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Exercise to the Rescue: An Analysis of Altered Metabolic Gene Regulation Post-exercise in Lean and Obese Individuals

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EXERCISE TO THE RESCUE: AN ANALYSIS OF ALTERED METABOLIC GENE

REGULATION POST-EXERCISE IN LEAN AND OBESE INDIVIDUALS

A Capstone Experience/Thesis Project

Presented in Partial Fulfillment of the Requirements for

the Degree Bachelor of Science with

Honors College Graduate Distinction at Western Kentucky University

By:

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*****

Western Kentucky University
2016

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ABSTRACT

The skeletal muscle of obese individuals exhibits a depressed ability to metabolize fats. Exercise training is thought to rescue this dampened ability to metabolize fats; mediated by a coordinated increase in the expression of a network of genes that regulate metabolism and fuel utilization. The purpose of this study is to determine the exercise-induced regulation of metabolically important genes in lean and obese individuals. Muscle biopsies (one pre-exercise/baseline and one immediately post-exercise) were obtained from 4 lean (BF% 24.4 ± 5.5; 23.5 yrs ± 1.9) and 13 obese (BF% 39.7 ± 2.4; 26.1 yrs ± 2.3), age-matched, relatively young subjects, free from overt disease, non-smokers, and not taking medications known to alter metabolism. RNA was isolated, quantified, reverse transcribed into cDNA, and evaluated using RT-PCR. The pre-exercise mRNA content of pyruvate dehydrogenase kinase 4 (*PDK4*) was significantly higher in the obese compared to the lean (P=0.04) and the mRNA content of peroxisome proliferator-activated receptor alpha (*PPARα*) was significantly lower (P=0.03). There were no differences in the mRNA content post-exercise. This suggests exercise tends to improve the expression of important metabolic genes in the skeletal muscle of the obese.

Keywords: skeletal muscle, metabolism, exercise, PPARs, PDK4, obese

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CHAPTER 1

INTRODUCTION

Obesity: Definition and Prevalence

In the United States, approximately 35 percent of adults and 17 percent of

children are considered obese—defined as a body mass index (BMI) greater than 30

kg/m^2 (Ogden, Carroll, Kit, & Flegal, 2014; Smith & Smith, 2016). Obesity is referred to

as an imbalance of caloric intake and outflow thought to be the result of a combination of

environmental, economic, genetic, and individual factors (Smith & Smith, 2016). As a

result, research interests have focused on interventions, such as change in diet or physical

activity, to alter these factors across a population (Flegal KM, Carroll MD, Ogden CL, &

Johnson CL, 2002).

In a clinical environment, obesity denotes excess adiposity rather than excess

weight (Krebs, Jacobson, & American Academy of Pediatrics Committee on Nutrition,

2003). Visceral adipose tissue is known to be strongly correlated with increased risk of

cardiovascular disease (Bouchi et al., 2016; Fox et al., 2007). However, body

composition is predominantly defined by BMI, which utilizes body weight (usually in

kilograms) and height (usually in meters) to quantify an individual's body composition

and place them into categories of obese and lean based on age and sex (Etchison et al.,

2011). BMI only takes into consideration relative body weight and does not consider

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overall body composition such as body fat or muscle mass (Etchison et al., 2011).

Therefore, a more accurate method of defining body composition is by assessing body fat

percentage. Using dual energy X-ray absorptiometry (DXA), body composition is

determined by measuring an individual's fat distribution based on density as well as

overall and regional body fat estimates (Pierce, 2014; Yang & Chang, 2016).

Obesity and Metabolic Health

Obesity tends to be strongly correlated to the development of insulin resistance,

metabolic syndrome, type 2 diabetes mellitus, and hypertension (Flegal KM et al., 2002).

Individuals who are severely obese tend to show altered fatty acid oxidation (FAO) levels

during fasting and decreased metabolic flexibility in response to insulin in skeletal

muscle tissue (Chomentowski, Coen, Radiková, Goodpaster, & Toledo, 2011).

Metabolic flexibility is a term used to refer to an organism's ability to alter energy

oxidation to energy availability (Galgani, Moro, & Ravussin, 2008). More specifically it

refers to a switch from increased lipid oxidation and high uptake of fatty acids during

fasting to decreased lipid oxidation and high uptake of glucose in the presence of insulin

(Kelley & Mandarino, 2000). Obese individuals with altered metabolic flexibility also

tend to have increased insulin resistance, although the physiology behind this correlation

is unclear (Chomentowski et al., 2011).

The mitochondria serves as the source of oxidative energy in eukaryotic cells

(Heilbronn, Gan, Turner, Campbell, & Chisholm, 2007). The numerous mitochondria in

human skeletal muscle allow for ample fatty acid oxidation (Chomentowski et al., 2011).

However, the skeletal muscle of obese individuals tends to demonstrate a diminished

mitochondrial capacity (Chomentowski et al., 2011). In order to understand this diminished capacity, research is concentrating on mitochondrial biogenesis (Heilbronn et al., 2007). Mitochondrial biogenesis, which is the process of developing new mitochondria in the cells, is mediated by both nuclear and mitochondrial encoded genomes (Heilbronn et al., 2007). The genomes operate in coordination with one another to produce numerous proteins involved in the uptake, transport, and oxidation of fatty acids not only during rest, but also during muscular activity (Civitarese, Hesselink, Russell, Ravussin, & Schrauwen, 2005).

Exercise and Metabolic Health

Research has shown the positive effects exercise has on the entire human body in terms of metabolism and energy expenditure (Catoire et al., 2012). Exercise has been shown to reduce cardiovascular disease, type 2 diabetes mellitus, and metabolic syndrome (Gaesser, 2007; Kodama et al., 2009; LaMonte, Blair, & Church, 2005). In the skeletal muscle, specifically, exercise increases cellular demands for oxygen, ATP, glucose, and fatty acids (Catoire et al., 2012). This is accompanied by changes in enzymatic activity and an increased capacity for FAO as a result of changes in gene expression (Catoire et al., 2012; Tunstall et al., 2002). Aerobic exercise training is known to alter expression of mRNA coding for important metabolic proteins and increase mitochondrial enzyme activity as well as increase expression of nuclear-encoded genes responsible for regulation of mitochondrial transcription in lean individuals (Egan, O'Connor, Zierath, & O'Gorman, 2013; Heilbronn et al., 2007). Genes demonstrating increased expression include peroxisome proliferator-activated receptor gamma

coactivator-1 alpha (*PGC-1α*) and mitochondrial transcription factor A (*TFAM*)

(Heilbronn et al., 2007).

However, the overall molecular mechanisms are complex and much is still

unknown in regards to the transcription and expression of many of the genes encoding for

proteins involved in increased FAO post-exercise training (Civitarese et al., 2005;

Tunstall et al., 2002). The specific effects of exercise training in obese individuals are

still under investigation. Researchers are working to determine the extent to which

exercise can reverse metabolic inflexibility and insulin resistance in the skeletal muscle

of these obese individuals (Heilbronn et al., 2007).

Critical Metabolic Genes

As mentioned above, *PGC-1α* is one of the critical genes involved in

mitochondrial activity (Heilbronn et al., 2007). This gene is expressed in a variety of

human tissue types including brown adipose tissue and skeletal muscle, both of which are

high in mitochondrial density (J. Lin et al., 2002). Multiple studies have found *PGC-1α*

to play a role in conditions such as obesity, diabetes mellitus, neuropathy, and

cardiomyopathy (J. D. Lin, Handschin, & Spiegelman, 2005).

Specifically, *PGC-1α* is a transcriptional coactivator vital to the coordination of

mitochondria biogenesis (Little, Safdar, Cermak, Tarnopolsky, & Gibala, 2010).

Research has shown that exercise tends to cause a temporary increase in both *PGC-1α*

transcription and mRNA expression in human skeletal muscle up to two hours post-

exercise (Henriette Pilegaard, Saltin, & Neufer, 2003). Studies have demonstrated that

activation of *PGC-1α* appears to facilitate the initial increase in mitochondrial production

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in response to exercise, while an increase in *PGC-1α* protein production aids in sustaining the production process (Wright et al., 2007). *PGC-1α* works by interacting with transcription factors bound to the promoter regions of target genes rather than binding DNA directly (Henriette Pilegaard et al., 2003). Once activated in the cytosol of the cell, *PGC-1α* moves to the nuclear membrane where it can interact with nuclear respiratory factors, estrogen-related receptor alpha, and myocyte enhancer factor 2 (J. D. Lin et al., 2005; Little et al., 2010).
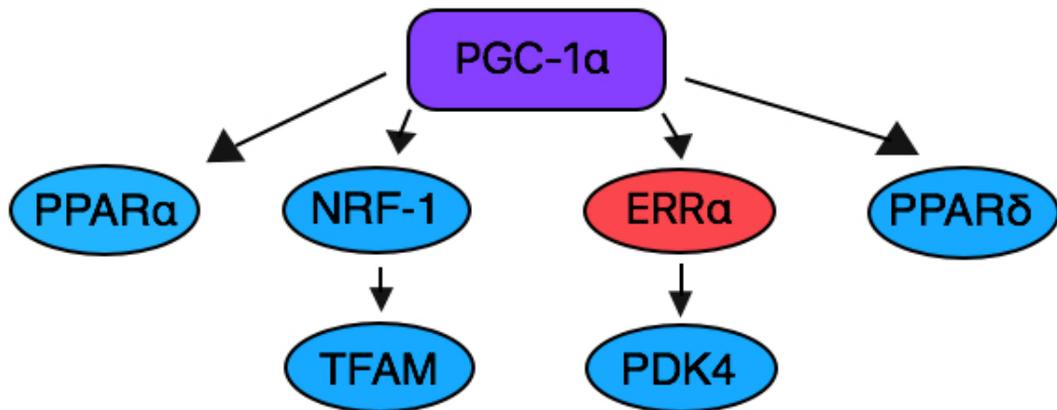
Figure 1: *PGC-1α* transcriptional coactivation. This figure illustrates the interactions of the proteins that result from transcription of the genes of interest. These proteins coactivate mitochondrial DNA transcription.

The induction of nuclear respiratory factor 1 (*NRF-1*) gene expression by *PGC-1α* is believed to play a role in activation of mitochondrial biogenesis and cellular respiration (Ventura-Clapier, Garnier, & Veksler, 2008). *NRF-1* protein functions primarily as a transcription regulator mediating nuclear and mitochondrial interactions by binding directly to mitochondrial DNA (mtDNA) (Baar, 2004; Scarpulla, 2002). A study conducted by Patti et al. (2003) demonstrated a correlation between decreased expression of *NRF-1*-dependent genes and type 2 diabetes mellitus associated insulin resistance.

This has created interest in further exploration of the gene's explicit function. In rat skeletal muscle, *NRF-1* mRNA expression was shown to increase following an acute bout of exercise (Murakami, Shimomura, Yoshimura, Sokabe, & Fujitsuka, 1998). Combined with the knowledge that exercise training increases the respiratory capacity of human skeletal muscle, these findings make further investigation of *NRF-1* expression advantageous (Baar, 2004).

Another downstream target of *PGC-1α* is mitochondrial transcription factor A (*TFAM*). The gene is encoded in the nucleus and the resultant protein is transported to the mitochondria to carry out its function (Gordon, Rungi, Inagaki, & Hood, 2001). This protein binds to mtDNA and plays a role in its protection and stabilization (Ichiseki et al., 2015). *TFAM* binds to enhancers along the mtDNA strand that are upstream of promoter sites (Kelly & Scarpulla, 2004). Expression of *TFAM* is regulated by *PGC-1α* via *NRF-1* and *NRF-2* (Finck & Kelly, 2006). In one study conducted by Short et al. (2003), abundance of mRNA encoding *PGC-1α*, *NRF-1*, and *TFAM* was shown to increase in response to exercise by 55, 15, and 85%, respectively, indicating these genes are prominent regulators of FAO in response to exercise training.

Two other peroxisome proliferator-activated receptors, *PPARα* and *PPARδ*, also play a role in FAO (Luquet et al., 2004). *PPAR*s are activated by lipid ligands such as prostaglandins and eicosanoids (Vega, Huss, & Kelly, 2000; Wagner & Wagner, 2010). Once activated, *PPARα* binds with *PGC-1α* to regulate transcription of enzymes involved in both FAO and the citric acid cycle including acyl coenzyme A (acyl-CoA) dehydrogenase (Baar, 2004). *PPARα* is found in high levels in tissues with great

oxidative energy demands such as muscle and brown adipose tissues while *PPARδ* is expressed abundantly in the skeletal muscle (Manio, Inoue, Fujitani, Matsumura, & Fushiki, 2016; Vega et al., 2000). Less is known about the function of *PPARδ*, also known as *PPARβ*, which is a ubiquitously expressed protein (Gilde et al., 2003). Researchers have demonstrated a positive correlation between *PPARδ* expression and increased FAO along with glucose uptake leading many to believe it could play a role in the management of metabolic disorders (Manio et al., 2016).

Another downstream target of *PGC-1α* is pyruvate dehydrogenase kinase 4 (*PDK4*) (Wende, Huss, Schaeffer, Giguère, & Kelly, 2005). *PDK4* is activated when *PGC-1α* binds estrogen-related receptor (*ERRα*) (Wende et al., 2005). *PDK4* is a mitochondrial protein that upregulates FAO by inhibiting the pyruvate dehydrogenase complex which increases glucose oxidation (Leem & Lee, 2016). In a study conducted by Pilegaard, Ordway, Saltin, & Neufer (2000), transcription of the *PDK4* gene was shown to increase in response to exercise demonstrating a shift from glycolysis to FAO following exercise training. This information makes this a gene of interest in further understanding metabolic flexibility in obese individuals.

In summary, our research has demonstrated that obesity is a growing health concern that tends to be correlated to the development of insulin resistance, metabolic syndrome, type 2 diabetes mellitus, and hypertension (Flegal KM et al., 2002). Obese individuals tend to show decreased fatty acid oxidation (FAO) during fasting and decreased metabolic flexibility in skeletal muscle tissue (Chomentowski et al., 2011). However, research has shown the positive effects exercise has on metabolism and energy

expenditure, specifically the effects of aerobic exercise training on mRNA expression and increased mitochondrial enzyme activity as well as increased expression of nuclear-encoded genes responsible for regulation of mitochondrial transcription in lean individuals (Catoire et al., 2012; Egan et al., 2013; Heilbronn et al., 2007). However, much is still unknown about the overall, complex mechanisms in regards to the transcription and expression of many of the genes encoding for proteins involved in increased FAO post-exercise training (Civitarese et al., 2005; Tunstall et al., 2002). The purpose of the present study is to determine if an acute bout of moderate-intensity exercise could favorably impact the expression of metabolically important genes in skeletal muscle from obese individuals.

CHAPTER 2

METHODS

This study involved the processing of samples previously collected at East

Caroline University (ECU) following approval by the ECU University and Medical

Center Institutional Review Board. Briefly, lean and obese subjects were briefed on all

study procedures and signed an informed consent prior to participation. Subjects

underwent a general health screening (BMI assessment, resting blood pressure and heart

rate, etc.) and completed a brief medical history questionnaire. Biological specimens

were collected at East Carolina University and skeletal muscle samples were processed at

Western Kentucky University (WKU) as part of this thesis project, upon receiving

approval from the WKU Biosafety Committee.

Participants wore a heart rate monitor and exercised at 60% of their heart rate reserve (HRR), which approximated 60% of their VO2max, for 60 minutes on a Monark cycle ergometer.

Muscle biopsies from the *vastus lateralis* were obtained. Approximately 100-150mg of skeletal muscle was obtained from the subjects. Two biopsies were performed on each subject:
1) pre-exercise/baseline and
2) immediately post-exercise.

Total RNA was isolated and quantified.
Quantification was done using a Bioanalyzer, RNA samples were also examined for quality.

Quality RNA was then reverse transcribed into cDNA.

RNA
Primer
Reverse Transcription
cDNA
PCR
Amplified DNA
Amplification
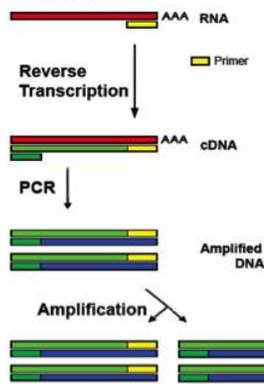
RT-PCR to assess gene expression. mRNA content was calculated using the comparative C_T method with a multiplexed endogenous control (B2M).

Figure 2: Study Workflow. This figure illustrates the overall methodology used to conduct the experiment.

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Subjects

Subject demographic and physical characteristics according to categorization by BMI are provided in Table 1. Subjects included 8 lean (BMI 23.1 kg/m^2 ± 0.7; 23.8 yrs ± 1.3) and 9 obese (34.2 kg/m^2 ± 1.1; 27.0 yrs ± 3.2) adult humans. According to Pierce et al. (2014), subjects were excluded if they did not fit within preferred BMI categories or exercised more than 3 days per week. Participants included both men and women, who were age-matched, relatively young, non-smokers that were not taking any medication known to alter their metabolism or pregnant (Pierce, 2014).

Participants were also categorized according to body fat percentage (BF%) using DXA values. When subjects were categorized as lean versus obese using BF%, 4 were classified as lean (BF% 24.4 ± 5.5; 23.5 yrs ± 1.9) and 13 were classified as obese (BF% 39.7 ± 2.4; 26.1 yrs ± 2.3). Participant demographics and anthropometric data are presented in Table 2 based on categorization by BF%.

Design

Briefly, subjects had height measured and weight assessed along with a dual-energy X-ray absorptiometry (DXA) scan (Prodigy, GE Lunar Corp., Madison, WI) prior to the pre-exercise muscle biopsy (Pierce, 2014). Following the pre-exercise muscle biopsy, participants wore a heart rate monitor and exercised at 60% of their heart rate reserve (HRR), which approximated 60% of their VO$_2$ max, for 60 minutes on a Monark Ergomedic 828E cycle ergometer (See Figure 2) (Monark Exercise, AB, Sweden) (Pierce, 2014). Resting serum levels of glucose and insulin were analyzed for basic blood chemistries (Pierce, 2014).

Muscle Biopsies

Approximately 100-150mg of skeletal muscle was obtained from the vastus lateralis of subjects by a percutaneous muscle biopsy (See Figure 2). A 30-45mg sample was immediately frozen in liquid nitrogen and stored at -80°C. An additional 30-45mg sample was minced in RNAlater and also stored at -80°C. Two muscle biopsies were performed on each subject: 1) pre-exercise/baseline and 2) immediately post-exercise.

RNA Isolation and Analysis

RNA was isolated for muscle samples using TRIzol® Reagent according to the manufacturer's protocol. Quantification of RNA samples was first determined using a NanoDrop Spectrophotometer. 2μL of each sample was run in duplicate and a mean value was calculated for concentration in ng/μL. The RNA samples were then quantified again and also qualified using the Agilent 2100 Bioanalyzer and RNA 6000 Nano Assay Kit (See Figure 2). The RNA Nano Chip was loaded with 1μL of each sample in duplicate along with 5μL RNA Nano Marker. In addition, each chip contained two wells of 9μL RNA Nano Gel-Dye Mix and 1μL RNA ladder. A mean concentration value was calculated for concentration in ng/μL. RNA quality was measured using a mean RNA Integrity Number (RIN).

RNA samples were reverse transcribed into cDNA using a High Capacity cDNA Reverse Transcription Kit (See Figure 2). 2μL of buffer, 0.80μL of dNTP mix (100mM), 2μL of random primers, 1μL of MultiScribe Reverse Transcriptase, and Nuclease-free H_2O were added to the RNA samples for the reaction. After being briefly centrifuged,

samples were placed in a thermal cycler under the following conditions: 10 minutes at 25°C, then 120 minutes at 37°C, then 5 minutes 85°C, and infinite hold at 4°C.

Real time polymerase chain reaction (RT-PCR) was used to evaluate the RNA expression of *PGC-1α* and its five downstream targets (*PPARδ, PPARα, NRF-1, TFAM, PDK4*). Optimization of the various genes was completed to determine optimal concentrations for amplification to occur. The cDNA samples were multiplexed using the endogenous control B2M. Samples were run in triplicate using TaqMan® Universal PCR Master Mix at a 2X concentration, gene expression assays at 20X concentrations, and Nuclease-free H_2O. PCR was run using the following cycle profile: 95°C for 10 minutes $\times$ 1 for denaturation, 95°C for 15 seconds $\times$ 40 for annealing, and 60°C for 1 minute $\times$ 1 for extension. The threshold cycle (C_t value) was recorded for each gene of interest for each sample (See Figure 2).

Statistical Analysis

Analysis of data between lean and obese demographics and metabolic gene expression was assessed using a T-test for independent samples using SPSS statistical software. Data is reported as mean $\pm$ SD and α-level was set a $p \leq 0.05$.

CHAPTER 3

RESULTS

Participant's Weight Status Classified by BMI

Subjects were categorized by BMI (lean: 23.1 kg/m^2 ± 0.7 versus obese 34.2

kg/m^2 ± 1.1, p<0.01). Anthropometric data are presented in Table 1. Difference in age

was insignificant (p=0.37). Differences in DXA (p=0.03), insulin levels (p<0.01), and

HOMA-IR (p<0.01) were all significant while difference in glucose levels (p=0.18) was

insignificant.

Table 1: Subject Demographics Based on Body Mass Index (BMI)

	Lean (n=8)	Obese (n=9)		p-value
Male/Female	4/4	6/3		n/a
Age (years)	23.8 ± 1.3	27.0 ± 3.2		0.37
BMI (kg/m2)	23.1 ± 0.7	34.2 ± 1.1	*	< 0.01
DXA (Region % Fat)	30 ± 3.8	41.4 ± 2.8	*	0.03
Glucose (mg/dL)	84.3 ± 2.3	89.1 ± 2.5		0.18
Insulin (uIU/mL)	6.8 ± 1.1	15.9 ± 1.4	*	< 0.01
HOMA-IR	1.4 ± 0.2	3.5 ± 0.4	*	< 0.01

Values are mean ± SD; *P<0.05 vs Lean group

Table 1: Basic demographic information and anthropometric data for study participants when categorized by BMI.

Participant's Weight Status Classified by BF%

Subjects were also categorized by body fat percentage (BF%) based on DXA-

assessed body composition (lean 24.4 ± 5.5 versus obese 39.7 ± 2.4; p=0.01). Difference

is age was insignificant (p=0.56) and there was a good distribution of males and females

(lean: 2/2 and obese: 8/5) in each group. However, the group numbers (lean: n=4 and

obese: n=13) were not equally distributed. BMI (p<0.01), Android (p=0.02), A/G ratio

(p=0.03), fasting insulin (p=0.03), and HOMA-IR (p=0.04) were significantly higher in

the obese subjects.

Table 2: Subject Demographics Based on Body Fat Percentage (BF%)				
	Lean (n=4)	Obese (n=13)		p-value
Male/Female	2/2	8/5		n/a
Age (years)	23.5 ± 1.9	26.1 ± 2.3		0.56
BMI (kg/m2)	22.0 ± 1.1	31.2 ± 1.5	*	< 0.01
DXA (Region % Fat)	24.4 ± 5.5	39.6 ± 2.4	*	0.01
Android (% Fat)	28.2 ± 5.6	50.7 ± 1.7	*	0.02
Gynoid (% Fat)	33.0 ± 7.5	44.0 ± 3.4		0.15
A/G Ratio	0.9 ± 0.1	1.2 ± 0.1	*	0.03
Glucose (mg/dL)	84.5 ± 4.5	87.5 ± 1.9		0.57
Insulin (uIU/mL)	6.2 ± 1.4	13.3 ± 1.5	*	0.03
HOMA-IR	1.3 ± 0.3	2.9 ± 0.4	*	0.04

Values are mean ± SD; *P<0.05 vs Lean group

Table 2: Subject demographics and anthropometric data based on categorization by body fat percentage (BF%). HOMA-IR refers to a homeostatic model assessment for insulin resistance determined using glucose and insulin values.

mRNA Analysis

To investigate potential differences in metabolic gene expression between lean

and obese individuals, that likely influence resting metabolism, mRNA content was

compared at baseline. Differences in baseline (pre-exercise) mRNA content between

lean and obese participants was assessed in two ways—1) based on BMI weight status

classification and 2) based on BF% weight status classifications. There were no

differences in the mRNA content of the genes of interest when groups were determined

by BF%, there was a difference in the pre-exercise mRNA content of peroxisome

proliferator-activated receptor alpha (*PPARα*), which was significantly lower (p=0.03) in

the obese compared to the lean (See Table 3 and Figure 3). Pre-exercise mRNA content

of pyruvate dehydrogenase kinase 4 (*PDK4*) was significantly higher in the obese

compared to the lean (p=0.04) (See Table 3 and Figure 4). All other mRNA content for

the other genes showed no difference pre-exercise. However, an acute bout of exercise

resulted in no differences in the expression of these genes (*PPARα, PDK4*) between lean

and obese individuals. There were no significant increases in mRNA content observed

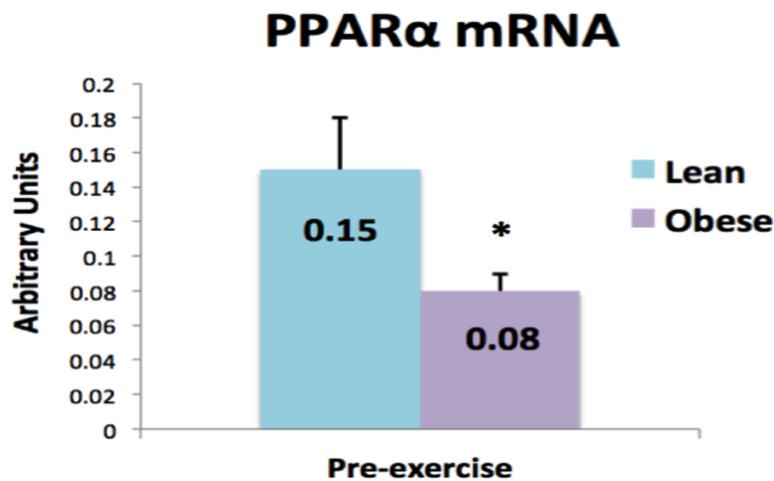immediately post-exercise for any of the genes analyzed.

Figure 3: Pre-exercise *PPARα* gene expression in lean and obese individuals categorized by BF%. Expression was significantly higher in lean individuals as compared to obese individuals.
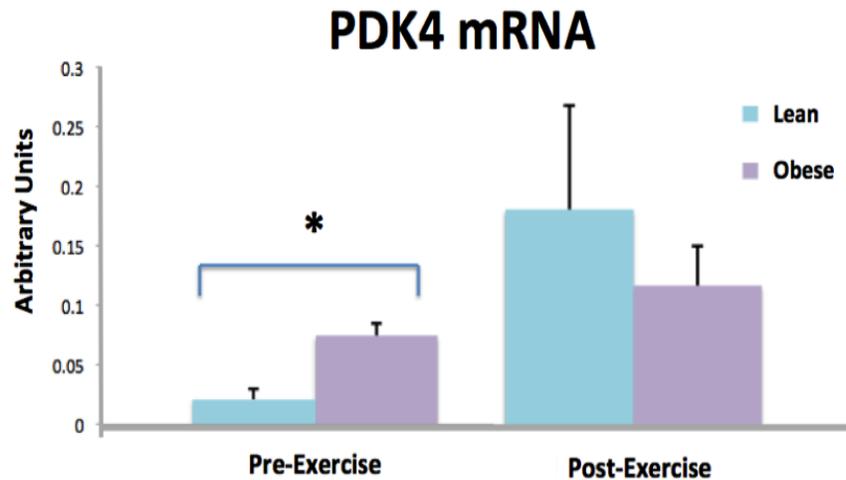
Figure 4: Pre- and post-exercise *PDK4* gene expression in lean and obese individuals categorized by BF%. Pre-exercise expression was significantly higher in obese individuals as compared to lean individuals. No significant difference was seen post-exercise.

Table 3: Gene Expression Pre- and Post-Exercise

	Lean (n=4)	Obese (n=13)		p-value
Male/Female	2/2	8/5		n/a
Age (years)	23.5 ± 1.9	26.1 ± 2.3		0.56
DXA (Region % Fat)	24.4 ± 5.5	39.6 ± 2.4	*	0.01
TFAM PreEx	0.21 ± 0.01	0.11 ± 0.03		0.18
TFAM PostEx	0.11 ± 0.03	0.12 ± 0.03		0.82
PDK4 PreEx	0.02 ± 0.01	0.07 ± 0.01	*	0.04
PDK4 PostEx	0.18 ± 0.09	0.12 ± 0.03		0.41
PGC-1α PreEx	0.07 ± 0.03	0.09 ± 0.02		0.71
PGC-1α PostEx	0.11 ± 0.05	0.09 ± 0.03		0.73
NRF-1 PreEx	0.10 ± 0.03	0.10 ± 0.02		0.95
NRF-1 PostEx	0.10 ± 0.02	0.14 ± 0.03		0.50
PPARδ PreEx	0.10 ± 0.02	0.11 ± 0.02		1.00
PPARδ PostEx	0.08 ± 0.03	0.21 ± 0.08		0.34
PPARα PreEx	0.15 ± 0.03	0.08 ± 0.01	*	0.03
PPARα PostEx	0.14 ± 0.05	0.11 ± 0.02		0.60

Values are mean ± SD; *P<0.05 vs Lean group

Table 3: Difference in gene expression based on results from RT-PCR of reverse-transcribed cDNA from RNA of subjects' muscle biopsies. Significant differences were found in *PDK4* expression pre-exercise and in *PPARα* expression pre-exercise.

CHAPTER 4

DISCUSSION

In the current study, exercise-induced differences in metabolic gene expression were examined in lean and obese individuals. The primary findings of this study were the following: 1) pre-exercise gene expression of pyruvate dehydrogenase kinase 4 (*PDK4*) was higher in obese compared to lean subjects and 2) pre-exercise gene expression of peroxisome proliferator-activated receptor alpha (*PPARα*) was lower in obese compared to lean subjects. These findings were observed when subjects were categorized by body fat percentage (BF%). No differences in metabolic gene expression were observed post-exercise. When subjects were categorized by body mass index (BMI), no difference in metabolic gene expression was observed pre- or post-exercise.

The observed higher levels of gene expression of *PDK4* in obese subjects corresponded with findings from other studies. *PDK4* is an enzyme known to inhibit the pyruvate dehydrogenase complex that stimulates oxidation of glucose for energy use (Leem & Lee, 2016). *PDK4* thus inhibits the breakdown of stored glucose. Lower levels of insulin along with increased levels of fatty acids promote *PDK4* expression (Huang, Wu, Bowker-Kinley, & Harris, 2002). This has been demonstrated in studies that show increased expression of *PDK4* in individuals with diabetes or insulin-resistance (Kwon, Huang, Unterman, & Harris, 2004; Rosa et al., 2003). Another study demonstrated

upregulation of *PDK4* following a high fat diet (Constantin-Teodosiu, Constantin, Stephens, Laithwaite, & Greenhaff, 2012). However, another study in transgenic mice in which the *PDK4* gene had been deleted showed improved glucose metabolism (Tao, Xiong, Harris, White, & Dong, 2013). In addition, a study conducted by Kwon et al. (2004) demonstrated that insulin led to a decreased expression of *PDK4* under normal physiological conditions allowing for utilization of stored glucose for energy. These studies in addition to the current study lead to the conclusion that the higher level of baseline expression of *PDK4* in obese individuals is related to insulin resistance. Although fasting insulin levels were significantly higher in the obese subjects (13.3 ± 1.5 µIU/mL vs. 6.2 ± 1.4 µIU/mL; $p=0.03$), it did not result in decreased *PDK4* expression.

The decreased levels of *PPARα* gene expression pre-exercise in obese subjects from the current study correspond to findings of other studies. *PPARα* is expressed most abundantly in tissues with high oxidative energy demands due to its role as a co-regulator of transcription of enzymes involved in FAO (Baar, 2004; Manio et al., 2016; Vega et al., 2000). In a study conducted by Costet et al. (1998), absence of this gene was shown to be highly correlated to obesity. Additionally, studies have been conducted that show decreased expression of *PPARα* to be positively correlated with reduced insulin sensitivity, diabetic conditions, and adiposity (Chou et al., 2002; Fu et al., 2003; Guerre-Millo et al., 2000; Kim et al., 2003). These findings in addition to the current study seem to show a positive correlation between *PPARα* gene expression and improved metabolic health.

The insignificant differences observed in all other genes pre- and post-exercise could be the result of several factors. This study was limited by the small sample size (n=17) and by the uneven distribution of participants when categorized by BF% (lean: n=4; obese: n=13). Another limit to the study was the time of muscle biopsy collection. In order to maintain minimal discomfort for participants, only one biological specimen was collected per subject immediately post-exercise. Collecting multiple muscle biopsies at varying post-exercise time intervals would have allowed for a more comprehensive examination of metabolic gene expression post-exercise.

In conclusion, the elevated levels of *PDK4* in obese individuals pre-exercise are consistent with findings in other studies that show elevated baseline expression with higher insulin levels. The baseline differences (between lean and obese) in *PPARα* expression appear to be consistent with a compromised metabolic function in obese individuals that then seems to be abolished with an acute bout of exercise. These findings indicate even a relatively mild, acute exercise stimulus may be helpful in improving the metabolic health of obese individuals.

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