ABSTRACT

Melanie Ann Sweazey. THE EFFECTS OF 10 DAYS OF ENDURANCE EXERCISE TRAINING ON GENE EXPRESSION REGULATING SKELETAL MUSCLE FATTY ACID OXIDATION IN OBESE AFRICAN AMERICAN VS. CAUCASIAN WOMEN. (Under the direction of Dr. Ronald N. Cortright, Ph.D.). Department of Exercise and Sport Science, December, 2005.

The prevalence of obesity and diabetes is greater in African-American (AAW) vs. Caucasian (CW) women. Although environmental factors may be influential, inherent biochemical differences also exist such as reductions in mitochondrial oxidation of fatty acids. The purpose was to determine the metabolic flexibility to expand the gene expression of key regulators of skeletal muscle lipid oxidation in response to exercise training in obese AAW and CW and we hypothesized that this expression would occur similarly in both AAW and CW. Biopsies of the vastus lateralis were obtained following a 12 h fast from 7 obese CW (BMI=38±2 kg/m^2) and 6 AAW (BMI=40±2) before and 4 h after acute exercise in the sedentary and trained (10 d of cycle ergometry) condition. Expression of PGC-1 α, CPT-1 β, UCP3, and PDK4 were assessed by quantitative real time PCR. Results demonstrated that PGC-1 α (master regulator of mitochondrial biogenesis) and PDK4 (reduces glucose utilization in favor of fatty acids) gene expression are elevated similarly in AAW and CW by endurance exercise in the untrained and trained condition. UCP3, which is expressed during times of elevated fatty acid oxidation is elevated post exercise in the untrained condition, but unlike CW, does not increase in AAW after exercise in the trained state. Only CW show elevated resting UCP3 gene expression following training. Changes in CPT-1 β (regulator of fatty acid

entry into the mitochondria) was not altered by acute exercise in either the untrained or trained condition and results were similar in both CW and AAW. However, only CW demonstrated a significant response in CPT-1 β expression 4 h post-exercise when comparing the trained vs. the untrained condition. In conclusion, AAW respond similarly to exercise with respect to the expression of some genes (PGC-1 α and PDK4) that promote or are associated with fatty acid oxidation; however, the failure by AAW to elevate basal UCP3 levels or increase expression of CPT-1 β following exercise in the trained condition suggests metabolic inflexibility in a portion of the adaptive response in fat metabolism to exercise training in this racial group of obese women.

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DEDICATION

I would like to dedicate my thesis to my parents, Jim and Peggy Sweazey. Without your love and support I don't think I could have made it this far. Thank you for your words of encouragement, continued guidance, and never ending love!

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LIST OF ACRONYMS

AAW: African American Women

CW: Caucasian Women

BMI: Body Mass Index = mass/ht^2 (kg/m^2)

NIDDM: Non-Insulin Dependent (type II or adult onset) Diabetes Mellitus. Characterized by a failure of the pancreatic β-cell to secreted insulin; usually follows chronic insulin resistance by skeletal muscle and is typically of the obese state.

ADA: American Diabetes Association

CS: Citrate Synthase. A Krebs (Citric Acid or tricarboxylic) cycle enzyme that results in the condensation of oxaloacetate and acetyl-CoA to form citric acid.

β-HAD: β-Hydroxyacyl Dehydrogenase. Used as a marker for beta-oxidation capacity. Catalyzes the conversion of β-hydroxyacyl-CoA to β-ketoacyl-CoA with the formation of one reducing equivalent of NADH.

PFK: Phosphofructokinase. Considered the rate limiting reaction in glycolysis. Catalyzes the conversion of fructose-6-phosphate to fructose-1, 6-biphosphate.

LCFA-CoA: Long Chain Fatty Acid-CoA. Activated form of a fatty acid required for triglyceride synthesis or transport into the mitochondria for oxidation.

TAG's: Triacylglycerols or Triglycerides. Storage form of neutral lipids in adipocytes and skeletal muscle. Composed of three fatty acids esterified to a (3 carbon) glycerol backbone.

ACS: acyl-CoA synthase. Mitochondrial outer membrane protein that activates long-chain fatty acids (i.e. palmitic acid) by esterification of coenzyme A to the fatty acid.

PDK4: Pyruvate dehydrogenase kinase. A key factor in skeletal muscle metabolism as it allows entry into the mitochondria for production of pyruvate. PDK4 is expressed in skeletal muscle.

PGC-1 α: Peroxisome Proliferator Activated Receptor Gamma Coactivator I. The master co-activator responsible for glucose homeostasis, lipid and energy homeostasis. PGC-1 α coactivates all the PPARs and is responsible for stimulating the rate-limiting step in transcription initiation

CPT-1 β: Carnitine Palmitoyltransferase-1 β. A specific member of the Carnitine-acyl transferases (CAT) which exchanges CoA with the carrier substance carnitine for transport of the activated palmitoyl-CoA into the mitochondrial matrix. Considered the rate-limiting step in the oxidation of fatty acids in skeletal muscle.

UCP3: Uncoupling proteins. Mitochondrial transporters found in the mitochondrial inner membrane. UCP3 is expressed primarily in skeletal muscle and is involved in the limitation of free radical cells as well as fatty acid metabolism.

PPARs: Peroxisome proliferators activated receptors. Members of the nuclear hormone receptor superfamily and act by regulating gene transcription. There are 3 forms

PPAR α- predominantly found in the liver, regulates fatty acid oxidation

PPAR β/PPAR δ- ubiquitous expression, responsible for fatty acid oxidation and thermogenesis

PPAR γ- found predominantly in adipose tissue, regulates adipocyte differentiation and function

CHAPTER I

INTRODUCTION

Obesity

Obesity has become a very common health problem in the United States and is threatening to become a global pandemic. It is estimated that nearly 1 in 2 adults are overweight or obese (Must, Spadano, Coakley, Field, Colditz, and Dietz, 1999). In addition to the health related effects of obesity, the economic costs associated with it continue to grow at epidemic proportions. Through treatment costs, lost wages, and direct health care costs, the economic burden on the US economy has been estimated at $70 billion dollars annually (Wickelgren, 1998). Diabetes related costs have been estimated at $98 billion dollars annually (Mokdad, Bowman Fordd, Vinicor, Marks, and Kpolan, 2001).

Traditionally, obesity has been thought of as a metabolic disease characterized by a slow and continuous process of energy imbalance (Chitwood, Brown, Lundy, and Dupper, 1995). However, more recently obesity has also been characterized as an impaired ability to utilize fat as a fuel. Specifically, subjects have been shown to manifest a reduced ability to oxidize fatty acids in skeletal muscle (Blaak & Saris, 2002; Kim, Hickner, Cortright, Dohm, and Houmard, 2000). This is extremely important as reductions in skeletal muscle oxidative capacity may lead to accumulation of bioactive lipids which is strongly associated with insulin resistance and diabetes (Kelley, Goodpaster, Wing, and Simoneau, 1999). It has been suggested that reductions in

skeletal muscle fatty acid oxidation capacity may be due to reduced size and functionality of the mitochondria (Kelley et al., 1999; Ritov, Menshikova, He, Ferrell, Goodpaster, and Kelley, 2005). However, the cellular mechanisms that can account for the reported differences in mitochondrial oxidative capacity/biogenesis are poorly understood. In addition to examining obesity related issues from a global standpoint, an emerging body of evidence suggests that obesity research should also focus on racial distinctions that may be relevant to the development of the disease and related pathologies. For example, African-American women (AAW) have a greater susceptibility to gain weight at an earlier age than Caucasian women (CW) (Burke, 1996). AAW also lose weight at a slower rate compared to CW who are at the same baseline weight (Foryet, 1995 & Kumanyika, 1994). Although racial group differences in income, education, and socioeconomic status contribute to these findings, it is not likely that they are the sole cause for the higher rate of obesity among AAW compared to CW (Kumanyika, Wilson, and Guilford-Davenport, 1993). Accordingly, inherent metabolic and/or physiological dysfunction has been suggested to significantly contribute to the predisposition of AAW to obesity (Kemper, Sargurt, Drane, Valois, and Hussey, 1994; Kumanyika et al., 1993; Kumanyaka, 1994; Streigel-Moore, Wilfley, Caldwell, Needham, and Brownwell, 1996). For example, many surveys report AAW engaging in significantly less physical activity compared to CW, therefore contributing to the greater state of obesity in this racial group (Weinsier, 2002).

Fat Oxidation

Skeletal muscle fat oxidation has been shown to be reduced in obese women, (Kim, et al., 2000). Again, it is likely that impairment of mitochondrial function is at least part of the mechanism associated with skeletal muscle defects in fatty acid oxidative capacity and in the development of obesity. Accordingly, mitochondrial oxidative enzymes including citrate synthase (CS) and β-hydroxyacyl dehydrogenase (β-HAD; β-oxidation) have been reported to be reduced in obese individuals, with promotion of excess fat storage rather than fat oxidation being the result (Colberg, Simoneau, Thaete, and Kelley, 1995; Simoneau, Colberg, Thaete, and Kelley, 1995; Simoneau & Kelley, 1997). These findings have only just begun to be replicated in AAW and comparisons are nearly absent in the literature. However, one study has recently demonstrated that in vitro rates of skeletal muscle oxidation are depressed in obese AAW vs. CW (Privette, 2002). These comparisons are important as results may help to identify risk factors and potential cause and effect mechanisms of obesity between AAW and CW, and in the propensity, prevalence, and severity in obesity related type II diabetes.

With regard to subcellular (mitochondrial) mechanisms that might contribute to a greater impairment in mitochondrial oxidation of fatty acids between AAW vs. CW, several genes involved in regulating energy homeostasis, mitochondrial function, and biogenesis are likely candidates. These could include several gene encoding proteins that regulate lipid delivery (LPL), mitochondrial fatty acid uptake (CPT-1), mitochondrial oxidative capacity (CS, UCP3/2) and biogenesis [PGC-1 α/β (key co-activators) and PPAR α/β/γ (transcription factors)]. Each has been previously determined to associate or

act as a marker for regulation/dysregulation of skeletal muscle lipid metabolism. Interestingly, the expression of these genes has also been shown to be responsive to positive physiological perturbations including endurance exercise training (Hildebrandt & Neufer, 2000; Pilegaard, Ordway, Saltin, and Neufer, 2000).

Metabolic Genes

PGC-1 α is the master co-activator responsible for glucose homeostasis, lipid and energy homeostasis, and is involved in pathogenic conditions including obesity and diabetes (Lin, Handschin, and Spiegelman, 2005). The coactivators integrate signaling pathways in the control of cellular and systemic metabolism. Additionally, PGC-1 α and β stimulate the expression of UCP2 and UCP3, which dissipate the proton gradient and lower mitochondrial membrane potential. This process is thought to reduce ROS production by the mitochondria, thus reducing oxidative stress, DNA damage, lipid peroxidation and oxidative protein modifications (Lin et al., 2005).

UCPs are mitochondrial transporters that are found in the mitochondrial inner membrane. UCP3 is expressed primarily in skeletal muscle and is involved in the limitation of free radical cells as well as fatty acid metabolism. UCPs in general, are responsible for controlling the level of respiration coupling (Rousset, Alves-Guerra, Mozo, Miroux, Cassard-Doulcier, Bouillaud, & Ricquier, 2004). UCP3 has been shown to increase the proton conductance of the inner membrane when activated by products of ROS metabolism. Additionally, evidence has shown that mild regulated uncoupling

caused by UCP3 attenuates mitochondrial ROS and protects against cellular damage (Esteves & Brand, 2005).

CPT-1 β is the rate limiting step for the carnitine palmitoyltransferase system and it is the enzyme responsible for controlling the transfer of long chain fatty acyl CoA into the mitochondria for oxidation (Saha & Ruderman, 2003). When in the catabolic state, the liver becomes a glucose producer, lipogenesis is slowed, and fatty acid oxidation is activated. The acute regulator of both skeletal muscle and liver fatty acid oxidation, acting allosterically and reversibly, is malonyl CoA. In the fed state, when insulin levels are low, malonyl CoA inhibits CPT-1 β, therefore limiting fatty acid oxidation. In the catabolic state, malonyl CoA levels are low, disinhibiting CPT-1 β, and thus increasing rates of fatty acid oxidation (Foster, 2004).

Pyruvate dehydrogenase complex (PDC) is a key factor in skeletal muscle metabolism as it is the entry for carbohydrate derived fuel into the mitochondria for oxidation. PDC catalyzes the oxidative decarboxylation of pyruvate to form acetyl-CoA. This links glycolysis to the tricarboxylic acid cycle and ATP production. PDC is regulated by the phosphorylation-dephosphorylation cycle where pyruvate dehydrogenase kinase (PDK) phosphorylates and inactivates the complex. PDK4 is the isoform expressed in skeletal muscle and PDK4 activity is increased with exercise (Pilegaard & Neufer, 2004). An increase in lipid delivery results in an increased expression of PDK4. In a study on Pima Indians who are prevalent in obesity and type 2 diabetes mellitus, changes in PDK4 mRNA expression in skeletal muscle was shown to correlate with an improvement in insulin sensitivity in morbidly obese patients who had

undergone bariatric surgery. This shows a link between PDK4 expression and fat mass. Additionally, the reversal of obesity and insulin resistance results in a reduction of PDK4 expression and thus facilitates glucose clearance, allowing appropriate use of glucose and lipid (Sugden, 2003).

Exercise training in lean individuals is recognized as an effective physiological method of expanding the skeletal muscle's mitochondrial oxidative capacity (biogenesis) and hence, the capacity to oxidize lipids (Holloszy, 2003; Spina, Chi, Hopkins, Nemeth, Lowry, and Holloszy, 1996). It has also been demonstrated that increased physical activity is associated with weight loss. If a similar fat oxidative response occurred in obese subjects, and in particular both AAW and CW, exercise may be an effective physiological treatment for expanding the oxidative capacity of the skeletal muscle of the these individuals. This also suggests that insulin action may be improved by reducing the accumulation of bioactive fatty acids (e.g. DAG, acetyl CoA) observed to be elevated with obesity and skeletal muscle insulin resistance (Lowell & Shulman, 2005). In support, at the whole body level, it has been reported that obese women increase their fat utilization through oxidation of non-plasma fatty acids in response to acute exercise (90 minutes at 54% max VO_2; Horowitz & Klein, 2000). Similarly, preliminary data from our laboratory demonstrate that as little as 10 days of endurance exercise training can increase *in-vitro* palmitate oxidation two-fold. This observation is true for both sedentary AAW and CW subjects.

Conclusion

Obesity is a growing epidemic in the United States and is characterized (at least in part) by an impaired mitochondrial capacity to oxidize fat as fuel. In addition to its link with diabetes, this impairment may at least partially explain the greater propensity toward obesity in AAW vs. CW. Several genes have been determined to regulate skeletal muscle lipid oxidation and altered gene expression may account for noticeable mitochondrial dysfunction leading to reductions in skeletal muscle lipid oxidation observed with obesity. Additional research is needed to determine whether these key regulatory genes are expressed differentially in obese AAW vs. CW and whether endurance exercise can enhance their expression and expand the skeletal muscle's capacity to oxidize lipids.

Problem and Purpose

Obesity is a global epidemic that has significantly increased the morbidity and mortality among individuals in Western society. This threat to health appears to be even larger among AAW when compared to CW. The physiological dysregulations and mechanisms involved have not been clearly elucidated, nor have physiological interventions been defined to counteract the growth and severity of obesity and related pathologies among AAW and CW.

Therefore, the purpose of this study is to examine potential mechanisms behind the expansion in skeletal muscle oxidative capacity following endurance exercise training. As AAW vs. CW possess a greater propensity toward obesity and a reduced capacity of skeletal muscle to oxidize fatty acids, comparisons will be made between

these races. Fortunately, both AAW and CW possess the metabolic capacity to increase the oxidation of fatty acids following aerobic exercise training. Accordingly, we will assess the expression of key metabolic genes known to be involved in skeletal muscles mitochondrial biogenesis and adaptations in oxidative capacity before and following acute exercise in the untrained and trained condition.

Research Hypothesis

Our global hypothesis is that obese/pre-diabetic women have an impaired ability to oxidize fatty acids in skeletal muscle. We propose that this dysregulation in lipid metabolism is due at least in part to impairment in skeletal muscle mitochondrial capacity. Endurance exercise training stimulates mitochondrial biogenesis which increases the ability of skeletal muscle to oxidize fatty acids. Based on preliminary data demonstrating that 10 days of endurance exercise training results in an increase in fatty acid oxidation in skeletal muscle in obese women, we hypothesize that the increase in fatty acid oxidation will be associated with an increase in gene expression responsible for the regulation of mitochondrial oxidative capacity/mitochondrial biogenesis.

Our secondary hypothesis is that in the sedentary state, obese AAW have a greater impairment in their skeletal muscle's fatty acid oxidation capacity compared to obese CW. However, following 10 days of endurance exercise training, AAW will also increase the expression of key genes known to regulate skeletal muscle mitochondrial capacity to oxidize fatty acids in skeletal muscle.

Delimitations

1. The subjects were recruited from the East Carolina University campus and the surrounding Pitt County/Greenville area via advertisement.
2. Subjects consisted of obese female, African-American and Caucasian women, pre-menopausal, between the ages of 20-45 years, Body Mass Index (BMI) of greater than 30 kg/m^2, and sedentary.
3. Subjects were sedentary at least six months prior to the study ($\leq$2 days/week at a moderate intensity).
4. Subjects were not on any medications that could affect whole body or skeletal muscle metabolism (e.g., synthetic thyroid replacement medications).
5. Subjects were non-smoking for at least 12 months prior to the study.
6. Subjects were under dietary monitoring 3 days prior and 3 days at the end of the training period.
7. African-American subjects were of second generation or greater; Caucasian subjects were of non-Hispanic, or oriental descent as determined by questionnaire.
8. Aerobic endurance training was delimited to 10 days of cycle ergometry at 75% VO_2 max for 60 minutes.

Limitations

1. The recruitment of subjects was limited by the nature of volunteerism and to individuals residing in and around Pitt County, North Carolina.
2. The training period was for 10 days only.
3. Subjects were obese. Non-obese subjects were not recruited.
4. Adherence and accuracy of participant self-reported diets 3 days prior to the study and then consumed identical meals during the last 3 days of training- done to control blood lipids and enzymatic activity.
5. Determination of gene expression, but not protein content for molecular determinants regulating rates of skeletal muscle fatty acid oxidation was limited to the vastus lateralis muscle.

Definition of Terms

1. BMI: [Body Mass Index] mass/ht^2 (kg/m^2)
2. Waist:Hip Ratio: Circumference measurement taken around the narrowest part of the natural waist and the widest portion of the hips/buttocks.
3. Minimal Waist: Circumference measurement taken around the narrowest part of the natural waist.
4. In vitro rates of skeletal muscle fatty acid oxidation: assessed from whole homogenates from vastus lateralis muscle biopsies by capturing CO_2 and acid soluble metabolites (ASM) following 30 minutes of incubation at 37°C.
5. Percutaneous muscle biopsy technique: 75-150 mg of vastus lateralis skeletal muscle obtained using a modification of the Bergstrom technique.
6. Sedentary: not engaged in physical activity for at least 6 months prior to the study; not exercising more than 2 days per week with a less than moderate intensity.
7. African-American: Second generation or greater as described by health history questionnaire
8. Obesity: BMI>30 kg/m^2, Minimal (Min) Waist:
9. Pre-Diabetes: Fasting glucose levels of 110-125 mg/dL
10. Diabetes: Fasting glucose levels of above 126 mg/dL (ADA guidelines)
11. Vastus Lateralis: lateral portion of the thigh musculature
12. Study conditions in the basal (pre-exercise) condition: Subjects remain fasted for 10-12 hours immediately before exercise in the untrained state

13. Study conditions in the recovery phase from acute exercise: Subjects remain fasted after arriving in the basal condition, performing 1 h of bicycle training, and returning 4 h post exercise for a second muscle biopsy, water consumption only

CHAPTER II

LITERATURE REVIEW

Overview of Obesity

Obesity has become an epidemic in the United States and worldwide. Obesity is defined as a body mass index (BMI) of 30 kg/m^2 or higher (Flegal, Carroll, Ogden, and Johnson, 2002; Mokdad et al., 2001). In the United States in the year 2000, the prevalence of obesity was 19.8%, reflecting a 61% increase since 1991. In a study by Mokdad et al., in 2001, approximately 56.4% of their participants were overweight, compared to 45% in 1991 (Mokdad, et al., 2001). According to Flegal et al. (2002), the age-adjusted prevalence of obesity was 30.5% in 1999-2000 and the prevalence of being overweight increased from 55.9% to 64.5% between 1988-1994, while extreme obesity increased from 2.9% to 4.7% (Flegal et al., 2002). Potential contributing factors that may be responsible for these alarming observations include reductions in physical activity and consumption of greater quantities of energy dense foods.

Obesity is a serious health threat as it is strongly related to physiological diseases including type II diabetes mellitus, hypertension, dyslipidemia, and cardiovascular disease (Kannel, 1996; Wickelgreen, 1998). Abbasi and colleagues (2002) conducted a study on the relationship between obesity, insulin resistance, and coronary heart disease risk. The results of their study indicated that 26% of the obese individuals (BMI>29.0 kg/m^2) were insulin-resistant. The results also provided significant information regarding the link between obesity and insulin resistance and the development of type II diabetes

(Abbasi, Brown, Lamendola, McLaughlin, & Reaven, 2002). Insulin resistance and hyperinsulinemia have also been shown to predict Coronary Artery Disease in non-diabetic individuals (Despres, Lamarche, Mauriege, Cantin, Lupien, and Dagenais, 1996). In addition to the negative physiological effects of obesity on glucose tolerance and cardiovascular disease, depression and anxiety, osteoarthritis, gallbladder disease, breast cancer, colon cancer, and endometrial cancer are also associated complications (Colditz, 1999; Kumanyika, 1987).

A recent study has determined that failure to treat and prevent obesity would someday significantly overwhelm the health care system (Must et al., 1999). The treatment costs, lost wages, and direct health care costs have been estimated at more than $70 billion dollars (Wickelgren, 1998) and diabetes related costs have been estimated at $98 billion dollars (Mokdad, et al., 2001).

Individuals who are classified as obese and overweight are advised to avoid additional weight gain and work toward the development of a healthy lifestyle, including smoking cessation, reduced saturated fat intake, reduced caloric intake, and increased physical activity (Flegal, Carroll, Kuczmarski, and Johnson, 1998).

Obesity in African American Women vs. Caucasian Women

The prevalence of obesity in the United States is higher among AAW compared to CW with nearly 48% of AAW being classified as overweight/obese. During infancy and childhood, AAW are not more overweight than CW. However, during adolescence and adulthood, body fat percentage is higher among AAW (Kumanyika, 1987).

According to Kuczmarski, and colleagues (1994), nearly one half of adult non-Hispanic black women are overweight in the United States (Kuczmarski, Flegal, Campbell, and Johnson, 1994).

There are many factors that might contribute toward the greater propensity of obesity in AAW including lifestyle, socioeconomic status, and diet (Kumanyika, 1987). AAW have reported less physical activity compared to CW, thus contributing to their higher prevalence for obesity (Weinsier, 2002). However, the differences in socioeconomic status, lifestyle, and education do not fully explain the increased incidence in AAW compared to CW (Chitwood et al., 1996).

Obesity and excess body fat are both a metabolic consequence, which occurs when energy storage exceeds energy expenditure. Longitudinal studies have identified several predictors of future weight gain, primarily in populations genetically predisposed to obesity. Low metabolic rates during rest, low 24-hour energy expenditures, low 24-hour fat oxidation, and insulin insensitivity constitute these indicators (Chitwood, et al., 1996).

With this in mind, several studies have identified that the resting energy expenditure (REE) of AAW is lower than CW. Jakicic and Wing (1998) compared the energy expenditures among AAW and CW using indirect calorimetry. There were no significant differences between the groups for age, weight, BMI, fat mass, fat free mass (FFM), percent body fat, dietary intake or physical activity. Metabolically active FFM was approximately 2 kg higher in the AAW than CW, although this difference was not statistically significant. Despite the higher lean mass, when corrected for FFM, AAW

still had a significantly lower REE by approximately 707 kJ/d (169 kcal/d) (Jakicic & Wing, 1998). This is consistent with previous studies as Chitwood et al. (1996) reported differences in REE in lean AAW vs. CW, with AAW being lower by approximately 753 kJ/d (180 kcal/d) and Foster et al. (1999) have reported a difference in overweight AAW, being lower by approximately 393 kJ/d (94 kcal/d) vs. CW. These and supporting studies suggest that AAW are less metabolically active compared to CW. Additionally, there has been evidence suggesting AAW are less successful than CW in weight loss programs, have smaller decreases in calorie intake, and have poorer attendance rates at exercise and nutrition programs (Jakicic et al., 1998). Additionally, Zurlo suggests a low rate of fat oxidation could contribute to weight gain, represented by a higher RER (Zurlo, Lillioja, Esposito-Del Puente, Nyomba, Raz, and Saad, 1990).

Weinsier et al. (2002) studied AAW and CW with respect to weight loss and the relationship to aerobic capacity, physical activity, energy expenditure, and likelihood for weight gain. In addition to having a lower rate of obesity, CW had a greater aerobic capacity than AAW regardless of whether they were overweight or of normal body weight and whether $VO_{2\,max}$ was adjusted for FFM or body weight (Weinsier et al., 2002). In the CW, weight loss improved fitness and increased activity, which led to an increase in activity energy-expenditure and a decrease in weight regain. However, in AAW, weight loss to a healthy body weight resulted in a decreased fitness level, decreased activity, decreased activity energy cost which in turn led to a decrease in activity energy-expenditure and therefore increased the probability for weight regain among the AAW subjects (Weinsier, et al., 2002).

Although AAW have a greater prevalence of obesity, they have a lower risk of developing some of the metabolic complications associated with obesity when compared to their Caucasian counterparts matched for BMI, body fat, and WHR. Basal fatty acid kinetics was found to be lower in obese AAW than in obese CW by Racette and colleagues (2000) when matched for percent body fat and WHR. Racette et al. also found the rate of fatty acid release into plasma was lower in AAW than CW at basal levels. It has been concluded that these differences in basal fatty acid kinetics is due to lipolysis of subcutaneous adipose tissue triglycerides which involve lipolytic regulatory mechanisms including the sympathetic nervous system activity, cortisol secretion, and adenosine and cytokine production (Racette, Horowitz, Mittendorfer, and Klein, 2000).

Excess obesity in AAW is not solely a result of excess caloric intake, low physical activity, inability to maintain a healthy diet, or lack of motivation (Kumanyika, 1987). Rather, physiological differences in skeletal muscle oxidative capacity and underlying genetic dysfunctions may also contribute significantly toward the differences between AAW and CW with respect to obesity. These are discussed below.

Obesity Associated with Metabolic Dysregulation

Obesity is a metabolic disease characterized in part by an impaired ability to utilize fat as a fuel. For example, participants in various studies have been shown to manifest a reduced ability to oxidize fatty acids in skeletal muscles (Blaak & Saris, 2002). Reductions in skeletal muscle oxidative capacity may lead to the accumulation of bioactive lipids which are associated with insulin resistance and diabetes (Shulman,

2000). In a previous study on genetically obese Zucker rats, fatty acid metabolism was evaluated as a contributor to the presence of muscle insulin resistance. The purpose of the study was to evaluate fatty acid uptake and disposal as modifiers of insulin sensitive muscle. Turcotte and colleagues (2001) found that fractional and total rates of palmitate uptake were 42 and 74% higher in obese than lean rats. The percentage of palmitate oxidized was not significantly different between the two groups. They found that pre- and post-perfusion muscle triglyceride levels were higher in both red and white muscle of obese rats, suggesting, that increased FA uptake and altered FA disposal to storage, may contribute to the development of muscle insulin resistance in obese rats (Turcotte, Swenberger, Zavitz Tucker, and Lee, 2001). An impairment in fatty acid oxidation has also been observed in humans (Kim et al., 2000). It has been proposed that a reduction in skeletal muscle fatty acid oxidation capacity is linked to reduced size and functionality of the mitochondria (Kelley et al., 2003).

Skeletal Muscle Fatty Acid Oxidation Regulatory Mechanisms

In addition to its role in glucose homeostasis, skeletal muscle is the major tissue site responsible for whole-body lipid oxidation where lipids delivered to the muscle are used as a fuel (Cortright, Muoio, and Dohm, 1997). During the fasted state, the predominant metabolic activity in skeletal muscle is via lipid oxidation of exogenous and endogenous fatty acids (Dagenais, Tancredi, and Zierler, 1976). Fatty acid oxidation contributes up to approximately 90% of the energy requirements in the resting muscle (Bulow, 1988; Dagenais et al., 1976). The role of skeletal muscle in fat accumulation is

essential as fatty acids that are not oxidized must be redistributed elsewhere. Skeletal muscle has been shown to store fatty acids as triglycerides; however, this is a limited process, and consequently non-oxidized fatty acids shift toward lipid accumulation in adipose tissue (Kelley et al., 1999; Bulow, 1988).

Skeletal muscle possesses a transmembrane and a postmembrane fatty acid transporter system. These transporters aid in clearing blood lipids, which are transported as albumin-bound fatty acids (adipocyte source), as VLDL triglycerides (liver source), or chylomicrons (gut source). The cellular fatty acids presented by the action of sarcolemmal specific lipases enter the cell by passive diffusion or active transport (e.g., CD36, FATPpm) and are then targeted toward oxidation in the mitochondria by cytosolic fatty acid binding proteins. Alternatively, fatty acids my also be esterified to glycerol units for storage (normally a limited process in skeletal muscle). Fatty acids bound for mitochondrial oxidation require both activation and transport into the mitochondrial matrix. Mitochondrial membrane bound long-chain acyl-CoA synthetase (ACS) activates cytosolic LCFA in an ATP dependent manner (Colemann & Lee, 2004) and subsequently gain access to the mitochondrial matrix β-oxidation machinery. Long chain acyl-CoA is impermeable to the mitochondrial membrane and thus requires a transport mechanism. The process is carnitine dependent and is initiated by carnitine palmitoyl transferase-1 β (CPT-1 β) which exchanges carnitine for CoA on the outer membrane. CPT-1 β is considered the rate-limiting step in fatty acid transport into the mitochondrial matrix and thus is rate limiting for FA oxidation. CoA is then re-exchanged for carnitine on the

matrix side of the mitochondria; a process which is catalyzed by the inner membrane protein CPT-II (Kerner & Hoppel, 1999).

Obesity is associated with skeletal muscle dysregulation of lipid metabolism. Some animal models have observed reduced oxidation of fatty acids by skeletal muscle. For example, using the Zucker rat model of obesity, it has been noted that the amount of lipids oxidized is much less in obese animals compared to lean animals, suggesting skeletal muscle lipid oxidation dysregulation (Bessesen, Rupp, and Eckel, 1995). In humans, Kelley and corkers (1999) have examined skeletal muscle activity in lean and obese individuals; specifically glycolytic and oxidative enzymatic activity. They found a decline in citrate synthase (CS) and β-HAD (β-oxidation) in obese, insulin-resistant subjects (Colberg et al., 1995; Simoneau, et al., 1995; Simoneau & Kelley, 1997). In support of these data in humans, significant reductions in skeletal muscle fatty acid oxidation have been demonstrated in obese vs. lean individuals (Kim et al., 2000).

Accumulation of Skeletal Muscle Lipids and Insulin Resistance

Unlike the liver, skeletal muscle has a limited capacity to store fatty acids in the form of triglycerides; therefore, reductions in lipid oxidation can result in accumulation of cytoxic levels of bioactive fatty acids which has been implicated in the etiology of skeletal muscle insulin resistance and progression toward diabetes. (Kelley, 2002; Shulman, 2000).

In brief, Shulman (2000) and others have proposed that mitochondrial dysfunction promotes the accumulation of various lipid metabolites such as long-chain acyl CoA

units, ceraminides, various diacylglycerol, etc. which can attenuate the insulin signaling cascade and consequent translocation of glucose transporters to the cell surface membrane halting the movement of glucose units in the mitochondria (Lowell & Shulman, 2005). Proposed mechanisms linking elevated bioactive lipids and skeletal muscle insulin resistance include PKC serine/threonine phosphorylation of the insulin receptor with consequent tyrosine kinase activity reduction following insulin binding (Lowell & Shulman, 2005).

Metabolic Dysregulation in African-American vs. Caucasian Women

As described earlier, income, education, and socioeconomic status are not the sole contributors to the higher incidence of obesity in AAW, suggesting there are metabolic and/or physiologic factors that predispose individuals to obesity (Kumanyika et al., 1993; Kuminyaka, 1994; Streigel-Moore et al., 1996). Additionally as mentioned, AAW exhibit lower resting rates of energy expenditure as well as physical activity energy expenditure. Most recently however, evidence is emerging that now suggest AAW also have lower rates of fat oxidation which certainly could contribute to the higher prevalence of obesity and diabetes among AAW (Kumanyika et al., 1993; Kuminyaka, 1994). In a study by Nicklas et al., it was found that resting metabolic rates, adjusted for lean body mass, were 5% lower in AAW compared to CW and oxidation was 17% lower (Nicklas, Berman, Davis, Dobovolny, and Dennis, 1999). Additionally, it has been found that the *in vivo* rate of fat oxidation in lean and obese AAW was approximately 50% of fat oxidation in CW (Privette, Hickner, Macdonald, Pories, and Barakat, 2003). These

results are significant, suggesting pre-obese AAW have a reduced ability to oxidize fatty acids, therefore, supporting the hypothesis that there is an underlying genetic component accountable for the racial differences between AAW and CW (Hickner et al., 2002; Chitwood et al., 1996). Additional support for these findings come from a study by Privette and colleagues who demonstrated that *in vitro rates* of skeletal muscle oxidation are depressed in obese AAW vs. CW (Privette et al., 2002).

Exercise and Fatty Acid Oxidation: Exercise and physical activity are key components in the treatment of obesity. When obese individuals engage in physical activity, they are often able to lose and maintain weight loss (Kayman, Bruvold, and Stern, 1990). Several studies have shown exercise training to be one of the few interventions that result in weight less and more specifically, increases lipid oxidation in the post absorptive state. Significant effects of endurance exercise on fat metabolism can be realized with as little as 7-10 days of training. Training regimens of this time frame result in increased post absorptive fat oxidation in lean subjects (Calles-Escandon, Goran, O'Connell, Nair, and Danforth, 1996). Additionally, following acute exercise, obese women used more fat as fuel by oxidizing non-plasma fatty acids (Van Baak, 1999). In conjunction with the previous support, our secondary hypothesis is that exercise training may reverse the initial decrease in lipid oxidation found in pre-obese AAW and may improve a preexisting dysfunction in skeletal muscle lipid metabolism in AAW.

Effects of Exercise Training on Skeletal Muscle Gene Expression: During exercise training, the expression of genes linked with lipid metabolism in human skeletal muscle is increased. Specifically, endurance exercise training results in many skeletal

muscle adaptations, including increased oxidative metabolism of fatty acids and carbohydrates. Many studies have shown that a single bout of sustained muscular activity results in the ability to transiently activate gene expression; including GLUT4, hexokinase, and uncoupling protein (UCP)-3 gene expression (Tunstall, Mehan, Wadley, Collier, Bonen, Hargreaves, and Cameron-Smith, 2002).

Tunstall et al. (2002) conducted a study to examine the transient and chronic changes in genes essential to fatty acid uptake and oxidation in skeletal muscle. Measurements of transient changes in gene expression were taken pre and post 9 days of exercise training. After 9 days of exercise, there was an increase in CPT-1 β gene expression; however, β-HAD gene expression remained unchanged after acute exercise and 3 hours post exercise. Also unchanged was the expression of PPARα and PGC-1 α genes, while PPARγ was significantly reduced with training (Tunstall, et al., 2002).

The impact of exercise intensity and duration on acute fiber-type specific transcriptional regulation of metabolic genes was evaluated in red and white gastrocnemius muscle from rats by Hildebrandt and colleagues (Hildebrandt, Pilegaard, and Neufer, 2003). In this study, rats engaged in one of three treadmill running protocols. The first consisted of low intensity exercise (LIE), approximately 50% $VO_{2\ max}$, the second LIE for 180 minutes, or the third, high intensity exercise (HIE), approximately 75% $VO_{2\ max}$ for 45 minutes. The results showed LIE for 45 minutes activated the transcription of pyruvate dehydrogenase kinase 4 (PDK4), uncoupling protein 3 (UCP3), heme oxygenase-1 (HO-1), and hexokinase II (HKII) genes within 1 hour post exercise in red gastrocnemius muscle. Additionally, in white gastrocnemius

muscle, LIE activated PDK4, UCP3, HKII, and lipoprotein lipase (LPL) transcription while the HO-1 remained unchanged. However, HO-1 transcription was much greater when LIE duration increased from 45 to 180 minutes. In the white gastrocnemius muscle, LIE for 180 minutes produced a more significant activation of PDK4, UCP3, HO-1, LPL, and CPT-1 genes. With all exercise conditions, within 24 hours post exercise, gene transcription returned to basal levels (Hildebrandt et al., 2003).

Using qRT-PCR-based nuclear run-on analysis, transcriptional activity of several genes can be analyzed from a single muscle biopsy allowing primary adaptive responses of human skeletal muscle to be examined, following exercise training. Pilegaard and colleagues have also determined that exercise training elicits many adaptive changes in skeletal muscle that improve metabolic efficiency. They further explored gene expression in human skeletal muscle during recovery from exercise, examining transcriptional activation of specific genes, including UCP3, PDK4, and HO-1. Additionally, transcriptional activity and mRNA content were determined for metabolic and regulatory genes. During cycling exercise training, participants engaged in a single 4-hour low-intensity cycling bout. Immediately post exercise HKII, LPL, and UCP3 genes were not elevated. However, during recovery, transcription of these genes steadily increased three to six-fold above control levels within 4-hours post-training. Also increased were mRNA levels for HKII, LPL, and UCP3 by 2-2.5 fold within 4-hours post-training. This exercise bout also elicited changes in the PDK4 gene. Upon completion of the exercise session, PDK4 was elevated >10 fold and continued to increase during recovery to nearly >20 fold after 2-4 hours recovery. Transcription of the

HO-1 gene also increased throughout the recovery period by 4.5 fold (Pilegaard et al, 2000). In all genes, activation of transcription returned to control levels within 22 hours following exercise.

Exercise is a unique stimulus as it can produce dramatic two and threefold increases in the expression of proteins with roles in glucose and fatty acid metabolism. Additionally, increases in GLUT-4, HKII, and LPL mRNA in skeletal muscle of humans have all increased following exercise. PDK4 was analyzed extensively as it increased immediately after exercise and continued to increase throughout the 4-hour recovery period (Pilegaard et al., 2000).

In another study by Pilegaard et al., endurance exercise was used to induce mitochondrial biogenesis in skeletal muscle. Peroxisome proliferator activated receptor co-activator 1 α (PGC-1 α), has been identified as a nuclear factor for activating genes required for mitochondrial biogenesis. Muscle biopsies were taken at rest, immediately post exercise and after 2, 6, and 24 hours of recovery. RT-PCR based nuclear run-on was used to analyze the transcriptional activity of selected genes. It was found that prolonged exercise induces a marked increase in transcription and mRNA content of transcription and mRNA content of PGC-1α in human skeletal muscle. Increases in the transcription of PGC-1α increased 3- to >100-fold and in PGC-1 α mRNA increases were from 3- to >20 fold (Pilegaard, Saltin, and Neufer, 2003).

Recent studies have demonstrated that when the PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator-1) system is induced, it will interact with DNA binding transcription factors including PPARs. In muscle, activation of

PPARs is intimately associated with the expression of nuclear genes necessary for mitochondrial biogenesis and fatty acid oxidation (Scarpulla, 2002; Vega, Huss, and Kelly, 2000). For example, PPARs have been identified to increase the expression of several genes such as CPT-1 β, and uncoupling proteins, (all mitochondrial enzymes) which are associated with elevations in skeletal muscle fatty acid oxidation. In contrast, in obese individuals, the skeletal muscle PPAR inducer PGC-1 α is lower compared to lean individuals (Larrouy, Vidal, Andreelli, Laville, and Langin, 1999), suggesting lower gene expression for proteins associated with mitochondrial biogenesis and heightened fatty acid oxidation. Unknown is whether a greater attenuation in the gene expression profile for mitochondrial oxidation of fatty acids occurs in sedentary AAW vs. CW and whether potential differences may contribute as a significant factor predisposing this racial group toward obesity. Along these same thoughts, it is presently unknown to what extent (if any) the gene expression profile is affected by endurance exercise training in AAW vs. CW. However, preliminary experiments from our lab demonstrating enhanced oxidative capacity in skeletal muscle from both AAW and CW following exercise training suggests an up regulation of the gene expression profile responsible for mitochondrial oxidation of lipids.

Conclusion

Obese AAW have a reduced capacity to oxidize fatty acids by skeletal muscle, which contributes to the greater incidence and severity of obesity and related diseases. From previous studies, it has been determined that endurance exercise training has the

ability to increase skeletal muscle's ability to oxidize fatty acids in both AAW and CW. The purpose of this study was to identify the key regulatory genes encoding the enzymes/proteins that play an essential role in skeletal muscle fatty acid oxidation in obese AAW and CW. An extension of this research will be to determine whether some or all of these genes are depressed in the basal (untrained state) condition before training in AAW vs. CW. We hypothesized that similar to obese CW, obese AAW will respond to endurance exercise training by upregulating the gene expression of some or all of the regulatory proteins associated with an increased capacity for skeletal muscle to oxidize fatty acids. As part of a larger study, comparisons between our obese AAW and CW subjects will be made with lean subjects of both races following the same exercise training protocol.

CHAPTER III

METHODS

Overview

The purpose of this study was to examine the potential mechanisms behind the expansion in skeletal muscle oxidative capacity following endurance exercise training. Specifically, we assessed the key regulatory metabolic genes known to be involved in skeletal muscle mitochondrial biogenesis and lipid metabolism. This was assessed before and following acute exercise in both the untrained and trained state.

Participants

The subjects consisted of seven Caucasian and six African-American females. They were obese individuals who had not smoked for a minimum of 1 year prior to the study. Each subject had to meet the following criterion: 1) As determined by a questionnaire African American were of second generation or greater, 2) 20-45 years old, 3) pre-menopausal, 4) had a BMI>30 kg/m^2, and 5) were sedentary women who had not exercised regularly for at least 6 months prior to the start of the study. All subjects were monetarily compensated for their participation in this study. Subjects were reimbursed $250 for their commitment of time and effort during the 10-days of endurance exercise training.

Recruitment of Subjects

Subjects were recruited using advertisements approved by the UMCIRB at East Carolina University. The ads were posted in the local Greenville newspaper as well as in the form of flyers that were hung throughout East Carolina University and the Greenville area. In addition, the study was posted on the Announce Bulletin at East Carolina University. Pregnant women, children <18 years old, the mentally disabled, and prisoners were not allowed to participate in this study.

The study protocol and informed documents were approved by the UMCIRB (# 04-0071) and conformed to the University, State of North Carolina, and Federal requirements for standard operating procedures (see consent form in the appendix). Prior to the initiation of any tests or measurements, an informed consent form was obtained in writing by one of the study investigators. During the preliminary visit, subjects signed the informed consent form only after the study had been described in all aspects by one of the study investigators. This was conducted through an in-person interview in clear language that could be understood by the subject. The interview and consent process took place at the Human Performance Lab or the FITT building on the same day, but before the personal history/nutrition questionnaires and preliminary physical assessments (body composition and VO_2 fitness testing) were performed. The investigators reviewed the study with each potential recruit, detailing the involved tests and procedures and associated benefits and risks. Copies of all the forms, including the medical history questionnaire, were provided for the recruit to examine. Any questions that the recruit had was encouraged and answered by the investigators. Telephone numbers were

provided for any additional questions that arose after the briefing. After the potential subject read the informed consent document, the investigators read the document to the interested subject (if requested) and allowed her to ask any questions as they went through the document. If the subject was illiterate, all documents were read to her by the investigator. The recruit was asked if she understood the study requirements and if she was ready to sign the informed consent. If so, she signed the document at that time. If not, she returned at a later date to sign it. It was made clear that she could withdraw from the investigation at any time without prejudice and that it was a voluntary study. The consent forms were read and signed (signature, date and time) by the subject, one of the investigators, and a witness. A final signature was made by the study principle investigator. Copies of both forms were provided to the research volunteer for her records. If potential participants were non-English speaking, informed consents were drafted and submitted to the IRB for approval prior to participant enrollment. Subjects were informed of their right to withdraw from the study at anytime and that the study was for research purposes only.

Procedures

The experimental design is depicted below. Briefly, on a day prior to the start of the study, subjects signed the informed consent, performed a VO_2 peak test on a cycle ergometer, underwent hydrostatic weighing for body composition, were assessed for their waist to hip ratio (WHR), and were counseled on their dietary recall information. Subjects were instructed to measure and record all food and drink for the three days prior

to the start of the study. Additionally, they were instructed to remain fasted for at least 10 hours prior to blood draws and muscle biopsies (see Figure 1).

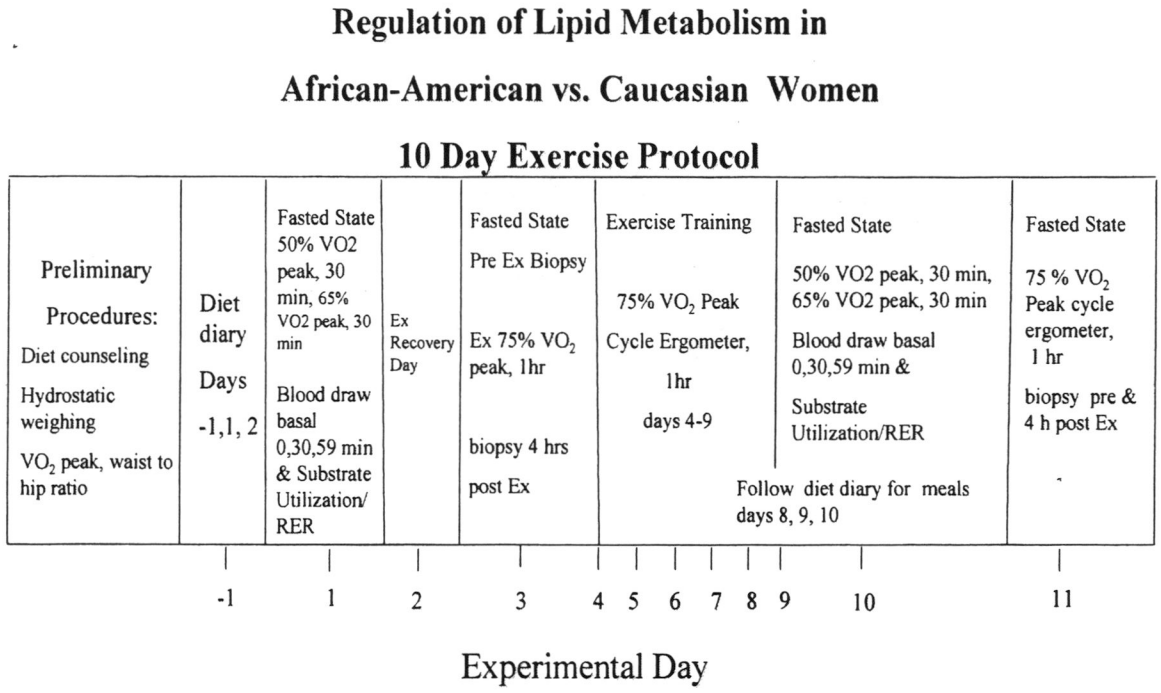

Figure 1: Experimental Design

On day 1 the subject arrived in the fasted state where she was fitted for a catheter in an antecubital vein. The catheter remained in her arm for a minimum of 30 minutes while the subject rested before the first blood sample was taken for basal measures. The subject then bicycled on the cycle ergometer at 50% of her VO_2 peak for the first thirty minutes and at 65% of her VO_2 peak for the remaining 30 minutes. At minutes 30 and 59 the second and third blood draws were taken. The volume of carbon dioxide (VCO_2) produced and oxygen consumed (VO_2) was collected over several minutes (see description of RER measurement below) in order to calculate the respiratory exchange

level (RER; VCO_2/VO_2). Subjects did not exercise on day 2 in order to minimize the effects of acute exercise on basal skeletal muscle gene expression. Therefore, on day 3 the subject arrived in the fasted state where she was prepped for a muscle biopsy of the vastus lateralis muscle. After the muscle biopsy was taken the subject performed one hour of biking at 75% VO_2 peak. Upon completion of the exercise bout, the subject returned 4 hours post exercise training (still in the fasted state; water consumption only) for a second muscle biopsy taken from the contralateral leg. On days 4-9 the subject trained for one hour on the cycle ergometer at 75% VO_2 peak. Training took place in the FITT training facility. On days 8, 9, and 10 the subject was instructed to consume meals exact in nature (constituents and amounts) as recorded earlier over the three days prior to the start of the study. This was to prevent any metabolic effects due to altered food consumption. Dietary intake was analyzed by standard nutritional assessment software (NutriQuest 2.1, McGraw-Hill Companies, Dubuque IA). On day 10 the subject was instructed to arrive in the fasted state for blood draws which were performed the same as on day 1. On day 11, and following a 10-12 h fast, the subject repeated the muscle biopsy procedures by having the third biopsy taken prior to, and the final biopsy taken 4 h post 60 min acute exercise on the contralateral leg.

Note: Data collected for analysis for the Respiratory Exchange Ratio will not be discussed, but will be reported in a subsequent report.

Biopsy Samples

Muscle biopsy samples from the vastus lateralis were snap frozen within 1-2 minutes of the biopsy with liquid nitrogen cooled tongs and stored in cryovials at -80 ° C until analysis for gene expression.

Pretest

Body composition was assessed using hydrostatic weighing. Minimal Waist to Maximal Hip Ratio (WHR) was determined by circumference measures. Height and weight was recorded to calculate body mass index (BMI expressed as kg/m^2). Weight was obtained pre and post exercise training to verify that significant weight loss did not occur. Subjects received information and training from a nutritionist or trained study investigator regarding portion sizes and how to select foods that were recorded in their food intake record. Subjects also performed a VO_2 peak test in the upright position to determine their fitness level and to establish the subsequent training workload.

Hydrostatic Weighing

Percent body fat of each subject was determined using hydrostatic weighing. This required the participant to sit in a chair that was attached to a scale, while remaining under the water, to chin level, and either expelling all air out of her lungs or inspiring as much air as possible, based on BMI. After the air was expired or inspired fully, a reading was taken from the scale before the subject breathed again. The subject performed this approximately six times and the average was used to determine body fat composition.

Specifically, if the subject's BMI was <35 kg/m^2, she performed the head above breathing in method, where she inspired as much air as possible. If her BMI was >35 kg/m^2 she performed the head above breathing out method, where she expired as much air as she could while remaining under the water, up to chin level. Additionally, the weight of the subject's bathing suit, chair weight, and water temperature were measured. Residual volume was also determined using a VO_2 max metabolic cart. Subjects breathed into a filter that was attached to the VO_2 max machine and the system then analyzed the subject's residual volume. If the subject performed the head above breathing in method, she also performed a pulmonary function test for vital capacity measures.

Dietary Records

Food consumption may have considerable effects on skeletal muscle lipid oxidation. Therefore, all subjects were required to consume a self-selected diet three days prior to exercise and repeat this diet over the last three days of the training period (beginning on day 8 and continuing through day 11). Subjects were instructed by a trained nutritionist or study investigator to measure and record all food items consumed for the three days preceding the study. Following the pretest measurements, subjects were trained to maintain a 3-day diary. Subjects were also provided with measuring cups to accurately measure the amounts of food consumed. This allowed us to determine the relative energy intake substrate component and nutrition portions for comparison among subjects. It was equally important that subjects were instructed to not drastically alter their normal dietary habits as this could artificially alter their metabolic response to

exercise and training. A second purpose of this dietary procedure was to control for the potential effects of drastically altered dietary practices at the end of the study. Therefore, subjects were advised to record and consume similar meals over the last three days of the study. This practice allowed us to more precisely isolate the impact of the exercise training protocol *per se* on muscle metabolism and subsequent changes in gene expression.

Peak Aerobic Capacity

A physician was present during each peak exercise test due to the increased risk of adverse affects and events occurring in individuals with risk factors, including obesity. A 12-lead electrocardiogram (ECG) monitor was used as cardiac activity was recorded proceeding, during, and following each peak exercise test. Each subject performed a VO_2 peak (L/min) test in the upright position on a cycle ergometer, the same mode in which the endurance exercise training took place. VO_2 peak was determined by measuring oxygen consumption (VO_2) during incremental exercise using an electronically braked cycle ergometer (Lode, Diversified, and Brea, CA). Oxygen consumption was measured with open circuit spirometry using a metabolic cart (ParvoMedics, OH). The VO_2 peak was used to set the workload for the subjects over the training period. Subjects' exercise intensity was maintained by heart rate throughout each training session using a Polar heart rate monitor.

Subjects were trained according to a modified protocol from our laboratory (Cox, Cortright, Dohm, and Houmard, 1999). Subjects were matched for aerobic capacity and

activity level prior to acceptance into the study and VO_2 peak was measured during incremental exercise using a cycle ergometer (Lode, Diversified, & Brea, CA). Oxygen consumption was measured via open circuit spirometry with a metabolic cart (ParvoMedics, OH). Additionally, a 12-lead electrocardiogram (ECG) recorded heart rate and activity, while VO_2 peak was used to set the workload. The exercise regimen consisted of ten consecutive days of exercise training for 60 minutes (ergometer cycling) at an exercise heart rate corresponding to 75% VO_2 peak. Cox et al. (1999) previously utilized a similar 7-day regimen in young and old subjects and reported improved aerobic capacity and insulin action without altering body composition. Our laboratory has also used a similar 10-day exercise protocol to increase VO_2 peak without altering body weight or composition in females (Hickner, Racette, Binder, Fischer, and Kohrt, 1999). The 10-day protocol was chosen to minimize changes in capillary density and body composition that occur with prolonged endurance training, while providing adequate stimulus for an endurance training effect. This includes increases in VO_2 peak, mitochondrial enzyme content, and insulin sensitivity. During the training period, each subject was intermittently monitored for oxygen consumption to ensure the workload was maintained at the predetermined intensity. A similar training protocol was successfully utilized with obese subjects in the past (Slentz et al., 2002). Body weight was assessed on day 1 and day 11 of the training period to demonstrate the absence of a weight loss effect due to the training regimen.

Exercise training took place in the FITT training facility located near the Human Performance Laboratory (HPL) at East Carolina University, Greenville, NC. The training

room is approximately 2,000 square feet housing eight motor-driven treadmills, four Stairmasters, three Precors, and eight cycle ergometers. This facility has been used to train subjects daily as part of ongoing studies.

Vastus Lateralis Muscle Biopsy Procedure

The procedure we used was a modification of the percutaneous biopsy procedure (Bergstrom, 1962). A typical sample consists of 75-100 mg of skeletal muscle; 10-20 mg of the muscle was fixed for fiber typing and determination of mitochondrial content, 20 mg was quick frozen in liquid nitrogen for determination of intramuscular fatty acid species. The remainder (~20-30 mg) was quick frozen for determination of gene expression using quantitative real-time PCR technology. Muscle biopsies were taken from the vastus lateralis. During the procedure, iodine was spread over the site of the biopsy to initially sterilize the area, followed by ethyl chloride as a topical anesthetic to desensitize the epidermal nerves and 4 cc sterile 1% lidocaine solution, was then injected into the area. The lidocaine was injected in the fascia surrounding the vastus lateralis. Once the site was numb, a small incision (~ 1-2 cm) was made using a scalpel blade. A sterilized biopsy needle was used to collect muscle tissue from the vastus lateralis. Once the muscle had been collected, pressure was applied to the site to end bleeding, the incision closed using a steri-strip and sealed against water using a tegaderm (3M Health Care, St. Paul, MN) bandage. Subjects were instructed to keep the area clean and dry for 3-4 days, and phone numbers were provided to all subjects for re-inspection of the biopsy if needed.

Gene Expression/ Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

As described, differences in the mitochondrial content and/or function may account for the differences in skeletal muscle fatty acid oxidation rates in AAW vs. CW. Therefore, we explored this potential mechanism by probing for gene expression of several genes/proteins known to regulate mitochondrial oxidation, and lipid metabolism. This mechanism may account for the impaired rates of fatty acid oxidation observed in AAW and CW. The level of gene expression was evaluated by Quantitative Real Time PCR (qRT-PCR). This work was conducted in collaboration with Dr. Darrell Neufer who is well established in this methodology (Pilegaard, Saltin, and Neufer, 2003). Approximately 20-30 mg of vastus lateralis muscle was quick frozen immediately after biopsy for subsequent isolation of total RNA. Isolation of RNA from skeletal muscle and quantification of RNA transcripts by reverse-transcriptase PCR were performed as previously described (Hildebrandt et al., 2000; Pilegaard et al., 2000), with the incorporation of real-time PCR technology (Pilegaard et al., 2003; Pilegaard et al., 2003). Real-time PCR represents a more accurate, efficient (384 well format) and cost effective means for quantification of RNA. All primers and probe sequences were derived from Gene Bank Sequence information using Primer Select software (Applied Biosystems) and had been given in detail elsewhere (Pilegaard et al., 2003; Pilegaard et al., 2003). All probes use MGB technology. Sufficient template was usually available for the analysis of at least 10-12 genes. The list of genes included in this application, represent the minimum analysis that was performed. Any new primer/probe sets designed during the course of these studies was verified to be specific for a single target gene by Blast Search

a method to search out new genes discovered with relevance to skeletal muscle metabolism. To account for potential changes in total mRNA with training, data was normalized to total mRNA content (quantification by picogreen (Molecular Probes) after reverse transcriptase reaction). β-actin and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA/total mRNA were also assessed for potential use as normalizing genes.

Blood Analysis

Subjects reported to the laboratory following an overnight fast (no food after 10:00 pm). On days 1 and 10, blood samples were drawn at minute 0 (baseline), at minute 30, ending the exercise period at 50% VO_2 peak, and at minute 59 ending the exercise period at 65% VO_2 peak. At each time point, serum, plasma, and EDTA treated tubes were collected. The total volume of blood collected per time point was approximately 15 milliliters, totaling 45 milliliters for the day. All samples were collected in test tubes, which were placed on ice for 15 minutes to allow for clotting prior to centrifugation at 3,000 g. Prior to each blood sample, approximately 1.5 milliliters of blood was drawn off and discarded to prevent contamination by saline in the venous line. In addition, the venous catheter was flushed with ~ 2 milliliters of sterile saline after time points 0 and 30 minutes. The blood vial was then aliquoted and immediately frozen at -80 ° C until further analysis.

Note: Blood collection as described above was conducted as part of a larger research project. Therefore, only samples in the pre-training condition were analyzed in the present study for determination of subject diabetic status.

All blood samples were obtained in room 3S-08, Brody School of Medicine according to the UMCIRB (#04-0071) approved protocol. All study personnel were trained in proper handling, disposal, and storage of human blood according to guidelines established by Prospective Health, ECU. All blood samples were drawn with biohazard protection for investigators and subjects. Proper disposal of biohazard waste was readily accomplished and maintained in all the laboratories utilized in this study. This procedure was repeated on day 10 of the study.

Insulin Action

Blood samples collected in the pre-training, overnight-fasted condition (10-12 hours) were measured by immunoassay (Access Immunoassay System, Beckman Coulter, and Fullerton, CA) and glucose with an oxidation method (YSI Model 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). Insulin action was determined by homeostasis model assessment (HOMA). The HOMA determination correlates highly with insulin action determined with a euglycemic-hyperinsulinemic clamp (Tanner, Koves, Cortright, Pories, Kim, and Kahn, 2002).

Data Storage

The raw data obtained from the biochemical analysis was stored on a computer hard drive, and backed up on CD ROM. Patient data sheets were coded, computer generated, and accessible by the P.I. or research technicians. Files were kept in a locked cabinet and demographic data was password protected, known only by the P.I. and

research technicians/assistants. Handling and storage of all subject data conformed to Federal and University HIPPA mandates.

Statistical Analysis

A two way (race X pre- to post- acute exercise) repeated measures analysis of variance (ANOVA) was used to compare mean differences for results obtained from quantitative real time PCR (skeletal muscle PGC-1 α, CPT-1 β PDK4, and UCP3 gene expression) under the untrained condition, and in a separate analysis, in the trained condition. Training status may also affect skeletal muscle expression of the genes studied. Therefore, two separate repeated measures ANOVA (race X training status) were employed to compare group mean differences in gene expression in the sedentary vs. the trained condition under 1) the pre-exercise/basal condition only or 2) the post-exercise condition only. A Two Way ANOVA (race X training status) was also used to analyze data collected in the rested condition pre- and post training for blood glucose and insulin values whole body substrate utilization (indirect calorimetry and blood variables). All data collected once during the study (e.g., body composition) was analyzed by One Way ANOVA. Significant F-ratios obtained by ANOVA were analyzed by appropriate *post hoc* assessment (Fisher's PLSD test). All data was expressed as mean ± SEM and threshold for statistical significance was established a priori as $P \leq 0.05$.

A separate Two Way ANOVA (race X training status) was also used to analyze comparisons between data collected in the rested (fasted, pre-exercise) and recovery (fasted, 4h post-exercise) conditions pre- and post training for whole body substrate

utilization (indirect calorimetry and blood variables). All data collected once during the study (e.g., body composition) was analyzed by One Way ANOVA. Significant F-ratios obtained by ANOVA were analyzed by appropriate *post hoc* assessment (Fisher's PLSD test). All data was expressed as mean ± SEM and threshold for statistical significance was established a priori as $P \leq 0.05$.

CHAPTER IV

RESULTS

Overview

The data demonstrates that 1) sedentary, obese AAW have a significantly lower VO_2 when compared for relative values 2) acute exercise (one day) causes a greater increase in AAW vs. CW at basal (resting) levels in the pre- acute state for PGC-1 α gene expression; post acute exercise results in increased PGC-1 α in the trained condition in both AAW and CW; AAW and CW had attenuated responses to acute exercise following 10 days of endurance exercise training 3) there were no significant differences in the resting condition with respect to PDK4, although following acute training PDK4 gene expression increased significantly in both AAW and CW; AAW and CW had attenuated responses to acute exercise following 10 days of endurance exercise training 4) UCP3 gene expression levels were similar between AAW and CW at basal levels; in the untrained state there was a significant increase for both time and race and in the trained/post exercise state, values increased in CW but not AAW 5) CPT-1 β had no effect following 10 days of endurance exercise training. Therefore, ten days of endurance exercise results in increases in PGC-1 α, PDK4, and UCP3 gene expression, indicating that following exercise training, mitochondrial biogenesis increases via PGC-1 α, serine phosphorylation of PDH and reduction in its activity and promotion of fatty acids as a substrate occur via PDK4, and the rate of rapid fatty acid β-oxidation increases

via UCP3 following endurance exercise training. It can also be determined that increased rates of oxidation are not due to an increase in CPT-1 β gene expression.

Subjects

The subjects consisted of 7 obese Caucasian (BMI>30kg/m^2) and 6 obese African-American women. All subjects were between the ages of 23-44 years and were pre-menopausal. All subjects were sedentary for at least 6 months prior to the start of the study, non-smoking, and non-diabetic. There were no significant differences between the mean ages of the two groups (P=0.4374; CW 35.0 ± 2.7; AAW 31.8 ± 2.8)

AAW had a slightly higher body mass index [kg of body weight/(ht. in meters)^2] than CW, although there was no significant difference between the groups (P=0.1946; CW =; CW = 38.0 ± 1.7; AAW = 40.3 ± 2.2) (CW however, had a slightly higher percent body fat via hydrostatic weighing (estimated via head above method) than the AAW (P=0.8673; CW = 42.5 ± 2.7; AAW = 43.1 ± 2.2).

In addition, relative and absolute VO_2 peak was measured via indirect calorimetry on a Lode cycle ergometer. There were no significant differences between the groups when compared in absolute terms (P=0.0650; CW = 2.1 ± 0.1; AAW = 1.8 ± 0.1), however, there were significant differences between the two groups when measured in relative terms (P=0.0087; CW = 20.7 ± 1.1; AAW = 16.7 ± 0.5).

Additionally, waist to hip ratio was significantly different as CW had a higher measure compared to their AAW counterparts (P=0.0519; CW = 0.8; AAW = 0.7).

Demographic data (age, weight, height, BMI, percent body fat, waist:hip, and VO_2 peak) are presented in Table 1.

Table 1. Participant Demographics

Obese Subjects	CW	AAW
Number of Subjects (N)	7	6
Age (yrs)	35±2.7	31.83±2.8
Height (m)	1.646±0.02	1.607±0.02
Weight (kg)	103.37±4.7	104.73±6.7
BMI (kg/m^2)	37.98±1.7	40.3±2.2
Percent Fat (hydro.)	42.54±2.7	43.13±2.2
Waist:Hip	0.8±0.03	0.74±0.01
VO^2 peak (L/min)	2.13±0.14	1.75±0.13
VO^2 peak (ml/kg/min)	20.7±1.1	16.7*±0.5

Subjects were not significantly different in BMI, percent fat, or absolute VO_2 peak. VO_2 peak was significantly higher in CW vs. AAW when measured in relative terms.
* Significant differences between CW and AAW at $P \leq 0.05$.

Blood Analysis

The insulin and glucose values are presented in Table 2.

Table 2. Blood Values: Insulin and Glucose

	Caucasian		African American	
	Pre	**Post**	**Pre**	**Post**
Insulin (µU/mL)	11.88±3.92	9.07±2.25	7.79±1.31	7.98±1.85
Glucose (mg/dL)	93.25±3.28	94.73±2.66	89.86±1.66	89.22±3.19

Fasting glucose and insulin values in pre-menopausal, obese CW and AAW pre- and post- 10 days of endurance exercise training at 75% VO_2 peak. Values are mean ±SEM.

Fasting, basal insulin values were similar (P=0.2806) pre- to post- exercise training in CW (11.9 ± 3.9 to 9.1 ± 2.3 μU/mL) and AAW (7.8 ± 1.3 to 8.0 ± 1.9 μU/mL). Fasting, basal glucose values were also similar (P=0.4396) pre- to post-exercise training in CW (93.3 ± 3.3 to 94.7 ± 2.7 mg/dL) and AAW (89.9 ± 1.7 to 89.2 ± 3.2 mg/dL). In both races fasting blood glucose and insulin levels were within normal values and did not significantly change and were used primarily for determination of non-diabetic status.

Nutritional Analysis

In order to control dietary intake, subjects were required to record their food intake three days prior to the start of training and the last three days of training. This was to verify that any and all changes found were due in part to a training effect and not changes in dietary consumption. Nutrition analyses were performed on 6 CW and 6 AAW three days prior to the start of the study and the last three days of the study. Subjects were instructed to eat identically on the last three days as they did the three days prior to the start of the study. They were instructed on how to measure and record all foods, drinks, and condiments. Additionally, they were instructed to provide brand names if possible.

The Caucasian women ate on average over the three days prior to the start of the study, 50.28% carbohydrate, 33.72% fat, and 16.00% protein (in grams, Carbohydrate = 267.4 ± 20.4; Fat = 77.2 ± 6.8; Protein = 80.2 ± 6.1) . In comparison, on the last three days of the study they consumed on average, 49.44% carbohydrate, 35.44% fat, and

15.11% protein (in grams, Carbohydrate = 229.2 ± 35.1; Fat = 74.1 ± 7.6; Protein = 70.6 ± 5.5). The AAW ate on average over the first three days prior to the start of the study, 42.89% carbohydrate, 37.11% fat, and 19.89% protein (in grams, Carbohydrate = 210.5 ± 29.7; Fat = 77.4 ± 8.6; Protein = 90.2 ± 8.0). In comparison, on the last three days of the study they consumed on average, 47.56% carbohydrate, 34.83% fat, and 17.61% protein (in grams, Carbohydrate = 208.8 ± 12.01; Fat = 71.4 ± 7.7; Protein = 79.1 ± 6.2). Differences between races during the three days prior to the start of the study were approximately 141.153 kcals higher in the CW group. Similarly, the CW had a 683.3 kcal higher total energy when compared to the AAW on the last three days of the study. When compared for total body weight, total calories (P=0.7209), carbohydrate (P=0.2036), fat (P=0.9409), and protein (P=0.1013) were all insignificant when compared for racial differences.

When compared for lean body mass, total calories (P=0.6414), carbohydrate (P=0.7360), fat (P=0.5341), and protein (P=0.7073) are also insignificant between races (in grams at baseline, CW carbohydrate = 4.7 ± 0.5; fat = 1.3 ± 0.2; protein = 1.4 ± 0.2; AAW carbohydrate =3.6 ± 0.5; fat = 1.3 ± 0.1; protein = 1.5 ± 0.1; in grams post-training, CW carbohydrate = 4.5 ± 0.6; fat = 1.3 ± 0.2; protein = 1.3 ± 0.1; AAW carbohydrate = 3.6 ± 0.3; fat = 1.2 ± 0.1; protein = 1.4 ± 0.1). The women in the Caucasian group ate similarly to the women in the African American group for all macronutrients as depicted in Table 3.

Table 3. Nutrition Analysis

Macronutrient	Caucasian Women		African American Women	
	Days -1,0,1	Days 8,9,10	Days -1,0,1	Days 8,9,10
ENERGY/TBM (kj/kg)	85.74±7.9	87.79±8.3	76.03±7.5	74.51±7.5
ENERGY/LBM (kj/kg)	160.0±24.7	145.03±16.1	133.28±12.4	130.04±12.1
CHO/TBM (gm/kg)	2.68±0.2	2.32±0.4	2.06±0.3	2.05±0.3
CHO/LBM (gm/kg)	4.66±0.5	4.45±0.6	3.58±0.5	3.59±.3
FAT/TBM (gm/kg)	0.79±0.1	0.75±0.1	0.74±0.1	0.70±0.1
FAT/LBM (gm/kg)	1.29±0.2	1.33±0.2	1.30±0.1	1.22±0.1
PRO/TBM (gm/kg)	0.82±0.1	0.72±0.1	0.86±0.1	0.91±0.1
PRO/LBM (gm/kg)	1.38±0.2	1.30±0.1	1.52±0.1	1.36±0.1

TBM=Total Body Mass, LBM=Lean Body Mass. Values are mean ± SEM.

Although most subjects adhered to their diet as per instructed, there were subjects who did not eat entirely the same. However, this could have been due in part to error in food measurement and portion size, financial means during the week, time to prepare similar foods, availability of similar foods, and NutriQuest software limitations. The primary difference most likely is due in part to inaccurate portion sizes and measurements. Subjects may have over or under estimated portion sizes if they did not have their measuring cups easily available, although they were instructed on proper measurement. Financial costs of certain foods may have prevented some subjects from preparing the same foods. Subjects may have had time conflicts that prevented them from eating similarly as well as availability and time to prepare the foods. It would have been ideal to have pre-made meals to provide participants with so that they would have eaten identically the last three days as they did the pre-three days. Additionally, limitations within the NutriQuest software include specific brand names and specific flavored foods such as cheese flavored grits in place of plain grits.

Racial Comparisons of the Effect of Acute Aerobic Exercise on Select Skeletal Muscle Gene Expression Regulating Fatty Acid Oxidation in the Untrained and Trained Condition

The genes that we focused on with respect to lipid metabolism included PGC-1 α, CPT-1 β, PDK4, and UCP3. Quantitative Real Time PCR was performed for all subjects at baseline and four hours post exercise during the acute phase (day 1) and in the trained state (day 10). Two way repeated measures analysis of variance [race X exercise condition (pre-and 4 h post acute exercise)] examined the effects of acute exercise on skeletal muscle PGC-1 α, CPT-1 β, PDK4, and UCP3 gene expression in untrained/sedentary African-American and Caucasian women in the fasted state. In a separate analysis, group comparisons were made prior to and 4 h after acute exercise in the trained condition.

Peroxisomal Proliferator-Activated Receptor γ Coactivator-1 α (PGC-1 α): Increased expression of muscle peroxisomal proliferators-activated receptor γ coactivator-1 α (PGC-1 α) is associated with mitochondrial biogenesis made possible by increased expression of enzymes acting on fatty acid oxidation, the tricarboxylic cycle, and oxidative phosphorylation (Knutti and Kralli, 2001). Therefore, we first compared the effects of race and acute aerobic exercise on PGC-1 α gene expression in our sedentary/untrained African-American and Caucasian subjects. Repeated measures ANOVA revealed a significant main effect for time (acute exercise; $P < 0.0001$), but not for race ($P = 0.8095$), nor did we note an interaction effect on this variable ($P = 0.8086$).

Thus, as depicted in Figure 2A, acute endurance exercise increased the expression of PGC-1 in both untrained African-American ($30.1 \pm 7.3_{\text{untrained/pre-exercise}}$ to $179.2 \pm 30.2_{\text{untrained/post-exercise}}$, arbitrary units relative to GAPDH; mean ± S.E.M) and untrained Caucasian women ($19.2 \pm 2.8_{\text{untrained/pre-exercise}}$ to $179.2 \pm 30.5_{\text{untrained/post-exercise}}$). African-American women demonstrated greater mean differences in PGC-1 α expression prior to acute endurance exercise compared to obese Caucasian women (56% greater in AAW), but *post hoc* analysis demonstrated that this difference was not statistically different (P = 0.1624) between the races. From these findings, we conclude that the gene expression of the PGC-1 α is similar in the rested condition regardless of race, but that both sedentary obese African-American and Caucasian women demonstrate a similar and significant response in the expression of this transcription factor coactivator following acute aerobic exercise. Next, we wished to observe the response of PGC-1 α to exercise training in both the rested and post exercise conditions (Figure 2 B). Repeated measures ANOVA demonstrated a significant (P = 0.0011) main effect for time (pre-post exercise), but not for race (P = 0.8018), nor was there an interaction between race and time (P = 0.5725). In the trained condition, mean differences in resting PGC-1 α gene expression were similar between our subject groups (AAW = $26.4 \pm 1.7_{\text{trained/pre-exercise}}$; CW = $41.2 \pm 13.6_{\text{trained/pre-exercise}}$; P=0.3374 by *post hoc* analysis). Similar to the untrained condition, both the African-American ($118.7 \pm 14.7_{\text{trained/post-exercie}}$) and Caucasian women ($111.8 \pm 25.7_{\text{trained/post-exercie}}$) demonstrated significant increases (P = 0.0011) in PGC-1 α gene expression following acute exercise in the trained condition.

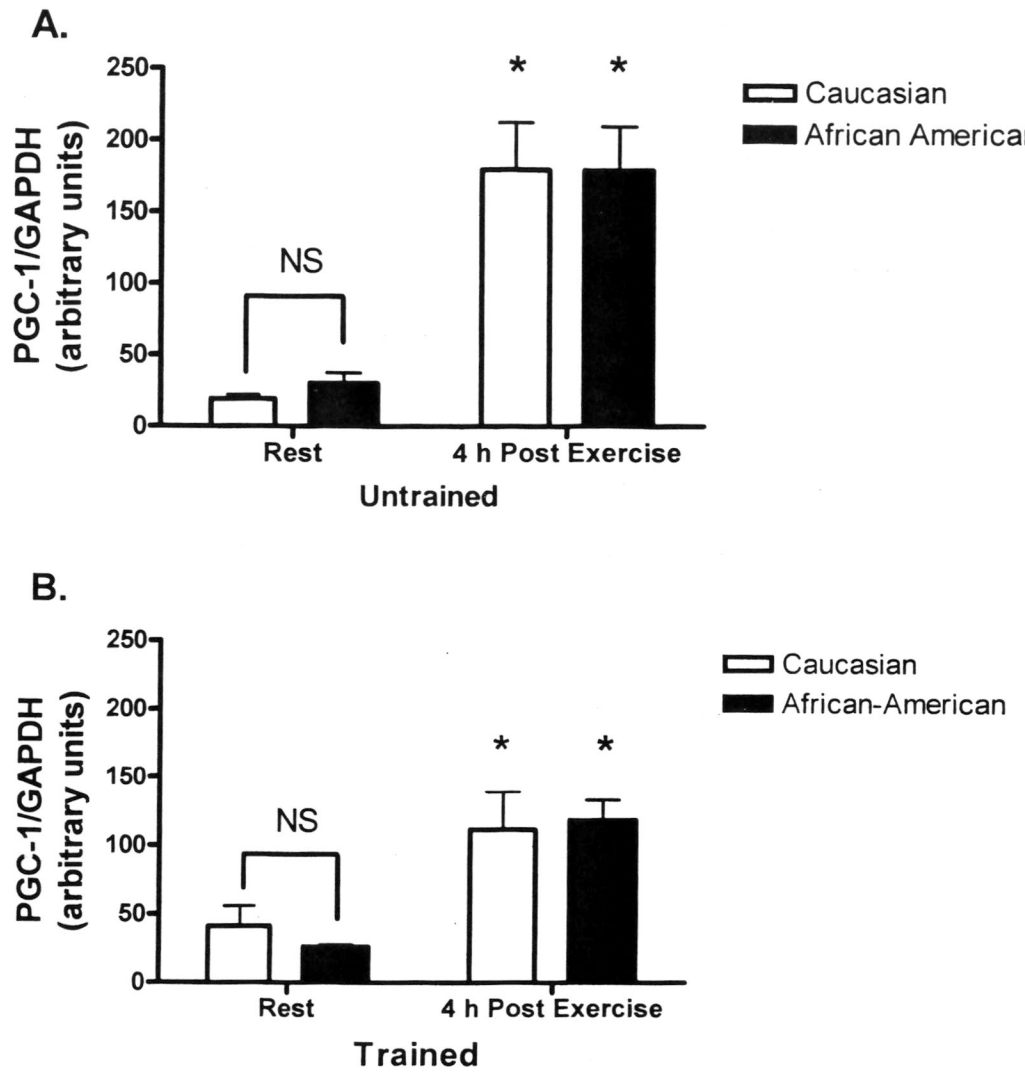

Figure 2. Racial comparisons on the effect of acute exercise on skeletal muscle peroxisomal proliferators-activated receptor γ coactivator-1 α (PGC-1 α) gene expression in the **A.** untrained and **B.** trained state. The subjects consisted of 7 obese Caucasian (BMI>30kg/m^2) and 6 obese African-American women. Acute exercise in both the untrained and trained conditions consisted of cycling on a Monark® bicycle at 75% VO_2 peak. For both the untrained and trained conditions, vastus lateralis muscle biopsies were obtained in the fasted (10 h) state prior to and 4 h post acute exercise. Exercise training consisted of 10 consecutive days of cycling at 75% VO_2 peak. In the rested state, post training biopsies were taken 24 h after the last exercise session. Quantitative real time PCR was used to assess the expression of PGC-1 gene expression prior to and post acute exercise. Values are mean ± S.E.M. **A.** * = significant main effect for time (post > pre exercise condition); $P < 0.0001$. **B.** * = significant main effect for time (post > pre exercise condition); $P = 0.0011$. **NS** = not statistically significant

Carnitine Palmitoyltransferase-1 β (CPT-1β): CPT-1 β catalyzes the conversion of the activated form of long chain fatty acids (long-chain-CoA) to its carnitine derivative. This represents the form of fatty acyl units required for transport to the mitochondrial matrix (Foster, 2004). As such, CPT-1 β is considered the rate limiting reaction in fatty acid oxidation. Earlier findings from our laboratory have demonstrated that CPT-1 β total content or activity is reduced in obese subjects (Kim et al., 2000). Evidence for further impairment in obese African-American vs. Caucasian women is lacking. As endurance exercise training is known to improve the mitochondrial capacity to oxidize fatty acids, we investigated to potential for CPT-1 β gene expression to be expanded in the trained state. Figure 3A depicts the results in the gene expression of CPT-1 β before and after acute exercise in the untrained state. Repeated measures ANOVA (race X pre-post exercise) indicated no interaction effect ($P = 0.10$) as well as non-significant differences (main effect for time, $P = 0.667$) in the expression of CPT-1 β post- (AAW = $94.3 \pm 11.7_{\text{untrained/pre-exercise}}$ vs. $85.9 \pm 9.6_{\text{untrained/post-exercise}}$; CW = $78.2 \pm 9.9_{\text{untrained/pre-exercise}}$, $92.2 \pm 5.3_{\text{untrained/post-exercise}}$; arbitrary units/GPDH, mean ± SEM.). In addition, significant differences were absent in the rested and post exercise conditions between the races (main effect for race, $P = 0.679$). Similar results (figure 3 B) were found for our subject groups in the trained state. Significant main effects for time ($P = 0.813$) and race ($P = 0.303$) as well as an interaction between these variables ($P = 0.675$) were absent. Mean values for the rested condition were $100.4 \pm 8.1_{\text{trained/pre-exercise}}$ for African-American vs. $111.3 \pm 18.5_{\text{trained/pre-exercise}}$ for Caucasian women (arbitrary units/GAPDH). Mean values for CPT-1 β gene expression were $98.4 \pm 9.2_{\text{trained/post-exercise}}$

vs. $118.3 \pm 9.0_{trained/post\text{-}exercise}$ in African-American and Caucasian women respectively, post exercise.

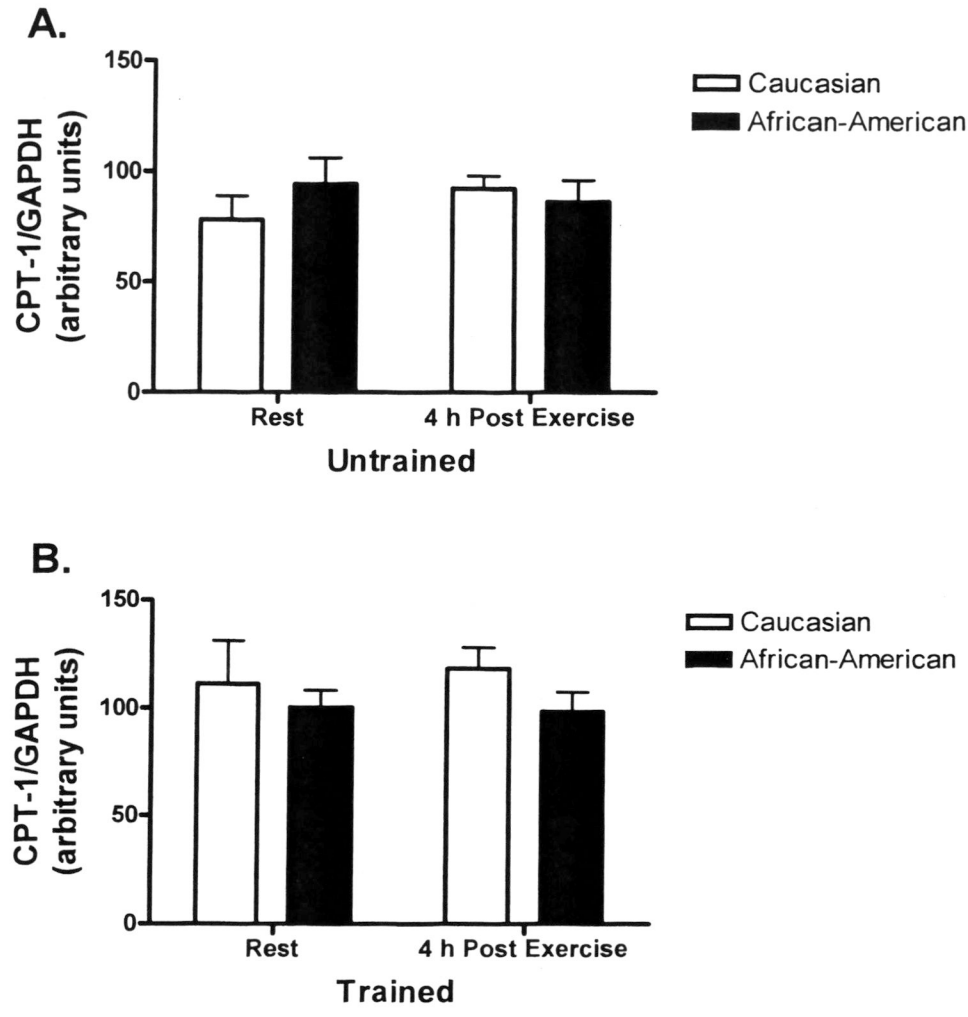

Figure 3. Racial comparisons on the effect of acute exercise on skeletal muscle carnitine palmitoyltransferase-1 β (CPT-1 β) gene expression in the **A.** untrained and **B.** trained state. The subjects consisted of 7 obese Caucasian (BMI>30kg/m2) and 6 obese African-American women. Acute exercise in both the untrained and trained conditions consisted of cycling on a Monark® bicycle at 75% VO_2 peak. For both the untrained and trained conditions, vastus lateralis muscle biopsies were obtained in the fasted (10 h) state prior to and 4 h post acute exercise. Exercise training consisted of 10 consecutive days of cycling at 75% VO_2 peak. In the rested state, post training biopsies were taken 24 h after the last exercise session. Quantitative real time PCR was used to assess the expression of CPT-1 gene expression prior to and post acute exercise. Values are mean ± S.E.M.

Pyruvate Dehydrogenase Kinase 4 (PDK4): Increased activity of pyruvate dehydrogenase kinase (PDK4 in muscle) results in serine phosphorylation of PDH, reduction in its activity and promotion of oxidation away from glucose and toward fatty acids as a substrate. This phenomenon occurs during prolonged aerobic exercise and is promoted in lean Caucasian women after training. Given the suppressed rates of fatty acid utilization noted in the obese state during acute exercise (Hickner et al., 2002) and the noted differences between AAW and CW, we chose to examine the gene expression of PDK4 before and after exercise in the untrained and trained condition in both groups. Figure 4 A depicts the results of acute exercise on PDK4 gene expression in the untrained state. The two way ANOVA [race X time (pre-post acute exercise)] demonstrated a significant main effect for time (P =0.0008) but the main effect for race (P = 0.5406) or interaction between the treatments (P = 0.8503) on the expression of this gene were not statistically different [(AAW = 51.1 ± 14.2 $_{\text{untrained/pre-exercise}}$; CW = 24.2 ± 7.4 $_{\text{untrained/pre-exercise}}$; arbitrary units relative to GAPDH, mean ± S.E.M.) and after (AAW = 181.4 ± 56.2 $_{\text{untrained/post-exercise}}$; CW = 166.2 ± 30.9$_{\text{untrained/post-exercise}}$)]. Thus, in the untrained state, there is a similar increase in PDK4 gene expression following 1 hour of endurance exercise and that both AAW and CW are responsive to the activity stimulus. Also noted, means differences in the pre-exercise expression of PDK4 were noted which was 211% higher in the AAW vs. CW. However, these differences were not statistically different by *post hoc* analysis (P = 0.11). Figure 4 B depicts the response of PDK4 gene expression following 10 days of endurance exercise training in both the rested and post exercise condition. Similar results occurred as in the pre-trained condition in that no differences

were observed between the races in the rested state following training, but both increased significantly [main effect of time, P = 0.005; no effect for race (P = 0.742) or interaction between the two variables (P = 0.3967)] were observed.

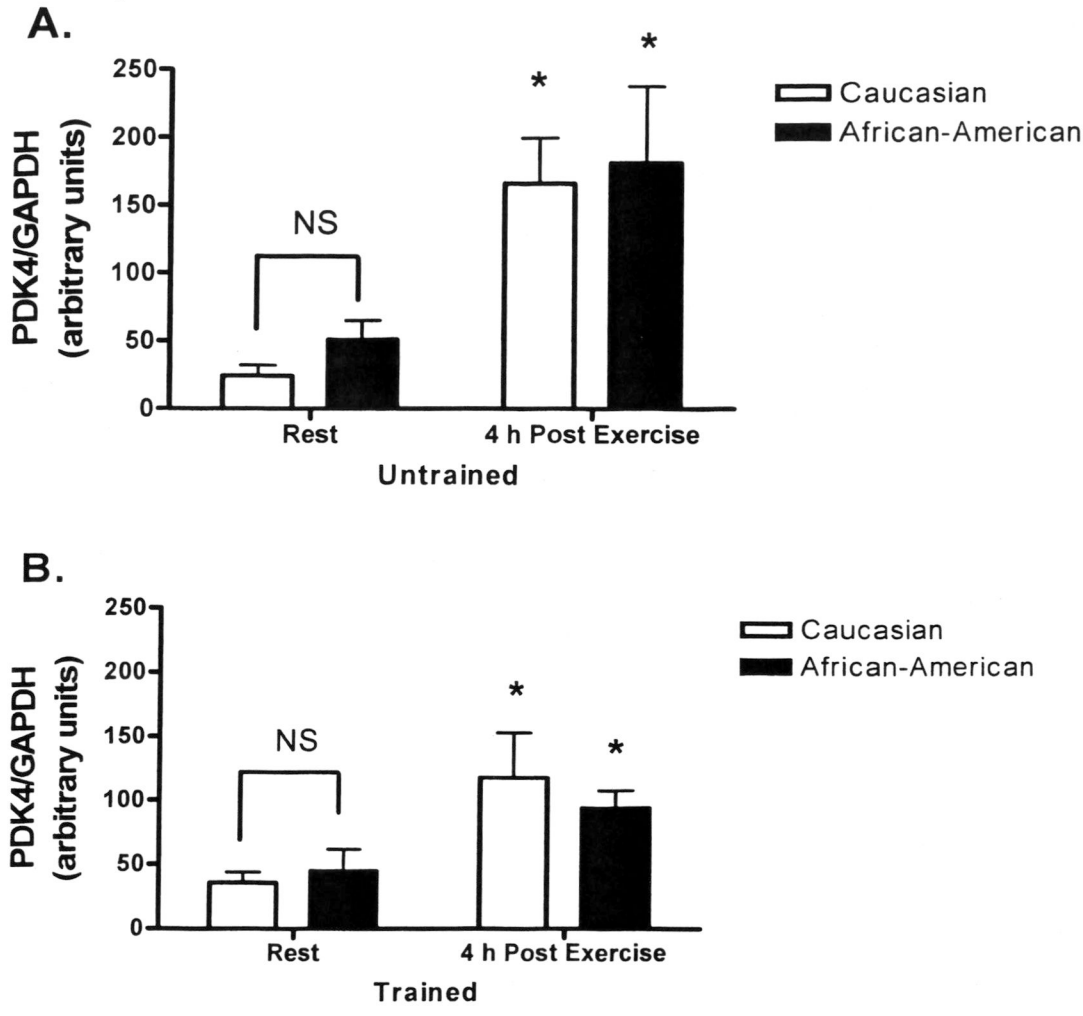

Figure 4. Racial comparisons on the effect of acute exercise on skeletal muscle pyruvate dehydrogenase kinase 4 (PDK4) gene expression in the **A.** untrained **B.** trained state. The subjects consisted of 7 obese Caucasian (BMI>30kg/m^2) and 6 obese African-American women. Acute exercise in both the untrained and trained conditions consisted of cycling on a Monark® bicycle at 75% VO_2 peak. For both the untrained and trained conditions, vastus lateralis muscle biopsies were obtained in the fasted (10 h) state prior to and 4 h post acute exercise. Exercise training consisted of 10 consecutive days of cycling at 75% VO_2 peak. In the rested state, post training biopsies were taken 24 h after the last exercise session. Quantitative real time PCR was used to assess the expression of PDK4 gene expression prior to and post acute exercise. Values are mean ± S.E.M.

A.* = significant main effect for time (post > pre exercise condition); P =0.0008.

B. * = significant main effect for time (post > pre exercise condition); P =0.005.

NS = not statistically different

Uncoupling Protein 3 (UCP3): It has been hypothesized that fatty-acid anions are exported to the cytosol by UCP3 in order to allow continued rapid fatty acid β-oxidation in the face of oversupply (as is probable in the obese state, and perhaps to a greater extent in AAW; Brand & Esteves, 2005). Therefore, we examined the potential for UCP3 gene expression to expand due to acute exercise in the pre-trained and trained condition and whether racial differences may exist between AAW and CW. In the untrained state (Figure 5 A), repeated measures ANOVA [race X time (pre-post exercise)] revealed significant P values for the main effect of time ($P < 0.0001$) and interaction between race and time ($P = 0.0022$). A significant difference was not noted for the main effect of race ($P = 0.1719$). ANOVA plus *post hoc* analysis indicated that mean values were significantly greater following acute exercise in the untrained state for both AAW (AAW = $59.9 \pm 10.8_{\text{untrained/pre-exercise}}$ vs. $93.4 \pm 9.8_{\text{untrained/post-exercise}}$; arbitrary units relative to GPDH; mean ± S.E.M.; $P = 0.01$) and CW (CW = $54.0 \pm 8.4_{\text{untrained/pre-exercise}}$ vs. $139.4 \pm 12.9_{\text{untrained/post-exercise}}$; $P = 0.0001$). *Post hoc* analysis also indicated that whereas the basal levels of UCP3 expression were similar in both races, mRNA levels for UCP3 were greater in the CW vs. the AAW ($P = 0.0187$) post exercise. A similar statistical analysis was performed for UCP3 expression before and after exercise following 10 days of endurance exercise training (Figure 5 B). An interaction ($P = 0.147$) and main effect for time ($P = 0.096$) were not noted, but a significant main effect for race ($P = 0.01$) was observed in the trained condition. *Post hoc* analysis indicated that before exercise in the trained condition, mean values for UCP3 gene expression were similar between CW vs. AAW, whereas *post hoc* analysis noted that values were greater ($P = 0.001$) in CW vs.

AAW following acute exercise in the trained state. Post exercise, the mean value was greater (49%) in CW vs. AAW and nearly reached statistical significance by *post hoc* analysis ($P = 0.08$; CW = 115.5 ± 18.2 to 171.6 ± 15.4 $_{\text{trained/pre-post exercise}}$; AAW = 80.1 ± 25.9 vs. 84.4 ± 11.8 $_{\text{trained/pre-post exercise}}$).

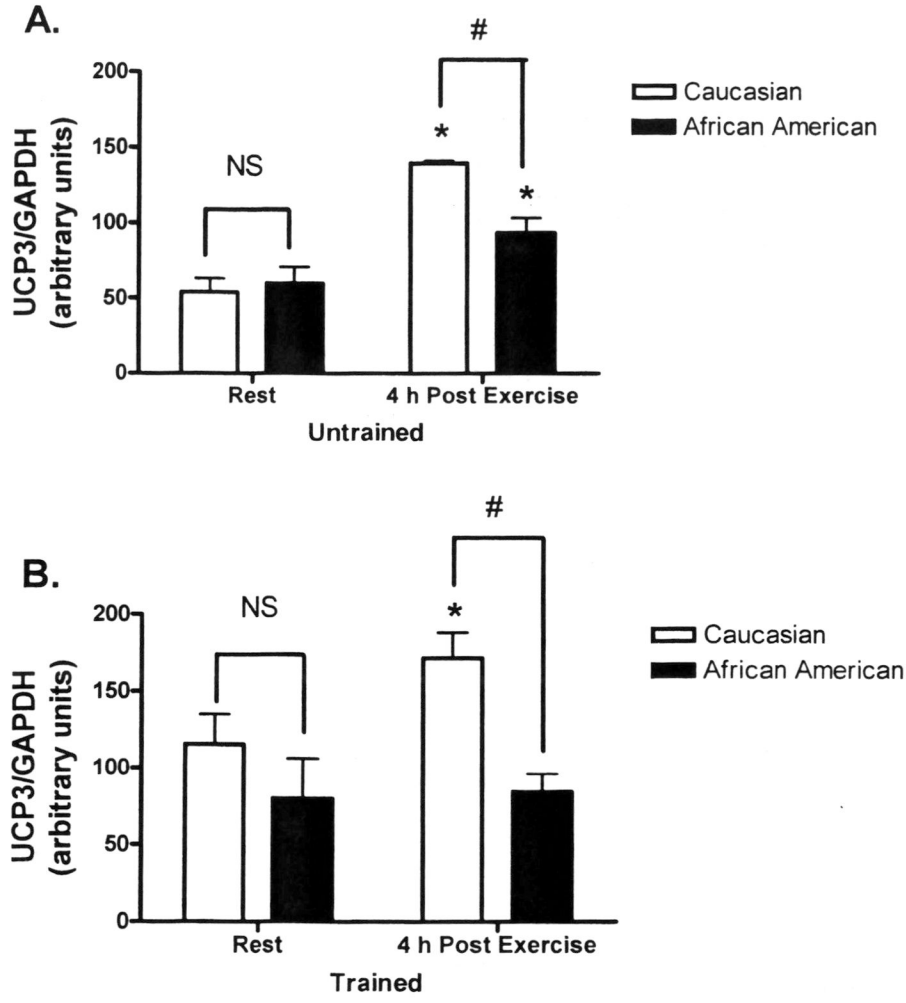

Figure 5. Racial comparisons on the effect of acute exercise on skeletal muscle uncoupling protein 3 (UCP3) gene expression in the **A.** untrained and **B.** trained state. The subjects consisted of 7 obese Caucasian (BMI>30kg/m^2) and 6 obese African-American women. Acute exercise in both the untrained and trained conditions consisted of cycling on a Monark® bicycle at 75% VO_2 peak. For both the untrained and trained conditions, vastus lateralis muscle biopsies were obtained in the fasted (10 h) state prior to and 4 h post acute exercise. Exercise training consisted of 10 consecutive days of cycling at 75% VO_2 peak. In the rested state, post training biopsies were taken 24 h after the last exercise session. Quantitative real time PCR was used to assess the expression of UCP3 gene expression prior to and post acute exercise. Values are mean ± S.E.M.
A. * = significant main effect for time (post > pre exercise condition); $P < 0.0001$; interaction effect (race X time) $P = 0.0022$. # = Fisher's PLSD post hoc analysis, CW > AAW; $P = 0.02$
B. * = significant main effect for time (post > pre exercise condition for CW only; $P = 0.08$. # = Fisher's PLSD post hoc analysis, CW > AAW post exercise; $P = 0.0011$.
NS = not statistically significant

Comparisons in Gene Expression Between the Trained and Untrained Condition

Training status may alter the basal and post-exercise training expression of each of the genes studied. Therefore, two separate repeated measures ANOVA (race X training status) was employed to compare group mean differences in gene expression in the sedentary vs. the trained condition under 1) the pre-exercise/basal condition only or 2) the post-exercise condition only.

Peroxisomal Proliferators-Activated Receptor γ Coactivator-1 α (PGC-1α): Non-significant differences for the main effects of race or training status and the interaction of each were observed for PGC-1 α gene expression when comparing the effect of training on basal expression of this gene. In contrast, ANOVA revealed a significant main effect ($P = 0.0145$) for training status when comparing the post exercise gene expression for PGC-1 α (AAW = $179.2 \pm 30.2_{\text{untrained/pre-exercise}}$ vs. $118.7 \pm 14.7_{\text{trained/pre-exercise}}$ = 51% reduction; CW = $179.2 \pm 30.5_{\text{untrained/post-exercise}}$ vs. $111.8 \pm 25.6_{\text{trained/post-exercise}}$ = 60% reduction; mean ± SEM, arbitrary units/GAPDH). Thus, the expression of PGC-1 α was lower 4 hours after acute exercise in both races following 10 consecutive day of endurance exercise training.

Carnitine Palmitoyltransferase-1β (CPT-1β): Non-significant differences for the main effects of race ($P = 0.87$) or training status ($P = 0.08$) and the interaction ($P = 0.21$) of each were observed for CPT-1 β gene expression when comparing the effect of training status on basal expression of this gene (AAW = $94.4 \pm 11.7_{\text{untrained/pre-exercise}}$ vs. $100.4 \pm 8.1_{\text{trained/pre-exercise}}$; CW = $78.2 \pm 9.9_{\text{untrained/pre-exercise}}$ vs. $111.3 \pm 18.5_{\text{trained/pre-exercise}}$; arbitrary units/GAPDH). When comparisons were made on gene expression of CPT-1 β

in the post- exercise condition before vs. after training, a significant effect (P = 0.0078) for training status but not for the main effect of race (P = 0.22) or and the interaction (P = 0.30) of each were observed (AAW = 85.9 ± $9.6_{\text{untrained/post exercise}}$ vs. 98.4 ± $9.2_{\text{trained/post-exercise}}$; CW = 92.2 ± $5.3_{\text{untrained/post-exercise}}$ vs. 118.3 ± $9.0_{\text{trained/post-exercise}}$. Post hoc analysis revealed that the increase in activation of CPT-1 β occurred in the CW only (P = 0.019). Therefore, it is plausible that increased CPT-1 β expression following endurance exercise in the trained state may reflect a more potent mitochondrial response to biogenesis factors (like PGC-1 β) in CW vs. AAW.

Pyruvate Dehydrogenase Kinase 4 (PDK4): Non-significant differences for the main effects of race (P = 0.10) or training status (P = 0.84) and the interaction (P = 0.51) of each were observed for PDK4 gene expression when comparing the effect of training on basal expression of this gene (AAW = 51.1 ± $14.2_{\text{untrained/pre-exercise}}$ vs. 44.9 ± $17.0_{\text{trained/pre-exercise}}$; CW = 24.2 ± $7.4_{\text{untrained/pre-exercise}}$ vs. 36.0 ± $7.4_{\text{trained/pre-exercise}}$; arbitrary units/GAPDH). Similarly, when comparisons were made on gene expression of PDK4 in the post exercise condition before vs. after training, non-significant differences for the main effects of race (P = 0.91) or training status (P = 0.07) and the interaction (P = 0.57) of each were observed (AAW = 181.4 ± $56.2_{\text{untrained/post-exercise}}$ vs. 93.8 ± $13.9_{\text{trained/post-exercise}}$; CW = 166.2 ± $30.9_{\text{untrained/post-exercise}}$ vs. 117.9 ± $32.5_{\text{trained/post-exercise}}$). Although differences were not statistically different, mean differences in PDK4 gene expression was reduced by 49% in African-American women and by 29% in Caucasian women 4 hours post exercise in the trained vs. untrained state.

Uncoupling Protein 3 (UCP3): A significant difference for the main effect of training status (P = 0.02) but not for race (P = 0.45) or the interaction (P = 0.19) of each were observed when comparing the effect of training on basal expression of UCP3 (AAW = 59.9 ± $10.8_{\text{untrained/pre-exercise}}$ vs. 80.1 ± $25.9_{\text{trained/pre-exercise}}$; CW = 54.0 ± $8.4_{\text{untrained/pre-exercise}}$ vs. 115.5 ± $18.2_{\text{trained/pre-exercise}}$; arbitrary units/GAPDH). Consequently, *post hoc* analysis indicated that only the Caucasian women demonstrated elevated (212%) UCP3 gene expression in the non-exercised condition following training (P = 0.03). In the post exercise condition, ANOVA revealed a main effect for race (P = 0.001) but not training status (P = 0.29). A statistically significant interaction effect between race and training status was nearly realized (P = 0.078). Thus, post exercise levels of UCP3 expression were greater in Caucasian vs. African-American women in both the untrained and trained condition (AAW = 93.4 ± $9.8_{\text{untrained/post-exercise}}$ vs. 84.4 ± $11.8_{\text{trained/post-exercise}}$; CW = 139.4 ± $12.9_{\text{untrained/post-exercise}}$ vs. 171.6 ± $15.4_{\text{trained/post- exercise}}$) but the levels did not increase in the post exercise condition due to training in either racial group.

Note: The data presented above was also expressed as the change (delta) in gene expression in the recovery phase following acute exercise compared to resting conditions and was performed for both the untrained and trained condition [i.e., gene expression (arbitrary units/GAPDH in recovery minus basal (rested) gene expression]. Subsequently, a 2 (race) by 2 (training status) repeated measures ANOVA was used to analyze the findings. The graphs and ANOVA results are presented for the reader in Appendix, but will not be the subject of discussion in the following chapter.

CHAPTER V

DISCUSSION

Overview

Obesity has reached epidemic proportions in the U.S. and is more prevalent in AAW vs. CW. Based on experiments examining metabolic dysfunction in obesity, it is believed that the etiology of this disease is due, at least in part, to an impaired mitochondrial capacity to oxidize fat as fuel. However, in lean subjects, the mitochondria's capacity to oxidize substrate for fuel is expanded by endurance exercise training, making chronic physical activity a plausible therapeutic intervention for obese subjects. Recently, our laboratory has demonstrated that skeletal muscle oxidation of fatty acids is elevated in both AAW and CW following 10 days of aerobic exercise training, but the factors that account for this finding are unknown (Cortright et al., 2004). Several genes have been determined to regulate skeletal muscle lipid oxidation and altered gene expression may account for noticeable mitochondrial dysfunction leading to reductions in skeletal muscle lipid oxidation observed with obesity. To date, it is undetermined whether these key regulatory genes are expressed differently in obese AAW vs. CW. The expression of genes responsible for skeletal muscle oxidative capacity have been shown to upregulate significantly following 4 hours of acute exercise in lean Caucasian subjects (Pilegaard, Ordway, Saltin, and Neufer, 2005), but whether endurance exercise training can enhance their expression is unknown for obese

individuals nor have specific genes responsible for fatty acid, carbohydrate oxidation in skeletal muscle been compared between races, either acutely or following endurance exercise training. Therefore, the purpose of these experiments was to examine the expression of several genes known to regulate proteins that are involved in mitochondrial biogenesis and oxidation and which may provide a potential mechanism behind the expansion in skeletal muscle oxidative capacity following endurance exercise training.

Because obesity is more prevalent in AAW we compared the gene expression response of obese AAW with obese CW before and following acute exercise in the untrained and trained condition. The genes selected for analysis by quantitative real time PCR were PGC-1 α, PDK4, UCP3, and CPT-1 β. The major findings of this study were **(1)** exercise training elicits a similar upregulation effect on PGC-1 α in both AAW and CW, suggesting a potential contribution by this protein toward mitochondrial biogenesis in each of these races. Interestingly, PGC-1 α was significantly reduced by 10 days of exercise training (4 hours post acute exercise) in both AAW and CW **(2)** Acute exercise did not elevate CPT-1 β gene expression in either the untrained or trained state for either AAW or CW. In addition, resting CPT-1 β expression was not statistically different in CW vs. AAW (P=0.079). Thus, the lower oxidative rates for fatty acid oxidation in AAW in the untrained/basal state can not be explained by lower CPT-1 β gene expression. However, in recovery form exercise in the trained condition, *post hoc* analysis revealed that the increase in CPT-1 β expression occurred in the CW only (P = 0.019). Therefore, it is plausible that increased CPT-1 β expression following endurance exercise in the trained state may reflect a more potent mitochondrial response to biogenesis factors (such

as PGC-1 α) in CW vs. AAW **(3)** acute exercise elevates PDK4 levels similarly in AAW and CW in both the untrained and trained state indicating increased serine phosphorylation of PDH, reduction in its activity and promotion of fatty acids as a substrate in either state of aerobic conditioning. No effect of training was observed in the expression of this gene either in the basal or post-exercise condition and **(4)** Significant increases in UCP3 gene expression occurred in both AAW and CW when measured 4 h into recovery from exercise in the untrained condition suggesting that obese AAW and CW are capable of increasing the activity of this protein which is speculated to be protective against cellular damage by excess ROS production. Interestingly, under these conditions, the rise in expression for UCP3 was greater for the CW than for the AAW. Following 10 days of exercise training, UCP3 gene expression increased in recovery from exercise, but only in the CW. Also, exercise training increased resting expression of this gene but only in the CW. Training had no effect on UCP3 gene expression in the recovery period for either the AAW or CW

Preliminary Evaluations

We were successful in our recruitment procedures in that subjects were similar in age, body weight, and level of aerobic fitness (i.e., individuals were sedentary and had similar VO_2 peak values). Subjects were also successfully recruited on the basis of BMI in that both racial groups were similar, with a value over 30 kg/m^2, indicating that both the AAW and CW were obese.

Exercise Training

Preliminary assessments and all training sessions were conducted in the Fitness Instruction Testing and Building (FITT) at East Carolina University. Measures of subjects' aerobic capacity were described by their VO_2 peak rather than their VO_2 max. In obese subjects, the peak values obtained from our cycle ergometer test may not necessarily reflect the true maximal aerobic capacity. Therefore, it was critical that we obtain peak work capacity via the mode of exercise that was prescribed to accurately set the intensity level at which to train our subjects. Aerobic capacity when measured on a cycle ergometer is often referred to as a peak test due to the quantity of skeletal muscle recruited compared to the amount required for the treadmill. It is well known that skeletal muscle is the major site of activity induced aerobic metabolism; therefore, by decreasing the amount of muscle mass available during our measurement, we would expect a lower maximum aerobic output for each person tested. Additionally, the training state of our subjects or rather, the lack of training, may have contributed to their inability to reach a true VO_2 max. Sedentary individuals typically do not have a high exercise intensity tolerance, particularly the discomfort of the bicycle seat. Initial assessments and exercise training was performed on a cycle ergometer, similar to the one they trained on during the ten days. The rationale for selecting this mode of exercise was to be certain that the vastus lateralis was recruited during the exercise session as the vastus lateralis was the location for the *in vitro* muscle biopsy experiments. Past studies have identified that cycling exercise, similar to that used in this study, sufficiently

recruits the vastus lateralis muscle and that this muscle group receives a sufficient stimulus to elicit an aerobic training effect.

In our subjects, there were no significant differences between races when compared in absolute terms, however there were significant differences between the groups when measured in relative terms. When compared for relative terms, the CW had a VO_2 peak of 20.7 ± 1.1 while the AAW had a VO_2 peak of 16.7 ± 0.5. Although these findings were significant, they do not have any physiological implications on gene expression. In sedentary women who are between the ages of 20-50 years old, a typical VO_2 max is 29.98 ml/kg/min, whereas active women between the same ages have a typical VO_2 max of 32.0 ml/kg/min (American College of Sports Medicine, 1990). This verifies that our subjects were in fact sedentary, and in most, below average sedentary levels.

All subjects experienced difficulty with the lack of comfort in the bike seat. Subjects trained on a Monark cycle ergometer with a gel seat to minimize discomfort as well as a Lifecycle upright bike with a wide seat. If possible, a larger seat may have allowed our subjects to have cycled longer during their peak VO_2 test, however, the seats used are the largest available. Additionally, the training workload was set at 75% of their VO_2 peak. Although it was difficult for each person to complete one hour at approximately 75% VO_2 peak, each participant was able to do so. This was very important as previous data from our laboratory indicated that the intensity and length of the training stimulus (75% VO_2 peak, 60 min/session, 10 continuous days) is sufficient to increase the capacity of the vastus lateralis to oxidize fatty acids as determined by our in

vitro oxidation protocol (Cortright, et al., 2004). To decrease level of boredom subjects were accompanied by a research assistant and had access to fans, water, music and television.

Body Composition

Body composition was measured in a hydrostatic weighing tank using both the head above and head below method, depending on BMI. The underwater hydrostatic weighing method is considered the gold standard. However, the head above method was used most frequently. Many of the subjects were not willing to completely submerge their head under the water while expelling all of their air (the process required for the underwater method). Additionally, the head below method not only requires the head to be below the water, it also requires the subject to bend at the waist, a task many of the subjects were not able to complete. The amount of abdominal fat was too large to allow subjects to lean over. Similarly, skinfold measurements using a skinfold caliper, were not utilized due to the inaccuracy of estimating percent body fat in obese individuals. Considerations to the head above method included a weighted chair, used to prevent subjects from floating to the surface while performing the test. In subjects who were able to perform the head below method (BMI<35kg/m^2), additional measures were required. The head above method required both vital capacity and total lung volume measurements, as subjects fully inspired air rather than fully expiring. An additional measure that was required during the head above method included a residual volume measurement.

Subjects body weight did not change significantly over the 10 days of exercise training, thus no weight loss effect occurred. This verifies any changes seen in gene expression was due in part to the training stimulus not weight loss. Based on minimal waist measurements both AAW and CW were well above "obese" classifications, as the AAW had an average minimal waist measure of 95.64 and the CW had an average minimal waist measure of 99.67. According to the American College of Sports Medicine, individuals with a minimal waist measure of 88 cm or greater are clinically classified as obese.

Menstrual Status and Physical Activity Questionnaires

Subjects were required to fill out questionnaires that assessed their current menstrual status and physical activity status. The purpose of the two questionnaires was primarily for screening. Subjects had to be both premenopausal and sedentary. To control for potential effects of estrogens on lipid metabolism, all subjects would have commenced the training protocol immediately post menses. This would verify that all subjects were at the mid-follicular stage of their menstrual cycle at the end of the training protocol. It is well known that blood estrogen levels are lowest during this phase of the menstrual cycle. It has also been observed via indirect calorimetry, that in vivo substrate utilization is not different between the luteal and follicular phases (Ashley, Bishop, Smith, Reneau, and Perkins, 2000). Additionally, although estrogen has wide fluctuations, there is a general increase in plasma estrogen as well as progesterone levels in the luteal phase. Hackney (1990), performed muscle biopsies of the vastus lateralis

muscle in 10 healthy females during the mid follicular and mid luteal phase and found a greater resting muscle glycogen content in the mid luteal phase, suggesting that a glycogen sparing effect occurs during the luteal phase (Ashley, Kramer, and Bishop, 2000). Ideally we would have had all subjects end training during the mid-follicular stage of their menstrual cycle, however, due to the constraints of this study and the 10-day protocol, this was not always feasible. However, in practice, the primary purpose of the menstrual status questionnaire was to screen for post menopausal women who would be excluded from the study.

Dietary Food Records

Subjects were instructed on the procedures and guidelines for measuring and recording their diets on the day established for assessment of their physical characteristics. At that time, each was provided with food record sheets and measuring cups to record their food intakes for three days prior to the start of the study and during the last three days of the study. Subjects were instructed to consume similar meals during the last three days of the study compared to that of the three days prior to the start of their training. This was to minimize the effect that dietary changes could have on gene expression and RER. Subjects were fasted (10-12 hours) for all muscle biopsies and blood draws. Each was also evaluated to confirm that caffeinated beverages were not consumed on the morning that biopsies and whole body assessment of substrate utilization were obtained or measured respectively. On days 1 and 10, RER was measured during the exercise session, and all subjects were analyzed for energy

expenditure including percent breakdown from fat, carbohydrate, and protein. Nutrition analysis was performed comparing total body mass and lean body mass for all participants for total energy (Joules), fat, carbohydrate and protein in grams per kilogram body weight. The analyses were expressed relative to total body mass and lean body mass. There were no significant differences between the CW and AAW when compared for total body weight and racial differences with respect to total energy consumption, carbohydrate, fat, and protein. Additionally, there were no significant differences between the CW and AAW when compared for lean body mass and racial differences with respect to total energy consumption, carbohydrate, fat, and protein. From the dietary assessment, we can conclude that the changes seen in gene expression were due to the exercise training stimulus, not alterations in food consumption.

Based on nutritional assessments, all subjects ate similarly that there were no significant differences between the three days prior to the start of the study and the last three days of the study. The significant effects that occurred with respect to the metabolic genes studied were due to the training stimulus, not alterations in dietary consumption.

Gene Expression

It is well known that exercise training elicits a number of adaptive changes in skeletal muscle which in turn improve metabolic efficiency. The exact molecular mechanisms responsible for these cellular adaptations are unknown. Unfortunately, there are few studies that compared the expression of skeletal muscle oxidative genes between

subjects before and after exercise training and there are no reports in the literature on the response of gene expression to aerobic exercise training in obese subjects or between AAW and CW. However, one study by Pilegaard et al. (2000) did explore the effects of exercise training on the expression of genes reported in the current manuscript in non-obese subjects and therefore, we will make reference to this data in our discussion of results where appropriate. These investigators examined the transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. Their study consisted of two separate training protocols. One protocol involved six untrained male subjects who completed 60-90 minutes of exhaustive one-legged knee extensor exercise for five consecutive days. On day 5, nuclei were isolated from biopsies taken from the vastus lateralis muscle in both the untrained and trained leg before exercise and from the trained leg immediately after exercise as well as after 15 min., 1 h, 2h, and 4 h of recovery. UCP3 and PDK4 were two genes examined for transcriptional activity. Additionally, the second protocol consisted of a single 4-h bout of cycling exercise with muscle biopsies taken at the previously mentioned time points. Skeletal muscle biopsies were obtained before and immediately after exercise as well as during the ensuing 4 h of recovery. Transcriptional activity and mRNA content were determined.

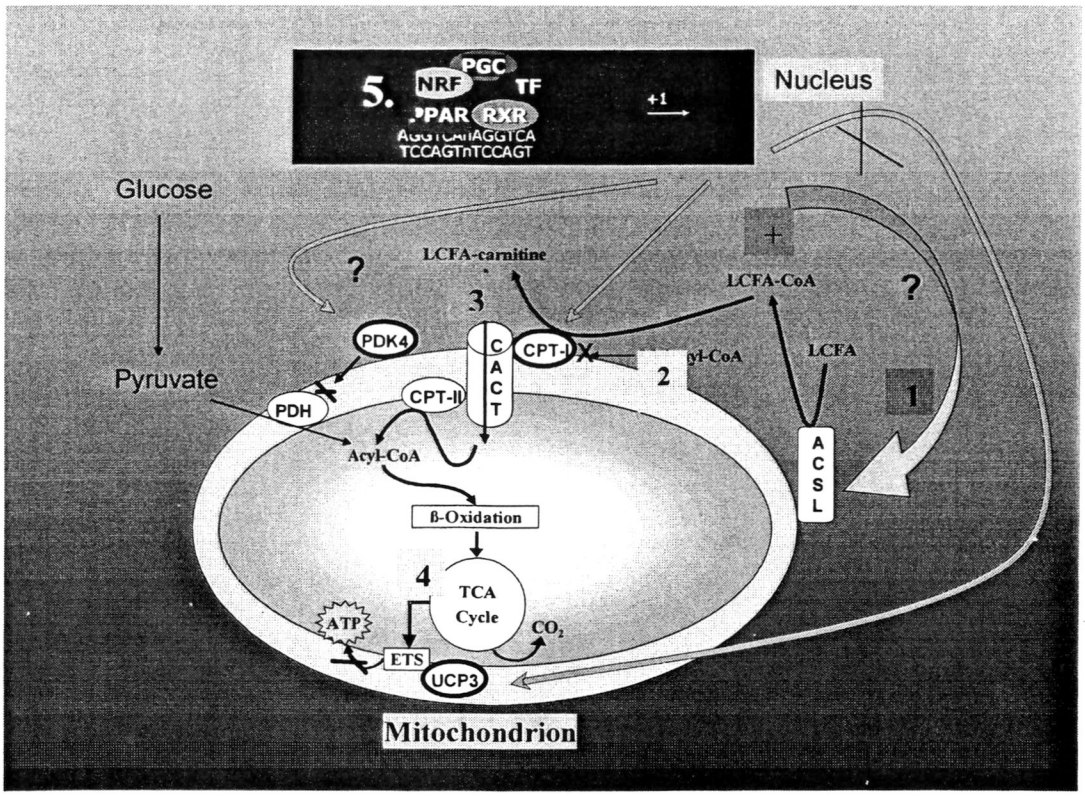

Figure 6. Summary of Metabolic Proteins Important to Skeletal Muscle Mitochondrial Oxidation of Energy Substrates. Fatty acids are presented to the mitochondria from storage sites within the muscle (intramuscular triacylglyerols) or from adipocyte derived triacylglycerols. Regardless of the source, fatty acids must be activated to their CoA derivative by long-chain acylCoA synthetase (ACSL) shown at site 1. This can now be acted upon by carnitine palmitoyltransferase-1 β (CPT-1; site 2) which exchanges carnitine for the CoA moiety. This step is considered rate limiting in the oxidation of fatty acids. The acylcarnitine product of CPT-1 is translocated to the matrix and reconverted to its CoA form by CPT-2 and then undergoes β-oxidation. The reducing equivalents NADH and $FADH_2$, derived from the TCA cycle, link oxidative and phosphorylation events in the electron transport chain (ETC) leading to the production of ATP. PGC-1 α is a master co-activator that interacts with several transcription factors leading to mitochondrial biogenesis and enhanced rates of fatty acid oxidation by the mitochondria. One mechanism includes enhanced expression of CPT-1. Glucose derived from the blood or from glycogen stores in the muscle are converted to pyruvate by the glycolytic pathway. Pyruvate is converted to acetyl-CoA by the pyruvate dehydrogenase complex (PDHC) when oxygen is present in sufficient quantity and complete oxidation leads to ATP production. When glucose or glycogen is depleted (fasting and starvation/diabetes) or under conditions of high fatty acid flux such as occurs during and in recovery from protracted endurance exercise, pyruvate dehydrogenase kinase 4 phosphorylates the PDHC complex and inhibits its activity (site 3). Pyruvate can then undergo gluconeogenesis for glucose production (liver), or in muscle, form lactate for export to the liver for gluconeogenesis and for maintenance of the NAD^+/NADH ratio needed to support glycolysis. In addition, muscle glycolysis is slowed to preserve glycogen and reduce the muscle cells reliance on carbohydrate. Obesity is associated with increased activity of PDK4 activity leading to increased oxidation of lipids and reductions in cytsolic metabolites believed to induce insulin resistance. Uncoupling protein 3 (UCP3), site 4, is believed to act as dissipater of the proton gradient in the ETC resulting in continued electron flow and reduction in free radical production (ROS) which can occur under conditions of over elevated flux of reducing equivalents derived from fatty acid oxidation.

Special Note to the Reader: According to Pilegaard et al. (2000), repeated (daily) expression of mRNA is hypothesized to represent the stimulus for elevated protein content required for enhancing the oxidative potential of skeletal muscle long known to occur with aerobic exercise training. As our protocol consisted of 10 days of consecutive aerobic exercise, the author will assume the stance that the expression of the measured genes involved in fatty acid oxidation by the mitochondria reflects this adaptation at the protein level.

Peroxisome Proliferator Activated Receptor γ Coactivator- 1 α (PGC-1α)

Background: Peroxisome Proliferator Activated Receptor Gamma Coactivator-1 α is a transcriptional coactivator capable of interacting with and modulating the activities of nuclear hormone receptors and transcriptional regulators through beta-oxidation, mitochondrial synthesis, and transcriptional factors. Coactivator refers to a protein or protein complex that increases the rate of transcription through interaction with transcription factors although it does not bind to DNA in a sequence-specific manner. Coactivators function as multiprotein complexes. The binding of coregulators can also be regulated by the binding of ligands to nuclear hormone receptors (Puigserver & Spiegelman, 2003).

As the master regulator of metabolism, PGC-1 α coactivates all the PPARs. PGC-1 α co-activators play a critical role in the maintenance of glucose, lipid and energy

homeostasis. They are involved in pathogenic conditions including obesity and diabetes (Lin et al., 2005).

PGC-1 α synthesis and activation are induced by glucagon or catacholamines via cyclic AMP. Additionally, CPT-1 is stimulated, as PGC-1 α plays a major role in the transition from fed to fasting metabolism in the liver (Foster, 2004). PGC-1 α mRNA is found in tissues that have high energy demands and are rich in mitochondria, including skeletal muscle, heart, brown fat, kidneys and liver. PGC-1 α levels are elevated by signals that relay metabolic needs and have been shown to enhance glucose uptake in muscle cells by activating the expression of the glucose transporter GLUT 4.

PGC-1 α is a co-regulator of gene expression responsible for stimulating the rate-limiting step in transcription initiation. They enhance transcription via modification of the chromatin structure on the target gene or by associating with the RNA polymerase machinery. As a coactivator of transcription, PGC-1 α requires a physical interaction with DNA-binding transcription factors followed by subsequent interactions with downstream effectors. However, interactions with downstream effectors takes place after PGC-1 α has been recruited to target promoters (Knutti & Kralli, 2001).

In skeletal muscle, PGC-1 α is induced with exercise training in both humans and rodents. Additionally, components of the ROS scavenging pathway are linked by PCG-1 α to mitochondrial oxidative metabolism, enabling cells to maintain normal redox status in response to changes in oxidative capacity (Lin et al., 2005). Due to the role of PGC-1 α and its control of energy homeostasis, it could potentially be a target for

pharmacological intervention for anti-obesity and diabetes control (Puigserver & Spiegelman, 2003).

PGC-1 α has a major impact on the NRF system, as it dramatically induces gene expression for NRF-1, NRF-2 and mtTFA when introduced into muscle cells. Additionally, the relationship of PGC-1 α on glucose uptake and metabolism is heavily studied as there are indications that rates of mitochondrial oxidation can affect glucose uptake (Puigserver & Spiegelman, 2003).

Summary and Interpretation of the Findings: PGC-1 α was shown to be similar between the races in basal expression and to be very responsive to aerobic exercise. We observed that the expression (by qRT-PCR) of the PGC-1 α gene was increased 9 fold in CW and 6 fold in AAW following 1 h of cycling exercise in the untrained condition. Exercise also increased the expression of the gene in the trained state with CW increasing by 2.7 fold and AAW by 4.7 fold above resting levels. In contrast, PGC-1 α levels were not elevated in the basal state following 10 days of exercise training. Interestingly, the expression was significantly reduced in the trained vs. untrained condition at 4 h post exercise. A definitive explanation is at present unavailable. However, it has been speculated by Pilegaard and colleagues (2000) that repeated exposure of skeletal muscle to elevated contractions leads to the transient expression of metabolic genes resulting in an elevation and stabilization of protein needed to express the exercise induced elevation in mitochondrial oxidative capacity. In this regard, an attenuated expression in PGC-1 α

in recovery from exercise may reflect a reduced need to heighten gene transcription in the face of previously induced elevations in protein content.

Given the positive role of PGC-1 α in mitochondrial biogenesis and oxidative capacity described above, we interpret these data as follows. First, it can now be stated that similar to lean subjects, obese subjects are capable of upregulating the expression of this important coactivator of transcription factors required for the enhancement of mitochondrial fatty acid oxidation. As an extension, AAW appear to be as responsive to the exercise stimulus as CW. By this gene alone, enhanced expression of PGC-1 α appears to be associated with the expansion of fatty acid oxidation in skeletal muscle in obese AAW and CW following 10 days of endurance exercise training as previously reported using this identical protocol (Cortright et al., 2004).

Carnitine Palmitoyltransferase-I β (CPT-1 β)

Background: Carnitine palmitoyltransferase-1 β, is the enzyme involved in the transport of long-chain fatty acids into the mitochondria (see Foster, 2004 for an excellent review on the role of the carnitine system in human metabolism). In the anabolic (fed) state, the liver stores glucose as glycogen and fatty acid synthesis is active. In the catabolic (fasted) state, the liver becomes a glucose producer, lipogenesis is slowed, and fatty acid oxidation is activated. In the fed state, insulin levels are elevated, glucagon is low, and malonyl CoA inhibits CPT-1 β therefore limiting fatty acid oxidation. In the catabolic state, malonyl CoA levels are lowered, CPT-1 β is disinhibited and fatty acid

oxidation rates increase (Foster, 2004). Overall, insulin is the hormone of anabolism while glucagon is the hormone of catabolism.

The rate limiting step in fatty acid oxidation is CPT-1 β, while the regulator is malonyl CoA (Foster, 2004). As the rate limiting step in fatty acid oxidation, CPT-1 β exchanges carnitine for CoA on the outer mitochondrial membrane. The fatty acid oxidation regulator, malonyl CoA, has been shown to diminish in exercising rat and possibly in human muscle as a result of the phosphorylation of acetyl CoA carboxylase (ACC). Malonyl CoA is directly controlled by two enzymes: it is synthesized by acetyl CoA carboxylase (ACC) and broken down by malonyl CoA decarboxylase (MCD) (Foster, 2004).

ACC activity decreases in response to lowered citrate as citrate is a positive allosteric activator of ACC and citrate is the source of acetyl CoA. ACC exists in two isoforms, ACC-1 and ACC-2. ACC-1 is found predominantly in liver and adipose tissue while ACC-2 is found in cardiac and skeletal muscle. AMP-activated protein kinase (AMPK) is the dominant inhibitor and the sequence is as follows: AMP kinase kinase (AMPKK) phosphorylates and activates AMP kinase, which phosphorylates and inhibits ACC. Long-chain fatty acyl CoA can also activate AMPKK through binding to the enzyme, therefore allowing fatty acids to lower ACC activity both directly and indirectly (Foster, 2004). Evidence also suggests that the phosphorylation of ACC is catalyzed by AMP-activated protein kinase (AMPK). AMPK activation results in the stimulation of skeletal muscle glucose uptake and fatty acid oxidation (Saha & Ruderman, 2003).

In addition to CPT-1 β, two additional enzymes help control fatty acid oxidation: carnitine palmitoyltransferase II (CPT-II) and carnitine:acylcarnitine translocase (CACT).

Summary and Interpretation of the Findings: In the untrained, the basal (rested) expression of CPT-1 (β) was similar between AAW and CW. Similar results were obtained in basal CPT-1 β levels following training in that differences were not seen between AAW and CW. Unlike PGC-1, CPT-1 β was not increased by acute exercise in either the untrained or the trained condition. However, when CPT-1 β expression was compared between the trained vs. the untrained state, values were observed to be higher post exercise (trained condition), but this was true only for the CW.

Obese AAW have been shown to oxidize fatty acids to a lesser extent than CW, although both races demonstrate suppressed rates compared to lean CW (Privette et al., 2003; Cortright et al., 2004). Fortunately, both AAW and CW elevate their capacity to oxidize fatty acids following endurance exercise training (Cortright et al., 2004). With this perspective, it is unfortunate that at this point that we do not have to capacity to make a conclusion on the interaction of obesity and expression of this gene. This data will be available from later experiments which will compare CPT-1 β gene expression in lean African-American with Caucasian women under the identical protocol. These data will allow us to determine if CPT-1 β expression is reduced in the obese state *per se* when compared to lean subjects and whether racial differences exist in both the basal and endurance exercise response. However, we would predict, if it could be assumed that its expression is indicative of mitochondrial content, that CPT-1 β levels would be lower in

the obese state based on the earlier findings by Kelley et al. (2003) and Ritov et al., (2004). The studies by Kelley, Ritov and colleagues both demonstrate that skeletal muscle from obese subjects are lower in mitochondrial content compared to lean subjects. This discrepancy is even larger when comparing obese-diabetic subjects to non-obese counterparts. Differences in mitochondrial content have not been compared between African-American and Caucasian women.

The observation that PGC-1 α, but not CPT-1 β, levels are elevated following acute exercise is somewhat surprising. Mitochondrial biogenesis has been shown to be under the control of PGC-1 α in skeletal muscle (Lin, Handschin and Spieglelman, 2005). Accordingly, (Koves, Noland, Bates, Henes, Muoio, and Cortright, 2005) have demonstrated, in primary muscle myotubes, that CPT-1 β expression is elevated by PGC-1α. As PGC-1 gene expression was elevated post exercise in both the untrained and trained condition, it appears that the time point (4h post exercise) was not long enough to realize the effect of increased PGC-1 expression on the down stream activation of CPT-1 β expression. Further studies are needed to see if CPT-1 β expression peaks later post-exercise under our experimental conditions and whether this is correlated with PGC-1 expression.

The finding that the post exercise CPT-1 β gene expression is higher following training in CW only is interesting. Based on earlier oxidation studies referred to above, we found similar increases in the oxidative response in both AAW and CW following 10 days of endurance exercise training. In other words, palmitate oxidation was elevated 24 hours post exercise in obese subjects from both races following training. Although the

present data is not conclusive, at this moment it appears that increased fasting CPT-1 β levels 4 h post exercise might (at least partially) explain these results in CW but not AAW. PGC-1 α elevations following acute exercise (but not basal levels) in the trained state may partially account for the elevations in fatty acid oxidation for both AAW and CW, with AAW elevating other aspects of mitochondrial biogenesis other than elevations in CPT-1 β gene expression. As a special note, we should remind the reader that although mRNA levels usually track well with the expression of proteins involved in mitochondrial oxidation, this may or may not be the case when making determinations on the effects of exercise and training between the races. Further data assessing mRNA stability and translation to protein for which these genes encode is required for more definitive statements on this topic.

In comparison to the Pilegaard study, CPT-1 β gene transcription was slightly elevated immediately after exercise in the one-legged knee extensor exercise protocol (~2-fold) however, they increased to greater than 3.5-fold above controls after 1 h of recovery. After 4-h of recovery, CPT-1 β mRNA levels were elevated nearly twofold throughout recovery, and by day 5, they were still approximately twofold, signifying a training response. After the prolonged exercise, 4-h low-intensity cycling, transcription and mRNA content of CPT-1 β were not influenced.

Pyruvate Dehydrogenase Kinase 4 (PDK4)

Background: Pyruvate Dehydrogenase Kinase 4 is an isoform specific to skeletal muscle (see Sugden for an excellent review on this topic). It is involved in the

decarboxylase reaction of pyruvate to acetyl CoA. As a carbon is decarboxylated from pyruvate, the end product is acetate. The ligand CoA and acetate form acetyl CoA.

PDK4 is responsible for attaching to Pyruvate Dehydrogenase (PDH) on the matrix transporter to allow entry into the mitochondria for the production of pyruvate, NADH, and CO_2. PDK4 controls the degree of phosphorylation of the PDH complex and plays a key regulatory role in the control of the flux of glucose metabolites into the mitochondria. It phosphorylates PDH at a specific serine residue to the inactive form and is sensitive to acute exercise (Pilegaard & Neufer, 2004).

PDK activity is highly regulated. PDK3 and PDK4 are the most distinct in their sequence. PDK4 is expressed in high levels in the heart, liver, kidney, and pancreatic islet. Its expression is increased during starvation, insulin resistance induced by high fat feeding, diabetes, and hyperthyroidism. It has been thought that PDK4 is a lipid status as it is a responsive PDK isoform, facilitates fatty acid oxidation through sparing pyruvate for oxaloacetate formation. In skeletal muscle the entry of pyruvate into the TCA cycle as oxaloacetate allows entry of acetyl-CoA into the TCA cycle through increase citrate formation. Citrate proceeds to act as a signal of fatty acid abundance and suppresses both glucose uptake and glycolysis. It has been predicted from this that PDK upregulation would allow continued uptake of long-chain fatty acyl-CoA into the mitochondria for oxidation as well as preventing long-chain fatty acyl-CoA accumulation in the cytoplasm (Sugden & Holness, 2003).

Summary and Interpretation of the Findings: Differences in the basal expression of PDK4 were not noted between AAW and CW in the untrained or the trained condition. PDK4 mRNA levels were also not altered in the basal condition following 10 days of training. In contrast, PKD4 gene expression was significantly elevated by acute exercise in both the untrained and trained condition, but changes and absolute levels were similar between the races. A decline in the mean values post exercise in the trained state was noted for both the obese AAW (49% decrease) and CW (29%) women and these differences nearly reached statistical significance ($P = 0.07$, main effect trained vs. untrained).

The pyruvate dehydrogenase complex (PDHC) catalyzes the oxidative decarboxylation of pyruvate to form acetyl-CoA and so links glycolysis with the Krebs cycle and oxidative metabolism of glucose derived carbons with ATP the bioenergetic result. When energy demands by the skeletal muscle cell are high, and/or glucose is scarce (fasting) or needs to be conserved (as with protracted exercise), the activity of the PDHC is reduced to conserve 3-C compounds for glucose synthesis (liver) or glycogen sparring (skeletal muscle and liver). In this context, it is not surprising that PDK4 gene expression was observed to be elevated post exercise in either the untrained or trained condition. As an explanation, the experimental conditions and bioenergetic state must be considered. First, we sampled our subjects 4 hours post exercise which would place the muscle in the recovery phase from the aerobic exercise bout. Furthermore, the subjects were not permitted to eat until after the biopsy was sampled. Under these bioenergetic conditions, it is well known that the muscle has shifted toward lipolysis, delivery and

oxidation of fatty acids as the predominate substrate to restore ATP-PC stores and glycogen used during exercise. This is important as it has been shown that increased lipid delivery and/or handling invariably results in the increased expression of PDK4. The same is true for PDK4 activity and level of expression in the fasting condition. Overall, an increased PDK expression/activity would serve to decrease glucose utilization and augment fatty acid oxidation, and perhaps may serve to reduce the elevated levels of lipids in the cytosol which could have negative effects (acute lipotoxicity) on cellular homeostasis.

It should also be considered that our subjects pedaled at 75% VO_2 peak, and thus a significant portion of required ATP for muscle contraction would be coming from glycogenolysis/glycolysis in addition to fatty acids derived from intramuscular stores or the blood. Accordingly, PDK4 activity will support this requirement as a high flux of pyruvate during exercise would serve to inhibit PDK4 via a feedforwad action to augment glucose oxidation. In recovery, the bioenergetic goals change. By phosphorylating the PDHC, available carbons from pyruvate (and amino acids) could be directed via gluconeogenesis toward glycogen reformation and fatty acids will be supplying most of the ATP for energy requirements in recovery, thus sparring muscle glycogen. The same could be said for production of pyruvate from lactate; that which is not oxidized by the mitochondria can be channeled toward glycogen reformation. It should be noted that gluconeogenesis is considered to be the major function of the liver, with the capacity of skeletal muscle for gluconeogenesis to be much less. Therefore, the value of pyruvate conversion to glucose and hence glycogen is questionable. However, a

reduction in the mass action of pyruvate into the mitochondria by reduced PDHC could still serve to reduce carbon flux through glycolysis in addition to reductions in catabolic hormones which stimulate glycogenolysis. In this regard, PDK4 could reflect the state of substrate availability and requirements for shifting toward anabolism of carbohydrates/glycogen.

In the trained condition, a mean drop in PDK4 expression could represent an adaptation in the bioenergetic response to endurance exercise. It is well accepted that following endurance training, the skeletal muscle is capable of utilizing lipid to a greater extent vs. the untrained state. In this study, we observed that the expression of PDK4 post training was reduced by 29% in our CW and 49% in the AAW ($P = 0.07$; main effect for time). This change was not statistically significant, but may be physiologically relevant. First, the change is in the direction predicted given the knowledge that the muscle has become more robust in terms of its mitochondrial bioenergetic capacity to utilize lipids and spare glycogen. Thus, a greater reliance on lipids during exercise, and concomitant less reliance on stored glycogen, would result in less glycogen "payback" in recovery. This would decrease the need to activate PDK4 as pyruvate flux from glycolysis is reduced. An alternative hypothesis may be simply that PDK4 protein content is already elevated to satisfactory levels and hence the need for expression of the gene is less. Unfortunately, we can not answer this question as protein content for PDK4 was not assessed in this study due to limitations in the amount of muscle obtained from our subjects. Thus, further studies of this nature are required to address this metabolic issue.

In the Pilegaard study, PDK4 showed a dramatic change, as transcription was increased by about fivefold immediately after exercise and remained elevated during the subsequent 2 h of recovery after the one-legged knee extension protocol. PDK4 mRNA stayed at control levels during the first 2 h of recovery and then increased to nearly eightfold above controls after 4 h of recovery. In the prolonged cycling exercise protocol, PDK4 elicited significant increases in transcription. Following the cycling bout, PDK4 was elevated by nearly >10-fold and continued to increase during recovery to >20-fold pre-exercise levels after 2-4 h of recovery. Additionally, PDK4 mRNA was 7 to 12 fold higher throughout the recovery period. The significant increase in transcription and mRNA content was much higher after the 4-h of cycling compared to the one-legged knee extensor exercise, indicating that PDK4 expression may progressively increase with increased exercise duration. Due to this increase, it would be expected that there would be a progressive inactivation of PDH and therefore provide a mechanistic explanation for the decline in carbohydrate oxidation that occurs during prolonged low-intensity exercise.

Uncoupling Protein (UCP3)

Background: Uncoupling proteins are involved in fatty acid metabolism and the regulation of fat content and is UCP3 found predominantly in skeletal muscle. Uncoupling proteins are mitochondrial transporters that are present in the inner membrane of the mitochondria. They belong to a family of anion mitochondrial carriers, where the mitochondria is the site of respiration. The outer mitochondrial membrane is permeable to small metabolites while the inner membrane is controlled to maintain the

electrochemical gradient via the mitochondrial respiratory chain, necessary for ATP synthesis. The inner membrane transports substrates including ADP, ATP, phosphate, oxoglutarate, citrate, glutamate, and malate. The citric acid cycle, fatty acid oxidation, steps of both urea synthesis and gluconeogenesis take place in the mitochondria. The energy produced from the mitochondrial respiration process is used for ATP synthesis via oxidative phosphorylation. The uncoupling proteins, along with mitochondrial transporters, appear to be responsible for the level of respiration coupling (Rousset, et al., 2004). Respiration is associated with the production of reactive oxygen species (ROS) where the oxygen molecule is able to accept an additional electron to create the superoxide ion. Mitochondria is responsible for a large production of ROS made in the cells and is involved in the activity of complexes I and III in the respiratory chain. However, ROS formation is dependent on the mitochondrial proton gradient and mitochondrial potential. UCPs may be the effectors involved in antioxidant defense mechanisms through mild uncoupling of respiration (Rousset et al., 2004).

There is strong evidence that mild uncoupling caused by UCP2 and UCP3 attenuates ROS production and protects against cellular damage as well as in the export of fatty acids (Brand & Esteves, 2005). UCP3s export fatty acids allowing increased rates of high fatty acid oxidation and protection against lipid toxicity. If fatty acid supply exceeds the oxidation rate, fatty acyl CoA can accumulate in the mitochondria therefore limiting further fatty acid β-oxidation. In order to prevent this, acyl CoA is hydrolyzed in the mitochondria to a fatty acid and free CoA. UCP2 and UCP3 increase the proton conductance of the mitochondrial inner membrane, however they must first be activated

by products of ROS metabolism, including fatty acids. UCPs continue to remain as important targets for the treatment of obesity and weight loss (Brand & Esteves, 2005).

Summary and Interpretation of the Findings: Analysis of the effects of endurance exercise and training on the expression of UCP3 in our AWW and CW yielded interesting results. With respect to the untrained condition, although there were no differences in UCP3 expression in the basal condition between the races, acute endurance exercise resulted in an increase in mRNA for both AAW and CW, when measured 4 hours post exercise. However, the elevation in expression was greater in the CW compared to the AAW. Following 10 consecutive days of aerobic training, the levels of UCP3 were increased following acute exercise, but now, the effect was only realized by the CW. Moreover, with respect to comparisons between subjects in the trained vs. untrained condition, the trained CW demonstrated greater basal levels of the gene which was not true for the AAW.

The physiological role for skeletal muscle UCP3 has been debated for some time now (see Dulloo et al., 2004) and several bioenergetic functions have been described, as discussed above. One important theme, regardless of the function (e.g., reduction in ROS production as above), is that their expression appears to be associated with changes in fatty acid flux within the cell. For example, in skeletal muscle, the increase in UCP3 expression is in line with the well-known fasting-induced shift in substrate utilization in favor of lipids as the predominant metabolic fuel and hence allowing the sparing of glucose for organs/tissues with an obligatory requirement for glucose, notably the brain.

In accordance, studies in animals (Cortright Zheng, Jones, Fluckey, DiCarlo, Grujic, Lowell, and Dohm, 1999) and humans (Noland, Hickner, Zheng, and Cortright, 2003) using aerobic exercise, also known to shift substrate mobilization and utilization from glycogen to lipids (if the duration is of moderate intensity and greater than 30 minutes of continuous activity) have demonstrated that UCP3 expression is significantly elevated post endurance exercise. For example, Noland et al. (2003) demonstrated that 60 minutes of acute cycling at 65-70% VO_2 peak increased UPC3 gene expression in vastus lateralis when measured 1 hour post exercise. However, mRNA levels increased in untrained but not trained subjects who had been cycling (> 100 km/week) regularly for months. It was speculated that the expanded mitochondrial capacity and hence ability to oxidize free fatty acids in the trained subjects attenuated the need to increase UCP3 activity. Thus, the presence of unmetabolized intracellular fatty acids (predicted to be higher in untrained subjects), which are known ligands for PPARs and other transcription factors, may be the factor that dictates UCP3 expression. This fits with the hypothesis that UCP3 expression is linked with reduced ROS production, which is known to be lower in trained vs. untrained individuals post exercise.

This study is the first to report that both obese AAW and CW significantly increase the expression of UCP3 levels following acute exercise in the untrained condition. These findings are consistent with the above discussion. However, our findings post exercise after 10 days of endurance exercise training does not fit with the previous discussion in that our trained CW demonstrated a significant increase in UCP3 expression 4h post cycling. That is, the Noland study would have predicted the absence of an

increase in these obese subjects following training. With regard to the above, it may be that 10 days of cycling was insufficient to elevate mitochondrial biogenesis to the point realized in others that exercise train for months or more. In this case, our subjects may be still at jeopardy for excess ROS production and therefore, UCP3 up regulates to compensate for elevations in fatty acid flux in the face of unchanged mitochondrial capacity yet to be developed later in training. Interestingly, the AAW demonstrated a reduced expression in UCP3 levels vs. CW in the untrained state and did not demonstrate elevations in UCP3 expression following acute exercise in the trained condition and thus appear to be "metabolically inflexible" at least with regard to this variable. It is unlikely that the argument of a greater induction in mitochondrial biogenesis occurred in AAW could be used as oxidation studies conducted earlier demonstrated similar expansion in the oxidation of palmitate in CW and AAW post 10 days of exercise training (Cortright et al., 2004). At this point it can only be speculated that perhaps AAW are more susceptible than CW to increasing ROS production as a result of increased fatty acid flux following acute aerobic exercise. The greater increase in UCP3 levels in the basal state following training in the CW only lends credence to this possibility.

Finally, it is at first glance discordant that when UCP3 mRNA is measured in the non-exercised condition in skeletal muscle from sedentary obese individuals, expression is similar to lean counterparts despite these individuals having elevated muscle lipid content (Vidal-Puig, 1998) and lower rates of fatty acid oxidation and fatty acid flux through the mitochondria (Cortright et al., 2004; Kim et al., 2000). This should act to elevate UCP3 message if free fatty acids were the stimulus as discussed. Two situations

could exist then; one that a lesion in the fatty acid induction of UCP3 gene expression exists in these obese individuals or two, that another signal downstream of free fatty acids exists. As stated by Lowell and others, that signal might be ROS themselves (Vidal-Puig, Zhang, Boss, Ido, Szczepanik, Mootha, Cortright, Muoio, and Lowell, 2000). Interestingly, in the same study by Vidal-Puig (1999), when obese subjects undergo weight loss (by 20%) and are weight stable, muscle UCP3 levels decline. Presuming that fatty acid flux would be reduced in skeletal muscle after 20% weight loss (reduction in intramuscular lipids), it appears again by this data that UCP3 gene expression most likely is responsive to the flux of fatty acids or their metabolites rather than serving a thermogenic purpose as is clearly demonstrated for UCP1 in rodent brown adipose tissue. As described, whether acting as a fatty acid anion carrier or a true uncoupler of the mitochondrial proton gradient, UCP3 does appear to be associated with reduced ROS production which may be its true physiological role.

Global Synopsis and Closing Remarks

The reader is encouraged to refer to the summary figure 6 for reference with respect to this discussion. Overall, we choose to examine the expression of 4 genes known to be responsive under metabolic conditions of heightened fatty acid flux and demand for ATP by skeletal muscle. Racial comparisons were made between obese African-American and Caucasian women for several important reasons: 1) Obese women demonstrate reductions in skeletal muscle fatty acid oxidation which is hypothesized to contribute to reduced utilization of lipids and muscle insulin resistance by impairment of

the insulin signaling cascade as discussed earlier 2) that obese African-American women are reported to have reduced rates of skeletal muscle fatty acid oxidation compared to CW which likely contributes to the exacerbated incidence and severity of obesity and diabetes in this racial group and 3) based on the finding from our laboratory that endurance exercise training increases the oxidation rate of exogenous palmitate, we wished to explore the skeletal muscle for possible gene/protein candidates that might help to explain our results. The four genes chosen, PGC-1 α, CPT-1 β, PDK4 and UCP3, have all been established to associate with changes in lipid metabolism and changes in the cellular bioenergetic demands in muscle, notably with aerobic exercise. By no means is this list comprehensive, and additional genes (e.g., Peroxisome Proliferator-Activated Receptors (PPARs), Cyclooxygenase (COX), Stearoyl-CoA Desaturase (SCD1), Acyl-CoA Synthetase Long-chain (ACSL) isoforms) are also being investigated in ongoing studies from our laboratory.

Overall, PGC-1 α, CPT-1 β, PDK4, and UCP3 gene expression are similar between AAW and CW in the basal (rested) condition before and after training. With respect to exercise, PGC-1 α, PDK4, and UCP3 expression is upregulated in recovery from aerobic exercise in both the untrained and trained state for both AAW and CW, the exception being a lack of response in UCP3 in AAW. The response to exercise training in basal levels of each gene is less pronounced, being elevated by training for UCP3 and only for CW. The rise in gene expression in recovery was attenuated for PGC-1 α levels in the trained compared to the untrained state for both AAW and CW, but was elevated for CPT-1 β but only in CW.

PGC-1 α was chosen because its functions provide a plausible molecular basis for the connection between environmental/hormonal stimuli and mitochondrial biogenesis and respiration when an organism has altered energy and thermogenic requirements. In short, PGC-1 α induces mitochondrial biogenesis by coactivating with transcription factors such as PPARs, NRF (nuclear respiratory factory) and RXR (retinoid receptor) proteins to induce elevated rates of transcription of mitochondrial proteins. One such protein is CPT-1 β, the regulatory step in fatty acid oxidation, as it is required for activated fatty acids to enter the mitochondrial matrix for β-oxidation. In particular, PGC-1 α upregulates and associates with PPARα coactivation leading to increased CPT-1 β expression (in addition to increases in proteins of β-oxidation and ETC gene/protein expression such as cytochrome c of complex IV). Globally, PGC-1 α increases the oxidation of fatty acids through its mitochondrial biogenesis properties. With respect to our findings, overall, both obese African-American and Caucasian women followed this paradigm and increased the expression of PGC-1 α following aerobic exercise. In contrast, PGC-1 α expression (again which was significantly elevated by acute exercise after training) was lower in the trained vs. untrained condition 4 h post exercise. This seems paradoxal, but it might be reasoned that protein levels had risen to sufficient levels needed to support mitochondrial biogenesis and so the elevations post exercise could be reduced to support more subtle increases in proteins of oxidation at they approached peak levels needed for energy transduction in the trained state.

CPT-1 β did not change in response to acute exercise in either the untrained or trained state. Our findings with CPT-1 β with acute exercise in the untrained condition

were similar to Pilegaard et al. (2000), but differed in the trained state in that Pilegaard et al. (2000) communicated significant increase in expression 4 h post exercise. Protocols differed in that they trained their subjects by one legged cycling and utilized 60-90 of exhaustive exercise for 5 consecutive days. Most importantly, subjects were non-obese. Experiments to confirm whether CPT-1 β regulation by exercise is altered in the obese state are required and certainly racial comparisons are warranted in light of the finding in the present study that CW but not AAW, elevate CPT-1 β expression in recovery from exercise once trained.

Our experiments demonstrated a robust increase in PDK4 expression in the recovery phase from exercise in both the untrained and trained condition and results were similar for AAW and CW. These results were similar to those of Pilegaard et al. (2000), the study which was referred to earlier. As described above, PDK4 is a major regulator of the PDHC activity and reduces its function by covalent (phosphorylation) modification. PDH4 is stimulated by a high $NADH/NAD^+$ and fatty acids. Therefore, high levels of mitochondrial fatty-acid oxidation leads to suppression of the PDHC due to high levels of acetyl-CoA (the PDHC is also attenuated by allosteric regulation by a high $NADH/NAD^+$ and by high levels of acetyl-CoA). This relationship between lipid and glucose metabolism may be significant when considering the state of skeletal muscle in the obese state. For example, skeletal muscle from obese subjects is characterized by lipid accumulation which is positively associated with insulin resistance and the progression toward type II diabetes. According to Lowell and Shulman (2005), fatty acids and/or their metabolites) may serve (as bioactive ligands) to activate particular

kinases (e.g, PKC) or phosphatases that disrupt the normal signaling cascade by insulin to translocate glucose transporters (Glut 40 to the cell surface membrane to facilitate glucose uptake. As compensation, activation of PDK4 by fatty acids (or their metabolites) may serve to compensate against this pathological development. As an explanation, PDK4 inhibition of the PDHC would serve to uncouple anaerobic and aerobic metabolism of glucose while at the same time promoting increased oxidation of fatty acids due to loss of competition for the TCA cycle by glucose derived pyruvate. This hypothesis fits our findings as aerobic exercise, which also raises fatty acid mobilization and oxidation, was associated with increased PDK4 gene expression. As stated earlier, future studies involving lean subjects under the identical protocol are needed for comparisons to visualize if this hypothesis bares credence when comparing obese vs. lean subjects. It will be interesting to determine if the elevation in PDK4 response occurs only to acute exercise. In obese subjects that would loose weight with a prolonged (e.g., 8 weeks) exercise program might demonstrate lower levels of PDK4 thus restoring the balance of glucose lipid utilization, in which case would be beneficial for glucose clearance from the blood by skeletal muscle. Equally informative will be the determination of the response in AAW and CW in order to better understand the equality or inequality of the expanded response in lipid utilization following long term endurance exercise training.

Finally, UCP3 gene expression was responsive to exercise in the untrained state in both AAW and CW, but increased only in CW post exercise in the trained state. This is also similar to findings by Pilegaard et al. (2000) with lean subjects. Thus obese subjects

apparently retain the ability to upregulate this gene, hypothesized to be protective against the accumulation of ROS. Obese CW may be at a slight advantage in this respect in the trained condition.

Overall recommendations are to repeat these experiments in lean AAW and CW for comparisons with the present findings and conduct analyses for protein content in all four groups. Applications and future studies are discussed immediately below.

Future Studies

As a perspective, our laboratory has obtained evidence from *in vitro* oxidation studies that in as little as 10 days of continuous aerobic exercise, rates of fatty acid oxidation are elevated in both obese African-American and Caucasian women. This occurs despite the observation that basal rates of palmitate oxidation are reduced in skeletal muscle from obese individuals and that obese African-American women are suppressed to an even greater extent that obese Caucasian women. A further interesting observation was obtained in that the greater reductions in the mitochondrial oxidation of lipids in obese African-American women occurred when palmitate, but not palmitoyl-CoA or palmitoyl-carnitine was used as substrate. This data suggests that the greater dysfunction in lipid oxidation observed in African-American women occurs at the level of acyl-CoA synthetase because only the oxidation of the inactivated form of the fatty acid was impaired.

The present data contributes some insight into the mechanisms by which obese African-American and Caucasian women can elevate the capacity of their skeletal muscle

to oxidize fatty acids during exercise and after training. In other words, several genes known to be involved in fatty acid oxidation (PGC-1 α with mitochondrial biogenesis; PDK4 which reduces glucose oxidation under conditions of energy production using fatty acids; CPT-1 β, the regulating step in mitochondrial oxidation of long-chain fatty acids;UCP3 which may have many functions such as control of ROS production and maintenance of oxidative-phosphorylation potential of the mitochondria) were altered in their expression after acute exercise in the untrained and trained condition as would be predicted for support and enhancement of skeletal muscle fatty acid oxidation. However, the scope of the study is limited to these four genes. Clearly, the expression of additional genes encoding proteins known to be inducible by exercise and training in lean Caucasian women need to be measured. Candidates would include transcription factors known to upregulate mitochondrial biogenesis and fatty acid oxidative such as the PPAR proteins (e.g., PPARα,γ,δ), proteins of the β-oxidative pathway such as long- and medium- chain CoA dehydrogenase (LACD, MCAD) and β-hydroxybutyrate dehydrogenase (β-HAD) and proteins of the TCA cycle (citrate synthase) and Electron Transport chain (cytochrome C oxidase, COX). In addition however, the genetic expression and inducibility by exercise and training of the known isoforms of the long-chain acyl CoA synthetases (ACSL 1-5) should be determined to observe a potential mechanism to explain the racial differences in fatty acid oxidation as described above. Understanding the plasticity of one or all of these proteins will shed important light on the mechanism by which chronic contractile activity elevates the oxidative capacity of skeletal muscle in obese African-American women. In this regard, the present study included a training

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APPENDIX A

UMCIRB APPROVAL

University and Medical Center Institutional Review Board
East Carolina University
Ed Warren Life Sciences Building • 600 Moye Boulevard • LSB 104 • Greenville, NC 27834
Office **252-744-2914** • Fax **252-744-2284** • **www.ecu.edu/irb**
Chair and Director of Biomedical IRB: Charles W. Daeschner, III, MD
Chair and Director of Behavioral and Social Science IRB: Susan L. McCammon, PhD

TO: Ronald Cortright, PhD, Dept of EXSS, ECU

FROM: UMCIRB

DATE: February 11, 2005

RE: Full Committee Approval for Continuing Review of a Research Study

TITLE: "Dysregulated Muscle Lipid Metabolism in African-Americans.""

UMCIRB #04-0071

The above referenced research study was initially reviewed by the convened University and Medical Center Institutional Review Board (UMCIRB) on **02-25-04.** The research study underwent a subsequent continuing review for approval on **02-09-05** by the convened UMCIRB. The UMCIRB deemed this **NIDDK** sponsored study **more than minimal risk** requiring a continuing review in 12 **months.**

The above referenced research study has been given approval for the period of **02-09-05** to **02-08-06**. The approval includes the following items:

- Protocol
- Informed consent for 8 weeks of Exercise Training (version 3/17/04)
- Informed consent for 10 days of Exercise Training (version 3/17/04)
- Advertisement (no version date)
- Personal History Form
- Activity and Dietary Recall
- Menstrual Cycle Recall
- Grant Application

The following UMCIRB members were recused for reasons of potential for Conflict of Interest on this research study:
None
NOTE: B. Hickner did not participate in the deliberation or vote on this study.

The UMCIRB complies with 45 CFR 46, 21 CFR 50, 21 CFR 56, ICH Guidelines, UMCIRB operating policies and procedures, institutional policies and other applicable federal regulations.

APPENDIX B

INFORMED CONSENT

Version 3-17-04

Informed Consent

Title of Project: Dysregulated Muscle Lipid Metabolism in African-Americans

Subtitle of Project: Effects of 10 Days of Exercise Training on Adipoctye Lipolytic Rate and Muscle Lipid Metabolism

Principal Investigator: Ronald N. Cortright, Ph.D
Co-Investigators: Robert C. Hickner, Ph.D.; Joseph Houmard, Ph.D.; Hisham Barakat, Ph.D.; and Joseph Gary, M.D.

Institution: Human Performance Laboratory, East Carolina University
Address: 371 Ward Sports Medicine Building, Greenville, NC
Telephone: (252) 328-4678 or 756-7735

This consent document may contain words that you do not understand. You should ask the study doctor or the study coordinator to explain any words or information in this consent form that you do not understand.

Introduction: I have been asked to participate in a research study being conducted by **Dr. Ronald N. Cortright, Dr. Robert C. Hickner, Dr. Joseph Houmard, Dr. Hisham Barakat, Dr. Joseph Gary** and fellow researchers at East Carolina University.

The **purposes** of this study are 1) to determine why African-American women have a greater tendency to gain weight and develop diabetes and 2) whether exercise can improve the ability of both African-American and Caucasian women to use fat for fuel.

Obesity has reached epidemic proportions in the United States and is threatening to become a global epidemic. Obesity represents a serious health threat because of the increased risk of developing chronic diseases such as diabetes and cardiovascular disease. According to recent estimates, the prevalence of obesity is greater among African-American than Caucasian women in the United States. African-American women gain weight at an earlier age and remain heavier than Caucasian women at the same age. This racial difference is important because obesity is strongly associated with skeletal muscle insulin resistance (inability of muscle to take in sugar from the blood) and supports the existing data demonstrating that African-American women have twice the incidence of type 2 diabetes compared with Caucasian women. Although environmental factors such as socioeconomic status, diet, and level of activity may influence the greater prevalence of obesity and diabetes, it is becoming increasingly evident that inherent physiological and biochemical differences underlie the increased incidence of these diseases in African-American women. We have demonstrated that obese African-American women have a reduced ability to release fat from sites of storage (the adipocyte) and to use fat by skeletal muscle to make energy when compared to Caucasian women of similar age and weight. This is fundamentally important because the reduced ability to release and "burn" fat can result in its increased accumulation within the fat and muscle cells, the latter which is strongly linked with insulin resistance in obese individuals. Newer information suggests that African-American women who are not obese may be more likely to gain weight when compared to non-obese Caucasian women. For example, our lab has noticed that non-obese African-American women have a greater difficulty using fat to make energy when they are at rest and during exercise. Despite the negative implications of these findings for health however,

Subjects Initials _____

UMCIRB
APPROVED
FROM 02.09.05
TO 02.08.06

Title of Project: Dysregulated Muscle Lipid Metabolism in African-Americans

the cellular mechanisms to explain this race/ethnic metabolic difference in the propensity toward obesity and diabetes has scarcely been studied in African-American women.

We hope to apply the knowledge gained from this research to better understand why obese women in general, and more so, African-American women, can not use fat as effectively for energy production as non-obese Caucasian women. These studies could lead to more specific (dietary, activity, or pharmacological) treatments for obesity and diabetes, especially for African-American women.

You should understand that you will be one of approximately 80 women (over 2 years) in the research study (ages 18 – 45 years). The study groups are comprised of 20 obese [body mass index (weight: kg/height: m^2) > 30] African-American, 20 obese Caucasian, 20 non-obese [body mass index (weight: kg/height: m^2 < 30] African-American and 20 non-obese Caucasian women.

The study will include 2 days of assessment and nutritional education and 10 days of aerobic exercise training. Your first visit will take approximately 90 minutes during which time we will determine your body composition (in terms of fat and lean weight) and your aerobic exercise capacity. On the same day, the researchers will also teach you how to select, measure, and record the foods that you eat for two-three day periods during the study. On another day of your choice, you will be asked to report to the office of Eastern North Carolina Radiology for a CT scan to determine your body's sites of trunk fat storage. The total hourly commitment for your participation in the study is approximately 15 hours (assessments = ~ 1.5 hours; CT scan = ~ 2 hours; 10 days of exercise = ~ 10 hours; 4 biopsies = ~ 2 hours).

On the first and last day of the exercise training protocol, you will be asked to report in the morning (after an overnight fast-no food after 10:00 PM the night before) to room 3S-08 in the Brody School of Medicine for the muscle biopsy procedures. The first biopsy will take place on day 1, before you exercise and the second will take place 2 hours after you exercise. You will perform your first and last exercise sessions in room 3S-08 in the Brody School of Medicine.

You will be asked to exercise on a bicycle for 9 days thereafter (total of 10 days of exercise training). The training sessions will take place in the exercise facility known as the FITT building, adjacent to Minges Collesium. Each exercise session will last 60 minutes. A trained exercise physiologist will assist you and will monitor your heart rate, blood pressure, and ability to use fat and carbohydrates for energy production during each exercise session.

On the last day of the study, you will report to the Brody School of Medicine (3S-08) and will be biopsied for the third and fourth muscle samples before and 2 hours after the last exercise session which will take place in the same room. From the small biopsy samples (~ 75 milligrams each; the size of a pencil eraser) the investigators will determine your muscle's ability to burn fat before and after exercise training. We will also determine which muscle genetic factors change (gene expression) because of exercise and training. This information will help us to improve drug and physical activity strategies for individuals prone to obesity and diabetes.

In order to determine the effects of a single or repeated bouts of exercise on your ability to release fat so that the muscle can burn it, on days 0 and 10, we will insert a small catheter into a vein in your arm and collect blood before, during, and after exercise. Each time we take blood, the total amount collected will

be small (about 40 milliliters = ~ 4 tablespoons; total = 80 milliliters or ~ 8 tablespoons for the entire study).

Details of each procedure are described below.

Plan and Procedures

My participation will involve:

The following are screening procedures and assessment of metabolism, fitness, and body composition:

1. **Preliminary Assessments: First Visit.**

- **Health History and Other Forms** (FITT building). During your first visit you will be asked to complete a health history questionnaire to help determine if you are suitable for this study (e.g., types of medications used if any). In addition, you will be asked to record the history of your recent menstrual cycle and whether you are currently taking birth control pills.

- **Diet Recording and Study Eating Habits.** Because different diets can affect the body's use of fat and carbohydrates as fuel, you will be asked to record your diet twice during the 10-day study. A trained nutritionist will explain how to measure the amount and select foods from a list provided to you based on what you normally eat. We will ask that you eat foods from the list and record your portion sizes for three days prior to the first exercise session. This procedure will allow us to determine each subject's amount of calories and diet composition. This way, we can rule out the possible variability in diet on the metabolism of your body. You will then be asked to eat the same or similar meals over days 7-10 of the study. These diets conform to nutritional health standards as suggested by the American Diabetic Association standards (60% carbohydrate, 25% fat, and 15% protein).

- **Body Composition** (FITT Building): **Hydrostatic weighing.** You will be asked to wear a bathing suit or other suitable clothing. You will be required to sit on a chair suspended in warm water. Your entire body will be in the water but you may choose to have your head submersed or not submersed. You will breathe out maximally for approximately 5 seconds. This is done so your weight in water and therefore the density of your body and percent body fat can be determined. You will surface immediately following the 5-second measurement, or when you need to breathe, whichever comes first if you elect to submerse your head. You will repeat this underwater procedure approximately 8 times, with approximately 30-60 seconds rest between each attempt. The results of the underwater weighing procedure will be confirmed by skinfold determination of body composition. Several sites on your body will be measured for skinfold thickness. This is a painless procedure that involves the use of a caliper that determines the thickness of the skin and fat located under the skin at the site. The information gained from both procedures will allow us to use equations to estimate your percent body fat and percent lean body weight.

- **Maximal Exercise Test.** Fitness test (FITT Building - Human Performance Laboratory). The procedure will determine your maximal ability to use the oxygen (air) you breath to make energy

from the food you eat. It will also allow the study investigators to set your workload for the submaximal exercise test described above. You will perform cycling (cyclists) exercise for ~10 minutes. You will begin cycling at light intensity (you will not breathe hard) for 2 minutes. The workload will be increased every 2 min. until you can no longer continue. This will allow us to determine your maximal exercise capacity.

2. **CT or CAT Scan.** Computed (Axial) Tomography (CT) is a routine method that provides very clear pictures of structures inside the body. The CT scan device uses sophisticated computers and a safe amount of X-rays. It will be used to assess the regional fat content in your trunk area. An appointment will be made for you at the Eastern Radiologists Inc. site (9 doctors Park; phone 754-5214). The visit will take approximately one hour. The CT scan device looks like a giant donut. You will be asked to lay down on a table and an instrument will be used to scan your middle body area. The test takes approximately 30 minutes. This information will help us to interpret the lipid metabolism data gained from your blood and muscle biopsies.

3. **Exercise Training.** Exercise training will occur over 10 consecutive days. You will be asked to come to the FITT building near Minges Coliseum at a time that is convenient for you. At least one of the study personnel will always be present. Your weight will be measured and you will be fitted with a heart rate monitor. Exercise will consist of pedaling a stationary bicycle for 60 minutes at approximately three-fourths of your maximal capacity as determined from your maximal aerobic capacity test taken at your first visit. We would like for you to pedal continuously for the entire 60 minutes, but if that becomes difficult on any day, you may stop exercising and resume as soon as you feel ready. Water will be provided for you throughout the exercise session. Occasionally, you will be fitted with a mouthpiece and nose clips so we can measure your consumption of oxygen and production of carbon dioxide. This will allow us to determine your utilization of fat as fuel for your working muscles. We will also monitor your heart rate and blood pressure each session. After the exercise session is finished, you will be encouraged to pedal at a very light workload to "cool down" and let your heart rate, blood pressure, and breathing return to near resting levels.

4. **The muscle biopsy procedure.** The biopsy procedure will take place at the Brody School of Medicine or the Human Performance Laboratory. The procedure will occur under sterile conditions and a physician will be available during the entire time of the procedure. You will report to room 3S-08 at 7:30 AM following an overnight fast (no food after 10:00 PM the night before). For this procedure, a small amount of anesthesia (3 cc of 1% Lidocaine) will be injected in a ½ inch area under the skin of my thigh. A small (1/4 inch) incision will then be made through the skin, fat, and fibrous layer that lies over the muscle. A biopsy needle (about ½ the width of a pencil) is then inserted through the incision ½ to 1 inch into the muscle. A small piece of muscle (1/2 the size of an erasure at the end of a pencil) is then clipped out with the biopsy needle. The needle is withdrawn and the muscle sample is prepared for analysis. **You will undergo two muscle biopsies on day 0 and two muscle biopsies on day 10** of the study. A separate incision will be made for each of the **four biopsies**. The first two biopsies will be taken from the left leg and the second two biopsies will be taken from the right leg. The muscle biopsies will be taken by Robert Hickner, Ph.D., Joseph Houmard, Ph.D., or Ronald N. Cortright, Ph.D. **The muscle samples will be assessed for the ability to metabolize fat and to control the metabolism of energy by assessing the levels of certain muscle factors (the expression of mitochondria and genes that regulate fat metabolism in skeletal muscle; e.g, PGC-1, PPARs, uncoupling proteins, etc.).**

5. **Blood samples** (a total of 8 samples for the entire study) will be obtained on the day of each biopsy (days 0 and 10). Blood will be drawn immediately prior to, twice during exercise, and once after exercise for analysis of fat release from stored sites (adipocytes). Blood will be drawn from a small catheter placed in your arm vein. On each occasion, the total amount of blood will be approximately 40 ml (~ 4 tablespoons) and the total amount of blood obtained for the entire study will be approximately 80 ml (~ 8 tablespoons). Blood will be drawn from a small catheter placed in your arm vein By determining blood born fat components known as glycerol and fatty acids, the investigators can determine the extent of lipolysis (fat release) that occurs before, during and after exercise. In addition, we will measure blood insulin and glucose as well as hormones known as catecholamines that are involved in the fat releasing process. Other fat metabolism related hormones such as Leptin, Adiponectin, Ghrelin (hormones released by the fat cells which are associated with the regulation of fat and blood sugar) will also measured. Determining blood lipids will also help us to determine the relationship between these blood variables and factors indicating your muscle's lipid metabolic capacity and diabetic status. The blood samples will be taken by Dr. Robert Hickner, Dr. Ronald Cortright, Dr. Joseph Houmard, or a trained research nurse either at the FITT building (Human Performance Lab) or the Brody School of Medicine.

Potential Risk and Discomforts

Certain risks and discomforts may be associated with this research. They include:

•The determination of body composition by hydrostatic (underwater) weighing is associated with minimal risk. Some individuals experience mild mental distress with their head submerged underwater. If this is the case, you will be assisted out of the water and will be allowed to perform a modified underwater weighing procedure at a later date with your head above the water at all time.

•The total amount of blood drawn for lipid metabolism measurements (~ 12 tablespoons) is very small compared to the total amount (about a gallon) of blood that you have. There is an extremely small risk of local bruising or infection associated with insertion of intravenous catheters (we draw blood through these) into your arm.

*Risks associated with the exercise protocols are dizziness, ventricular arrhythmia (odd heart beats), and in very rare instances death. These risks are very small, with an incidence of fewer than 1 in 10,000 deaths in patients who are known to, or suspected of, having heart disease. The risk is expectedly much smaller than this in a group of younger, healthy subjects. To minimize this risk, we will have a physician present and a heart (ECG) monitoring device will also be used during the exercise tests. The physician will be trained to recognize heart problems during exercise and trained to revive people in the event of serious heart problems during the exercise test. The exercise tests will be stopped if you feel dizzy, are having chest pain, are having serious shortness of breath, or ask that the test be ended. The test will also be stopped if the physician detects (from the ECG) heart function that is not normal. All of the necessary emergency equipment (including crash cart for heart problems) will be in the room.

• Dr. Joseph Gary, M.D. or other attending physicians will be provided medical coverage for the maximal exercise test and the muscle biopsies performed at the Brody School of Medicine or the Human Performance Laboratory. With respect to the muscle biopsy procedure, there is a small risk of hematoma (bruise) or infection around the biopsy site. This risk will be minimized by using sterile procedures and applying pressure to the biopsy site for 10 minutes, or until bleeding is stopped if longer than 10 minutes, following biopsy. A steri-strip (thin bandage) will be applied over the incision and will remain in place for at least 4 days to close the incision during healing. A pressure wrap will also be

placed around the biopsied limb and will remain for 8 hours following biopsy. There is an extremely remote risk of allergic reaction to the Lidocaine anesthesia. This risk will be minimized by using subjects who have had prior exposure to Lidocaine or Novocaine anesthesia; this precaution should eliminate this risk. Dr. Joseph Gary, M.D. (or other physicians associated with the study) will initiate any medical treatment necessary during or following any adverse event from the biopsy procedure.

• The CT (CAT) scan procedure is a safe test involving the exposure of the subject to amounts of radiation (X-ray) that is deemed safe for children and adults. However, the x-ray exposure is not appropriate for unborn children. Therefore, subjects who are pregnant will be excluded from the study.

Exclusions

To the best of your knowledge, you are not allergic to Novocaine. For example, you have not had an allergic reaction to an injection at the dentist's office. To your knowledge, you do not possess any condition which would result in excessive bleeding. You do not have known kidney disease, and you do not have know heart disease (i.e., had a heart attack). Other exclusion criteria include: individuals who are ill or taking medications, individuals who are known diabetics, individuals who currently smoke, African-Americans that are not of at least second generation African-American decent, individuals who are pregnant, and individuals who are exercise training or who have exercise trained regularly within the last 6 months.

Potential Benefits

1) You will receive information concerning your health risk due to your level of obesity and insulin resistance.
2) You will benefit from gaining knowledge of your body composition and aerobic fitness level.
3) You may receive muscle and cardiovascular-respiratory benefits from exercise training for 10 days.
4) You will receive information about their skeletal muscle fiber type.
5) Society and medical science may benefit from gaining the knowledge resulting form this investigation.

Termination of Participation

Your participation in this research study may be terminated without your consent if the investigators believe that these procedures will pose unnecessary risk to you. You may also be terminated from participation if you do not adhere to the study protocol.

Cost and Compensation

The policy of East Carolina University does not provide for compensation or medical treatment for subjects because of physical or other injury resulting from this research activity. However, every effort will be made to make the facilities of the School of Medicine available for treatment in the event of such injury.

Title of Project: Dysregulated Muscle Lipid Metabolism in African-Americans

You will receive $250.00 for your time and efforts for participating in the exercise and muscle biopsy procedures. You will receive, free of charge, the body composition and maximal aerobic capacity analysis. The remuneration is prorated as follows:

1. $50.00 per muscle biopsy (maximum 4 biopsies total)
2. $50.00 for exercise training for 10 days

You do not give up any legal rights as a research participant by signing this consent form.

Confidentiality

Only the investigators associated with this study will have access to the data obtained. The data gathered from the study will be stored on a computer hard drive which will be accessible only by the investigators or technical staff. Numeric coding will protect the identity of the subjects. No identifying information will be released. The information and insights gained from the study may be presented at scientific conferences and/or published. In both instances, you will not be identified by name.

Voluntary Participation

The nature and purpose of the procedures, the known risks involved, and the possibility of complications have been explained to you. No guarantee of assurance has been given by anyone as to the results that may be obtained. You know that being in this study is of your own free will. You know that you can decide not to be in this study after you have already started. You may stop at any time without losing benefits that you would have received before being in the research study.

Persons to Contact with Questions

The investigators will be available to answer any questions concerning this research, now and in the future. You may contact the investigators, **Ronald Cortright Ph.D.** (work: 328-4678 or home: 756-7735), **Robert Hickner, Ph.D.** (work: 328-4677 or home: 353-5556), or **Joe Houmard, Ph.D.** (work: 328.4688/328-4617). Drs. Cortright, Hickner and Houmard are found in the Human Performance Laboratory, Ward Sports Medicine Building, ECU. Also, if questions arise about your rights as a research subject, you may contact the Chairman of the University and Medical Center Institutional Review Board at phone number 252-816-2914 (days).

Research Participant Authorization To Use And Disclose Information

Federal laws require that researchers and health care providers protect your identifiable health information. Federal laws also require that researchers get your permission to use collected health information for research. The identifiable information we will collect from subjects in this research project will include:

*General Medical History including: Family health history, medications, nutrition, physical activity levels, menstrual history, nutritional history, and body weight history.

Title of Project: Dysregulated Muscle Lipid Metabolism in African-Americans

*Muscle biopsy information, body composition information, blood levels of insulin, glucose, and other compounds related to muscle and fat cell lipid metabolism.

The members of our research team that will have access to your information will include the Principle investigator, co-investigators, as well as technical and nursing personnel involved in this project. Information about you will be used and released in such a way that will protect your identity as much as possible; however, confidentiality cannot be absolutely guaranteed. We will only share your information with those individuals listed above. If we need to share information with other individuals other than those listed, we will request your permission a second time.

You will be given a signed copy of your authorization to release medical information for your records. You can limit the amount and type of information that is shared and you must make this request in writing; however, the researcher is able to use any and all information collected prior to the request not to disclose information. Although you can limit the release of your medical information, withholding some information may cause you to become ineligible for this research project. Because research information continues to be looked at after a study is finished, it is difficult to say when the use of your information will stop. There is currently not an expiration date for the use and disclosure of your information for this study.

If you have questions related to the sharing of information, please call Ronald N. Cortright, Ph.D. at 252-328-4678. You may also telephone the University and Medical Center Institutional Review Board at 252-744-2914. In addition, if you have concerns about confidentiality and privacy rights, you may phone the Privacy Officer at Pitt County Memorial Hospital at 252-847-6545 or at East Carolina University at 252-744-2030.

FUTURE TESTING OF BLOOD/MUSCLE SAMPLES

Upon termination of this study, the blood and muscle samples collected for this study will be stored for up to 7 years to research scientific questions specifically related to obesity, the effects of exercsie, muscle/fat cell lipid metabolism and insulin resistance/diabetes in African-American vs. Caucasian women. You will continue to be the owner of the samples and retain the right to have the sample material destroyed at any time during this study by contacting the study principal investigator Ronald N. Cortright, at 252.328.4678. During this study, the samples will be stored with number identifiers only; however, the number identifier will be linked to a specific name and will be kept on file in the possession of the principal investigator. The linked file will be stored password protected on the Principal Investigator's computer with CD backup. No other individuals will have access to these identifying materials unless the principal investigator is required by law to provide such identifying information. Data will not be publicly available and participants will not be identified or linked to the samples in publication. If a commercial product is developed from this research project, you will not profit financially from such a product. Furthermore, there are no plans for the investigators to profit financially from such a product.

Title of Project: Dysregulated Muscle Lipid Metabolism in African-Americans

CONSENT TO PARTICIPATE

I have read all of the above information, asked questions and have received satisfactory answers in areas I did not understand. (A copy of this signed and dated consent form will be given to the person signing this form as the participant or as the participant authorized representative.)

Participant's Name **(PRINT)** **Signature** **Date** **Time**

Guardian's Name **(PRINT)** **Signature** **Date** **Time**

WITNESS: I confirm that the contents of this consent document were orally presented, the participant or guardian indicates all questions have been answered to his or her satisfaction, and the participant or guardian has signed the document.

Witness's Name **(PRINT)** **Signature** **Date**

PERSON ADMINISTERING CONSENT: I have conducted the consent process and orally reviewed the contents of the consent document. I believe the participant understands the research.

Person Obtaining consent **(PRINT)** **Signature** **Date**

Principal Investigator's **(PRINT)** **Signature** **Date**

UMCIRB APPROVED
FROM 02.09.05
TO 02.08.06

APPENDIX C

PERSONAL HISTORY FORM

PERSONAL HISTORY FORM

PLEASE PRINT AND FILL OUT COMPLETELY

1. Name: _____ Date: _____
SS#_____ Phone: (home) _____ (work) _____
Address:
_____
City: _____ State _____ Zip _____
Email:_____

2. Employer: _____
Occupation: _____

3. Date of Birth: _____ Sex: _____ Age: _____ Race: _____

4. General Medical History Circle one

Any medical complaints presently? (if yes, explain) .... yes no
_____
_____
_____

Any major illnesses in the past? (if yes, explain) ..... (date) _____ yes no
_____
_____
_____

Any hospitalization or surgery? (if yes, explain) ..... (date) _____ yes no
_____
_____
_____

Have you ever had an EKG (electrocardiogram) ? ..... (date) _____ yes no

Are you diabetic? ....If yes, at what age did you develop diabetes: _____
yes no

Are you currently taking any medications? .....
yes no

Medication	Dosage	Reason	Times taken per day

5. Family History

	Age if alive	Age of death	Cause of death
Father	_____	_____	_____
Mother	_____	_____	_____

Do you have a family history of: (Blood relatives only: give age of occurrence if applicable)
Relationship Age of occurrence
--High blood pressure .... yes no
_____
--Heart attack.....yes no
_____
--By-pass surgery.....yes no
_____
--Stroke.....yes no
_____
--Diabetes.....yes no
_____
--Gout.....yes no
_____
--Obesity.....yes no
_____

6. Tobacco History (check one)

_____ None
_____ Quit months/years ago
_____ Cigarette
_____ Snuff
_____ Chewing tobacco
_____ Pipe

Total years of tobacco use? _____

Cigarette history
_____ 1-10 daily
_____ 11-20 "
_____ 21-30 "
_____ 31-40 "
_____ more than 40

Snuff history
_____ < 0.5 cans daily
_____ 0.5-2.5 cans "
_____ > 2.5 cans "

Chewing history
_____ < 0.5 pouches daily
_____ 0.5-2.5 pouches "
_____ > 2.5 pouches "

7. Weight History
What do you consider a good weight for you? _____ Weight at age 21? _____
Highest weight since age 21? _____ Weight one year ago? _____
Weight now? _____

8. Cardio-Respiratory History
Any heart disease now?.....yes no
Any heart disease in the past?.....yes no
Heart murmur?.....yes no
Occasional chest pains?.....yes no
Chest pains on exertion?.....yes no
Fainting?.....yes no
Daily coughing?.....yes no
Cough that produces sputum?..... yes no
High blood pressure?.....yes no
Shortness of breath --at rest.....yes no
Lying down.....yes no
Sleeping at night.....yes no
After 2 flights of stairs.....yes no

9. Muscular History
Any muscle injuries or illnesses now?..... yes no
Any muscle injuries in the past?..... yes no
Muscle pain at rest?..... yes no
Muscle pain on exertion?..... yes no

10. Bone-Joint History
Any bone or joint (including spinal) injuries or illnesses now?..... yes no
Any bone or joint (including spinal) injuries or illnesses in the past?..... yes no
Ever had painful joints?..... yes no
Ever had swollen joints?..... yes no
Flat feet?..... yes no

11. Physical Activity Survey

a. Compared to a year ago, how much regular physical activity do you currently get? (Check One)

____ much less ____ somewhat less ____ about the same
____ somewhat more ____ much more

b. For the last three months, have you been exercising on a regular basis?

____ yes ____ no

c. What type of exercise or physical activity do you currently do or have done regularly in the past?

(For example: walking, swimming, weight lifting, gardening, etc.)

_____
_____
_____

d. On the average, how many days per week do you exercise? _____

e. How long do you exercise each time? For how many minutes? _____

f. How hard do you exercise on a scale from 1 to 5: with 1 being easy and 5 being very hard?

____ 1 ____ 2 ____ 3 ____ 4 ____ 5

g. Do you ever check your heart rate (pulse) to determine how hard you are exercising?

____ yes ____ no

h. What aerobic activity or activities would you prefer in a regular exercise program for yourself?

____ Walking and/or running ____ Tennis ____ Bicycling
____ Racquetball ____ Swimming ____ Basketball
____ Aerobic dance ____ Stationary cycling ____ Soccer
____ Stair climbing ____ Rowing _____Other

12. Alcohol History

Do you ever drink alcoholic beverages? Yes ____ No ____
If yes, what is your approximate intake of beverages per week?
Beer _____ Wine _____ Mixed Drinks _____

13. Sleeping Habits

Do you ever experience insomnia (trouble sleeping)? Yes ____ No ____
If yes, approximately how often?_____

How many hours of sleep do you usually average per night?_____

14. Education

Please indicate the highest level of education completed.

____ Grade School ____ Junior High ____ High School

____ College ____ Graduate ____ Postgraduate

Please indicate degree earned (i.e. B.A., M.S., Ph.D.)_____

15. Family Physician

Name: _____

Address: _____

Telephone: _____

Signature: _____

Date: _____

APPENDIX D

PARTICIPANT INSTRUCTION SHEET

INSTRUCTIONS FOR PARTICIPANTS

Preliminary: Date: _____ Time: _____
Location: FITT Building (see enclosed map)
Please call _____ if there are any changes: _____
Please allow about 2 hours

You will be asked to:
1. Read and sign consent form
2. Fill out a health history form
3. Perform a lung volumes test (to measure Residual volume)
4. Have your body fat measured in the hydrostatic tank
5. Have your aerobic capacity (VO_{2peak}) measured through a mouthpiece you will be breathing through during your exercise test

Please Remember:
* Bring exercise clothes (and shoes) and a swimsuit to change into
* Be hydrated and not hungry (have something small to eat about 1-2 hours before coming)
* Be on time or call ahead

Biopsy and Blood Draw: Date: _____ Time: _____
Location: Brody School of Medicine 3S08 (see enclosed map)
Please call _____ if there are any changes: _____
Please allow about 1 hour

You will be asked to:
1. Be fasted for at least 8 hours prior to your appointment time.
2. Have a muscle biopsy (includes Lidocaine and cold spray to numb a small portion of your thigh, followed by a small incision and the collection of a small piece of your vastus lateralis muscle). No stitches will be necessary, a steri-strip will be used to aid in the healing process.
3. A blood draw, collecting 15 mL of blood

Please Remember:
* To wear or bring shorts to change into
* Be fasted. Do not eat or drink anything (NO COFFEE! water is okay) after 10 pm the night prior.
* If your first exercise session is scheduled immediately after the biopsy, please bring something to munch on and drink.

Training Days:

Location: FITT Building (see enclosed map)
Please call _____ if there are any changes: _____
Please allow about 1 hour and 30 minutes each day

You will be asked to:

1. Exercise for 60 minutes continuously on a cycle ergometer
2. Exercise 10 days consecutively

Please Remember:

* Wear exercise clothes and shoes
* Be well hydrated and make sure you have eaten something small in the past 2 hours

If you have any questions or cannot get in touch with the indicated person for your appointment please call these other numbers:
Courtney File: 258-1541
Melanie Sweazey: 607-351-3009
Jasper Evans: 902-8862 OR 744-2934

APPENDIX E

DIRECTIONS TO THE F.I.T.T. (FITNESS, INSTRUCTION, TESTING, AND TRAINING) BUILDING AND BRODY SCHOOL OF MEDICINE (BSOM)

DIRECTIONS TO THE F.I.T.T. BUILDING

- Get on to Charles Boulevard (See Map to find out how to get on Charles Blvd from where you live.)
- Go down Charles Blvd. until you see Ficklen Drive (Ficklen Drive is across from Pirates Place)
- Take a left onto Ficklen Drive
- You will take 2nd right turn off of Ficklen Drive directly after the bus stop and commuter parking lot. (once you make this right you will be heading up a small hill)
- The Minges Building will be straight ahead of you at the top of the hill
- We are the one-story, off white building (**Building 189**) to the right of Minges
- You may park in any available spots near the building
- We are the FITT Building, and the building number is 189.

DIRECTIONS TO ROOM 3S08: BRODY SCHOOL OF MEDICINE

- Park in the visitors lot directly in front of the Brody School of Medicine Outpatient Entrance
- Enter through the two large sliding glass doors and walk straight past the small waiting room and reception desk.
- Take your first right down the hallway and walk to the end.
- At the end of the hallway, take a left and continue to the end of that hallway.
- At the end of this hallway, take another left and walk toward the sign hanging from the ceiling that says exit to the Medical School.
- When you get to this sign, there will be a hallway on your right, turn down it and walk through the double brown doors that will be in front of you.
- When you walk through the double door, to your right you will see a sign for the elevators.
- Take these elevators to the third floor and walk down the hall until you get to room **3S08**. There will be a sign next to the door.

APPENDIX F

VO_2 PEAK CYCLE PROTOCOL

VO_{2peak} Cycle Protocol

Subject I.D. Number: _____ DOB: _____

Date: _____ RHR: _____

RBP: _____

Min	Watts	HR	BP	RPE	Comments
1	40				
2	40				
3	40				
4	60				
5	60				
6	60				
7	80				
8	80				
9	80				
10	100				
11	120				
12	140				
13	160				
14	180				
15	200				
16	220				
17	240				
18	260				
19	280				

APPENDIX G

TRAINING SESSION TEMPLATE

ID #: _____ Session: _____

Date: _____ Pre HR: _____ Pre BP: _____ Wt: _____

Max VO_2: _____ L/min 75% _____ L/min Max HR: _____

_____ ml/kg/min 75% _____ml/kg/min Target HR: _____

Warm-up 5 minutes

Minute	HR	VO2	RER
10	_____	_____	_____
30	_____	_____	_____
50	_____	_____	_____

Notes/Comments:

APPENDIX H

ACTIVITY QUESTIONNAIRE

ACTIVITY QUESTIONNAIRE

Please circle one number (0-7) in Part A which best describes your general activity for the last year.
Please complete the answers to Part B as accurate as possible.

PART A

DO NOT PARTICIPATE REGULARLY IN PROGRAMMED RECREATION SPORT OR HEAVY PHYSICAL ACTIVITY.

0 - Avoid walking or exertion, eg, always use elevator, drive whenever possible instead of walking
1 - Walk for pleasure, routinely use stairs, occasionally exercise sufficiently to cause heavy breathing or perspiration.

PARTICIPATED REGULARLY IN RECREATION OR WORK REQUIRING MODEST PHYSICAL ACTIVITY, SUCH AS GOLF, HORSEBACK RIDING, CALISTHENICS, GYMNASTICS, TABLE TENNIS, BOWLING, WEIGHT LIFTING, YARD WORK.

2 - 10 to 60 minutes per week
3 - Over one hour per week.

PARTICIPATE REGULARLY IN HEAVY PHYSICAL EXERCISE SUCH S RUNNING OR JOGGING, SWIMMING, CYCLING, ROWING, SKIPPING ROPE, RUNNING IN PLACE OR ENGAGING IN VIGOROUS AEROBIC ACTIVITY TYPE EXERCISE SUCH AS TENNIS, BASKETBALL OR RACQUETBALL

4 - Run less than one mile per week or spend less than 30 minutes per week in comparable physical activity.
5 - Run 1 to 5 miles per week or spend 30 to 60 minutes per week in comparable physical activity.
6 - Run 5 to 10 miles per week or spend 1 to 3 hours per week in comparable physical activity.
7 - Run over 10 miles per week or spend over 3 hours per week in comparable physical activity

Part B
Indicate the number of miles completed on average for the last year in the following activities.

1) Number of miles ran per week _____ .

2) Number of miles cycling per week _____ .

3) Number of miles swam per week _____ .

4) Are you a member of any collegiate team yes/no If yes indicate which team _____

APPENDIX I

MENSTRUAL CYCLE RECALL

MENSTRUAL CYCLE RECALL

Please answer these questions as best you can and return them along with your informed consent:

1. Are you taking birth control pills_____yes_____no?
2. When was your first day of your last menstrual cycle?_____
3. How many days is your cycle?_____

APPENDIX J

FOOD DIARY

Food Diary

Date: _____ **Interviewer:**_____

Name: _____ **ID #:**_____

Meal/Time	Food Item	Amount	Description/Preparation

APPENDIX K

DELTA GRAPH FOR PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR γ COACTIVATOR-1 A

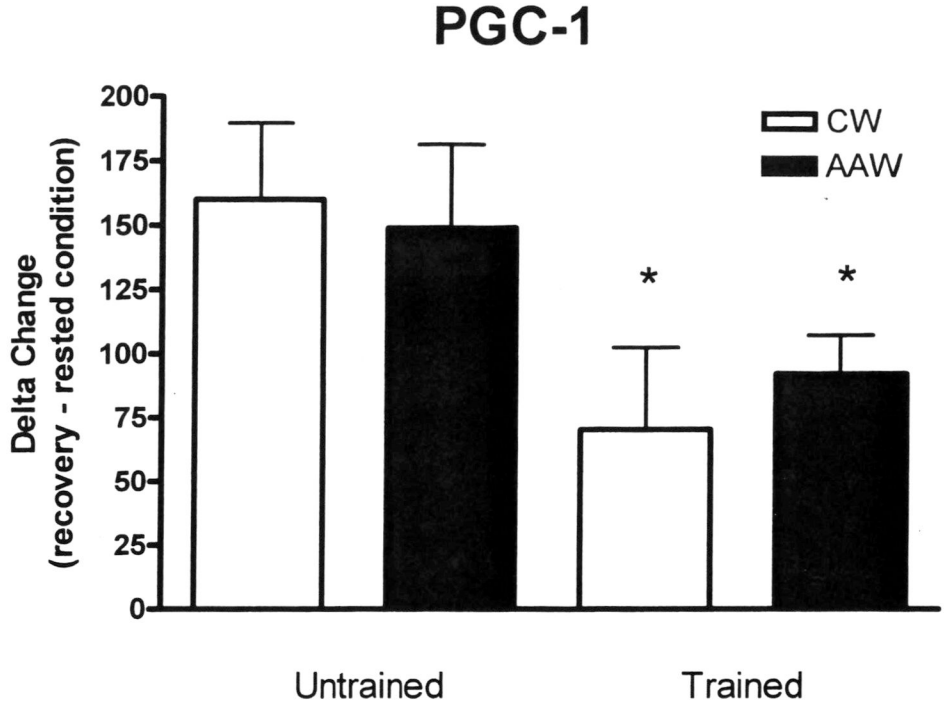

Figure 7: Racial comparisons on the delta change in skeletal muscle peroxisomal proliferator-activated receptor γ coactivator-1 α (PGC-1 α) gene expression in the untrained and trained state. The subjects consisted of 7 obese (BMI>30kg/m^2) Caucasian (CW) and 6 obese African-American women (AAW). Exercise consisted of 60 min of cycling on a Monark ergometer at 75% VO_2 peak. In both the untrained and trained condition, vastus lateralis muscle biopsies were obtained in the fasted (10 h) state immediately before (rested condition) and 4 h after acute exercise (recovery condition). Quantitative real time PCR was used to assess the expression of PGC-1 gene expression. Values are mean ± S.E.M. Original data (arbitrary units/GADPH) was transformed as the qRT-PCR recovery value minus the qRT-PCR value in the rested, fasted condition and then analyzed using a 2 (training status) X 2 (race) repeated measures ANOVA. * = significant main effect for time; P = 0.0112. *The results indicate that the increase in PGC-1 in recovery from acute exercise was less (main effect for training status, P=0.0112) after training, but the difference was not racially specific.*

APPENDIX L

DELTA GRAPH FOR CARNITINE PALMITOYLTRANSFERASE-1 β

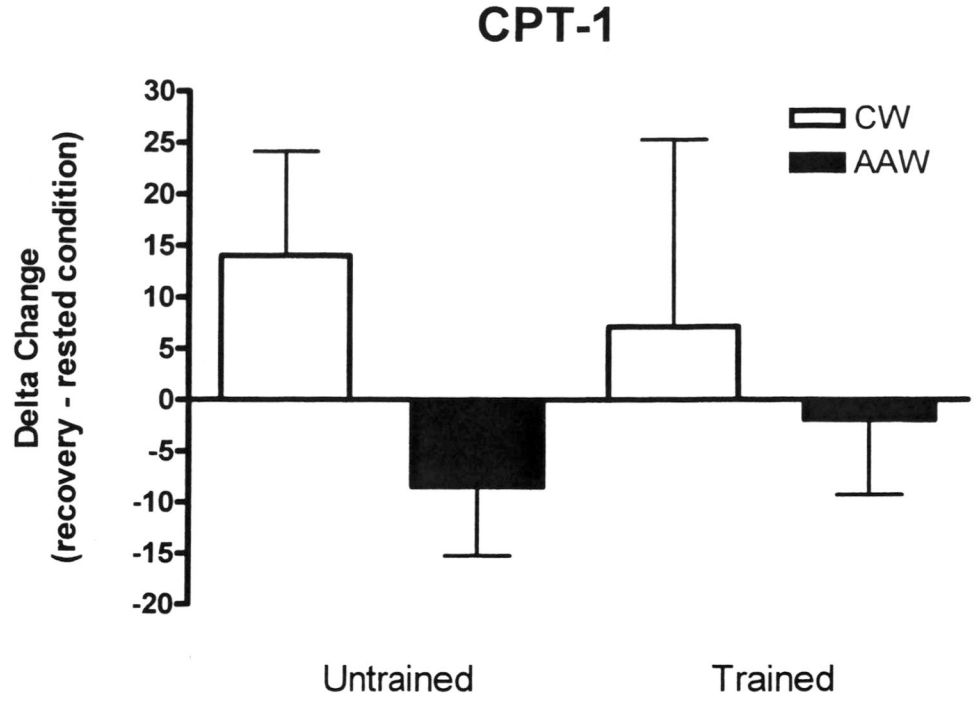

Figure 8: Racial comparisons on the delta change in skeletal muscle carnitine palmitoyltransferase-1 β (CPT-1 β) gene expression in the untrained and trained state. The subjects consisted of 7 obese (BMI>30kg/m^2) Caucasian (CW) and 6 obese African-American women (AAW). Exercise consisted of 60 min of cycling on a Monark ergometer at 75% VO_2 peak. In both the untrained and trained condition, vastus lateralis muscle biopsies were obtained in the fasted (10 h) state immediately before (rested condition) and 4 h after acute exercise (recovery condition). Quantitative real time PCR was used to assess the expression of CPT-1 β. Values are mean ± S.E.M. Original data (arbitrary units/GADPH) was transformed as the qRT-PCR recovery value minus the qRT-PCR value in the rested, fasted condition and then analyzed using a 2 (training status) X 2 (race) repeated measures ANOVA. Values are mean ± S.E.M. *Conclusion - There was an increase in the mean values for CPT-1 gene expression in recovery from exercise in both the untrained and trained condition, but this occurred in CW only. However, the increase was minor and not statistically different. AAW demonstrated a small, non-significant decline in CPT-1 gene expression in recovery from exercise in both the untrained and trained condition. An effect of training status was not noted.*

APPENDIX M

DELTA GRAPH FOR PYRUVATE DEHYDROGENASE KINASE 4

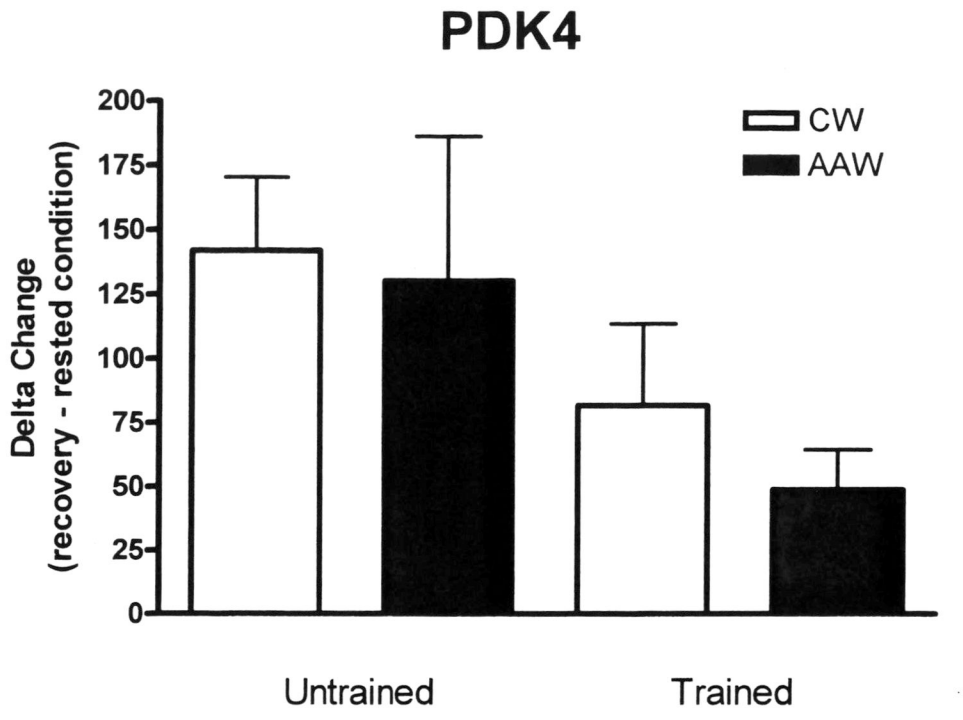

Figure 9: Racial comparisons on the delta change in skeletal muscle gene expression of pyruvate dehydrogenase kinase 4 (PDK4) the untrained and trained state. The subjects consisted of 7 obese (BMI>30kg/m^2) Caucasian (CW) and 6 obese African-American women (AAW). Exercise consisted of 60 min of cycling on a Monark ergometer at 75% VO_2 peak. In both the untrained and trained condition, vastus lateralis muscle biopsies were obtained in the fasted (10 h) state immediately before (rested condition) and 4 h after acute exercise (recovery condition). Quantitative real time PCR was used to assess the expression of PDK4. Values are mean ± S.E.M. Original data (arbitrary units/GADPH) was transformed as the qRT-PCR recovery value minus the qRT-PCR value in the rested, fasted condition and then analyzed using a 2 (training status) X 2 (race) repeated measures ANOVA. Values are mean ± S.E.M. *Overall Conclusion - The mean increase in PDK4 gene expression in recovery from exercise was lower following training for both AAW and CW, but the decline was not statistically significant (P = 0.08).*

APPENDIX N

DELTA DATA FOR UNCOUPLING PROTEIN 3

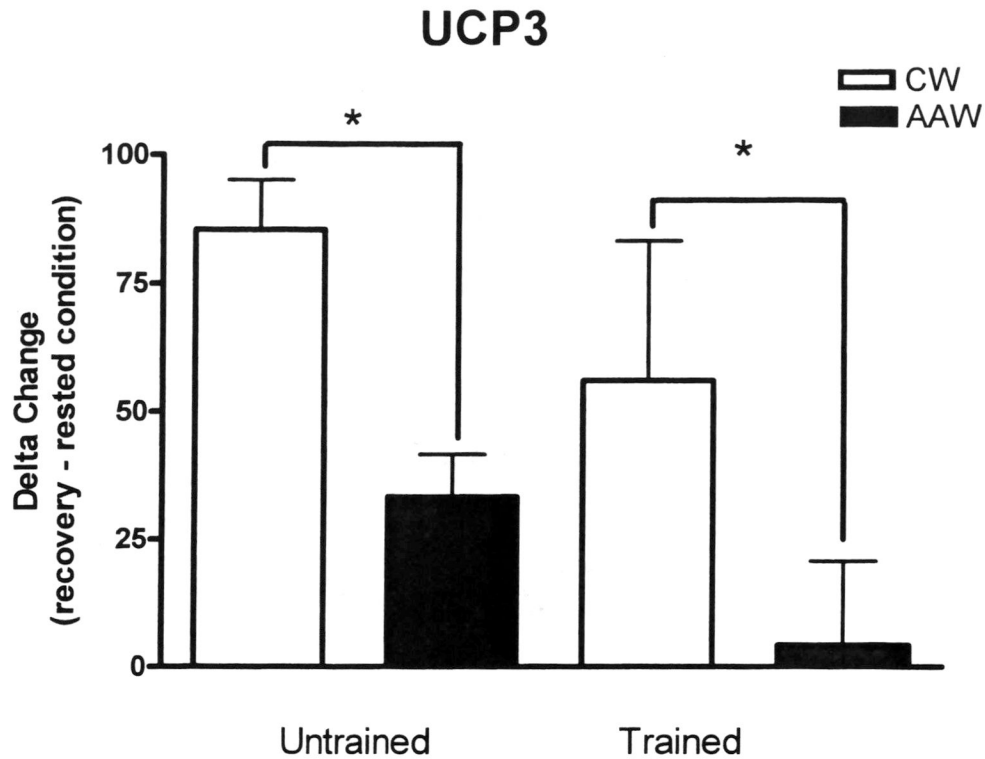

Figure 10: Racial comparisons on the delta change in skeletal muscle gene expression of uncoupling protein 3 (UCP3) in the untrained and trained state. The subjects consisted of 7 obese (BMI>30kg/m^2) Caucasian (CW) and 6 obese African-American women (AAW). Exercise consisted of 60 min of cycling on a Monark ergometer at 75% VO_2 peak. In both the untrained and trained condition, vastus lateralis muscle biopsies were obtained in the fasted (10 h) state immediately before (rested condition) and 4 h after acute exercise (recovery condition). Quantitative real time PCR was used to assess the expression of UCP3. Original data (arbitrary units/GADPH) was transformed as the qRT-PCR recovery value minus the qRT-PCR value in the rested, fasted condition and then analyzed using a 2 (training status) X 2 (race) repeated measures ANOVA. Values are mean ± S.E.M. * = significant main effect for race; P = 0.0252. *Overall Conclusion – The increase in UCP3 gene expression in recovery from exercise was significantly less in AAW vs. CW in both the untrained and trained condition (main effect for race, P=0.025). The main effect for training (P=0.08) and the interaction of race X training status was not statistically significant.*