7293*	-0.0597
HDL_{2B}-C	-0.1030	-0.1266	0.7829*	-0.1204
HDL_{2A}-C	-0.1359	-0.002	0.8684*	-0.2549
HDL_3-C	-0.1451	-0.7219*	-0.0211	0.2778
HDL PRT	-0.1754	-0.4277	0.6058*	0.0223
HDL_{2B} PRT	-0.1008	-0.1247	0.7843*	-0.1199
HDL Density	0.0388	-0.2654	-0.7385*	0.1938
Exercised, Trained State				
AMPK	0.6968†	-0.5136	-	-0.5837†
COX-IV	0.5844†	0.18542	-0.3063	-0.2143
F_1 ATPase	-0.2380	0.3603	-0.4288	0.8909*
TC	0.0722	-0.6212*	0.3955	-0.2903
LDL-C	-0.1116	-0.5435†	0.5581	-0.2958
HDL-C	0.2035	-0.6259*	0.7704*	-0.4098
HDL_{2B}-C	0.1955	-0.5693†	0.7904*	-0.3206
HDL_{2A}-C	0.1390	-0.5559†	0.8956*	-0.357
HDL PRT	0.1960	-0.6293*	0.6739*	-0.4642
HDL_{2B} PRT	0.2081	-0.5672†	0.7958*	-0.3234
CRP	-0.1509	0.5672†	-0.1213	-0.4659
Insulin	-0.3020	0.6007*	-0.0593	-0.0388
HDL Density	-0.2611	0.532†	-0.7561*	0.196

Correlations for which any one of PPARα, PPARδ, PGC-1α, or AMPKα showed (*, $P<0.05$) or approached (†, $P<0.10$) significance are shown.

Discussion

In the current study we have demonstrated enhanced expression of PPARs following aerobic exercise in overweight and obese men and women. We further reveal for the first time the potential role of PPARδ and AMPKα in the regulation of blood lipids and lipoproteins following exercise. The primary finding of this study was that a single session of aerobic exercise stimulates enhanced expression of PPARδ and PGC-1α in overweight and obese men and women; whereas, AMPKα and PPARα expression were significantly augmented only after 12 wks of exercise training, in the exercised/trained state. These effects appear to mediate enhanced expression of proteins involved in oxidative and lipoprotein-lipid metabolism. Our findings show that the induction of these proteins occurred when exercise training involved either ATM or LTM type exercise and regardless of gender. These data provide new mechanistic insights as to how acute exercise and exercise training mediate oxidative adaptation and regulate blood lipid and lipoprotein concentrations in obese men and women.

More specifically, we have shown that acute exercise induced an increase in PPARδ protein content in vastus lateralis muscle of untrained, overweight and obese men and women. The fact that the highest observed PPARδ content was seen in the exercised/trained condition is evidence of a potential additive effect of acute exercise and exercise training on the expression of this protein (**Figure 12A&C**). To our knowledge we are the first to report an acute exercise-induced increase in PPARδ protein content in humans, particularly in overweight and obese men and women. Others have shown increased PPARδ protein content after training (69), but the authors

did not make clear that they controlled for the timing of the last exercise session. Though our data suggest the possibility of an additive effect of acute exercise and exercise training on PPARδ protein content (**Figure 12A&C**), the difference between the exercised/untrained and exercised/trained states did not reach significance.

Previous work has demonstrated that overexpression of PPARδ induces a muscle fiber-type transition to mitochondria rich type I myofibers and enhanced endurance performance (220). Other studies have shown that PPARδ stimulation results in the expression of genes involved in oxidative metabolism, which can lead to reduced adiposity and normalization of several metabolic parameters associated with obesity (138-139). Fritz et al. (70) have previously demonstrated increased PPARδ protein expression with a tendency toward an increase in mRNA expression following a 4 month low-intensity, self-supervised endurance exercise program in type 2 diabetics. In addition, an acute session of exhaustive cycling exercise has been demonstrated to increase PPARδ mRNA content in healthy, normal weight men (142, 221). In the present study, we expand on those findings by demonstrating that acute exercise induces the enhanced expression of PPARδ protein in overweight and obese men and women without the need to achieve exhaustion. Moreover, our data suggest that the previous observations by Fritz et al. (69) may be explained as a response to the most recent exercise session rather than an adaptive response to the training stimulus.

We also confirm previous findings (98) by demonstrating the upregulation of PPARα protein content following a regimen of aerobic exercise training (**Figure 12A&B**). However, despite previously described increased PPARα mRNA content following a

single session of exercise we were not able to confirm a significant increase in PPARα protein following acute exercise. Thus, our data suggest that exercise training over a period of weeks may be necessary to evoke a significant rise in PPARα protein content in obese men and women. Indeed, our results indicate at least a marginal acute rise in PPARα protein that in our subjects simply did not reach statistical significance (**Figure 12A&B**), suggesting the accumulation of repeated exercise bouts may be necessary to elicit a significant enhancement in the expression of this protein.

We also show that exercise-induced increases of PGC-1α content appear to be mediated primarily by acute exercise (**Figure 13A&C**). Our data corroborate previously published results demonstrating the induction of PGC-1α expression following acute exercise in both human and rodent models (1, 4, 75, 130, 146). Other researchers have also implicated exercise training as a stimulus for enhanced expression of PGC-1α (172, 194, 204). However, to our knowledge we are the first to assess the effects of acute exercise and exercise training on the expression of PGC-1α protein in a single study. Given that we measured an increase in PGC-1α expression following just one session of exercise with no significant additive effects observed after exercise training (exercised/trained condition), we suggest that exercise-induced augmentations of PGC-1α protein content are largely mediated by the most recent session of exercise, and not an adaptive effect of exercise training. Regardless, PGC-1α has been demonstrated to mediate adaptive effects consistent with enhanced mitochondrial biogenesis and oxidative capacity (147, 195). It appears the influence of an exercise regimen on the expression of PGC-1α is limited to the response to the metabolic perturbations of the

most recent exercise session, rather than an adaptive mechanism stimulated by exercise training.

Similar to PPARα, we have demonstrated augmented AMPKα protein content in the exercised/trained condition but not after a single session of exercise in the untrained state (**Figure 13A&B**). Our data suggests that while acute exercise may induce the activation of AMPKα, enhanced AMPKα expression was only observed in the trained/exercised state, suggesting the importance for a chronic stimulus to induce this effect. Acute exercise has previously been demonstrated to induce enhanced AMPK activity, measured by phosphorylation at Thr172, which was observed immediately post-exercise and returned to baseline by 2 h after exercise (131). Exercise trained individuals express greater content of AMPKα_1 but similar expression of the other AMPK subunits as sedentary individuals (158). Our data describing the influence of an exercise training intervention on AMPKα expression are consistent with the findings of Sriwijitkamol et al. (194) in the obese Zucker rat, and of Clark et al. (34) in humans. However, our findings differ from those of Leick et al. (131) who showed no influence of either acute exercise or exercise training on AMPKα protein content in PGC-1α knockout mice. Their findings may indicate the importance of PGC-1α in mediating this adaptive effect on AMPKα.

While the induction of PPARα, PPARδ, PGC-1α, and AMPKα may not each be individually required to elicit the oxidative adaptations in skeletal muscle observed with exercise training, the systematic blunting of any one of these proteins significantly diminishes these adaptations (131, 172, 195). Understanding the stimuli required to

induce activation and expression of these proteins is paramount to our ability to maximize the adaptive effects of exercise with regards to mitochondrial biogenesis and oxidative capacity. Our data suggest that while acute aerobic exercise is sufficient to stimulate the expression of PPARδ and PGC-1α, exercise training is required for augmented expression of PPARα and AMPKα in overweight and obese humans.

PPARδ has previously been demonstrated to elicit enhanced expression of proteins involved in oxidative metabolism and cholesterol transport (151, 160). To test whether exercise-induced expression of PPARδ and related proteins (PPARα, PGC-1α, and AMPKα) elicited an effect on previously described transcriptional targets, we elected to assess the influence of exercise on proteins involved in both of these processes. As PGC-1α is recognized as the master regulator of mitochondrial biogenesis, and stimulation of PPARδ has been purported to augment oxidative capacity (139), we also elected to assess the influence of our exercise regimen on markers of mitochondrial biogenesis. Similar to the induction of PPARδ and PGC-1α with exercise in our sample of overweight and obese humans, we measured an increase in the expression of CD36/FAT following a single session of exercise, an increase which was maintained after 12 wks of exercise training (**Figure 14A&B**). CPT-I protein content was significantly augmented in the exercise/trained state, and was accompanied by a small though not significant increase in CPT-I content following a single session of exercise (**Figure 14A&C**). Our data regarding CD36/FAT and CPT-I are indicative of PPARδ mediated enhancements in oxidative protein expression, similar to that previously reported in skeletal muscle of humans treated with pharmacological PPARδ agonists

(192). Interestingly, we did not detect an influence of exercise or exercise training on F_1 ATPase expression, which is thought to be a target of PGC-1α independent of its influence on PPAR. We have further utilized the expression of COX-IV protein as a previously validated indicator of mitochondrial biogenesis (26, 97, 171). From our assessment of COX-IV expression, we demonstrate augmented mitochondrial biogenesis in the exercised/trained state in our sample of overweight and obese men and women (**Figure 14A&D**). Together, our findings indicate the viability of exercise to augment oxidative function, fatty acid transport and mitochondrial biogenesis in overweight and obese humans. Our data are further suggestive of PPAR and PGC-1α mediation of these events. These novel findings demonstrate the capacity of our sample of overweight and obese men and women to adapt to the stresses of an exercise regimen and further reveal important mechanisms by which these adaptations occur.

In order to better understand how exercise and PPAR may influence cholesterol transport and metabolism we have also assessed the influence of our exercise protocol on the expression of lipoprotein metabolism and transport proteins: ABCA1, LDLR, and LPL. The induction of these proteins in skeletal muscle represents the primary means by which PPARδ has been proposed to influence blood cholesterols (74). We measured upregulated LPL expression following a single session of aerobic exercise in the untrained state, and this elevation persisted in the exercised/trained state (**Figure 15**). Contrastingly, we observed no detectable influence of our exercise protocol on the expression of either ABCA1 or LDLR. Our findings that exercise did not influence ABCA1 content, despite enhanced expression of PPARs -α and -δ, contradict previous

studies indicating that the administration of a PPARδ agonist upregulated ABCA1 expression (160, 192). Sprecher et al. (192) demonstrated no influence of PPARδ activation on LPL expression in humans. However, they suggested an increase in LPL activity due to the lipolytic effects observed with PPARδ agonism. Increased LPL activity, as suggested by Sprecher et al. (192), would be consistent with previous observations by our laboratory following acute exercise (81). The augmented LPL expression measured in this study supports the enhanced capability of the muscle to catabolize and absorb lipoprotein-associated lipid.

To our knowledge, the first study to link the specific activation of PPARδ to changes in blood lipids and lipoproteins was performed by Oliver et al. (160). They reported that obese rhesus monkeys treated with the PPARδ agonist GW501516 exhibited increased HDL-C concentrations along with reduced VLDL-TAG and LDL-C concentrations compared to vehicle treated animals. In addition, the decrease in LDL-C concentration was mediated by a reduction in LDL particle number and concomitant reduction in the relative amount of small LDL particles (i.e., LDL_3-C and LDL_4-C). Their work has been previously expanded by Sprecher et al. (191-192) in humans. We expanded on these results in our present study by examining the possibility that skeletal muscle PPARδ may mediate previously described responses of blood lipids and lipoproteins to acute exercise and exercise training. Considering that exercise-mediated changes in blood lipids and lipoproteins are dependent upon caloric expenditure, and that previous results demonstrated altered blood lipid and lipoprotein concentrations across the vascular bed of skeletal muscle (113), we hypothesized that skeletal muscle energy metabolism is a

key component to altered blood lipids. Considering the previous findings by Oliver et al. (160) and Sprecher et al. (191-192), who also demonstrated the influence of PPARδ agonism on skeletal muscle proteins involved in lipoprotein transport and metabolism, we hypothesized that skeletal muscle PPARδ is a primary regulatory point for exercise-induced changes in blood lipids and lipoproteins.

Our data demonstrate that without exercise (resting/untrained condition) PPARδ is significantly negatively correlated with the concentration of TC and LDL-C (**Table 5**). This relationship between PPARδ and TC persisted following training while the relationship with LDL-C approached significance ($P<0.1$) in the exercised/trained state (**Table 5**). In the resting/untrained state PPARδ is negatively correlated with the concentration and particle number of small LDL-C fractions LDL_3-C and LDL_4-C. These findings are consistent with the findings of Oliver et al. (160) regarding LDL-C. Interestingly, while PPARδ content was enhanced following acute exercise, the correlation between PPARδ and TC and LDL-C was no longer significant the exercised/untrained state. Our findings suggest that any influence of PPARδ on LDL-C requires a consistent long term stimulus to affect changes in this lipoprotein fraction. However, further research is necessary to better define the role of PPARδ in the regulation of total cholesterol and LDL-C.

Perhaps the most surprising finding of our study was that in overweight and obese men and women, skeletal muscle AMPKα protein content was positively correlated with HDL-C and its subfractions, regardless of exercise status (**Table 5**). This correlation persisted across both forms of HDL_2-C (HDL_{2a}-C and HDL_{2b}-C) and across HDL

particle numbers suggesting that AMPKα related alterations in HDL are mediated by particle number. To our knowledge, we are the first to show such a connection between AMPKα and HDL-C with exercise or obesity. While AMPK is a known stimulus for the oxidation of fatty acids, little is known to link skeletal muscle AMPK to lipoprotein and cholesterol metabolism. An examination of previously published literature reveals that hepatic AMPK stimulation can inhibit cholesterol synthesis by phosphorylation and inhibition of HMG-CoA reductase (38). AMPK is activated by statin treatment, which also targets the inhibition of HMG-CoA Reductase (198). This mechanism provides a link between AMPK and cholesterol metabolism; however, it may not explain the link between skeletal muscle AMPK and HDL-C. Previous research has also shown that obese Zucker rats treated with AICAR, an activator of AMPK, exhibit increased HDL-C and reduced TAG concentration (22). In addition, genetic variants in the α2 subunit of AMPK have been associated with alterations in HDL-C (189) and apolipoprotein AI (ApoAI, the primary protein component of HDL-C) concentrations (224). Interestingly, ApoAI has previously been demonstrated, in both skeletal muscle (88) and endothelial cells (53), to stimulate AMPK phosphorylation at Thr172, a site consistent with the activation of AMPK. Therefore, while the exact mechanism remains unknown it is apparent that HDL-C and AMPK are engaged in a feedback relationship.

In summary, we have shown that a single session of aerobic exercise induced enhanced expression of PPARδ and PGC-1α, whereas, enhanced expression of PPARα and AMPKα was only observed in the exercised/trained state. We further demonstrate that neither mode (ATM or LTM) of aerobic exercise training nor gender influences this

response in overweight and obese men and women. We corroborate the work of others (160, 192), in showing the potential role of PPARδ in the regulation of blood concentrations of TC and LDL-C, and expand on these findings by showing that PPARδ may be a primary mechanism for previously observed exercise training induced changes in these blood cholesterols. Interestingly, we reveal for the first time that AMPK appears to be involved in the regulation of HDL-C and its subfractions following exercise, and that this regulation appears to occur by modulating the particle number of these cholesterol fractions. Our data provide a new understanding as to how oxidative adaptation to exercise is mediated and by what mechanism exercise mediates changes in blood cholesterols.

CHAPTER IV

HIGH VOLUME RESISTANCE EXERCISE ENHANCES PPARδ AND PGC-1α PROTEIN EXPRESSION, BUT NOT MARKERS OF MITOCHONDRIAL BIOGENESIS IN THE OBESE ZUCKER RAT

Introduction

The prevalence of obesity and diabetes has risen to epidemic proportions in the United States (27). These disease states are often associated with dysregulated metabolism including reduced glucose uptake, altered protein turnover, and dysregulated lipid metabolism. In particular, obesity is associated with increased rates of incomplete mitochondrial β-oxidation, an inability to switch to carbohydrate fuel supplies, the accumulation of intramuscular acylcarnitines, and a reduction of overall mitochondrial content (95, 120). The peroxisome proliferator-activated receptors (PPARs) are a family of nuclear receptors involved in the regulation of lipid metabolism and glucose homeostasis which have been implicated in the treatment of several diseases. PPARδ, in particular, appears to play a critical role in metabolic adaptation in skeletal muscle. In addition, the transcriptional coactivator PPARγ coactivator-1α (PGC-1α) has been referred to as a "master regulator of mitochondrial biogenesis" (147). Due to their roles in metabolism, any intervention which may target enhanced content and function of PPARδ and PGC-1α could potentially ameliorate or repair previously mentioned metabolic diseases.

The PPAR family members show a tissue specific distribution. PPARγ is primarily expressed in adipose tissue; however, PPARs -α and -δ are present in tissues with high metabolic activity, including liver and muscle. Due to its relatively ubiquitous expression, PPARδ was once believed to serve as a redundant mechanism for the other PPAR isoforms. In some of the first work to document the function of PPARδ, Muoio et al. (152) described what appeared to be compensatory regulation by PPARδ in skeletal muscle when PPARα was knocked out in mice. Since then it has come to be accepted that the primary PPAR isoform in skeletal muscle is PPARδ. When animals are bred to overexpress PPARδ, they exhibit an increase in oxidative myofiber content (220) and a general increase in muscle oxidative capacities similar to that observed with exercise training (199). Despite such findings, the characterization of PPARδ's response to exercise is incomplete. Mahoney, et al. (142) and Watt, et al. (221) have previously considered the effect of an acute bout of cycle exercise on PPAR mRNA expression in humans. In both studies, PPARs -α and -δ exhibited increased mRNA expression at 3 hours post exercise, however, protein content was not assessed in these studies. The PPARδ response to exercise training has been reported in relatively few studies, and results are mixed with either no change (175) or increased (70) PPARδ protein content. Also, stimulation of PPARδ with a synthetic agonist has been shown to alter blood lipids and lipoproteins, including increases in HDL-cholesterol and decreases in triacylglycerol (TAG) and LDL-cholesterol concentrations (160). However, it is not known whether this response may be mediated by PPARδ.

In contrast, PGC-1α has been characterized more extensively. Originally identified and named for its function to stimulate PPARγ in brown adipose tissue (167), PGC-1α has since been identified as a coactivator of many transcription factors including all three PPAR isoforms and NRFs 1 and 2. PGC-1α has been identified as a master regulator of mitochondrial biogenesis. A relative consensus exists in the literature that PGC-1α mRNA and protein expression are enhanced with both acute exercise and exercise training (4, 71, 146, 172). Sriwijitkamol et al. (194) have also previously demonstrated that seven weeks of exercise training induced greater PGC-1α protein expression in obese Zucker rats. Moreover, RE elicits enhanced expression of PGC-1α mRNA (48). We have expanded on these previous works by describing the influence of a short term RE protocol on PGC-1α protein and mitochondrial biogenesis in the obese Zucker rat.

In addition, due to its role in mitochondrial biogenesis, PGC-1α is considered to be tightly coupled to fatty acid oxidation (FAO). However, previous work in lean and obese women showed that PGC-1α was uncoupled from FAO in obesity (97). To date, it is not known if the discordance between PGC-1α expression and mitochondrial FAO with obesity is the result of the inability of PGC-1α to stimulate mitochondrial biogenesis or other disruptions with mitochondrial FAO.

The purpose of this study was to test the hypothesis that purported markers of mitochondrial biogenesis respond similarly to short term, high volume resistance exercise in lean and obese Zucker rats. To test this hypothesis, we explored the influence of obesity and high volume resistance exercise on PPARδ and PGC-1α, and whether changes in PPARδ and PGC-1α are associated with a response of downstream

target proteins and indices of mitochondrial biogenesis. We further sought to understand whether obesity and RE influence blood lipids and lipoproteins, and how PPARδ and PGC-1α relate to blood lipid concentrations. Results from this study will improve our understanding of metabolic dysfunction in obesity and how RE may potentially ameliorate such dysfunction.

Methods

Animals

Lean (*fa*/-, n=16) and obese (*fa*/*fa*, n=14) Zucker rats were purchased (Charles River Laboratories, Wilmington, MA) at 16 weeks of age, and all methods were approved by the Institutional Animal Care and Use Committee of Texas A&M University. All animals were singly housed in a secure, temperature and humidity controlled environment and maintained on a constant 12:12 h light-dark cycle with food and water provided *ad libitum*. Animals were assigned to one of four conditions: lean-sedentary (SED, n=8), lean-resistance exercised (RE, n=8), obese-SED (n=6), and obese-RE (n=8). Prior to the study, animals were weighed and analyzed for body composition by dual energy x-ray absorptiometry (DXA) scans. To perform scans, animals were anesthetized using a ketamine (Ketaset; 37.5 mg/kg BW) and medetomidine (Domitor; 0.25 mg/kg BW) cocktail administered by intraperitoneal (i.p.) injection and immediately awakened post-scan by administration of antisedan. Using these data animals were matched such that body weight and composition were similar within lean and obese phenotypes (**Table 6**).

Table 6: Body weight and composition of lean and obese Zucker rats in SED or RE treatment groups.

		Number	Body Weight (g)	%Body Fat	Lean Mass (g)	Fat Mass (g)
Lean	SED	8	384.3±24.1	16.2±3.6	312.5±21.0	60.6±16.1
	RE	8	383.0±17.0	16.2±1.5	312.5±15.2	60.3±6.9
Obese	SED	6	587.0±97.8	62.8±2.8	209.3±37.3	354.6±69.1
	RE	8	585.8±55.3	60.0±3.0	228.5±35.4	340.0±22.1

No significant differences were seen between SED and RE treatments within each phenotype. Phenotypes were significantly different for body weight, % body fat, lean mass, and fat mass; $P<0.05$. Data are mean ± SE.

Operant conditioning and resistance exercise

All animals were operantly conditioned to the RE protocol over a period of two weeks, consisting of 6 total sessions. The exercise protocol has been previously described (67) and resembles that of a traditional "squat" performed by humans in a weight room. Briefly, rats were taught to press an illuminated lever in a specially designed cage to avoid a brief foot shock stimulus (<3 mA, 60 Hz; 1-5 V). This movement facilitates a full extension and flexion of the hindlimb. During the final two conditioning sessions, a non-weighted Velcro vest was placed over the scapulae and the animals were required to perform the same movement. Once operantly conditioned, animals performed the exercise with little or no requirement for shock.

The experimental protocol consisted of 4 RE sessions (RE1-RE4) over an 8-day period, and was progressive in nature, with weight and repetitions increased with each session such that during RE3 and RE4 animals performed an average of 88 repetitions per session. During the RE sessions, the Velcro vest was placed over the scapulae and

additional weight added to the vest. All RE animals received the same absolute amount of additional overload such that during RE4 animals performed 80g (18 repetitions), 130g (16 repetitions), 180g (14 repetitions), 230g (30 repetitions), and 280g (6 repetitions). SED animals experienced only normal cage activity during the experiment. The total number of shocks administered to each RE animal was recorded and SED animals were given the average number of shocks received by the RE animals. Operant conditioning and experimental RE sessions were all separated by 48-72 h and conducted during the animals' dark cycle. Muscles were harvested and animals sacrificed on the 9^{th} day.

To measure mitochondrial protein synthesis rates in skeletal muscle, an i.p. 99.9% deuterium oxide (^2H_2O) bolus injection (20ul/g BW) was administered to each animal 24 h prior to sacrifice (approximately 8 hours prior to last exercise session), and 4% ^2H_2O was provided *ad libitum* in the drinking water throughout the last day. The use of ^2H_2O as a metabolic label allows for assessing long-term biosynthesis of macromolecules in a variety of species under free-living conditions (25), including obese rodent models (3). An important distinction between the ^2H_2O method and traditional *in vivo* precursor-product protocols is that ^2H_2O does not rely on amino acid transporters for intracellular enrichment, and measurements can be done over long periods of time in non-fasted states. As a result, a 24 h enrichment period with ^2H_2O ultimately gives a more realistic assessment of *cumulative* protein synthesis rates, since it accounts for daily activity- and sleep-patterns and allows for normal food consumption (72).

Tissue and blood collection

Consistent with previous research (64) all tissue samples were collected 16 h following exercise. Fasting blood samples (12 h fast) were collected from the lateral saphenous vein 16 h following RE3 for analysis of serum lipids and cholesterols. To allow for a better comparison of the fasting blood samples to the post RE4 condition, the total volume of work (weight * repetitions) completed during RE3 and RE4 was approximately matched. Blood samples were again collected by cardiac puncture at the time of sacrifice (post-RE4). All blood samples were collected in vacutainers containing either EDTA for plasma separation or a gel with a clot activator for serum separation, and frozen at -80°C until analysis.

On the morning of muscle harvest, rat chow was withdrawn 4 h prior to muscle excision and the animals were transported to the core laboratory. Approximately 16 h following RE4, animals were anaesthetized using ketamine (Ketaset; 37.5 mg/kg BW) and medetomidine (Domitor; 0.25 mg/kg BW) by i.p. injection. Two mL of whole blood were collected by cardiac puncture; the hindlimb muscles were quickly harvested and the animal euthanized. Fat, blood, and connective tissue were removed from muscles before snap-freezing in liquid nitrogen, and were subsequently pulverized before storing at -80°C until further analyses. Each group (lean RE, lean SED, obese RE and obese SED) was represented on any given experimental day, and selected in a random order to eliminate potential bias.

Assessment of blood lipids and lipoproteins

All lipid and lipoprotein analyses were performed using serum collected after RE3 and RE4. Analysis of serum nonesterified fatty acids (NEFA), total cholesterol, LDL-cholesterol and HDL-cholesterol was performed colorimetrically using commercially available kits (Wako Diagnostics, Richmond, Va). All lipid and lipoprotein concentrations are expressed in mmol/L.

Isolation of protein and western blot analysis

All muscle tissue analyses were performed using mixed gastrocnemius. Western blot analysis of proteins was performed as previously described with minor modifications (56). Briefly, tissue was weighed, powdered at the temperature of liquid nitrogen, and then homogenized in cold buffer (25 mM HEPES, 4 mM EDTA, 25 mM benzamidine, 1 µM leupeptin, 1 µM pepstatin, 0.15 µM aprotinin, 2 mM phenylmethylsulfonyl fluoride, pH 7.4). The homogenate was then centrifuged (10,000 *g* for 30 min at 4°C). Protein concentration of the supernatant was determined according to the method described by Smith et al. (186). Samples were stored at -80°C until sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

Before gel electrophoresis, an aliquot of the supernatant was diluted in an equal volume of buffer (125 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 200 mM DTT, and 0.002% bromophenol blue). Protein was then separated by electrophoresis across an 8% polyacrylamide gel and transferred to nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ). Membranes were then incubated in blocking solution (containing 5% nonfat dried milk in Tris-buffered saline) at room temperature. Following blocking,

membranes were incubated with rabbit anti-PPARδ, PGC-1α, CPT-Iβ, FAT/CD36, F_1 ATPase (Santa Cruz Biotechnology, Santa Cruz, CA), phospho-LKB1 (Ser 428), AMPKα, phospho-AMPKα (Thr172), cytochrome c oxidase IV (COX-IV), and α-actinin (Cell Signaling Technology, Danvers, MA). The membranes were then washed and incubated again with anti-rabbit IgG coupled to horseradish peroxidase (Cell Signaling) and developed using chemiluminescence (Alpha Innotech, FluorChem SP, San Leandro, CA). Absorbance was normalized to a protein standard (obtained from rat quadriceps) loaded on each gel and expressed as normalized absorbance units (AU).

Isolation of skeletal muscle mitochondria

For the analysis of mitochondrial fractional protein synthesis rates (mtFSR), mitochondrion were isolated from mixed gastrocnemius muscle based on a previously described procedure with modifications (173). Enrichments of sub fractions were confirmed with immunoblotting using COX-IV (mitochondrial), and α-actinin (myofibrillar), specific antibodies. A recent study by the Endocrine Research Unit at Mayo Clinic College of Medicine reported a 75% purity of the sub-fraction containing subsarcolemmal mitochondria with a similar isolation procedure (105). The protocol used to isolate contractile proteins and intermyofibrillar mitochondria previously has been confirmed to yield robust enrichments as evidenced by electron microscopy (225).

Frozen gastrocnemius muscle (100 mg) was thawed and gently homogenized with 50 slow and compressive strokes of a glass-pestle (Dounce homogenization) in ice-cold mitochondrial isolation buffer 1 (10 mM Hepes, 200 mM Sucrose, 50 mM Mannitol, 2 mM EDTA disodium salt, Sigma P8340 protease inhibitor cocktail, pH 7.4). The

homogenate was centrifuged at 600 x g for 10 min at 4°C, followed by careful removal of the supernatant (cytosolic proteins and subsarcolemmal mitochondria) from the pellet (myofibrillar, nuclear, and stromal proteins, intermyofibrillar mitochondria). Subsarcolemmal mitochondria were obtained by centrifugation of the supernatant at 10,000 x g for 10 min (4°C) and were stored in mitochondrial isolation buffer 2 (50 mM Hepes, 5 mM EGTA, 1 mM ATP, 100 mM KCl, 5 mM $MgSO_4$, Sigma P8340 protease inhibitor cocktail, pH 7.4) until combined with the intermyofibrillar (IMF) mitochondria for assessment of protein synthesis. The remaining supernatant, containing cytosolic proteins, was precipitated with 95% ethanol (50% [v/v] in total solution) during low-speed centrifugation at 700 x g for 10 min (4°C) and thereafter dried.

The pellet produced by the centrifugation of the original homogenate was suspended in mitochondrial isolation buffer 3 (100 mM KCl, 50 mM Tris, 5 mM MgCl2 hexahydrate, 1 mM EDTA disodium salt, 10 mM β-glycerophosphate disodium salt, 50 mM NaF, 1.5% BSA, Sigma P8340 protease inhibitor cocktail, pH 7.5) and centrifuged at 650 x g for 3 min at4°C. The resulting pellet was homogenized in the same buffer with a glass pestle using shear and compression to release the IMF mitochondria, followed by centrifugation at 650 g for 4 min at 4°C to collect the organelle-rich supernatant. IMF mitochondria were then pelleted by centrifugation at 10,000 x g for 10 min (4°C) and combined with the SS mitochondrial fraction. The mitochondrial-rich sub-fractions were washed in isolation buffer 2 and ethanol, followed by high-speed centrifugation and drying in preparation for protein synthesis measurements.

Assessment of mitochondrial protein synthesis rates by deuterium oxide

^2H_2O enrichment of body water/plasma. The assessment of mtFSR was performed as described previously (72), with the modification that the mitochondrial subfraction was the primary source of protein assessed. Briefly, labeling (^2H) of body water was assessed by exchange with acetone as described by Yang, et al. (235). The reaction occurred with 20 µL of a sample/standard, 2 µL of 10N NaOH and 4 µL of a 5% (vol/vol) solution of acetone in acetonitrile for 24 h. Acetone was removed by the addition of 0.6 ml of chloroform and 0.5 g Na_2SO_4. The samples were mixed and 0.1 ml of the chloroform transferred to a GCMS vial. The samples were analyzed using an Agilent 5975C-MSD equipped with an Agilent 7890 GC system (GCMS), and a HP-5ms capillary column (30 m × 0.25 mm × 0.25 µm). The following temperature program were used: 60ºC initial, increased by 20°C/min to 100ºC, increased by 50ºC/min to 220ºC, and held for 1 min. The sample was injected at a spit ratio of 40:1 with a helium flow of 1 mL/min. Acetone eluted at (1.7 min). The mass spectrometer was operated in electron impact mode (70eV). Selective ion monitoring of mass-to-charge ratios (*m/z*) 58 (M) and 59 (M + 1) was conducted using a dwell time of 10 ms per ion. All plasma samples were measured twice (prepared on two separate occasions) and an average value of the two runs was used for all calculations.

^2H-alanine enrichment in skeletal muscle. Enrichment of the mitochondrial-rich fraction was determined by measuring protein-bound ^2H-alanine (E_A) as previously described (72). Briefly, about 0.030 g of mitochondrion was homogenized on ice in 0.3 ml of a 10% (wt/vol) TCA and centrifuged at 800 x g at 4°C for 15 min. The supernatant

was discarded and the protein pellet washed 3 additional times with TCA. The protein pellet was then dissolved in 6 N HCl (0.13 mL/0.01 g tissue, 200 uL) and reacted at 100ºC for 18 h. Homogenates were centrifuged at 14,000 rpm at 4ºC for 30 min. An aliquot (50 uL) of the hydrolysate was dried for 1 h at 110 ºC and thereafter derivatized with a 3:2:1 (vol:vol:vol) ratio (0.1 mL) of methyl-8 reagent (Pierce, Rockford, IL), methanol and acetonitrile for 1 h at 70. The resulting methyl-8 /^{2}H-alanine derivative was transferred to a GC/MS vial and analyzed with an Agilent 5975C VL MSD equipped with an Agilent 7890A GC system (HP-5ms capillary column, 30 m x 0.25 mm x 0.24 µm) to determine ^{2}H-labeling of protein-bound alanine. The initial temperature of the column program was set at 90°C and held for 5 min, increased by 5°C/min to 130°C, which was further increased at a rate of 40°C/min to 240 °C and held for 5 min, all steps performed at a constant helium flow of 1 mL/min. Peak abundances of ions 99 and 100 were extracted from chromatograms and M+1/M ratios were used to calculate % enrichment of protein-bound alanine using a regression formula generated by ^{2}H-alanine standards (R^2 = 0.999). ^{2}H-labeling of alanine were readily detected in all sub-fractions using a 20:1 split ratio for mixed proteins, 10:1 split ratio for myofibrillar and cytosolic proteins, and 5:1 split ratio for mitochondrial proteins. All samples were analyzed three times, which included complete re-preparation of the frozen hydrolysate on each occasion, to account for variations in preps as well as GC/MS analysis. An average value of the three runs was used for all FSR calculations.

Calculations. Fractional synthesis rates of mixed, myofibrillar, cytosolic, and mitochondrial proteins were calculated using the equation

$$E_A \times [E_{BW} \times 3.7 \times t\ (h)]^{-1} \times 100$$

where E_A represents amount of protein-bound ^{2}H-alanine (mole % excess), E_{BW} is the quantity of ^2H_2O in body water (mole % excess), and 3.7 represents the exchange of ^{2}H between body water and alanine [i.e. 3.7 of 4 carbon-bound hydrogens of alanine exchange with water, (55)]. This equation assumes that ^{2}H-labeling of body water equilibrates with free alanine more rapidly than alanine is incorporated into newly made protein and that protein synthesis is linear over the study (231).

Statistical analysis

The independent factors in this study were exercise (RE vs. SED), and phenotype (lean vs. obese). Dependent variables of interest included protein content of PPARδ, PGC-1α, and select target proteins (CD36/FAT, CPT-I, COX-IV, and F_1 ATPase), phosphorylation of AMPK and LKB1, mtFSR, and serum lipid and lipoprotein concentrations. An exercise by phenotype (2X2) ANOVA was employed as the global analysis for each dependent variable of interest. The comparison-wise error rate, α, was set at 0.05 for all statistical tests. When significant F ratios were found, a Fisher's LSD post hoc analysis was used to distinguish differences among means. Correlation analyses were performed using Pearson's product-moment correlations for PPARδ, PGC-1α, and AMPK to target proteins and serum lipids and lipoproteins. All data were analyzed using the Statistical Analysis System (SAS, version 9.13, Cary, NC) and expressed as mean ± SE.

Results

Serum lipid and lipoprotein concentrations are greater in obese compared to lean Zucker rats.

Serum lipid and lipoprotein concentrations for lean and obese Zucker rats are shown in **Table 7**. Because RE did not alter serum lipids and lipoproteins in either the fasted (RE3) or fed (RE4) state, data were collapsed across exercise treatment for each phenotype. Serum NEFA concentrations were greater in obese compared to lean rats. Total cholesterol was greater in obese rats, driven by greater HDL-cholesterol concentrations in this phenotype. No differences were seen in LDL-cholesterol concentrations between phenotypes.

Table 7: Serum lipids and lipoproteins of lean and obese Zucker rats.

	Lean	Obese
Post-RE3		
NEFA (mmol/L)	0.85±0.06	1.46±0.07*
Total Cholesterol (mmol/L)	2.61±0.43	7.25±0.48*
LDL-Cholesterol (mmol/L)	1.40±0.12	1.36±0.13
HDL-Cholesterol (mmol/L)	2.27±0.59	7.47±0.65*
Post-RE4		
NEFA (mmol/L)	0.44±0.05	0.64±0.06*
Total Cholesterol (mmol/L)	2.88±0.43	4.43±0.48*
LDL-Cholesterol (mmol/L)	1.57±0.17	1.97±0.19*
HDL-Cholesterol (mmol/L)	1.59±0.33	3.05±0.36*

Serum lipids were measured 16 h after RE3 (12 h fast) and RE4 (4 h fast). No significant effect of exercise treatment was seen, therefore data are collapsed across exercise. *P<0.05 compared to lean. Data are mean ± SE.

Resistance exercise yields enhanced PPARδ content and has a differential effect on PGC-1α content in lean and obese Zucker rats.

In this study we have shown that resistance exercise results in greater expression of PPARδ protein regardless of phenotype (**Figure 16A&B**). Furthermore, we show that PGC-1α content is greater following resistance exercise in obese animals but not in lean animals (**Figure 16A&C**).

Phosphorylation of AMPK is enhanced following RE in lean but not obese Zucker rats.

Resistance exercise did not influence phosphorylated LKB1 (Ser 428), however, lean animals displayed a greater amount of phosphorylated LKB1 than did obese (**Figure 17A&B**). We have shown that despite no effect of phenotype or RE on the content of AMPKα, there was greater than a two-fold increase in the phosphorylation of AMPKα at Thr172 in lean, but not obese Zucker rats following RE (**Figure 17A&C**). Post-RE AMPK phosphorylation in the lean Zucker was also more than two-fold greater than AMPK phosphorylation of the obese Zucker rat regardless of exercise treatment.

Changes in expression of CPT-I and F_1 ATPase are concomitant with PPARδ and PGC-1α, respectively.

We did not observe a significant effect of RE on the content of CD36/FAT, but lean animals exhibited a greater content of CD36 than obese (**Figure 18A&B**). Similar to PPARδ, greater content of CPT-I was measured in both lean and obese RE animals compared to SED. Lean animals also showed greater total expression of CPT-I than obese littermates (**Figure 19A&B**). F_1 ATPase was differentially affected by RE based

on phenotype; similarly to PGC-1α, F_1 ATPase content was greater in obese RE animals compared to SED (**Figure 19A&D**), whereas no effect of RE on this protein was seen in lean animals (**Figure 19A&D**).

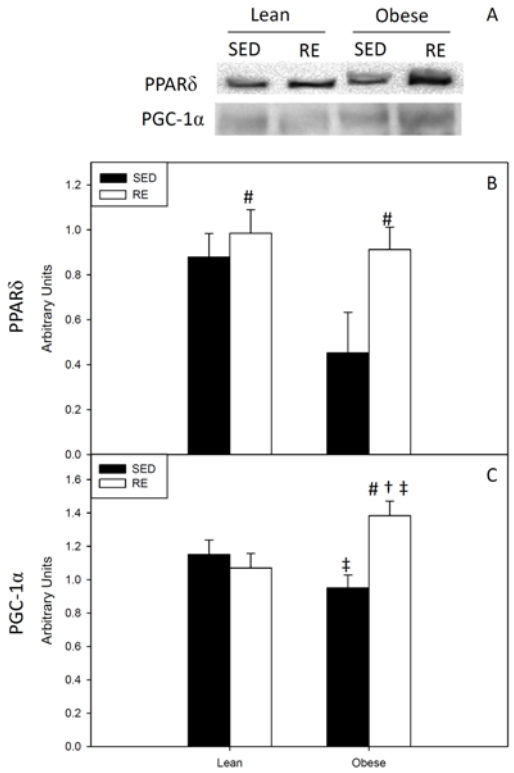


Figure 16: Expression of PPARδ and PGC-1α in lean and obese Zucker rats in SED or RE treatments. A: Sample immunoblot analysis of PPARδ and PGC-1α. B: Quantification of the relative abundance of PPARδ. C: Quantification of the relative abundance of PGC-1α. *P<0.05 Compared to lean. #P<0.05 Different between treatments within phenotype. †P<0.05 Different between phenotype within treatment. ‡P<0.05 Different between phenotype and treatment. Data are mean ± SE.

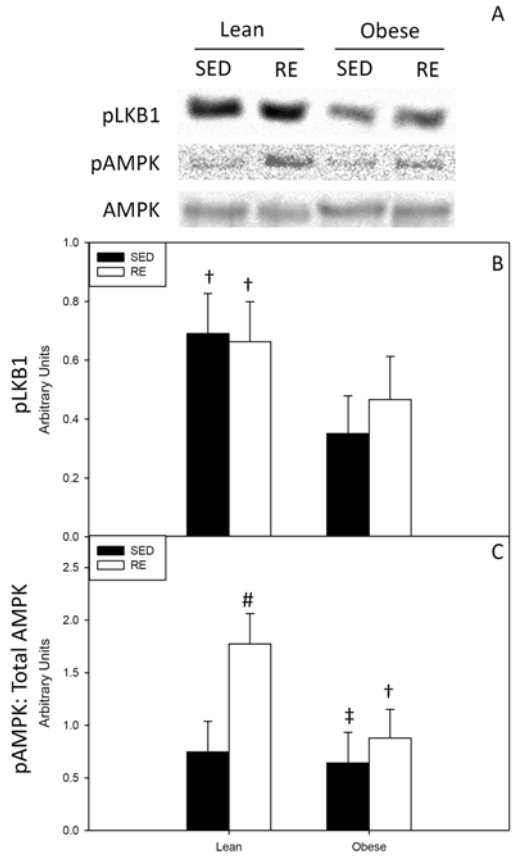


Figure 17: Phosphorylation of LKB1 and AMPKα at Thr172 in lean and obese Zucker rats in SED or RE treatments. A: Sample immunoblot analysis of pAMPK and AMPK. B: Quantification of phosphorylated LKB1. C: Quantification of the relative phosphorylation of AMPK. *P<0.05 Compared to lean. #P<0.05 Different between treatments within phenotype. †P<0.05 Different between phenotype within treatment. ‡P<0.05 Different between phenotype and treatment. Data are mean ± SE.

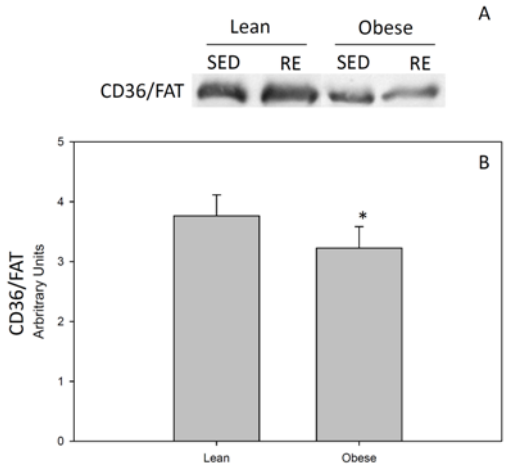


Figure 18: Expression of CD36/FAT in lean and obese Zucker rats in. No effect of exercise treatment was seen. Therefore, data have been collapsed across exercise. A: Sample immunoblot analysis of CD36/FAT. B: Quantification of the relative abundance of CD36/FAT. *P<0.05 Compared to lean. Data are mean ± SE.

Mitochondrial biogenesis is not enhanced following RE in obese Zucker rats despite enhanced PGC-1α protein content.

To assess mitochondrial biogenesis we examined COX-IV protein content and mitochondrial protein synthesis rates in skeletal muscle using $^{2}H_2O$. COX-IV expression was two-fold greater in the SED state in obese animals compared to lean (**Figure 19C**). RE repressed COX-IV expression in obese Zucker rats such that a difference in the expression of COX-IV between phenotypes was no longer seen (**Figure 19C**). We demonstrated enhanced mtFSR following RE in lean, but not obese, Zucker rats (**Figure 20**).

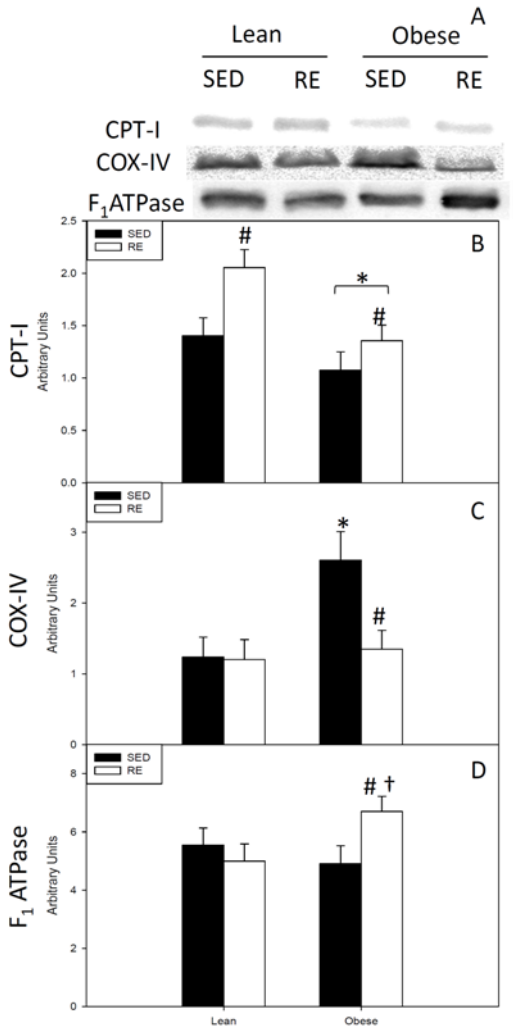


Figure 19: Expression of PPARδ and PGC-1α target proteins in lean and obese Zucker rats in SED or RE treatments. A: Sample immunoblot analysis of CPT-I, COX-IV, and F1 ATPase. B: Quantification of the relative abundance of CPT-I. C: Quantification of the relative abundance of COX-IV. D: Quantification of the relative abundance of F1 ATPase. *P<0.05 Compared to lean. #P<0.05 Different between treatments within phenotype. †P<0.05 Different between phenotype within treatment. ‡P<0.05 Different between phenotype and treatment. Data are mean ± SE.

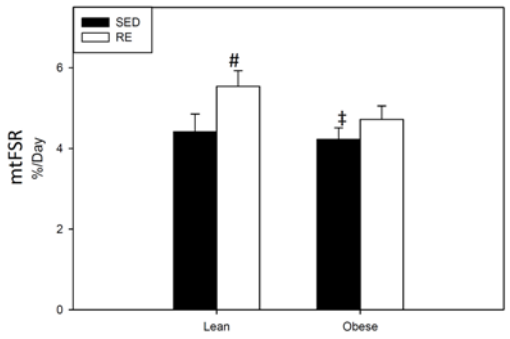


Figure 20: Mitochondrial protein synthesis in lean and obese Zucker rats in SED or RE conditions. Figure is adapted from that in Nilsson et al. (manuscript in review). #P<0.05 Different between treatments within phenotype. ‡P<0.05 Different between phenotype and treatment. Data are mean ± SE.

Expression of CPT-I and COX-IV are correlated with expression of PPARδ, and serum HDL-cholesterol concentrations are correlated with PPARδ in the SED state.

Correlational data are shown in **Table 8**. Significant correlational findings were: PPARδ was positively correlated with post-RE4 NEFA concentrations in lean animals, PPARδ and AMPK were both positively correlated with CPT-I protein content, and PPARδ was positively correlated with post-RE4 HDL-cholesterol concentrations in the SED state. Furthermore, PPARδ was consistently negatively correlated with COX-IV content.

Table 8: Pearson's product-moment correlation matrix of PPARδ, PGC-1α, and AMPKα to serum lipids and lipoproteins and other skeletal muscle proteins measured.

		PPARδ	PGC-1α	AMPK
Lean (n=16)				
4-NEFA	r	0.74094	-0.29710	0.28284
	sig.	0.0010	0.4045	0.3630
CPT-I	r	0.56521	0.31704	0.60774
	sig.	0.0555	0.4442	0.0623
COX-IV	r	-0.78400	-0.88679	0.10221
	sig.	0.0073	0.1132	0.8472
Obese (n=14)				
CD36	R	0.58596	0.22220	0.29689
	sig.	0.0453	0.5372	0.3753
CPT-I	R	0.79768	0.3023	0.60769
	sig.	0.0033	0.3959	0.0473
Resistance Exercise (n=16)				
CPT-I	R	0.62227	-0.15450	0.61571
	sig.	0.0307	0.6915	0.0437
COX-IV	R	-0.64160	-0.44725	-0.36673
	sig.	0.0455	0.4501	0.4184
Sedentary (n=14)				
4-NEFA	r	0.72051	0.08780	0.14883
	sig.	0.0037	0.7974	0.6443
4-TC	r	0.67911	0.15452	-0.38821
	sig.	0.0076	0.6501	0.2124
4-HDL	r	0.6571	-0.04117	-0.26412
	sig.	0.0107	0.9043	0.4068
CPT-I	r	0.45790	0.71193	0.62693
	sig.	0.1567	0.0314	0.0524
All Animals (n=30)				
4-NEFA	r	0.52906	0.12214	0.13497
	sig.	0.0032	0.5979	0.5295
4-TC	r	0.26019	0.50586	0.11146
	sig.	0.1728	0.0193	0.6041
4-LDL	r	0.11144	0.49126	0.11212
	sig.	0.5649	0.0237	0.6019
4-HDL	r	0.38702	0.19164	0.12452
	sig.	0.0381	0.4053	0.5021
CPT-I	r	0.53179	0.14589	0.60404
	sig.	0.0090	0.5635	0.0037
COX-IV	r	-0.61310	-0.29595	-0.13775
	sig.	0.0068	0.4064	0.6536

Correlations for which any one of PPARδ, PGC-1α, or AMPKα showed significance ($P<0.05$) are shown.

Discussion

The primary findings of this study were that high volume RE increased PPARδ protein content in lean and obese Zucker rats. However, PGC-1α protein content was elevated following RE in obese, but not lean, Zucker rats. Furthermore, the content of CPT-I and F_1 ATPase followed similar patterns to PPARδ and PGC-1α, respectively, indicating an effective functional response of these oxidative control-point proteins to exercise. High volume RE has previously been demonstrated to enhance muscle oxidative potential (203) and fat metabolism (161). Here we provide evidence this effect may be mediated by PPARδ and PGC-1α. We have also shown a significant positive correlation between PPARδ muscle protein content and blood HDL-cholesterol in the SED animals, suggesting PPARδ may be important in the regulation of this cholesterol fraction in the sedentary state. Interestingly, the elevated expression of PGC-1α in the obese Zucker rat in response to exercise did not result in an increase in mtFSR or COX-IV. Conversely, mtFSR was elevated in lean animals after exercise, even though PGC-1α protein content was unchanged. These results suggest that increased mitochondrial biogenesis in response to exercise is not dependent on changes in PGC-1α expression.

To our knowledge, we are the first to show that short-term high volume RE is capable of elevating PPARδ and PGC-1α protein content in skeletal muscle, and to link elevated expression of these proteins to the metabolic adaptations that occur following exercise. Previous work has demonstrated that overexpression of PPARδ induces a muscle fiber-type transition to mitochondria rich type I myofibers and enhanced endurance performance (220). Other studies have shown that PPARδ stimulation results

in the expression of genes involved in oxidative metabolism, and can lead to reduced adiposity and normalization of several metabolic parameters associated with obesity (138-139). Fatty acids (e.g., NEFA) and their derivatives are ligands for the activation of all three PPAR isoforms (8), and PPARδ has been called a "fatty acid sensor" since its activity is highly inducible by fatty acids (168). In our study, PPARδ content was strongly correlated with serum NEFA concentrations in the lean Zucker rat. However, this association between serum NEFA and PPARδ was not seen in the obese Zucker rat, which may suggest an inability of NEFA to fully activate PPARδ in the obese animal. In addition, we have shown that PPARδ content is positively correlated with HDL-cholesterol in the sedentary state, which may indicate a role of PPARδ in the regulation of HDL-cholesterol concentrations in the absence of exercise. However, it is likely that exercise mediated PPARδ regulation of serum lipid and lipoprotein concentrations requires a long term training regimen which should be further explored.

Furthermore, in contrast to findings in previous studies (194), PGC-1α was not significantly suppressed in the sedentary state in obese Zucker rats. Similar to previous work with treadmill training (194), we demonstrated that high volume RE enhances PGC-1α protein content in the obese Zucker rat, but not in the lean. The present study suggests that the ability of the obese Zucker rat to respond to an exercise stimulus is congruent with enhanced muscle oxidative potential.

It is important to acknowledge the importance of AMP-activated protein kinase (AMPK) in the stimulation of PPARδ and PGC-1α. In fact, Kramer et al. (122) have shown that PPARδ stimulated increases in FAO are abolished when AMPKα has been

silenced. Further evidence also shows that phosphorylation of AMPKα is a potent stimulator of PGC-1α transcription (101). In our study we observed that lean, but not obese animals, exhibited enhanced phosphorylation of AMPKα at Thr172 following RE. These findings are consistent with those of a previous study in which an attenuation of AMPK phosphorylation at Thr172 followed moderate intensity exercise in obese and type 2 diabetic humans compared to lean controls (193). Our data suggest that the ability to activate AMPK following an exercise stimulus in the obese may be impaired. This is in stark contrast to our data showing that PGC-1α protein was enhanced following RE in obese animals rather than lean, as it is believed that AMPK phosphorylation is an important stimulus of PGC-1α transcription (101). To further understand these upstream effects, we have also examined the influence of phenotype and exercise on LKB1, an important AMPK kinase. Our data reveal that lean animals exhibit greater levels of phosphorylated LKB1 (Ser 428), and that RE had no effect on the level of phosphorylation in either phenotype. We should note that the assessment of signaling responses in our present study was conducted 16 h following the last exercise bout, which may not be the optimal time point to assess LKB1 and AMPK activation responses (phosphorylation) to exercise in either phenotype.

To identify the functional significance of PPARδ and PGC-1α activation it was necessary to understand the influence of our RE stimulus on proteins that are known transcriptional targets of PPARδ and PGC-1α. Although we found a small phenotypic effect on the expression of CD36/FAT, whereby, lean animals exhibited greater expression of this protein, RE had no influence on its expression. This observation is

consistent with previously published data in which RE had no effect on CD36 mRNA in nonobese humans (236). However, the primary effect of exercise on this PPARδ target appears to be a subcellular relocalization rather than altered total expression (96). Our findings related to the effect of exercise on CD36 are in contrast to studies using pharmaceutical PPARδ agonists where CD36 content is enhanced (192). The slight depression of CD36 expression in the obese Zucker rat compared to lean is not consistent with reports showing no phenotypic differences (89), and should be further explored as a potential mechanism contributing to metabolic dysfunction in the obese.

In the present study CPT-I, a known rate-limiting element of FAO, revealed greater protein content in both lean and obese animals after RE compared to SED conditions, suggesting that RE facilitated CPT-I expression in the present study. This finding is consistent with another report in which elevated CPT-I mRNA content was measured following endurance exercise (130). The altered expression of CPT-I is thought to be mediated by PPARδ, since the PPARδ agonist GW501516 facilitated CPT-I expression induced in humans (192) and cultured human cells (122). We found the expression pattern of CPT-I to be correlated with changes in PPARδ protein content, and this association persisted regardless of phenotype in the present study. These results support the notion that high volume RE stimulates PPARδ leading to enhanced ability to oxidize fatty acids via enhanced expression of PPARδ targets.

To further expand on the notion that RE facilitates an improvement in FAO, we assessed the effect of exercise and phenotype on the F_1 ATPase (a subunit of ATP synthase). The F_1 ATPase is an important mitochondrial metabolic protein that is

thought to be a target of PGC-1α, independent of its stimulation of PPARδ. A recent study by Garcia-Roves et al. (71) demonstrated that the expression of PGC-1α, PPARδ, and ATP synthase are elevated after swim training. In the present study, we demonstrated that RE resulted in elevated protein content of PGC-1α in obese Zucker rats, and this elevation was concomitant with elevated F_1 ATPase. Lean rats did not exhibit altered expression of either protein following resistance exercise. At this time, we cannot account for the phenotypic differences in these responses, but are confident that exercise induced alterations of PGC-1α are accompanied by altered expression of specific proteins responsible for mitochondrial substrate delivery and oxidation.

Recognizing that PGC-1α is considered the master regulator of mitochondrial biogenesis, we have employed both conventional (COX-IV) and novel (mtFSR) approaches to the assessment of mitochondrial biogenesis. We have previously used ^2H_2O as a stable isotope label allowing the assessment of fractional synthesis rates over the course of 24 h in mixed muscle (72). By employing this methodology to isolated mitochondria we were able to assess cumulative mtFSR in the present study. Previous studies have assessed mtFSR (19, 84, 225); however, our laboratory is the first to assess mtFSR based on a cumulative 24 h measurement. Furthermore, we are the first to employ the assessment of mtFSR for the study of PGC-1α induced mitochondrial biogenesis. In the present study, we demonstrated that mitochondrial rates of synthesis are similar between phenotypes, but that RE facilitated an increase in mtFSR only in the lean animals. The observation that exercise can elevate mtFSR is supported by Wilkinson et al. (225), who demonstrated increased mtFSR following exercise in

relatively untrained, healthy individuals. Guillet et al. (84) found elevated mtFSR following resistance exercise, which is consistent with our present work. It is perplexing, however, that we did not measure enhanced mtFSR following exercise in obese animals, even though PPARδ and PGC-1α proteins were elevated in this phenotype in response to the exercise regimen. Our data suggest that the role of PGC-1α on mitochondrial biogenesis is not mediated by altered mtFSR, given that we do not observe any association between the expression of PGC-1α and mtFSR, regardless of phenotype.

A more conventional way to explore mitochondrial biogenesis is by examining changes in COX-IV expression (26, 97, 171) and/or activity (135). In our study COX-IV expression was two-fold greater in obese SED animals compared to lean counterparts. Furthermore, in the present study we did not find any association with changes in COX-IV and RE that would suggest the induction of mitochondrial biogenesis. However, it should be noted that our study was conducted over 9 days, whereas prolonged training may produce different results.

Previous findings have shown that exercise-induced COX-IV expression is likely a result of stimulated PGC-1α and PPARδ (71). There are two primary points to observe from our data. First, the abundance of COX-IV in obese SED animals is indicative of greater mitochondrial volume. We have noted in our studies that mitochondrial protein is about 31% greater per unit wet muscle mass in obese animals than lean (Nilsson et al., manuscript in review). Taken together these data suggest a higher mitochondrial density in obese animals than lean, in contradiction to previous work suggesting reduced

mitochondrial content in the obese (95). Second, unlike mtFSR, COX-IV was not enhanced following RE in lean animals. Our data suggest that the use of a single marker as an indicator of mitochondrial biogenesis should be viewed with caution. Combined with our findings for PPARδ and PGC-1α protein response and markers of mitochondrial biogenesis, our data indicate that in the obese Zucker rat PGC-1α content is uncoupled from mitochondrial biogenesis, whether it is assessed on the basis of COX-IV expression or mtFSR. Thus, we corroborate the work of others (97) who reported that FAO may be uncoupled from PGC-1α with obesity. Our study offers an explanation for this previous observation as the coupling of FAO and PGC-1α likely relies upon PGC-1α induced mitochondrial biogenesis. The failure to induce mtFSR suggests a mechanism for metabolic dysfunction in the obese.

In summary, high volume resistance exercise induced expression of PPARδ and PGC-1α content in the obese Zucker rat. Our data suggest that PPARδ may be important in the regulation of blood HDL-cholesterol concentrations in the sedentary state; however, this relationship is not apparent 16 h following short term, high volume RE. PPARδ and PGC-1α appear to mediate enhanced specific expression of substrate delivery and oxidative proteins following RE, including CPT-I and F_1 ATPase. However, such stimulation of PPARδ and PGC-1α does not result in increased mitochondrial biogenesis, as assessed by COX-IV expression or mtFSR, in the obese Zucker rat. This suggests that improved metabolism following exercise with obesity is due to enhanced substrate delivery and ATP production rather than elevated mitochondrial volume. These results have important implications related to our

understanding of mitochondria-mediated control of metabolism with obesity, as well as how this control is affected by exercise.

CHAPTER V

CONCLUSIONS

These studies were carried out with the primary goal to enhance understanding of the regulation of lipid metabolism in obesity, and how exercise influences dyslipidemia and lipid metabolism in the obese. The first study was designed to further comprehend how the obese respond to exercise and exercise training with respect to blood lipids and lipoproteins. The second study was devised to better understand how lipid metabolism is regulated in skeletal muscle of obese individuals following exercise and exercise training and how this regulation may influence blood lipids and lipoproteins. The third study was employed to develop a greater understanding of differences in regulation of lipid metabolism between lean and obese phenotypes and to achieve an improved mechanistic understanding of how exercise regulates lipid metabolism and oxidative function.

Exercise and Blood Lipids and Lipoproteins with Obesity

The first study was conducted to test the hypothesis that both acute exercise and exercise training would elicit anti-atherogenic effects on blood lipids and lipoproteins in overweight and obese men and women. Additionally, the influence of exercise training on the transient response to acute exercise was tested. Data from this study showed that exercise training sufficient to elicit the expenditure of 1500 $kcal \cdot wk^{-1}$ appears to be more efficacious to elicit anti-atherogenic effects on blood lipids and lipoproteins with obesity than a single session of exercise, and that this effect occurred regardless of the mode of

exercise training employed. Interestingly, how HDL-C is modified differs between men and women. In men, an increase in total HDL-C concentration was measured; however, in women a shift in HDL-C subfractions was observed. This shift involved a reduction in the smaller, more dense HDL_3-C subfraction and augmentation of the larger, less dense HDL_2-C subfraction and was measured with a concomitant reduction in HDL-C mean density. This shift in HDL-C subfractions is consistent with enhanced reverse cholesterol transport and a reduction in atherogenic risk (91, 177, 201).

Conversely, a single bout of exercise with the expenditure of 400 kcal had little effect on blood lipids and lipoproteins regardless of exercise training status in this study. Acute exercise did induce a reduction in the ratio of TC: HDL-C in men, however, an increase in hsCRP and NEFA (in women) was also observed. In addition, exercise training did not affect the transient response to a single session of exercise in the sample of overweight and obese men and women.

These findings support the hypothesis that exercise training would elicit changes in blood lipid and lipoprotein concentrations and particle numbers consistent with reduced atherogenic risk. However, acute exercise of 400 kcal did not prove to be a potent stimulus of these effects. Perhaps due to the lack of effect of a single session of exercise on these CVD risk markers, no influence was observed of exercise training on transient responses to acute exercise.

Exercise and PPARs with Obesity

The second study explored the possibility that PPARs are a primary mediator of oxidative adaptation to exercise and serve as a principal mechanism for alterations in

lipids and lipoproteins following exercise. Therefore, the following hypotheses were tested: 1) acute exercise and exercise training would augment skeletal muscle content of PPARδ and related proteins (PPARα, PGC-1α, and AMPKα), 2) enhanced PPARδ content would be associated with augmented content of target proteins involved in fatty acid and lipoprotein transport and metabolism, and 3) PPARδ content would be correlated with blood concentrations of lipids and lipoproteins at rest and following exercise.

In this study, skeletal muscle content of PPARδ and PGC-1α was enhanced following a single bout of aerobic exercise. In addition, content of PPARα and AMPKα was enhanced following 12 wks of exercise training. These data demonstrate the roles of acute exercise and exercise training in augmenting the content of proteins involved in metabolic regulation and adaptation. Enhanced expression of proteins involved in oxidative function and adaptation and lipoprotein metabolism, which are transcriptional targets of PPAR, was measured. Moreover, mitochondrial biogenesis, as assessed by COX-IV content, was enhanced following exercise training. Similar to results in the first study, exercise training-induced adaptations occurred regardless of the mode of exercise training employed. With regards to the regulation of blood lipids and lipoproteins, PPARδ content was correlated with the serum concentration of total and LDL-cholesterol, whereas AMPKα content was correlated with the concentration of HDL-C and its subfractions.

These data support the hypothesis that exercise augments the expression of PPARδ, PPARδ-related proteins, and transcriptional targets of PPARδ. However, while a single

session of aerobic exercise enhanced expression of PPARδ and PGC-1α, a significant influence of exercise on PPARα and AMPKα was only seen in the exercised, trained condition. Additionally, exercise did augment the protein content of purported PPARδ transcriptional targets. While a relationship was observed between PPARδ and total and LDL-C concentrations this effect was not seen with respect to HDL-C. Therefore, while PPAR may be involved in the regulation of blood lipids and lipoproteins further research is necessary to better define such regulation. These data indicate the importance of skeletal muscle in the regulation of lipid and lipoprotein concentrations. Furthermore, PPARδ and AMPKα appear to serve as primary mediators of this function of skeletal muscle.

Exercise and PPAR in the Lean and Obese Zucker Rat

In the third study, the influence of obesity and high volume resistance exercise on lipid and lipoprotein metabolism was explored in a rodent model. High volume RE was chosen in this study as a model of the ATM exercise utilized in the first two studies. The rationale for using RE is that ATM exercise may induce a hypertrophic response (82) which is not consistent with other forms of aerobic exercise. In contrast, high volume RE induces similar hypertrophic responses to ATM exercise while also showing the potential to induce oxidative adaptations similar to that seen with aerobic exercise (161, 203).

Therefore, in this study the following hypotheses were tested: 1) high volume RE would enhance PPAR and PGC-1 content in lean and obese Zucker rats, 2) these effects would be associated with augmented content of PPAR and PGC-1 target proteins

involved in FA transport and metabolism, as well as, enhanced mitochondrial biogenesis, and 3) PPARδ content would be correlated with blood concentrations of lipids and lipoproteins at rest and following exercise in both phenotypes.

In study 3, high volume RE augmented PPARδ expression, regardless of phenotype. In comparison, high volume RE induced the expression of PGC-1α in obese animals only. Perhaps the most novel finding of this study was that despite enhanced PGC-1α content, obese Zucker rats showed no significant exercise-induced mitochondrial biogenesis. These data demonstrate that despite the ability to induce signaling conducive of mitochondrial biogenesis and the expression of proteins involved in oxidative function (such as CPT-I and F_1 ATPase), obese animals exhibit an impairment in the ability to exhibit mitochondrial biogenesis. It is not known why obese animals appear resistant to such a response. However, given an already elevated mitochondrial volume measured in the obese animals, such a response may not be necessitated by the exercise paradigm. The measures of mitochondrial biogenesis used in this study are reliant upon the induction of protein synthesis (COX-IV expression and mtFSR). However, obese animals may exhibit an impaired ability to induce enhanced rates of protein synthesis. Previous observations have suggested already elevated rates of protein synthesis in obese Zucker rats compared to lean in the basal state; therefore, basally elevated protein synthesis may impair the ability to induce augmented protein synthesis with the exercise paradigm (68).

These data support the hypothesis that high volume RE would elicit augmented PPARδ and PGC-1α content in the obese Zucker rat. However, PGC-1α was not

augmented by high volume RE in lean littermates. Similarly, high volume RE yielded concurrent enhancements in target proteins involved in FA transport and oxidative metabolism. By contrast, these data do not support the hypothesis that these responses would result in mitochondrial biogenesis in the obese Zucker rat, nor was a significant relationship between PPARδ and blood lipids and lipoproteins observed.

Comprehensive Conclusions

Primary findings of these studies include: 1) exercise-induced changes in lipid and lipoprotein concentrations in the obese appear to be primarily mediated by exercise training, 2) PPARδ and AMPKα expression are induced by exercise and appear to serve as regulatory points for lipid and lipoprotein concentrations at rest and following exercise, and 3) despite induction of PPARδ and PGC-1α following exercise, the obese Zucker rat failed to undergo significant mitochondrial biogenesis. In the second study the most consistent finding regarding the role of skeletal muscle in lipid and lipoprotein concentrations was that AMPKα was correlated with HDL-C and its subfractions in all conditions. What makes this more intriguing is that the primary influence of exercise on cholesterol in the sample of obese humans was an exercise training effect on HDL-C and its subfractions. Similarly, AMPKα expression was induced only after exercise training. That these effects occurred in parallel provides further evidence for the impact of AMPKα on HDL-C. In addition, the relationship observed in study two between PPARδ, AMPKα and blood cholesterols in obese humans was not present in the Zucker rat. While mechanisms related to the discrepant findings between studies are unclear, it may represent species-specific differences for the role of skeletal muscle in regulating

blood cholesterols in humans and rats. Alternatively, the induction of AMPKα expression and the exercise-induced alterations in HDL-C in the human studies occurred after 12 wks of exercise training; whereas, observations from the third study were obtained after four sessions of exercise in 9 days. Therefore, it is possible that a longer term training protocol, even in rats, may demonstrate similar relationships as seen in this sample of humans. While a similar relationship between PPARδ and cholesterol was hypothesized despite species differences in cholesterol metabolism, these differences should be considered. Very simply, humans are LDL-C animals, that is the primary cholesterol fraction in human serum is LDL-C, whereas, rats are HDL-C animals. These species differences may be reflective not only of hepatic differences in cholesterol metabolism but also in differences in the metabolism and transport of cholesterol by peripheral tissues, such as skeletal muscle.

Similarly, induction of mitochondrial biogenesis was observed in the sample of overweight and obese human subjects but not in the obese Zucker rat. As discussed, the measures of mitochondrial biogenesis utilized in both models are based on enhanced protein content and synthesis. It is possible that the obese Zucker rat is already operating at a maximum rate of protein synthesis and therefore may not be capable of eliciting enhanced mtFSR (68). However, a single bout of exercise did not induce a significant enhancement in mitochondrial biogenesis in the sample of human subjects, this effect occurred only after exercise training. Therefore, it is also likely that to observe such a response a longer term stimulus would be necessary in the Zucker rat. In addition, while there are recognized differences in the exercise paradigm, the high

volume RE employed in the third study did induce enhanced mtFSR in the lean Zucker rat. Therefore, an effect of mitochondrial biogenesis can be expected with this protocol.

Finally, in the obese Zucker rat enhanced expression of F_1 ATPase (subunit of ATP synthase) was measured following exercise. This effect was not seen in the human subjects. This disparity may be a primary difference in the exercise paradigm. While many of the effects measured were consistent between the exercise paradigms regarding the enhancement of oxidative function, the exercise protocol used in the third study likely involves a more explosive muscle contraction than the treadmill paradigms used in the human studies. This may, in fact, necessitate the induction of F_1 ATPase, to provide a higher rate of ATP production with the high volume RE paradigm but not with the treadmill paradigms.

Significance

These studies enhance understanding of the regulation of skeletal muscle energy metabolism and its link to disease, in particular obesity. The presented data demonstrating the efficacy of exercise training to improve CVD risk factors in overweight and obese men and women better enable practitioners to utilize aerobic exercise as a therapeutic agent in this population. These effects appear to occur regardless of the mode of exercise training utilized, ATM or LTM. Additionally, these data provide evidence of how lipid and lipoprotein concentrations are regulated both at rest and following exercise. By demonstrating the potentially important role of skeletal muscle PPARδ and AMPKα expression in regulating lipids and lipoproteins, strategies may be developed to target these proteins in an effort to improve CVD risk. In addition,

data demonstrating the influence of high volume RE in the lean and obese Zucker rat improve the understanding of how metabolism may be dysregulated in the obese condition. The demonstration that PGC-1α content is uncoupled from mitochondrial biogenesis in these animals may explain previous findings that PGC-1α is uncoupled from FAO in obese humans.

Limitations and Delimitations

Delimitations.

The delimitations of the human studies include: otherwise healthy, overweight and obese adult men and women, nonsmokers who are not taking medications known to alter lipid metabolism, acute exercise of 400 kcal, exercise training of 1500 kcal·wk^{-1} using either LTM or ATM modes, fasting blood and muscle samples obtained after three days without exercise or 24 h post-exercise, blood lipid and lipoprotein concentrations adjusted for shifts in plasma volume following acute exercise, the content of the proteins measured. The delimitations of study 3 (obese Zucker rat) include: lean and obese Zucker rats, the high volume RE model employed, the content of the proteins that were measured, phosphorylation of AMPKα and LKB1, and concentration of the blood lipids and lipoproteins measured (TC, HDL-C, LDL-C, NEFA), and assessment at 16 h post-exercise.

Furthermore, these studies are delimited to the assessment of the protein content of the targets of interest. As a transcription factor, the direct effect of PPAR, and of PGC-1α as a transcriptional coactivator, is on the transcription of DNA to mRNA, rather than mRNA translation to protein. Therefore, the most direct measure of the effect of PPAR

would have been to assess mRNA content of target genes. However, the measurement of protein of these target genes provides a greater look at the functional effect of upregulating the PPAR pathway following an exercise stimulus.

Limitations.

Limitations of the human studies include: all subjects resided in the same geographical area, dietary and activity analysis was based on self-reported information, findings relate only to otherwise healthy physically inactive overweight and obese men and women, self-reported exercise abstinence during sample collection periods, and subject compliance. Limitations of study 3 (obese Zucker rat) include: findings are limited to male lean and obese Zucker rats, findings cannot be extrapolated beyond the short term of the exercise protocol, findings are specific to the exercise protocol and to assessment of samples at 16 h post-exercise.

In addition, due to the descriptive nature of these studies, the described relationship between PPAR, PGC-1α and AMPKα with target proteins and blood lipids and lipoproteins is merely indicative of a regulatory role of these proteins on the described effects. To prove this cause/effect relationship it would be necessary to inhibit the PPAR, PGC-1α and AMPKα pathways in an effort to blunt the observed effects on known targets. A reduced or eliminated effect would then directly indicate the necessity for these proteins in mediating oxidative function, and blood lipids and lipoproteins.

Summary

In summary, in this dissertation the ability of exercise to induce metabolic adaptations in the obese which are likely mediated by PPAR has been demonstrated.

The induction of significant changes in blood lipids and lipoproteins in the obese appears to be primarily regulated by the performance of an exercise training program. Together with previous data, the current data suggest that the attainment of such a training adaptation requires the expenditure of at least 1500 kcal·wk^{-1}. In both human and rat models of obesity exercise induced augmented expression of PPARδ and PGC-1α following a single bout of exercise. In the human model exercise training further increased expression of PPARα and AMPKα following exercise training. These findings were seen with a concomitant induction of proteins involved in oxidative transport and function in both models. This indicates the ability of both the obese rat and human to adapt to exercise in a manner consistent with enhanced oxidative function which is likely mediated by PPAR. The data presented in this dissertation describe the likely regulation of blood lipids and lipoproteins by skeletal muscle, and more specifically by PPARδ and AMPKα.

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APPENDIX A

INFORMED CONSENT

Molecular Basis for the Lipid Response to Acute Exercise Before and After Exercise Training

You have been asked to participate in a research study of the effects of acute exercise and blood lipids and lipoproteins. You were contacted through posted notices or word-of-mouth. Your participation is completely voluntary, and that you were selected as a possible participant because of your physical conditions that are suitable for this study. Twenty people will be recruited to participate in this study. The purpose of this study is: 1) to analyze the way your body responds to acute exercise and exercise training; and 2) to investigate a potential mechanism for how this occurs.

You will be asked to perform several procedures requiring a total of about 4 hours in the laboratory to test your physical fitness and health. These tests may require that you visit the laboratory on two separate days. You will be asked to perform a graded exercise test (GXT) by walking or running on a land-based, motorized treadmill until you are exhausted. While you are doing this test, you will be asked to have electrodes attached to your chest to measure the activity of your heart through an electrocardiogram (ECG), you will be asked to breathe through a mouthpiece connected to a machine to measure the amount of oxygen your body is using, and your blood pressure will be measured. After the GXT, you will be asked to have an earlobe stick with a small lancet like ones used for finger sticks so that about two drops of blood can be collected to measure a substance in your blood (lactic acid) produced by exercise. You will be asked to also be asked to perform tests to measure your muscle strength, endurance, and flexibility. Your body bone density and body fat will be measured by lying at rest wearing exercise clothing in a DEXA (Dual Energy X-ray Absorptiometry) scanning machine. This machine will scan the body with a small amount of X-ray radiation. The radiation exposure is comparatively less than the amount of natural radiation you would be subjected to flying in an airplane from Houston to Dallas. Anytime you feel uncomfortable in the machine you can remove yourself from it. You will be asked to also have circumference measures taken on parts of your body, including your hip and abdomen. You will be asked to breathe through a mouthpiece attached to a machine to measure your breathing capacity and the health of your lungs. During these tests a local physician will be present and consult with each individual prior to exercise as well as review each subject's ECG and make an assessment as to your cardiovascular risk, if the physician determines that you are at risk for cardiovascular disease you will be referred to a cardiologist and removed from the study at least until the time that the cardiologist gives written consent for your participation.

At least one week after these measures have been taken, you will be asked to complete an acute session on a motorized treadmill. During these exercise sessions, your heart rate, blood pressure, and oxygen uptake will be measured. Twenty-four hours before and again 24 hours following the acute exercise session the researchers will take blood and muscle samples. First, you will be asked to have about 3 to 4 teaspoons (15 to 20 ml) of blood drawn from a vein in your arm so that your blood fats

and cholesterol can be measured. Also at these times, you will be asked to give a small piece of muscle from your thigh muscle. The amount of muscle that will be taken is about the size of a pea. Muscle samples will be stored for measurement of factors related to your muscles use of fats and cholesterol, leftover sample will be stored by the AESL for potential future use as relates to the context of this study. These samples will be used to measure cellular mechanisms and factors related to lipids and cholesterol before and after exercise. Blood and muscle samples will be stored by the AESL for potential future measurements related to the context of this experiment.

Once you complete the exercise sessions on the water treadmill, you will be assigned at random to complete an exercise training program on either the land-based treadmill or the water treadmill. The training program will last 12 weeks, and require you to train 3 times per week for about 20 minutes to an hour each day. You will have a personal trainer from the Applied Exercise Physiology Laboratory staff assigned to supervise each training session, and each session will be personalized for you. You will be asked to walk or run on your assigned treadmill for each training session until you expend about 500 kilocalories of energy. The physical effort required to complete each exercise session will be easy to moderate at the beginning, but will steadily increase from moderate to hard as you get in better physical condition.

After 12 weeks of training, you will be asked to repeat all the measures taken at the beginning of the study, including blood sampling, maximal effort GXT, body composition, lung tests, and assessment of my muscle strength, endurance, and flexibility. Values obtained from these measurements will be used to compare to your pre-training values to see how well the training program worked. In addition you will be asked to repeat the acute exercise session and subsequent muscle biopsy.

The physical exertion required of you in this study will range from easy to maximal effort. During exercise there are physical risks to you including: muscle and bone strains and sprains, abnormal blood pressure, fainting, abnormal heart beats, shortness of breath, and in rare instances heart attack.

The data collected during this study is confidential and the names of all the subjects will be entered as a code in data analysis to ensure the confidentiality. The records of this study will be kept private. No identifiers linking you to the study will be included in any sort of report that might be published. Research records will be stored securely and only Nicholas P. Greene and his research collaborators will have access to the records. Your decision whether or not to participate will not affect your current relations with Texas A&M University. If you decide to participate, you are free to refuse any situations that may be objectionable. You can withdraw at any time without your relations with the university, job, benefits, etc., being affected. You can contact Stephen F. Crouse at 112 Netum Steed Laboratory, (979) 845-3999 (s-crouse@tamu.edu) with any questions about this study. If Stephen F. Crouse is not available, you can contact the department head, Robert Armstrong.

This research has been reviewed by the Institutional Review Board – Human Subjects in Research, Texas A&M University. For research-related problems or questions regarding subjects' rights, you can contact the Institutional Review Board through Ms. Angelia M. Raines, Director of Research Compliance, Office of Vice President for Research at (979) 458-4067 (araines@vprmail.tamu.edu).

Please be sure you have read the above information, asked questions and received answers to your satisfaction. You will be given a copy of the consent form for your records. By signing this document, you consent to participate in the study.

Signature of Participant: _____ Date:_____

Signature of Investigator:_____ Date:_____

APPENDIX B

ACTIVITY RECORD AND COMPLIANCE FORM

Name:_____ Age:_____ Ht:_____ Wt:_____

Address: _____ Phone:_____(W) _____
_____(H)
_____ Occupation:_____
_____

DIRECTIONS: This Seven Day Physical Activity Record is designed to measure your habitual physical activities over the course of one week. You are asked to record your sleep habits as well as the physical activities you participated in over the course of the past seven days; include both occupational and leisure-time physical activities.

1. **BEFORE READING ANY FURTHER, PLEASE REVIEW ATTACHMENT 1 FOR EXAMPLES OF LIGHT, MODERATE, HARD, AND VERY HARD PHYSICAL ACTIVITIES!**

2. **DO NOT RECORD LIGHT ACTIVITIES.** See Attachment 1 for examples of LIGHT ACTIVITIES. Most of you will spend the majority of your waking hours in light activity. For example, a laboratory worker may be on their feet all day and may feel "fatigued", but the energy cost is in the "light" category. However, we need you to record the number of hours you spend sleeping.

3. For all other physical activities, which may be classified as moderate, hard, or very hard, **DOCUMENT ONLY THE TIME ACTUALLY SPENT PERFORMING THE ACTIVITY**: Include both occupational and leisure-time activities. For example, the laboratory worker in the illustration given above may spend a number of hours stocking shelves with supplies, which would likely be moderate exercise. It is unlikely, however, that they would spend an 8 hour day performing this task, and time should be subtracted for lunch, breaks, etc. Similarly, being at the pool for 2 hours but swimming for 15 minutes should be recorded as 15 minutes, not 2 hours.

4. For this record to be representative of your normal physical activity habits, it is critical that the week's activities be "normal" for you. For example, a week in which you take a holiday or a few days vacation would clearly NOT be a "normal" week for you. **IF THE UPCOMING WEEK'S ACTIVITIES WILL NOT REPRESENT YOUR NORMAL ACTIVITY PATTERNS, THEN PLEASE DO NOT COMPLETE THIS FORM - WAIT FOR A WEEK**

THAT WILL REFLECT YOUR NORMAL PHYSICAL ACTIVITY PATTERNS. Note that a week is not necessarily Sunday through Saturday, but may be any consecutive 7 day period.

5. Use the record forms beginning on the next page to record; (1) the physical activity, (2) the total hours/minutes spent performing the activity, (3) and rate how hard you worked at the particular physical activity. Use the following scale to rate how hard you worked.

6. Return this completed record to the laboratory staff at your next laboratory visit.

SCALE TO RATE HOW HARD YOU WORK

1 - Barely breaking a sweat; breathing just slightly elevated.

2 - Moderate sweating; breathing significantly above normal, but could talk normally.

3 - Heavy sweating; breathing very heavy to nearly winded, could NOT talk normally.

PLEASE GO TO THE NEXT PAGE TO BEGIN YOUR SEVEN DAY ACTIVITY RECORD

*From: Blair et al., Assessment of habitual physical activity by a seven day recall in a community survey and controlled experiments. Am. J. Epidem. 122:794-804, 1985.

DAY ONE

Date:_____ Day of Week:_____

Activity	TOTAL TIME (Hours:Minutes)	HOW HARD (1,2,3)
Sleeping, including naps		

DAY TWO

Date:_____ Day of Week:_____

Activity	TOTAL TIME (Hours:Minutes)	HOW HARD (1,2,3)
Sleeping, including naps		

DAY THREE

Date:_____ Day of Week:_____

Activity	TOTAL TIME (Hours:Minutes)	HOW HARD (1,2,3)
Sleeping, including naps		

DAY FOUR

Date:_____ Day of Week:_____

Activity	TOTAL TIME (Hours:Minutes)	HOW HARD (1,2,3)
Sleeping, including naps		

DAY FIVE

Date:_____ Day of Week:_____

Activity	TOTAL TIME (Hours:Minutes)	HOW HARD (1,2,3)
Sleeping, including naps		

DAY SIX

Date:_____ Day of Week:_____

Activity	TOTAL TIME (Hours:Minutes)	HOW HARD (1,2,3)
Sleeping, including naps		

DAY SEVEN

Date:_____ Day of Week:_____

Activity	TOTAL TIME (Hours:Minutes)	HOW HARD (1,2,3)
Sleeping, including naps		

CONCLUDING QUESTIONS

1. Would you say that during the past week you were (check one):

_____less active than usual
_____about as active as usual
_____more active than usual

2. Which statement most nearly describes your attitude toward leisure-time physical activity?

_____I absolutely detest physical activity and exertion of any type.
_____I do not enjoy physical activity or exertion of any type.
_____I do not like activities which make me sweat, but I do like some types of light activities.
_____I enjoy light physical activity of many types, and occasionally like hard physical activity.
_____I thoroughly enjoy all types of physical activities, even those which are hard and very hard.

3. When you have time off from work (weekends/vacations) or during work breaks (lunch, etc), how often do you participate in physical activities, including recreational sports, which would be considered moderate to very hard?

_____Never
_____Seldom

_____Sometimes/Irregularly
_____Frequently/Regularly
_____Almost Always

THANK YOU VERY MUCH FOR YOUR TIME AND ACCURACY IN COMPLETING THIS QUESTIONNAIRE. THIS INFORMATION IS INDISPENSABLE FOR OUR STUDY, AND WE THANK YOU FOR YOUR WILLINGNESS TO COOPERATE IN COMPLETING THIS FORM.

ACTIVITY COMPLIANCE

NAME:_____
DATE:_____

1. My activity level (**has / has not**) changed from the last activity record submitted.

2. My activities have changed as follows:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Printed Name _____

Signature _____

APPENDIX C

3 DAY DIET RECORD AND COMPLIANCE FORM

APPLIED EXERCISE SCIENCE LABORATORY
HYDROWORX PROJECTS
THREE DAY DIET RECORD

Name:_____ Age:_____ Ht:_____ Wt:_____

DIRECTIONS: This Three Day Diet Record is designed to measure your food intake over the course of three consecutive days. Please make sure that ONE recorded day is a weekend. Because of this requirement, this record should be filled out **Thursday, Friday, Saturday OR Sunday, Monday, Tuesday**.

1. Records should be kept over a time period that best represents your "normal" eating patterns for 2 weekdays and one weekend day. For example, if Monday is a work holiday, it is unlikely that you will eat as you normally would.

2. Record **ALL** food and drink (**including water**) that you consume on each day. Record both the type of food or drink and the amounts consumed.

3. Please be as specific on foods and amounts as possible. For example, if you eat a turkey sandwich, please record the type of bread (white, whole wheat, rye, etc), number of slices of meat, and any additional items (cheese, tomato, mayonnaise, etc). Also include brand names of items when possible. For help in determining what is considered a serving, see the serving size chart on page 2 for some common food items.

4. Page 3 shows a sample day of the diet record. Please read this to help you become familiar with the recording format.

5. If you have any questions about filling out the record, please contact laboratory staff for assistance.

6. Return this record to the laboratory staff once it is complete.

Please do not change your diet in any way during the course of the study. Maintain normal eating habits, please do not begin a "diet". If you travel, don't worry, these changes from normal are only temporary.

Serving Size Chart

1 Cup cereal flakes or 1 baked potato = size of a fist

½ cup cooked rice, pasta or potato = size of an ice cream scoop

1 pancake = size of a CD

1 cup of salad greens or 1 medium fruit = size of a baseball

½ cup fresh fruit or vegetables = size of a standard light bulb

¼ cup dried fruit = 1 large egg

3 oz. meat, fish, poultry = size of a deck of cards

2 Tbsp peanut butter = size of a golf ball

1 ½ oz. cheese = 4 stacked dice or 2 cheese slices; 1 tsp margarine, butter and spreads = 1 dice

<u>DAY 1</u> **Date: __________** **Day of Week:____________________**

Food Eaten	**# of servings or amount**	**Food Eaten**	**# of servings or amount**

DAY 2 **Date: _________** **Day of Week:___________________**

Food Eaten	**# of servings or amount**	**Food Eaten**	**# of servings or amount**

<u>DAY 3</u> **Date: _________** **Day of Week:___________________**

Food Eaten	**# of servings or amount**	**Food Eaten**	**# of servings or amount**

DAY 1 **Date: SAMPLE** **Day of Week: _SAMPLE DAY__**

Food Eaten	# of servings or amount	Food Eaten	# of servings or amount
Breakfast			
coffee (caffinated)	1-8oz cup		
w/ half&half	2 Tbsp		
Raisin Bran cereal	1 cup		
w/ 1% milk	1 cup		
Multivitamin	1 vitamin		
Lunch			
Turkey sandwich (homemade)			
w/ turkey deli meat	3 slices		
w/ Kraft American cheese	1 slice		
w/ Lite mayo	2 Tbsp		
w/ whole wheat bread	2 slices		
apple	1 medium		
Lay's potato chips	1 snack bag		
Sprite	12oz can		
Snacks			
water	20oz bottle		
Nature's Own honey granola bar	2 bars		
Hershey's Kisses	3 kisses		
Dinner			
McDonald's Big Mac			
w/ cheese and mayo			
french fries	medium		
Bluebell Vanilla Ice Cream	2 scoops		
w/ chocolate syrup	2 Tbsp		

DIET COMPLIANCE

NAME:_____
DATE:_____

1. My diet (**has / has not**) changed from the last diet record submitted.

2. My diet changed as follows:

   _____
   _____
   _____
   _____
   _____
   _____
   _____
   _____
   _____
   _____
   _____
   _____
   _____
   _____
   _____

Printed Name _____

Signature _____

APPENDIX D

ACUTE EXERCISE WORKSHEET

PRE-EXERCISE DATA

Name: _____Date: _____Age: _____ Height: ___in

Medications/Doses: _____

Target:

Exercise1: Speed___mph Grade__% Energy expenditure _____kcal

Exercise2: Speed____mph Grade__% RHR_____bpm

Exercise3: Speed____mph Grade__% RSBP_____mmHg RDBP___mmHg

EXERCISE DATA

Note: Collect and record data (ie: VO_2, HR, RER, RPE) throughout the 1st minute of warm-up, and during the exercise periods indicated below. Adjust the treadmill elevation as necessary to maintain the prescribed VO_2.

Time (min)	Speed (mph)	Grade (%)	HR (bpm)	RPE	VO_2 (L/min)	RER	Comments
Warmup 0-3	3.0	0					
Exercise 3-5							
10-12							
20-22							
30-32							
40-42							
Cool Down							
0-3	2.2	0					
5-7							

POSTEXERCISE DATA

Duration _____

Total Kcal _____

APPENDIX E

SAMPLE TRAINING LOG

12 WEEK EXERCISE LOG
Texas A&M University Applied Exercise Science Lab

NAME:_____ PHONE:_____
VO_2MAX: _____

SPOT CHECK VO_2
WEEK: 1 DATES: _____ - _____ INTENSITY: 50-60% VO_2: _____
TARGET SPEED: JET:

TARGET KCAL: 250	TARGET HR: _____	TARGET TIME: _____
DATE: _____	DATE: _____	DATE: _____
PRE-WT: _____	PRE-WT: _____	PRE-WT: _____
RBP: ___/___	RBP: ___/___	RBP: ___/___
RHR: _____	RHR: _____	RHR: _____
EXER HR: _____	EXER HR: _____	EXER HR: _____
RPE: _____	RPE: _____	RPE: _____
SPEED: _____	SPEED: _____	SPEED: _____
JET: _____	JET: _____	JET: _____
TIME: _____	TIME: _____	TIME: _____
POST-WT: _____	POST-WT: _____	POST-WT: _____
POST-BP: ___/___	POST-BP: ___/___	POST-BP: ___/___
POST-HR: _____	POST-HR: _____	POST-HR: _____
PAIN: _____	PAIN: _____	PAIN: _____

COMMENT: _____
_____

WEEK: 2 DATES: _____ - _____ INTENSITY: 60-65% VO_2: _____

	TARGET SPEED:	JET:
TARGET KCAL: 300	TARGET HR: _____	TARGET TIME:_____
DATE: _____	DATE: _____	DATE: _____
PRE-WT: _____	PRE-WT: _____	PRE-WT: _____
RBP: ___/___	RBP: ___/___	RBP: ___/___
RHR: _____	RHR: _____	RHR: _____
EXER HR: _____	EXER HR: _____	EXER HR: _____
RPE: _____	RPE: _____	RPE: _____
SPEED: _____	SPEED: _____	SPEED: _____
JET: _____	JET: _____	JET: _____
TIME: _____	TIME: _____	TIME: _____
POST-WT: _____	POST-WT: _____	POST-WT: _____
POST-BP: ___/___	POST-BP: ___/___	POST-BP: ___/___
POST-HR: _____	POST-HR: _____	POST-HR: _____
PAIN: _____	PAIN: _____	PAIN: _____

COMMENT: _____
_____

WEEK: 3 DATES: _____ - _____ INTENSITY: 65-70% VO_2: _____

	TARGET SPEED:	JET:
TARGET KCAL: 350	TARGET HR: _____	TARGET TIME: _____
DATE: _____	DATE: _____	DATE: _____
PRE-WT: _____	PRE-WT: _____	PRE-WT: _____
RBP: ___/___	RBP: ___/___	RBP: ___/___
RHR: _____	RHR: _____	RHR: _____
EXER HR: _____	EXER HR: _____	EXER HR: _____
RPE: _____	RPE: _____	RPE: _____
SPEED: _____	SPEED: _____	SPEED: _____
JET: _____	JET: _____	JET: _____
TIME: _____	TIME: _____	TIME: _____
POST-WT: _____	POST-WT: _____	POST-WT: _____
POST-BP: ___/___	POST-BP: ___/___	POST-BP: ___/___
POST-HR: _____	POST-HR: _____	POST-HR: _____
PAIN: _____	PAIN: _____	PAIN: _____

COMMENT: _____
_____

APPENDIX F

ZUCKER RAT EXERCISE LOG

Rat #: _____ Cohort:_____ Type: Lean or Obese

Session	Shocks	Repetitions			Notes
		Weight	Pred Reps	Actual Reps	
OC1		N/A	50		
Date:					
OC2		N/A	50		
Date:					
OC3		N/A	50		
Date:					
OC4		NoRT	50		
Date:					
OC5		NoRT	50		
Date:					
OC6		NoRT	35-40		
Date:					
		80g	10-15		

Rat #: _____ Cohort:_____ Type: Lean or Obese

Session	Shocks	Repetitions			Notes
		Weight	Pred Reps	Actual Reps	
RT1		30	14		
		80	12		
Date:		130	10		
		180	8		
		230	6		
RT2		80	16		
		80	16		
Date:		130	10		
		180	10		
		230	8		
RT3		80	18		
		130	16		
Date:		180	14		
		180	14		
		230	8		
RT4		80	18		
		130	16		
Date:		180	14		
		180	14		
		230	8		

APPENDIX G

SAMPLE STATISTICAL CODE AND RAW DATA FOR CHAPTER II

Variable Definitions:

Independent Variables

Unique Identifier	**ID**
Gender (M F)	**GENDER**
Age (years)	**AGE**
Height (inches)	**HEIGHT**
Treadmill Type (LAND or WATER)	**MODE**
Timepoint	**TIMEPT**
Training Time Point (PRE or POST)	**TRAINING**
Acute Exercise (Rest or 24)	**EX**

Dependent Variables

Total Cholesterol St Joseph's (mg/dL)	**TCS**
HDL Cholesterol St Joseph's (mg/dL)	**HDLS**
LDL Cholesterol St Joseph's (mg/dL)	**LDLS**
Triglycerides St Joseph's (mg/dL)	**TAGS**
Glucose St Joseph's (mg/dL)	**GLUCOSES**
Total Cholesterol Spectracell Analysis (mg/dL)	**TCSPE**
Triglycerides Spectracell Analysis (mg/dL)	**TAGSPE**
VLDL-Cholesterol Spectracell Analysis (mg/dL)	**VLDLSPE**
LDL-Cholesterol Spectracell Analysis (mg/dL)	**LDLSPE**
Remnant Lipoprotein Spectracell Analysis (mg/dL)	**RLPSPE**
IDL-Cholesterol Spectracell Analysis (mg/dL)	**IDLSPE**
LDL3-Cholesterol Spectracell Analysis (mg/dL)	**LDL3SPE**
LDL4-Cholesterol Spectracell Analysis (mg/dL)	**LDL4SPE**
HDL-Cholesterol Spectracell Analysis (mg/dL)	**HDLSPE**
HDL2b-Cholesterol Spectracell Analysis (mg/dL)	**HDL2BSPE**
HDL2a-Cholesterol Spectracell Analysis (mg/dL)	**HDL2ASPE**
HDL3-Cholesterol Spectracell Analysis (mg/dL)	**HDL3SPE**
Total Cholesterol: HDL-C ratio Spectracell Analysis	**TCHDLSPE**
VLDL-Cholesterol Particle Number Spectracell	**VLDLPRT**
LDL-Cholesterol Particle Number Spectracell	**LDLPRT**
Remnant Lipoprotein Particle Number Spectracell	**RLPPRT**
LDL3-Cholesterol Particle Number Spectracell	**LDL3PRT**
LDL4-Cholesterol Particle Number Spectracell	**LDL4PRT**
HDL-Cholesterol Particle Number Spectracell	**HDLPRT**
HDL2b-Cholesterol Particle Number Spectracell	**HDL2BPRT**
LDL Mean Density Spectracell Analysis (g/cm^3)	**LDLDENS**

Lipoprotein (a) Spectracell Analysis (mg/dL)	**LPaSPE**
C-Reactive Protein Spectracell Analysis (mg/dL)	**CRPSPE**
Insulin Spectracell Analysis (uIU/mL)	**INSULIN**
HDL Mean Density Spectracell Analysis (g/cm^3)	**HDLDENS**

Repeated Measures ANOVA

Data were analyzed using the Statistical Analysis System (SAS) version 9.2. SAS process for data analysis used was PROC MIXED. By use of PROC MIXED the best statistical fit to the repeated-measures effects was able to be chosen for each dependent variable of interest. Sample code:

PROC MIXED;
CLASS ID GENDER ACUTEEX Train;
MODEL TCS= GENDER ACUTEEX Train GENDER*ACUTEEX GENDER*Train Train*ACUTEEX GENDER*Train*ACUTEEX ;
REPEATED ACUTEEX*Train/ SUB=ID TYPE=CS R RCORR ;
LSMEANS GENDER ACUTEEX Train GENDER*ACUTEEX GENDER*Train Train*ACUTEEX GENDER*Train*ACUTEEX / PDIFF=ALL ADJUST=TUKEY ;
RUN;

QUIT;

The above code allows for the analysis of any main or interactive effects of gender, acute exercise, and exercise training. The statement "REPEATED" allows for the system to model the statistic based on the repeated-measures variables indicated, for this analysis: ACUTEEX and Train. The statement TYPE on this line of code provides a place to input methods of modeling for the covariance matrix of the repeated variables. The following two lines set up post hoc analysis across all conditions in the MODEL statement. Differences here are determined across all levels (PDIFF=ALL) using the Tukey-Kramer method.

Raw data of all blood variables after adjustment for changes in plasma volume subsequent to each of the experimental acute exercise sessions.

Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	TCS	HDLS	LDLS
1	JG1	M	40	68.0	Water	1	Rest	Pre	259.000	45.0000	178.000
2	JG1	M	40	68.0	Water	2	24	Pre	210.955	29.4355	.
3	JG1	M	40	68.0	Water	3	Rest	Post	194.000	30.0000	.
4	JG1	M	40	68.0	Water	4	24	Post	163.947	34.1162	.
5	RK2	F	51	64.5	Water	1	Rest	Pre	203.000	58.0000	132.000
6	RK2	F	51	64.5	Water	2	24	Pre	204.344	56.1945	131.802
7	RK2	F	51	64.5	Water	3	Rest	Post	183.000	50.0000	94.000
8	RK2	F	51	64.5	Water	4	24	Post	170.741	53.7162	96.881
9	GB3	M	39	73.0	Water	1	Rest	Pre	147.000	47.0000	84.000
10	GB3	M	39	73.0	Water	2	24	Pre	149.130	49.7101	89.275

Obs	TAGS	GLUCOSES	TCSPE	TAGSPE	VLDLSPE	LDLSPE	RLPSPE	IDLSPE	LDL3SPE	LDL4SPE
1	180.000	88.000	290.000	167.000	32.0000	200.000	57.0000	50.0000	35.0000	11.0000
2	.	80.457	225.378	226.065	44.3496	129.516	41.7985	35.3227	32.0847	10.4006
3	.	83.000	193.000	204.000	40.0000	97.000	29.0000	23.0000	25.0000	10.0000
4	.	86.238	163.000	160.157	31.2732	78.657	27.4825	21.7965	22.7441	6.6337
5	66.000	91.000	218.000	93.000	18.0000	141.000	57.0000	50.0000	12.0000	4.0000
6	80.716	88.889	227.026	115.863	23.2952	146.004	58.3401	50.8816	15.0193	5.0064
7	196.000	87.000	218.000	136.000	28.0000	128.000	56.0000	50.0000	12.0000	7.0000
8	102.636	86.330	213.906	118.943	23.9805	126.617	59.4715	51.7978	12.4698	4.7961
9	79.000	105.000	125.000	61.000	12.0000	71.000	23.0000	21.0000	9.0000	4.0000
10	48.696	96.377	139.594	64.826	12.7826	82.072	29.2174	26.1739	9.3333	3.6522

Obs	HDLSPE	HDL2BSPE	HDL2ASPE	HDL3SPE	TCHDLSPE	VLDLPRT	LDLPRT	RLPPRT	LDL3PRT
1	59.0000	18.0000	5.0000	35.0000	4.91525	121.000	1361.00	242.000	329.000
2	51.5122	17.8576	5.2003	28.4544	4.37524	169.745	936.05	177.594	304.167
3	56.0000	23.0000	5.0000	28.0000	3.44643	154.000	726.00	125.000	238.000
4	53.0697	19.9011	4.7384	28.4302	3.07143	121.302	585.66	116.564	214.174
5	59.0000	26.0000	11.0000	23.0000	3.69492	70.000	879.00	243.000	115.000
6	57.6249	25.6451	10.4215	21.5583	3.93972	88.889	925.68	247.256	143.041
7	61.0000	31.0000	12.0000	19.0000	3.57377	109.000	807.00	236.000	115.000
8	62.3492	30.6950	12.4698	19.1844	3.43077	93.044	791.36	254.193	114.147
9	42.0000	14.0000	5.0000	23.0000	2.97619	47.000	465.00	99.000	85.000
10	44.7391	16.4348	6.8986	21.4058	3.12018	48.696	528.55	123.768	88.261

Obs	LDL4PRT	HDLPRT	HDL2BPRT	LDLDENS	LPaSPE	CRPSPE	INSULIN	HDLDENS
1	135.000	11753.00	1660.00	1.0323	9.6000	0.30000	15.100	1.101
2	128.535	9982.58	1601.29	1.0336	9.8118	0.11774	7.261	1.098
3	118.000	10279.00	2077.00	1.0339	8.8000	0.23000	8.800	1.093
4	85.291	10141.05	1799.63	1.0343	7.5814	0.31273	12.415	1.096
5	53.000	10149.00	2317.00	1.0300	8.4000	0.05000	5.400	1.090
6	62.325	9777.84	2308.06	1.0305	7.2542	0.06130	7.459	1.089
7	90.000	9774.00	2757.00	1.0306	6.1000	0.05000	14.400	1.085
8	58.512	9980.67	2773.10	1.0306	5.9472	0.06715	5.180	1.085
9	47.000	8184.00	1223.00	1.0310	8.3000	0.10000	11.000	1.100
10	45.652	8294.49	1475.07	1.0307	6.8986	0.14203	7.609	1.095

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	TCS	HDLS	LDLS
11	GB3	M	39	73.0	Water	3	Rest	Post	159.000	50.0000	97.000
12	GB3	M	39	73.0	Water	4	24	Post	162.487	49.8427	101.679
13	RR5	F	42	64.5	Water	1	Rest	Pre	247.000	69.0000	152.000
14	RR5	F	42	64.5	Water	2	24	Pre	247.295	66.2214	163.484
15	RR5	F	42	64.5	Water	3	Rest	Post	229.000	65.0000	143.000
16	RR5	F	42	64.5	Water	4	24	Post	226.246	60.5308	144.877
17	DD7	F	58	63.0	Land	1	Rest	Pre	189.000	57.0000	112.000
18	DD7	F	58	64.0	Land	2	24	Pre	191.119	61.7759	112.934
19	DD7	F	58	65.0	Land	3	Rest	Post	186.000	59.0000	110.000
20	DD7	F	58	66.0	Land	4	24	Post	190.651	59.0342	116.133

Obs	TAGS	GLUCOSES	TCSPE	TAGSPE	VLDLSPE	LDLSPE	RLPSPE	IDLSPE	LDL3SPE	LDL4SPE
11	62.000	94.000	130.000	59.000	12.0000	78.000	26.0000	23.0000	9.0000	4.0000
12	56.821	95.698	134.575	56.821	10.9654	82.739	25.9182	22.9276	9.9685	3.9874
13	128.000	96.000	259.000	132.000	26.0000	169.000	53.0000	46.0000	20.0000	7.0000
14	88.985	90.020	239.018	85.363	13.6582	167.519	44.8029	40.5606	20.9011	6.8291
15	107.000	96.000	237.000	103.000	20.0000	159.000	59.0000	53.0000	17.0000	5.0000
16	106.177	93.277	234.185	104.192	20.8385	156.785	48.6231	43.6615	15.8769	5.9538
17	99.000	73.000	194.000	67.000	12.0000	112.000	29.0000	26.0000	15.0000	7.0000
18	82.046	86.872	188.417	62.645	10.7143	109.942	27.8957	24.9999	14.3822	7.5289
19	83.000	90.000	183.000	59.000	10.0000	106.000	32.0000	30.0000	14.0000	7.0000
20	75.486	90.971	189.684	60.002	9.6777	114.197	31.9365	30.0010	15.4844	7.7422

Obs	HDLSPE	HDL2BSPE	HDL2ASPE	HDL3SPE	TCHDLSPE	VLDLPRT	LDLPRT	RLPPRT	LDL3PRT
11	40.0000	14.0000	6.0000	20.0000	3.25000	44.000	515.00	110.000	87.000
12	41.8679	13.9560	4.9843	22.9276	3.21429	40.871	545.28	111.648	94.701
13	63.0000	26.0000	9.0000	27.0000	4.11111	101.000	1109.00	223.000	194.000
14	57.8402	24.1087	8.1742	25.4538	4.13238	52.770	1099.90	190.386	198.664
15	59.0000	25.0000	11.0000	2.0000	4.01695	77.000	1000.00	251.000	159.000
16	56.5615	23.8154	7.9385	23.8154	4.14035	78.392	1009.18	206.400	148.846
17	70.0000	28.0000	11.0000	31.0000	2.77143	44.000	756.00	123.000	141.000
18	67.7604	29.7297	9.6525	28.3783	2.78063	40.540	745.17	118.726	136.100
19	67.0000	28.0000	10.0000	30.0000	2.73134	38.000	704.00	136.000	133.000
20	65.8086	29.0332	9.6777	28.0654	2.88235	36.775	765.51	135.488	146.134

Obs	LDL4PRT	HDLPRT	HDL2BPRT	LDLDENS	LPaSPE	CRPSPE	INSULIN	HDLDENS
11	47.000	7705.00	1224.00	1.0307	9.9000	0.11000	6.000	1.097
12	46.852	8030.65	1259.03	1.0307	11.7629	0.11962	7.377	1.099
13	87.000	11149.00	2353.00	1.0307	.	0.09000	15.800	1.092
14	84.846	10282.94	2168.75	1.0306	5.8978	0.06208	5.898	1.092
15	60.000	10012.00	2255.00	1.0305	.	0.09000	8.700	1.090
16	75.415	9966.74	2178.12	1.0301	.	0.06946	7.938	1.092
17	87.000	12550.00	2524.00	1.0309	75.9000	0.17000	7.000	1.094
18	93.629	11800.16	2670.84	1.0309	73.2624	0.21235	6.564	1.091
19	83.000	11982.00	2500.00	1.0311	75.9000	0.14000	8.000	1.092
20	96.777	11509.73	2607.18	1.0311	74.9057	0.22259	8.807	1.091

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	TCS	HDLS	LDLS
21	BB8	M	49	70.0	Water	1	Rest	Pre	198.000	49.0000	136.000
22	BB8	M	50	69.0	Water	2	24	Pre	186.236	45.8122	124.490
23	BB8	M	51	68.0	Water	3	Rest	Post	234.000	53.0000	151.000
24	BB8	M	52	67.0	Water	4	24	Post	223.333	49.4009	139.969
25	JC9	M	35	75.0	Water	1	Rest	Pre	173.000	46.0000	112.000
26	JC9	M	35	75.0	Water	2	24	Pre	176.895	48.5382	113.256
27	JC9	M	35	75.0	Water	3	Rest	Post	180.000	46.0000	110.000
28	JC9	M	35	75.0	Water	4	24	Post	179.419	43.5876	113.531
29	SD11	F	59	62.0	Land	1	Rest	Pre	218.000	79.0000	130.000
30	SD11	F	59	62.0	Land	2	24	Pre	226.136	80.9112	135.889

Obs	TAGS	GLUCOSES	TCSPE	TAGSPE	VLDLSPE	LDLSPE	RLPSPE	IDLSPE	LDL3SPE	LDL4SPE
21	66.000	97.000	167.000	63.000	10.0000	122.000	19.0000	17.0000	20.0000	7.0000
22	80.669	86.645	153.371	55.572	9.0628	105.866	15.5363	12.9469	17.4285	6.4735
23	151.000	103.000	204.000	79.000	15.0000	133.000	26.0000	22.0000	23.0000	7.0000
24	168.786	107.035	203.779	101.889	20.5837	131.736	27.7880	22.6421	30.8756	9.2627
25	74.000	85.000	169.000	73.000	14.0000	109.000	23.0000	20.0000	21.0000	6.0000
26	74.425	100.312	161.902	66.120	11.9728	103.225	19.8467	16.5030	21.7883	6.4718
27	120.000	91.000	177.000	78.000	15.0000	115.000	27.0000	23.0000	19.0000	5.0000
28	110.490	90.216	177.391	80.080	16.2186	115.558	27.3690	23.3143	19.2596	6.0820
29	46.000	87.000	194.000	46.000	6.0000	115.000	15.0000	14.0000	24.0000	8.0000
30	48.754	89.210	224.580	60.580	8.9210	133.400	22.0950	19.3979	22.5099	5.6015

Obs	HDLSPE	HDL2BSPE	HDL2ASPE	HDL3SPE	TCHDLSPE	VLDLPRT	LDLPRT	RLPPRT	LDL3PRT
21	35.0000	11.0000	5.0000	19.0000	4.77143	39.000	852.00	83.000	191.000
22	38.5420	11.3534	5.1788	22.0098	3.97933	34.857	745.94	65.730	166.318
23	55.0000	18.0000	5.0000	32.0000	3.70909	59.000	929.00	112.000	219.000
24	51.4593	18.5253	5.1459	27.7880	3.96000	78.218	965.38	118.356	292.289
25	47.0000	16.0000	6.0000	25.0000	3.59574	52.000	760.00	98.000	198.000
26	46.7045	16.2873	7.4425	22.9748	3.46651	46.381	738.86	84.133	207.096
27	47.0000	16.0000	7.0000	24.0000	3.76596	59.000	786.00	114.000	181.000
28	46.6286	15.2050	5.0683	26.3553	3.80435	60.820	788.63	115.558	183.473
29	73.0000	31.0000	12.0000	30.0000	2.65753	23.000	827.00	66.000	231.000
30	82.2597	39.2108	17.2196	25.8293	2.73014	34.232	918.03	93.359	213.689

Obs	LDL4PRT	HDLPRT	HDL2BPRT	LDLDENS	LPaSPE	CRPSPE	INSULIN	HDLDENS
21	84.000	6921.00	964.00	1.0316	.	0.21000	10.100	1.096
22	79.673	7712.38	1017.83	1.0316	.	0.19918	9.860	1.099
23	84.000	10958.00	1607.00	1.0323	.	0.25000	27.200	1.100
24	120.415	9849.31	1624.06	1.0332	.	0.28817	23.877	1.097
25	71.000	9059.00	1417.00	1.0326	10.4000	0.45000	15.300	1.098
26	79.818	8785.42	1463.70	1.0329	10.8941	0.61482	11.002	1.096
27	63.000	8904.00	1437.00	1.0318	11.3000	0.60000	10.000	1.097
28	69.943	9192.93	1333.98	1.0319	12.1640	0.62847	12.265	1.101
29	93.000	12739.00	2781.00	1.0324	7.3000	0.09000	5.600	1.092
30	69.501	13292.26	3528.97	1.0317	9.2322	0.18672	5.705	1.086

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	TCS	HDLS	LDLS
31	SD11	F	59	62.0	Land	3	Rest	Post	227.000	85.0000	126.000
32	SD11	F	59	62.0	Land	4	24	Post	229.002	84.7408	130.138
33	KN12	M	29	71.0	Land	1	Rest	Pre	190.000	34.0000	125.000
34	KN12	M	30	71.0	Land	2	24	Pre	193.046	32.8814	120.919
35	KN12	M	31	71.0	Land	3	Rest	Post	187.000	32.0000	102.000
36	KN12	M	32	71.0	Land	4	24	Post	189.409	32.1811	112.174
37	TS16	M	37	72.5	Land	1	Rest	Pre	138.000	68.0000	63.000
38	TS16	M	37	72.5	Land	2	24	Pre	138.749	68.3972	62.535
39	TS16	M	37	72.5	Land	3	Rest	Post	154.000	70.0000	69.000
40	TS16	M	37	72.5	Land	4	24	Post	151.682	71.1975	67.070

Obs	TAGS	GLUCOSES	TCSPE	TAGSPE	VLDLSPE	LDLSPE	RLPSPE	IDLSPE	LDL3SPE	LDL4SPE
31	80.000	90.000	222.000	79.000	14.0000	131.000	25.0000	22.0000	18.0000	6.0000
32	72.635	85.750	207.817	78.688	15.1323	178.561	26.2293	23.2028	17.1499	6.0529
33	154.000	126.000	180.000	85.000	17.0000	122.000	27.0000	23.0000	21.0000	6.0000
34	197.289	109.251	167.377	86.658	17.2893	113.070	25.5627	22.8049	19.4107	5.9399
35	265.000	134.000	181.000	80.000	15.0000	127.000	26.0000	23.0000	27.0000	7.0000
36	227.107	93.785	175.617	81.832	15.6308	120.449	25.7449	22.9865	18.3892	6.4362
37	33.000	89.000	115.000	41.000	8.0000	51.000	11.0000	9.0000	9.0000	6.0000
38	37.130	82.077	120.965	41.136	7.9145	54.620	10.4550	8.5985	9.8687	5.0809
39	73.000	89.000	146.000	61.000	13.0000	66.000	16.0000	13.0000	11.0000	6.0000
40	65.006	95.962	145.490	67.070	14.4459	61.911	14.4459	12.3822	11.3503	6.1911

Obs	HDLSPE	HDL2BSPE	HDL2ASPE	HDL3SPE	TCHDLSPE	VLDLPRT	LDLPRT	RLPPRT	LDL3PRT
31	77.0000	35.0000	16.0000	25.0000	2.88312	54.000	889.00	107.000	176.000
32	74.6526	35.3086	17.1499	22.1940	2.78378	58.511	806.05	112.988	166.455
33	41.0000	13.0000	3.0000	25.0000	4.39024	64.000	841.00	114.000	200.000
34	36.9121	12.5162	3.6064	20.7895	4.53448	66.824	775.37	108.191	184.560
35	39.0000	13.0000	3.0000	22.0000	4.64103	59.000	891.00	111.000	255.000
36	39.5368	13.7919	3.6778	22.9865	4.44186	61.604	817.40	111.255	176.536
37	56.0000	27.0000	10.0000	20.0000	2.05357	31.000	377.00	46.000	90.000
38	58.4307	28.2383	11.7252	18.3695	2.07023	30.290	396.70	44.947	93.802
39	67.0000	32.0000	12.0000	23.0000	2.17910	49.000	472.00	66.000	108.000
40	68.1019	33.0191	11.3503	23.7325	2.13636	55.720	449.89	62.943	111.440

Obs	LDL4PRT	HDLPRT	HDL2BPRT	LDLDENS	LPaSPE	CRPSPE	INSULIN	HDLDENS
31	75.000	12582.00	3150.00	1.0312	10.2000	0.13000	6.100	1.087
32	80.705	11946.43	3177.78	1.0315	11.1979	0.21185	7.566	1.085
33	77.000	8282.00	1198.00	1.0320	.	0.79000	46.800	1.101
34	73.188	7209.52	1126.45	1.0324	.	.	24.290	1.099
35	85.000	7605.00	1198.00	1.0329	5.0000	0.38000	105.700	1.099
36	76.315	7754.73	1224.72	1.0316	4.5973	0.44134	91.119	1.099
37	72.000	9314.00	2393.00	1.0324	.	0.02000	.	1.086
38	62.535	9426.11	2540.47	1.0321	.	0.02931	.	1.085
39	68.000	11111.00	2863.00	1.0321	.	0.05000	.	1.086
40	70.166	11289.44	2954.18	1.0324	.	0.05159	.	1.086

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	TCS	HDLS	LDLS
41	CM19	M	35	65.0	Land	1	Rest	Pre	161.000	44.0000	100.000
42	CM19	M	35	65.0	Land	2	24	Pre	151.664	45.7069	86.220
43	CM19	M	35	65.0	Land	3	Rest	Post	163.000	40.0000	88.000
44	CM19	M	35	65.0	Land	4	24	Post	152.480	37.1426	84.060
45	GJ20	M	51	68.0	Land	1	Rest	Pre	200.000	33.0000	127.000
46	GJ20	M	52	68.0	Land	2	24	Pre	191.242	32.3797	128.507
47	GJ20	M	53	68.0	Land	3	Rest	Post	206.000	38.0000	142.000
48	GJ20	M	54	68.0	Land	4	24	Post	215.087	39.7083	150.009
49	MP22	F	51	67.0	Water	1	Rest	Pre	.	.	.
50	MP22	F	52	67.0	Water	2	24	Pre	.	.	.

Obs	TAGS	GLUCOSES	TCSPE	TAGSPE	VLDLSPE	LDLSPE	RLPSPE	IDLSPE	LDL3SPE	LDL4SPE
41	87.000	98.000	152.000	77.000	15.0000	103.000	34.0000	30.0000	11.0000	4.0000
42	98.685	104.918	150.937	81.649	16.5168	99.932	29.1901	25.1388	14.0237	4.3629
43	173.000	94.000	162.000	110.000	23.0000	104.000	27.0000	24.0000	16.0000	6.0000
44	154.435	93.834	144.661	98.721	20.5262	87.969	22.4811	19.5488	15.6390	4.8872
45	200.000	92.000	187.000	103.000	21.0000	124.000	26.0000	21.0000	43.0000	15.0000
46	152.792	89.044	191.647	110.799	22.5646	123.447	31.1654	25.7014	41.2841	11.3329
47	131.000	94.000	203.000	90.000	18.0000	135.000	24.0000	20.0000	41.0000	10.0000
48	129.052	100.374	205.160	84.932	15.4421	136.773	24.2662	20.9572	45.2234	13.2361
49	.	.	.	.	.	.	.	.	.	.
50	.	.	.	.	.	.	.	.	.	.

Obs	HDLSPE	HDL2BSPE	HDL2ASPE	HDL3SPE	TCHDLSPE	VLDLPRT	LDLPRT	RLPPRT	LDL3PRT
41	33.0000	10.0000	4.0000	19.0000	4.60606	59.000	665.00	142.000	104.000
42	34.5918	10.8034	4.3629	19.4254	4.36336	63.366	666.90	123.616	132.965
43	36.0000	11.0000	4.0000	21.0000	4.50000	88.000	706.00	115.000	151.000
44	35.1878	10.7518	3.9098	20.5262	4.11111	79.172	608.94	96.766	144.661
45	42.0000	15.0000	6.0000	21.0000	4.45238	80.000	985.00	111.000	409.000
46	45.5339	17.1005	6.2736	22.1598	4.20889	87.020	949.13	132.554	392.603
47	50.0000	20.0000	6.0000	24.0000	4.06000	68.000	1014.00	103.000	388.000
48	54.0474	18.7512	6.6181	28.6782	3.79592	58.459	1059.99	104.786	431.277
49	.	.	.	.	.	.	.	.	.
50	.	.	.	.	.	.	.	.	.

Obs	LDL4PRT	HDLPRT	HDL2BPRT	LDLDENS	LPaSPE	CRPSPE	INSULIN	HDLDENS
41	51.000	6648.00	864.00	1.0303	7.9000	.	21.400	1.100
42	54.017	6836.29	970.23	1.0311	7.3754	0.83103	23.165	1.098
43	77.000	7125.00	1004.00	1.0318	9.1000	0.58000	12.500	1.099
44	66.466	7141.16	958.87	1.0321	8.1127	0.57669	21.797	1.100
45	181.000	7875.00	1350.00	1.0344	46.6000	0.15000	4.800	1.096
46	139.637	8482.46	1542.08	1.0344	45.3315	0.25297	5.161	1.092
47	119.000	9223.00	1767.00	1.0338	46.6000	0.10000	5.900	1.092
48	163.245	10315.34	1643.48	1.0342	47.4294	0.12133	6.397	1.095
49	.	.	.	.	.	.	.	.
50	.	.	.	.	.	.	.	.

Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	TCS	HDLS	LDLS
51	MP22	F	53	67.0	Water	3	Rest	Post	.	.	.
52	MP22	F	54	67.0	Water	4	24	Post	.	.	.
53	GA24	M	49	69.5	Land	1	Rest	Pre	163.000	37.0000	88.000
54	GA24	M	49	69.5	Land	2	24	Pre	153.502	37.1214	91.299
55	GA24	M	49	69.5	Land	3	Rest	Post	189.000	38.0000	124.000
56	GA24	M	49	69.5	Land	4	24	Post	194.804	40.2312	131.281
57	HW14	F	.	.	Land	1	Rest	Pre	183.000	43.0000	102.000
58	HW14	F	.	.	Land	2	24	Pre	170.893	44.7577	101.722
59	HW14	F	.	.	Land	3	Rest	Post	.	.	.
60	HW14	F	.	.	Land	4	24	Post	.	.	.

Obs	TAGS	GLUCOSES	TCSPE	TAGSPE	VLDLSPE	LDLSPE	RLPSPE	IDLSPE	LDL3SPE	LDL4SPE
51	.	.	.	.	.	.	.	.	.	.
52	.	.	.	.	.	.	.	.	.	.
53	189.000	108.000	154.000	137.000	29.0000	87.000	32.0000	25.0000	18.0000	7.0000
54	126.413	108.354	140.359	101.733	21.3699	82.169	31.0014	26.5869	16.1528	5.5180
55	133.000	103.000	171.000	107.000	22.0000	111.000	35.0000	31.0000	21.0000	8.0000
56	115.400	111.165	168.336	82.580	15.8807	113.282	32.8202	28.5853	19.0569	7.4110
57	192.000	89.000	173.000	77.000	15.0000	101.000	28.0000	24.0000	17.0000	6.0000
58	123.084	88.498	167.841	72.223	13.7325	99.993	31.6356	27.9736	14.5463	6.0016
59	.	.	.	.	.	.	.	.	.	.
60	.	.	.	.	.	.	.	.	.	.

Obs	HDLSPE	HDL2BSPE	HDL2ASPE	HDL3SPE	TCHDLSPE	VLDLPRT	LDLPRT	RLPPRT	LDL3PRT
51	.	.	.	.	.	.	.	.	.
52	.	.	.	.	.	.	.	.	.
53	38.0000	13.0000	4.0000	21.0000	4.05263	113.000	622.00	137.000	173.000
54	36.8204	12.2400	4.2138	20.3666	3.81199	82.269	566.85	131.430	153.502
55	38.0000	12.0000	4.0000	22.0000	4.50000	85.000	766.00	147.000	200.000
56	40.2312	13.7633	4.2349	22.2330	4.18421	60.347	777.10	137.633	183.158
57	56.0000	19.0000	8.0000	28.0000	3.08929	59.000	692.00	117.000	162.000
58	54.1161	17.5979	4.9844	31.6356	3.10150	52.895	670.35	134.273	138.342
59	.	.	.	.	.	.	.	.	.
60	.	.	.	.	.	.	.	.	.

Obs	LDL4PRT	HDLPRT	HDL2BPRT	LDLDENS	LPaSPE	CRPSPE	INSULIN	HDLDENS
51	.	.	.	.	.	.	.	.
52	.	.	.	.	.	.	.	.
53	87.000	7494.00	1169.00	1.0335	22.4000	0.17000	14.200	1.098
54	68.223	7174.46	1102.61	1.0330	23.4768	.	17.156	1.098
55	96.000	7561.00	1099.00	1.0327	27.0000	0.04000	8.600	1.099
56	88.932	7837.67	1212.23	1.0322	29.6440	0.02117	10.058	1.098
57	74.000	10569.00	1748.00	1.0321	15.4000	0.45000	8.800	1.096
58	74.257	10714.38	1576.69	1.0319	15.4618	0.46792	7.222	1.101
59	.	.	.	.	.	.	.	.
60	.	.	.	.	.	.	.	.

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	TCS	HDLS	LDLS
61	PR15	F	.	.	Water	1	Rest	Pre	172.000	54.0000	97.000
62	PR15	F	.	.	Water	2	24	Pre	175.999	50.7480	102.576
63	PR15	F	.	.	Water	3	Rest	Post	.	.	.
64	PR15	F	.	.	Water	4	24	Post	.	.	.
65	KE23	F	.	.	Land	1	Rest	Pre	187.000	43.0000	125.000
66	KE23	F	.	.	Land	2	24	Pre	188.403	42.5427	126.615
67	KE23	F	.	.	Land	3	Rest	Post	.	.	.
68	KE23	F	.	.	Land	4	24	Post	.	.	.
69	CR21	M	.	.	Water	1	Rest	Pre	214.000	35.0000	138.000
70	CR21	M	.	.	Water	2	24	Pre	210.819	34.2448	132.698

Obs	TAGS	GLUCOSES	TCSPE	TAGSPE	VLDLSPE	LDLSPE	RLPSPE	IDLSPE	LDL3SPE	LDL4SPE
61	104.000	99.000	172.000	61.000	11.0000	104.000	25.0000	23.0000	14.0000	7.0000
62	113.373	105.815	181.613	77.310	14.5766	109.378	34.6598	30.8807	14.5766	5.2908
63	.	.	.	.	.	.	.	.	.	.
64	.	.	.	.	.	.	.	.	.	.
65	94.000	91.000	201.000	76.000	13.0000	136.000	49.0000	44.0000	13.0000	5.0000
66	96.228	94.202	182.934	68.271	11.4460	123.374	39.9091	36.9716	13.1680	5.4698
67	.	.	.	.	.	.	.	.	.	.
68	.	.	.	.	.	.	.	.	.	.
69	203.000	91.000	259.000	204.000	40.0000	168.000	46.0000	38.0000	33.0000	10.0000
70	221.521	92.033	267.002	197.764	38.0973	179.785	49.4409	42.3779	30.3922	9.8454

Obs	HDLSPE	HDL2BSPE	HDL2ASPE	HDL3SPE	TCHDLSPE	VLDLPRT	LDLPRT	RLPPRT	LDL3PRT
61	58.0000	22.0000	7.0000	29.0000	2.96552	40.000	705.00	104.000	136.000
62	57.7664	22.1348	7.5582	27.9654	3.14393	56.147	720.19	146.845	138.207
63	.	.	.	.	.	.	.	.	.
64	.	.	.	.	.	.	.	.	.
65	53.0000	20.0000	6.0000	27.0000	3.79245	50.000	859.00	206.000	126.000
66	48.1138	17.5235	5.1659	25.4243	3.80211	43.556	789.07	169.158	124.589
67	.	.	.	.	.	.	.	.	.
68	.	.	.	.	.	.	.	.	.
69	52.0000	15.0000	5.0000	31.0000	4.98077	152.000	1181.00	195.000	313.000
70	49.1198	14.1260	5.6718	29.3221	5.43573	146.610	1232.81	209.749	288.940

Obs	LDL4PRT	HDLPRT	HDL2BPRT	LDLDENS	LPaSPE	CRPSPE	INSULIN	HDLDENS
61	83.000	10741.00	1942.00	1.0310	46.1000	0.20000	11.000	1.096
62	64.785	10658.17	1992.13	1.0311	50.1002	0.30233	14.037	1.094
63	.	.	.	.	.	.	.	.
64	.	.	.	.	.	.	.	.
65	67.000	9886.00	1794.00	1.0297	24.4000	.	7.600	1.096
66	67.866	9152.76	1572.05	1.0304	25.6269	.	8.103	1.097
67	.	.	.	.	.	.	.	.
68	.	.	.	.	.	.	.	.
69	127.000	10512.00	1363.00	1.0329	18.5000	0.56000	24.600	1.102
70	121.997	9953.45	1269.20	1.0321	20.7609	0.65279	17.443	1.101

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	TCS	HDLS	LDLS
71	CR21	M	.	.	Water	3	Rest	Post	.	.	.
72	CR21	M	.	.	Water	4	24	Post	.	.	.

Obs	TAGS	GLUCOSES	TCSPE	TAGSPE	VLDLSPE	LDLSPE	RLPSPE	IDLSPE	LDL3SPE	LDL4SPE
71	.	.	.	.	.	.	.	.	.	.
72	.	.	.	.	.	.	.	.	.	.

Obs	HDLSPE	HDL2BSPE	HDL2ASPE	HDL3SPE	TCHDLSPE	VLDLPRT	LDLPRT	RLPPRT	LDL3PRT
71	.	.	.	.	.	.	.	.	.
72	.	.	.	.	.	.	.	.	.

Obs	LDL4PRT	HDLPRT	HDL2BPRT	LDLDENS	LPaSPE	CRPSPE	INSULIN	HDLDENS
71	.	.	.	.	.	.	.	.
72	.	.	.	.	.	.	.	.

APPENDIX H

SAMPLE STATISTICAL CODE AND RAW DATA FOR CHAPTER III

Variable Definitions:

Independent Variables

Unique Identifier	**ID**
Gender (M F)	**GENDER**
Age (years)	**AGE**
Height (inches)	**HEIGHT**
Treadmill Type (LAND or WATER)	**MODE**
Timepoint	**TIMEPT**
Training Time Point (PRE or POST)	**TRAINING**
Acute Exercise (Rest or 24)	**EX**

Definition of TIMEPT variable

TIMEPT has 4 levels corresponding to each combination of acute exercise and training times such that:

- 1 = pretraining, resting (immediate pre-ex for blood variables, 3 days pre-ex for muscle)
-2= pretraining, 24 h postexercise
-3= posttraining, resting (immediate pre-ex for blood variables, no muscle biopsy attained
-4= posttraining, 24 h postexercise for blood and muscle

Dependent Variables

Peroxisome Proliferator-Activated Receptor δ	**PPARD**
PPARδ "Relative Phosphorylation"	**PPARDPHO**
PPARδ "Total Phosphorylation"	**TOTALPHOS**
AMP-activated Protein Kinase α	**AMPK**
PPARγ Coactivator-1α	**PGC1a**
F1 ATPase (primary subunit of ATP Synthase)	**F1ATPase**
Carnitine-Palmitoyl Transferase-I	**CPTI**
FAT/CD36 (Fatty Acid Translocase)	**CD36**
Cytochrome C Oxidase-IV	**COXIV**
Peroxisome Proliferator-Activated Receptor α	**PPARα**
ATP Binding Cassette A1	**ABCA1**
Lipoprotein Lipase	**LPL**
LDL Receptor	**LDLR**

Repeated Measures ANOVA

Data were analyzed using the Statistical Analysis System (SAS) version 9.2. SAS process for data analysis used was PROC MIXED to determine change in content of the above listed proteins due to the exercise intervention. By use of PROC MIXED the best statistical fit to the repeated-measures effects was able to be chosen for each dependent variable of interest. Because only one muscle sample was obtained after exercise training the analysis for protein content was based on the TIMEPT variable for repeated measures, defined above. Sample code:

```
PROC MIXED;
CLASS ID  GENDER  TIMEPT;
MODEL LPL= TIMEPT  ;
REPEATED TIMEPT/ SUB=ID TYPE=CSH RCORR ;
LSMEANS TIMEPT  / PDIFF=ALL ADJUST=TUKEY ;
RUN;

QUIT;
```

The above code allows for the analysis of any main or interactive effects of gender, acute exercise, and exercise training. The statement "REPEATED" allows for the system to model the statistic based on the repeated-measures variables indicated, for this analysis: TIMEPT. The statement TYPE on this line of code provides a place to input methods of modeling for the covariance matrix of the repeated variables. The following two lines set up post hoc analysis across all conditions in the MODEL statement. Differences here are determined across all levels (PDIFF=ALL) using the Tukey-Kramer method.

Correlations

For the assessment of correlation correlation of PPARδ, PPARα, PGC-1α, and AMPKα to blood lipid and lipoprotein variables the PROC CORR process was used.

Blood lipid and lipoprotein raw data presented in Appendix G

This process enabled the assessment of Pearson's Product-Moment correlations on all variables of interest. Sample Code:

```
PROC SORT;
BY TIMEPT;

PROC CORR;

BY TIMEPT;
   VAR PPARD PPARDPHO TOTALPHOS AMPK PGC1a PPARa ABCA1 LPL;

   WITH  VO2PEAKR PPARD PPARDPHO TOTALPHOS AMPK PGC1a F1ATPase
CPTI CD36 COXIV PPARa ABCA1 LPL TCS       HDLS  LDLS  TAGS  GLUCOSES
```

```
        TCSPE     TAGSPE    VLDLSPE   LDLSPE    RLPSPE
        IDLSPE    LDL3SPE   LDL4SPE   HDLSPE    HDL2BSPE
        HDL2ASPE  HDL3SPE   TCHDLSPE  VLDLPRT   LDLPRT
        RLPPRT    LDL3PRT   LDL4PRT   HDLPRT    HDL2BPRT
        LDLDENS   LPaSPE    CRPSPE    INSULIN   HDLDENS NEFA;
RUN;

QUIT;
```

To assess correlations at each collection period in which blood and muscle samples were each obtained it was first necessary to sort data by variable TIMEPT. Therefore, prior to running PROC CORR, PROC SORT was run for this variable. This was followed by PROC CORR which allowed the assessment of correlations at each level of TIMEPT for protein variables of interest with all protein and blood variables.

Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	PPARD	PPARDPHO	TOTALPHOS	AMPK
1	JG1	M	40	68.0	Water	1	Rest	Pre	.	.	.	0.50617
2	JG1	M	40	68.0	Water	2	24	Pre	.	.	.	0.58025
3	JG1	M	40	68.0	Water	3	Rest	Post	.	.	.	.
4	JG1	M	40	68.0	Water	4	24	Post	.	.	.	1.14815
5	RK2	F	51	64.5	Water	1	Rest	Pre	1.0600	0.38679	0.41	.
6	RK2	F	51	64.5	Water	2	24	Pre	3.9200	0.30867	1.21	.
7	RK2	F	51	64.5	Water	3	Rest	Post	.	.	.	.
8	RK2	F	51	64.5	Water	4	24	Post	0.7700	0.24675	0.19	.
9	GB3	M	39	73.0	Water	1	Rest	Pre	1.6400	0.22561	0.37	0.60000
10	GB3	M	39	73.0	Water	2	24	Pre	1.3300	0.62406	0.83	0.70000
11	GB3	M	39	73.0	Water	3	Rest	Post	.	.	.	.
12	GB3	M	39	73.0	Water	4	24	Post	3.4100	0.06452	0.22	1.17500
13	RR5	F	42	64.5	Water	1	Rest	Pre	0.5000	0.38000	0.19	1.12500
14	RR5	F	42	64.5	Water	2	24	Pre	2.6700	0.12360	0.33	1.35000
15	RR5	F	42	64.5	Water	3	Rest	Post	.	.	.	.
16	RR5	F	42	64.5	Water	4	24	Post	2.2600	0.08850	0.20	1.67500
17	DD7	F	58	63.0	Land	1	Rest	Pre	2.4300	0.35802	0.87	.
18	DD7	F	58	64.0	Land	2	24	Pre	1.3300	0.42105	0.56	.
19	DD7	F	58	65.0	Land	3	Rest	Post	.	.	.	.
20	DD7	F	58	66.0	Land	4	24	Post	2.0100	0.36318	0.73	.
21	BB8	M	49	70.0	Water	1	Rest	Pre	1.1000	0.23636	0.26	.
22	BB8	M	50	69.0	Water	2	24	Pre	1.7800	0.19663	0.35	.
23	BB8	M	51	68.0	Water	3	Rest	Post	.	.	.	.

Obs	PGC1a	F1ATPase	CPTI	CD36	COXIV	PPARa	ABCA1	LPL	LDLR
1	0.77647	4.9605	0.98139	0.63868	1.63750	1.29730	1.17857	0.85553	0.95279
2	1.21765	4.0855	1.01270	0.79414	1.72750	1.32432	1.02679	1.44724	0.55365
3	.	.	.	.	.	.	.	.	.
4	0.85490	1.9539	1.26693	0.67719	1.75750	2.31532	1.14286	2.07161	0.52361
5	0.94118	2.4243	1.23580	0.63158	1.04125	1.68468	1.38393	1.66960	0.69099
6	1.48431	4.4441	1.00864	0.74561	1.17600	1.15315	0.98214	1.96859	0.57082
7	.	.	.	.	.	.	.	.	.
8	1.39216	4.2730	.	0.85088	1.37400	1.54054	.	.	.
9	1.19391	11.3684	0.89474	1.61078	0.95333	1.21429	0.89286	1.11368	0.99379
10	1.56233	9.1278	0.96053	2.22607	1.40333	1.23469	0.84821	1.33702	0.74534
11	.	.	.	.	.	.	.	.	.
12	1.69252	6.8947	0.89912	2.18822	1.45000	1.23469	0.87500	1.12575	0.66460
13	1.01662	8.4211	0.88596	1.00426	1.11688	1.38776	0.92857	1.10060	1.07453
14	1.31302	7.6617	0.96053	1.36291	1.41125	1.54082	1.01786	1.13883	0.93789
15	.	.	.	.	.	.	.	.	.
16	1.47368	8.2632	.	1.51529	1.64625	2.20408	1.00893	1.35211	0.81988
17	1.03704	7.9792	0.71397	0.86239	0.91950	0.75862	0.90323	0.56470	1.42553
18	1.81893	6.7083	0.83885	1.09174	0.73100	0.51724	0.70968	0.76178	0.93617
19	.	.	.	.	.	.	.	.	.
20	1.52675	8.9792	.	0.84404	1.09850	0.60920	0.74194	0.41045	1.01064
21	1.12346	9.3854	0.21569	1.04587	1.15050	0.71264	0.48387	0.36075	0.75532
22	1.10288	13.8229	0.43137	1.27523	1.41450	0.79310	0.89516	0.61782	1.21809
23	.	.	.	.	.	.	.	.	.

Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	PPARD	PPARDPHO	TOTALPHOS	AMPK
24	BB8	M	52	67.0	Water	4	24	Post	0.2000	0.35000	0.07	.
25	JC9	M	35	75.0	Water	1	Rest	Pre	2.3800	0.21008	0.50	0.66667
26	JC9	M	35	75.0	Water	2	24	Pre	.	0.35989	6.46	1.33333
27	JC9	M	35	75.0	Water	3	Rest	Post	.	.	.	.
28	JC9	M	35	75.0	Water	4	24	Post	4.6900	0.25800	1.21	0.88889
29	SD11	F	59	62.0	Land	1	Rest	Pre	0.2100	0.42857	0.09	1.66667

30	SD11	F	59	62.0	Land	2	24	Pre	0.8000	0.38750	0.31	2.33333
31	SD11	F	59	62.0	Land	3	Rest	Post	.	.	.	.
32	SD11	F	59	62.0	Land	4	24	Post	0.4400	0.43182	0.19	4.00000
33	KN12	M	29	71.0	Land	1	Rest	Pre	1.5000	0.58667	0.88	0.35772
34	KN12	M	30	71.0	Land	2	24	Pre	1.3700	0.33577	0.46	0.53659
35	KN12	M	31	71.0	Land	3	Rest	Post	.	.	.	.
36	KN12	M	32	71.0	Land	4	24	Post	8.0600	0.37345	3.01	1.32520
37	TS16	M	37	72.5	Land	1	Rest	Pre	2.9300	0.40956	1.20	1.47967
38	TS16	M	37	72.5	Land	2	24	Pre	4.1500	0.39036	1.62	0.93496
39	TS16	M	37	72.5	Land	3	Rest	Post	.	.	.	.
40	TS16	M	37	72.5	Land	4	24	Post	5.0200	0.34462	1.73	1.57724
41	CM19	M	35	65.0	Land	1	Rest	Pre	2.8600	0.26224	0.75	0.23810
42	CM19	M	35	65.0	Land	2	24	Pre	3.9500	0.32152	1.27	0.36905
43	CM19	M	35	65.0	Land	3	Rest	Post	.	.	.	.
44	CM19	M	35	65.0	Land	4	24	Post	8.6700	0.32641	2.83	0.76190
45	GJ20	M	51	68.0	Land	1	Rest	Pre	0.2000	0.35374	1.56	1.17857
46	GJ20	M	52	68.0	Land	2	24	Pre	2.2800	0.26754	0.61	0.96429

Obs	PGC1a	F1ATPase	CPTI	CD36	COXIV	PPARa	ABCA1	LPL	LDLR
24	1.25103	12.3542	0.62745	1.28440	1.30900	0.79310	0.88710	0.78149	1.11170
25	0.62745	8.3115	0.89773	.	1.65000	.	0.62617	0.59302	1.75000
26	0.82353	4.9180	1.18182	.	1.35833	.	0.92523	1.17442	1.65385
27	.	.	.	.	.	.	.	.	.
28	0.69935	6.8852	0.87500	.	2.24833	.	0.66355	1.08140	1.55769
29	0.43791	7.9180	0.61072	.	0.96875	.	0.53271	1.17110	1.18269
30	0.35294	7.9344	1.07241	.	1.10375	.	0.50467	0.81395	1.37500
31	.	.	.	.	.	.	.	.	.
32	0.30719	7.9016	1.39746	.	0.84500	.	0.47664	.	2.08654
33	1.33333	20.0833	1.04967	1.03712	0.83400	1.34673	0.69828	0.48528	0.66964
34	1.65714	18.6667	0.96026	1.23242	0.80200	3.69347	1.00862	0.63347	0.65774
35	.	.	.	.	.	.	.	.	.
36	.	.	.	.	.	.	.	.	.
37	1.68571	21.3333	1.05629	0.98969	0.78550	.	0.86207	0.54791	0.56250
38	1.60000	29.0833	1.35430	1.27835	0.94850	1.32663	0.65517	0.62663	0.72619
39	.	.	.	.	.	.	.	.	.
40	2.11429	24.9167	1.70199	1.50773	1.50225	2.03518	0.91379	0.78439	0.79464
41	1.33673	18.0000	.	1.47191	0.91733	1.16854	0.68462	1.15163	1.19512
42	1.23469	18.0909	.	1.40449	0.85983	1.04494	0.67692	1.11905	1.33740
43	.	.	.	.	.	.	.	.	.
44	1.67347	25.9545	.	1.65169	1.12750	1.38202	0.80000	1.56391	1.32114
45	1.15306	27.0000	.	1.34140	0.90175	1.10112	0.78462	1.59398	1.54065
46	1.57143	21.1364	.	1.46126	1.14100	1.06742	0.90000	1.82331	1.65041

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	PPARD	PPARDPHO	TOTALPHOS	AMPK
47	GJ20	M	53	68.0	Land	3	Rest	Post	.	.	.	.
48	GJ20	M	54	68.0	Land	4	24	Post	3.5900	0.34540	1.24	0.76190
49	MP22	F	51	67.0	Water	1	Rest	Pre	0.4025	0.35404	0.57	0.13830
50	MP22	F	52	67.0	Water	2	24	Pre	0.7550	0.30464	0.92	0.26596
51	MP22	F	53	67.0	Water	3	Rest	Post	.	.	.	.
52	MP22	F	54	67.0	Water	4	24	Post	.	.	.	.
53	GA24	M	49	69.5	Land	1	Rest	Pre	1.6225	0.33128	2.15	0.28723
54	GA24	M	49	69.5	Land	2	24	Pre	3.6775	0.38953	5.73	0.53191
55	GA24	M	49	69.5	Land	3	Rest	Post	.	.	.	.
56	GA24	M	49	69.5	Land	4	24	Post	3.0300	0.37129	4.50	0.28723
57	HW14	F	.	.	Land	1	Rest	Pre	0.8125	0.23692	0.77	0.07879
58	HW14	F	.	.	Land	2	24	Pre	0.5575	0.22870	0.51	0.16364
59	HW14	F	.	.	Land	3	Rest	Post	.	.	.	.
60	HW14	F	.	.	Land	4	24	Post	.	.	.	.
61	PR15	F	.	.	Water	1	Rest	Pre	.	.	.	0.49091
62	PR15	F	.	.	Water	2	24	Pre	.	.	.	0.92727
63	PR15	F	.	.	Water	3	Rest	Post	.	.	.	.
64	PR15	F	.	.	Water	4	24	Post	.	.	.	.
65	KE23	F	.	.	Land	1	Rest	Pre	.	.	.	.
66	KE23	F	.	.	Land	2	24	Pre	.	.	.	.
67	KE23	F	.	.	Land	3	Rest	Post	.	.	.	.
68	KE23	F	.	.	Land	4	24	Post	.	.	.	.
69	CR21	M	.	.	Water	1	Rest	Pre	.	.	.	.

Obs	PGC1a	F1ATPase	CPTI	CD36	COXIV	PPARa	ABCA1	LPL	LDLR
47	.	.	.	.	.	.	.	.	.
48	1.63265	16.4091	.	.	0.94825	1.88764	0.76154	1.89223	1.03659
49	2.22222	14.4000	0.73832	0.85000	0.71625	0.53642	1.21739	0.81614	1.01802
50	4.85185	18.6000	0.87850	1.55000	1.27500	1.12583	1.44928	1.11031	1.40541
51	.	.	.	.	.	.	.	.	.
52	.	.	.	.	.	.	.	.	.
53	3.03704	55.2000	1.10280	1.20000	1.06800	1.09272	1.68116	0.67892	1.74775
54	5.44444	60.4000	1.08411	1.28750	1.60850	0.96689	1.97101	0.91839	1.43243
55	.	.	.	.	.	.	.	.	.
56	4.14815	53.0000	1.20561	1.06250	1.16600	1.03974	2.15942	0.85112	1.19820
57	2.25000	40.5000	0.87654	0.68182	0.91000	0.36585	0.54667	0.53621	0.67811
58	3.08333	56.1667	1.01235	1.09917	1.09000	0.34146	0.58667	0.47845	0.55794
59	.	.	.	.	.	.	.	.	.
60	.	.	.	.	.	.	.	.	.
61	6.91667	.	.	0.55785	.	1.97561	0.61333	0.92672	.
62	9.33333	.	.	0.47107	.	3.07317	0.70667	0.95086	.
63	.	.	.	.	.	.	.	.	.
64	.	.	.	.	.	.	.	.	.
65	.	.	.	.	.	.	.	.	.
66	.	.	.	.	.	.	.	.	.
67	.	.	.	.	.	.	.	.	.
68	.	.	.	.	.	.	.	.	.
69	.	.	.	.	.	.	.	.	.

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TIMEPT	ACUTEEX	TRAIN	PPARD	PPARDPHO	TOTALPHOS	AMPK
70	CR21	M	.	.	Water	2	24	Pre	.	.	.	.
71	CR21	M	.	.	Water	3	Rest	Post	.	.	.	.
72	CR21	M	.	.	Water	4	24	Post	.	.	.	.

APPENDIX I

SAMPLE STATISTICAL CODE AND RAW DATA FOR CHAPTERS II AND III
DEMOGRAPHICS, PHYSIOLOGIC ADAPTATION, DATA AND ACTIVITY

Variable Definitions:

Independent Variables

Unique Identifier	**ID**
Gender (M F)	**GENDER**
Age (years)	**AGE**
Height (inches)	**HEIGHT**
Treadmill Type (LAND or WATER)	**MODE**
Timepoint	**TIMEPT**
Training Time Point (PRE or POST)	**TRAINING**
Acute Exercise (Rest or 24)	**EX**

Dependent Variables

Descriptive Variables

Resting Energy Expenditure (kcal/day)	**RMR**
Peak Measure VO2 (ml/min)	**VO2PEAKA**
Peak Measure VO2 (ml/kg/min)	**VO2PEAKR**
Bruce GXT time (sec)	**BRUCTIME**
Body Weight (lbs)	**BODYWT**
Body Mass (kg)	**BODYMASS**
Chest (skinfolds) (mm)	**CHEST**
Axilla (skinfolds) (mm)	**AXILLA**
Triceps (skinfolds) (mm)	**TRICEP**
Subscapula (skinfolds) (mm)	**SUBSCAP**
Abdominals (skinfolds) (mm)	**ABDOM**
Suprailiac (skinfolds) (mm)	**SUPRAIL**
Thigh (skinfolds) (mm)	**THIGH**
Sum of skinfold measurements (mm)	**SUM**
Body Density (Skinfolds)	**BODYDENS**
Body Mass Index	**BMI**
Body Fat % (Skinfold)	**PERCFTSK**
Waist Circumference (inches)	**WAIST**
Hip Circumference (inches)	**HIP**
Waist : Hip Ratio	**WHRATIO**
Body Fat % (DEXA)	**PERCFTDX**
% Android Fat (DEXA)	**ANDROID**
% Gynoid Fat (DEXA)	**GYNOID**

Android : Gynoid Ratio (DEXA)	**AGRATIO**
Lean Body Mass (g) (DEXA)	**LBM**
Left arm percent fat (DEXA)	**LAPERFAT**
Left leg percent fat (DEXA)	**LLPERFAT**
Left trunk percent fat (DEXA)	**LTRPRFAT**
Left total percent fat (DEXA)	**LTOPRFAT**
Right arm percent fat (DEXA)	**RAPERFAT**
Right leg percent fat (DEXA)	**RLPERFAT**
Right trunk percent fat (DEXA)	**RTRPRFAT**
Right total percent fat (DEXA)	**RTOPRFAT**
Arms percent fat (DEXA)	**ARMPRFAT**
Legs percent fat (DEXA)	**LEGPRFAT**
Trunk percent fat (DEXA)	**TRUPRFAT**
Left arm fat mass (g) (DEXA)	**LAFAT**
Left leg fat mass (g) (DEXA)	**LLFAT**
Left trunk fat mass (g) (DEXA)	**LTRFAT**
Left total fat mass (g) (DEXA)	**LTOFAT**
Right arm fat mass (g) (DEXA)	**RAFAT**
Right leg fat mass (g) (DEXA)	**RLFAT**
Right trunk fat mass (g) (DEXA)	**RTRFAT**
Right total fat mass (g) (DEXA)	**RTOFAT**
Arms fat mass (g) (DEXA)	**ARMFAT**
Legs fat mass (g) (DEXA)	**LEGFAT**
Trunk fat mass (g) (DEXA)	**TRUFAT**
Left arm lean mass (g) (DEXA)	**LALEAN**
Left leg lean mass (g) (DEXA)	**LLLEAN**
Left trunk lean mass (g) (DEXA)	**LTRLEAN**
Left totat lean mass (g) (DEXA)	**LTOLEAN**
Right arm lean mass (g) (DEXA)	**RALEAN**
Right leg lean mass (g) (DEXA)	**RLLEAN**
Right trunk lean mass (g) (DEXA)	**RTRLEAN**
Right total lean mass (g) (DEXA)	**RTOLEAN**
Arms lean mass (g) (DEXA)	**ARMLEAN**
Legs lean mass (g) (DEXA)	**LEGLEAN**
Trunk lean mass (g) (DEXA)	**TRULEAN**
Fat Mass (g) (DEXA)	**FATMASS**
Bone Mineral Content (g) (DEXA)	**BMC**
Bone Mineral Density (DEXA)	**BMD**
Daily Activity and Dietary Intake	
Caloric Expenditure through Daily Activity (kcal)	**ACTIVITY**
Recommended Dietary Caloric Intake (kcal)	**RECTOTCAL**
Actual Dietary Caloric Intake (kcal)	**ACTTOTCAL**
Dietary Carbohydrate Intake (g)	**CHO**
Dietary Total Fat Intake (g)	**TOTFAT**

Dietary Monounsaturated Fatty Acid Intake (g)	**MONO**
Dietary Polyunsaturated Fatty Acid Intake (g)	**POLY**
Dietary Saturated Fatty Acid Intake (g)	**SAT**
Dietary Protein Intake (g)	**PRO**

Data were analyzed using the Statistical Analysis System (SAS) version 9.2. SAS process for data analysis used was PROC MIXED. By use of PROC MIXED the best statistical fit to the repeated-measures effects was able to be chosen for each dependent variable of interest. Sample code:

```
PROC MIXED;
CLASS ID  GENDER TRAINING;
MODEL BMI= GENDER  TRAINING GENDER*TRAINING  ;
REPEATED TRAINING/ SUB=ID TYPE=UN R RCORR ;
LSMEANS GENDER  TRAINING GENDER*TRAINING / PDIFF=ALL
ADJUST=TUKEY ;
RUN;

QUIT;
```

The above code allows for the analysis of any main or interactive effects of gender, acute exercise, and exercise training. The statement "REPEATED" allows for the system to model the statistic based on the repeated-measures variables indicated, for this analysis: TRAINING. The statement TYPE on this line of code provides a place to input methods of modeling for the covariance matrix of the repeated variables. The following two lines set up post hoc analysis across all conditions in the MODEL statement. Differences here are determined across all levels (PDIFF=ALL) using the Tukey-Kramer method.

Body composition and physiologic adaptation measures

Obs	ID	GENDER	AGE	HEIGHT	MODE	TRAINING	RMR	VO2PEAKA	VO2PEAKR	BRUCTIME	BODYWT	BODYMASS
1	JG1	M	40	68.0	Water	PRE	2041	2809	35.10	625	175.5	79.800
2	JG1	M	40	68.0	Water	POST	2035	3111	39.40	660	174.0	79.100
3	RK2	F	51	64.5	Water	PRE	1295	2344	27.90	443	184.0	83.600
4	RK2	F	51	64.5	Water	POST	1453	2434	28.60	488	187.0	85.000
5	GB3	M	39	73.0	Water	PRE	1301	3530	36.60	609	213.0	96.800

Obs	CHEST	AXILLA	TRICEP	SUBSCAP	ABDOM	SUPRAIL	THIGH	SUM	BODYDENS	BMI	PERCFTSK	WAIST
1	46.0	40.5	33.0	41.0	49.5	23.0	48.5	281.5	0.97800	26.7400	53.0000	37.5
2	35.5	26.5	25.5	33.5	40.0	22.5	40.5	224.0	1.00300	26.5100	41.3600	36.0
3	31.5	45.0	36.0	30.0	34.5	27.5	50.0	254.5	0.97100	31.1600	56.4200	35.0
4	34.5	41.0	29.5	36.5	36.5	26.0	49.0	253.0	0.97200	31.6700	56.0800	35.0
5	53.5	42.5	38.0	43.5	44.5	31.0	56.0	309.0	0.96700	28.1600	58.6300	43.0

Obs	HIP	WHRATIO	PERCFTDX	ANDROID	GYNOID	AGRATIO	LBM	LAPERFAT	LLPERFAT	LTRPRFAT	LTOPRFAT
1	40.0	0.94	29.5	39.8	34.3	1.16	54133	21.0	27.6	34.3	29.5
2	39.0	.	28.9	38.3	32.4	1.18	53391	20.7	25.4	34.9	28.9
3	46.0	0.76	44.6	46.4	52.1	0.89	44534	39.6	51.3	42.8	44.4
4	45.5	0.77	44.8	46.4	50.9	0.91	45039	38.2	51.3	43.2	44.9
5	45.0	0.96	42.0	51.1	41.3	1.24	53617	30.0	40.4	47.6	42.2

Obs	RAPERFAT	RLPERFAT	RTRPRFAT	RTOPRFAT	ARMPRFAT	LEGPRFAT	TRUPRFAT	LAFAT	LLFAT	LTRFAT	LTOFAT
1	21.1	27.6	34.3	29.4	21.0	27.6	34.3	938	3440	6698	11433
2	20.7	25.4	34.9	28.9	20.7	25.4	34.7	945	3143	6370	10792
3	39.5	51.3	42.9	44.9	39.6	51.3	42.9	1674	7722	8373	18298
4	38.3	51.3	43.2	44.6	38.3	51.3	43.2	1548	7852	8509	18289
5	29.7	40.5	47.6	41.9	29.9	40.4	47.6	1478	6735	11008	19589

Obs	RAFAT	RLFAT	RTRFAT	RTOFAT	ARMFAT	LEGFAT	TRUFAT	LALEAN	LLLEAN	LTRLEAN	LTOLEAN	RALEAN
1	973	3516	6364	11169	1911	6957	13062	3526	9037	12831	27335	3647
2	978	3199	6393	10880	1923	6342	12764	3626	9218	11877	26602	3736
3	1518	7812	7920	17622	3192	15534	16294	2552	7326	11173	22930	2320
4	1379	8074	8217	18211	2926	15926	16726	2499	7456	11205	22459	2221
5	1383	6525	10860	19292	2861	13260	21868	3446	9918	12105	26847	3267

Obs	RLLEAN	RTRLEAN	RTOLEAN	ARMLEAN	LEGLEAN	TRULEAN	FATMASS	BMC	BMD
1	9237	12201	26798	7173	18274	25032	22603	3018	1.262
2	9370	11931	26790	7362	18589	23808	21672	3143	1.288
3	7410	10555	21604	4873	14736	21728	35920	2869	1.241
4	7675	10810	22579	4721	15131	22015	36500	2928	1.254
5	9603	11931	26771	6713	19521	24036	38881	3630	1.308

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TRAINING	RMR	VO2PEAKA	VO2PEAKR	BRUCTIME	BODYWT	BODYMASS
6	GB3	M	39	73.0	Water	POST	1432	3550	37.50	647	209.0	95.000
7	RR5	F	42	64.5	Water	PRE	1317	1891	24.90	432	169.0	76.800
8	RR5	F	42	64.5	Water	POST	1104	2175	28.74	491	166.5	75.700
9	DD7	F	58	63.0	Land	PRE	1252	2150	28.10	397	169.0	76.800
10	DD7	F	58	64.0	Land	POST	1382	2360	31.60	544	164.5	74.800

Obs	CHEST	AXILLA	TRICEP	SUBSCAP	ABDOM	SUPRAIL	THIGH	SUM	BODYDENS	BMI	PERCFTSK	WAIST
6	40.5	35.5	36.5	35.0	37.5	34.5	51.5	271.0	0.98300	27.6300	50.6900	41.5
7	34.5	45.5	43.0	42.5	46.0	31.5	58.0	301.0	0.95000	28.6200	66.6500	36.5
8	39.5	43.5	35.5	37.5	41.5	33.5	52.0	283.0	0.95900	28.2000	62.4200	35.5
9	29.5	34.5	30.0	28.5	37.0	24.5	49.0	233.0	0.98000	30.0000	52.0100	35.0
10	38.0	35.0	31.5	32.0	34.5	20.0	41.5	232.5	0.98000	28.3000	51.9000	32.5

Obs	HIP	WHRATIO	PERCFTDX	ANDROID	GYNOID	AGRATIO	LBM	LAPERFAT	LLPERFAT	LTRPRFAT	LTOPRFAT
6	46.0	0.90	41.2	49.9	44.5	1.12	53541	28.2	39.6	47.2	41.3
7	41.0	0.89	45.6	49.5	50.8	0.97	39988	44.9	46.3	47.6	45.4
8	40.5	0.88	44.0	49.4	50.2	0.98	40484	44.1	45.6	45.4	44.0
9	43.5	0.80	45.8	47.8	51.0	0.94	39449	36.6	48.3	47.9	46.0
10	41.0	0.79	44.6	47.1	51.7	0.91	39339	40.3	47.6	45.8	44.6

Obs	RAPERFAT	RLPERFAT	RTRPRFAT	RTOPRFAT	ARMPRFAT	LEGPRFAT	TRUPRFAT	LAFAT	LLFAT	LTRFAT	LTOFAT
6	28.1	39.6	47.1	41.1	28.2	39.6	47.1	1332	6327	10828	18937
7	44.9	46.3	47.6	45.7	44.9	46.3	47.6	1844	5910	8697	16974
8	44.1	45.6	45.4	44.0	44.1	45.6	45.4	1801	5398	8451	16080
9	36.5	48.3	47.9	45.6	36.6	48.3	47.9	1039	6289	8887	16582
10	40.3	47.6	45.8	44.5	40.3	47.6	45.8	1431	5533	8462	15823

Obs	RAFAT	RLFAT	RTRFAT	RTOFAT	ARMFAT	LEGFAT	TRUFAT	LALEAN	LLLEAN	LTRLEAN	LTOLEAN	RALEAN
6	1311	6591	10179	18548	2643	12919	21007	3392	9669	12134	26915	3348
7	1802	5451	8866	16530	3646	11361	17563	2259	6863	9587	20373	2209
8	1814	5144	8332	15731	3615	10542	16783	2282	6450	10184	20436	2299
9	1054	6228	8975	16752	2093	12517	17862	1803	6729	9676	19471	1831
10	1477	5673	8221	15811	2908	11206	16683	2116	6090	10025	19618	2187

Obs	RLLEAN	RTRLEAN	RTOLEAN	ARMLEAN	LEGLEAN	TRULEAN	FATMASS	BMC	BMD
6	10069	11432	26626	6739	19739	23566	37485	3688	1.282
7	6329	9759	19615	4468	13192	19346	33503	2836	1.232
8	6147	10035	20048	4581	12598	20219	31811	2652	1.216
9	6659	9776	19978	3633	13389	19452	33334	2817	1.205
10	6245	9739	19720	4304	12335	19763	31634	2740	1.200

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TRAINING	RMR	VO2PEAKA	VO2PEAKR	BRUCTIME	BODYWT	BODYMASS
11	BB8	M	49	70.0	Water	PRE	1761	3110	31.30	585	219.5	99.800
12	BB8	M	50	69.0	Water	POST	1649	3200	32.30	645	219.0	99.500
13	JC9	M	35	75.0	Water	PRE	1394	3440	26.70	481	284.0	129.100
14	JC9	M	35	75.0	Water	POST	2093	3840	29.90	570	282.0	128.200
15	SD11	F	59	62.0	Land	PRE	.	1565	23.40	429	147.5	67.000

Obs	CHEST	AXILLA	TRICEP	SUBSCAP	ABDOM	SUPRAIL	THIGH	SUM	BODYDENS	BMI	PERCFTSK	WAIST
11	47.5	46.0	34.5	47.5	41.5	19.5	55.0	291.5	0.97100	31.5600	56.3300	42.5
12	26.5	30.5	22.0	36.5	30.0	20.5	33.0	199.0	1.01100	32.4100	37.7700	42.0
13	52.0	50.0	41.5	42.0	57.0	28.0	57.5	328.0	0.95900	35.5700	62.1300	48.5
14	.	.	.	.	.	.	.	.	.	.	.	.
15	25.5	37.0	30.0	26.5	35.5	38.5	52.5	245.5	0.97400	27.0300	54.8800	31.0

Obs	HIP	WHRATIO	PERCFTDX	ANDROID	GYNOID	AGRATIO	LBM	LAPERFAT	LLPERFAT	LTRPRFAT	LTOPRFAT
11	43.0	0.99	38.2	49.7	39.7	1.25	59031	31.5	32.0	45.1	38.0
12	44.0	0.95	38.7	49.6	40.1	1.24	58292	29.1	32.2	46.2	38.7
13	48.0	1.01	42.5	53.2	41.1	1.29	69817	31.1	36.8	49.5	42.7
14	.	.	40.9	52.5	37.7	1.39	71605	30.2	34.8	48.4	41.0
15	41.0	0.76	38.4	34.1	48.0	0.71	39583	43.1	47.2	32.9	38.4

Obs	RAPERFAT	RLPERFAT	RTRPRFAT	RTOPRFAT	ARMPRFAT	LEGPRFAT	TRUPRFAT	LAFAT	LLFAT	LTRFAT	LTOFAT
11	31.5	32.0	45.2	38.4	31.5	32.0	45.1	1729	4847	11162	18294
12	29.0	32.2	46.2	38.7	29.1	32.2	46.2	1481	4757	11711	18450
13	30.9	36.8	49.5	42.3	31.0	36.8	49.5	1849	6870	16809	26053
14	30.0	34.8	48.4	40.7	30.1	34.8	48.4	2011	6774	15714	24985
15	43.1	47.2	32.8	38.4	43.1	47.2	32.9	1443	5639	4909	12373

Obs	RAFAT	RLFAT	RTRFAT	RTOFAT	ARMFAT	LEGFAT	TRUFAT	LALEAN	LLLEAN	LTRLEAN	LTOLEAN	RALEAN
11	1727	4728	11296	18166	3456	9575	22458	3758	10287	13585	29860	3748
12	1518	4746	11648	18388	2998	9503	23358	3612	10003	13643	29192	3708
13	2078	7223	15831	25555	3927	14093	32640	4106	11787	17144	35026	4642
14	2133	6991	14962	24524	4143	13765	30675	4657	12671	16721	35940	4971
15	1431	5545	4975	12305	2874	11184	9884	1909	6302	10025	19840	1890

Obs	RLLEAN	RTRLEAN	RTOLEAN	ARMLEAN	LEGLEAN	TRULEAN	FATMASS	BMC	BMD
11	10026	13717	29172	7506	20313	27302	36460	3843	1.346
12	9973	13579	29100	7320	19976	27222	36838	3954	1.412
13	12385	16165	34791	8747	24172	33309	51608	3930	1.331
14	13070	15920	35665	9627	25741	32640	49509	4112	1.343
15	6195	10171	19743	3798	12497	20196	24678	2090	1.059

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TRAINING	RMR	VO2PEAKA	VO2PEAKR	BRUCTIME	BODYWT	BODYMASS
16	SD11	F	59	62.0	Land	POST	.	1709	26.00	478	145.0	65.900
17	KN12	M	29	71.0	Land	PRE	1791	2680	22.60	449	261.0	118.600
18	KN12	M	30	71.0	Land	POST	2168	3020	25.60	526	260.0	118.200
19	TS16	M	37	72.5	Land	PRE	.	3020	30.20	548	220.0	100.000
20	TS16	M	37	72.5	Land	POST	.	3820	39.00	662	216.0	98.200

Obs	CHEST	AXILLA	TRICEP	SUBSCAP	ABDOM	SUPRAIL	THIGH	SUM	BODYDENS	BMI	PERCFTSK	WAIST
16	20.5	16.5	27.5	26.0	24.5	16.5	35.0	166.5	1.01100	26.5800	37.6900	32.0
17	39.5	41.5	41.5	56.0	41.0	34.5	50.0	304.0	0.97200	36.4800	56.1700	50.0
18	34.5	34.0	31.5	40.5	35.0	23.5	39.5	238.5	1.00000	36.3400	42.9200	50.0
19	26.5	19.5	15.5	23.5	19.0	15.5	36.5	156.0	1.03400	29.4900	27.9600	44.0
20	.	.	.	.	.	.	.	.	.	.	.	.

Obs	HIP	WHRATIO	PERCFTDX	ANDROID	GYNOID	AGRATIO	LBM	LAPERFAT	LLPERFAT	LTRPRFAT	LTOPRFAT
16	41.0	0.78	40.0	36.6	49.2	0.74	37849	41.1	48.0	36.4	40.0
17	47.0	1.06	47.4	58.4	46.6	1.25	59313	41.8	41.4	53.9	47.3
18	46.0	1.09	30.0	58.1	47.6	1.22	58878	41.0	43.6	53.3	47.7
19	44.0	1.00	37.6	31.0	37.7	0.82	65254	27.9	36.1	31.6	32.3
20	.	.	37.8	29.5	34.7	0.85	65653	25.3	33.9	31.0	31.0

Obs	RAPERFAT	RLPERFAT	RTRPRFAT	RTOPRFAT	ARMPRFAT	LEGPRFAT	TRUPRFAT	LAFAT	LLFAT	LTRFAT	LTOFAT
16	41.1	47.9	36.4	40.0	41.1	48.0	36.4	1389	5341	5696	12768
17	41.8	41.4	53.9	47.6	41.8	41.4	53.9	2287	7353	16514	26834
18	41.0	43.6	53.3	47.7	41.0	43.6	53.3	2515	7357	16587	27094
19	27.9	36.1	31.7	32.5	27.9	36.1	31.6	1304	6489	7350	15532
20	25.2	33.9	31.0	30.9	25.3	33.9	31.0	1204	6005	7214	14732

Obs	RAFAT	RLFAT	RTRFAT	RTOFAT	ARMFAT	LEGFAT	TRUFAT	LALEAN	LLLEAN	LTRLEAN	LTOLEAN	RALEAN
16	1364	5386	5307	12470	2753	10727	11003	1989	5791	9964	19113	1951
17	2519	7111	16505	26673	4806	14464	33019	3182	10403	14150	29896	3513
18	2561	7358	16062	26603	5076	14715	32649	3618	9515	14511	29660	3679
19	1385	6812	7202	15737	2689	13301	14552	3367	11471	15881	32516	3577
20	1292	6154	6863	14656	2496	12159	14076	3549	11712	16063	32842	3835

Obs	RLLEAN	RTRLEAN	RTOLEAN	ARMLEAN	LEGLEAN	TRULEAN	FATMASS	BMC	BMD
16	5847	9268	18735	3940	11638	19231	25238	2192	1.065
17	10067	14113	29416	6696	20470	28264	53506	3530	1.418
18	9510	14071	29218	7297	19025	28582	53698	3747	1.403
19	12041	15548	32739	6944	23513	31428	31270	3330	1.163
20	12001	15270	32811	7384	23713	31333	29388	3311	1.168

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TRAINING	RMR	VO2PEAKA	VO2PEAKR	BRUCTIME	BODYWT	BODYMASS
21	CM19	M	35	65.0	Land	PRE	1864	2363	22.50	417	231.0	105.000
22	CM19	M	35	65.0	Land	POST	1888	2596	25.10	465	228.0	103.600
23	GJ20	M	51	68.0	Land	PRE	1579	3017	29.60	500	187.0	85.000
24	GJ20	M	52	68.0	Land	POST	1363	3349	40.10	662	184.0	83.600
25	MP22	F	51	67.0	Water	PRE	1711	1990	15.90	289	276.0	125.500

Obs	CHEST	AXILLA	TRICEP	SUBSCAP	ABDOM	SUPRAIL	THIGH	SUM	BODYDENS	BMI	PERCFTSK	WAIST
21	45.5	35.5	41.5	42.5	40.0	45.0	49.5	299.5	0.97200	38.5200	56.0600	49.0
22	33.5	32.5	31.5	35.0	32.5	34.0	41.5	240.5	0.99700	38.0200	43.9800	47.0
23	40.5	23.5	30.5	27.5	42.5	22.5	40.5	227.5	0.99800	28.4900	43.5000	38.0
24	31.0	23.0	26.5	24.0	33.0	13.0	35.0	185.5	1.01642	28.0400	35.4200	37.0
25	38.5	51.5	36.5	49.0	41.0	37.5	54.5	308.5	0.94600	43.3200	69.0300	48.5

Obs	HIP	WHRATIO	PERCFTDX	ANDROID	GYNOID	AGRATIO	LBM	LAPERFAT	LLPERFAT	LTRPRFAT	LTOPRFAT
21	48.0	1.02	42.6	56.4	47.2	1.20	56775	39.5	43.9	46.1	43.6
22	46.0	1.02	47.4	57.4	47.0	1.22	52239	39.3	43.9	52.7	47.6
23	42.0	0.90	32.2	42.1	34.2	1.23	54542	24.3	26.9	38.9	32.3
24	41.0	0.90	30.4	41.2	32.5	1.27	55417	21.6	25.6	36.9	30.4
25	52.5	0.92	46.9	54.2	46.4	1.17	64087	49.0	45.8	49.0	46.8

Obs	RAPERFAT	RLPERFAT	RTRPRFAT	RTOPRFAT	ARMPRFAT	LEGPRFAT	TRUPRFAT	LAFAT	LLFAT	LTRFAT	LTOFAT
21	39.4	43.9	46.1	43.6	39.5	43.9	46.1	2044	6910	12228	21658
22	39.3	43.9	52.7	47.2	39.3	43.9	52.7	1839	6698	14300	23309
23	24.3	26.9	38.8	32.2	24.3	26.9	38.9	1023	3465	8136	13032
24	21.6	25.6	36.9	30.4	21.6	25.6	36.9	981	3165	7860	12453
25	49.0	45.8	49.0	47.0	49.0	45.8	49.0	3016	8956	16464	29099

Obs	RAFAT	RLFAT	RTRFAT	RTOFAT	ARMFAT	LEGFAT	TRUFAT	LALEAN	LLLEAN	LTRLEAN	LTOLEAN	RALEAN
21	2185	7054	12493	22241	4229	13964	24721	3132	8843	14284	27967	3355
22	2022	6964	14141	23747	3861	13663	28441	2839	8555	12845	25639	3126
23	1124	3459	7954	12914	2147	6923	16089	3181	9396	12801	27342	3503
24	1000	3246	7226	11770	1982	6412	15087	3562	9189	13459	28477	3641
25	2756	8849	15460	27565	5772	17805	31924	3142	10614	17108	33034	2873

Obs	RLLEAN	RTRLEAN	RTOLEAN	ARMLEAN	LEGLEAN	TRULEAN	FATMASS	BMC	BMD
21	9026	14592	28808	6487	17869	28875	43899	2286	1.311
22	8898	12708	26600	5965	17454	25553	47056	2762	1.249
23	9375	12519	27200	6684	18771	25230	25946	3498	1.262
24	9424	12383	26940	7203	18613	25842	24223	3480	1.257
25	10485	16059	31054	6014	21099	33167	56664	2764	1.354

Obs	ID	GENDER	AGE	HEIGHT	MODE	TRAINING	RMR	VO2PEAKA	VO2PEAKR	BRUCTIME	BODYWT	BODYMASS
26	MP22	F	52	67.0	Water	POST	1593	2439	21.10	382	254.5	115.700
27	GA24	M	49	69.5	Land	PRE	1864	2970	30.10	508	217.5	98.900
28	GA24	M	49	69.5	Land	POST	1479	2830	30.50	599	204.5	93.000
29	HW14	F	.	.	Land	PRE	.	2170	22.52	.	212.0	96.364
30	HW14	F	.	.	Land	POST	.	.	.	.	.	.

Obs	CHEST	AXILLA	TRICEP	SUBSCAP	ABDOM	SUPRAIL	THIGH	SUM	BODYDENS	BMI	PERCFTSK	WAIST
26	30.5	35.5	32.0	40.0	32.5	20.5	40.5	231.5	0.98172	39.9437	51.3089	46.0
27	41.5	43.5	33.5	42.5	42.0	17.5	50.5	271.0	0.98000	31.7200	52.0600	42.5
28	28.5	21.5	13.0	33.0	34.0	18.0	15.0	163.0	1.02706	29.8286	30.7587	39.0
29	.	.	.	.	.	.	.	.	.	.	.	.
30	.	.	.	.	.	.	.	.	.	.	.	.

Obs	HIP	WHRATIO	PERCFTDX	ANDROID	GYNOID	AGRATIO	LBM	LAPERFAT	LLPERFAT	LTRPRFAT	LTOPRFAT
26	50.0	0.92	46.4	54.0	46.6	1.16	59715	47.1	46.4	48.1	46.2
27	44.5	0.96	30.2	43.1	31.0	1.39	66394	21.6	22.1	39.6	30.0
28	41.5	0.94	28.0	41.9	27.9	1.50	64288	21.9	19.6	36.1	27.9
29	.	.	.	.	.	.	.	.	.	.	.
30	.	.	.	.	.	.	.	.	.	.	.

Obs	RAPERFAT	RLPERFAT	RTRPRFAT	RTOPRFAT	ARMPRFAT	LEGPRFAT	TRUPRFAT	LAFAT	LLFAT	LTRFAT	LTOFAT
26	47.1	46.4	48.1	46.6	47.1	46.4	48.1	2652	8276	14772	26364
27	21.5	22.1	39.6	30.4	21.6	22.1	39.6	1200	3671	8752	13957
28	21.7	19.7	36.1	28.0	21.8	19.6	36.1	1155	2840	8224	12588
29	.	.	.	.	.	.	.	.	.	.	.
30	.	.	.	.	.	.	.	.	.	.	.

Obs	RAFAT	RLFAT	RTRFAT	RTOFAT	ARMFAT	LEGFAT	TRUFAT	LALEAN	LLLEAN	LTRLEAN	LTOLEAN	RALEAN
26	2903	7933	14018	25307	5555	16209	28790	2974	9550	15963	30684	3257
27	1247	3502	9619	14793	2446	7172	18371	4343	12947	13365	32507	4544
28	1167	2780	8082	12354	2322	5620	16308	4131	11649	14539	32513	4199
29	.	.	.	.	.	.	.	.	.	.	.	.
30	.	.	.	.	.	.	.	.	.	.	.	.

Obs	RLLEAN	RTRLEAN	RTOLEAN	ARMLEAN	LEGLEAN	TRULEAN	FATMASS	BMC	BMD
26	9155	15140	29031	6231	18705	31103	51671	2816	1.350
27	12311	14652	33887	8887	25258	28017	28750	3738	1.271
28	11348	14286	31775	8330	22997	28825	24942	3620	1.288
29	.	.	.	.	.	.	.	.	.
30	.	.	.	.	.	.	.	.	.

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Obs	ID	GENDER	AGE	HEIGHT	MODE	TRAINING	RMR	VO2PEAKA	VO2PEAKR	BRUCTIME	BODYWT	BODYMASS
31	PR15	F	.	.	Water	PRE	.	2222	14.92	.	327.6	148.909
32	PR15	F	.	.	Water	POST	.	.	.	.	.	.
33	KE23	F	.	.	Land	PRE	.	1980	18.15	.	240.0	109.091
34	KE23	F	.	.	Land	POST	.	.	.	.	.	.
35	CR21	M	.	.	Water	PRE	.	2540	18.55	.	301.2	136.900

Activity and Dietary Records

Obs	ID	GENDER	AGE	HEIGHT	MODE	TRAINING	ACTIVITY	RECTOTAL	ACTTOTAL	CHO	TOTFAT	MONO	POLY	SAT	PRO
1	JG1	M	40	68.0	Water	PRE	2527	2906	2540	327	86	22	12	27	102
2	JG1	M	40	68.0	Water	POST	2891	2696	1551	220	51	15	6	18	60
3	RK2	F	51	64.5	Water	PRE	2905	2295	1594	224	52	14	7	20	65
4	RK2	F	51	64.5	Water	POST	2826	2350	2593	306	114	22	13	42	92
5	GB3	M	39	73.0	Water	PRE	3115	3110	2313	312	82	12	3	32	93
6	GB3	M	39	73.0	Water	POST	3083	3030	3235	554	84	7	4	26	78
7	LG4	F	34	65.5	Water	PRE	2623	2452	2040	264	67	13	8	26	102
8	LG4	F	34	65.0	Water	POST	2588	2406	1434	155	64	16	8	25	70
9	RR5	F	42	64.5	Water	PRE	2472	2304	1419	186	53	10	5	20	56
10	RR5	F	42	64.5	Water	POST	2453	2258	1535	165	76	11	4	36	49
11	DD7	F	58	63.0	Land	PRE	2684	2149	1770	141	99	14	2	34	82
12	DD7	F	58	64.0	Land	POST	2627	2131	972	103	34	5	2	11	62
13	BB8	M	49	70.0	Water	PRE	3345	2950	2069	227	94	28	10	28	91
14	BB8	M	50	69.0	Water	POST	3242	2921	2072	250	75	17	6	31	102
15	JC9	M	35	75.0	Water	PRE	4426	3590	3870	479	153	48	12	50	157
16	JC9	M	35	75.0	Water	POST	4185	3388	3065	367	145	7	3	31	78
17	SD11	F	59	62.0	Land	PRE	2292	1997	3270	362	149	14	7	49	100
18	SD11	F	59	62.0	Land	POST	2414	1991	2364	304	108	34	28	21	66
19	KN12	M	29	71.0	Land	PRE	4021	3450	2893	420	104	31	11	35	88
20	KN12	M	30	71.0	Land	POST	3864	3479	1285	181	41	8	2	15	60
21	TS16	M	37	72.5	Land	PRE	3407	3095	2449	331	82	22	7	35	107
22	TS16	M	37	72.5	Land	POST	3294	3086	3918	476	150	28	14	50	135
23	CM19	M	35	65.0	Land	PRE	3611	3110	2515	397	78	7	5	22	70
24	CM19	M	35	65.0	Land	POST	3439	3088	2533	332	115	16	14	31	58
25	GJ20	M	51	68.0	Land	PRE	2972	2698	2834	349	94	23	15	45	63
26	GJ20	M	52	68.0	Land	POST	2952	2653	2028	218	70	13	5	22	59
27	GA24	M	49	69.5	Land	PRE	3304	2918	2863	310	117	35	16	32	148
28	GA24	M	49	69.5	Land	POST	3287	2860	2766	246	129	48	29	35	130

APPENDIX J

SAMPLE STATISTICAL CODE AND RAW DATA FOR CHAPTER IV

Variable Definitions:

Independent Variables

Animal Number	**animal**
Treatment Group	**group**
Phenotype (Lean vs obese)	**phenotype**
Gel for Western blot analysis	**gel**
Exercise condition (RE vs SED)	**exercise**

Dependent Variables

Muscle Protein Expression (Relative Abundance)

F1 ATPase (ATP Synthase)	**F1ATPASE**
FAT/CD36	**CD36**
p38 MAPK	**p38**
CPT-I	**CPT-I**
p38γ MAPK	**p38gamma**
PPARδ	**ppard**
PGC-1α	**PGC1a**
AMPKα	**AMPK**
AMPK phosphorylation (Thr 172)	**pAMPK**
AMPK relative phosphorylation	**pAMPKTOTAL**
Cytochrome C Oxidase-IV	**COX**
PPARδ	**PPARd**
LKB1 phosphorylation (Ser 428)	**pLKB1**

Blood Lipids and Lipoproteins

NEFA (post RE3)	**threeNEFA**
NEFA (post RE4)	**fourNEFA**
Total cholesterol (Post RE3)	**threeTC**
Total cholesterol (Post RE4)	**fourTC**
LDL-cholesterol (Post RE3)	**threeLDL**
LDL-cholesterol (Post RE4)	**fourLDL**
HDL-cholesterol (Post RE3)	**threeHDL**
HDL-cholesterol (Post RE4)	**fourHDL**

ANOVA

Data were analyzed using the Statistical Analysis System (SAS) version 9.2. SAS process for data analysis used was PROC MIXED to determine change in content of the above listed proteins and concentration of blood lipids and lipoproteins due to the exercise intervention and differences between lean and obese phenotypes. By use of PROC MIXED we are able to place a repeated measure on "gel" to statistically account for differences between Western blot membranes in normalizing the data. Sample code:

```
PROC MIXED DATA = WORK.SORTTempTableSorted
        METHOD=REML;
        CLASS animal phenotype exercise gel         ;
        MODEL AMPK=       phenotype exercise phenotype*exercise
                /         HTYPE=3;
REPEATED gel / SUBJECT=animal*gel TYPE=UN;
LSMEANS phenotype*exercise / PDIFF=ALL ADJUST=TUKEY ;
RUN;
QUIT;
```

The above code allows for the analysis of any main or interactive effects of the exercise intervention or phenotype. The statement "REPEATED" allows for the system to model the statistic based on the repeated-measures variables indicated, for this analysis: "gel". This repeat allowed the statistical system to adjust for differences in Western blot gels of intensity of densitometry. The statement TYPE on this line of code provides a place to input methods of modeling for the covariance matrix of the repeated variables. The following two lines set up post hoc analysis across all conditions in the MODEL statement. Differences here are determined across all levels (PDIFF=ALL) using the Tukey-Kramer method. Fisher's LSD is also automatically printed with the output.

Correlations

For the assessment of correlation of PPARδ, PGC-1α, and AMPKα to blood lipid and lipoprotein variables the PROC CORR process was used. This process enabled the assessment of Pearson's Product-Moment correlations on all variables of interest. Sample Code:

```
PROC SORT;
BY Phenotype;

PROC CORR;
BY Phenotype;
  VAR      F1    CD36  p38    CPTI  p38g  COXIV      pAMPK
      PPARd      AMPKa      PGC1a pAMPKTOTAL;

  WITH     threeNEFA  fourNEFA   threeTC    fourTC thretwoTC
      fourtwoTC  LDLHDLthree1   LDLHDLfourone  threeLDL
      fourLDL    threeHDL   fourHDL    F1    CD36  p38   CPTI  p38g
      COXIV      pAMPK      PPARd      AMPKa      PGC1a
      pAMPKTOTAL;
```

To assess correlations it was first necessary to sort data by variable Phenotype or exercise. Therefore, prior to running PROC CORR, PROC SORT was run for either or both of these variables. This was followed by PROC CORR which allowed the assessment of correlations at each level of phenotype, exercise or phenotype*exercise for protein variables of interest with all protein and blood variables.

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Obs	Animal	Phenotype	Exercise	gel	three NEFA	four NEFA	threeTC	fourTC	thretwo TC	fourtwo TC
1	121	Lean	EX	1	1.15203	0.39287	86.082	82.077	98.273	101.838
2	122	Lean	EX	1	0.83007	0.48628	114.272	99.164	120.427	107.540
3	123	Lean	EX	2	0.88933	0.61057	90.322	96.200	101.128	105.436
4	124	Lean	EX	2	0.66925	0.71897	119.499	95.053	143.428	109.595
5	125	Lean	EX	3	0.87102	0.48780	84.109	128.629	67.950	83.482
6	126	Lean	EX	3	0.88067	0.40905	95.029	116.290	79.028	73.693
7	127	Lean	EX	4	0.95176	0.33558	73.192	88.521	62.126	55.619
8	128	Lean	EX	4	1.11366	0.30385	133.044	142.955	112.750	126.811
9	111	Lean	SED	1	0.70484	0.40577	106.093	95.126	123.753	123.126
10	112	Lean	SED	1	0.76385	0.31125	101.243	92.284	103.164	106.834
11	113	Lean	SED	2	1.08266	0.43738	110.138	101.679	120.398	126.539
12	114	Lean	SED	2	0.82065	0.49156	70.426	114.857	90.915	103.559
13	115	Lean	SED	3	0.66372	0.59202	84.985	74.882	77.405	63.117

Obs	LDLHDLthree1	LDLHDLfourone	three LDL	fourLDL	three HDL	fourHDL	F1	CD36
1	133.409	80.875	54.0394	42.217	79.370	38.657	.	.
2	186.555	147.026	75.6939	65.085	110.861	81.941	4.40964	2.55734
3	122.771	117.299	23.9266	57.579	98.844	59.720	4.19853	2.49735
4	195.696	122.025	63.4846	52.803	132.212	69.222	6.79412	2.71958
5	108.987	111.261	51.2949	60.254	57.692	51.007	4.13616	1.99625
6	118.703	102.734	54.7750	51.342	63.928	51.392	6.54920	2.96629
7	93.828	85.304	41.1947	47.652	52.634	37.652	5.71111	1.90909
8	177.671	149.397	59.3783	77.269	118.293	72.128	5.60000	2.98182
9	144.225	140.339	43.9038	57.217	100.321	83.123	6.43373	2.20926
10	153.536	109.371	60.4888	57.068	93.048	52.303	.	.
11	183.331	157.690	65.7393	72.866	117.592	84.824	5.17647	1.25397
12	104.754	120.520	49.3855	60.249	55.369	60.271	4.08088	2.87302
13	119.521	99.019	45.3965	41.166	74.124	57.852	5.40618	1.71536

Obs	p38	CPTI	p38g	COXIV	pAMPK	PPARd	AMPKa	PGC1a	p AMPKTOTAL
1	.	.	.	.	.	.	0.71186	0.76032	.
2	1.98649	1.95918	4.38158	1.72973	1.34043	1.65813	1.27119	0.73878	1.05447
3	1.63478	1.45789	6.96774	.	0.29091	0.53149	2.00000	1.31906	0.14545
4	2.01739	1.11579	5.74194	.	0.69091	1.01385	0.57143	0.83564	1.20909
5	1.84211	1.38265	6.03846	1.08475	0.88095	1.19477	0.84932	0.60403	1.03725
6	2.42105	1.18878	5.07692	1.18644	1.56349	1.75294	1.00685	1.87752	1.55286
7	.	2.88462	5.96154	1.43561	0.49116	0.70446	.	1.56842	.
8	.	.	7.50000	0.57955	0.41768	0.63507	.	1.24211	.
9	1.91892	1.37415	4.32895	1.45946	0.54255	0.81938	1.20339	1.05117	0.45085
10	.	.	.	.	.	.	1.18644	1.22980	.
11	1.53913	1.47368	8.61290	.	0.36364	0.57431	1.57143	1.48343	0.23140
12	1.66957	1.42105	5.06452	.	1.69091	1.57683	1.07143	1.49586	1.57818
13	2.10526	1.03061	6.34615	1.79661	0.57937	0.58954	1.19863	0.59899	0.48336

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Obs	Animal	Phenotype	Exercise	gel	three NEFA	four NEFA	threeTC	fourTC	thretwo TC	fourtwo TC
14	116	Lean	SED	3	0.83519	0.24670	118.116	140.955	103.270	133.687
15	117	Lean	SED	4	0.85498	0.38560	142.701	177.799	124.081	117.366
16	118	Lean	SED	4	0.43117	0.42848	84.747	132.783	.	87.593
17	131	Obese	EX	3	1.84693	0.69153	216.079	129.357	153.800	85.307
18	141	Obese	EX	1	1.47261	0.63710	243.844	189.734	355.079	243.582
19	142	Obese	EX	1	1.29213	0.45319	248.298	284.012	319.626	287.397
20	143	Obese	EX	2	1.64293	0.62011	848.843	446.602	993.374	546.148
21	144	Obese	EX	2	1.13893	1.11825	183.681	170.734	283.528	208.843
22	145	Obese	EX	3	1.46648	1.01544	462.663	331.172	428.452	245.778
23	147	Obese	EX	4	1.40664	0.24899	258.532	27.769	267.239	17.480
24	148	Obese	EX	4	1.19484	1.08833	231.510	219.935	255.268	211.954
25	132	Obese	SED	1	2.06117	0.35486	411.109	156.763	578.199	187.845
26	133	Obese	SED	2	0.90366	0.57445	208.622	94.284	263.196	129.593

Obs	LDLHDLthree1	LDLHDLfourone	three LDL	fourLDL	three HDL	fourHDL	F1	CD36
14	165.220	122.005	76.4037	73.434	88.817	48.571	7.63043	2.42322
15	168.419	170.319	49.3236	87.679	119.096	82.640	6.73333	1.67273
16	93.932	116.381	51.7786	66.247	42.154	50.134	6.68889	2.18182
17	194.523	107.252	80.7472	54.747	113.775	52.505	7.90847	2.70037
18	319.427	300.343	55.8708	101.445	263.557	198.898	8.31024	1.09256
19	384.891	255.273	87.3759	138.113	297.515	117.161	5.97289	1.99598
20	912.499	489.943	68.5886	186.032	843.910	303.910	6.34559	1.41270
21	232.309	231.658	31.5265	60.075	200.782	171.583	7.16176	2.23810
22	608.699	300.094	48.9484	116.169	559.751	183.925	4.02632	1.69288
23	263.991	22.271	46.0823	14.620	217.908	7.651	4.77778	1.61818
24	286.847	229.895	44.3064	81.922	242.540	147.972	6.37778	1.74545
25	537.614	190.122	22.3226	84.299	515.292	105.823	.	.
26	362.990	130.111	94.1479	57.688	268.842	72.424	4.12500	2.51323

Obs	p38	CPTI	p38g	COXIV	pAMPK	PPARd	AMPKa	PGC1a	p AMPKTOTAL
14	2.63158	0.81122	5.76923	0.98305	1.23810	1.29412	0.75342	1.71141	1.64329
15	.	1.61538	7.88462	0.69318	0.23978	0.44734	.	1.10000	.
16	.	3.03846	8.84615	1.16288	0.47182	0.74845	.	1.61579	.
17	2.15789	0.84694	6.42308	1.16949	1.42063	1.54118	0.66438	2.15940	2.13828
18	2.06757	1.01361	4.43421	3.17838	0.51064	0.66688	1.00000	0.92101	0.51064
19	2.31081	1.65306	3.88158	1.28649	0.75532	0.80250	1.18644	2.13824	0.63663
20	2.27826	1.36316	5.90323	.	0.85455	0.81864	1.57143	1.60083	0.54380
21	1.84348	1.03684	5.06452	.	0.87273	0.88917	1.00000	1.51796	0.87273
22	2.47368	1.08163	5.53846	0.64407	0.58730	0.71242	1.15068	0.75000	0.51039
23	.	1.92308	4.03846	1.56061	0.52597	0.74783	.	0.75263	.
24	.	1.19231	6.53846	1.06439	0.33260	0.58984	.	1.22632	.
25	.	.	.	.	.	.	0.93220	1.16427	.
26	1.82609	1.48947	4.03226	.	0.65455	0.66373	1.50000	1.11464	0.43636

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Obs	Animal	Phenotype	Exercise	gel	three NEFA	four NEFA	threeTC	fourTC	thretwo TC	fourtwo TC
27	135	Obese	SED	2	1.62182	0.77967	339.827	296.455	343.527	254.936
28	136	Obese	SED	3	1.70763	0.42553	200.056	41.156	235.202	25.072
29	137	Obese	SED	3	1.63015	0.65486	414.320	118.890	368.268	90.017
30	146	Obese	SED	1	1.19159	0.36551	203.127	183.549	273.815	199.358

Obs	LDLHDLthree1	LDLHDLfourone	three LDL	fourLDL	three HDL	fourHDL	F1	CD36
27	366.567	318.191	38.8255	94.545	327.742	223.646	6.46324	2.49206
28	206.219	30.428	37.6769	18.019	168.542	12.410	4.01945	1.65918
29	402.701	156.442	55.0584	85.947	347.642	70.495	6.35698	2.00749
30	251.911	261.776	42.1617	86.863	209.749	174.913	4.77711	1.34004

Obs	p38	CPTI	p38g	COXIV	pAMPK	PPARd	AMPKa	PGC1a	p AMPKTOTAL
27	1.73913	0.87895	5.61290	.	0.12727	0.27330	0.85714	1.44337	0.14848
28	1.94737	1.16327	6.34615	3.28814	0.46825	0.49673	1.23973	1.33389	0.37771
29	1.57895	0.88776	6.19231	2.13559	0.70635	1.13595	0.50685	1.44463	1.39361
30	2.10811	1.02041	4.57895	1.29730	0.50000	0.25625	0.88136	0.77648	0.56731

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EDUCATION

2002	B.S.	University of South Carolina	Exercise Science
2005	M.S.	University of South Carolina	Exercise Science
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HONORS AND AWARDS

2010 Student Research Manuscript Award, Third Place. Texas Chapter of the American College of Sports Medicine Annual Meeting, Houston, TX

2009 Student Research Presentation Award, Doctoral Category, Second Place. Texas Chapter of the American College of Sports Medicine Annual Meeting, Tyler, TX

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PUBLICATIONS

Nicholas P. Greene, Bradley S. Lambert, Elizabeth S. Greene, Aaron F. Carbuhn , John S. Green, Stephen F. Crouse. Comparative Efficacy of Water and Land Treadmill Training for Overweight or Obese Adults. *Medicine & Science in Sports & Exercise* 41: 1808-1815, 2009.

<u>Accepted for Publication</u>

Nicholas P. Greene, Elizabeth S. Greene, Aaron F. Carbuhn, John S. Green, Stephen F. Crouse. VO_2 Prediction and Cardiorespiratory Responses During Underwater Treadmill Exercise. Submitted for publication in *Research Quarterly for Sport and Exercise*, accepted for publication March 14, 2010.