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# Comparison of the Effects of Energy Flux on Metabolic Conditions and Satiety in Young Adults

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by

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# DEDICATION

To my parents, Molly and George and my family and friends for their unwavering support towards my pursuit of higher education.

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# ABSTRACT

Within the United States, 68% of all adults have been classified as overweight to obese, as defined by 25 $kg/m^2 \leq$ BMI $\leq$ 35 $kg/m^2$. Research has shown that overweight/obese individuals can still gain health benefits through participation in aerobic activity without losing weight. These improvements include improved glucose control, CRF, HDL and quality of life. More research is necessary to understand the health benefits associated with a chronic aerobic intervention under weight stable conditions. We therefore examined the health benefits of an aerobic 6-month intervention under weight stable conditions on body composition, cholesterol and measurements of appetite and satiety on young, previously sedentary adults.

This dissertation is comprised of three study aims that were designed to 1) determine a possible association between an increase in energy expenditure and body composition and fitness; 2) examine the association between changes in energy expenditure and body composition to produce alterations in blood lipids and C-reactive protein; 3) analyze an association between changes in energy expenditure and body composition on objective and subjective measurements of appetite. Data used in these studies was drawn from the Energy Flux Study, a randomized controlled trial completed in Columbia, SC involving young, previously sedentary adults (n=81). We determined energy expenditure over a 10-day wear period using SenseWear mini armbands at baseline and upon intervention completion. Body composition (primarily fat mass and fat

free mass) were measured using a dual X-ray absorptiometry scanner (DXA), percent body fat was determined using %BF= (total fat/body weight). Graded exercise tests (GXT) used a modified Bruce protocol in which participants worked to volitional fatigue on a motorized treadmill. Blood was collected from an antecubital vein in either EDTA (1.25 mg/dL) and/or aprotinin (500 TU/mL) tubes (dependent on analyses). The exercise intervention had participants complete between 3-6 exercise sessions per week for approximately one hour at 70-75% $VO_{2max}$ HR for 6 months. Throughout the intervention, participants were required to maintain their weight ±3% of their baseline body weight.

Study 1 found female participants showed improvements in their body composition with increases in vigorous activity only. While no other significant changes were seen in body composition through increases in energy expenditure, those who participated in an exercise program increased their cardiorespiratory fitness.

Study 2 found a decrease in fat mass through participation in an aerobic intervention will improve cholesterol levels as compared to changes in EE. This was further identified in those who displayed a beneficial change in their lipid profile. Those individuals who improved their lipid profile showed a greater decrease in FM as compared to the group who worsened their lipid profile.

Study 3 found a decrease in fasting and non-fasting leptin in male and female exercise participants from baseline to follow-up. Females showed a significant decrease in fat mass and percent body fat at 6-months. Changes in FM were significantly associated with

fasting and non-fasting leptin in males only. Limited significance is seen in subjective measures for appetite and hunger.

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## LIST OF ABBREVIATIONS

ACSM ........ American College of Sports Medicine

BF ........ Body fat

BMI ........ Body Mass Index

CHD ........ Coronary Heart Disease

CRF ........ Cardiorespiratory fitness

CRP ........ C-reactive protein

DXA ........ Dual X-ray absorptiometry

ECG ........ Electrocardiogram

EE ........ Energy expenditure

EI ........ Energy intake

FEX ........ Female exercise participants

GXT ........ Graded exercise test

HDL-C ........ high density lipoprotein-cholesterol

HR ........ Heart rate

kg ........ kilogram

LDL-C ........ low density lipoprotein-cholesterol

MEX ........ Male exercise participants

MVPA ........ Moderate-to-vigorous activity

RPE ........ Rate of perceived exertion

TC ........ Total cholesterol

TDEE ........ Total daily energy expenditure

TG ........................................................................................................................ Triglyceride

VAS....................................................................................................... Visual Analogue Scale

VLDL-C..................................................................... Very low density lipoprotein-cholesterol

VPA......................................................................................... Vigorous physical activity

# CHAPTER ONE

## OVERALL INTRODUCTION

Over the past two decades, obesity has nearly doubled; affecting both children and adults, no matter the race or the sex of the individual (99). While this prevalence has currently plateaued, the financial burden (31) and increased likelihood of several chronic diseases (18) continues to place a major burden on many health professionals. Obesity has been defined as a mismatch in human physiology and the surrounding environment (67). The internal regulation of energy homeostasis has been termed energy balance, or the regulation of energy expenditure and energy intake. Environmental factors play key roles in decreasing levels of energy expenditure (by means of physical activity) in occupation (20) and household management (4). These shifts in energy balance due to lifestyle choices in either energy expenditure and/or energy intake may lead to weight gain. A positive energy balance, which is an increase in energy intake over energy expenditure, constitutes weight gain over a duration of time. Conversely, a negative energy balance, or higher energy expenditure than energy intake promotes weight loss.

Energy balance is able to be maintained at different rates of energy expenditure and energy intake, which is known as energy flux. High energy flux pairs increased energy expenditure with caloric intake, while a low energy flux is defined as low energy expenditure and low energy intake. More than 50 years ago, Jean Mayer defined the benefits of high energy flux as a tool for weight maintenance (58). Within his research, he discovered males were able to better regulate energy intake in more active occupations compared to more sedentary individuals. The importance of the participation in high levels of physical activity has also been studied within the National Weight Control Registry. Within this sample, those who were able to successfully maintain their weight over one year duration (72% of the sample) reported physical activity levels above the American College of Sports Medicine (ACSM) recommendation of 1000 kcal/week (38). This dissertation consists of three studies that have been developed to better understand several health parameters associated with participation in a 6-month aerobic intervention under weight stable conditions. These studies were designed to analyze 1) the relationship between increased energy expenditure with body composition and fitness 2) the association with energy expenditure and body composition with blood lipids and C-reactive protein (CRP) 3) the association with energy expenditure and body composition on objective and subjective levels of appetite and hunger.

## Statement of the Problem

Obesity, or the accumulation of fat mass is associated with an increased risk with several chronic diseases such as stroke, type II diabetes, cardiovascular disease and some cancers. It is important to understand the associations with an aerobic exercise intervention while maintaining weight in adults.  In order to investigate this problem further research is needed to understand the effects of increased energy expenditure on body composition, blood lipids and assessments of appetite.

## Scope of the Study

The overall goal of these studies is to 1) identify possible effects of energy expenditure on body composition in addition to cardiorespiratory fitness, 2) demonstrate an association of energy expenditure and body composition on metabolic markers, and 3) understand the prospective association of increased energy expenditure on satiety levels using objective and subjective measurements.

Specific Aims and Research Questions

*Paper 1*

Evaluating the association of energy expenditure with body composition and fitness, under weight stable conditions among adult males and females.

Aims:

A. Association between change in energy expenditure and body composition.

B. Association between change in energy expenditure and cardiorespiratory fitness.

C. Analyzing separately for sex to determine any potential differences.

*Paper 2*

Evaluating the association of change in energy expenditure and body composition with metabolic markers under weight stable conditions in adult males and females.

Aims:

A. Association between change in energy expenditure and body composition with metabolic markers.

B. Determine any differences in metabolic markers between sex and compare exercisers vs. controls.

C. Evaluate the difference in those who improved their lipid profile with those who worsened their lipid profile throughout the intervention.

*Paper 3*

Evaluating the prospective association of energy flux on satiety levels in adult males and females.

Aims:

A. Association between energy expenditure and body composition with changes in fasting and non-fasting leptin and ghrelin during an aerobic exercise intervention.

B. Association between energy expenditure and body composition with changes in fasting and non-fasting VAS scores (question 1-4) during an aerobic exercise intervention.

C. Determine a difference in sex within each of the objective and subjective score changes throughout the intervention.

# CHAPTER TWO

## LITERATURE REVIEW

Obesity has a variety of definitions, however it is easily defined as the mismatch between human physiology and the environment in which we live (67). This definition includes not only the caloric intake and expenditure on a day-to-day basis, but also the conflicts of the present day environment. Obesity has become a major health concern not only for those adults and children living in the United States, but also for many countries across the globe (49). Overweight and obese adults have an abnormal or excessive fat accumulation and have been defined as a body mass index (BMI) score greater than 25 $kg/m^2$ (18). This rise is becoming a pivotal health burden, as overweight and obesity increase the likelihood of certain diseases such as coronary heart disease, type II diabetes, stroke and some forms of cancer (18). Even with continued concern for this epidemic, projections show by 2030, more than half of U.S. adults may be obese (100). The imbalance between energy intake and energy expenditure (i.e. energy balance), is the underlying cause for the continued rise in weight gain. Energy balance is comprised of an assortment of variables including biological, environmental and behavioral which can disrupt body weight regulation, or energy balance (40).

Throughout the day, there is a constant shift in energy intake, or calories consumed and energy expenditure, or the calories burned from either participation in physical activity or daily living. As humans, our physiology encourages the matching of energy intake and expenditure to promote energy homeostasis.  A positive energy balance or higher energy intake than energy expenditure, results in weight gain, whereas a negative energy balance is associated with expenditure exceeding intake.

In addition to the growing rise in overweight and obese adults in the United States, it has been well documented that more than half of all adults are not meeting the 2008 Physical Activity Guidelines (18). Many occupations require sedentary and sitting behavior as opposed to moderate-intensity activities, decreasing occupation-related energy expenditure by more than 100 calories in men and women (20). In addition, less time allocated to household management with the development of technology has considerably lowered physical activity and substantially increased sedentary behavior (4). The combined increase of occupational and household sedentary behavior has developed a continued reduction in physical activity for several decades.

## Components of Energy Balance

Energy balance is built upon the interaction of energy stores, energy expenditure and energy intake. These components hold a complex relationship and cannot be thought of as separate entities. Energy expenditure encompasses resting energy expenditure, thermic effect of food and non-resting energy expenditure (through daily living and physical activity) (35). Energy expenditure at rest comprises two-thirds of total energy expenditure in a sedentary population, which can be determined from body composition (35). Fat free mass accounts for most of the resting energy expenditure (47, 71), however based off the composition of each individual, this greatly varies. Energy intake is the consumption of all foods and beverages per day and it greatly affected by physiological constraints including body composition that influence appetite and satiety. The National Academy of Science established dietary references for macronutrients (60). As this literature review discusses an average age of 27 years within the EFS, each recommendation is based off an age group between 19-30 years. Recommendations include: 130 grams of carbohydrates/day, total fiber of 38 g/day in males and 25 g/day in females, total fat between 20-35 g/day in males and females and 56 g/day of protein in males and 46 g/day in females. The question still remains as to whether caloric intake or energy expenditure (by means of physical activity) holds a higher responsibility within the energy balance equation to account for obesity.

### Energy Flux

Energy balance can be maintained at different rates, depending on energy expenditure and energy intake. This turnover rate is referred to as energy flux and was defined several decades ago by Mayer et al (58). Within this study, Mayer saw that those whose occupation demanded higher energy expenditure consumed the same amount of calories as those classified in a sedentary occupation, assuming similar body composition. Mayer found that being more sedentary does not down regulate caloric intake. Furthermore, more recently it was shown that those who expended and consumed high levels of energy were able to maintain their weight over the duration of one year compared to more sedentary individuals (83). The ability to maintain a high energy flux, that is the pairing of high expenditure and intake, may facilitate weight maintenance for long periods of time. Further understanding of the levels of energy flux can define various health outcomes associated with increased energy expenditure.

### Weight Loss and Weight Maintenance

The American Heart Association recommends weight loss in overweight/obese patients to reduce the risk of cardiovascular disease (51). While many interventions focus on PA and/or diet with weight loss, limited research exists regarding weight maintenance. Weight loss companies push for strict caloric reduction and prove to be very costly. It has been reported that about half of all able to lose weight, were able to maintain the weight loss for at least one year through a combination of PA and diet (69, 96). To help combat the differing ideas, The National Weight Control Registry was established to further identify weight maintenance strategies from those who were able to maintain at least 30 pounds of weight loss (98). Of this sample, the most common strategies for weight maintenance included: high levels of physical activity and low calorie and fat intake (90). It is important to note, weight maintenance was accomplished by the combination of dietary alterations and increase energy expenditure through physical activity. Therefore, energy expenditure and energy intake are two very interdependent variables when discussing weight loss and weight maintenance.

More research is necessary to define the benefits of exercise under weight stable conditions. Swift et al. reviewed a collection of clinical trials for exercise training in which little to no weight was lost throughout the intervention period (89). Even without weight loss, health benefits such as improved cardiovascular respiratory fitness (CRF), glucose control, endothelial function, lipoprotein particle size, high density lipoprotein (HDL-C) and quality of life improved due to participation in an exercise program (89). In addition, more recent research compared two forms of weight loss (dietary or exercise) and an exercise group without weight loss to identify metabolic efficiency and fat distribution (75, 77). These findings determined that even without weight loss, a decrease was seen in abdominal fat in males and females, overall reducing future risk of obesity.

Body Composition and Aerobic Exercise

Alterations in body composition from exercise have been well documented. A recent review compared the effects of aerobic and resistance training in overweight/obese individuals. Schwingshackl et al. found that those classified as overweight (BMI>25 $kg/m^2$), who participated in an aerobic training intervention and showed improvements in $VO_{2max}$ had greater reductions in body weight, waist circumference and fat mass when compared to a resistance training intervention (81). However it is critical to point out the changes in fitness may be due to reductions in body weight as results are commonly reported to body weight.

Chronic aerobic training has been reviewed in a 16 month training study in which participants completed aerobic exercise and body composition measurements (28). As compared to the control participants, those who completed 16 months of aerobic training had a significant decrease in body weight, fat mass and BMI as compared to those in the control group. Differing results were seen for males and females. Exercising males were in negative energy balance (350 kcal/day), lost an average of 5.2 kilograms (kg) with about 96% of weight loss coming from body fat (28). Similarly, exercising females were in a negative energy balance (200 kcal/day) and while they did not show comparable weight loss, exercising females maintained weight and had a 3% decrease in total body fat as compared to controls (28). Therefore, chronic exercise prescriptions have large effects on body composition in overweight males and females, but weight loss may differ between sex.

Body composition cannot be solely determined by an aerobic intervention. A recent study conducted by Ross et al. completed a 12-week intervention in which participants were either placed in a diet induced weight loss group, exercise induced weight loss group or an exercise group without weight loss (75, 77). This study is of critical clinical significance by its ability to encompass both the effects of energy intake and expenditure measured over duration of time. Male participants in either the diet or exercise-induced weight loss groups showed reductions in abdominal obesity, visceral fat, and waist circumference (75). Similarly, female participants in the diet induced and exercise without weight loss groups showed a decrease in total and abdominal fat as compared to the controls (77). More research is necessary to determine other health benefits such as lipids and metabolic alterations under chronic aerobic training in weight stable conditions.

A review of the association between energy expenditure and body fatness found a linear association with exercise and body fatness. As exercise energy expenditure increases, body fatness decreases. However, this relationship was only seen in short term studies (76). Fundamental research determined the association between physical fitness and mortality. Blair et al. identified those who had a lower fitness level were placed at a higher risk of death as compared to more fit males and females (11). Coinciding with this review, much research has been placed on the idea in which body fatness has had an effect on mortality. However, recent evidence has further defined the fact that body fatness and fitness are related. Barry et al. stated that those who are unfit, or a low CRF have twice the risk of death regardless of ones BMI (7). Furthermore, those classified as either overweight, or obese but who are deemed fit, have similar mortality risk as those in the same fitness category who are normal weight (7).

Impacts of Dyslipidemia

Cholesterol is a key component to a functioning system, as it is present in cell membranes and is a precursor to steroid hormones (63). Lipoproteins, formed from lipids and proteins carry cholesterol throughout the blood stream and are identified as three major classes: very low density lipoprotein (VLDL-C), low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) (63). While cholesterol is a significant component of the physiological system, any disruption of homeostasis within this system can be associated with risk for chronic disease. Dyslipidemia is defined as increased fasting concentrations of total cholesterol (TC), LDL-C and triglycerides (TG), in addition to decreased concentrations in HDL-C (63) and has been linked as one of the most modifiable risk factors for coronary heart disease (94). High levels of serum total cholesterol are identified as a major risk factor for the development of coronary heart disease (1, 24).

Many patients who present with dyslipidemia are initially given medication to resolve their heightened lipid levels. While at times, pharmacological therapy may be the only option, it is imperative to provide resources to those who may be able to make lifestyle alterations. The Third Report of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) recommends lifestyle therapy, such as alterations in diet and exercise, for patients who fall into an intermediate range of cardiovascular heart disease (CHD) risk (63).

Cholesterol levels have also been identified with activity level in male and females. Previous investigators have identified favorable blood analyses with more active groups, as compared with more inactive individuals (30),(9). Blair et al. compared nutrient intake in regular runners and those who participated in more sedentary behavior (9). Results showed that those in the running groups (males and females) consumed about 500-600 more kcals/day than the controls. However, the runners showed lower cholesterol levels.  Interestingly, a greater significance was seen in females as compared to males. Furthermore, triglycerides were lower for both sexes and HDL-C were higher in runners. In addition, the diet of the running group and control group were very similar in composition, particularly those nutrients that could affect lipids. Therefore, the alterations in cholesterol may be associated with chronic exercise (9). More specifically, it has been shown that high levels of LDL-C and/or low levels of HDL-C will increase the likelihood of developing coronary artery disease (91). In addition it was determined that high levels of HDL-C have an antiatherogenic effect, or the protection from lining of the arteries, however high levels of LDL-C and VLDL-C are positively correlated with coronary heart disease (61).

Cholesterol has also been associated with body composition, more specifically fat distribution. A review by Després et al. studied the association between body fat distribution and plasma lipoproteins (26) and identified the relationship between excess trunk fat and high plasma triglyceride levels. Furthermore, Després identified a link in gender, as males generally accumulate more trunk fat than females (26). This further leads to potential sex differences in metabolic syndrome and should be considered.

<u>Exercise and Satiety</u>

Media sources have provided the public with copious information relating to an increase in exercise will lead to an increase in caloric consumption. From the energy balance equation, many believe that an increase in energy expenditure (by means of physical activity), will trigger an increased intake in order to regulate energy balance. Studies have shown that an increase in energy intake is not necessarily a response after exercise, even under circumstances of high energy expenditure or graded exercise programs (50, 88). After a bout of exercise, there is a slow compensation action (by means of caloric intake) for the energy expended (15). More research is necessary to understand the effect of satiety on males and females when completing varying levels of energy expenditure.

Signaling mechanisms, primarily, leptin and ghrelin are released in response to either feeding or fasting. Satiety signals are triggered through a variety of hormones, however two of the most influential hormones include insulin and leptin (87). As further reviewed by Stensel, leptin is released from the adipose tissue and assists with energy balance regulation by alerting the body when satiety has been reached. This was furthered reviewed by Blundell et al. in which leptin has been researched to play a key role in appetite by adipose tissue and is required in a negative feedback loop for the regulation of adipose tissue (13). With this idea in mind, leptin has been included within appetite control models in which leptin is the main signal to the brain indicating the energy stores of the body (6, 62). Conversely, hormones within the body not only regulate satiety and inhibit meal consumption, some are necessary to trigger appetite stimulation. This primary hormone is known as ghrelin, which is released from the gastric cells in the stomach (87). Ghrelin holds a unique characteristic, as it is able to enhance appetite, therefore making it a critical component when considering caloric intake in the energy balance equation. Ghrelin levels rise before meals and decreases after meals, making them a large player in intake regulation (23). A recent review by Adams et al. further described ghrelin's relationship to the energy balance equation noting that ghrelin potentially can induce weight gain by stimulating the desire to eat in addition to decreasing fat utilization (2, 22).

A recent review discussed the relationship between the drive to eat and mechanisms that control human appetite. Eating is a patterned activity, which was initially brought on by the availability of food. Currently, food is entirely unrestricted (in most areas), shifting the research focus to the triggers and inhibitions of satiety (92). In accordance with Tremblay et al., several models were discussed to better understand appetite control. Some of the classic theories surround one macronutrient to adjust for the homeostasis within the body, such as the lipostatic theory (using body fat mass), aminostatic theory (amino acids and metabolites are critical agents), the glucostatic theory (triggered by a decrease in glucose availability) and most recently a theory relating to carbohydrate stores (92). While much research has identified energy intake as the primary determinant of increased body weight over the past few decades (48), there continues to be conflicting evidence as the primary  source of weight gain (20).

Several triggers are associated with the desire or response to consume a meal. Physiologically, the hypothalamus plays the strongest role as a regulator for eating behavior, receiving all neural, metabolic and endocrine signals from the periphery (57). Peripheral signals constitute primarily the gastrointestinal tract, however signals from the pancreas and adipose tissue contribute to hormonal signaling (39). Satiation, or the process which causes one to terminate consumption, may be initiated by neural input from gastric distention to the brain, mainly after a meal has been consumed (87). Once this response is triggered, a variety of hormones are released, altering the system of satiety, or complete fullness. As previously stated, there is limited acute compensation for an increase in expenditure linked with intake, that is, becoming sedentary does not downregulate energy intake. However, within long term research there appears to be partial compensation.

A recent review by Martins et al. compared the impact of acute exercise and the association with appetite. The effects of acute training have been well documented within the review and state that an acute response is lacking when related to hunger or energy intake (57). Even in those working at high intensities, the acute exercise response shows no effect on appetite (50). The acute response is associated with a variety of differences such as gender, body weight and eating behavior (57). Firstly, several studies have shown no effect in energy intake after an acute bout of exercise in men, however woman have been shown to increase caloric intake, to the degree in which exercise energy expenditure is negated by caloric consumption (41, 70). Comparing high and low exercise intensity, Imbeault et al. saw no difference measured in hunger and fullness levels immediately after either high- or low-intensity exercise, however these results were only consistent in males (41). Conversely, females were randomized into either a high intensity, low intensity or control group in a study conducted by Pomerleau et al. Results from this study discovered that as intensity increased, energy intake increased within the meal following the exercise session (70).

In conclusion, there are a multitude of components which are associated with changes in energy intake and energy expenditure. The regulation of energy balance, or energy homeostasis is highly complicated. Obesity is primarily due to a combination of decreased activity and increased energy intake, or positive energy balance. It is important to place significance on participating in routine physical activity to decrease mortality and improve overall quality of life. Weight loss is only one potential component within an exercise program but other exercise-induced changes have crucial impact on health as well. Overall, these studies further researched the effects of an increase in energy expenditure over a 6-month duration on health outcomes under weight stable conditions. These exercised-induced changes include potential alterations in body composition, in which we specifically looked at fat and fat free mass in addition to improvements in fitness. Secondly, this study analyzed if changes in body composition and energy expenditure were able to improve upon 6-month blood lipids. Finally, this study looks to further understand a potential association with an increase in energy expenditure on objective and subjective measurements. By researching the exercise-induced effects of a chronic aerobic program, we may have a better understanding of the health benefits of aerobic physical activity without weight loss.

CHAPTER THREE

GENRAL METHODOLOGY

Enrollment process and inclusion/exclusion criteria

The three studies in this dissertation used data collected in the Energy Flux Study (EFS), a randomized controlled trial for sedentary, healthy males and females completing 26-weeks of aerobic exercise intervention. All participants were recruited between October 2012 and November 2013 and were from the greater Columbia, South Carolina area. Interested participants initially completed an online screener to ensure preliminary criteria were met. Upon completion, participants were invited to attend an orientation session to review study protocol and procedures and confirm all inclusion/exclusion criteria. Inclusion criteria included: BMI >25 kg/$m^2$ and < 35 kg/$m^2$ and between the ages of 21 and 45. In addition, participants needed a fasting glucose measurement of <126 mg/dL and have been taking all prescribed medications for three or more months. Furthermore, participants needed to be able to participate in a fairly strenuous physical exercise program and provide consent before beginning the research study. Exclusion criteria included: planning on participating or currently participating in a weight loss intervention/exercise program, planning to have weight loss surgery, or a weight change of > 5 pounds in the last 12 months. Further, a history of depression, anxiety or panic with medications started within the last three months, TC ≥ 240 mg/dL with a LDL-C ≥ 190 mg/dL or TG levels of > 300 mg/dL were excluded from participation. Additional exclusion criteria included currently taking any medications such as hormone replacement therapy, beta blockers, allergy shots or systematic corticosteroids and any significant cardiovascular disease (including but not limited to serious arrhythmias, cardiomyopathy, congestive heart failure, stroke or transient ischemic cerebral attacks, peripheral vascular disease with intermittent claudication, acute, chronic, or recurrent thrombophlebitis, myocardial infarction, hypertension or an abnormal stress test). Additionally, anyone with a medical history with the presence of significant conditions of disease that may interfere with the study, someone pregnant or actively trying to become pregnant, or any females who gave birth within the last 12 months were excluded from the study. Furthermore, anyone scoring >90$^{th}$ percentile in the Brief Symptom Inventory (BSI), planning to move from the area in the next 8 months or any other medical, psychiatric or behavioral factors that would interfere with the ability to participate or follow intervention protocol were ineligible to participate in the EFS.

Study Design

The EFS consisted of baseline measurement visits in addition to follow up visits at 3 and 6 months. In addition, participants were randomized into either an exercise or control group at a 5:1 ratio to complete an intervention protocol lasting 26 weeks. Laboratory visit #1 consisted of a graded treadmill exercise test to determine cardiorespiratory fitness and assess body composition. Upon participant arrival, resting blood pressure, weight and height in addition to a dual-energy X-ray absorptiometry (DXA) scan to measure body composition. Subsequently, participants completed a modified Bruce protocol with a 12-lead electrocardiogram (ECG) in which the participants worked to fatigue. Laboratory visit #2 included measurements of height, weight, waist and hip circumferences and a resting metabolic rate test. Subsequently, under fasting conditions, a blood sample was collected in addition to a fasting glucose measurement. At the end of this visit, participants were given a food log to complete for two days in addition to a SenseWear mini armband to wear for three days to assess PA. Laboratory visit #3 included height, weight, two separate blood draws, a series of questionnaires assessing satiety levels and the consumption of a breakfast sandwich. After participants ate until they reached satiation, the remaining food was weighed (to be used at the post assessment period). In addition, participants were given a food log to complete for four days, including the day of the breakfast.

The intervention consisted of treadmill based exercise with a 3 minute warm-up and cool-down at a self-selected speed and incline. Those randomized into the exercise intervention were given a personalized exercise prescription to achieve an intensity of 70-75% $HR_{max}$. All randomized groups were expected to maintain their weight throughout the 26 week intervention period (±3%). Exercise participants were given a 4-6 week ramp-up phase before beginning their exercise dose based off participants comfort and experience with a treadmill. At the start of their training program, participants began with a weekly exercise energy expenditure goal of 10 kcal/kg/week. Exercise intensity was monitored via a heart rate monitor (Polar, FT1™, Lake Success, NY, USA) and checked every five minutes by trained staff to ensure participants were exercising at the desired intensity. Energy expenditure from each exercise session was based on metabolic equations and estimated using the individual's weight, duration and speed/grade of the treadmill (3). Metabolic calculations were used in place of armband data so that exercise prescriptions could be calculated prior to the participants arrival of each exercise session. In addition, participants were weighed at the beginning of every exercise session, to ensure weight maintenance (±3% of baseline weight). All study protocols were approved by the University of South Carolina Institutional Review Board, and informed consent was obtained from each participant prior to data collection.

## Randomization and Incentive

Participants were randomly assigned to a high exercise group (targeting 35 kcal/kg/week), moderate exercise group (17.5 kcal.kg/week) or non-exercise control group, matched for age and sex, randomized at a ratio of 3-3-1.

In order to facilitate retention and adherence, participants are promised $1000 upon completion of the study in the sequence of: $150 for baseline, $350 for completing three month measurements and $500 upon the completion of six month measurements. Those participants randomized to an exercise training group will receive an additional $400 throughout the six months of training to compensate for the additional food consumed to remain weight stable ($200 at three months and $200 at six months). Throughout the follow-up assessment periods, participants also will also receive a water bottle, t-shirt and a sandwich container. Upon study completion, each participant will be given a one-on-one counseling session to review their measurements, dietary information and training sessions.

## Measurements

Height (cm) and weight (kg) was measured with the participants wearing surgical scrubs and in bare feet using a wall mounted stadiometer (Model s100, Ayrton Crop., Prior Lake, MN, USA) and an electric scale (HealthometerH model 500 KL, McCook, IL, USA). At least two measurements to the nearest 0.1 cm and 0.1 kg, respectively were performed. A third measurement was taken if the difference between the initial measurements was greater than 0.4 cm and 0.1 kg for height and weight, respectively. The average of two measurements was used to calculate body mass index (BMI, $kg/m^2$). Fat mass and fat free mass were measured with a Lunar fan beam dual X-ray absorptiometry (DXA) scanner (GE Healthcare model 8743, Waukesha, WI, USA). In addition, waist and hip circumference were measured using a spring-loaded tape measure. Waist circumference was measured midway between the costal margin and the iliac crest with the participant standing with their feet together and their arms parallel to the floor. Hip circumference was measured around the widest point of the greater trochanter with the participant standing in the same position as described above. The average of three measurements to the nearest 0.1 cm was used for waist and hip circumference.

Cardiorespiratory fitness (CRF) was assessed by trained exercise physiologists. Before completing the GXT, a 12-lead Electrocardiogram (ECG) was placed on each participant. The GXT was administered as a modified Bruce protocol on a motorized treadmill (Full Vision., Newton, KS). The modified Bruce consisted of two minute stages and began at a speed of 1.7 miles per hours (mph) and 0%, then progressed to 1.7 mph and 5%, 2.5 and 10%, 3.4 and 12% and 4.2 an 16%. After the $4^{th}$ minute, this protocol is synonymous to the Bruce Protocol. Participants worked until they reached volitional fatigue, in which they selected to stop the treadmill. During the test participants were asked to rate their perceived exertion (RPE) based off the Borg scale of perceived exertion and blood pressure was measured during the final 30 seconds of each stage. Criteria for a maximal test required at least two of the following variables: a heart rate or

VO2 plateau with increasing workload, a respiratory quotient ≥ 1.15 and a RPE rating ≥ 17.

Blood draws were completed during the measurement visits (baseline, 3 month and 6 month) in addition to the appetite assessment visits. During the measurement visits, blood was drawn from the antecubital vein and blood samples were collected in tubes containing EDTA (1.25 mg/ml). For the appetite assessment, one hour before and one hour after the fixed meal was consumed blood samples were collected in tubes containing EDTA (1.25 mg/ml) and aprotinin (500 TIU/ml) (Kallikrein inhibitor unit, Phoenix, Burlingame, USA). All samples were immediately centrifuged for 10 minutes and subsequently stored in a -80 Centigrade freezer until analyses.

<u>Appetite Assessment</u>: Participants arrived after a 12-hour fast and received a standard fixed-component breakfast meal consisting of a sandwich with egg and cheese on white bread and water, coffee or tea with the option of artificial sweetener. Participants were instructed to eat until they reached satiation. Sandwiches were weighed prior to serving. If a sandwich was partially consumed, the remaining amount was weighed. Total amount of sandwich consumed (in grams), along with amount of water, coffee, tea and, if applicable, sweetener, were recorded. Participants also completed a series of questionnaires assessing hunger and palatability using self-rated visual analog scales (VAS) (25) and satiation through the a Satiety Quotient Questionnaire (SQ) (34).

<u>Questionnaires</u>: Participants completed two food logs, for either two days (appetite assessment visit) or four days (Laboratory visit #2) in which they were asked to record all food and drink consumed. These logs included any consumption prior to the morning of

the visit. Secondly, for the appetite assessment visit hunger and palatability was assessed before the fixed breakfast, immediately following the completion of the breakfast, 30- and 60-minutes post-breakfast via a validated self-rating 100 centimeter visual analogue scale (VAS) scale. Each rating was converted to a score in centimeters, allowing the comparison of hunger and satiety based off the following four questions: “how hungry do you feel”; “how satisfied do you feel”; “how full do you feel”; “how much do you think you can eat”. The SQ questionnaire was completed immediately following the fixed breakfast sandwich.

## STUDY 1 METHODOLOGY

Purpose: This study will address Aim#1 which is to identify a possible association between energy expenditure on body composition after a 6-month aerobic intervention. In addition, this study aims to analyze the increase in physical fitness through the participation in routine aerobic activity, under weight stable conditions.

### Research questions

Research question 1.1: Under weight stable conditions, is there a linear association between change in energy expenditure and change in body composition when adjusting for age, sex and baseline energy expenditure/body composition assessments?

Research question 1.2: Is there a difference in sex when energy expenditure changes are used to identify body composition upon completion of a 6-month aerobic intervention without weight loss?

Research question 1.3: When weight is required to be maintained, is there a change in cardiorespiratory fitness during an aerobic intervention in males and females?

Study Design: This study has a longitudinal design.

Study population

This analysis will include sedentary healthy males (51.9%) and females who participated in the Energy Flux Study and completed all measurement and intervention components associated with their assigned group (n=81).

Study measurements

For this analysis, body composition was calculated using DXA scans, percent body fat was then calculated by: %Body Fat = fat mass/ body weight. Energy expenditure was measured by means of a SenseWear mini armband to determine intensities of energy expenditure per day. CRF was also assessed by means of a modified Bruce protocol on a motorized treadmill in which participants worked to fatigue. All measurements were conducted at baseline, 3-months into the intervention and upon study completion.

Statistical Analysis

Statistical analyses were first run using paired t-test to determine differences from baseline to intervention completion for EE, fitness and body fat. Next, linear regression models were completed to determine if changes from baseline to 6-months for energy expenditure were associated with alterations in body composition. Several models were used to identify if changes in total daily EE (TDEE), moderate-to-vigorous EE (MVPA), moderate EE and vigorous EE were associated with body composition at follow-up. Each model used within the analysis adjusted for age, sex, baseline EE and baseline body composition. Energy expenditure was measured through slope values (as measured from baseline, 3-month and 6-month assessments). Statistical significance was set as $p<.05$.

STUDY 2 METHODOLOGY

Purpose: This study will address Aim#2 which plans to identify the possible effects of a 6-month exercise intervention on blood lipids in young adults. Changes from baseline to 6-months in energy expenditure (total daily, moderate-to-vigorous and vigorous physical activity and body composition (fat mass and fat free mass) will be reviewed to determine a possible association in blood lipids and C - reactive protein.

Research Questions

Research question 2.1: What is the association of change in energy expenditure and body composition with metabolic markers at follow-up adjusted for age, sex and baseline energy expenditure/body composition?

Research question 2.2: When males and females are analyzed separately, is there a difference in metabolic markers when adjusting for age and baseline assessments?

Research question 2.3: Using a lipid risk variable, is there a difference in either energy expenditure or body composition between those who improved their lipid risk and those who worsened their risk?

Study design: This study has a longitudinal design.

Study population

This analysis will include previously sedentary healthy males and females who participated in the EFS and completed all measurement and intervention components (n=64). Participants within this analysis (63.5% male) had an average age of 31.7 ± 7.4 and BMI of 27.8 ± 2.8 kg/m$^2$.

Study measurements

For this study, blood draws were taken under a fasting state before and upon the completion of a 26 week intervention. Samples were placed in -80 degrees Centigrade and sent in entirety to LabCorp for analysis upon completion of the study. Participants were given SenseWear mini armbands to monitor energy expenditure. Expenditure data was classified based on the intensity, or total daily, moderate-to-vigorous and vigorous. In addition, DXA full body scans were completed at baseline, 3M into the intervention and upon intervention completion.

Statistical Analysis

Paired sample t-tests were used to determine significant change from baseline through study completion. Linear regression models were used to determine if energy expenditure and body composition influenced blood markers and CRP throughout an aerobic 6-month intervention. Results were analyzed separately for sex and adjusted for age and baseline measurements. Slopes were calculated based on individual regression analyses and used within each regression model. Factor analysis calculated the lipid risk variable which included total cholesterol, triglycerides, and LDL/HDL ratio. Statistical significance was set as $p<.05$.

## STUDY 3 METHODOLOGY

Purpose: This study will address Aim#3 which uses objective measurements by means of leptin and ghrelin and subjective measurements through appetite questionnaires to further understand a possible association with the participation of a 6-month exercise intervention on appetite.

### Research questions

Research question 3.1: What is the association between a change in energy expenditure and body composition on fasting and non-fasting leptin and ghrelin values after completion of a 6-month aerobic intervention?

Research question 3.2: What is the association between a change in energy expenditure and body composition on fasting and non-fasting VAS questionnaires after completion of a 6-month aerobic intervention?

Research Question 3.3: Do these possible associations differ when analyzed separately for sex?

Study design: This study will use a longitudinal design.

### Study population

This analysis included previously sedentary healthy males (52.6%) and females (n=76) with a mean age of 30.8 ± 7.1 years and BMI of 28.0 ± 2.9 kg/$m^2$. All included participants completed all intervention, assessment and blood draw components of the EFS.

Study measurements

For this study differences in baseline and 6 month satiety levels are determined from all four questionnaires used (fasted and 60 minutes post consumption). Additionally, blood samples were completed pre/post intervention in a fasted and non-fasted state and analyzed for leptin and ghrelin at Pennington Biomedical Center, Louisiana.

Statistical Analysis

Correlations between VAS questionnaires (questions 1-4) with leptin and ghrelin were run at baseline and 6-month measurements, each correlation was run separately for fasting and non-fasting conditions. Dependent t-tests were run to determine a significant change in either fasting or non-fasting samples from baseline to 6-months for VAS questionnaires, leptin and ghrelin. In addition, linear regression models were run to determine if a change in energy expenditure or body composition was responsible for a change in leptin, ghrelin or a change in VAS scores (questions 1-4) at follow-up. Models were adjusted for age, sex, baseline blood/VAS score and baseline body composition (FM or FFM). Each regression models were run for male exercisers, female exercisers and controls.

# CHAPTER FOUR

# ASSOCIATION OF ENERGY EXPENDITURE WITH BODY COMPOSITION AND FITNESS UNDER WEIGHT STABLE CONDITIONS[1]

[1]DeMello MM, Drenowatz C, Prasad VK, Durstine JL, Blair SN, Hand GA. To be submitted to *Obesity Reviews*.

**Abstract**

<u>Objective</u>: The purpose of this study was to evaluate the association between energy expenditure and body composition during a 6-month aerobic intervention in which weight was maintained.

<u>Methods</u>: Previously sedentary males and females between the ages of 21-45 were recruited to participate. Participants were randomized into either an exercise group or a non-exercise control group. Those within the exercise group completed 3-6 aerobic exercise sessions per week for approximately one hour. Participants in the exercise and control group were expected to maintain their weight ±3% of their baseline body weight. Energy expenditure was measured by a SenseWear mini armband and DXA scans were completed at baseline and 3 and 6 month follow-up.

<u>Results</u>: A total of 81 males (51.9%) and females completed all measurement visits. Energy expenditure (TDEE, MVPA and vigorous PA) in addition to CRF significantly increased in males and females who were randomized into an exercise group as compared to non-exercise controls. A significant increase in VPA was associated with an increase in fitness at 6-months, after adjusting for age and sex, no other significant changes in EE on fitness were detected. In females, increase in vigorous PA was associated with beneficial changes in body composition. No other significant associations between change in TDEE, MVPA, or vigorous PA and body composition were observed in females.

<u>Conclusion</u>: Based on our findings, an increase in TDEE or MVPA through a 6-month aerobic intervention does not alter body composition (FM or FFM) if EI is required to increase in order to maintain body weight in young adults.

Keywords: energy expenditure, body composition, cardiorespiratory fitness, weight maintenance.

**Introduction**

The rise in the prevalence of chronic diseases in the United States population has, in the least part, been associated with a reduction in physical activity levels (73, 82). More than half of all adults are not meeting the 2008 Physical Activity Guidelines (18). Many occupations require high amounts of sedentary and sitting behavior as opposed to moderate-intensity activities, decreasing occupation-related energy expenditure by more than 100 calories/day in males and females (20). In addition, less time is allocated to household management due to technological advances, substantially increasing sedentary leisure behavior within the home (4). These reductions in energy expenditure have created an imbalance between energy intake and expenditure, resulting in a gradual upward shift in body weight, which has detrimental effects on various chronic diseases such as type II diabetes, stroke and some forms of cancer (99).

An increase in physical activity has the potential to also alter body composition, primarily fat mass and fat free mass. These alterations come about through participating in high levels of aerobic intensity, increasing duration of the session, or resistance training. Irving et al. compared training intensity and found high intensity interval training was the best way to reduce % body fat and fat mass (42), however no differences were seen when comparing high and low intensities in untrained overweight females (33). However, significant alterations in body composition, more specifically a reduction in total and visceral fat was seen in middle-age lean and obese men who underwent a moderate-intensity intervention for 3 months (55). Within a similarly related population, a dose response relationship is seen between exercise duration and central obesity, as measured through abdominal and suprailiac skinfolds (84). While results from these interventions follow a similar pattern, the multiple methods of measurement of body composition (hydrostatic weighing, skinfolds and BodPod) limits the relationship between exercise and body fat loss location. Therefore more research using objective, gold-standard measurement is necessary to define the relationship of exercise and body composition in males and females.

Several aerobic interventions have determined the differences in physical activity and segmentation of body composition, however many of these interventions produced a change in weight. However, it is essential to analyze how physical activity is able to alter body composition under weight stable conditions. Therefore, the purpose of this study is to identify a possible association between a change in energy expenditure and body composition after a 6-month aerobic intervention under weight stable conditions. In addition, this study aims to analyze the increase in physical fitness through the participation in routine, aerobic activity, even when weight is maintained.

**Methods**

Males and females between the ages of 21-45 were recruited from the greater Columbia, South Carolina area by means of social media and e-mail listings. Criteria for participation ensured a variety of the general public free from major acute or chronic diseases. Inclusion criteria consisted of overweight or obese individuals ($25\ kg/m^2 < BMI < 35\ kg/m^2$) and be able to participate in a fairly strenuous physical exercise program. Exclusion criteria removed those who were already participating in an exercise or weight loss program, planned to have weight loss surgery or had weight fluctuations more than 5 pounds in the last month. Those with any history of depression, anxiety or panic that were not stable on medications were not allowed to participate. Those with total cholesterol levels ≥240 mg/dL, LDL-C ≥ 190 mg/dL or triglyceride levels > 300 mg/dL were excluded from participation. Additional exclusion criteria consisted of prescription of medications such as hormone replacement therapy, beta blockers or systematic corticosteroids in addition to any significant cardiovascular disease or disorders. Furthermore, this study recruited sedentary individuals, as defined by <7,000 steps per day with no more than ten minutes of vigorous activity (93). Finally, participants were not invited to participate if they were planning on moving out of the area within the next 8 months.

Participants attended orientation and completed a series of baseline measurements before randomization during a two-week period. The study protocol was approved by the University of South Carolina Institutional Review Board and participants signed the written informed consent prior to any measurements taken. During orientation, participant Body Mass Index (BMI) was confirmed and a resting blood pressure was taken to ensure each individual was able to participate in an exercise program. To ensure participant safety, if two resting blood pressure measurements were ≥ 150 mmHg systolic or ≥90 mmHg diastolic, the participant was deemed ineligible for the study. Baseline 1 (B1) consisted of a graded exercise test in which participants worked to maximal fatigue. Due to recruitment of sedentary individuals, participants completed a modified Bruce protocol, as previously described (37). Before beginning the fitness test, each participant was prepped for an electrocardiogram (ECG) to monitor heart activity throughout the test. In addition, body composition was measured by means of a dual X-ray absorptiometry (DXA) scanner (GE Healthcare model 8743, Waukesha, WI, USA).

Randomization and Intervention

Upon successful completion of baseline testing and confirmation of inclusion criteria, participants were randomized into an exercise group or a non-exercise control group based on participants age and sex. Participants within the exercise group came to the Clinical Exercise Research Center at the University of South Carolina 3-6 times per week for approximately one hour sessions. All exercise sessions were completed on treadmills and were under supervision of trained research staff. Before the start of each exercise session, participants were given a Polar heart rate (HR) monitor to ensure they were within the required intensity of 70-75% of their $VO_{2max}$ HR as determined by their baseline GXT. Trained staff recorded participant HR every five minutes to ensure this criteria was met.

In addition, all participants were expected to maintain their weight throughout the 6 month intervention. Controls did not complete any aerobic training, however they came into the lab monthly to ensure weight maintenance. In order to be classified as weight stable, participants were expected to remain within ±3% of their baseline body weight. If their weight was not within this range for at least two weeks, individuals met with the staff registered dietician to discuss healthy options to alter their weight to meet the requirements of the study.

Energy Expenditure

Participants were given SenseWear mini armbands to wear for a period of ten days during their baseline and 6-month visits. The armband uses tri-axial accelerometry, galvanic skin response, heat flux, skin temperature and near body temperature to estimate energy expenditure per minute and has been shown to provide an accurate estimation of energy expenditure in free-living adults (46). Participants were instructed to wear the monitor for 24-hours per day except during periods when it had the potential to get wet, such as showering or swimming.

Body Composition

Body composition was measured using a Lunar fan beam dual X-ray absorptiometry (DXA) scanner (GE Healthcare model 8743, Waukesha, WI, USA). Total body scans were completed, percent body fat (BF) was subsequently measured as (%BF=fat mass/body weight).

Follow-Up

Participants were followed up three months into the intervention and then upon completion of the study. All groups completed the same measurements as completed at baseline. In addition, those in the exercise group also completed a GXT one month into their exercise intervention to adjust for any cardiovascular adaptations. Exercise speed/grade was adjusted if HR became consistently lower for one week of exercise sessions.

Statistical Analyses

Statistical analyses were first run using paired t-test to determine differences from baseline to intervention completion for EE, fitness and body fat. Next, linear regression models were completed to determine if change from baseline to 6-months for energy expenditure caused alterations in body composition. Several models were used to identify if changes in total daily EE (TDEE), moderate-to-vigorous EE (MVPA), moderate EE and vigorous EE (VPA) were associated with body composition at follow-up. Each model used within the analysis adjusted for age, sex, baseline EE and baseline body composition. Energy expenditure was measured through slope values (as measured from baseline, 3-month and 6-month assessments). Analyses were completed using SPSS version 22, with $p<0.05$ for statistical significance.

**Results**

A total of 81 males (51.9%) and females completed all measurement and intervention components of the EFS. The mean age was 31.4 ± 7.1 with almost half of the participants being Caucasian (48.1%) and African American (32.1%) followed by Asian/Asian Americans (16.0%). Eighty-four percent of participants who completed the study were classified as overweight ($25\ kg/m^2 \leq BMI \leq 30\ kg/m^2$), with the remaining classified as obese ($BMI>30\ kg/m^2$). Mean compliance (days/week) for baseline, 3-month and 6-month armband wear time was 77.4% for all participants within the study.

Those randomized to the exercise group significantly increased their $VO_{2max}$ peak or cardiorespiratory fitness (CRF) in males ($p<0.01$) and females ($p<0.05$). Non-exercising controls did not significantly change their CRF.

Table 4.1 shows changes in body composition and energy expenditure at baseline and upon completion of the intervention. In exercisers, significant increases were observed in TDEE, MVPA and vigorous PA in both males and females. Interestingly, no significant changes in body composition were seen in males either in the exercise group or the control group. However, female exercising participants showed significant changes in total fat mass ($p<0.01$) and percent fat mass ($p<0.01$), despite a constant body weight. Body composition at follow-up was significantly associated with change in vigorous activity throughout the 6M intervention, as seen in Table 4.2. In females only, an increase in Total ($p<.01$) and Trunk FFM ($p<.01$) was shown with an increase in vigorous PA. Additionally, a decrease in android ($p<.05$), gynoid ($p<.05$) and percent FM ($p<.01$) was seen through increases in VPA, however these significant changes were only seen in females. However, when weight change was placed into the regression model, only trunk FFM was significant for females. No significant changes in body composition were seen in males in either the first or second model with vigorous activity.

Furthermore, no significant associations were detected for subsequent body composition with change in total daily energy expenditure, moderate-to-vigorous activity or moderate activity only.

**Discussion**

Our findings show that even though significant increases in energy expenditure were demonstrated throughout a 6 month exercise intervention, changes in body composition were seen only in women, which was associated with a change in VPA. Even though male participants not only showed an increase in energy expenditure, but also a significant increase in CRF, no changes were found in body composition.

The significant differences in changes in body composition through physical activity are striking. When the group was analyzed separately for sex, and included non-exercise controls, only the males had a significant increase in fitness. However, only the females showed a decrease in FM and increase in FFM through changes in VPA. Possible reasons for this to occur may include females typically have higher body fat percentages as compared to males, therefore allowing for a greater possibility for change. Males and females had a similar change in vigorous activity from baseline to the end of the intervention. Males increased their vigorous activity by 50.5 ± 98.4 kcals/day while females increased their vigorous activity by 42.2 ± 89.5 kcal/day. It is curious, that even with a lower kcal/day, VPA is associated with a reduction in body composition in females only. Recent research has lacked a change in body composition without a weight loss component tied to the intervention (8). This result within this analysis requires further attention to understand this relationship.

It is important to note, that even though these individuals did not alter their body composition, both males and females within the two exercise groups showed significant improvements in their CRF. Wei et al. demonstrated the relationship of low cardiorespiratory fitness as a predictor of cardiovascular disease and all-cause mortality in males (95), similar results from the same data set were shown in females (10). A review of physical activity for the prevention of obesity highlighted the benefits of routine physical activity independent of body weight (44). Jakicic et al. stated those individuals who are overweight or obese but also consistently improve their physical activity levels are still able to gain benefits such as increasing CRF and decreasing the chances of cardiovascular disease. A recent meta-analysis discussed the joint association between physical fitness (CRF) and Body Mass Index (BMI), in which it was determined mortality was dependent on CRF and not BMI (7). Therefore, improved CRF has a protective effect against risk of death, even in overweight to obese individuals. Therefore, while participants did not alter their weight, the increase in health benefits through CRF should not be overlooked.

Additionally, while each participant was measured to ensure weight maintenance, there was no direct intervention to ensure participants maintained their weight. Recently, several recommendations have been issued to highlight the amount of physical activity necessary to either maintain weight loss or lose weight. The American College of Sports Medicine states that over 250 min/wk of participation in moderate intensity PA is essential to maintain weight loss (27). More information is necessary to evaluate intensity and duration of weight maintenance within this population.

This study has several strengths and limitations that should be discussed. Strengths of this research include the use of objective measurements (body composition, EE and fitness) at multiple time points. Additionally, all exercise sessions were monitored by trained staff to ensure participants maintained their required intensity and dose. Lastly, participants were successfully able to maintain their weight (±3%), allowing for the analyses of an increase in energy expenditure on health outcomes under weight stable conditions. A few limitations exist within this study design. Firstly, the sample recruited for this study was primarily recruited from the South Carolina campus and was of moderate-to-high socioeconomic status, therefore not a true representation of the general public. A large incentive may have encouraged those to participate outside of looking to participate in the study. Finally, the lack of dietary analyses hinders an explanation for the lack of change in body composition. With the constant need to maintain weight for 6 months, some may have turned to more calorically dense foods, limiting their choices for a healthy, balance diet. By selecting choices that potentially limit alterations in body composition i.e. fat and fat free mass, large changes may not be seen.

## Conclusion

In conclusion, female participants within this study showed improvements in their body composition with increases in vigorous activity only. While no other significant changes were seen in body composition through increases in energy expenditure, it is critical to note those who participated in an exercise program (both males and female groups) increased their cardiorespiratory fitness. More research is necessary to define duration and intensity to manipulate body composition under weight stable conditions.

Table 4.1: Baseline descriptives of male and female participants within the EFS, values shown for pre/post intervention.

| Characteristic | Male (n=42) | | Female (n=39) | |
|---|---|---|---|---|
| | Baseline | 6-Month | Baseline | 6-Month |
| *Anthropometric* | | | | |
| Age, y | 31.3 ± 6.5 | | 30.9 ± 7.8 | |
| Weight, kg | 87.3 ± 13.8 | 86.9 ± 13.5 | 74.7 ± 8.6 | 74.6 ± 8.8 |
| BMI, kg/m$^2$ | 27.7 ± 3.0 | 27.6 ± 3.0 | 28.2 ± 2.7 | 28.2 ± 2.9 |
| $VO_{2peak}$ (ml/kg/min) | 33.3 ± 7.1 | 36.2 ± 7.7** | 25.1 ± 4.7 | 26.3 ± 6.0 |
| *Body Composition* | | | | |
| Total Fat Mass, kg | 26.8 ± 7.9 | 26.5 ± 7.8 | 29.9 ± 4.9 | 29.3 ± 5.5 |
| Total Fat Free Mass, kg | 60.9 ± 7.8 | 60.8 ± 7.9 | 45.6 ±5.7 | 46.0 ± 5.7 |
| Percent Fat Mass | 30.3 ± 5.4 | 30.2 ± 5.5 | 39.9 ± 4.0 | 39.1 ± 4.3** |
| *Energy Expenditure* | | | | |
| Total Daily, kcal/day | 2829.0 ± 398.1 | 2953.6 ± 505.5** | 2229.4 ± 228.1 | 2361.9 ± 304.2** |
| MVPA, kcal/day | 456.8 ±188.9 | 653.8 ± 315.5** | 256.4 ± 100.0 | 423.5 ± 232.2** |
| Vigorous, kcal/day | 15.4 ± 24.3 | 66.5 ± 102.7** | 7.4 ± 12.0 | 49.2 ± 95.0** |

Data is expressed as group means ± SD. MVPA= moderate-to-vigorous energy expenditure, vigorous activity is expressed as the combination of vigorous and very vigorous activity. *indicates significance (p<.05) between baseline and 6M. ** indicates significance (p<0.01) between baseline and 6M.

Table 4.2: Linear regression model for vigorous energy expenditure and body composition throughout the intervention.

| | Males (n=42) | | Females (n=38) | |
|---|---|---|---|---|
| | Beta | p-value | Beta | p-value |
| ***ΔTDEE*** | | | | |
| Total FM (kg) | .025 | .491 | .075 | .220 |
| Total FFM (kg) | .000 | .993 | .038 | .342 |
| Trunk FM (kg) | .032 | .378 | .072 | .304 |
| Trunk FFM (kg) | .023 | .635 | -.206 | .658 |
| Percent FM (%) | .009 | .837 | .065 | .367 |
| ***ΔMVPA*** | | | | |
| Total FM (kg) | -.009 | .788 | -.002 | .972 |
| Total FFM (kg) | -.027 | .499 | .028 | .475 |
| Trunk FM (kg) | .016 | .636 | .015 | .826 |
| Trunk FFM (kg) | .048 | .329 | -.011 | .859 |
| Percent FM (%) | -.026 | .529 | -.012 | .865 |
| ***ΔVPA*** | | | | |
| Total FM (kg) | .021 | .571 | -.112 | .071 |
| Total FFM (kg) | -.002 | .968 | .120 | .002 |
| Trunk FM (kg) | .049 | .189 | -.103 | .147 |
| Trunk FFM (kg) | .089 | .086 | .232 | .000 |
| Percent FM (%) | .024 | .604 | -.184 | .009 |

Model: 6M body composition = age + baseline body composition + ΔEE + Baseline EE

# CHAPTER FIVE

# ASSOCIATION OF ENERGY EXPENDITURE AND BODY COMPOSITION WITH METABOLIC MARKERS UNDER WEIGHT STABLE CONDITIONS[2]

[2]DeMello MM, Drenowatz C, Prasad VK, Durstine JL, Blair SN, Hand GA. To be submitted to *Medicine & Science in Sports & Exercise*.

## Abstract

Introduction: A large percentage of overweight and obese individuals live within the United States. While a reduction in body weight may help reduce the risk of several types of chronic diseases, the importance of an exercise program without weight loss should not be overlooked. The purpose of this study is to identify the possible association between changes in energy expenditure and body composition on blood markers in previously sedentary male and female adults.

Methods: Males and females between the ages of 21-45 years with a BMI range of overweight to obese ($25kg/m^2 \leq BMI \leq 35kg/m^2$) were recruited to participate in the EFS. Baseline assessments lasted approximately two weeks with B1 including a GXT and DXA total body scan and B2 consisting of a RMR, fasting blood draw and energy expenditure through a 10-day SenseWear armband assessment period. Upon completion of baseline testing, participants were randomized into an exercise group or non-exercise control group. The intervention consisted of aerobic treadmill training for a period of 6 months. Participants within the control and exercise groups were required to maintain their weight (±3%) throughout the 6-month duration. Follow-up testing was completed upon intervention completion.

Results: A total of 64 (62.5% male) completed the intervention and all measurement visits. Linear regression models included changes in energy expenditure with body composition to determine any changes in blood lipids and CRP values throughout the intervention. Overall, a decrease in fat mass during an aerobic training intervention was associated with a decrease in TC and LDL-C in males and a decrease in TG and VLDL-C in females. Furthermore, those who improved upon their lipid profile showed a greater decrease in fat mass as compared to those who worsened their profile. No significant changes in EE were detected in either model.

Conclusion: Even without significant weight loss, participation in a 6-month aerobic intervention was associated with changes in blood lipids in males and females.

Keywords: cholesterol, energy expenditure, body composition, weight maintenance, CRP, energy balance, energy flux

**Introduction**

It is well documented that prevalence of overweight and obesity in adults has increased from 1999 through 2014 within the United States (65), with levels increasing around the globe. This high prevalence is cause for concern as overweight/obesity increase the likelihood of certain diseases such as coronary heart disease, type II diabetes, stroke and some forms of cancer (18). Participation in routine, aerobic activity to improve cardiorespiratory fitness has been shown to decrease the risk of mortality, even in overweight to obese individuals (72).

While the American Heart Association recommends weight loss in obese individuals to decrease the risk of developing cardiovascular disease risk factors (51), the importance of aerobic physical activity under weight stable conditions should not be overlooked. Several benefits have been identified in clinical exercise interventions which showed little to no weight loss (89). A review done by Swift et al. highlighted the benefits of exercise even when no weight loss or modest weight loss (<5 kilogram (kg)) was reported (89). Swift reviewed several clinical trials in which overweight/obese individuals completed an exercise intervention and reported little to no weight loss. Through these interventions, participants saw improvements in cardiorespiratory fitness, glucose control, endothelial function, lipoprotein particle size, high density lipoprotein and improved quality of life (89).

Cholesterol plays a vital role within the functioning bodily system, primarily seen through its role within cell membranes and as a precursor to steroid hormones (63). The effect of exercise on lipoproteins has been well documented with both acute (85) and chronic effects (53). A clinical exercise trial by Kraus et al. showed a relationship between an increase in exercise duration with an improvement in blood lipids in participants undergoing an aerobic intervention under weight stable conditions (53). Furthermore, c-reactive protein (CRP), a marker of inflammation has been identified as a predictor of cardiovascular disease in males and females (19). Hamer noted that CRP is greatly decreased with weight loss, not solely exercise training. Furthermore, an inverse relationship between physical activity and CRP levels has been identified (36). More research is necessary to determine the association with a high controlled aerobic intervention and the possible effects on blood markers in males and females.

The purpose of this study is to identify the possible effects of a 6-month exercise intervention on blood lipids and CRP through changes in energy expenditure and body composition in young adults.

**Methods**

Males and females with a Body Mass Index (BMI) between 25 and 35 kg/m$^2$ and between the ages of 21-45 were recruited to participate in the EFS from the greater Columbia, South Carolina area. Those interested initially completed an online screener to confirm eligibility for participation. Inclusion/exclusion criteria allowed for a selection of individuals free from chronic disease (i.e. serious arrhythmias, cardiomyopathy, congestive heart failure, stroke or transient ischemic cerebral attacks, peripheral vascular disease with intermittent claudication, acute, chronic or recurrent thrombophlebitis and Stage II or III hypertension, myocardial infarction and/or an abnormal response to exercise) within the general population. Further exclusion criteria included any medications for depression, anxiety, hormone replacement, beta blockers or systemic corticosteroids. Any females pregnant or planning on becoming pregnant were excluded. Those with total cholesterol levels ≥240 mg/dL, LDL-C ≥ 190 mg/dL or triglyceride levels > 300 mg/dL were excluded from participation. The Energy Flux Study (EFS) recruited sedentary individuals with a sedentariness defined as <7,000 steps per day with no more than 10 minutes of vigorous activity (93). Finally, participants wishing to participate in the EFS also needed access to the internet, have no intentions of moving from the area for the next 8 months and able to participate in a rigorous exercise program.

Eligible participants, based on the online screener, were contacted by recruitment staff to complete a second screener and then scheduled for orientation at the University of South Carolina. The study protocol was approved by the University of South Carolina Institutional Review Board and participants signed written informed consent prior to taking any measurements. During orientation, physical measurements such as height, weight and resting blood pressure were taken (BMI and healthy resting BP) to confirm eligibility.

Baseline testing took approximately two weeks to complete. Baseline 1 (B1) consisted of anthropometric measures and a graded treadmill exercise test. Height and weight were recorded in bare feet and surgical scrubs. Participants were each prepped with a 12-lead electrocardiogram (ECG) to monitor heart activity throughout the fitness test. Subsequently, participants completed a modified Bruce protocol on a motorized Trackmaster treadmill (Full Vision, Inc., Newton, KS). The protocol consisted of two minute stages in which participants worked to volitional fatigue, as previously described (37). Additionally, fat mass and fat free mass was measured with a Lunar fan beam dual X-ray absorptiometry (DXA) scanner (GE Healthcare model 8743, Waukesha, WI, USA) prior to the fitness test. Baseline 2 (B2) was conducted under a 12-hour fast and 24 hour abstention from physical activity. Anthropometric measurements such as height, weight, and waist and hip circumference were completed with participants wearing surgical scrubs and in bare feet. Subsequently, participants lay in a supine position to complete a resting metabolic rate (RMR) test in a quiet and dimly lit room, lasting approximately 45 minutes. Following the RMR, a blood draw was completed in which blood was taken from the antecubital vein in tubes containing EDTA (1.25 mg/mL) and immediately centrifuged for 10 minutes. Samples were then stored at – 80 degrees Centigrade. All samples were shipped simultaneously and analyzed for C-reactive protein and a lipid panel (total cholesterol, triglycerides, high-density lipoprotein (HDL-C), very low density lipoprotein (VLDL-C), and low density lipoprotein (LDL-C).

Energy Expenditure

Participants were given a SenseWear Mini Armband ® to measure energy expenditure over a period of 10 days. They were instructed to wear the armband for 24-hours and only remove it during activities where it might get wet such as taking a shower or swimming. Participant’s expenditure was analyzed for total daily (TDEE), moderate-to-vigorous activity (MVPA), vigorous energy expenditure (VPA) and sedentary expenditure per day.

Randomization and Intervention

Upon completion of B1 and B2, participants were randomized into either an high flux (target 35 kcal/kg/week), moderate flux (17.5 kcal/kg/week) or a non-exercise control group. Those within the high flux group attended 5-6 session/week for approximately one hour. Moderate flux participants came in for 3 sessions for an hour duration.

The intervention consisted of treadmill based exercise in which participants worked at 70-75% of their $VO_{2max}$ heart rate (HR) based on B1 GXT. Exercise participants were given a 4-6 week ramp-up phase to familiarize themselves with the treadmill and each exercise session. Before each session, participants were given a heart rate monitor (Polar, FT1™, Lake Success, NY, USA) and heart rate was checked every five minutes to ensure they were working at the appropriate intensity. Energy expenditure was calculated based on metabolic equations calculated from the individuals weight, duration and speed/grade of the treadmill (3). By using metabolic equations, research staff were able to properly prescribe the exercise duration and speed/grade prior to each session.

Participants were expected to maintain their weight throughout the intervention ±3% of their baseline body weight. Participants were weighed at the start of every exercise session to ensure they remained within range, if they did not meet this requirement for more than 2 weeks, they met with our registered dietician to discuss healthy options to remain within maintenance range. Those placed in the control group met with research staff monthly to be weighed to ensure maintenance criteria was met.

Follow-Up

Follow-up measurements were conducted half way through the intervention and upon completion of the study for each participant. Measurements from B1 and B2 were repeated three months into the intervention and upon completion of the intervention for both groups. Additionally, at the follow-up visits participants were given a SenseWear monitor to wear for a period of ten days and only remove during time of bathing/swimming. Blood was analyzed at baseline and at the completion of the study (6-month) for this paper.

Statistical Analyses

Data was analyzed for all participants who successfully completed the intervention in addition to completing a blood draw at both pre and post intervention. A paired sample t-test was used to determine significant change from baseline to intervention completion. Linear regression models were used to determine if energy expenditure and body composition (specifically fat mass and fat free mass) influenced blood markers throughout a 6-month aerobic intervention. Results were analyzed separately for sex. Since each variable was accurately measured at baseline, 3-months and 6-months, slopes were calculated based on individual regression analyses and used within each regression model. Factor analysis calculated the lipid risk variable which included total cholesterol, triglycerides and LDL/HDL ratio at baseline and 6-months. Two groups were created via median split from the difference in the lipid risk variable from baseline to 6-months. One-way analysis of variance was initially used for baseline variable to ensure no distinct differences existed between each group. Finally, analysis of covariance was used to determine differences in body composition and energy expenditure between groups, adjusting for sex and age. Statistical analyses were completed using SPSS version 22 with $p<.05$ for statistical significance.

**Results**

For the purpose of this paper, a subsample of the clinical exercise trial was used (n=64) to include those who completed the intervention, assessments and pre/post blood draw. Reasons blood was not collected include failure to successfully draw blood due to fasting and/or dehydration or participant refusal. This sample included males (62.5% ) and females with an average age of 31.4 ± 7.3 and BMI of 27.7 ± 2.7 kg/$m^2$ successfully completed both pre and post intervention blood draws. Participant descriptives are shown in Table 5.1. There was no significant change in weight in either males ($p=.242$) or females ($p=.388$), therefore the criteria was met for weight maintenance throughout the 6 month intervention. Males and females both saw a significant increase in total daily (TDEE), moderate-to-vigorous (MVPA) and vigorous energy expenditure from baseline to intervention completion. However, only female participants saw significant changes in fat mass ($p=.02$) and percent fat mass ($p<0.01$).

Blood analyses are shown in Table 5.2. Blood results at baseline were within normal range as a group means, however some participants measured outside of normal ranges. At baseline, 14 participants had a total cholesterol (TC) value as ‘borderline high’ and ‘high’, 20 participants low density lipoprotein-cholesterol (LDL-C) was considered ‘borderline high’ and ‘high, with one participant’s LDL-C classified as ‘very high’ (59). Furthermore, 8 participants were classified as having between ‘borderline high’ and ‘high’ triglycerides (TG) and 18 participants had ‘low’ high density lipoprotein-cholesterol (HDL-C) at baseline (59). No significant change was seen in blood markers in male participants from baseline to post intervention. However, females showed a significant decrease in triglycerides by 16.0 ± 26.7 mg/dL, and LDL-C by 3.0 ± 5.4 mg/dL, HDL-C increased by 3.0 ± 4.6 mg/dL. At baseline, males and females significantly differed in LDL-C and HDL-C blood variables, with males having a higher LDL-C and lower HDL-C as compared to females. No other blood marker differed among sex at baseline.

Linear regression models showed a significant change in blood markers with changes in FM as opposed to changes in energy expenditure. It was found that changes in blood lipids were independent from changes in energy expenditure. However, a significant increase in SED was associated with an increase in C-reactive protein (.45, $p<.05$), in males but not in females. An increase in fat mass resulted in an increase in TC ($\beta=.31$, $p<.05$), LDL-C ($\beta=.34$, $p<.01$) and lipid risk ($\beta=.22$, $p<.05$) in males and an increase in triglycerides ($\beta=.30$, $p<.05$) and VLDL-C ($\beta=.30$, $p<.05$) in females, with no significance seen in changes in TDEE. Change in MVPA was not significantly associated with any blood markers at follow-up, however, an increase in fat mass resulted in an increase in TC ($\beta=.31$, $p<.05$) and HDL-C ($\beta=.33$, $p<.05$) in males, with no change in females.  In the VPA model, an increase in FM showed an increase in TC ($\beta=.35$, $p<.01$) and LDL-C ($\beta=.33$, $p<.01$) in males and an increase in triglycerides ($\beta=.30$, $p<.05$) and VLDL-C ($\beta=.31$, $p<.05$) in females. With sedentary time, an increase in FM resulted in significant increases in TC ($\beta=.30$, $p<.05$) and LDL-C ($\beta=.32$, $p<.05$) in males and an increase in triglycerides ($\beta=.30$, $p<.05$) and VLDL-C ($\beta=.302$, $p<.05$) in females.

Change in lipid risk was analyzed pre/post intervention and a median split created two groups. Group 1 represents those individuals who improved their lipid profile throughout the intervention and Group 2 represented those individuals who worsened their lipid profile. Analysis of covariance determined group differences between energy expenditure and body composition. No significance was seen in either change in TDEE, MVPA, VPA or SED. However, FM decreased significantly in Group 1 as compared to Group 2, as shown in Figure 5.1. No significant changes were seen in FFM.

**Discussion**

Energy Expenditure and Body Composition

All participants significantly increased their total daily, MVPA and vigorous energy expenditure throughout the intervention. Body composition changes were only significant in females, with a decrease in total fat mass and percent body fat from baseline until completion of the intervention.

Blood Lipids

Throughout the 6-month intervention, blood lipids changed pre to post in males and females separately, even without weight loss. Body composition changes, particularly changes in FM showed significant modifications in female participants. In male participants, an increase in FM was associated with an increase in TC and LDL-C. These increases in cholesterol can lead to other diseases as LDL-C has been associated with an increase with the risk for atherogenesis (56, 86). Female participants showed significance primarily with a positive relationship between fat mass and TG and VLDL-C. It is important to highlight the fact that energy expenditure did not affect the follow-up blood lipid outcomes. Change in body composition, primarily FM had a stronger association with lipids than alterations in EE in male and female participants.

C-reactive Protein

No significance was found in changes in TDEE, MVPA or VPA with CRP at follow-up. Significance was only seen in male participants during sedentary energy expenditure, that is an increase in sedentary EE resulted in increased CRP values. Due to the requirement of weight maintenance, the lack of significant change in CRP agrees with previous research (19). Interestingly, within the literature, there is a strong inverse relationship between participation in physical activity and a decrease in inflammatory markers such are CRP (36, 68). However, changes in CRP throughout an exercise intervention are conflicting when body weight is considered. Interestingly, this study saw an increase in CRP values, however tis was not a significant increase at follow-up. As this study was not designed with CRP as a main outcome, more research is necessary to understand the potential shift in CRP values under weight stable conditions.

Lipid Risk Factor

Analyses from changes in FM and FFM are compared in one group who displayed beneficial change in their lipid profile and a second group who worsened their lipid profile during the 6-month intervention. The group with an improved lipid profile during the exercise intervention, showed a significantly greater decline in FM than those who worsened their lipid profile with no changes seen in FFM in either group. These results further show that change in FM is critical to alter blood lipids under weight stable conditions. While energy expenditure did not significantly affect biomarkers, it is critical to note that alterations in FM were most likely induced through the participation in an exercise intervention.

Strengths and Limitations

The primary strength of the EFS is the objective measurements at multiple time points throughout the intervention. These measure include, changes in energy expenditure, body composition and blood analyses. All exercise sessions took place within the clinical laboratory at the University of South Carolina and were monitored by trained research staff to ensure participants were reaching proper intensity. Training intensity was also re-established following the 1-month and 3-month fitness assessments. The main limitation of the EFS is limited knowledge of dietary assessment. Participants were only required to fill out food logs three times during the intervention. Food logs have been criticized as an assessment tool due to self-report, however since they are low cost and reduce participant burden, they were used within this study. Further limitations include the lack of diversity within this sample. All participants lived in South Carolina and had a relatively high-to-moderate socioeconomic status, limiting the scope of a general population. Additionally, the lack of chronic disease with a generally healthy lipid profile only allows for a limited change, if any. Assessing those individuals who are able to show improvements (those with a chronic disease, or very poor lipid profile) will allow for a better understanding of an intervention on blood markers.

## Conclusion

The primary finding from this randomized, controlled exercise trial in previously sedentary individuals is a decrease in fat mass will improve cholesterol levels. The significance of a decrease in FM was also depicted in the group who improved their lipid profile throughout the intervention. Changes in EE did not significantly improve blood lipids, however it is critical to note that without participating in an aerobic intervention, alterations in body composition (decrease in FM) may not have occurred. Results from this study are conflicting in regards to CRP. A change in sedentary EE was significantly associated with CRP in male participants only, more research is necessary to understand the effects of exercise without weight loss on CRP levels in adults. Overall, a 6-month aerobic intervention allowed for an improvement in blood lipids without the loss of body weight in young adults.

Table 5.1: Baseline descriptives of all completed intervention and measurement participants in the EFS (n=64).

| Variable: | Baseline | | 6 Month | |
|---|---|---|---|---|
| | **Male** | **Female** | **Male** | **Female** |
| Males | 62.5% | | | |
| Age | 31.2 ± 6.6 | 31.9 ± 8.4 | | |
| *Anthropometry* | | | | |
| Height (cm) | 176.9 ± 8.5 | 162.5 ± 6.0 | 177.0 ± 8.5 | 162.3 ± 6.2 |
| Weight (kg) | 86.8 ± 14.0 | 73.6 ± 7.2 | 86.4 ± 13.6 | 73.3 ± 7.4 |
| BMI ($kg/m^2$) | 27.6 ± 3.0 | 27.8 ± 2.2 | 27.5 ± 3.0 | 27.8 ± 2.3 |
| *Body Composition* | | | | |
| Total FM (g) | 26.4 ± 7.9 | 29.9 ± 4.5 | 26.2 ± 7.8 | 29.1 ± 5.0* |
| Total FFM (g) | 60.8 ± 7.9 | 44.5 ± 4.8 | 60.7 ± 8.0 | 44.8 ± 5.1 |
| Fat Mass (%) | 30.1 ± 5.3 | 40.6 ± 3.8 | 30.0 ± 5.6 | 39.5 ± 4.4↓ |
| *Energy Expenditure* | | | | |
| Total Daily (kcal/day) | 32.8 ± 3.1 | 30.5 ± 2.5 | 34.7 ± 4.9↓ | 32.2 ± 3.7* |
| MVPA (kcal/day) | 5.5 ± 2.5 | 3.8 ± 1.4 | 7.8 ± 4.0↓ | 6.0 ± 3.4↓ |
| Vigorous (kcal/day) | 0.2 ± 0.3 | 0.1 ± 0.2 | 0.6 ± 1.1* | 1.1 ± 1.8* |

Note. Reported as means ± SD unless prevalence is reported. *p-value <0.05 ↓p-value<0.01
FM= fat mass, FFM= fat free mass, MVPA= moderate-to-vigorous physical activity

Table 5.2: Cholesterol and C-reactive protein at baseline and 6-months.

| Blood Variable | Males (n=40) | | | Females (n=24) | | |
|---|---|---|---|---|---|---|
| | Baseline | 6-Month | p-value | Baseline | 6-Month | p-value |
| Total Cholesterol | 183.1 ± 32.1 | 185.2 ± 34.2 | .63 | 170.6 ± 34.5 | 166.6 ± 33.3 | .34 |
| Triglycerides | 99.9 ± 45.7 | 105.4 ± 55.0 | .36 | 90.9 ± 55.6 | 74.9 ± 42.8 | .01 |
| HDL-C | 42.6 ± 10.5 | 44.0 ± 9.8 | .30 | 53.0 ± 12.6 | 56.0 ± 13.3 | .01 |
| LDL-C | 121.0 ± 31.7 | 120.0 ± 30.5 | .80 | 99.5 ± 29.7 | 95.6 ± 30.4 | .30 |
| VLDL-C | 20.0 ± 9.2 | 21.1 ± 11.0 | .33 | 18.0 ± 11.2 | 15.0 ± 8.5 | .01 |
| CRP | 1.4 ± 2.8 | 1.2 ± 1.3 | .62 | 1.4 ± 1.6 | 2.5 ± 3.5 | .06 |

Note. Reported as means ± SD, all values reported as mg/dL.
HDL-C = high density lipoprotein-cholesterol, LDL-C= low density lipoprotein-cholesterol, VLDL-C= very low density lipoprotein-cholesterol, CRP= C-reactive protein.

Table 5.3: Linear regression for energy expenditure and body composition to determine blood markers.

| | Males | | Females | |
|---|---|---|---|---|
| Blood Markers | ΔTDEE | ΔFM | ΔTDEE | ΔFM |
| Total Cholesterol | -.083 | .314* | .110 | -.112 |
| Triglycerides | -.209 | -.001 | .161 | .298* |
| HDL-C | .156 | .146 | -.036 | -.101 |
| LDL-C | -.076 | .337$^{\downarrow}$ | .097 | -.140 |
| VLDL-C | -.203 | .002 | .145 | .301* |
| Lipid Risk | -.167 | .221* | .175 | .007 |
| | ΔMVPA | ΔFM | ΔMVPA | ΔFM |
| Total Cholesterol | .021 | .310* | -.186 | -.226 |
| Triglycerides | -.078 | -.026 | -.062 | .265 |
| HDL-C | .082 | .158 | .048 | -.060 |
| LDL-C | -.004 | .325* | -.133 | -.277 |
| VLDL-C | -.076 | -.022 | -.066 | .264 |
| Lipid Risk | -.023 | .197 | -.119 | -.116 |
| | ΔVigorous | ΔFM | ΔVigorous | ΔFM |
| Total Cholesterol | -.202 | .348$^{\downarrow}$ | .194 | -.117 |
| Triglycerides | -.171 | .021 | .115 | .300* |
| HDL-C | -.205 | .193 | -.046 | -.116 |
| LDL-C | -.097 | .336* | .194 | -.177 |
| VLDL-C | -.165 | .023 | .120 | .305* |
| Lipid Risk | -.111 | .222 | .202 | -.058 |
| | ΔSedentary | ΔFM | ΔSedentary | ΔFM |
| Total Cholesterol | .136 | .304* | -.048 | -.097 |
| Triglycerides | .057 | -.006 | .050 | .296* |
| HDL-C | .076 | .126 | -.066 | -.112 |
| LDL-C | .106 | .324* | .000 | -.103 |
| VLDL-C | .054 | -.002 | .067 | .302* |
| CRP | .450* | .017 | .334 | .267 |
| Lipid Risk | .052 | .240 | .051 | .038 |

Note. All blood samples are measured in mg/dL. Value indicates beta for variable, *p-value<0.05, $^{\downarrow}$p-value<0.01. HDL-C= high density lipoprotein-cholesterol, LDL-C= low density lipoprotein-cholesterol, VLDL-C= very low density lipoprotein-cholesterol, CRP= C-reactive protein, TDEE= total daily energy expenditure, MVPA= moderate-to-vigorous physical activity, FM= fat mass
Model: 6M blood marker = sex + age + ΔEE + ΔBody Composition + Baseline energy expenditure + Baseline body composition + baseline blood marker.

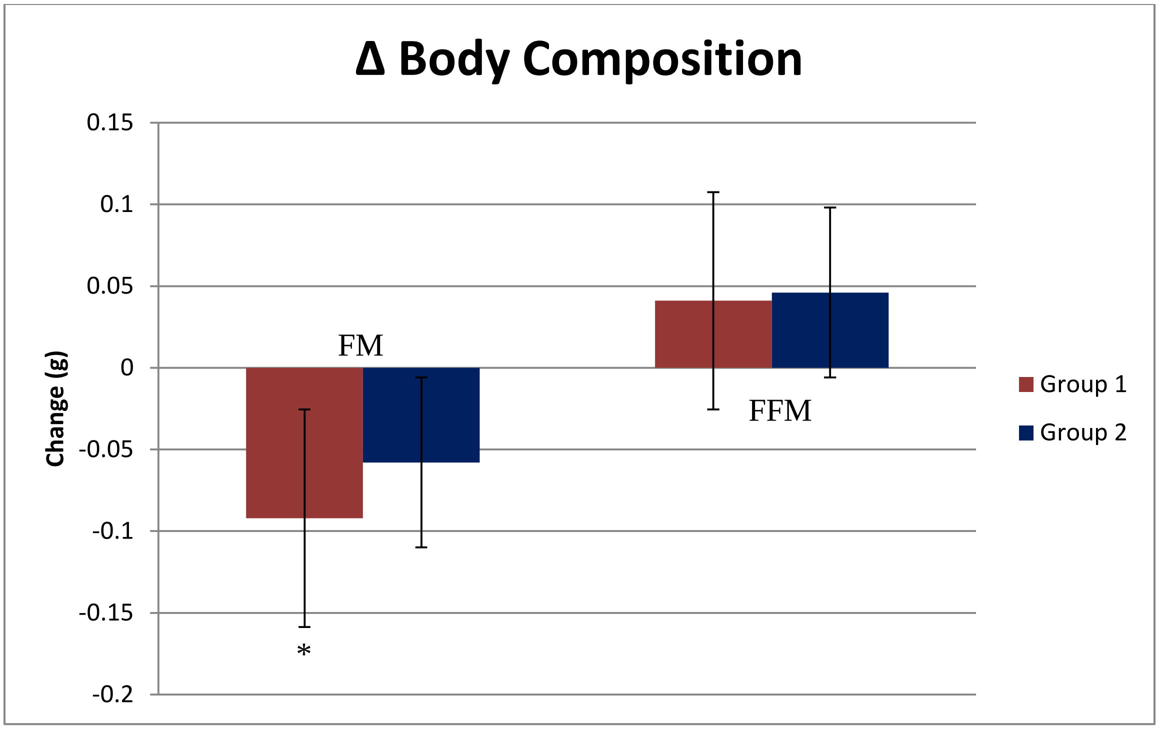


Figure 5.1: Change in body composition between 2 lipid profile groups.

Note. Group 1 improved upon their lipid profile, Group 2 worsened their lipid profile. Change is determined from baseline to 6-months. *p<0.05

CHAPTER SIX

PROSPECTIVE ASSOCATION OF ENERGY FLUX AND SATIETY[3]

[3]DeMello MM, Drenowatz C, Prasad VK, Durstine JL, Blundell JE, Blair SN, Hand GA. To be submitted to the *American Journal of Clinical Nutrition*.

**Abstract**

Introduction: More than one third of all adults are classified as obese. There remains uncertainty whether physical activity causes an increase in appetite. The purpose of this study is to use blood markers and subjective appetite questionnaires to further understand a possible association between energy expenditure and body composition on appetite during a 6-month exercise intervention.

Methods: Participants completed two weeks of baseline measurements including a DXA, blood draws and an appetite assessment. During the appetite assessment, participants completed a series of fasted and non-fasted questionnaires (VAS) relating to levels of satiety in addition to blood draws. Blood was analyzed for leptin and ghrelin measurements under fasting and non-fasting conditions. Participants also received a SenseWear mini armband to measure energy expenditure over a period of 10 days at baseline and 6 months follow-up. Upon randomization, participants were placed into an exercise intervention or non-exercise control group. Follow-up measurements including the appetite assessment were completed at the end of the intervention.

Results: 76 male (52.6%) and female participants within the exercise and control group completed the 6-month intervention. Changes in leptin/ghrelin and VAS questionnaires were measured through linear regression models using either body composition (FM) or EE (TDEE, MVPA, vigorous).

Conclusion: Leptin significantly decreased from baseline to 6-months in male and female exercise participants. Change in EE and body composition at follow-up showed limited difference in leptin/ghrelin and VAS questionnaires.

Keywords: energy balance, exergy expenditure, appetite, body composition, weight maintenance

## Introduction

More than one-third of all adults living in the United States have been classified as obese, with similar trends across the globe (65). Recent data has shown 63% of all adults have attempted to lose weight over the past 12 months (64). An underlying mechanism relating to obesity prevalence is the concept of energy balance, or the relation of energy expenditure and energy intake. Expenditure and intake are two main components resulting in possible weight fluctuation dependent on lifestyle choices. Weight loss is commonly induced through dietary restriction or increasing physical activity. A positive energy balance, or a greater energy intake than expenditure results in weight gain, while negative energy balance (expenditure exceeds intake) results in weight loss.

Dietary restriction is cited as the most common way to lose weight (97), (54), however physical activity interventions should not be overlooked. Obese patients trying to lose weight, regardless of their weight loss goals are encouraged to incorporate a form of exercise into their routine to gain cardiovascular health benefits (38). Furthermore, the National Weight Control Registry stated those individuals who found success in weight maintenance cited high levels of physical activity and low fat intake as their tools to prevent weight regain (17). Therefore, while there is much debate regarding the sole culprit of obesity, the combination of increased physical activity paired with a restricted diet, is ultimately critical with weight loss and/or weight maintenance goals.

Furthermore, much research has explored the coupling effects of exercise-induced energy deficits with appetite. The comparison of exercise with appetite has been studied in both short and long term interventions. Over the short term, exercise is a more effective way to produce negative energy balance as compared to dieting (57) which may partially be due to a loss of energy intake compensation after a bout of exercise (16). However, over long term exercise interventions, it takes considerable time for energy intake to adjust to the rising levels of energy expenditure in order to maintain energy homeostasis (14). Therefore, the coupling effect of energy intake and expenditure has raised much controversy over using physical activity as a means of weight loss. This randomized, controlled trial evaluated a possible chronic association with exercise and appetite in young adults independent of weight loss. The purpose of this study was to use objective measurements by means of leptin and ghrelin and subjective measurements through appetite questionnaires to further understand a possible association with the participation of a 6-month exercise intervention on appetite.

**Methods**

Sedentary males and females between the ages of 21-45 were recruited to participate in the Energy Flux Study (EFS) from the greater Columbia, South Carolina area by means of flyers and social media. Interested participants initially completed an online screener to confirm inclusion/exclusion criteria. Inclusion criteria included a Body Mass Index (BMI) score of 25 - 35 $kg/m^2$ (overweight to obese), previously sedentary (<7,000 steps/day with no more than 10 minutes of vigorous activity)(93) and the ability to participate in a fairly rigorous exercise intervention. Exclusion criteria eliminated those who had cancer within the past 5 years of diagnosis or recently had surgery. Additionally, those with a history of depression and/or anxiety who were treated with medication were excluded. Those adults who were currently taking hormone replacement therapy, beta blockers, allergy shots of systematic corticosteroids were not allowed to participant. Finally, anyone planning on moving from the Columbia, SC area in the next 8 months, planning to get pregnant or had any other medical, psychiatric or behavioral factors that potentially count interfere with study participants were excluded.

Baseline Assessments

Upon meeting all inclusion/exclusion criteria, participants were scheduled to attend an orientation session. Orientation included a 25 minute presentation of the overall study design. The study protocol was approved by the University of South Carolina Institutional Review Board and participants subsequently signed the informed consent. Baseline assessments (Baseline 1, Baseline 2 and Appetite Assessment) were completed over a course of two weeks. Baseline 1 (B1) consisted of a graded treadmill exercise test (GXT) in which participants worked to fatigue. Prior to the test, participants completed a total body Lunar fan beam dual X-ray absorptiometry (DXA) scan (GE Healthcare model 8743, Waukesha, WI, USA) in which fat mass and fat free mass was measured. The GXT consisted of a modified Bruce protocol consisting of two minute stages in which either the speed, grade or both increased, as previously described (37). Subsequently, participants attended the second baseline assessment, however it was required to be two days after B1 due to the criterion of abstaining from activity.

Baseline 2 was conducted following a 12 hour fast and abstention from physical activity for a 24 hour period. First, participants completed a resting metabolic rate (RMR) test, lasting approximately 45 minutes as previously described (37). Finally, participants were given a SenseWear mini armband to wear for a period of ten days to assess total daily energy expenditure. Participants were told to wear the monitor 24 hours per day and to only remove it during times when it might get wet, such as showering or swimming. Upon completion of armband wear, participants were confirmed sedentary based on previously mentioned criteria. If participants were not deemed “sedentary”, they were excluded from the study. Anthropometric measurements such as height and weight were also taken twice during both baseline assessments. A third measurement was taken if height and weight measurements were greater than 0.4 centimeters (cm) and/or 0.1 kilogram (kg) respectively.

Appetite Assessment

The final baseline measurement included an appetite assessment held in the clinical lab at the University of South Carolina on Saturdays between 8:00 – 11:00 am. The flow of the appetite assessment is depicted in Figure 3.1. Those who were lactose intolerant (that is could not consume cheese) were excluded. Participants arrived to the laboratory 12 hours fasted. Blood was drawn from the antecubital vein in tubes containing EDTA (1.25 mg/dL) and aprotinin (500 TU/mL) (Kallikrein inhibitor unit, Phoenix, Burlingame, USA). Samples were immediately centrifuged for 10 minutes and subsequently stored in a -80 Centigrade freezer for analyses.

Next, participants were seated in a classroom to complete the first set of questionnaires (in a fasted state). Questionnaires consisted of visual analogue scales (VAS) in which participants marked their response on a 100 cm line based of their level of hunger and satiety. Each rating was converted to a score in millimeters, allowing comparison of hunger and satiety based off the following four questions: Question 1:

“how hungry do you feel”; Question 2: “how satisfied do you feel”; Question 3: “how full do you feel”; Question 4:“how much do you think you can eat”.

Upon completion of the first set of questionnaires (VAS Series 1), participants were moved to a second classroom for a fixed component breakfast which consisted of eggs, white bread and cheese (Subway®, 720 calories). The nutrient composition of the fixed meal was 21.1% from protein, 48.9% from carbohydrate, and 30.5% from fat. In addition, they were given the option of coffee, tea or water with the choice of artificial sweetener. During baseline, the amount of food was determined individually. Participants were instructed to eat until they reached a comfortable level of satiation, with no time requirement. Once they claimed they were finished to the research staff, the portion of food remaining was weighed, if no food remained the entire amount was recorded in grams (g).

Next, participants completed another set of questionnaires immediately following meal consumption (VAS Series 2) and then 30-min (VAS Series 3) and 60-min (VAS Series 4) post meal consumption. After the completion of VAS Series 4, participations were escorted back to the phlebotomy room for a final blood draw. Blood was collected as previously described. Leptin and ghrelin were analyzed at each assessment visit for both fasted and non-fasting values.

Randomization and Intervention

Upon completion of baseline assessments, participants were randomized into either high flux (target 35 kcal/kg/week), moderate flux (17.5 kcal/kg/week) or a non-exercise control group. Those within the high flux group attended 5-6 session/week for approximately one hour. Moderate flux participants came in for 3 sessions for an hour duration.

The intervention consisted of treadmill based exercise in which participants worked at 70-75% of their $VO_{2max}$ heart rate (HR) based on the baseline GXT. Exercise participants were given a 4-6 week ramp-up phase to familiarize themselves with the treadmill and each exercise session. Before each session, participants were given a heart rate monitor (Polar, FT1™, Lake Success, NY, USA) and heart rate was checked every five minutes to ensure they were working at the appropriate intensity. Energy expenditure was calculated based on metabolic equations calculated from the individuals weight, duration and speed/grade of the treadmill (3). By using metabolic equations, research staff were able to properly prescribe the exercise duration and speed/grade prior to each session.

Participants were expected to maintain their weight throughout the intervention ±3% of their baseline body weight. Participants were weighed at the start of every exercise session to ensure they remained within range, if they did not meet this requirement for more than 2 weeks, they met with our registered dietician to discuss healthy options to remain within maintenance range. Those placed in the control group met with research staff monthly to be weighed to ensure maintenance criteria was met.

Follow-Up Measurements

Similar assessments (GXT, RMR and DXA) were measured upon completion of the intervention. In addition, a post-intervention appetite assessment was completed as previously described; however participants consumed the same weight of food as they ate during the pre-training assessment. This comparison allowed for the determination of the dose effect of exercise on measured variables per content of the meal.

Statistical Analysis

Correlations between VAS questionnaires (questions 1-4) with leptin and ghrelin were run at baseline and 6-month measurement points, each correlation was run separately for fasting and non-fasting conditions. Dependent t-tests were run to determine a significant change in either fasting or non-fasting samples from baseline to 6-months for each VAS questionnaire, leptin and ghrelin. In addition, linear regression models were run to determine if a change in energy expenditure or body composition was associated with a change in leptin, ghrelin or a change in VAS scores (questions 1-4). All models were adjusted for age, sex, baseline values (either EE or body composition) and baseline measurements of the dependent variable. Each regression model was run to compare males and females within the exercise group. VAS Series 4 were used for analyses as the blood samples were completed during the same time point. Statistical analyses were completed using SPSS version 22 with $p<.05$ for statistical significance.

**Results**

A total of 76 males (52.6%) and females completed the EFS and all intervention components and assessments. Within this sample, 14 participants were non-exercise controls (42.9% males), with the remainder within the exercise group (54.8% males). The mean age of the total sample was 30.8 ± 7.1 with most of the sample being Caucasian (50.0%) and African Americans (30.3%), followed by Asian/Asian Americans (15.8%). Eighty-three percent of participants who completed the study were classified as overweight (25 kg/$m^2$ ≤ BMI ≤ 30 kg/$m^2$) with the remaining classified as obese (BMI > 30 kg/$m^2$).

Baseline and 6-month characteristics are shown in Table 3.1. Weight did not change within any group throughout the intervention. However, significant body composition changes were seen in female exercise participants (FEX). FEX significantly decreased percent body fat (-1.14 ±1.8 %) and total FM (-1.00 ± 1.9 g) during the intervention, while males showed no significant changes. FEX also increased TDEE significantly, while male exercise participants (MEX) showed no change. No significant changes were shown in controls.

Leptin and Ghrelin

Fasting and non-fasting values for leptin and ghrelin are shown in Table 3.2. MEX and FEX significantly decreased both fasting and non-fasting leptin values from baseline to 6 months. No significant change was seen in ghrelin, with no significant differences seen in leptin or ghrelin within control participants. Change in EE in MEX and FEX (TDEE, MVPA or Vigorous) was not associated with blood markers at follow-up after adjusting for age and baseline measures. However, in MEX participants, there was a significant association with change in FM with fasted leptin ($\beta$=.285, $p<.01$) and non-fasted leptin ($\beta$=.226, $p<.01$) values. In addition, no significance was seen in ghrelin measurements within MEX or FEX models analyzing change in body composition or EE. Baseline TDEE was significantly associated with fasted leptin at follow-up. Additionally, baseline MVPA and baseline FM was significantly associated with fasted and non-fasting leptin within the respected models at follow-up.

VAS Questionnaires

Changes in EE and body composition for VAS questions 1-4 are shown in Table 3.2. Significance in response from baseline to 6-months within each question was only seen in question 1(“how hungry do you feel”). MEX significantly increased their fasting response to hunger (i.e. were more hungry at the follow-up assessment). In addition, FEX also became more hungry (question 1) at follow-up under non-fasting conditions. Changes in EE were not associated with questions 1-4 in either MEX or FEX participants. However, change in FM was associated with an increase in question 3 related to the level of fullness, in FEM ($\beta$=.351, p<.05). That is, females felt fuller at follow-up as compared to baseline. MEX did not significantly differ with body composition changes with any VAS questions at follow-up. Control participants showed no significant different in changes in VAS questions with changes in EE or body composition.

**Discussion**

A significant decrease in fasting and non-fasting leptin was seen in exercise participants at follow-up with no significance change in controls. Results within the FEX group (direct relationship between fat mass and leptin) compliments the literature (5, 21, 78). A recent review by Asarian et al showed levels of leptin are highly correlated with body fat mass (5), however the research is conflicting in males. A study comparing the effect of an exercise intervention in obese males identified that exercise training decreases leptin independent of changes in body fat (66). These results are similar to the findings within this study. MEX did not significantly change their body fat, however participation in an aerobic exercise intervention could be reasoning behind decreases in leptin levels. Pasman et al. stated the interdependent relationship needs to be further researched specifically with fat depot in relation to leptin metabolism to further define the relationship of leptin in overweight/obese individuals.

In the present study, a change in FM and leptin was found in MEX only. No other significance in EE or FFM was determined in either group. Kraemer et al. reviewed the association with leptin under long term exercise interventions (52). In this review, he found several studies with a decrease in fasting leptin levels after participation in an aerobic exercise intervention. Similar results were also seen in those who participated in the exercise intervention, however while the FEM does not showed a linear association, this group still had a significant decrease in leptin. A potential explanation as to why exercise only participants did not see a more significant decrease in fasting leptin is due to the requirement to maintain weight throughout the intervention. The constant need to increase caloric intake may not have allowed for results more consistent within the literature.

When each model was reviewed to show changes in TDEE, MVPA and vigorous activity, no significant changes were seen. However, baseline TDEE and baseline MVPA was significant within MEX models, indicating that the 6-month intervention within this group may not be long enough to determine a significant impact. With a longer duration, there is potential to see a more significant change in feelings of hunger measured through the VAS scales or a potential change in ghrelin. While the EFS population is solely overweight to obese, research has shown no difference in leptin secretion in obese and never-obese participants (5, 74).

FEX participants showed a direct relationship with the measure of fullness (question 3) and FM. Blundell recently discussed the concept of FM having an inhibitory influence on intake under normal weight conditions, primarily due to insulin and leptin sensitivity (12). However, with an increase in fat mass, leptin and insulin resistance are weakened, ultimately preventing FM from suppressing intake, allowing for overeating to occur. (12). While FEX did significantly decrease percent BF, a greater loss may be required to decrease the feeling of fullness. Furthermore, there is much debate regarding the validity of self-report data. Even with significant decreases in leptin fasting and non-fasting values, MEX and FEX reported little to no change in hunger, satiety, fullness or overall consumption. More research is necessary to define the effects on eating between sexes (5).

This randomized controlled trial has a few strengths and limitations associated with the precision of measurement outcomes. A major limitation of the study is that participants were only offered a breakfast sandwich from Subway to analyze satiety. Consumption of an egg and cheese sandwich may have limited the degree of accuracy associated with meal enjoyment in some of the participants. In order to better assess satiety and hunger, using a buffet style breakfast to fully grasp participants consumption based off their individual choices would allow for a stronger analysis of each VAS question. A major strength is objectively measuring each variable, that is body composition (DXA), blood markers and EE (SenseWear arm monitor). Furthermore, these measurements were completed at multiple time points. All exercise session were completed within the training facility and each participant was supervised to ensure they were exercising at the appropriate intensity. Additionally, participants were able to maintain their weight throughout the intervention, allowing for analyses under weight stable conditions.  Future research should determine difference in fat deposits, such as abdominal fat in response to changes in leptin.

**Conclusion**

Exercise plays a vital role within a healthy lifestyle. Participation in routine physical activity will increase the energy expenditure component of energy balance, influencing appetite and energy intake. Females significantly decreased their FM and percent body fat, while males saw no significant reduction. Furthermore, MEX and FEX decreased their fasting and non-fasting leptin levels from baseline to follow-up. Changes in FM are significantly associated with fasting and non-fasting leptin in males only. The reductions in FM seen through decreases in leptin levels is consistent with previous research, however no associations were seen with changes in EE. Possibly, with the significant baseline EE variables within each model, a longer intervention duration is necessary to fully understand the effects of EE on leptin and ghrelin measurements. Minimal changes in subjective assessments on hunger are not surprising, as self-report data has been criticized for accuracy specifically when measuring energy intake (79, 80). Future research is necessary to investigate the mechanisms associated with an aerobic intervention lasting longer than 6-months to continue to assess and association with exercise and hunger/appetite. Furthermore, more research is necessary to analyze the difference between sex in blood markers with changes in EE and/or FM to critically analyze the relation between exercise and levels of satiety and hunger.

Table 6.1: Participant characteristics throughout the 6-month intervention.

| | **Exercise** | | | | **Controls** (n=14) | |
|---|---|---|---|---|---|---|
| | Males (n= 34) | | Females (n= 28) | | | |
| *Characteristics* | Baseline | 6-Month | Baseline | 6-Month | Baseline | 6-Month |
| Age | 30.7 ± 6.3 | | 31.0 ± 8.3 | | 30.7 ± 6.8 | |
| Height (cm) | 178.0 ± 8.7 | | 162.6 ± 6.1 | | 168.5 ± 8.2 | |
| Weight (kg) | 88.8 ± 13.9 | 88.2 ± 13.6 | 74.6 ± 8.5 | 73.9 ± 9.0 | 78.9 ± 12.5 | 79.8 ± 11.3 |
| BMI ($kg/m^2$) | 27.9 ± 3.1 | 27.7 ± 3.0 | 28.2 ± 2.6 | 28.1 ± 2.9 | 27.7 ± 3.1 | 28.0 ± 2.9 |
| *Body Composition* | | | | | | |
| Total FM (g) | 27.3 ± 8.4 | 27.0 ± 8.4 | 29.9 ± 4.7 | 28.9 ± 5.5† | 27.9 ± 6.4 | 28.1 ± 6.2 |
| Total FFM (g) | 61.6 ± 6 | 61.6 ± 7.8 | 45.2 ± 6.2 | 45.6 ± 6.1 | 52.4 ± 9.2 | 52.4 ± 9.0 |
| Body Fat (%) | 30.3 ± 5.8 | 30.1 ± 6.0 | 40.1 ± 4.0 | 39.0 ± 4.5* | 35.3 ± 5.8 | 35.2 ± 6.2 |
| *Energy Expenditure (kcal/day)* | | | | | | |
| Total Daily | 2861.7 ± 364.0 | 2961.4 ± 481.5 | 2201.0 ± 215.5 | 2380.9 ± 319.0* | 2495.7 ± 483.6 | 2598.6 ± 592.6 |
| MVPA | 474.0 ± 192.8 | 662.8 ± 311.8* | 252.4 ± 103.9 | 487.5 ± 233.4* | 339.4 ± 156.5 | 390.5 ± 309.3 |
| Vigorous | 17.1 ± 26.6 | 78.9 ± 111.3* | 8.7 ± 13.9 | 58.7 ± 107.4† | 6.0 ± 7.1 | 9.4 ± 16.0 |

Note. Group means ± SD. *p-value<0.01 †p-value<0.05. BMI= Body Mass Index, MVPA= moderate-to vigorous activity, cm= centimeters, kg= kilograms, m=meters.

Table 6.2: Appetite Assessment blood markers and questionnaires for exercise participants only.

| | Males (n=34) | | Females (n=28) | |
|---|---|---|---|---|
| | Baseline | 6-Month | Baseline | 6-Month |
| ***Fasting*** | | | | |
| Leptin (ng/dL) | 15.2 ± 11.1 | 12.7 ± 8.2↓ | 39.2 ± 16.4 | 35.0 ± 17.2* |
| Ghrelin (pg/dL) | 766.2 ± 236.5 | 756.6 ± 302.5 | 898.2 ± 209.5 | 935.0 ± 314.7 |
| VAS 1.1 | 52.7 ± 20.2 | 64.6 ± 20.2* | 43.4 ± 23.8 | 52.8 ± 26.5 |
| VAS 1.2 | 26.1 ± 16.1 | 20.7 ± 17.5 | 22.8 ± 18.0 | 21.0 ± 15.6 |
| VAS 1.3 | 12.8 ± 13.7 | 14.6 ± 15.7 | 15.4 ± 16.9 | 15.9 ± 16.9 |
| VAS 1.4 | 68.6 ± 20.1 | 75.1 ± 13.6 | 56.9 ± 21.5 | 60.9 ± 18.8 |
| ***Non-Fasting*** | | | | |
| Leptin (ng/dL) | 13.7 ± 10.2 | 11.3 ± 7.0* | 33.3 ± 16.0 | 29.5 ± 16.4* |
| Ghrelin (pg/dL) | 681.0 ± 270.5 | 690.4 ± 279.0 | 809.5 ± 235.3 | 820.7 ± 264.9 |
| VAS 4.1 | 29.2 ± 23.5 | 24.3 ± 18.1 | 13.2 ± 16.4 | 22.0 ± 24.0↓ |
| VAS 4.2 | 67.0 ± 20.3 | 70.9 ± 19.5 | 70.7 ± 25.9 | 73.1 ± 22.2 |
| VAS 4.3 | 66.6 ± 19.5 | 67.2 ± 23.7 | 71.6 ± 25.2 | 71.9 ± 23.9 |
| VAS 4.4 | 36.5 ± 22.5 | 36.7 ± 23.0 | 22.0 ± 22.5 | 26.9 ± 24.1 |

Note. Group means ± SD. *p-value<0.01, ↓p-value<0.05
VAS measurements reported in centimeters (cm). VAS= visual analogue scale

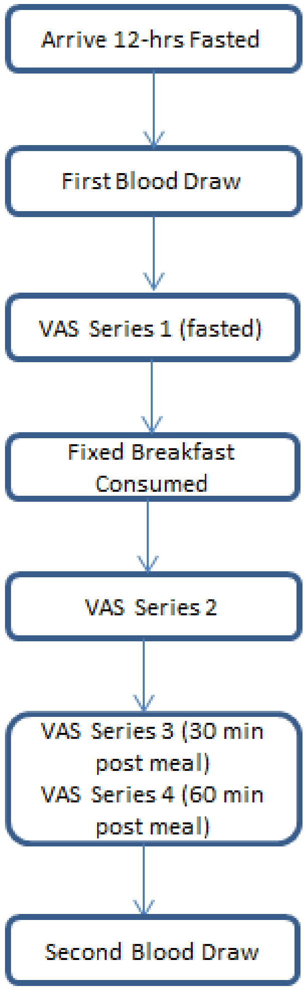


Figure 6.1: Appetite assessment flow chart for the EFS.
Note. VAS= visual analogue scale.

# CHAPTER SEVEN

## OVERALL DISCUSSION

These studies analyzed several associations between body composition (primarily FM and FFM), energy expenditure (TDEE, MVPA and VPA) and metabolic blood markers as well as measures of appetite. Data was drawn from the Energy Flux Study, a randomized controlled trial completed in Columbia, SC. These analyses researched a multitude of health parameters relating to weight maintenance during an exercise intervention. Major findings of this dissertation include 1) Participation in a 6-month aerobic training intervention lead to minimal changes in body composition in males and females when body weight was maintained. However, significant increases in CRF were seen in those who participated in the aerobic intervention. 2) A decrease in fat mass through participation in an aerobic intervention will improve cholesterol levels as compared to changes in EE. Furthermore, those individuals who improved their lipid profile showed a greater decrease in FM as compared to the group who worsened their profile. 3) Female exercisers showed significant decreases in FM and percent BF at follow-up, with no significant changes in males. Furthermore, males and females showed a decrease in fasting and non-fasting leptin after completion of a 6-month aerobic intervention under weight stable conditions.

The American College of Sports Medicine provided guidelines for adults to successfully maintain body weight over a long period of time (27). Donnelly et al. reported high levels of moderate intensity PA (250-300 min/week) will allow for weight maintenance within the adult population. Many clinical interventions have used a variety of exercise durations and intensities to further understand a recommended exercise dose for weight maintenance, however limitations exist for PA, as much for the data is self-report (89). However, within several clinical interventions exercising at least 200 min/week has stronger weight maintenance benefits compared to those who completed 150-199 min/week and those who exercised less than 150 min/week (43, 45). Therefore, it is greatly understood that high levels of physical activity are essential to improve health and allow for successful weight maintenance over longer periods of time. The EFS researched the effects of a 6-month aerobic intervention (high levels of PA) on metabolic markers, body composition and appetite.

The first aim analyzed the possible association of energy expenditure and body composition. Significant changes were seen through increases in vigorous activity, resulting in a decrease in FM in female participants. Ross et al. documented the dose-response relationship between physical activity and total fat mass (76). Within this review, those studies which implemented a short term (≤16 weeks) exercise intervention had a positive relationship with fat mass loss and exercise, while no relationship was observed in long term studies (≥26 weeks). Therefore, Ross believed while a relationship exists, the effects of increased energy expenditure over a long term intervention impedes further. Similarly, Ross compared exercise groups with and without weight loss (77). Within these findings, there were substantial reductions in total and abdominal fat in females. These findings, in addition to reductions in fat mass from the EFS provide strong support for the recommendation of routine exercise as a strategy for adiposity reduction in overweight to obese females.

Furthermore, the second aim of this study reviewed an increase in energy expenditure and body composition on blood lipids following completion of an aerobic intervention. Within this sample, a greater decrease in FM will better improve cholesterol as compared to changes in energy expenditure. Previous research has shown those who are active individuals gain a more favorable cholesterol panel as compared to sedentary individuals (9, 30). Furthermore, fat distribution has been highly reviewed with cholesterol levels within adult populations (26). Excessive trunk fat is positively associated with an increase in plasma triglycerides. Instead of using pharmaceutical agents to lower cholesterol, many should aim to improve lifestyle changes, such as improvements in PA to decrease their cholesterol levels. The combination of nutritional supplements with an exercise prescription has been shown to decrease TC, LDL-C, and TG concentrations in addition to improving HDL-C (94). While this analysis did not highlight the importance of increasing EE to improve upon blood lipids, the necessity of routine PA should not be overlooked (29, 32).

Finally, the third aim was to further understand the relationship of chronic aerobic activity with objective and subjective measurements of appetite. Decreases in leptin levels under fasting and non-fasting conditions with significant decreases in FM and percent BF were seen in females, with no significant body composition changes in males. These changes in female participants are consistent with the literature (5), (21) (78) which showed a direct relationship with FM and leptin levels. However, other mechanisms, such those who are more active have lower leptin concentrations as compared to more sedentary need to be considered as well (52). Furthermore, changes in FM decreased fasting and non-fasting leptin at follow-up pairs nicely to the relationship between leptin and fat mass. Interestingly, no significant differences were seen in changes in EE, however since several of the baseline EE measurements were significant within the model, it may indicate a longer intervention (> 6 months) is required to interpret significant change.

**Conclusion**

To the best of our knowledge, the research done within this dissertation is novel and provides a link to associations in the participation of an aerobic exercise program under weight stable conditions. There are several strengths of this RCT, primarily the use of objective measurements at multiple time points. Assessments of body composition, blood markers, energy expenditure and cardiorespiratory fitness used objective measurements at baseline, 3-months and 6-months thus allowing for increased accuracy of measurement and data analysis. Furthermore, all exercise sessions were monitored to ensure participants were maintaining the required intensity. All participants were overweight to obese and confirmed as sedentary using accelerometers at baseline.

Finally, all participants within the study maintained their weight throughout the 6-month intervention. There are also a few limitations to consider. The recruited sample is primarily from the University of South Carolina campus in Columbia, SC. Majority of participants were Caucasian and African American and had a moderate to high socioeconomic status. Therefore, participants in the EFS are not a true representation of the general public. In addition, dietary intake reported through self-report food logs is a weakness which is thoroughly assessed through the validation of self-report. A final limitation of this study includes using a Subway sandwich to assess hunger during the pre/post appetite assessment. While this reduced staff burden, it may not have led to the most accurate representation of satiety.

In conclusion, weight loss is not required during an exercise program in order to gain health benefits in adults (89). Additionally, it is more feasible to not place a strong focus on weight loss, but instead focus on the benefits of exercise under a regimented exercise program. Those who are overweight and obese have the ability to gain several benefits from chronic physical activity. A common variable seen within this dissertation is the effect of exercise on a decrease in FM through participation in an aerobic intervention. Within male and female populations, a decrease in FM was able to lower cholesterol levels in addition to decreasing leptin all under weight stable conditions. This finding is of critical significance since so many adults are failing with weight loss interventions. Placing a higher value of increasing health and fitness through aerobic activity as opposed to weight loss will hopefully encourage active lifestyles.

# REFERENCES


1. The Lipid Research Clinics Coronary Primary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA*. 1984;251(3):365-74.
2. Adams CE, Greenway FL, Brantley PJ. Lifestyle factors and ghrelin: critical review and implications for weight loss maintenance. *Obes Rev*. 2011;12(5):e211-8.
3. American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription*. 8 ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2010.
4. Archer E, Shook RP, Thomas DM et al. 45-Year trends in women's use of time and household management energy expenditure. *PLoS One*. 2013;8(2):e56620.
5. Asarian L, Geary N. Sex differences in the physiology of eating. *Am J Physiol Regul Integr Comp Physiol*. 2013;305(11):R1215-67.
6. Badman MK, Flier JS. The gut and energy balance: visceral allies in the obesity wars. *Science*. 2005;307(5717):1909-14.
7. Barry VW, Baruth M, Beets MW, Durstine JL, Liu J, Blair SN. Fitness vs. fatness on all-cause mortality: a meta-analysis. *Prog Cardiovasc Dis*. 2014;56(4):382-90.
8. Beavers KM, Beavers DP, Nesbit BA et al. Effect of an 18-month physical activity and weight loss intervention on body composition in overweight and obese older adults. *Obesity (Silver Spring)*. 2014;22(2):325-31.
9. Blair SN, Ellsworth NM, Haskell WL, Stern MP, Farquhar JW, Wood PD. Comparison of nutrient intake in middle-aged men and women runners and controls. *Med Sci Sports Exerc*. 1981;13(5):310-5.
10. Blair SN, Kampert JB, Kohl HW et al. Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. *JAMA*. 1996;276(3):205-10.
11. Blair SN, Kohl HW, Paffenbarger RS, Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA*. 1989;262(17):2395-401.
12. Blundell JE, Caudwell P, Gibbons C et al. Role of resting metabolic rate and energy expenditure in hunger and appetite control: a new formulation. *Dis Model Mech*. 2012;5(5):608-13.
13. Blundell JE, Gibbons C, Caudwell P, Finlayson G, Hopkins M. Appetite control and energy balance: impact of exercise. *Obes Rev*. 2015;16 Suppl 1:67-76.
14. Blundell JE, King NA. Effects of exercise on appetite control: loose coupling between energy expenditure and energy intake. *Int J Obes Relat Metab Disord*. 1998;22 Suppl 2:S22-9.
15. Blundell JE, King NA. Exercise, appetite control, and energy balance. *Nutrition*. 2000;16(7-8):519-22.

16. Cameron JD, Goldfield GS, Riou M, Finlayson GS, Blundell JE, Doucet É. Energy depletion by diet or aerobic exercise alone: impact of energy deficit modality on appetite parameters. *Am J Clin Nutr*. 2016.
17. Catenacci VA, Odgen L, Phelan S et al. Dietary habits and weight maintenance success in high versus low exercisers in the National Weight Control Registry. *J Phys Act Health*. 2014;11(8):1540-8.
18. Center of Disease Control and Prevention Web Site [Internet] Atlanta (GA): Adult Obesity Causes and Consequences; [cited 2015 July 1]. Available from http://www.cdc.gov/obesity/adult/causes.html
19. Church TS, Earnest CP, Thompson AM et al. Exercise without weight loss does not reduce C-reactive protein: the INFLAME study. *Med Sci Sports Exerc*. 2010;42(4):708-16.
20. Church TS, Thomas DM, Tudor-Locke C et al. Trends over 5 decades in U.S. occupation-related physical activity and their associations with obesity. *PLoS One*. 2011;6(5):e19657.
21. Considine RV, Sinha MK, Heiman ML et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med*. 1996;334(5):292-5.
22. Cummings DE. Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiol Behav*. 2006;89(1):71-84.
23. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*. 2001;50(8):1714-9.
24. Dattilo AM, Kris-Etherton PM. Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. *Am J Clin Nutr*. 1992;56(2):320-8.
25. de Graaf C. The validity of appetite ratings. *Appetite*. 1993;21(2):156-60.
26. Després JP, Moorjani S, Lupien PJ, Tremblay A, Nadeau A, Bouchard C. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis*. 1990;10(4):497-511.
27. Donnelly JE, Blair SN, Jakicic JM et al. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc*. 2009;41(2):459-71.
28. Donnelly JE, Hill JO, Jacobsen DJ et al. Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: the Midwest Exercise Trial. *Arch Intern Med*. 2003;163(11):1343-50.
29. Durstine JL, Grandjean PW, Davis PG, Ferguson MA, Alderson NL, DuBose KD. Blood lipid and lipoprotein adaptations to exercise: a quantitative analysis. *Sports Med*. 2001;31(15):1033-62.
30. Durstine JL, Pate RR, Sparling PB, Wilson GE, Senn MD, Bartoli WP. Lipid, lipoprotein, and iron status of elite women distance runners. *Int J Sports Med*. 1987;8 Suppl 2:119-23.
31. Finkelstein EA, Trogdon JG, Cohen JW, Dietz W. Annual medical spending attributable to obesity: payer-and service-specific estimates. *Health Aff (Millwood)*. 2009;28(5):w822-31.

32. Gordon B, Chen S, Durstine JL. The effects of exercise training on the traditional lipid profile and beyond. *Curr Sports Med Rep*. 2014;13(4):253-9.
33. Grediagin A, Cody M, Rupp J, Benardot D, Shern R. Exercise intensity does not effect body composition change in untrained, moderately overfat women. *J Am Diet Assoc*. 1995;95(6):661-5.
34. Green SM, Delargy HJ, Joanes D, Blundell JE. A satiety quotient: a formulation to assess the satiating effect of food. *Appetite*. 1997;29(3):291-304.
35. Hall KD, Heymsfield SB, Kemnitz JW, Klein S, Schoeller DA, Speakman JR. Energy balance and its components: implications for body weight regulation. *Am J Clin Nutr*. 2012;95(4):989-94.
36. Hamer M. The relative influences of fitness and fatness on inflammatory factors. *Prev Med*. 2007;44(1):3-11.
37. Hand GA, Shook RP, Paluch AE et al. The energy balance study: the design and baseline results for a longitudinal study of energy balance. *Res Q Exerc Sport*. 2013;84(3):275-86.
38. Haskell WL, Lee IM, Pate RR et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Circulation*. 2007;116(9):1081-93.
39. Hellström PM, Geliebter A, Näslund E et al. Peripheral and central signals in the control of eating in normal, obese and binge-eating human subjects. *Br J Nutr*. 2004;92 Suppl 1:S47-57.
40. Hill JO. Understanding and addressing the epidemic of obesity: an energy balance perspective. *Endocr Rev*. 2006;27(7):750-61.
41. Imbeault P, Saint-Pierre S, Alméras N, Tremblay A. Acute effects of exercise on energy intake and feeding behaviour. *Br J Nutr*. 1997;77(4):511-21.
42. Irving BA, Davis CK, Brock DW et al. Effect of exercise training intensity on abdominal visceral fat and body composition. *Med Sci Sports Exerc*. 2008;40(11):1863-72.
43. Jakicic JM, Marcus BH, Gallagher KI, Napolitano M, Lang W. Effect of exercise duration and intensity on weight loss in overweight, sedentary women: a randomized trial. *JAMA*. 2003;290(10):1323-30.
44. Jakicic JM, Otto AD. Physical activity considerations for the treatment and prevention of obesity. *Am J Clin Nutr*. 2005;82(1 Suppl):226S-9S.
45. Jakicic JM, Winters C, Lang W, Wing RR. Effects of intermittent exercise and use of home exercise equipment on adherence, weight loss, and fitness in overweight women: a randomized trial. *JAMA*. 1999;282(16):1554-60.
46. Johannsen DL, Calabro MA, Stewart J, Franke W, Rood JC, Welk GJ. Accuracy of armband monitors for measuring daily energy expenditure in healthy adults. *Med Sci Sports Exerc*. 2010;42(11):2134-40.
47. Johnstone AM, Murison SD, Duncan JS, Rance KA, Speakman JR. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. *Am J Clin Nutr*. 2005;82(5):941-8.
48. Katan MB, Ludwig DS. Extra calories cause weight gain--but how much? *JAMA*. 2010;303(1):65-6.

49. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond)*. 2008;32(9):1431-7.
50. King NA, Lluch A, Stubbs RJ, Blundell JE. High dose exercise does not increase hunger or energy intake in free living males. *Eur J Clin Nutr*. 1997;51(7):478-83.
51. Klein S, Burke LE, Bray GA et al. Clinical implications of obesity with specific focus on cardiovascular disease: a statement for professionals from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: endorsed by the American College of Cardiology Foundation. *Circulation*. 2004;110(18):2952-67.
52. Kraemer RR, Chu H, Castracane VD. Leptin and exercise. *Exp Biol Med (Maywood)*. 2002;227(9):701-8.
53. Kraus WE, Houmard JA, Duscha BD et al. Effects of the amount and intensity of exercise on plasma lipoproteins. *N Engl J Med*. 2002;347(19):1483-92.
54. Kruger J, Galuska DA, Serdula MK, Jones DA. Attempting to lose weight: specific practices among U.S. adults. *Am J Prev Med*. 2004;26(5):402-6.
55. Lee S, Kuk JL, Davidson LE et al. Exercise without weight loss is an effective strategy for obesity reduction in obese individuals with and without Type 2 diabetes. *J Appl Physiol (1985)*. 2005;99(3):1220-5.
56. Leon AS, Sanchez OA. Response of blood lipids to exercise training alone or combined with dietary intervention. *Med Sci Sports Exerc*. 2001;33(6 Suppl):S502-15; discussion S28-9.
57. Martins C, Morgan L, Truby H. A review of the effects of exercise on appetite regulation: an obesity perspective. *Int J Obes (Lond)*. 2008;32(9):1337-47.
58. Mayer J, Roy P, Mitra KP. Relation between caloric intake, body weight, and physical work: studies in an industrial male population in West Bengal. *Am J Clin Nutr*. 1956;4(2):169-75.
59. Medicine, American College of Sports. ATP III Classification of LDL, Total and HDL Cholesterol (mg/dL). In. Baltimore, MD 2010, p 48.
60. Medicine, Dietary Reference Intakes for Energy, Carbohydrate, Fober, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids (Macronutrients). In. Washington, DC: The National Academies Press; 2005.
61. Miller NE, Hammett F, Saltissi S et al. Relation of angiographically defined coronary artery disease to plasma lipoprotein subfractions and apolipoproteins. *Br Med J (Clin Res Ed)*. 1981;282(6278):1741-4.
62. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. *Nature*. 2006;443(7109):289-95.
63. National Cholesterol Education Program (NCEP) Expert Panel on Detection Ea, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*. 2002;106(25):3143-421.
64. Nicklas JM, Huskey KW, Davis RB, Wee CC. Successful weight loss among obese U.S. adults. *Am J Prev Med*. 2012;42(5):481-5.

65. Ogden CL, Carroll MD, Fryar CD, Flegal KM. Prevalence of Obesity Among Adults and Youth: United States, 2011-2014. *NCHS Data Brief*. 2015;(219):1-8.
66. Pasman WJ, Westerterp-Plantenga MS, Saris WH. The effect of exercise training on leptin levels in obese males. *Am J Physiol*. 1998;274(2 Pt 1):E280-6.
67. Peters JC, Wyatt HR, Donahoo WT, Hill JO. From instinct to intellect: the challenge of maintaining healthy weight in the modern world. *Obes Rev*. 2002;3(2):69-74.
68. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. *J Appl Physiol (1985)*. 2005;98(4):1154-62.
69. Pi-Sunyer X, Blackburn G, Brancati FL et al. Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: one-year results of the look AHEAD trial. *Diabetes Care*. 2007;30(6):1374-83.
70. Pomerleau M, Imbeault P, Parker T, Doucet E. Effects of exercise intensity on food intake and appetite in women. *Am J Clin Nutr*. 2004;80(5):1230-6.
71. Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J Clin Invest*. 1986;78(6):1568-78.
72. Ricketts TA, Sui X, Lavie CJ, Blair SN, Ross R. Addition of Cardiorespiratory Fitness Within an Obesity Risk Classification Model Identifies Men at Increased Risk of All-cause Mortality. *Am J Med*. 2015.
73. Rook A. An investigation into the longevity of Cambridge sportsmen. *Br Med J*. 1954;1(4865):773-7.
74. Rosenbaum M, Nicolson M, Hirsch J et al. Effects of gender, body composition, and menopause on plasma concentrations of leptin. *J Clin Endocrinol Metab*. 1996;81(9):3424-7.
75. Ross R, Dagnone D, Jones PJ et al. Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Ann Intern Med*. 2000;133(2):92-103.
76. Ross R, Janssen I. Physical activity, total and regional obesity: dose-response considerations. *Med Sci Sports Exerc*. 2001;33(6 Suppl):S521-7; discussion S8-9.
77. Ross R, Janssen I, Dawson J et al. Exercise-induced reduction in obesity and insulin resistance in women: a randomized controlled trial. *Obes Res*. 2004;12(5):789-98.
78. Ryan AS, Elahi D. The effects of acute hyperglycemia and hyperinsulinemia on plasma leptin levels: its relationships with body fat, visceral adiposity, and age in women. *J Clin Endocrinol Metab*. 1996;81(12):4433-8.
79. Schoeller DA, Bandini LG, Dietz WH. Inaccuracies in self-reported intake identified by comparison with the doubly labelled water method. *Can J Physiol Pharmacol*. 1990;68(7):941-9.
80. Schoeller DA, Thomas D, Archer E et al. Self-report-based estimates of energy intake offer an inadequate basis for scientific conclusions. *Am J Clin Nutr*. 2013;97(6):1413-5.
81. Schwingshackl L, Dias S, Strasser B, Hoffmann G. Impact of different training modalities on anthropometric and metabolic characteristics in overweight/obese subjects: a systematic review and network meta-analysis. *PLoS One*. 2013;8(12):e82853.

82. Services, U.S. Department of Health and Human. [Internet] Physical Activity Guidelines Advisory Committee Report, 2008. [cited 2016 April 7]. Available from: http://www.health.gov/paguidelines/.
83. Shook R, Hand G, Drenowatz C et al. Low levels of physical activity are associated with dysregulation of energy intake and fat mass gain over 1-year. *American Journal of Clinical Nutrition*. In Review.
84. Slentz CA, Duscha BD, Johnson JL et al. Effects of the amount of exercise on body weight, body composition, and measures of central obesity: STRRIDE--a randomized controlled study. *Arch Intern Med*. 2004;164(1):31-9.
85. Slentz CA, Houmard JA, Johnson JL et al. Inactivity, exercise training and detraining, and plasma lipoproteins. STRRIDE: a randomized, controlled study of exercise intensity and amount. *J Appl Physiol (1985)*. 2007;103(2):432-42.
86. Sniderman AD, Pedersen T, Kjekshus J. Putting low-density lipoproteins at center stage in atherogenesis. *Am J Cardiol*. 1997;79(1):64-7.
87. Stensel D. Exercise, appetite and appetite-regulating hormones: implications for food intake and weight control. *Ann Nutr Metab*. 2010;57 Suppl 2:36-42.
88. Stubbs RJ, Sepp A, Hughes DA et al. The effect of graded levels of exercise on energy intake and balance in free-living men, consuming their normal diet. *Eur J Clin Nutr*. 2002;56(2):129-40.
89. Swift DL, Johannsen NM, Lavie CJ, Earnest CP, Church TS. The role of exercise and physical activity in weight loss and maintenance. *Prog Cardiovasc Dis*. 2014;56(4):441-7.
90. Thomas JG, Bond DS, Phelan S, Hill JO, Wing RR. Weight-loss maintenance for 10 years in the National Weight Control Registry. *Am J Prev Med*. 2014;46(1):17-23.
91. Tran ZV, Weltman A. Differential effects of exercise on serum lipid and lipoprotein levels seen with changes in body weight. A meta-analysis. *JAMA*. 1985;254(7):919-24.
92. Tremblay A, Bellisle F. Nutrients, satiety, and control of energy intake. *Appl Physiol Nutr Metab*. 2015;40(10):971-9.
93. Tudor-Locke C, Leonardi C, Johnson WD, Katzmarzyk PT, Church TS. Accelerometer steps/day translation of moderate-to-vigorous activity. *Prev Med*. 2011;53(1-2):31-3.
94. Varady KA, Jones PJ. Combination diet and exercise interventions for the treatment of dyslipidemia: an effective preliminary strategy to lower cholesterol levels? *J Nutr*. 2005;135(8):1829-35.
95. Wei M, Kampert JB, Barlow CE et al. Relationship between low cardiorespiratory fitness and mortality in normal-weight, overweight, and obese men. *JAMA*. 1999;282(16):1547-53.
96. Weiss EC, Galuska DA, Kettel Khan L, Gillespie C, Serdula MK. Weight regain in U.S. adults who experienced substantial weight loss, 1999-2002. *Am J Prev Med*. 2007;33(1):34-40.
97. Williamson DF, Serdula MK, Anda RF, Levy A, Byers T. Weight loss attempts in adults: goals, duration, and rate of weight loss. *Am J Public Health*. 1992;82(9):1251-7.

98. Wing RR, Hill JO. Successful weight loss maintenance. *Annu Rev Nutr*. 2001;21:323-41.
99. World Health Organization Web site [Internet] Washington (D.C.): Fact sheet on obesity and overweight; [cited 2015 July 1]. Available from http://www.who.int/mediacentre/factsheets/fs311/en/.
100. Wright JD, Wang CY. Trends in intake of energy and macronutrients in adults from 1999-2000 through 2007-2008. *NCHS Data Brief*. 2010;(49):1-8.