

Abstract

Regulation of Lipolysis By Perilipin: Influence of Obesity and Exercise Training

By: Emily Ann Johnson

June 2010

Director: Robert C. Hickner

Department of Exercise and Sport Science

Obesity is the result of excess energy storage due to an imbalance between energy storage and energy utilization. Excess energy is stored as triacylglycerol (TAG) in adipose tissue in various regions throughout the body. In order to reduce excess fat stores, one must increase the utilization of fat stores for energy. Lipolysis is the process where TAG are hydrolyzed to provide free fatty acids as an energy substrate to other organs during times of energy demand. Blunted catecholamine stimulated lipolysis has been well documented in obese adults. The purpose of this study was to evaluate the effects of obesity and exercise training on lipolysis and lipolytic protein expression in abdominal subcutaneous adipose tissue (SCAT) in men.

Lean endurance trained (n=10), lean sedentary (n=10), and obese sedentary (n=8) men were recruited for this study. Abdominal SCAT was obtained using needle aspiration. Western blots were used to determine the protein content of adipose triglyceride lipase (ATGL), comparative gene identification 58 (CGI-58), hormone sensitive lipase (HSL), and perilipin. Microdialysis was used to evaluate abdominal SCAT lipolysis *in vivo*. Two probes were inserted into abdominal SCAT. One probe served as a control (perfused with a Ringer solution) while the second probe was

perfused with isoproterenol, followed by a mixture of isoproterenol and phentolamine. Ethanol was added to the perfusates to measure local blood flow.

Unstimulated, resting lipolysis was higher in exercise trained compared to obese men. β -adrenergic stimulation increased lipolysis above baseline in exercise trained and sedentary men, but not in obese men. Blood flow was not different in exercise trained, sedentary, and obese men during any treatment. Perilipin protein content was lower in the sedentary compared to exercise trained men but was not different than obese men. There were no differences in ATGL, HSL, or CGI-58 protein content in any of the groups.

In response to 8 weeks of exercise training in the obese group, unstimulated, resting lipolysis did not change. β -adrenergic stimulated lipolysis did not stimulate lipolysis before exercise training but increased above baseline after exercise training. Lipolysis during a 60 minute acute exercise bout (30 minutes at 70% VO_{2max} and 30 minute of intervals (2 minutes at 85% VO_{2max} and 4 minutes at 50% VO_{2max})) was not different from baseline before training but was higher than baseline after exercise training. Adipose tissue blood flow was lower than baseline during an acute exercise bout after exercise training. There were no other differences in blood flow during any treatment. ATGL and perilipin protein content in subcutaneous SCAT increased in response to an acute exercise bout before exercise training. Resting ATGL protein content increased in response to 8 weeks of exercise training. Resting Perilipin protein content tended to be higher at rest after 8 weeks of exercise training.

Based on our results, exercise training increases perilipin protein content in abdominal subcutaneous adipose tissue of lean men, which appears to be a key

component for a reduced β -adrenergic responsiveness in sedentary compared to lean men. It also appears that acute exercise increases ATGL and perilipin in obese men. Eight weeks of exercise training increased ATGL and tended to increase perilipin in obese men. ATGL and perilipin are likely the key components to increasing β -adrenergic lipolysis in abdominal subcutaneous adipose tissue of obese men after exercise training.

Regulation of Lipolysis By Perilipin: influence of Obesity and Exercise Training

A Dissertation

Presented to

The Faculty of the Department of

Exercise and Sport Science

East Carolina University

In Partial Fulfillment

Of the Requirements for the Degree

Doctor of Philosophy in Bioenergetics

By

Emily Ann Johnson

June 29.2010

©Copyright 2010

Emily Ann Johnson

Regulation of Lipolysis By Perilipin: Influence of Obesity and Exercise Training

By:

Emily Ann Johnson

Approved By:

Director of Dissertation

Robert C. Hickner, PhD

Committee Member

Timothy P. Gavin, PhD

Committee Member

Robert M. Lust, PhD

Committee Member

Jacques Robidoux, PhD

Dean of the College of Health
and Human Performance

Glen G. Gilbert, PhD

Dean of the Graduate School

Paul Gemperline, PhD

Chair of the Department of Exercise
and Sport Science

Stacey R. Altman, JD

Table of Contents

LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
ABBREVIATIONS.....	x
CHAPTER I: Introduction.....	1
CHAPTER II: The effects of obesity and exercise training status on lipolysis and lipolytic proteins	
Abstract.....	11
Introduction.....	17
Materials and Methods.....	25
Results.....	31
Discussion.....	33
CHAPTER III: Exercise increases catecholamine stimulated lipolysis, ATGL, and perilipin.	
Abstract.....	38
Introduction.....	40
Materials and Methods.....	43
Results.....	49
Discussion.....	61
CHAPTER IV: Summary and Conclusions.....	67
CHAPTER V: References.....	70
APPENDIX A: IRB Approval.....	78

List of Tables

Table 2.1 Subject characteristics in lean exercise trained, lean sedentary, and obese sedentary men.....	24
Table 2.2 Blood chemistry values in lean exercise trained, lean sedentary and obese sedentary men.....	25
Table 2.3 Nutritional intake of exercise trained, lean sedentary, and obese sedentary men.....	26
Table 2.4 Correlations between lipolytic protein content and lipolysis in lean exercise trained, lean sedentary, and obese sedentary men.....	27
Table 2.5 Correlations between lipolytic protein content in lean exercise trained, lean sedentary, and obese sedentary men.....	28
Table 3.1 Subject characteristics in obese men before and after 8 weeks of exercise training.....	53
Table 3.2 Blood chemistry values in obese men before and after 8 weeks of exercise training.....	54
Table 3.3 Nutritional intake in obese men before and after 8 weeks of exercise training.....	55

List of Figures

Figure 1.1 Schematic of lipolysis regulation.....	10
Figure 2.1 Dialysate glycerol concentrations in lean exercise trained, lean sedentary, and obese sedentary men.....	29
Figure 2.2 Ethanol outflow/inflow ratios in lean exercise trained, lean sedentary, and obese sedentary men.....	30
Figure 2.3 Adipose triglyceride protein levels in lean exercise trained, lean sedentary, and obese sedentary men.....	31
Figure 2.4 Comparative gene identification 58 protein levels in lean exercise trained, lead sedentary, and obese sedentary men.....	32
Figure 2.5 Hormone sensitive lipase protein levels in lean exercise trained, lean sedentary, and obese sedentary men.....	33
Figure 2.6 Perilipin protein levels in lean exercise trained, lean sedentary, and obese sedentary men.....	34
Figure 3.1 Dialysate glycerol concentrations in obese men before and after 8 weeks of exercise training.....	56
Figure 3.2 Ethanol outflow/inflow ratios in obese men before and after 8 weeks of exercise training.....	57
Figure 3.3 Adipose triglyceride protein levels in obese men before and after 8 weeks of exercise training.....	58
Figure 3.4 Comparative gene identification 58 protein levels in obese men before and after 8 weeks of exercise training.....	59
Figure 3.5 Hormone sensitive lipase protein levels in obese men before and after 8 weeks of exercise training.....	60
Figure 3.6 Perilipin protein levels in obese men before and after 8 weeks of exercise training.....	61

Abbreviations

Acute Exercise	ACEX
Adenosine Triphosphate	ATP
Adenylate Cyclase	AC
Adipose Triglyceride Lipase	ATGL
Analysis of Variance	ANOVA
Atrial Natriuretic Peptide	ANP
Baseline	BASE
Beta Adrenergic Receptors	β -AR
Body Mass Index	BMI
Carbohydrate	CHO
Comparative Gene Identification 58	CGI-58
Cyclic adenosine monophosphate	cAMP
Cyclic guanosine monophosphate	cGMP
Diacylglycerol	DAG
Ethanol Outflow:Inflow Ratio	EtOH O/I
Exercise Training	ET
Fat	FAT
Free Fatty Acids	FFA
Glucose	GLU
High Density Lipoprotein	HDL
Hormone Sensitive Lipase	HSL
Insulin	INS
Isoproterenol	ISO
Isoproterenol and Phentolamine	IP

Lean, Exercise Trained Men	ET
Lean, Sedentary Men	SED
Low Density Lipoprotein	LDL
Monoacylglycerol	MAG
Obese, sedentary men	OB
Protein	PRO
Protein Kinase A	PKA
Protein Kinase G	PKG
Subcutaneous Adipose Tissue	SCAT
Total Cholesterol	TC
Triacylglycerol	TAG
Triglycerides	TG

Chapter I

Introduction

Obesity is an ever growing concern in the United States. In a 2007-2008 survey, 31.6% of United States citizens were considered to be obese, defined as having a body mass index (BMI) greater than 30 kg/m². Obesity incidence among men has increased 4.7 percentage points since 1999-2000 (35). Obesity is the result of a number of factors such as genetics, physical inactivity, poor nutrition choices, compulsive eating patterns, and a stressful lifestyle (13). Obesity is a significant health risk, as it is associated with metabolic disorders such as Type 2 diabetes and insulin resistance as well as cardiovascular disorders such as hypertension and coronary artery disease.(26)

Obesity is the result of excess energy storage. Excess energy is stored as triacylglycerol (TAG) in adipose tissue in various regions throughout the body. However, subcutaneous adipose tissue (SCAT) is the largest reservoir of TAG. Excess energy storage is the result of a lipolytic imbalance, that is, energy is being stored at a greater rate than it is being utilized (22).

TAG supply free fatty acids (FFA) for energy generation in times of need such as during fasting and during exercise (14). FFA and glycerol are the products of lipolysis, or TAG hydrolysis. FFA released from adipocytes enter the circulation where they can be utilized by other organs to produce adenosine triphosphate (ATP) via β -oxidation. (2).

Lipolysis (See Figure 1.1)

Lipolysis is hormonally regulated by the catecholamines epinephrine and

norepinephrine, which are potent stimulators of lipolysis. Catecholamines stimulate lipolysis by binding to G_s -coupled β -adrenergic receptors (β -AR) located on the adipocyte membrane (1, 2, 26). β -adrenergic stimulation increases cyclic adenosine monophosphate (cAMP) which, in turn, activates protein kinase A (PKA). PKA phosphorylates perilipin and hormone sensitive lipase (HSL) (26).

Perilipin is a lipid droplet coating protein which binds comparative gene identification 58 (CGI-58) in the unstimulated state. Upon PKA phosphorylation, CGI-58 loses its affinity for perilipin and translocates to activate adipose triglyceride lipase (ATGL). ATGL demonstrates a high specificity for TAG and is the primary enzyme responsible for TAG hydrolysis. HSL shows some specificity for TAG but HSL has a ten-fold specificity for diacylglycerol (DAG). Monoacylglycerols (MAG) are hydrolyzed by monoglyceride lipase (MGL). FFA and glycerol are then released by the adipocyte into the interstitial space and circulation to be delivered to peripheral tissues (56).

Lipolysis is also stimulated via the atrial natriuretic peptide (ANP) pathway which acts independently of catecholamines. ANP binds to natriuretic peptide A which increases cyclic guanosine monophosphate (cGMP), thus activating protein kinase G (PKG). PKG phosphorylates HSL (26) and perilipin (32). ANP increases during exercise, allowing for lipolysis to increase in a catecholamine independent manner (26).

Lipolysis is inhibited by insulin and via the α -adrenergic pathway. Insulin a potent antilipolytic hormone that decreases lipolysis in times of energy storage such as in the fed or resting state. Insulin dephosphorylates HSL and also activates phosphodiesterase which results in lower cAMP levels. Catecholamines can bind to α -

adrenergic receptors which are coupled to G_i proteins causing a decrease in cAMP levels due to adenylate cyclase (AC) inhibition (22).

Lipolytic Proteins

ATGL is most highly expressed in brown and white adipose tissue but is also found in testis, cardiac, and skeletal muscle (51). ATGL has a high specificity for TAG and little to no specificity for DAG (28, 51). ATGL knockout mice exhibit increased adipose tissue stores and lipid droplet size (41) and reduced TAG hydrolase activity (39). ATGL knockout mice do not have an impaired basal lipolytic rate (16) but basal lipolysis appears to be reduced in human adipocytes under ATGL inhibition (28). β -adrenergic stimulated lipolysis is significantly lower in ATGL knockout mice than wild type mice (16). A reduction in β -adrenergic stimulated lipolysis indicates an impaired ability to release FFA for energy during times of need such as during exercise or fasting (21). Huijsman et al (2009) found that ATGL knockout mice have lower maximal running capacity and overall lower running endurance (20). Thus, ATGL is not only important to initiate TAG hydrolysis to maintain a lipolytic balance, but is crucial for energy production during exercise.

CGI-58 is primarily located on the lipid droplet membrane, though a small amount can be found in the cytosol. CGI-58 activates ATGL thus CGI-58 plays an essential role in the initiation of lipolysis (15, 55). A rare autosomal recessive disease in humans referred to as Chanarian-Dorman syndrome (CDS) is characterized by mutated CGI-58. Those with CDS have an excessive TAG accumulation. (55). CGI-58 is bound to unphosphorylated perilipin on the lipid droplet membrane (53). In those with CDS, the

interaction between perilipin and CGI-58 is disrupted, and therefore hinders CGI-58 association with the lipid droplet membrane. (55). Once perilipin is phosphorylated by PKA, CGI-58 dissociates from perilipin, translocates, and associates with ATGL to allow for activation (15). CGI-58 is not a lipase itself but it does improve the ATGL enzymatic activity. Without CGI-58, lipolysis is hindered while TAG content increases (54). CGI-58 is essential to the functionality of TAG hydrolysis.

HSL is a cytosolic protein that is directly phosphorylated by PKA, which results in activation of HSL and promotes HSL translocation to the lipid droplet. HSL translocation is directed by perilipin (51) and perilipin phosphorylation is required for HSL to translocate to the lipid droplet (31). HSL knockout mice do not show a reduction in TAG hydrolase activity or MAG accumulation (40). HSL knockout mice have reduced white adipose tissue stores despite no differences in intracellular TAG levels compared to wild type mice (18). However, DAG accumulates in white adipose tissue of HSL knockout mice, indicating that HSL has a high specificity for DAG (17). White adipose tissue incubated with a HSL inhibitor resulted in an accumulation of DAG and a reduction in basal and stimulated FFA release. Like ATGL, HSL is necessary to provide FFA for energy during aerobic exercise. HSL-null mice have a reduced capacity for aerobic exercise. HSL-null mice compensate for the lack of substrate provided by lipolysis by utilizing liver glycogen stores. HSL-null mice have reduced liver glycogen stores indicating a substrate shift from FFA to carbohydrates. This shift contributes to the reduced aerobic capacity in HSL-null mice (12).

Perilipin is a primary target for catecholamine signaling and is essential for ATGL, CGI-58, and HSL to completely and efficiently hydrolyze triglycerides. Though perilipin is required for stimulated lipolysis, perilipin does not possess any enzymatic activities. (52). Perilipin null mice are lean and have lower overall fat mass than wild type mice (30, 33). Perilipin null mice have a higher basal lipolytic activity in adipocytes than wild type mice which largely contributes to the lower fat mass and lower adipocyte size in perilipin null mice (45, 46). Perilipin null mice have an attenuated lipolytic response to isoproterenol stimulation. Stimulated lipolysis remains impaired when adipocytes are activated with forskolin, an AC agonist; this indicates that the deficiency is downstream of the β -adrenergic receptor. When cells were stimulated with isoproterenol, a nonspecific β -AR agonist, there was no increase in HSL associated with the lipid droplet in perilipin null mice where HSL is clearly associated with the lipid droplet in wild type mice (45) Thus, perilipin is required for HSL to associate with the lipid droplet in order for HSL to participate in lipolysis.

Obesity

Obese individuals have a reduced capacity for catecholamine stimulated lipolysis (24, 38). This phenomenon has been explained a number of ways. De Glisezinski et al. (1998) determined that the antilipolytic influence of α_2 -AR was the major contributing factor to decreased lipolysis in obese men (8). Reynisdottir et al. (1994) found that obese women have a reduced β_2 -AR binding capacity while β_1 -AR binding capacity was not different between obese and lean women (38). Langin et al. (2005) found that β_2 -AR density was similar in lean and obese preadipocytes but lower HSL in obese individuals is to blame as it is the rate limiting enzyme during stimulated lipolysis (28) since perilipin

is essential for HSL translocation to the lipid droplet surface (45). It is possible that the lipolytic defect observed in human obesity is the result of the interplay between perilipin and associated lipases.

Lower lipolytic protein contents have been observed in human obesity. ATGL (42) and HSL are lower in obese than lean men and women. Despite the elevated basal lipolysis in obese individuals, stimulated lipolysis in the obese was severely attenuated in mature adipocytes *in vitro* (28). Perilipin protein content has been shown to be much higher in the lean than the obese individuals (47). At present, little is known about the effects of obesity on CGI-58. CGI-58 mRNA has been shown to be similar in lean and obese SCAT (42). However, more work needs to be done to clarify the effects of obesity on CGI-58, particularly given what is known about Chanarian-Dorman syndrome. To date, no studies have evaluated all lipolytic proteins (ATGL, CGI-58, HSL, and perilipin) in human SCAT at one time.

Exercise Training

Not only are obese individuals at a disadvantage at rest due to stimulated lipolytic impairment, but they are at a disadvantage during exercise as well. Catecholamine stimulation of the β -adrenergic pathway is a primary source of FFA which provide a substrate for ATP production during exercise (56). Resistance to stimulated lipolysis creates a challenge for obese individuals to improve their lipolytic imbalance.

Aerobic exercise training improves β -AR stimulated lipolysis in overweight and obese individuals (8, 9, 43). Increased β -adrenergic responsiveness is crucial for

shifting the lipolytic imbalance in the obese in order to reduce adiposity and improved overall health. Though the connection between improved lipolytic responsiveness and exercise training is well-established, the mechanisms behind this occurrence are not. Specifically, the roles of lipolytic proteins are not clear.

Physical activity may impact perilipin content; however perilipin's response to exercise training is unclear. Perilipin protein content in SCAT depots in ET rats was reported to be similar to that of untrained rats; however, perilipin was higher in epididymal fat tissue after exercise training (36). Perilipin function plays a critical role in modulating lipolysis through regulation of lipolytic proteins. As physical activity and exercise training are used to maintain body fat balance and cardiovascular/metabolic health, the response of perilipin and associated proteins to these stimuli needs to be determined (11).

HSL null mice have a reduced exercise capacity simply due to a lack of FA mobilization. This demonstrates the importance of HSL during submaximal exercise (12). ET has been shown to increase HSL in rat SCAT (36) while HSL phosphorylation is up regulated during exercise in SCAT (49, 49, 50). Specifically, β -adrenergic stimulation results in an increase in phosphorylation of HSL Ser⁵⁶³ and Ser⁶⁶⁰, corresponding with an increase in SCAT lipolysis (36). However, HSL does not appear to change in response to exercise training in muscle (3).

ATGL and CGI-58 are essential for energy utilization and exercise capacity (5), yet few studies have examined the effects of exercise training on ATGL and CGI-58. In muscle, ATGL increases in response to exercise training while CGI-58 remains

unchanged (3). Research studies evaluating the effects of ATGL and CGI-58 in adipose are needed to clarify the role of these lipolytic proteins in β -AR stimulated lipolysis, the contribution of FFA to working muscles for fatty acid oxidation, and overall impact on adiposity.

Statement of the Problem

Obesity significantly contributes to the development of insulin resistance, Type 2 Diabetes, and cardiovascular disorders. The cause of obesity is a lipolytic imbalance, that is, excess energy is not being utilized for energy production at a rapid enough rate. Additionally, the ability to mobilize TAG is, despite no impairment in basal lipolysis in vitro, reduced under catecholamine stimulation which is a significant problem given that catecholamines are the greatest activator of lipolysis.

Lipolysis is carried out by a cascade involving perilipin, ATGL, CGI-58, and HSL. These proteins directly influence the rate at which TAG is hydrolyzed in humans. Evidence shows that obesity affects these lipolytic proteins and that these proteins may be largely responsible for the reduced ability to mobilize TAG in the obese and sedentary state. However, the effect of obesity and exercise training on lipolytic proteins is not clear since not studies to date have evaluated all lipolytic proteins (ATGL, CGI-58, HSL, and perilipin) in relation to obesity and exercise training in human SCAT.

Hypotheses

Resting and stimulated lipolysis, perilipin, and lipolytic proteins are lower in obese individuals but can be increased in response to exercise training.

Specific Aims

Our aim is to evaluate differences in lipolysis and lipolytic proteins in lean exercise trained men, lean sedentary men, and obese sedentary men. We also intend to evaluate lipolysis and lipolytic protein content in obese men before and at the end of eight weeks of endurance exercise training.

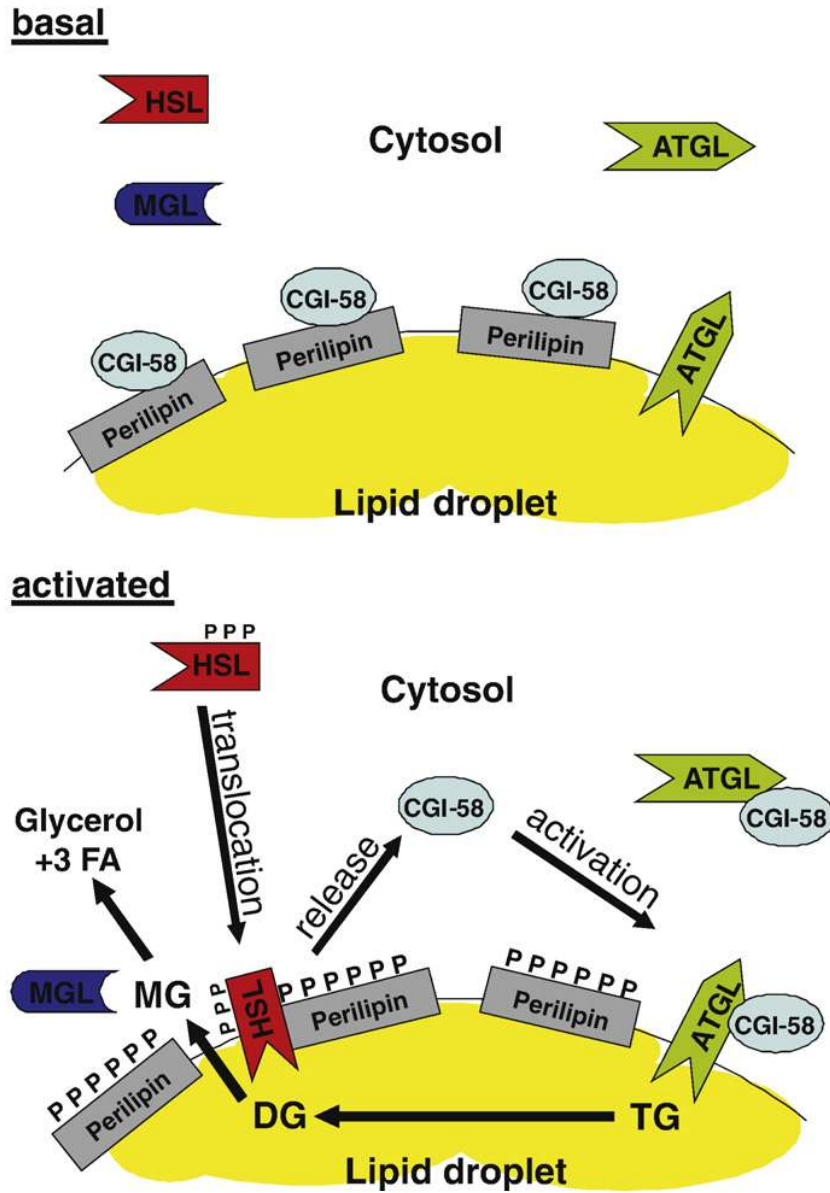


Figure 1.1. Proposed model for the regulation of lipolysis (57).

Chapter II

The Effects of Obesity on Lipolysis and Lipolytic Proteins

Abstract

Emily A. Johnson. The Effects of Obesity on Lipolysis and Lipolytic Proteins (under the direction of Robert C. Hickner). Department of Exercise and Sport Science. June 2010

Lipolysis is the process where TAG are hydrolyzed to provide free fatty acids as an energy substrate to other organs during times of energy demand. Blunted catecholamine stimulated lipolysis has been well documented in obese adults. The purpose of this study was to evaluate the effects of obesity and exercise training status on lipolysis and lipolytic protein expression in abdominal subcutaneous adipose tissue (SCAT) in men. Lean endurance trained (ET, n=10), lean sedentary (SED, n=10), and obese sedentary (OB, n=10) men were recruited for this study. Abdominal SCAT was obtained using needle aspiration. Western blots were used to determine the protein content of adipose triglyceride (ATGL), comparative gene identification 58 (CGI-58), hormone sensitive lipase (HSL), and perilipin. Microdialysis was used to evaluate abdominal SCAT lipolysis *in vivo*. Two probes were inserted into abdominal SCAT. One probe served as a control (perfused with a Ringer solution) while the second probe was perfused with isoproterenol (ISO) and a mixture of isoproterenol and phentolamine (IP). Ethanol was added to the perfusates to measure blood flow. Unstimulated, resting lipolysis was higher in ET than OB. ISO stimulated lipolysis was higher in ET and SED compared to baseline (BASE). Lipolysis did not increase further in response to IP in any of the groups. Ethanol outflow/inflow was not different in any group during any treatment. Perilipin was lower in the SED than ET but not different than OB. There were no differences in ATGL, HSL, or CGI-58 in any of the groups. Based on our results, it

appears that exercise training increases perilipin protein expression in abdominal SCAT in lean men. This may be the key component for a reduced β -adrenergic responsiveness in SED compared to lean. This may be the key component for a reduced β -adrenergic responsiveness in SED compared to lean.

Introduction

Obesity, defined as excess fat storage, is a significant health risk as it is associated with metabolic disorders such as Type 2 diabetes and insulin resistance as well as cardiovascular disorders such as hypertension and coronary artery disease (26, 28). Excess fat is stored as triacylglycerols (TAG) in subcutaneous adipose tissue (SCAT). TAG are broken down in times of need, such as during exercise and fasting conditions, to provide various body tissues with energy substrate..

Lipolysis is the process where TAG are hydrolyzed to provide free fatty acids (FFA) as an energy substrate to other organs during times of energy demand (1). FFA enter the circulation and are taken in by other organs to generate ATP via β -oxidation (1, 2). Lipolysis is largely regulated by catecholamines which bind to G_s -coupled β -adrenergic receptors (β -AR), leading to stimulation of the lipolytic cascade (2).

Blunted catecholamine stimulated lipolysis has been well documented in obese adults (22, 23, 37) and is, in part, attributed to lower expression of β_2 -AR, an increased antilipolytic effect of α_2 -AR, and/or decreased HSL expression (28). However, exercise training increases stimulated lipolysis (10).

It has been shown that HSL has a high specificity for diacylglycerol (DAG), indicating that an undetermined protein is responsible for TAG hydrolysis. In recent years, adipose triglyceride lipase (ATGL) has been identified as the primary TAG hydrolase. ATGL knockout mice do not have an impaired basal lipolytic rate (16) but basal lipolysis appears to be reduced in human adipocytes under ATGL inhibition (28). ATGL and HSL work with one another to hydrolyze TAG into DAG and

monoacylglycerol (MAG) (1). Perilipin has been identified as a director of stimulated lipolysis. Upon phosphorylation by PKA, perilipin releases the ATGL activator comparative gene identification 58 (CGI-58) (15) and promotes HSL translocation to the lipid droplet surface (45). It is not clear at this time how ATGL, CGI-58, HSL, and perilipin are affected by obesity and/or exercise training status. It is also unclear if these proteins are responsible for the blunted catecholamine stimulated lipolysis seen in obesity or contribute to increased catecholamine stimulated lipolysis in response to exercise training. The purpose of this study was to evaluate the effects of obesity and exercise training status on lipolysis and lipolytic protein expression in SCAT in men.

Materials and Methods

Subjects

Lean endurance trained (ET, n=10), lean sedentary (SED, n=10), and obese sedentary (OB, n=10) men were recruited for this study. Lean subjects had a body mass index (BMI) less than 25 kg/m² and obese subjects had a BMI greater than 28 kg/m² but less than 40 kg/m². Endurance trained subjects participated in endurance physical activity for at least 30 minutes per day, 3 times or more per week, for the last 3 months. Sedentary subjects participated in regular physical activity for less than 20 minutes per day and less than 1 day per week and were weight stable for at least 6 months prior to testing. Individuals that were excluded from this study included those with Type 1 or Type 2 diabetes, insulin resistance, coronary artery disease, hypertension or any condition that may interfere with exercise including arthritis, Parkinson's disease, and stroke. Individuals who smoked were excluded from this study. Individuals taking prescription medications that alter lipid metabolism or blood flow were also excluded. Subjects were instructed to not use vitamin supplements at least 48 hours prior to microdialysis and SCAT biopsy procedures.

Study Design

At least 1 week prior to the clinical investigation, subjects underwent anthropometric measurements and a maximal aerobic capacity test. A clinical investigation took place at the Brody School of Medicine the morning after a 12 hour fast. Subjects were instructed to avoid heavy physical activity and alcohol for at least 24 hours prior to testing. On this day, subjects underwent a microdialysis experiment, an

abdominal SCAT biopsy, and a venous blood sample. All measurements are described in detail below. Subjects were also asked to log all food and drink consumption three days prior to the clinical investigation.

Anthropometric Measurements

Height was measured on a mounted stadiometer. Body weight was measured on a calibrated, electronic scale to the nearest 0.1lb and converted to kilograms. BMI was calculated as body mass in kilograms divided by height in square meters. Minimum waist, umbilical, and maximum waist circumferences were measured in all participants as outlined by the American College of Sports Medicine (4). Waist to hip ratio was calculated as the minimum waist circumference divided by the maximum waist circumference. Body composition (body fat percentage, fat mass, fat free mass and lean body mass) was analyzed by whole body scans using dual energy x-ray absorptiometry (DEXA, Lunar USA).

VO_{2max} Test

Subjects performed a VO_{2max} test on an electric treadmill using a ParvoMedics TrueMax metabolic cart (Anaheim, CA). Prior to each exercise test, the metabolic cart was calibrated according to the manufacturer's indications. A tank consisting of 16% oxygen and 4% carbon dioxide was used for gas calibration. Flow rate was calibrated using a 3 liter syringe. Heart rate was monitored throughout the test with a heart rate monitor (Polar, Lake Success, NY). Expiratory gases were collected throughout the test and recovery. A headgear piece was used to stabilize the mouthpiece, and noseclips were used to prevent any expiratory gases from escaping through the nose. All participants completed the Bruce exercise protocol (4). Heart rate was recorded every

minute of the test and recovery. A test was deemed successful if three of the following four criteria were met: maximal heart rate is within 20 beats per minute (bpm) of the age predicted maximal heart rate ($220 - \text{age in years}$), the rate of perceived exertion (RPE) reaches or exceeds 17, the respiratory exchange ratio met or exceeded 1.15, and the participant reached volitional exhaustion (4). If three of the four previous criteria were not met, the test was repeated to ensure accurate results.

Adipose Tissue Biopsy

Abdominal SCAT was obtained using needle aspiration. Local anesthesia (5cc of 1% Lidocaine) was injected into the abdominal SCAT. Fat tissue (approx. 0.5-1 mg) was aspirated from the abdominal SCAT using a 16g needle attached to a 60cc syringe for suction. The tissue was placed in microcentrifuge vials and subjected to centrifuge at 1500xg for 10 minutes. The infranatant was drawn off and discarded. Adipose tissue was frozen and stored in a -80°C freezer.

Microdialysis

Microdialysis provides an effective technique for evaluating localized abdominal SCAT lipolysis *in vivo* (43). Two 10mm, 20kDa cutoff microdialysis probes (CMA Elite, CMA, Microdialysis, Stockholm, Sweden) were inserted into the abdominal SCAT, 2-3 cm apart, using an 18 gauge needle under sterile conditions. The insertion sites were sprayed with ethyl chloride followed by insertion of microdialysis probes. The probes were allowed to equilibrate with a Ringer solution consisting of 5mM ethanol and saline at $2.0\mu\text{l}/\text{min}$ using a CMA/102 microinfusion pump (CMA/Microdialysis, Stockholm, Sweden) for 60 minutes. After equilibration, the probes were perfused for 30 minutes

the Ringer solution to measure unstimulated lipolysis. Then, one probe was perfused for 30 minutes with a Ringer to serve as a control while the second was perfused with 1 μ M of isoproterenol (ISO) to measure β -adrenergic simulated lipolysis. Probe #2 was then perfused with the Ringer solution with 0.1mmol/l phentolamine and 1 μ M ISO (IP). Dialysate glycerol was measured using the CMA Microdialysis Analyzer (CMA/Microdialysis, Stockholm Sweden). Data for each probe and stage was averaged.

Ethanol was added to the perfusates to measure blood flow. Inclusion of 5mM ethanol in the perfusate has been shown to effectively detect nutritive blood flow changes around the microdialysis probe in skeletal muscle (34). The ethanol outflow: inflow ratio is inversely related to blood flow and was calculated as: Ethanol Outflow:Inflow=[Ethanol_{dialysate}]/[Ethanol_{perfusate}]. Ethanol concentrations was determined using the Victor3 Multilabel counter (PerkinElmer, Wellesley, MA) (19).

Biochemical Analysis

Venous blood samples were centrifuged at 4°C for 15 minutes then stored at 4°C. Samples were shipped on ice and plasma samples analyzed for glucose (GLU), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), and insulin (INS) (LabCorp, Burlington, NC). Low density lipoproteins (LDL) were calculated as: TC-HDL-(TG/5). HOMA was calculated as (INS x GLU)/450.

Western Blot Analysis

Abdominal SCAT samples were homogenized as described by Wang et al (48). SCAT was manually homogenized on ice in glass vials with protein lysis buffer (5% SDS, 50mM HEPES, 2 mM EDTA, 50 mM NaF, 1mM benzamidine, and 1mL/5mL

buffer Protease Inhibitor Cocktail Set III (Calbiochem, San Diego, CA), pH=7.4). SCAT was homogenized in a ratio of 200 μ L buffer per 100mg of tissue. Homogenates were incubated at 37°C and were vortexed every ten minutes for a total of one hour. Homogenates were then centrifuged for 15 minutes at 14,000g at room temperature. The supernatant was separated from the fat cake and pellet. The supernatant was saved and the pellet and fat cake were discarded. Wang et al found that an additional extraction of the remaining fat cake does not yield any additional protein (48).

Protein concentration of the homogenate was measured using a Bioinchnonic Acid Protein Assay Kit (Pierce-23225, Pierce Laboratories, Rockford, IL). 40 μ g of tissue added to each well of a 10% polyacrylamide gel (Bio-Ray, Hercules, CA) and separated by SDS-PAGE. Proteins were transferred to polyvinylidene fluoride membranes. Membranes were blocked with a 5% non-fat milk solution for 1 hour. Primary antibodies for perilipin (Sigma Aldrich, St. Louis, MO), ATGL, HSL, CGI-58, and β -actin (Novus Biologicals, Littleton, CO) were applied in a 1:5000 ratio and incubated overnight at 4°C. Membranes were washed in TBST and the secondary antibody was applied for one hour. Membranes were rewashd with TBST/TBS and an ECL solution was applied for 5 minutes. Blots were detected using an enhanced chemiluminescence system. Autoradiographs were quantified using Gel Pro Analyzer software (MediaCybernetics, Bethesda, Maryland). Blots were stripped using Restore[™] Western Blot Stripping Buffer (Pierce Biotechnology, Rockford, IL) and reprobod for the aforementioned programs and for β -actin (Sigma-Aldrich Corp), a strong indicator of DNA content (25). Protein values are reported in relation to β -actin.

Statistical Analysis

Data are expressed as mean±standard error. Data were considered significant at $p<0.05$. A two-way mixed plot factorial analysis of variance (ANOVA) was used to compare demographic information, and expression of perilipin, HSL, ATGL, and CGI-58 between ET, SED, and OB men. A 3x3 repeated measures ANOVA (participant category x probes) was used to analyze microdialysis data. Correlations analysis was used to determine relationships between lipolysis and lipolytic proteins.

Results

Subject characteristics are presented in Table 2.1. None of the subjects were taking any medications, vitamins, or supplements. None had ever been diagnosed with cardiovascular or metabolic disorders. None suffered from skeletal-muscular conditions that prevented the completion of the maximal exercise test. The OB group had a higher weight ($p < 0.001$), BMI ($p < 0.001$), body fat percentage ($p < 0.001$), and fat mass ($p < 0.001$) than the ET and SED groups. Waist to hip ratio was lower in SED than ET and OB ($P < 0.05$). OB had a lower relative VO_{2max} than ET and SED ($p < 0.05$). ET had a higher relative VO_{2max} than SED ($p < 0.05$). ET had a higher absolute VO_{2max} than SED and OB ($p < 0.05$). Time to exhaustion during the VO_{2max} test was lower in OB than ET and SED ($p < 0.05$). Time to exhaustion was higher in ET than SED ($p < 0.05$). No other significant findings were observed.

Biochemical Results

Biochemical results are listed in Table 2.2. TG were higher in OB than ET ($p < 0.05$). LDL were higher in ET than SED ($p < 0.05$). No other significant findings were observed.

Nutritional Analysis

Macronutrient intake, calculated from 3 day diet logs, is listed in Table 2.3. There were no differences in caloric intake, carbohydrate, fat, or protein consumption in ET, SED, or OB.

Dialysate Glycerol

Basal. All dialysate glycerol levels are found in Figure 2.1. Unstimulated glycerol was lower in OB ($37.96 \pm 8.6 \mu\text{mol/L}$) than ET ($61.81 \pm 6.9 \mu\text{mol/L}$; $p < 0.05$). SED glycerol ($51.50 \pm 9.2 \mu\text{mol/L}$) was not different than ET or OB.

β -adrenergic stimulation. ISO stimulated glycerol was higher in ET ($134.40 \pm 10.0 \mu\text{mol/L}$) than in SED ($87.28 \pm 11.7 \mu\text{mol/L}$; $p < 0.05$) and OB ($46.11 \pm 9.3 \mu\text{mol/L}$; $p < 0.001$). There was no difference in ISO stimulated glycerol levels between SED and OB. Glycerol levels were higher than baseline in response to ISO in ET ($p < 0.001$) and SED ($p < 0.05$). ISO stimulated glycerol was not different than baseline in OB.

Beta-adrenergic stimulation and alpha-receptor blockade. IP stimulated glycerol (Figure 2.1) was higher in ET ($143.21 \pm 24.5 \mu\text{mol/L}$) than in SED ($87.00 \pm 9.5 \mu\text{mol/L}$; $p = 0.05$) and OB ($63.43 \pm 12.9 \mu\text{mol/L}$; $p < 0.05$). There was no difference in IP stimulated glycerol levels in SED and OB. IP glycerol levels were higher than baseline in ET ($p < 0.05$) and SED ($p < 0.05$). IP stimulated lipolysis was not different than baseline in OB. IP glycerol levels were not different than ISO stimulated glycerol in any of the groups.

Ethanol Outflow:Inflow (EtOH O/I)

EtOH O/I (shown in figure 2.2) during nonstimulated conditions was not different between the groups (ET: 0.71 ± 0.02 ; SED: 0.69 ± 0.04 ; OB: 0.71 ± 0.05). EtOH O/I during ISO conditions (ET: 0.59 ± 0.03 ; SED: 0.68 ± 0.04 ; OB: 0.73 ± 0.05) and IP conditions (ET: 0.64 ± 0.02 ; SED: 0.73 ± 0.04 ; OB: 0.70 ± 0.05) was not different between the groups (IP

data not shown). There were no differences in EtOH O/I in responses to any of the treatments in any group.

Protein Expression

As shown in figure 2.3, ATGL protein expression was not different between ET (0.32 ± 0.1 AU/Beta Actin), SED (0.15 ± 0.04 AU/Beta Actin; $p=0.14$), and OB (0.17 ± 0.05 AU/Beta Actin; $p=0.21$).

CGI-58 was not different between ET (0.60 ± 0.2) and SED (0.24 ± 0.06 AU/Beta Actin; $p=0.15$) or OB (0.26 ± 0.1 AU/Beta Actin; $p=0.19$). There was also no difference between SED and OB ($p=0.88$) as illustrated in Figure 2.4.

HSL, presented in figure 2.5, was not different between ET (0.39 ± 0.2 AU/Beta Actin), SED (0.25 ± 0.04 AU/Beta Actin), and OB (0.22 ± 0.06 AU/Beta Actin).

Shown in Figure 2.6, perilipin was higher in ET (1.62 ± 0.6 AU/Beta Actin) than SED (0.55 ± 0.09 AU/Beta Actin; $p=0.05$). There was no difference between ET and OB (0.95 ± 0.2 AU/Beta Actin) or between SED and OB.

Correlations

Correlations are shown in Table 2.4 and 2.5. We did not observe any significant relationships between lipolysis and lipolytic protein content. We did observe significant correlations between lipolytic protein content.

	ET	SED	OB
N	10	10	10
Age (yrs)	27±2.8	22±1.4	23±0.9
Height (cm)	178.0±2.3	179.9±2.8	178.0±3.2
Weight (kg)	75.4±2.6	73.5±3.9	103.7±9.1 ^{ET**, SED**}
Body Mass Index (kg/m²)	23.8±2.6	22.7±0.9	32.5±2.4 ^{ET**, SED**}
Body Fat (%)	17.9±2.1	18.5±2.5	34.9±2.1 ^{ET**, SED**}
Fat mass (kg)	12.9±1.5	13.5±2.6	37.0±6.2 ^{ET, SED}
Lean Body Mass (kg)	58.6±2.1	57.1±1.5	59.9±1.8
Waist:Hip	0.9±0.02	0.8±0.01 ^{ET, OB}	0.9±0.02
VO_{2max} (ml/kg/min)	52.6±2.7	45.0±1.7 ^{ET, OB}	34.8±1.5 ^{OB}
VO_{2max} (L/min)	4.0±0.1 ^{SED, OB}	3.3±0.2	3.5±0.2
Time to Exhaustion (min)	13.7±0.8	11.9±0.4 ^{ET, OB}	8.8±0.6 ^{ET}

Table 2.1: Subject Characteristics. ^{ET}: different from ET group, p<0.05. ^{ET**}: different from ET group, p<0.001. ^{SED}: different from SED group, p<0.05. ^{SED**}: different from SED group, p<0.001. ^{OB}: different from OB group, p<0.05. ^{OB**}: different from OB group, p<0.001. All data presented as Mean±SE.

	ET	SED	OB
GLU (mg/dl)	89.20	91.90±2.6	92.29±3.5
TC (mg/dl)	160.20±10.5	146.40±11.1	187.14±14.0
TG (mg/dl)	70.80±9.7	79.80±11.3	146.40±38.9 ^{ET}
HDL (mg/dl)	48.00±2.5	55.30±6.9	42.29±5.2
LDL (mg/dl)	98.04±8.6	77.73±13.9	115.57±9.8 ^{SED}
INS (uIU/ml)	4.11±1.9	3.63±0.8	8.51±2.2
HOMA	0.94±0.5	0.83±0.2	1.98±0.6

Table 2.2: Plasma biochemical results. ^{ET}: different from ET group, p<0.05. ^{SED}: different from SED group, p<0.05. ET n=10, SED n=10, OB n=8. Data is presented as mean±SE.

	kcal/day	CHO (g)	FAT (g)	PRO (g)
ET	2051.44±354.1	234.24±33.0	91.26±27.4	95.57±16.7
SED	2317.44±59.1	241.18±41.54	97.17±14.7	101.78±13.71
OB	2331.49±506.1	241.16±46.8	76.80±8.7	122.75±43.4

Table 2.3: Nutritional intake in lean exercise trained (ET), lean sedentary (SED), and obese sedentary (OB) men. There were no significant differences in caloric intake (kcal/day), carbohydrate (CHO), fat (FAT), or protein (PRO) consumption between the three groups. ET n=10, SED n=10, OB n=8. Data are expressed as mean±SE.

	Ringer	ISO	IP
ATGL	0.06	0.13	-0.17
CGI-58	0.12	0.01	0.04
HSL	0.07	0.10	-0.06
Perilipin	0.003	0.16	-0.10

Table 2.4: Correlations between lipolytic proteins and lipolysis in abdominal subcutaneous adipose tissue in lean exercise trained (n=10), lean sedentary (n=10), and obese men (n=10). There were no significant correlations between lipolysis under unstimulated (Ringer), isoproterenol stimulated (ISO) and isoproterenol and phentolamine stimulated (IP) conditions with adipose triglyceride lipase (ATGL), comparative gene identification 58 (CGI-58), hormone sensitive lipase (HSL) and perilipin.

	ATGL	CGI-58	HSL	Perilipin
ATGL		0.58**	0.50*	0.76**
CGI-58	0.58**		0.87**	0.17
HSL	0.50*	0.87**		0.02

Table 2.5: Correlations between lipolytic proteins and lipolysis in abdominal subcutaneous adipose tissue in lean exercise trained (n=10), lean sedentary (n=10), and obese men (n=10). We observed significant correlations between adipose triglyceride lipase (ATGL) and comparative gene identification 58 (CGI-58), ATGL and hormone sensitive lipase (HSL), ATGL and perilipin, and HSL and CGI-58. *p<0.05. **p≤0.001.

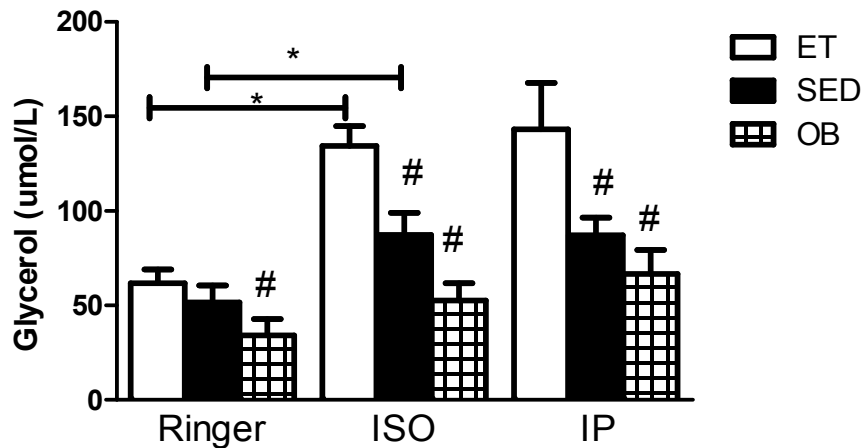


Figure 2.1: Dialysate glycerol concentrations in abdominal subcutaneous adipose tissue of lean sedentary (SED, n=10), lean trained (ET, n=10) and obese sedentary (OB, n=10). Resting, unstimulated dialysate glycerol was lower in obese sedentary men (SED) than in lean exercise trained men (ET). Under isoproterenol stimulation (ISO), dialysate glycerol increased above unstimulated conditions in ET and lean sedentary men (SED). Dialysate glycerol under ISO stimulation was higher in ET than in SED and OB. Under ISO and phentolamine stimulation (IP), glycerol levels were higher than baseline in ET and SED. Glycerol levels did not increase above ISO stimulation in any of the groups. * $p < 0.05$; different from Ringer Probe. #: $p < 0.05$; different from ET. Data are expressed as means \pm SE.

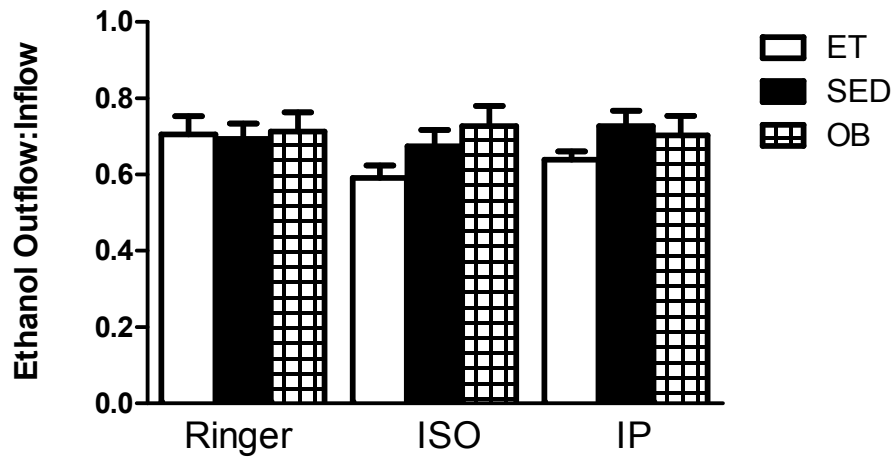


Figure 2.2: Ethanol outflow:inflow ratios in abdominal subcutaneous adipose tissue in lean exercise trained (ET, n=10), lean sedentary (SED, n=10) and obese sedentary (OB, n=10) men. Ethanol outflow:inflow ratio was not different during any treatment (ringer, isoproterenol (ISO), isoproterenol and phentolamine (IP)) and were not different between groups (lean exercise trained (ET), lean sedentary (SED) and obese sedentary (OB) men. Data are expressed as mean \pm SE.

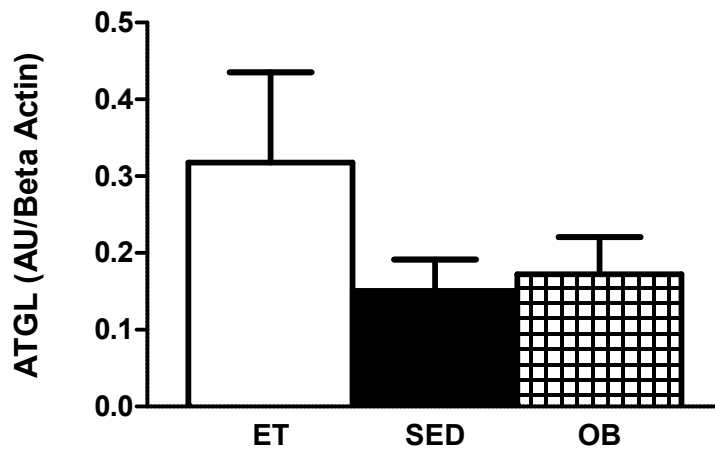


Figure 2.3: Adipose triglyceride lipase (ATGL) protein content in subcutaneous adipose tissue in lean exercise trained (ET, n=10), lean sedentary (SED, n=10), and obese sedentary (OB, n=10) men. ATGL expression was not different between lean exercise trained (ET), lean sedentary (SED), and obese sedentary (OB) men. Data are expressed as mean±SE.

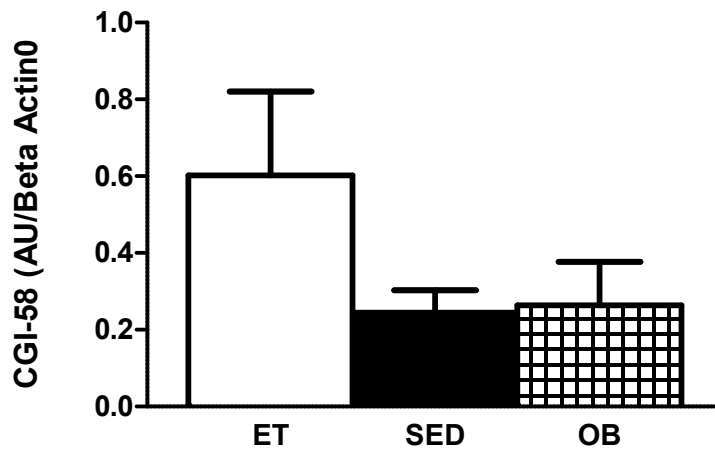


Figure 2.4: Comparative gene identification 58 (CGI58) expression in subcutaneous adipose tissue in lean exercise trained (ET, n=10), lean sedentary (SED, n=10), and obese sedentary (OB, n=10) men. CGI-58 was not different between lean exercise trained (ET), lean sedentary (SED), and obese sedentary (OB) men. Data are expressed as mean \pm SE.

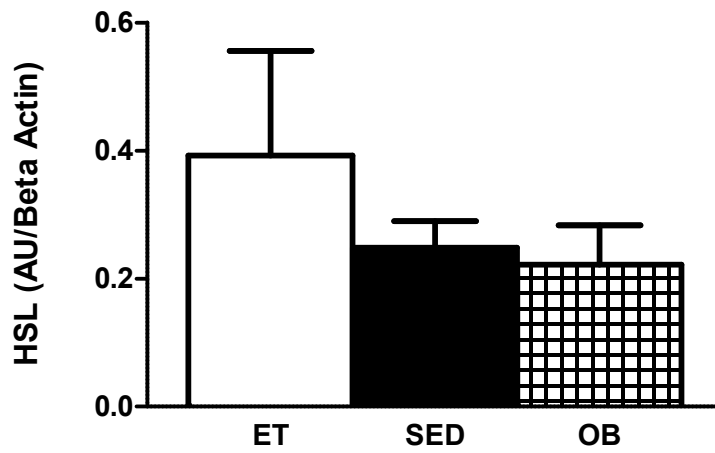


Figure 2.5: Hormone sensitive lipase (HSL) expression in abdominal subcutaneous adipose tissue in lean exercise trained (ET, n=10), lean sedentary (SED, n=10), and obese sedentary (OB, n=10) men. HSL was not different between lean exercise trained (ET), lean sedentary (SED), and obese sedentary (OB) men. Data are expressed as mean±SE.

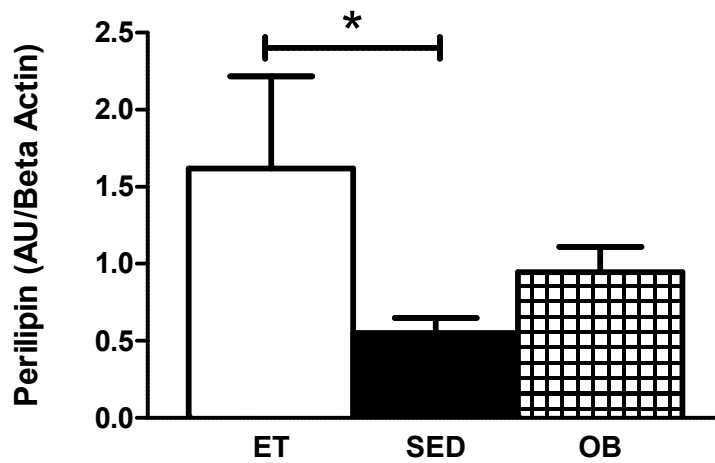


Figure 2.6: Perilipin protein content in abdominal subcutaneous adipose tissue in lean exercise trained (ET, n=10), lean sedentary (SED, n=10), and obese sedentary (OB, n=10) men.. Perilipin was significantly lower in SED than ET men. There was no differences between ET and OB or SED and OB. * $p < 0.05$. Data are expressed as mean \pm SE.

Discussion

We determined that lean ET and lean sedentary men (SED) have an increased lipolysis in response to β -adrenergic stimulation. Lipolysis in OB men did not respond to β -adrenergic stimulation. These findings support the previous work of others in that obese individuals have a reduced capacity for catecholamine stimulated lipolysis (24, 38). We did not observe further increases in lipolysis with the addition of phentolamine (an α -AR antagonist). This supports the contention that β -adrenergic stimulation of lipolysis is impeded in obese (23, 24, 28, 29) but does not agree with previous studies reporting the antilipolytic action of α -AR is responsible for defects in lipolysis (8).

Additionally, we observed a higher lipolytic rate in ET than SED in response to β -adrenergic stimulation, indicating that a sedentary lifestyle may also hinder stimulated lipolysis. Others have found that indeed exercise training does increase lipolytic response to β -adrenergic stimulation (43). We believe that a significant defect exists in the β -adrenergic pathway that hinders stimulated lipolysis but that this defect can be, at least in part, reversed in response to exercise training. The increase in stimulated lipolysis was higher in ET compared to SED and OB indicating that exercise plays a substantial role in increasing response to β -adrenergic stimulation. We do not believe that an increase in insulin (a potent antilipolytic hormone) sensitivity is responsible for our findings since plasma insulin and insulin sensitivity were not different between groups.

We observed a decreased lipolysis at rest in OB men compared to ET. Again, this is likely due to a resistance to β -adrenergic stimulated lipolysis and not due to an antilipolytic effect from insulin or α -adrenergic pathway. This further illustrates the defect

in human obesity; obese men are not able to mobilize FFA from SCAT at rest or in times of need such as during exercise as effectively as their lean and/or exercise trained counterparts. Though we did not observe a significant relationship between lipolysis and lipolytic protein content, we did observe significant relationships among the four lipolytic proteins measured. This may indicate that ATGL, CGI-59, HSL, and perilipin protein content are regulated in concert.

We evaluated the differences in essential lipolytic proteins in ET, SED, and OB as well in an attempt to clarify where the defects in the β -adrenergic pathway are located. We did not observe any differences in ATGL, CGI-58, or HSL among groups. Perilipin, however, was lower in the SED compared to the ET group and were not different in SED and OB. Based on our results, it appears that exercise training increases perilipin protein expression in lean men. This may be the key component for a reduced β -adrenergic responsiveness in SED compared to ET. As previously determined, perilipin null mice have a 75% lower glycerol release from adipocytes than wild type mice (45). It may be that the increase in perilipin content increases HSL translocation to the lipid droplet (51) and/or enables lipases' ability to hydrolyze TAG by fragmenting the lipid droplet in order to increase the surface area of the lipid droplet (6). It is also possible that an increased perilipin content increases HSL translocation to the lipid droplet and/or CGI-58 release. However, one limitation to this study is lipolytic protein activity was not evaluated. It is possible that the activity levels of each protein will clarify and help explain our results. Lipolytic protein activity evaluation is a necessary avenue to be explored in future studies.

Atrial natriuretic peptide (ANP) may play a role in the increases in stimulated lipolysis in exercise trained men. ANP, released in response to atrial stretching, activates cGMP which, in turn, phosphorylates perilipin and HSL (32). Response to ANP stimulated lipolysis increases in response to aerobic exercise training and is therefore a potential target for future studies. (32).

In the present study, we identify exercise as a potentially crucial element to a stimulated lipolytic response to β -adrenergic activation due to perilipin upregulation. It appears that exercise training increases lipolysis in response to β -adrenergic stimulation. This effect may be more important in obese men due to a lower lipolysis at rest as well as a blunted lipolytic response to stimulation. In the present study, we determined that perilipin may respond to exercise training and therefore increase lipolysis. However, the precise mechanisms of perilipin's action should be investigated further in future investigations.

Chapter III

The Effects of Exercise Training on Lipolysis and Lipolytic Proteins in Human Obesity

Abstract

Emily A. Johnson. The Effects of Exercise Training on Lipolysis and Lipolytic Proteins in Human Obesity (under the direction of Robert C. Hickner). Department of Exercise and Sport Science. June 2010.

Lipolysis is the process where TAG are hydrolyzed to provide free fatty acids as an energy substrate to other organs during times of energy demand such as exercise. Blunted catecholamine stimulated lipolysis has been well documented in obese adults yet the mechanisms of how this defect can be alleviated by exercise is not clear. It is the purpose of this study to evaluate the effects of 8 weeks of exercise training on lipolysis and key lipolytic proteins (ATGL, CGI-58, HSL, and perilipin) in subcutaneous abdominal adipose tissue (SCAT) of obese men. Eight obese, sedentary men participated in this study. Abdominal SCAT was obtained using needle aspiration. Western blots were used to determine the protein content of ATGL, CGI-58, HSL, and perilipin. Microdialysis was used to evaluate abdominal SCAT lipolysis *in vivo*. Two probes were inserted into abdominal SCAT. One probe served as a control (perfused with a Ringer solution). The second probe was perfused with isoproterenol (ISO) and a mixture of isoproterenol and phentolamine (IP) to evaluate beta-adrenergic regulation of lipolysis. Ethanol was added to the perfusates to measure local microvasculature exchange. Unstimulated, resting lipolysis did not change in response to ET. ISO stimulated lipolysis increased from baseline (BASE) before and after ET. The response to ISO stimulation was higher after ET than before ET. Lipolysis did not increase further from ISO in response to IP before or after ET. Lipolysis was higher than BASE during

an acute exercise bout (ACEX) after ET but not before ET. Ethanol outflow/inflow ratio was lower than BASE during ACEX after ET. There were no other differences in ethanol outflow/inflow during any treatment. ATGL increased in response to ACEX before ET and in response to ET. Perilipin was higher after ACEX before ET and tended to be higher at rest after ET. Based on our results, it appears that ACEX increases ATGL and perilipin in abdominal subcutaneous adipose tissue of obese men. ET increased ATGL protein content, and tended to increase perilipin, protein content in abdominal SCAT. ATGL and perilipin may be the key components to increasing β -adrenergic lipolysis in abdominal subcutaneous adipose tissue of obese men after endurance training.

Introduction

Obesity rates among men have increased 4.7% in the last ten years and is on the rise (35). Obesity is the result of an energy surplus; excess energy is stored as triacylglycerols (TAG) in adipose tissue. Obesity is a significant health risk, as it is associated with metabolic disorders such as Type 2 Diabetes and insulin resistance as well as cardiovascular disorders such as hypertension and coronary artery disease.(26).

Lipolysis, or TAG hydrolysis, releases FFA from adipocytes which enter the circulation where they can be taken up and utilized by other organs for energy production (2). There are a number of proteins that help facilitate the lipolytic process. Perilipin is a lipid droplet coating protein which binds comparative gene identification 58 (CGI-58) protein in the unstimulated state. Upon PKA phosphorylation, CGI-58 loses affinity for perilipin and translocates to activate adipose triglyceride lipase (ATGL) which resides on the lipid droplet surface. ATGL demonstrates a high specificity for TAG and is the primary enzyme responsible for TAG hydrolysis. HSL shows some specificity for TAG but HSL has a ten-fold specificity, as compared to ATGL, for diacylglycerol (DAG). Monoacylglycerols (MAG) are hydrolyzed by monoglyceride lipase (MGL). FFA and glycerol are then released by the adipocyte into circulation where they are delivered to peripheral tissues (56).

It has been reported that catecholamine stimulated lipolysis is blunted in the obese state (24, 38). This is of particular concern in the obese population: with reduced circulating free fatty acid (FFA) availability during exercise, the ability to generate adenosine triphosphate (ATP) in muscle is hindered (2, 14).

A reduced β_2 -adrenergic receptor density (38) and lower hormone sensitive lipase (HSL) content (28) have been offered as explanations for reduced lipolysis in obese individuals during exercise. However, in recent years, other proteins, such as ATGL and CGI-58 have been identified as critical lipolytic components. These developments have caused the reasons for a lipolytic defect in obesity to be less certain. However, perilipin, ATGL and HSL protein content and CGI-58 mRNA have been shown to be lower in obese (28, 42). Given the role of these essential proteins, it seems logical that these proteins contribute to the reduction in catecholamine stimulated lipolysis, yet this premise has not been clarified in humans. To date, no studies have evaluated the content all of the lipolytic proteins in obese human SCAT.

Exercise training has been shown to improve catecholamine stimulated lipolysis in obese and overweight individuals (8, 9, 43). However, given the recent advancements in our understanding of the lipolytic cascade, it is unknown if exercise training has any effect on ATGL, CGI-58, HSL, or perilipin in adipose tissue of obese humans. In a previous study in human skeletal muscle, Alsted et al (2008) found that ATGL protein content was increased in response to 8 weeks of exercise training in overweight males while CGI-58 AND HSL remained unchanged (3). Despite these findings, the effects of exercise training on the aforementioned proteins in human abdominal SCAT is, at this time, unknown. Therefore, it is the purpose of this study to evaluate the effects of 8 weeks of exercise training on stimulated lipolysis and key lipolytic proteins (ATGL, CGI-58, HSL, and perilipin) in obese men. We believe that lipolysis and lipolytic protein content is lower in obese but exercise trained individuals have an increased lipolysis and lipolytic protein content.

Materials and Methods

Subjects

Obese sedentary (OB, n=8) men were recruited for this study. Participants had a body mass index (BMI) greater than 30 kg/m² but less than 40 kg/m². Participants participated in regular physical activity for less than 20 minutes per day and less than 1 day per week and were weight stable for at least 6 months prior to testing. Individuals that were excluded from this study included those who smoked or had Type 1 or Type 2 diabetes, insulin resistance, coronary artery disease, hypertension or any condition that may interfere with exercise including arthritis, Parkinson's disease, and stroke. Individuals taking prescription medications that alter lipid metabolism or blood flow were also excluded. Participants were instructed to not use vitamin or other nutritional supplements at least 48 hours prior to microdialysis and SCAT biopsy procedures.

Study Design

At least 1 week prior to the clinical investigation, participants underwent anthropometric measurements and a maximal aerobic capacity test. Anthropometric measurements and the maximal aerobic capacity test were repeated 4 weeks into, and during the final week of exercise training (ET). A clinical investigation took place before and within 24 hours of the final exercise training bout, at the Brody School of Medicine the morning after a 12 hour fast. Participants were instructed to avoid heavy physical activity and alcohol. On these days, participants underwent a microdialysis experiment, abdominal SCAT biopsies, and a venous blood sample. All measurements are

described in detail below. Subjects were also asked to log all food consumption three days prior to each clinical investigation.

Anthropometric Measurements

Height was measured on a mounted stadiometer. Body weight was measured on a calibrated, electronic scale to the nearest 0.1lb and converted to kilograms. BMI was calculated as body mass in kilograms divided by height in square meters. Minimum waist, umbilical, and maximum waist circumferences was measured in all participants as outlined by ACSM (4). Waist to hip ratio was calculated as the minimum waist circumference divided by the maximum waist circumference. Body composition (body fat percentage, fat mass, fat free mass and lean body mass) was analyzed by whole body scans using dual energy x-ray absorptiometry (DEXA, Lunar USA).

VO_{2max} Test

Participants performed a VO_{2max} test prior to, 4 weeks into, and in the last week of exercise training, on an electric treadmill using a ParvoMedics TrueMax metabolic cart (Anaheim, CA). Prior to each exercise test, the metabolic cart was calibrated according to the manufacturer's indications. A tank consisting of 16% oxygen and 4% carbon dioxide was used for gas calibration. Flow rate was calibrated using a 3 liter syringe. Heart rate was monitored throughout the test with a heart rate monitor (Polar, Lake Success, NY). Expiratory gases were collected throughout the test and recovery. A headgear piece was used to stabilize the mouthpiece, and noseclips were used to prevent any expiratory gases from escaping through the nose. All participants completed the Bruce exercise protocol (4). Heart rate was recorded every minute of the test and recovery. A test was deemed successful if three of the following four criteria

were met: maximal heart rate is within 20 beats per minute (bpm) of the age predicted maximal heart rate ($220 - \text{age in years}$), the rate of perceived exertion (RPE) reaches or exceeds 17, the respiratory exchange ratio met or exceeded 1.15, and the participant reached volitional exhaustion (4). If three of the four previous criteria were not met, the test was repeated to ensure accurate results.

Exercise Training

Subjects exercised for the 8 weeks on a treadmill, cycle ergometer, and/or elliptical trainer. Subjects progressed from 3 exercise sessions/week (weeks 1 and 2) and progressed to 4 sessions/week (weeks 3-5, and 8) and 5 sessions/week (weeks 6-7). Subjects exercised at a heart rate corresponding to 65-70% $VO_{2\max}$ for the first 30 minutes. The second 30 minutes of exercise included intervals at a heart rate corresponding to 80-85% $VO_{2\max}$ (2 minute work intervals during weeks 1-2, 3 minutes during weeks 3-5 and 8, 4 minutes during weeks 6-7) with 2-4 minutes of recovery (4 minutes weeks 1-2, 3 minutes weeks 3-5 and 8, 2 minutes weeks 6-7) at a heart rate corresponding to 45-50% $VO_{2\max}$. The intensity of each exercise sessions was determined by their individual $VO_{2\max}$ assessment.

Each subject was monitored during every exercise session by a trained exercise professional. Subjects wore a heart rate monitor (Polar, Lake Success, NY) throughout each exercise session and heart rate was measured regularly to ensure exercise was being performed at the prescribed intensity; adjustments to workload were made if the participants' heart rate fell out of the prescribed range. All subjects remained weight stable throughout the intervention. Weight was measured once a week during the 8 weeks of ET.

Acute Exercise Bout

Participants completed an acute exercise bout before and immediately after 8 weeks of exercise training. Dialysate glycerol was measured during the exercise bout and abdominal SCAT biopsies were taken before and immediately following the exercise bouts. Subjects exercised at the same absolute workload during the first and last exercise bout.

Adipose Tissue Biopsies

Abdominal SCAT biopsies were collected prior to, and immediately following, the microdialysis experiments. Abdominal SCAT was obtained using needle aspiration. Local anesthesia (5cc of 1% Lidocaine) was injected into the abdominal SCAT. Fat tissue (approx. 0.5-1 mg) was aspirated from the abdominal SCAT using a 16g needle attached to a 60cc syringe for suction. The tissue was placed in microcentrifuge vials and subjected to centrifuge at 1500xg for 10 minutes. The infranatant was drawn off and discarded. Adipose tissue was frozen and stored in a -80°C freezer. Abdominal SCAT biopsies were collected prior to and following the microdialysis experiments.

Microdialysis

Microdialysis provides an effective technique for evaluating localized abdominal SCAT lipolysis *in vivo* (44) Microdialysis was performed prior to and following 8 weeks of exercise training. Two microdialysis probes (CMA Elite, CMA, Microdialysis, Stockholm, Sweden) were inserted into the abdominal SCAT, 2-3 cm apart, using an 18 gauge needle under sterile conditions. The insertion sites were sprayed with ethyl chloride followed by insertion of microdialysis probes. The probes were allowed to equilibrate with a Ringer solution consisting of 5mM ethanol and saline at 2.0µl/min using a

CMA/102 microinfusion pump (CMA/Microdialysis, Stockholm, Sweden) for 60 minutes. After equilibration, the probes were perfused for 30 minutes with Ringer solution to measure unstimulated lipolysis. Then, one probe was perfused for 30 minutes with a Ringer to serve as a control while the second was perfused with Ringer containing 1 μ M of isoproterenol (ISO) to measure β -adrenergic stimulated lipolysis. Probe #2 was then perfused with the Ringer solution with 0.1mmol/l phentolamine and 1 μ M isoproterenol (IP). Glycerol was measured using CMA/600 Microdialysis Analyzer (CMA/Microdialysis, Stockholm Sweden). Data for each probe and stage were averaged.

Ethanol was added to the perfusates to measure local microvascular exchange. Inclusion of 5mM ethanol in the perfusate has been shown to effectively detect nutritive blood flow changes around the microdialysis probe in skeletal muscle (34). The ethanol outflow: inflow ratio is inversely related to local nutritive blood flow and was calculated as: Ethanol Outflow:Inflow=[Ethanol_{dialysate}]/[Ethanol_{perfusate}]. Ethanol concentrations were determined using the Victor3 Multilabel counter (PerkinElmer, Wellesley, MA) (19).

Biochemical Analysis

Venous blood samples were centrifuged at 4°C for 15 minutes then stored at 4°C. Samples were shipped on ice and plasma samples analyzed for glucose (GLU), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), and insulin (LabCorp, Burlington, NC). Low density lipoproteins (LDL) were calculated as: TC-HDL-(TG/5). HOMA was calculated as (GLUxINS)/450.

Western Blot Analysis

Abdominal SCAT samples were homogenized as described by Wang et al (48). SCAAT was manually homogenized on ice in glass vials with protein lysis buffer (5% SDS, 50mM HEPES, 2 mM EDTA, 50 mM NaF, 1mM benzamidine, and 1mL/5mL buffer Protease Inhibitor Cocktail Set III (Calbiochem, San Diego, CA), pH=7.4). SCAAT was homogenized in a ratio of 200 μ L buffer per 100mg of tissue. Homogenates were incubated at 37°C and were vortexed every ten minutes for a total of one hour. Homogenates were then centrifuged for 15 minutes at 14,000g at room temperature. The supernatant was separated from the fat cake and pellet. The supernatant was saved and the pellet and fat cake were discarded. Wang et al found that an additional extraction of the remaining fat cake does not yield any additional protein (48).

Protein concentration of the homogenate was measured using a Bioinchnonic Acid Protein Assay Kit (Pierce-23225, Pierce Laboratories, Rockford, IL). 40 μ g of tissue added to each well of a 10% polyacrylamide gel (Bio-Ray, Hercules, CA) and separated by SDS-PAGE. Proteins were transferred to polyvinylidene fluoride membranes. Membranes were blocked with a 5% non-fat milk solution for 1 hour. Primary antibodies for perilipin (Sigma Aldrich, St. Louis, MO), ATGL, HSL, CGI-58, and β -actin (Novus Biologicals, Littleton, CO) were applied in a 1:5000 ratio and incubated overnight at 4°C. Membranes were washed in TBST and the secondary antibody was applied for one hour. Membranes were rewashd with TBST/TBS and an ECL solution was applied for 5 minutes. Blots were detected using an enhanced chemiluminescence system. Autoradiographs were quantified using Gel Pro Analyzer software (MediaCybernetics, Bethesda, Maryland). Blots were stripped using Restore™ Western Blot Stripping Buffer (Pierce Biotechnology, Rockford, IL) and reprobred for the aforementioned proteins and

for β -actin (Sigma-Aldrich Corp), a strong indicator of DNA content (25). Protein values are reported in relation to β -actin.

Statistical Analysis

Data are expressed as mean \pm standard error. Data were considered significant at $p < 0.05$. Multiple Paired sample t-tests were performed to analyze differences between pre and post exercise training (demographics, expression of perilipin, HSL, ATGL, and CGI-58). A 2x2 (pre/post training x probe) repeated measured analysis of variance (ANOVA) was used to analyze lipolysis at rest and during exercise. A 2x3 (pre/post training x probe) repeated measures ANOVA was used to analyze lipolysis at rest, during ISO stimulation, and during IP stimulation.

Results

Subject Characteristics

Subject characteristics are listed in Table 3.1. There were no significant differences in body composition (weight, BMI, body fat percentage, lean body mass, and waist to hip ratio) after, as compared to before, 8 weeks of exercise training. There was no change in VO_{2max} (relative or absolute); however, time to exhaustion during the VO_{max} test was longer after, compared to before, exercise training ($P=0.001$).

Blood Chemistry

Blood chemistry characteristics are listed in Table 3.2. There were no significant differences in any of the parameters measured in response to exercise training.

Nutritional Analysis

Nutritional analysis calculated from 3 day diet logs are listed in Table 3.3. There were no differences in caloric intake, carbohydrate, fat, or protein consumption before, as compared to after, 8 weeks of ET.

Glycerol

Resting, unstimulated dialysate glycerol levels did not change in response to 8 weeks of ET (PRE ET: 35.71 ± 8.6 , POST ET: 34.63 ± 7.2 $\mu\text{mol/L}$, $p=0.93$).

Dialysate glycerol did not change in response to ISO from baseline (resting, unstimulated, BASE) before 8 weeks of ET (BASE: 35.71 ± 8.6 , ISO: 46.11 ± 9.2 $\mu\text{mol/L}$, $p=0.16$). Dialysate glycerol increased from BASE in response to ISO after 8 weeks of ET (BASE: 34.63 ± 7.2 , ISO: 71.29 ± 15.9 $\mu\text{mol/L}$, $p=0.05$).

Dialysate glycerol did not increase further (as compared to ISO) with IP before ET (ISO: 46.11 ± 9.2 , IP: 63.43 ± 12.9 $\mu\text{mol/L}$, $p=0.39$) or after ET (ISO: 71.29 ± 15.9 , IP: 91.42 ± 19.6 $\mu\text{mol/L}$, $p=0.52$, Figure 3.1).

Dialysate glycerol was higher than BASE during an acute exercise bout (ACEX: 113.69 ± 17.7 ; $p=0.01$) before ET. Dialysate glycerol was not different than BASE during ACEX (106.08 ± 25.5 ; $p=0.06$) after ET. There was no difference in dialysate glycerol during ACEX before and after ET.

Ethanol Outflow/Inflow

Ethanol outflow/inflow ratios (Eth O/I) are shown in figure 3.2. Before ET, Eth O/I was not different from BASE (0.71 ± 0.05) in response to ISO (0.73 ± 0.05), IP (0.70 ± 0.05), or Ac. Ex (0.67 ± 0.03).

After ET, Eth O/I was not different from BASE (0.78 ± 0.03) in response to ISO (0.79 ± 0.06) and IP (0.65 ± 0.05 , data not shown). Eth O/I was lower than BASE during ACEX (0.63 ± 0.02 ; $p=0.003$).

There were no differences in Eth O/I in any of the treatments before ET compared to after ET.

Protein Content

ATGL expression is detailed in figure 3.1. ATGL increased significantly in response to ACEX before ET (PRE ACEX: 0.16 ± 0.06 , POST ACEX: 2.74 ± 1.0 AU/beta, $p=0.04$). ATGL did not change in response to ACEX after ET (PRE ACEX: 1.23 ± 0.5 ,

POST ACEX: 3.31 ± 1.5 AU/beta, $p=0.22$). Resting ATGL increased in response to 8 weeks of ET (PRE ET: 0.16 ± 0.06 , POST ET: 1.23 ± 0.5 AU/beta, $p=0.05$).

CGI-58 did not respond to AC before ET (PRE ACEX: 0.27 ± 0.1 , POST AC: 0.74 ± 0.5 AU/beta, $p=0.34$) or after ET (PRE ACEX: 0.89 ± 0.6 , POST ACEX: 1.90 ± 1.2 AU/beta, $p=0.34$). Resting CGI-58 did not change in response to 8 weeks of ET (PRE ET: 0.27 ± 0.1 , POST ET: 1.90 ± 1.2 AU/beta, $p=0.43$).

HSL did not respond to ACEX before ET (PRE ACEX: 0.22 ± 0.09 , POST ACEX: 0.30 ± 0.08 AU/beta, $p=0.54$) or after exercise training (PRE ACEX: 0.41 ± 0.2 , POST ACEX: 1.11 ± 0.6 AU/beta, $p=0.34$). Resting HSL did not change in response to 8 weeks of ET (PRE ET: 0.22 ± 0.09 , POST ET: 0.41 ± 0.2 AU/beta, $p=0.35$).

Perilipin increased significantly in response to an AC before ET (PRE ACEX: 0.83 ± 0.2 , POST ACEX: 3.46 ± 1.1 AU/beta, $p=0.05$). Perilipin did not change in response to AC after ET (PRE ACEX: 2.47 ± 0.7 , POST ACEX: 8.68 ± 3.3 AU/beta, $p=0.11$). Resting perilipin increased in response to 8 weeks of ET (PRE ET: 0.83 ± 0.2 , POST ET: 2.47 ± 0.7 AU/beta, $p=0.056$).

	PRE ET	POST ET
Age (yrs)	22.5±0.9	
Height (cm)	177.0±3.2	
Weight (kg)	96.2±5.2	96.2±5.1
Body Mass Index (kg/m²)	30.7±1.2	30.7±1.4
Body Fat (%)	34.9±2.1	34.0±2.6
Fat mass (%)	32.7±3.4	31.7±3.6
Lean Body Mass (kg)	59.9±1.8	60.9±2.3
Waist:Hip	0.9±0.02	0.9±0.03
VO_{2max} (ml/kg/min)	34.8±1.5	38.0±3.4
VO_{2max} (L/min)	3.5±0.2	3.6±0.3
Time to Exhaustion (min)	8.8±0.6	11.1±0.5*

Table 3.1: Subject Characteristics. Time to exhausting during the VO_{2max} test was higher after 8 weeks of exercise training (ET). *p=0.001 Data are expressed as mean±SE Data are expressed as mean±SE

	PRE ET	POST ET
GLU (mg/dl)	92.3±3.5	82.2±2.9
TC (mg/dl)	187.1±14.0	193.7±14.6
TG (mg/dl)	146.4±38.9	153.5±22.4
HDL (mg/dl)	42.3±5.2	43.0±2.3
LDL (mg/dl)	115.6±9.8	119.5±12.0
INS (uIU/ml)	8.5±2.2	8.5±1.9
HOMA	2.0±0.6	1.6±0.4

Table 3.2: Biochemical characteristics. There were no differences in blood chemistry in response to 8 weeks of ET. Data are expressed as mean±SE.

	kcal/day	CHO (g)/day	FAT (g)/day	PRO (g)/day
PRE ET	2331.5±506.1	241.2±46.8	76.8±8.7	122.8±43.4
POST ET	1877.8±184.9	219.3±25.4	75.4±8.6	85.2±13.2

Table 3.3: Nutritional analysis before and after 8 weeks of exercise training (ET). There were no significant differences in caloric intake (kcal/day), carbohydrate (CHO), fat (FAT), or protein (PRO) consumption before and after ET. Data are expressed as mean±SE.

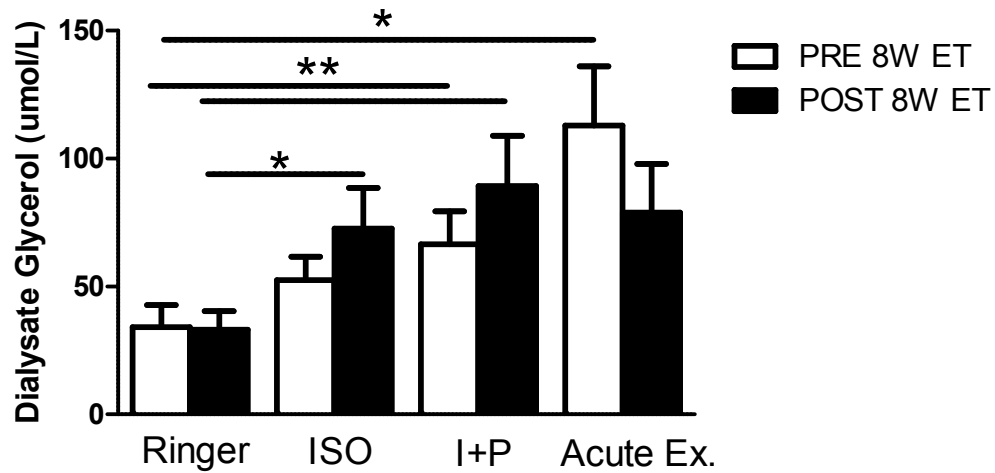


Figure 3.1: Dialysate glycerol concentrations before and after 8 weeks of exercise training (ET) in abdominal subcutaneous adipose tissue in obese (n=8) men. Unstimulated glycerol was not different after 8W ET. Dialysate glycerol was higher than BASE after ET. Dialysate glycerol was higher than BASE during exercise before 8W ET. *p<0.05; different from BASE. Data are expressed as mean±SE.

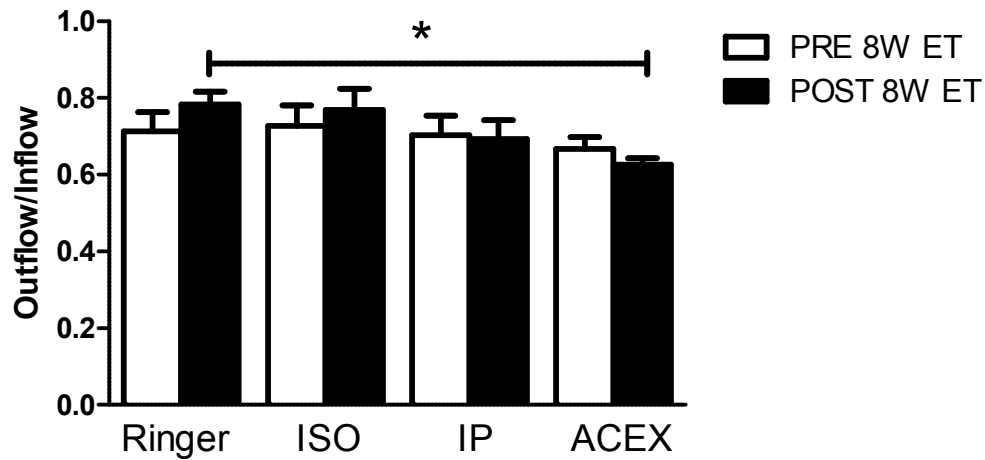


Figure 3.2: Ethanol outflow/Inflow ratios (Eth O/I) before and after 8 Weeks of exercise training (ET) in abdominal subcutaneous adipose tissue in obese (n=8) men. Eth O/I was not different among any of the treatments before ET. Eth O/I during ISO was not different than BASE after ET. Eth O/I during acute exercise (ACEX) was lower than BASE after ET. There were no differences before and after ET among any of the treatments. *p=0.003; different from BASE. Data are expressed as mean±SE.

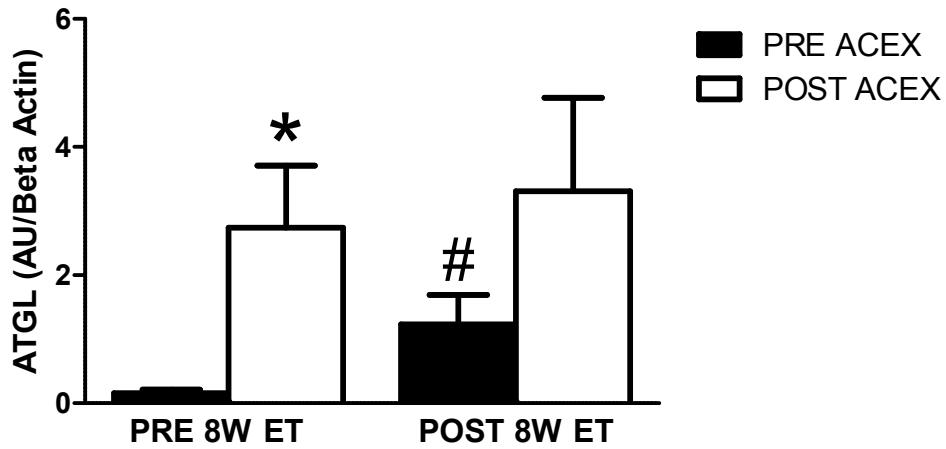


Figure 3.3: Adipose triglyceride (ATGL) protein content in response to an acute exercise bout (ACEX) and 8 weeks of aerobic exercise training (ET) in abdominal subcutaneous adipose tissue in obese (n=8) men. ATGL increased after ACEX before, but not after, ET. Resting ATGL increased in response to ET. * $p < 0.05$; different from PRE ACEX. #: $p < 0.05$; different from PRE ACEX, PRE 8W ET. Data are expressed as mean \pm SE

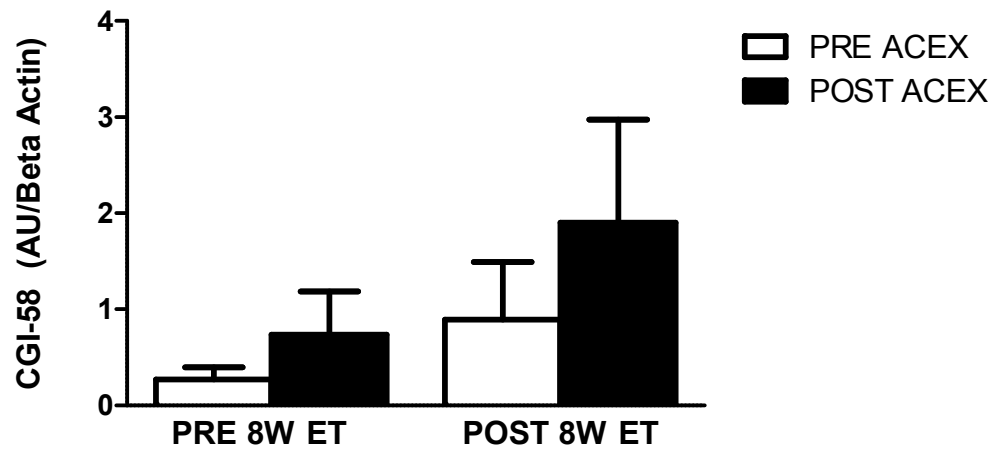


Figure 3.4: Comparative gene identification 58 (CGI-58) expression in response to an acute exercise bout (ACEX) and 8 weeks of exercise training (ET) in abdominal subcutaneous adipose tissue in obese (n=8) men. CGI-58 expression was not different after an acute exercise bout before or after ET. Resting CGI-58 expression was not different in response to ET. Data are expressed at mean \pm SE.

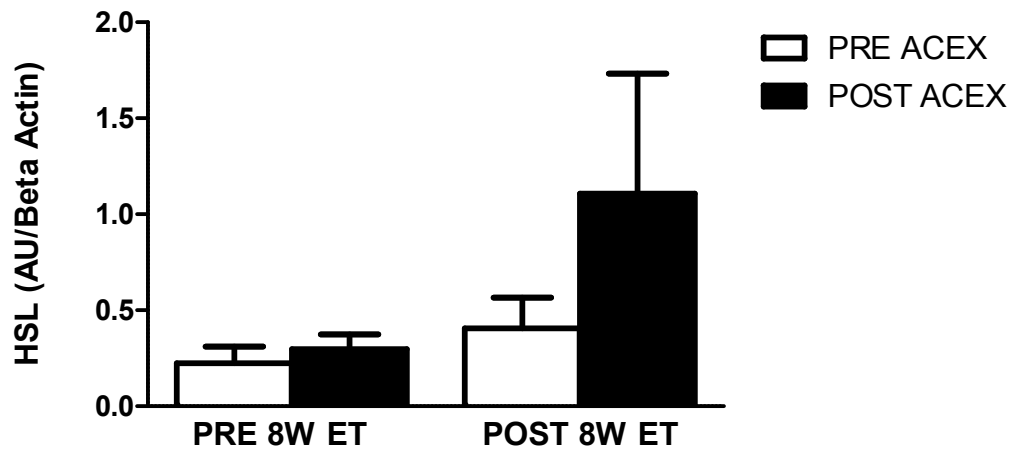


Figure 3.5: Hormone sensitive lipase (HSL) expression before and after 8 weeks of exercise training (ET) in abdominal subcutaneous adipose tissue in obese (n=8) men. HSL content did not increase after an acute exercise bout (ACEX) before or after ET. Resting HSL expression did not change in response to 8 weeks of ET. Data are expressed as mean \pm SE.

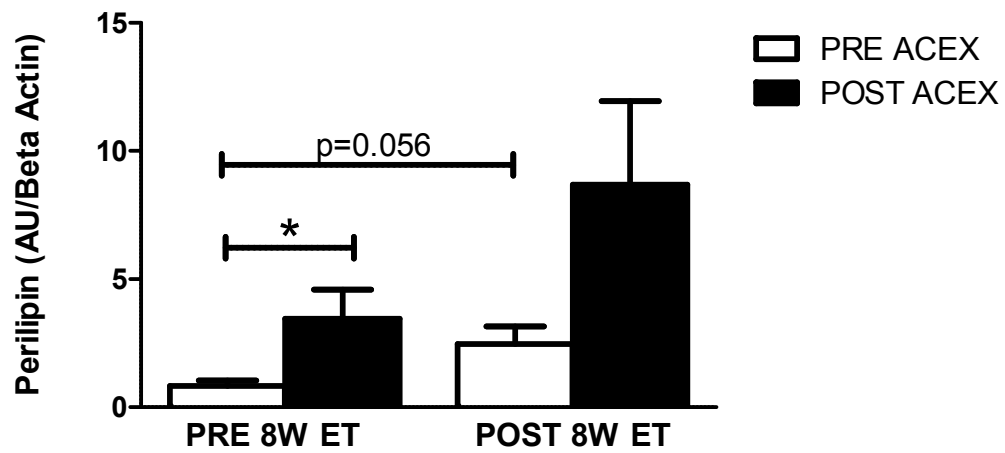


Figure 3.6: Perilipin expression before and after 8 weeks of exercise training (ET) in abdominal subcutaneous adipose tissue in obese (n=8) men. Perilipin expression significantly increased after acute exercise bout (ACEX) before but not after ET. Resting perilipin expression tended to be higher in response to ET ($p=0.056$). $*p=0.05$. Data are expressed as mean \pm SE.

Discussion

We have previously demonstrated that obese men have a blunted lipolytic response to β -adrenergic stimulation compared to lean men. Additionally, we found that sedentary men have a reduced lipolytic response to β -adrenergic stimulation compared to their aerobically trained counterparts (unpublished data). These findings indicate that exercise training may be an effective method to increase β -adrenergic stimulated lipolysis. It was therefore the purpose of this study to investigate the effects of acute exercise and exercise training on lipolysis and the lipolytic proteins ATGL, CGI-58, HSL, and perilipin. At present, this is the only study to evaluate the effects of endurance training and acute exercise on these lipolytic proteins in the SCAT tissue of obese men. Eight weeks of aerobic exercise training (ET) increased β -adrenergic stimulated lipolysis in the SCAT of obese men in this study. Lipolysis increased during an acute exercise bout was higher than baseline before ET but was not different than baseline after ET. Subjects exercised at the same absolute workload before and after ET. Therefore, subjects were exercising at a lower stimulus relative to their maximal aerobic capacity after ET which may explain the lack of increase in lipolysis during acute exercise. Additionally, ATGL protein content in SCAT obtained under fasting, resting conditions increased, and perilipin tended to be higher, after 8 weeks of aerobic ET. Previous studies also concluded that exercise training improves β -adrenergic stimulated lipolysis (9, 43), which agree with the current findings. However, we extend these findings further by finding increases in ATGL, and likely perilipin, which may contribute to increases in β -adrenergic stimulated lipolysis.

In the present study, lipolysis did not increase further in response to α -adrenergic blockade before or after ET. Others believe that the antilipolytic action of α -AR is partially responsible for lower exercise-induced lipolysis in obese individuals, but that exercise training decreased the α -adrenergic suppression of lipolysis (8). We did not find that stimulated lipolysis was hindered by α -adrenergic action in our subjects and therefore, decreases in lipolysis were due to defects in the β -AR stimulated pathway and/or alternative pathways. Additionally, insulin is a potent antilipolytic hormone (2), however, insulin was not different after exercise training. Therefore we don't believe that increases in lipolysis are due to a decrease in the antilipolytic action of insulin.

We found that resting ATGL protein content increased in subcutaneous abdominal adipose tissue in response to ET. ATGL has previously been shown to increase in skeletal muscle in response to 8 weeks of exercise training in overweight men. Interestingly, CGI-58 and HSL did not change in response to ET in skeletal muscle, which agrees with our results in abdominal SCAT (3).

ATGL is hormonally regulated, is essential for providing FFA during exercise, and is required for optimal exercise performance (20). Based on our findings, it seems that ATGL is indeed a key component to lipolysis during exercise. We are confident that exercise training does increase ATGL expression in obese men and that our results are not merely due to an acute elevation in ATGL because an acute exercise bout after ET did not increase ATGL expression. The increase in ATGL is likely a significant component to the improvement in β -adrenergic stimulated lipolysis given that ATGL-null mice have a reduced capacity for catecholamine stimulated lipolysis (16). In the current

study, increased ATGL is likely responsible for the increase in β -adrenergic stimulated lipolysis.

We found that resting perilipin tended ($p=0.056$) to be higher after ET. We also detected an increase in perilipin content in response to an acute bout of exercise prior to 8 weeks of ET, demonstrating the importance of perilipin during exercise. In addition, perilipin protein content tended ($p=0.10$) to increase in response to an acute exercise bout after ET. Though perilipin null mice are leaner than wild-type mice and resist diet-induced obesity, they also have a reduced capacity for stimulated lipolysis (46). In response to PKA stimulation, perilipin coated lipid droplets fragment and disperse throughout the cytosol of adipocytes while lipid droplets lacking perilipin do not fragment at all (6). Perilipin is an essential lipolytic component during stimulated conditions, and likely has a significant function during exercise given the increased expression in response to acute exercise in this study.

It is possible that lipolytic protein activity differs in response to acute exercise and/or exercise training, although we did not measure lipolytic protein activities in this study. The measurement of lipolytic protein activity is a potential area of investigation in future studies.

We believe that increases in ATGL and perilipin are significant contributors to an increased response to β -adrenergic stimulated lipolysis after ET. We are confident that the changes observed in lipolysis, ATGL, and perilipin are due to the isolated effects of exercise and ET and not a result of body composition changes because fat mass and lean body mass were not different after, compared to before, 8 weeks of ET.

Additionally, it is possible that the increases in ATGL and perilipin content at rest are a result of the last exercise bout although subjects were tested at least 12 hours after their last exercise bout.

Other pathways that were not explored in this particular study could play a role in our findings as well. Atrial natriuretic peptide (ANP), released in response to an increase in end diastolic volume (as seen during exercise), promotes lipolysis through the cGMP-dependent phosphorylation of perilipin and HSL (7). Aerobic ET has been shown to increase dialysate glycerol during ANP infusion *in vivo* and has been shown to increase lipolysis with greater efficiency than isoproterenol in isolated adipocytes (32). Therefore, studies evaluating the role of ANP in SCAT lipolysis in relation to perilipin and HSL, in particular, are needed.

Additionally, tumor necrosis factor alpha (TNF α) has been shown to increase lipolysis by inhibiting the antilipolytic effects of insulin, the G_i pathway, and adenosine. However, TNF α has also been shown to degrade perilipin which increases basal lipolysis *in-vitro*. The effects of TNF α on lipolysis and perilipin *in vivo* need to be clarified. TNF α production is increased in obese SCAT and may contribute to a lower stimulated lipolysis in obese SCAT that should be evaluated in future studies (27).

In conclusion, we found, that 8 weeks of aerobic ET increased β -AR stimulated lipolysis in abdominal SCAT in obese men. It seems that the increase in β -AR stimulated lipolysis is largely a result of an increase in ATGL expression and may also be explained by an increase in perilipin. However, future studies should focus on lipolytic protein activity as well as the influence of ANP and TNF α on lipolysis. These

findings may further elucidate the mechanism of training-induced alterations in lipolysis in obese individuals.

Chapter IV

Summary and Conclusions

Our purpose in the present investigations was to determine if lipolysis is hindered in obese and/or sedentary men and if so, can this hindrance be attributed to adipose triglyceride lipase (ATGL), comparative gene identification 58 (CGI-58), hormone sensitive lipase (HSL), and/or perilipin. We believe that 8 weeks of exercise training will increase lipolysis and lipolytic protein content in abdominal SCAT in obese men.

In aim #1, we found that lean exercise-trained men (ET) increased dialysate glycerol release in response to isoproterenol significantly over lean sedentary men (SED). This indicates that exercise training may have a significant impact on stimulated lipolysis. Additionally, isoproterenol stimulated lipolysis was higher in SED (and ET) than in obese sedentary men (OB) indicating a blunted lipolytic response to β -adrenergic stimulation in the obese state. Additionally, resting unstimulated lipolysis in OB was lower than ET men as well. OB are in need of triacylglycerol (TAG) mobilization to reduce adipose tissue stores since obesity is a significant health risk, as it is associated with metabolic and cardiovascular disorders (26). Lipolysis is also hindered in OB under stimulated conditions; this may lead to reduced lipolysis during times of need such as during exercise. To help explain what may be causing this defect in sedentary and obese individuals, we measured lipolytic protein content in ATGL, CGI-58, HSL, and perilipin, which are all involved with lipolysis downstream of β -adrenergic receptors (β -AR). ATGL, CGI-58, and HSL were not different in any of the groups. Perilipin, however, was lower in SED compared to ET but was not different between SED or ET and OB. Perilipin may play a larger role in exercise and exercise training

than originally thought in that exercise training may increase perilipin content in men. Perilipin enhances lipases' ability to hydrolyze TAG by fragmenting the lipid droplet in order to increase the surface area of the lipid droplet (6). Perilipin also increases HSL translocation to the lipid droplet which is essential during stimulated conditions (51). It is possible that an increase in perilipin content enhances the ability to fragment lipid droplets and is able to recruit more HSL to the lipid droplet surface in trained as compared to sedentary and obese men. The activity of perilipin (and other lipolytic proteins) was not measured in this study and is a potential direction to be explored in future studies.

In aim #2 we studied the effects of 8 weeks of exercise training on lipolysis and lipolytic proteins in obese men. Unstimulated resting lipolysis did not change in response to exercise training. Lipolysis was higher during isoproterenol stimulation after, as compared to before, exercise training. It appears that aerobic exercise training does increase stimulated lipolysis. We also found that lipolysis increased in response to an acute exercise bout before exercise training but not after exercise training when the acute exercise bout was performed at the same absolute intensity before and after training. However, blood flow was higher during an exercise exercise bout after ET. A higher blood flow may account for the reduced dialysate glycerol observed during exercise after ET. We found that ATGL and perilipin increased in response to an acute exercise bout before ET while CGI-58 and HSL did not change. Catecholamine stimulation of the β -adrenergic pathway is a primary source of FFA which provides a substrate for ATP production during exercise as shown by the increase in lipolysis during the acute exercise bout (56). In the present study, we observed an increase in β -

adrenergic stimulated lipolysis. This may be attributed to an increase in ATGL and perilipin since, in this study, ATGL increased and perilipin tended to increase (though not significantly) in response to exercise training. CGI-58 and HSL did not change in response to exercise training.

In conclusion, we found that obese individuals have a blunted lipolytic response under stimulated conditions but exercise training improves this defect. The precise components in the lipolytic cascade responsible for this improvement may likely be ATGL and perilipin since perilipin is reduced in lean sedentary men compared to lean exercise trained men. Furthermore, ATGL increases and perilipin tends to increase in response to an acute exercise bout and in response to exercise training. We demonstrate for the first time that ATGL and perilipin are likely responsible, in part, for a decreased stimulated lipolytic response in sedentary obese men, but that this defect may be alleviated with exercise.

Chapter V

References

1. **Ahmadian M, Duncan RE and Sul HS.** The skinny on fat: lipolysis and fatty acid utilization in adipocytes. *Trends Endocrinol.Metab.* 20: 9: 424-428, 2009.
2. **Ahmadian M, Wang Y and Sul HS.** Lipolysis in adipocytes. *Int.J.Biochem.Cell Biol.* 42: 5: 555-559, 2010.
3. **Alsted TJ, Nybo L, Schweiger M, Fledelius C, Jacobsen P, Zimmermann R, Zechner R and Kiens B.** Adipose Triglyceride Lipase in human skeletal muscle is upregulated by exercise training. *Am.J.Physiol.Endocrinol.Metab.* 2008.
4. **American College of Sports Medicine.** *ACSM's Guidelines for Exercise Testing and Prescription.* Philadelphia, PA: Lippincott Williams & Wilkins, 2005, p. 366.
5. **Arner P and Langin D.** The role of neutral lipases in human adipose tissue lipolysis. *Curr.Opin.Lipidol.* 18: 3: 246-250, 2007.
6. **Brasaemle DL.** Thematic review series: adipocyte biology. The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis. *J.Lipid Res.* 48: 12: 2547-2559, 2007.
7. **Carmen GY and Victor SM.** Signalling mechanisms regulating lipolysis. *Cell.Signal.* 18: 4: 401-408, 2006.

8. **De Glisezinski I, Crampes F, Harant I, Berlan M, Hejnova J, Langin D, Riviere D and Stich V.** Endurance training changes in lipolytic responsiveness of obese adipose tissue. *Am.J.Physiol.* 275: 6 Pt 1: E951-6, 1998.
9. **de Glisezinski I, Moro C, Pillard F, Marion-Latard F, Harant I, Meste M, Berlan M, Crampes F and Riviere D.** Aerobic training improves exercise-induced lipolysis in SCAT and lipid utilization in overweight men. *Am.J.Physiol.Endocrinol.Metab.* 285: 5: E984-90, 2003.
10. **Despres JP, Bouchard C, Savard R, Tremblay A, Marcotte M and Theriault G.** Level of physical fitness and adipocyte lipolysis in humans. *J.Appl.Physiol.* 56: 5: 1157-1161, 1984.
11. **Ducharme NA and Bickel PE.** Lipid droplets in lipogenesis and lipolysis. *Endocrinology* 149: 3: 942-949, 2008.
12. **Fernandez C, Hansson O, Nevsten P, Holm C and Klint C.** Hormone-sensitive lipase is necessary for normal mobilization of lipids during submaximal exercise. *Am.J.Physiol.Endocrinol.Metab.* 295: 1: E179-86, 2008.
13. **Gade W, Gade J, Collins M, Schmit J and Schupp N.** Failures of feedback: rush hour along the highway to obesity. *Clin.Lab.Sci.* 23: 1: 39-50, 2010.
14. **Granneman JG and Moore HP.** Location, location: protein trafficking and lipolysis in adipocytes. *Trends Endocrinol.Metab.* 19: 1: 3-9, 2008.
15. **Granneman JG, Moore HP, Granneman RL, Greenberg AS, Obin MS and Zhu Z.** Analysis of lipolytic protein trafficking and interactions in adipocytes. *J.Biol.Chem.* 282: 8: 5726-5735, 2007.

16. **Haemmerle G, Lass A, Zimmermann R, Gorkiewicz G, Meyer C, Rozman J, Heldmaier G, Maier R, Theussl C, Eder S, Kratky D, Wagner EF, Klingenspor M, Hoefler G and Zechner R.** Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science* 312: 5774: 734-737, 2006.
17. **Haemmerle G, Zimmermann R, Hayn M, Theussl C, Waeg G, Wagner E, Sattler W, Magin TM, Wagner EF and Zechner R.** Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. *J.Biol.Chem.* 277: 7: 4806-4815, 2002.
18. **Harada K, Shen WJ, Patel S, Natu V, Wang J, Osuga J, Ishibashi S and Kraemer FB.** Resistance to high-fat diet-induced obesity and altered expression of adipose-specific genes in HSL-deficient mice. *Am.J.Physiol.Endocrinol.Metab.* 285: 6: E1182-95, 2003.
19. **Hickner RC, Rosdahl H, Borg I, Ungerstedt U, Jorfeldt L and Henriksson J.** The ethanol technique of monitoring local blood flow changes in rat skeletal muscle: implications for microdialysis. *Acta Physiol.Scand.* 146: 1: 87-97, 1992.
20. **Huijsman E, van de Par C, Economou C, van der Poel C, Lynch GS, Schoiswohl G, Haemmerle G, Zechner R and Watt MJ.** Adipose triacylglycerol lipase deletion alters whole body energy metabolism and impairs exercise performance in mice. *Am.J.Physiol.Endocrinol.Metab.* 297: 2: E505-13, 2009.
21. **Imbeault P, Tremblay A, Despres J and Mauriege P.** Beta-Adrenoceptor-Stimulated Lipolysis of Subcutaneous Abdominal Adipocytes as a Determinant of Fat Oxidation in Obese Men. *Eur.J.Clin.Invest.* 30: 4: 290-296, 2000.
22. **Jaworski K, Sarkadi-Nagy E, Duncan RE, Ahmadian M and Sul HS.** Regulation of Triglyceride Metabolism. * IV. Hormonal regulation of lipolysis in adipose tissue. *Am.J.Physiol.Gastrointest.Liver Physiol.* 293: 1: G1-4, 2007.

23. **Jocken JW and Blaak EE.** Catecholamine-induced lipolysis in adipose tissue and skeletal muscle in obesity. *Physiol.Behav.* 2008.
24. **Jocken JW, Goossens GH, van Hees AM, Frayn KN, van Baak M, Stegen J, Pakbiers MT, Saris WH and Blaak EE.** Effect of beta-adrenergic stimulation on whole-body and abdominal subcutaneous adipose tissue lipolysis in lean and obese men. *Diabetologia* 51: 2: 320-327, 2008.
25. **Kern PA, Di Gregorio G, Lu T, Rassouli N and Ranganathan G.** Perilipin expression in human adipose tissue is elevated with obesity. *J.Clin.Endocrinol.Metab.* 89: 3: 1352-1358, 2004.
26. **Langin D.** Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. *Pharmacol.Res.* 53: 6: 482-491, 2006.
27. **Langin D and Arner P.** Importance of TNFalpha and neutral lipases in human adipose tissue lipolysis. *Trends Endocrinol.Metab.* 17: 8: 314-320, 2006.
28. **Langin D, Dicker A, Tavernier G, Hoffstedt J, Mairal A, Ryden M, Arner E, Sicard A, Jenkins CM, Viguerie N, van Harmelen V, Gross RW, Holm C and Arner P.** Adipocyte lipases and defect of lipolysis in human obesity. *Diabetes* 54: 11: 3190-3197, 2005.
29. **Large V, Reynisdottir S, Langin D, Fredby K, Klannemark M, Holm C and Arner P.** Decreased expression and function of adipocyte hormone-sensitive lipase in subcutaneous fat cells of obese subjects. *J.Lipid Res.* 40: 11: 2059-2066, 1999.
30. **Martinez-Botas J, Anderson JB, Tessier D, Lapillonne A, Chang BH, Quast MJ, Gorenstein D, Chen KH and Chan L.** Absence of perilipin results in leanness and reverses obesity in *Lepr(db/db)* mice. *Nat.Genet.* 26: 4: 474-479, 2000.

31. **Miyoshi H, Souza SC, Zhang HH, Strissel KJ, Christoffolete MA, Kovsan J, Rudich A, Kraemer FB, Bianco AC, Obin MS and Greenberg AS.** Perilipin promotes hormone-sensitive lipase-mediated adipocyte lipolysis via phosphorylation-dependent and -independent mechanisms. *J.Biol.Chem.* 281: 23: 15837-15844, 2006.
32. **Moro C, Pillard F, De Glisezinski I, Harant I, Riviere D, Stich V, Lafontan M, Crampes F and Berlan M.** Training enhances ANP lipid-mobilizing action in adipose tissue of overweight men. *Med.Sci.Sports Exerc.* 37: 7: 1126-1132, 2005.
33. **Mottagui-Tabar S, Ryden M, Lofgren P, Faulds G, Hoffstedt J, Brookes AJ, Andersson I and Arner P.** Evidence for an important role of perilipin in the regulation of human adipocyte lipolysis. *Diabetologia* 46: 6: 789-797, 2003.
34. **Newman JM, Di Maria CA, Rattigan S and Clark MG.** Nutritive blood flow affects microdialysis O/I ratio for [(14)C]ethanol and (3)H(2)O in perfused rat hindlimb. *Am.J.Physiol.Heart Circ.Physiol.* 281: 6: H2731-7, 2001.
35. **Ogden CL, Carroll MD, Curtin LR, Lamb MM and Flegal KM.** Prevalence of high body mass index in US children and adolescents, 2007-2008. *JAMA* 303: 3: 242-249, 2010.
36. **Petridou A, Tsalouhidou S, Tsalis G, Schulz T, Michna H and Mougios V.** Long-term exercise increases the DNA binding activity of peroxisome proliferator-activated receptor gamma in rat adipose tissue. *Metabolism* 56: 8: 1029-1036, 2007.
37. **Reynisdottir S, Wahrenberg H, Carlstrom K, Rossner S and Arner P.** Catecholamine resistance in fat cells of women with upper-body obesity due to decreased expression of beta 2-adrenoceptors. *Diabetologia* 37: 4: 428-435, 1994.

38. **Reynisdottir S, Wahrenberg H, Carlstrom K, Rossner S and Arner P.** Catecholamine resistance in fat cells of women with upper-body obesity due to decreased expression of beta 2-adrenoceptors. *Diabetologia* 37: 4: 428-435, 1994.
39. **Schoiswohl G, Schweiger M, Schreiber R, Gorkiewicz G, Preiss-Landl K, Taschler U, Zierler KA, Radner FP, Eichmann TO, Kienesberger PC, Eder S, Lass A, Haemmerle G, Alsted TJ, Kiens B, Hoefler G, Zechner R and Zimmermann R.** Adipose triglyceride lipase plays a key role in the supply of the working muscle with fatty acids. *J.Lipid Res.* 51: 3: 490-499, 2010.
40. **Schweiger M, Schreiber R, Haemmerle G, Lass A, Fledelius C, Jacobsen P, Tornqvist H, Zechner R and Zimmermann R.** Adipose triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue triacylglycerol catabolism. *J.Biol.Chem.* 281: 52: 40236-40241, 2006.
41. **Smirnova E, Goldberg EB, Makarova KS, Lin L, Brown WJ and Jackson CL.** ATGL has a key role in lipid droplet/adiposome degradation in mammalian cells. *EMBO Rep.* 7: 1: 106-113, 2006.
42. **Steinberg GR, Kemp BE and Watt MJ.** Adipocyte triglyceride lipase expression in human obesity. *Am.J.Physiol.Endocrinol.Metab.* 2007.
43. **Stich V, de Glisezinski I, Galitzky J, Hejnova J, Crampes F, Riviere D and Berlan M.** Endurance training increases the beta-adrenergic lipolytic response in subcutaneous adipose tissue in obese subjects. *Int.J.Obes.Relat.Metab.Disord.* 23: 4: 374-381, 1999.
44. **Stich V, Hejnova J, Sulkovicova H, Hainer V and Kunesova M.** Microdialysis in investigation of adipose tissue metabolism. *Sb.Lek.* 99: 3: 227-234, 1998.

45. **Sztalryd C, Xu G, Dorward H, Tansey JT, Contreras JA, Kimmel AR and Londos C.** Perilipin A is essential for the translocation of hormone-sensitive lipase during lipolytic activation. *J.Cell Biol.* 161: 6: 1093-1103, 2003.
46. **Tansey JT, Sztalryd C, Gruia-Gray J, Roush DL, Zee JV, Gavrilova O, Reitman ML, Deng CX, Li C, Kimmel AR and Londos C.** Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proc.Natl.Acad.Sci.U.S.A.* 98: 11: 6494-6499, 2001.
47. **Wang Y, Sullivan S, Trujillo M, Lee MJ, Schneider SH, Brolin RE, Kang YH, Werber Y, Greenberg AS and Fried SK.** Perilipin expression in human adipose tissues: effects of severe obesity, gender, and depot. *Obes.Res.* 11: 8: 930-936, 2003.
48. **Wang Y, Sullivan S, Trujillo M, Lee MJ, Schneider SH, Brolin RE, Kang YH, Werber Y, Greenberg AS and Fried SK.** Perilipin expression in human adipose tissues: effects of severe obesity, gender, and depot. *Obes.Res.* 11: 8: 930-936, 2003.
49. **Watt MJ, Holmes AG, Pinnamaneni SK, Garnham AP, Steinberg GR, Kemp BE and Febbraio MA.** Regulation of HSL serine phosphorylation in skeletal muscle and adipose tissue. *Am.J.Physiol.Endocrinol.Metab.* 290: 3: E500-8, 2006.
50. **Watt MJ, Holmes AG, Steinberg GR, Mesa JL, Kemp BE and Febbraio MA.** Reduced plasma FFA availability increases net triacylglycerol degradation, but not GPAT or HSL activity, in human skeletal muscle. *Am.J.Physiol.Endocrinol.Metab.* 287: 1: E120-7, 2004.
51. **Watt MJ and Steinberg GR.** Regulation and function of triacylglycerol lipases in cellular metabolism. *Biochem.J.* 414: 3: 313-325, 2008.

52. **Yamaguchi T.** Crucial role of CGI-58/alpha/beta hydrolase domain-containing protein 5 in lipid metabolism. *Biol.Pharm.Bull.* 33: 3: 342-345, 2010.
53. **Yamaguchi T, Omatsu N, Matsushita S and Osumi T.** CGI-58 interacts with perilipin and is localized to lipid droplets. Possible involvement of CGI-58 mislocalization in Chanarin-Dorfman syndrome. *J.Biol.Chem.* 279: 29: 30490-30497, 2004.
54. **Yamaguchi T, Omatsu N, Morimoto E, Nakashima H, Ueno K, Tanaka T, Satouchi K, Hirose F and Osumi T.** CGI-58 facilitates lipolysis on lipid droplets but is not involved in the vesiculation of lipid droplets caused by hormonal stimulation. *J.Lipid Res.* 48: 5: 1078-1089, 2007.
55. **Yen CL and Farese RV,Jr.** Fat breakdown: a function for CGI-58 (ABHD5) provides a new piece of the puzzle. *Cell.Metab.* 3: 5: 305-307, 2006.
56. **Zechner R, Strauss JG, Haemmerle G, Lass A and Zimmermann R.** Lipolysis: pathway under construction. *Curr.Opin.Lipidol.* 16: 3: 333-340, 2005.
57. **Zimmermann R, Lass A, Haemmerle G and Zechner R.** Fate of fat: The role of adipose triglyceride lipase in lipolysis. *Biochim.Biophys.Acta* 2008.

Appendix A

IRB Approval



University and Medical Center Institutional Review Board
East Carolina University • Brody School of Medicine
600 Moyer Boulevard • Old Health Sciences Library, Room 1L-09 • Greenville, NC 27834
Office 252-744-2914 • Fax 252-744-2284 • www.ecu.edu/irb
Chair and Director of Biomedical IRB: L. Wiley Nifong, MD
Chair and Director of Behavioral and Social Science IRB: Susan L. McCammon, PhD

TO: Robert Hickner, PhD, Department of EXSS, ECU, 363 Ward Sports Medicine Bldg.
FROM: UMCIRB *[Signature]*
DATE: October 5, 2009
RE: Full Committee Approval for Continuing Review of a Research Study Requiring Modifications
TITLE: Exercise Training and Caloric Restriction: Influence on Lipolysis and Associated Proteins

UMCIRB #08-0493

The above referenced research study was initially reviewed by the convened University and Medical Center Institutional Review Board (UMCIRB) on 10/8/08. The research study underwent a subsequent continuing review for approval on 9/23/09 by the convened UMCIRB. Requested modifications were prescribed and received final approval on 10/1/09 by the UMCIRB Chairperson. The UMCIRB deemed this unfunded study more than minimal risk requiring a continuing review in **12 months**. Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The investigator must adhere to all reporting requirements for this study.

The above referenced research study has been given approval for the period of **9/23/09 to 9/22/10**. The approval includes the following items:

- Continuing Review Form (dated 9/13/09)
- Protocol Summary (dated 3/20/09)
- Informed consent (dated 9/28/09)
- Medical History Questionnaire
- Advertisement

The following UMCIRB members were recused for reasons of potential for Conflict of Interest on this research study: R. Hickner

NOTE: The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting: None

The UMCIRB applies 45 CFR 46, Subparts A-D, to all research reviewed by the UMCIRB regardless of the funding source. 21 CFR 50 and 21 CFR 56 are applied to all research studies under the Food and Drug Administration regulation. The UMCIRB follows applicable International Conference on Harmonisation Good Clinical Practice guidelines.

Filename: Dissertation-E
Directory: C:\Users\Emily Johnson\Documents
Template: C:\Users\Emily
Johnson\AppData\Roaming\Microsoft\Templates\Normal.dotm
Title: -
Subject:
Author: Emily Johnson
Keywords:
Comments:
Creation Date: 7/9/2010 11:20:00 AM
Change Number: 7
Last Saved On: 7/15/2010 9:16:00 AM
Last Saved By: Emily Johnson
Total Editing Time: 39 Minutes
Last Printed On: 7/15/2010 9:17:00 AM
As of Last Complete Printing
Number of Pages: 91
Number of Words: 15,844 (approx.)
Number of Characters: 90,313 (approx.)