The Effects of Exercise Training and Dietary Supplementation on Fat Metabolism and Body Composition in Obese Women

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Reduced energy expenditure and impaired fat oxidation are critical factors associated with obesity. Although much is known about the effects of exercise training on fat metabolism in normal weight, healthy women, considerably less is known about the potential benefits of exercise on fat metabolism in obesity. **PURPOSE**: 1) To determine the effects of aerobic exercise on fat metabolism and body composition in previously sedentary obese women. 2) To examine whether a dietary supplement purported to increase metabolism will elicit further improvements in fat metabolism and body composition when combined with exercise. **METHODS:** 15 Obese (BMI > 30 kg/m2) premenopausal women aged 36 ± 7 years completed a 16- week intervention consisting of moderate exercise. Women were randomized into either a dietary supplement group or placebo; all participated in the exercise training intervention. Pre and post intervention, all subjects underwent a DXA, graded exercise test, and indirect calorimetry to measure energy expenditure and fat oxidation at rest and during exercise (treadmill walking at 55% VO2 max). **RESULTS:** VO_2 max improved on average by 11% from 50.9 ± 8.2 to 56.1 ± 8.1 ml/kgFFM/min; ($p<0.01$). There was a significant weight loss overall (85.5 ± 9.7 to 83.2 ± 10.1kg; $p< 0.05$) but there was no significant difference between intervention groups. However, the analysis revealed a significant weight loss in the supplement group (89.9 ± 10.9 to 87.4 ± 12.0 kg; $p<0.05$), while the Placebo group did not quite reach significant differences (80.5 ± 5.0 ± to 78.3 ± 5.9, $p=0.06$). The amount of weekly structured

exercise (kcal per week) was strongly associated with greater reductions in waist circumference (R^2=0.77; P<0.05). There was an exercise-training induced increased rate of fat oxidation during 60 minutes of sub-maximal exercise (0.30 ± 0.06 to 0.34 ± 0.12 g/min; p<0.05). There was, however, no change in resting metabolic rate (RMR) or resting fat oxidation. **CONCLUSION:** Exercise training increases the reliance on fatty acids for energy during physical activity in obese women. Exercise training also improved body composition. These improvements were not affected by the dietary supplement.

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PREFACE

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One thing that I have learned form this experience is determination. With many things like research and life there is no instant gratification. It takes time, patience and hard work to achieve your goals.

> ``You have powers you never dreamed of. You can do things you never thought you could do. There are no limitations in what you can do except the limitations in your own mind as to what you cannot do. Don't think you cannot. Think you can". – Darwin P. Kingsley.

Finally, I dedicated this paper to my Mom and Dad, who inspire me daily to become the best complete person I can be. Thank you, I love you.

1.0 CHAPTER 1

1.1 INTRODUCTION

1.1.1 Rationale

Obesity is a primary risk factor for cardiovascular disease and type 2 diabetes mellitus. Approximately two thirds of Americans are either overweight or obese (Ogden et al, 2006). Reduced energy expenditure and impaired fat oxidation are critical factors associated with obesity and also with a sedentary lifestyle and physical inactivity (Goodpaster et al., 2003). Thus, identifying optimal exercise programs and regimens in obese women to increase fat metabolism and reduce body fat has tremendous public health implications. Although several studies have demonstrated that aerobic exercise training such as walking or cycling improves fat metabolism in healthy lean men and women, it is not clear whether obese individuals derive this same benefit from exercise. Goodpaster and colleagues (2002) found that physical fitness might have a more potent influence on fat metabolism during exercise than obesity per se. This suggests that obese individuals participating in aerobic exercise training programs may indeed enhance their fat metabolism. This hypothesis was directly addressed in the proposed study.

The marketing and use of popular “fat burning” supplements has also raised the question of whether these products are effective in their ability to increase fat metabolism and reduce

body fat. Previous studies have examined some of the individual ingredients in the proposed dietary supplement and have shown positive results. Green tea has been shown to exert a direct inhibition of gastric and pancreatic lipases as well as a stimulation of thermogenesis in vitro (Chantre and Lairon D, 2002). Caffeine has also been reported to increase fat metabolism in humans during exercise (Chesley et al, 1998). In addition, calcium has been reported to modulate adiposity in humans independent of caloric restriction in obese humans (Zemel, 2003). However, a systematic Placebo-controlled study has not been conducted to determine the effects of these combined supplements on fat metabolism. Thus, another purpose of this study will be to examine whether a popular dietary supplement, Thermo Dynamx ® tablets (Experimental and Applied Science, Inc., Golden, CO), which contains caffeine, green tea extract and calcium, may provide further improvements in fat metabolism and body composition in obese women when superimposed on exercise.

1.1.2 Purpose

The purpose of this study was twofold: 1) to examine the effect of aerobic exercise on fat metabolism and body composition in previously sedentary obese women and 2) to examine whether a popular over-the-counter Ephedra-free dietary supplement purported to increase metabolism will elicit further improvements in fat metabolism and body composition when combined with exercise. The overall goal of the study was to determine whether aerobic training with or without a dietary supplement would improve fat metabolism and reduce body fat in obese women. This proposed study may be useful to help define exercise interventions strategies to prevent obesity and associated diseases in premenopasual women.

1.1.3 Research Hypotheses

1) It was hypothesized that aerobic exercise would improve fat metabolism and reduce body fat in obese women.
2) It was hypothesized that obese women consuming a current popular dietary supplement, when combined with regular exercise, would have additional improvements in fat metabolism and body composition compared to those who exercise with Placebo.

1.1.4 Significance

Recent evidence suggests that fat oxidation by skeletal muscle is impaired in obesity and is linked to insulin resistance. The significance of this study is to expand our knowledge of fat metabolism in obese women. We know that endurance training can improve the capacity to oxidize fatty acids in normal weight individuals but it is less clear whether exercise training enhances fat oxidation in obese persons. Moreover, it is not known whether dietary supplements might offer any additional improvement over any exercise training benefit. Therefore, examining the effects of exercise on fat oxidation may result in better strategies for prevention and treatment of obesity and associated diseases.

2.0 CHAPTER 2

2.1 BACKGROUND -OBESITY EPIDEMIC

Obesity is among our nation's most pressing public health problem. This problem is shared with numerous developing nations therefore the World Health Organization has declared obesity a "global epidemic." The prevalence of obesity has increased dramatically in both adults and children in the United Stated in the past three decades. It has been reported that about thirty percent of our population today is obese (Flegal et al., 2002). A study released in 2003 stated that the prevalence of body mass index (BMI) of > 30 has doubled between the years 1986- 2000 (Sturm, 2003). It has also been reported that women (34%) are more likely to be obese compared to men (27.7%). These statistics are most likely due to the environmental conditions that we now live in with constant availability of high fat food and with little need to be physically active.

Obesity is defined as the accumulation of excess body fat. Its represents the long-term results of positive energy balance. Obesity is a risk factor for cardiovascular disease, certain cancers (gastric, colon and breast cancers), diabetes and mortality. Obesity can exacerbate other chronic conditions such as hypertension, dyslipedemia, and musculoskeletal problems. Obesity is not a single disorder but a heterogeneous group of conditions with multiple causes. Therefore; the regulatory mechanisms leading to the development of obesity are still not well understood.

There is evidence, however, that exercise or increased energy expenditure plays an important role in obesity prevention and treatment.

2.1.1 Energy Balance

Ensuring optimal health and maintaining a healthy body weight is accomplished through sound dietary practices and participation in regular amounts of physical activity. Energy balance is the difference between the number of kilocalories that one eats (energy intake) and the number of kilocalories that an individual burns (energy expenditure). Obesity is caused by long-term positive energy balance, i.e. ingesting more energy than expended over a long period of time causes obesity. The excess calories that are consumed lead to accumulation of body fat either by being stored as fat or preventing the mobilization and oxidation of endogenous fat. To maintain body weight a balance or steady state between energy intake and energy expenditure must take place. In general, ingesting 3500kcals more than expended will lead to a gain of approximately 1lb.of fat. Any alterations in the intake or expenditure of energy can shift the energy balance thus lead to a change in body weight.

When one considers energy balance in humans under physiological conditions, fat is a key nutrient that maintains a chronic imbalance between intake and oxidation, thus directly contributing to the increase in adipose tissue. The concept of the fat balance in addition to the energy balance offers a new framework for understanding the pathogenesis of obesity.

Although the concept of energy and fat balance seems simple, it is rather complex. Energy intake is influenced by many factors; environment, behavioral, biological and genetic influences. Energy intake is controlled by hunger, appetite and satiety (McMurray and Hackney, 2005). Hunger and satiety appear to be genetic and physiological in nature receiving input from

many areas of the body (e.g. blood glucose, endogenous opiates, neurotransmitters of the gastrointestinal system and leptin). An individual's appetite appears to be psychological or environmental (learned response and cultural) (McMurray and Hackney, 2005). Energy intake is also difficult to measure.

Energy expenditure consists of resting metabolic rate (RMR), the thermic effects of food (TEF), and physical activity. Resting metabolic rate is the energy expended by an individual at rest under fasting and comfortable conditions. This accounts for approximately 60-70% of daily energy expenditure (Ravusissin et al., 1986). Thermic affect of food is about 5-15 % of daily energy expended. It can be effected by the size and composition of the meal ingested.

The most variable component of daily energy expenditure is physical activity or exercise. Sedentary adults exhibit a range of physical activity that is about 20-30 % of total energy expenditure, whereas in very active individuals this can account for large proportion of total calories. Energy expenditure can be measured with more accuracy in a laboratory but with a greater burden to the subject. Therefore, quantification of total energy balance is complicated.

2.1.2 Weight Loss – Caloric Restriction and Physical Activity

Millions of Americans experience illnesses that can be improved or prevented through weight loss and/or regular physical activity. There are limited choices that an individual can perform to attempt to induce weight loss. Weight reduction and exercise are common nonpharmalogical interventions for the treatment of obesity and associated diseases.

Weight loss by caloric restriction has been shown to be best for short -term weight loss. A Meta analysis review of literature was conducted over a 25-year period focusing on caloric restriction. This analysis established that obese people lost approximately 11 kg in a 15-week

period but it also reported the average weight regained in a one year was 35% of their loss (Miller et al., 1997). This Meta analysis demonstrates that caloric restriction is not a long-term solution for weight loss. Decreases in resting metabolic rate (RMR) with caloric restriction may be one key. Dietary restriction can decrease RMR, which could eventually inhibit weight loss and can lead to weight regain (Thompson et al, 1996). Energy balance is key to weight maintenance. Research has demonstrated that exercise promotes energy balance while providing favorable alterations to obesity related co-morbidities and mortality (Hansen et al. 2005). Several studies have suggested that exercise alone does not play a major role in weight loss but has positive effects on weight maintenance and weight regain.

The literature supporting the effects of exercise on resting metabolic rate is inconclusive. Stiegler and Cunliffe (2006) reported that exercise prevented a decrease in resting metabolic rate. Some other investigators stated that they only observed an impact on RMR with exercise when the exercise was at a significant exercise intensity and duration (Mole et al., 1989). The prevalent hypothesis is that exercise should assist to preserve muscle mass, which in turn should help to sustain RMR. This hypothesis, however, has not been adequately tested.

2.2 SUBSTRATE OXIDATION

2.2.1 Fat Oxidation

An individual has an abundance of fats to be oxidized and used for energy. Fat is a primary source of energy during resting and sub-maximal exercise conditions. Lipid substrates are found in circulation as fatty acids derived from adipose tissue lipolysis and from diet as fatty acids,

triglycerides or within lipoproteins (e.g. chylomicrons, VLDL). (Hansen et al., 2005). There are also small local sources located in the muscle beds that are available as substrate when needed and can contributed substantially to total energy metabolism. These sources are called intramuscular triglycerides (van Loon et al. 2003).

Fat or triglycerides are broken down to fatty acids and glycerol by hormone sensitive lipase (HSL). Hormone sensitive lipase is inhibited by insulin and activated by catecholamines and growth hormone. The glycerol then can be used to make glucose or it can be metabolized through glycolysis. Free fatty acids will then enter the blood stream to be used as fuel in β-oxidation. They may be used as a precursor in the production of cholesterol. Fatty acids are activated by using adenosine triphosphate (ATP) and coenzyme A (CoA) in order to enter the mitochondria for oxidation. In the mitochondria, β- oxidation occurs by cleaving off two carbon atoms at a time forming acetyl – coA. For a 16-carbon fatty acid such as palmitate it will yield 129 ATPs. (McArdle et al. 1996).

2.2.2 Substrate Oxidation in Healthy Normal Weight Individual

Upon the onset of exercise, increased energy demands are met primarily through the oxidation of two main fuel substrates, carbohydrates and fats. Many factors greatly influence the relative utilization of these fuels (i.e. the exercise bout duration, intensity, and trained status of individual). It has been demonstrated that higher aerobic or anaerobic energy needs are primary from carbohydrates metabolisms whereas low to moderate aerobic exercise is primarily elicited by the oxidation of fats.

In a normal healthy individual performing light to moderate exercise, fatty acids are released from triglycerides in muscle itself as free fatty acids (FFA). These FFA are bound to

blood albumin as well as triglycerides in the muscle itself. At the initiation of exercise there is an initial drop in plasma FFA concentration due to the increase in the active muscles. Then this is followed by an increase in FFA from the adipose tissue via hormonal stimulation. The stimulation is from both the sympathetic nervous system and from a decrease in insulin (McArdle, et al., 1996).

Romijn and colleagues (1993) examined both men and women and the effects of exercise intensity and fat oxidation using tracer techniques. These investigators found that at an exercise intensity of 65% VO_2 max will elicit the highest rates of fat oxidation (0.7g/min) followed by 25% and 85% VO_2 (0.4 and 0.5g/min respectively). Another study looked at exercise intensity and fat oxidation using indirect calorimetry. This study concluded that fat oxidation rates appeared to be high over a large range of exercise intensities but there was one point that the investigators referred to as Fatmax (maximal amount of fat oxidation). After the Fatmax was achieved the investigators observed a drop in fat oxidation in all their subjects. These authors concluded that Fat oxidation max was 64± 4% VO_2 max or 74 ± 3% Heart Rate Max. (Achten et al., 2002).

2.2.3 Impaired Fat Metabolism

In some individuals fat metabolism can be impaired. Free fatty acids (FFA) levels are elevated in most obese subjects. Physiological elevations in plasma FFA concentrations inhibit insulin stimulated peripheral glucose uptake. An imbalance between uptake and oxidation of plasma FFA could lead to accumulation of lipids in the skeletal muscle. This has been show to be strongly associated to insulin resistance (Mensink et al., 2005). Sedentary overweight

individuals have been shown to exhibit a significant altered balance in substrate oxidation and impaired fat oxidation during exercise compared to controlled subjects (Dumortier et al., 2005).

Kelley et al. (1999) measured substrate oxidation with indirect calorimetry across the leg in both obese and lean subjects and found that there was suppression in lipid oxidation in the obese subjects (higher RQ). These obese subjects also exhibited suppression in skeletal muscle CPT-1 (caritine palmitoyltransferase –1) activity and oxidative enzyme activity compared to the lean subjects. Similar results have been found measuring fatty acid oxidation via tracer methodology in obese and lean men during moderate intensity exercise (Goodpaster et al., 2002). These obese subjects established a trend for lower plasma fatty acid oxidation per kilogram fat free mass and significantly lower percent of plasma free fatty acid uptake oxidized compared with the lean group. The main finding in the obese group was that they oxidized significantly more (50%) non-plasma free fatty acids than the lean group. (Goodpaster et al., 2002)

Mittendorfer et al. (2004) measured lipolysis and oxidation of free fatty acids in lean, overweight and obese individuals during a 90-minute bout of exercise. These investigators concluded that as the percent body weight increased there was a decrease in the oxidation of plasma free fatty acids and in increase in non-plasma free fatty acids.

2.2.4 Substrate Oxidation and Exercise Training

It has been well demonstrated that endurance training in normal healthy individuals can alter the capacity to oxidize fatty acids. Many studies report at a given submaximal level of exercise lipid oxidation is higher and glucose, lactate and insulin concentrations are lower after exercise training. Training induced lipolytic activity is most significant improved by adaptations that occur within the muscular and cardiovascular systems. Aerobic training increases blood flow

and oxygen delivery to the muscles. These are both key elements in lipid metabolism. In the muscle itself there are adaptations to aerobic training. There is an increase in mitochondria (both size and content) as well as an increase in fatty acid transport proteins (e.g. carnitine palmitoyltransferase). Both of these increase the ability to use fats for energy (McMurray, 2005). Chronic aerobic exercise decreases lactate formation at any given workload. Less lactate reduces the body's inhibitory effects on β- oxidation. Exercise training concomitantly reduces total carbohydrate oxidation as well.

Sial et al. (1998) demonstrated that in the elderly, exercise training increases total fat oxidation without changes in lipolysis or the availability of FFA. Much of the research examining substrate metabolism during sub-maximal exercise has been limited to normal weight healthy college age men, examining different training intensities that produce the greatest fat oxidation during an acute bout of aerobic activity. There has been relatively little focus on the effects of exercise training on different populations (e.g. obesity).

2.2.5 Substrate Oxidation in Obese Individuals

Obese individuals have excessive fat stores that are potentially available for release. Endurance training stimulates the mobilization and oxidation of fatty acids from endogenous triglycerides, therefore exercise may be the best stimulus with which to mobilize and oxidize these excessive fat stores. Some evidence suggests that impaired fat oxidation in obesity is not observed during sub-maximal exercise. Sub-maximal exercise is a good model to examine muscle fat oxidation. During sub-maximal exercise skeletal muscle burns ~80% of fat. At rest the liver, kidney and heart a big role in fat oxidation while skeletal muscle only burns ~18-20% of fat.

Exercise also has an effect on substrate utilization in obese subjects. With low intensity exercise training researchers have found that there is an increase in fat oxidation in these obese subjects (Dumortier et al., 2005). Kanaley et al. (2001) examined the rates of substrate oxidation in lean and obese women during a short duration at high exercise intensity after a 16-week exercise training intervention. In this study fat and carbohydrate oxidation was measured using indirect calorimetry during a 30-minute bout of exercise in 8 non-obese (Non-Ob), 11 lower body obese (LBO0 and 12 upper body obese women (UBO). The authors concluded that the obese women had significantly greater fat oxidation at 30 minutes of exercise than the non-obese women (Non-OB 23.5 μmol/ kgFFM/min, LBO 35.2 μmol/ kgFFM/min, UBO 33.2 μmol/ kgFFM/min, $P<0.001$). In this research study the investigators did not see any significant changes in rest or exercise fat oxidation after the 16- week exercise intervention. The authors suggested that the 16 week duration of the exercise intervention may not have been long enough to induce changes or those changes in weight or body composition need to occur for significant changes in fat oxidation to be observed.

Therefore, the question remains whether exercise training can increase the reliance on fats as an energy source in obese persons. An additional question is whether there may be other means to enhance fat metabolism in obese women.

2.3 SUBSTRATE OXIDATION MEASURING TECHNIQUES

2.3.1 Indirect Calorimetry

Much of the study of exercise physiology involves the measurement of energy metabolism. Measurements of the oxygen and carbon dioxide contents of expired air together with either the inspired or expired breathing volume, provide the basic data for determining respiratory gas exchange, oxygen uptake and inferring the body's rate of energy expenditure. Indirect calorimetry is used to estimate rates of whole body lipid and CHO oxidation (energy expenditure) by measuring oxygen consumption (VO_2) and carbon dioxide production (VCO_2). Because of the inherent differences in chemical composition of carbohydrates and lipids different amounts of oxygen are needed to completely oxidize the molecule's carbon and hydrogen atoms to the carbon dioxide and water end products. Thus, the amount of carbon dioxide produced in relation to oxygen consumed varies somewhat depending on the substrate metabolized (McArdle, 1996). This ratio of metabolic gas exchange is termed the respiratory quotient or RQ.

RQ is defined as the following: RQ= CO_2 produced ÷ 0_2 consumed

The RQ is useful to use as a general guide to the macronutrient mixture catabolized for energy. During complete oxidation of glucose molecule 6 molecules of oxygen are consumed and six molecules of carbon dioxide are produced. The overall equation for this reaction is as followed:

$$C_6H_{12}0_6 + 6\ 0_2 \rightarrow 6\ CO_2 + 6\ H_20$$

Because the gas exchange in the above reaction is equal the RQ is =1.0 for carbohydrate metabolism.

The chemical composition of lipids differs from CHO. Lipids contain fewer oxygen atoms in proportion to hydrogen atoms then CHO. Therefore when palmitic acid is oxidized to carbon dioxide and water, 16 carbon dioxide molecules are produced for every 23 molecules consumed. The equation is as followed:

$$C_{16}H_{32}0_2 + 23\ 0_2 \rightarrow 16\ CO_2 + 16\ H_2O$$

$$RQ = 16\ CO_2 \div 23\ 0_2 = 0.70$$

RQ is based an assumption. This assumption is that the exchange of oxygen and carbon dioxide measured at the lungs reflect the actual gas exchange from nutrient catabolism at the cellular level. This assumption is thought to be reasonably valid at steady state exercise.

Using indirect calorimetry to measure substrate oxidation provides information on whole body processes but does not provide detailed information on the underlying metabolic processes. To examine the source of the fatty acid oxidation other techniques are required. Infusions of labeled fatty acids in the blood and measurement of the label in the breath CO_2 can provide information about oxidation of plasma fatty acids. Using infusions of labeled isotopes, whole body fat oxidation can be divided into plasma derived fat oxidation, and intramuscular triglyceride oxidation.

2.4 DIETARY SUPPLEMENTATION TO ENHANCE FAT METABOLISM

In recent year's dietary supplementation have become more popular The FDA reported that in1996 consumers spent more than 6.5 billion dollars in dietary supplements. Today there are

many types of dietary supplementations. One popular category of supplements that has been on the rise in the last few years is "fat burners". These types of supplements are marketed to consumers for them to take to enhance their body's ability to burn fat. There are many different companies and ways that these companies are going about targeting the enhancement of fat metabolism.

2.4.1 Calcium

Calcium has been shown to play a pivot role in metabolism. Recent researches have suggested that higher intake of calcium; lower body fat and/ or body weight and reduce weight gain in midlife. A study by Lin et al. (2000) investigating dairy calcium, changes in body composition and exercise intervention over a two year time frame in young women concluded that subjects taking a high calcium intake, corrected by a total energy intake and a lower vitamin A intake, gained less weight and body fat over two years in randomized exercise intervention trial.

Calcium has also been reported to modulate adiposity in humans independent of caloric restriction in obese humans (Zemel et al 2003). A 12-week multi-center trial of 68 overweight and obese adults consuming a reduced-calorie diet, the participants who consumed 3 servings of dairy a day lost more body fat compared to those who ate an equal amount of calcium through supplements or a low-dairy diet. All participants lost weight and body fat, but people on the high-dairy diet lost nearly twice as much body fat, more abdominal fat as well as more inches around the waist compared to the other groups (Zemel et al 2004).

2.4.2 Green Tea

Research studies suggest that green tea extract may enhance metabolism and facilitate fat oxidation. Some researchers speculate that the substances in green tea known as polyphenols, specifically the catechins, are responsible for the herb's fat-burning effect. The healthful properties of green tea are largely attributed to polyphenols, chemicals with potent antioxidant properties. In fact, the antioxidant effects of polyphenols appear to be greater than vitamin C.

Polyphenols contained in teas are classified as catechins. Green tea contains six primary catechin compounds: catechin, gallaogatechin, epicatechin, epigallocatechin, epicatechin gallate, and apigallocatechin gallate (also known as EGCG). EGCG is considered to be the most active component in green tea and is the best researched of all the green tea polyphenols. Green tea contains roughly 30% to 40% polyphenols and black tea contains only 3% to 10% polyphenols. Green tea also contains alkaloids including caffeine, theobromine, and theophylline. These alkaloids provide green tea's stimulant effects.

There have been some clinical trials conducted by the University of Geneva, in Switzerland that have indicate that green tea appears to raise metabolic rates and increase fat oxidation. In addition to caffeine, green tea contains catechin polyphenols that raise thermogenesis and hence overall energy expenditure. Green tea has been shown to exert a direct inhibition of gastric and pancreatic lipases and stimulates thhermogenesis (Chantre P, 2002).

2.4.3 Caffeine

Caffeine consumption is inexpensive and has little or no acute adverse effects on health and is socially acceptable. Researchers for years have been investigating caffeine and its effects on

sports performance. The most consistent finding in this literature is that caffeine can increase time to exhaustion during sub-maximal exercise lasting 30-60 minutes. Caffeine has also been shown to improve both speed and power (Magkos and Kavouras, 2004).

Caffeine has been reported to increase fat metabolism in humans during exercise (Chesley, 1998). It has been found in mice that caffeine impacts peroxisome proliferators activator receptor (PPAR) especially the PPAR-α. PPAR-α regulation is important for gene expression in liver, heart and adipose tissue. This nuclear receptor is vital for lipid metabolism because it is needed to oxidize fatty acids for energy. This is where observations have been found that suggest PPAR is required to change lipid metabolism in response to caffeine (Aoyama et al 1998). Some investigators have found that caffeine causes body weight to be reduced (Dulloo et al 1999).

In a double-blinded study examining the effects of oral caffeine on energy expenditure in healthy subjects who are habitual caffeine drinkers, the investigators concluded that caffeine increased energy expenditure dose dependently (100mg, 200mg, 400mg group) (Astrup et al., 1990). Researchers have also found that after the ingestion of caffeine there is an increase in metabolic rate of approximately 7%. (Koot and Deurenberg 1995). This increase in metabolic rate has been seen to last up to three hours post ingestion of caffeine (Koot and Deurenberg 1995).

2.5 CONCLUSION

Obesity is associated with impaired fat oxidation. However, the effect of exercise training on fat oxidation in obese premenopausal women has not been well established. In addition, there is

little information regarding the role of dietary supplementation and substrate oxidation during exercise training in obese.

3.0 CHAPTER 3

3.1 METHODS

3.1.1 Subjects

Premenopausal mildly obese (BMI 30 to 38 KG/M^2) women (age 18-45 y) research subjects were recruited and medically screened to determine adequate health, stable weight, and no contraindications to exercise. Fliers and newspaper ads recruited all subjects. . Exclusionary criteria for this study were primarily related to chronic diseases or medications that may alter substrate metabolism or otherwise confound the results of this study. In addition, being too physically active was also ground for exclusion. Women in this age group are typically a segment of the population most likely to engage in dieting for weight loss. Men were excluded from this study at this time due to previous research showing inherent gender differences in fatty acid metabolism. Children were excluded from this study based on the purpose of this study, which is to determine changes in fat metabolism following two different exercise protocols and a popular over-the-counter Ephedra-free dietary supplement in obese middle-aged women. Dietary supplementation is discouraged in children who are still growing, and there is insufficient data regarding safety of the Ephedra- free dietary supplement in children.

Upon completion of an informed consent document (Appendix A) and health screening questionnaire (Appendix B) all women were asked to perform the following procedures before and after the respective interventions: 1) A fasting blood draw for determination of lipid profiles, electrolytes, blood glucose and serum caffeine levels, a urine pregnancy test was also performed; 2) A physical exam conducted by a study physician to make sure all subjects are in good health; 3) Indirect calorimetry to determine resting metabolic rate (RMR) 4) A graded treadmill exercise test to determine maximal aerobic capacity (physical fitness); 5) A dual energy x-ray absorptiometry (DEXA) scan to measure fat mass and fat free mass; 6) Open circuit spirometry to determine their resting metabolic rate; and 7) One hour of treadmill walking at 55% of maximal aerobic capacity using indirect calorimetry to determine exercise energy expenditure, and rates of fatty acid oxidation during exercise. Then all women were randomized into one of two groups:

1. Aerobic training only receiving a dietary supplement, Thermo Dynamx ® in tablet form.
2. Aerobic training only receiving Placebo.

3.1.2 Screening Visit

At the screening visit, subjects underwent a through medical exam. A health history questionnaire was administered which assessed current and past medical history and medications. A fasting blood draw was obtained examining complete blood count, general chemistry profile and fasting lipid profile.

3.1.3 Indirect Calorimetry

Indirect calorimetry was used to determine resting energy expenditure. It was also used to quantify carbohydrates and fat oxidation. Indirect calorimetry was performed for 30 minutes following an overnight fast using an open canopy system (Delta Trac, Yorba Linda, CA). Prior to testing, calibration of both gas and pressure were completed. Subjects arrived at approximately 7:00AM and laid comfortably on a bed for 20 minutes prior to measuring. Subjects were told to refrain from fidgeting and sudden movements, also to minimize activity prior to attending this test i.e. elevator instead of stairs. This test was performed for 35 minutes in order to estimate carbohydrates and fat oxidation from expired O_2 and CO_2 measurements. The first five minutes of data collection were discarded due to lack of reliability. After five minutes steady state is achieved and the average rates of resting fat and carbohydrate oxidation were measured during the remaining 30 minutes of measurement period. The equations C = 4.55 Vco2 – 3.21 Vo2 – 2.87 (where C is grams of carbohydrates) and F = 1.67 Vo2 – 1.67 Vco2 – 1.92(where F are grams of fat per minute) developed by Frayn (1983) were used to determine rates of resting fat and carbohydrate oxidation in g· min-[1.]

3.1.4 Dual Energy X-Ray Absorptiometry (DEXA)

Whole body DXA (GE Lunar Prodigy Bone Densitometer) scans were performed for body composition analysis. Whole body DXA measured whole body and regional (trunk, head, and limbs) bone mass, lean mass, and fat mass. Manufacturer's recommendations for patient positioning were performed (scan protocols). DXA scan analyses were conducted following the scans. Study participants disrobed and wore a hospital gown to minimize artifacts. Whole body

scans were acquired with the subject supine and aligned with scanner table as prescribed by the manufacturer.

The amount of radiation exposure received from a DXA scan is about 0.002 rem. A rem is a unit of radiation. This amount of radiation is a small part (0.3 rem) of the average whole body radiation exposure that each member of the public receives per year from radiation exposure that is recognized as being totally free of the risk of causing genetic defects (cellular abnormalities) or cancer. The risk associated with the amount of radiation exposure received from this procedure is considered to be low and comparable to other everyday risks. All woman of childbearing potentially, had a urine pregnancy test performed prior to the DXA scan. If the test is positive, that person was excluded from the DXA scan.

The Obesity and Nutrition Research Center staff monitored machine maintenance and repair as well as any software upgrades. Although the scanner is usually calibrated during maintenance, small changes in measured fat mass are common. In the event of scanner maintenance, precision studies were performed using the traveling whole body phantom to ensure consistency of measurement. Should changes in software or machine occur, the scanner would be calibrated with the new software to ensure the consistency of measurements.

We conducted centralized review of all whole body scans. DXA scan printouts were reviewed by Bret Goodpaster, PhD, Associate Director of the Metabolism Core of the ONRC, to evaluate proper patient positioning and scan analysis. If improper patient positioning is detected, the scanner will be marked in the database to permit this to be taken into account. If improper analysis is detected, the scans will be reanalyzed. Any artifacts in bone or soft tissue will be recorded. Dr. Goodpaster has extensive experience in interpreting DXA scans.

3.1.5 Anthropometric Measures

Height was measured to the nearest 0.5 cm using a stadiometer with the subject not wearing shoes. When measuring the height, the subject would stand without shoes, heels together, back as straight as possible, heels, buttocks, shoulders and head touching the wall, and looking straight ahead. Body weight was measured to the nearest 0.2 kg on a balance-beam scale with subjects fasted and wearing minimal clothing. The balance scale was calibrated daily by using a known weight. The scale was also calibrated each time before use by putting the beam weight on zero, and seeing whether the beam balances out. If not, a screwdriver was used on the moveable tare weight to adjust the beam weight. Waist circumference was measured on the right side of the body with a tension regulated fiberglass tape. Two measurements were taken on each subject, averaging those within 1cm of one another. The waist was measured at the level of the umbilicus (American College of Sports Medicine Guidelines, 2005).

3.1.6 Grade Exercise Test (GXT) to Measure Maximum Aerobic Capacity (VO_{2Max})

As part of the screening process, in order to ascertain whether subjects are sedentary upon entry into the study, a VO_2 Max Test was performed. This VO_2 Max Test was repeated at the completion of the intervention.

Subjects performed the VO_2 max test at the Obesity Nutrition Research Center (ONRC) Exercise Physiology Lab to determine physical fitness and target workload used for exercise training. For this study all subjects that are on beta blockades are excluded due to the fact we are using heart rate as a variable for their exercise training. This test generally lasts 8-10 minutes and is conducted using a standard incremental protocol with a treadmill (Quinton, Q- Stress

System). All graded exercise tests (GXT) were supervised by an Advance Cardiac Life Support trained exercise physiologist. The GXT continued until volitional exhaustion or one of the established criteria for VO_2 max has been reached using American College of Sports Medicine Guidelines: an RER >1.15, HR max ≥ age predicated (220-age) or a plateau in the VO_2 work rate curve (American College of Sports Medicine Guidelines, 2005). Heart rate, blood pressure and EKG were recorded prior to, during, and immediately following this test. Subjects breathed through a mouthpiece connected to a two way breathing valve (Hans Rudolph, Kansas City, MO) during the test, and expired air was collected into a mixing chamber interfaced to a computerized metabolic cart (Sensor Medics cs 2900) to measure expiratory flow and expired air for CO_2 and O_2 fractions. The metabolic cart analyzed the data for O_2 consumption (VO_2) every 30 seconds.

Individuals were excluded from further participation if signs and/or symptoms occur prior to the test or during exercise testing and were referred to their PCP for further care. The exercise test was stopped if the subject has any of the following: a) a positive ECG (> 2mm ST segment depression), b) signs or symptoms of cardiovascular decompensate (hypotensive response to exercise), c) onset of angina or angina like symptoms, d) shortness of breath, e) change in heart rhythm, f) signs of poor perfusion (light-headedness). The ONRC is equipped with cardiac emergency equipment (crash cart and Zoll Def.).

3.1.7 Rates of Fatty Acid Oxidation

Research subjects performed one hour of walking at 55% of their pre-determined maximal aerobic capacity (VO_2max) before and after the intervention in order to quantify plasma and non-plasma fatty acid oxidation. This test was conducted at the same absolute intensity at post testing. The rationale for quantification of these two distinct components of fat metabolism is

that the intervention may have different effects on plasma and non-plasma fat oxidation (Goodpaster et al.,2002), which may have important implications for improvements in metabolic dysfunction, including insulin resistance, in obese women (Goodpaster and Kelley,2002). This was accomplished using a combination of gas exchange indirect calorimetry and stable isotope dilution as described in a recent publication by Dr. Goodpaster (Goodpaster et al.,2002). The information regarding plasma and non-plasma fat oxidation using stable isotope methodology was not being addressed in this project.

At least one week following the VO_2max test, subjects were given a standard dinner consisting of 10 kcal/kg; 50% carbohydrate, 30% fat, 20% protein and then fasted until completion of this part of the study. It has been shown by previous research that recent diet effects substrate oxidation. We had all subjects replicate the same diet at post testing. They were instructed to avoid strenuous physical activity for two days prior to the study and to eat at least 200 g of carbohydrate for the three days preceding the study to ensure adequate glycogen stores for the exercise bout. Subjects were asked to record food intake in a diary for the three days prior to this exercise bout so that they can replicate their diet during the three days preceding the post exercise-training bout of exercise. This diary consisted of each research subject writing down on a piece of paper everything that they ate and drank the three days prior.

At ~ 7:00 A.M. blood samples were collected in a heated dorsal hand vein before tracer infusion for basal determination of plasma glucose, FFA, insulin, epinephrine, cytokines, norepinephrine, glycerol, and background isotope enrichment. A resting breath sample was collected to determine the background C13/C12 ratio. . As part of the parent study from which this thesis project was based, a continuous infusion of 0.08 μmol•kg-1•min-1 of [1-13C] palmitate (99% enriched, Isotech, Inc., Miamisburg, OH) bound to 5% human albumin was

started with a calibrated syringe pump (Harvard Apparatus, Natick, MA) at the onset of exercise to achieve isotopic equilibrium. A priming dose of 18 μmol•kg-1 NaH13CO3 was given prior to [1-^{13}C] palmitate infusion to shorten the time required to achieve equilibration of recovered expired $^{13}CO_2$. Exact infusion rates were calculated by measuring the concentrations of the isotopes in each infuscate. Again this information was collected but will not be presented in this dissertation.

Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured from whole body gas exchange indirect calorimetry to calculate rates of total lipid and carbohydrate oxidation during exercise.

3.1.8 Blood Lipid Profile

Blood samples were collected pre and post intervention on all subjects. The samples were taken by veinipuncture from the antecubital vein into Vactutainer tubes. All subjects were fasted for 8-10 hours prior. The specimens were observed for a milky chylomicron layer after it stands overnight in the refrigerator at 4 °C (39.4°F). Plasma total cholesterol and plasma triglycerides were measured by enzymatic methods. High-density lipoprotein (HDL) was measured enzymatically after precipitation of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) from plasma.

3.2 EXERCISE TRAINING PROTOCOL

A 16-week program of exercise training was used. All subjects received the same exercise training protocol. Participants were encouraged to exercise daily, with a minimum being 4 sessions weekly. The three main choices for exercise were cycling on a stationary bicycle, rowing or walking/jogging. Subjects choose the type of exercise they participate in and can alternate the type of exercise they perform. Subjects were given instructions on proper stretching and warm-up. All sessions were start with 5 minutes of stretching. In at least one exercise session per week, a graduate student in exercise physiology, trained in the protocol and motivational techniques, joined the participants. This was done to monitor target exercise intensity and to encourage research subject. The exercise physiologist also measured blood pressure and heart rate to monitor changes that may occur from the dietary supplement. Aerobic exercise training was conducted at the Exercise Facility of the Obesity Nutrition Research Center Laboratory. Subjects were also instructed to use the wireless heart rate monitor (Polar Heart rate Monitor) provided by the study to record exercise duration and mean heart rate. The mean heart rate was used for estimation of weekly caloric expenditure.

3.2.1 Aerobic Training

Training intensity and duration was recorded for each exercise session in the participant's personal exercise log and were quantified for by the total number of calories expended during the exercise bout. Intensity was adjusted weekly examining subjects' heart rate to compensate for increasing fitness, so in effect, they performed more work as they become more physically fit while maintaining their heart rate at a given proportion of their maximum as described below.

The duration began at 30 minutes of 60-70% HR max during weeks 1-8 and further increase up to 45 minutes and 75% HR max respectively during weeks 8-16 of the training intervention. Safety and care was taken during each exercise session in addition to providing water to prevent undue thermoregulatory stress.

Subjects were required to come to a minimum of one supervised exercise session per week. Subjects were encouraged to exercise daily with a minimum being four sessions weekly. Participants were encouraged to use the exercise facility to perform their unsupervised workouts as well.

3.2.2 Dietary Supplementation

Subjects were randomized to either the dietary supplement or Placebo groups in a double-blind fashion. Both the dietary supplement and the Placebo were provided in capsular form by Experimental and Applied Science, (EAS). They were all coded, which was indicating Placebo or Supplement. This information was kept until completion of the study. The dietary supplement is designed to enhance fat metabolism, primarily through actions of Green Tea, caffeine and calcium. A breakdown of the supplement is provided in the table below (Table 1). Caffeine is one of the main ingredients in the dietary supplement. The maximum amount of caffeine from the dietary supplement was 400 mg. One 8 ounce cup of coffee contains ~150mg (National Soft Drink Association). Thus the maximum amount of caffeine to be consumed in this study is equivalent to 2 to 3 cups of coffee per day. I was in charge of dispensing the dietary supplement or Placebo to all participants. I was also in charge of storage and accountability of the supplements.

There are some risks associated with this dietary supplement, including specific drug-drug interactions. Each individual was specifically instructed to use this Supplement (or Placebo) according to the products label. Specific supplement ingredients are provided in the table below for a 3-capsule serving. Specifically, research subjects were asked to consume either product or Placebo (double-blinded administration) exactly five days per week and no more. Research subjects was instructed to take the dietary supplement twice daily with food due to caffeine content, at least 4 hours between servings and 1 hour before exercise. They began the supplement regime with one capsule twice daily for two weeks, then 2 capsules twice daily for two weeks, then 3 capsules twice daily for 3 weeks. This was the halfway point in the 16-week intervention at which time they will halt the supplement for two weeks (They will continue, however, with the exercise component of the intervention). After this two-week hiatus they resumed the dietary supplement, consuming 3 capsules twice daily for the remaining 6 weeks of the 16 -week intervention. This titration schedule was used due to the fact that the dietary supplement recommends on its product label that this supplement not be used more than 12 weeks in a row without two weeks of discontinuing the supplement, therefore the investigators feel that using this product as described above was safer for the research subjects. After 16 weeks of dietary supplementation, subjects came to the GCRC within one week for assessments of the outcome parameters (post-testing). This allowed us to ask questions concerning any adverse reactions to halting the supplement such as headache, agitation, etc that may be associated with acute caffeine withdraw.

Table 1. Ingredients of the Dietary Supplement

PROPOSED THERMO DYNAMX			
Product Form	Capsule		
Bottle Count	120 Capsules		
Serving Size:	3 Capsules		
Servings Per Container:	40		
Amount Per Serving			%DV*
Calcium (as milk calcium)	200	mg	20%
Vitamin B6 (as Pyridoxine Hydrochloride)	0.5	mg	25%
Folic Acid	100	µg	25%
Vitamin B12 (as cyanocoblamin)	1.5	µg	25%
Vitamin D (as cholecalciferol)	80	IU	20%
Tea Complex (Green Tea, White Tea, Oolong Tea)	900	mg	†
EGCG	150	mg	
Green Mate	500	mg	†
Caffeine (as Caffeine anyhdrous, Green Tea, White Tea, Oolong Tea, Green Mate)	200	mg	†
Bioperine® (Piper nigrum fruit extract)	5	mg	†

3.2.3 Dietary Control

All subjects met with a registered dietitian who instructed them to maintain their normal dietary patterns throughout the 16 weeks. The subjects were also instructed to keep at least two days a week of dietary records to determine if there are any changes in dietary patterns. This was done to ensure there were no significant changes in fat composition occurred in their diets.

3.3 STATISTICAL ANALYSIS

Statistical analyses were performed using JMP (Cary, NC) software, with statistical significance defined as $p \leq 0.05$. Data was initially analyzed to provide descriptive information on subject characteristics (age, body weight, BMI etc.) physical fitness, fat metabolism and body composition. Analyses were conducted to determine if the data were normally distributed before conducting additional analyses.

Differences in physical characteristics (height, weight, VO_2Max, total fat mass, total fat free mass, RMR, fat metabolism) before and after training were examined using paired t- tests. Repeated measures analysis (ANOVA) (group x time) was used to assess differences in relative and absolute body composition, physical fitness and fat oxidation across treatment groups. Appropriate transformations (non-parametric ANOVA for change score) were performed on outcomes that were not normally distributed.

Linear regression analysis was used to examine the relationship between changes in fat metabolism and body composition. The strength of a relationship between two variables was measured using the Pearson coefficient of linear correlation

3.3.1 Power Analysis

The sample size estimates were based only on the first aim of this study since we had prior data to estimate effect sizes for this aim. In order to detect a significant ($P<0.05$) change in fat oxidation with intervention, 15 subjects in each group were expected to complete an exercise training program, conservatively estimating a 25% dropout rate. This calculation was based on a paired t-test with a two-sided significance level set at $P=0.05$, with a power of 0.80 to detect a

30% change in fat oxidation pre-post training. We are hypothesizing that the dietary supplement would result in further enhancements of fat oxidation (Hypotheses2). Using the variance (SD) in the *change* in fat oxidation from a previous study in which 24 subjects improved fat oxidation by 0.20 (0.38) mg/kg fat free mass/min, we estimate that a 0.20 (0.38) difference in the *change* in fat oxidation in treatment versus Placebo (n=30 in each group) would provide a power of 0.54. These power computations are an estimate of the sample size based on a standard analysis with the goal of designing the study to see an effect using straightforward analyses.

4.0 CHAPTER 4

4.1 RESULTS

4.1.1 Subjects

Seven women who randomized into the Placebo group and eight who were randomized into the Supplement group completed the study. Unfortunately, 8 individuals dropped out of the study during the course of the intervention. This was an unexpectedly high rate of participant drop out (35% drop out rate). Six of these participants were withdrawn due to lack of compliance with the intervention. One participant did not complete follow-up testing due a cold virus induced illness and one participant withdrew due to family relocation. Table 2 depicts the characteristics of both those women who completed the intervention and those who completed baseline testing but who did not complete the intervention or post-intervention testing. There were no differences in racial composition, level of obesity assessed by either BMI or proportion of body fat, or in physical fitness (VO_2max).

The Placebo and Supplement groups did not differ at baseline with respect to age or physical fitness (Table 2). BMI and waist circumference were higher at baseline in the Supplement group, although the proportion of body fat did not differ between the groups at baseline (Table 2).

Participants expended an average of 898 ± 565 kcals per week during an average of three days per week of structured exercise. There was no difference between groups in caloric expenditure during their recorded exercise sessions (812 ± 599 in Supplement and 997 ± 555 Kcal/week in Placebo). However, there was a wide variance in weekly exercise expenditure among all participants (321 to 2206 kcals per week). On average participants reported taking the pill 5 days per week twice daily.

Table 2. Subject Descriptive Baseline Characteristics

	Placebo (n=7)	***Supplement (N=8)***	***Overall completers (n=15)***	***Non-completers (N=8)***
Age	36.7 ± 7.2	38.2 ± 6.6	36.8 ± 7.2	32 ± 9.5
Race	5 C/ 2 AA	6C/ 2 AA	11C/4 AA	5C/2AA/1AS
Height (cm)	163.0 ± 5.6	164.9 ± 6.1	163.7 ± 5.7	160.9 ± 6.03
Weight (kg)	80.5 ± 5.0	89.8 ± 10.9	85.5 ± 9.7	87.1 ± 10.0
BMI (kg/m^2)	30.4 ± 0.6	33.0 ± 2.4 **	32.0 ± 2.3	33.1 ± 2.8
BF %	45.2 ± 4.5	48.5 ± 3.6	47.0 ± 4.2	47.7 ± 3.0
Waist Circumference (in)	34.9 ± 1.6	38.1 ± 3.6*	36.6 ± 3.2	39.4 ± 2.9
VO_2max (ml/FFMkg/min)	51.0 ± 10.8	50.9 ± 4.9	50.9 ± 7.9	53.2 ± 8.3

Abbreviations: C= Caucasian, AA= African American and A= Asian.
Values are means ± SD, * =Difference Between Placebo and Supplement Group ($p< 0.05$), ** = Difference Between Placebo and Supplement Group ($p<0.01$)

4.1.2 Resting Energy Metabolism

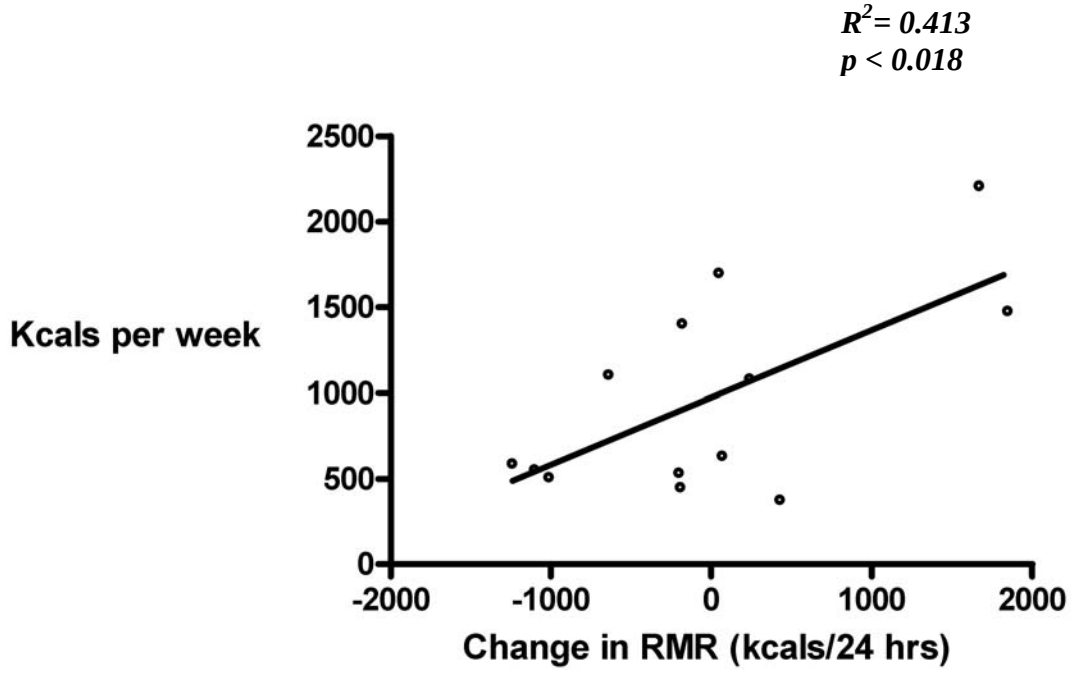
Resting metabolic rate measured by indirect calorimetry did not change in either Placebo or Supplement group (Table 3). Moreover, the energy derived from fat calories during resting conditions did not change in either group. However, when both groups were combined, the amount of energy expended during weekly structured physical activity was positively correlated with greater increases in resting metabolic rate (R^2=0.41, $P<0.05$) (Figure 1) and the proportion

of fat calories expended at rest (R^2=0.33, P<0.05) (Figure 2). Thus, these data strongly suggest a potential dose-response association between the amount of physical activity performed and improvements in resting energy metabolism.

Table 3. Resting Energy Metabolism

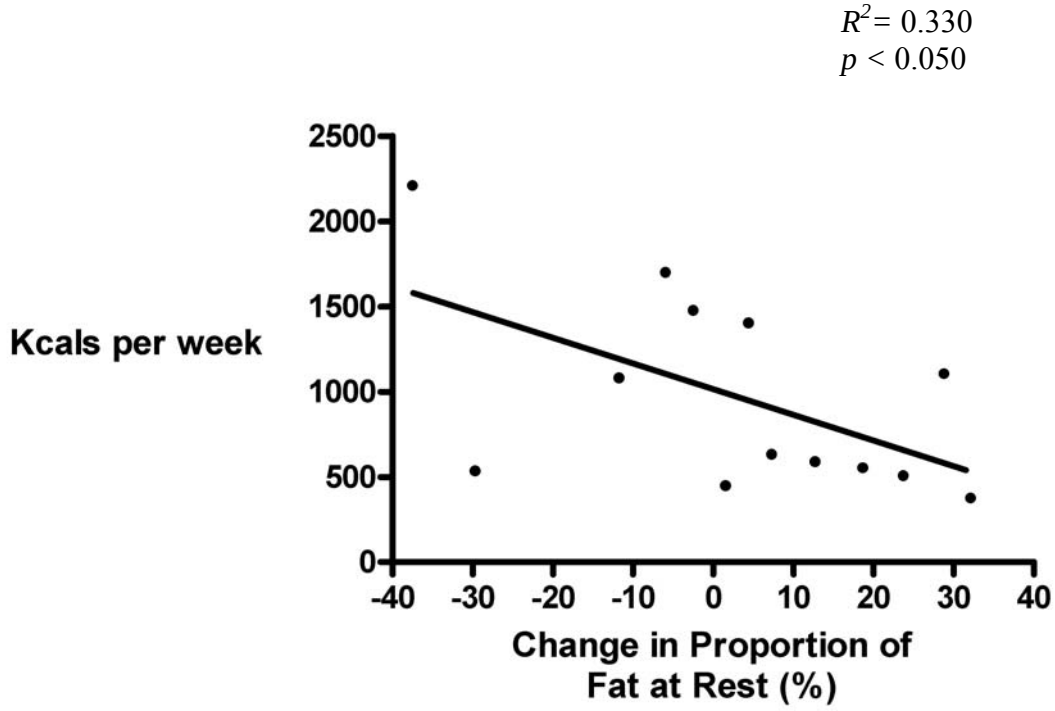
	Placebo		Supplement		Overall	
	Pre	Post	Pre	Post	Pre	Post
RMR (Kcal/24 hours	1507 ± 138	1489 ± 228	1517 ± 127	1516 ± 154	1512 ± 211	1493 ± 202
Resting fat oxidation (g/min)	0.07 ± 0.01	0.05 ± 0.02	0.06 ± 0.03	0.06 ±0.03	0.06 ± 0.02	0.07 ± 0.02
% Fat oxidation	56.3 ± 7.7	48.5 ± 9.2	52.8 ± 10.6	53.2 ± 12.0	54. ± 13.8	49.8 ± 14.1

Abbreviations: RMR=Resting Metabolic Rate; FFM= Fat Free Mass; % fat oxidation= the proportion of resting energy expenditure as fat. Values are means ± SD,



Abbreviations: RMR = Resting Metabolic Rate; Kcals= calories
The plot displays changes in resting metabolic rate and calories expended per week.

Figure 1. Correlation Between Resting Metabolic Rate and Weekly Calories Expended



Abbreviations: Kcals= Calories expended
The plot displays changes in proportion of fat at rest and calories expended per week.

Figure 2. Correlation Between Proportion of Fat Calories at Rest and Weekly Calories Expended

4.1.3 Body Composition Response to Intervention

The exercise intervention resulted in a significant loss of body weight when both Placebo and Supplement groups were combined together. This loss of body weight could be completely accounted for by the loss of body fat since lean mass was completely preserved in these women (Table 4). There were no differences between Placebo and Supplement group with respect to weight or body fat change. However, Supplement group lost a significant amount of weight, while the weight loss in Placebo Group did not quite reach statistical significance (P=0.06).

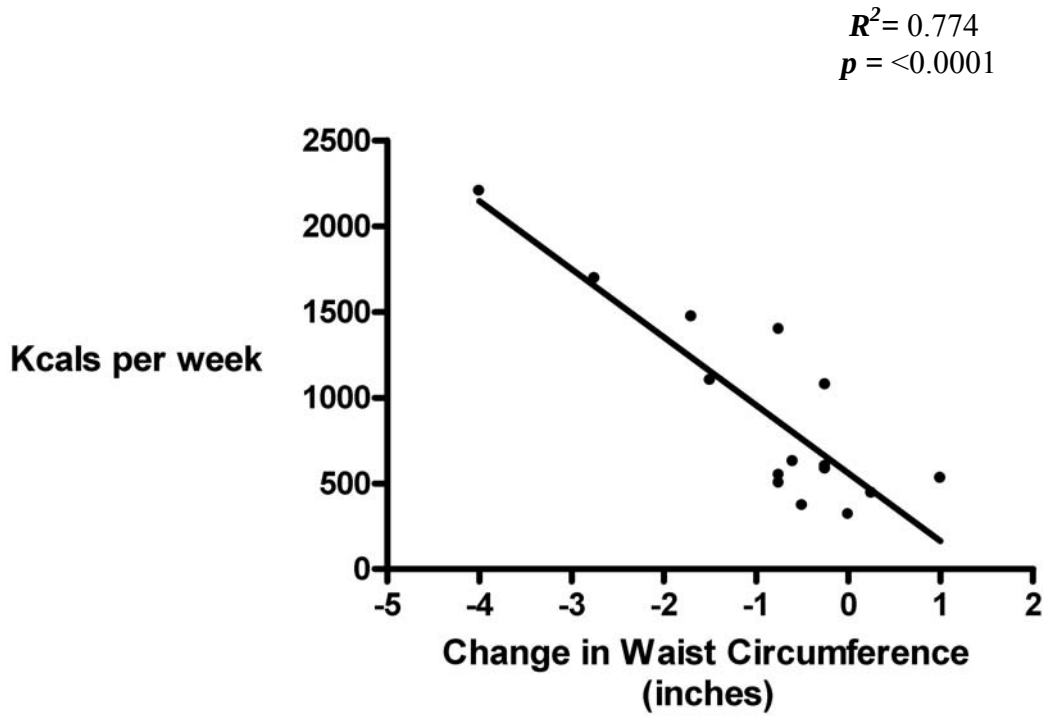
However, with respect to the loss of body fat, the loss of fat was significant in the Placebo group but not in the Supplement group (P=0.06).

The exercise intervention had an overall positive effect on abdominal adiposity as reflected by a significant reduction in waist circumference. Waist circumference as a marker of abdominal obesity decreased in the Placebo group. Unfortunately, this reduction did not reach statistical significance in the Supplement group. Interestingly, the amount of weekly structured exercise was strongly associated with greater reductions in waist circumference (R^2=0.77; P<0.0001) (Figure 3). Thus, those who performed more exercise experienced greater reductions in abdominal adiposity.

Table 4. Changes in Body Composition With Intervention

	Placebo		Supplement		Overall	
	Pre	Post	Pre	Post	Pre	Post
Weight (kg)	80.5 ± 5.0	78.3 ± 5.9	89.9 ± 10.9	87.4 ± 12.0*	85.5 ± 9.7	83.2 ± 10.1*
FM (kg)	34.7 ± 4.5	32.7 ± 4.8*	41.9 ± 7.4	40.0 ± 8.4	38.6 ± 7.1	36.6 ±7.7 *
FFM (kg)	42.0 ± 3.6	42.0 ± 3.6	44.7 ± 5.0	44.6 ± 5.4	43.4 ± 4.5	43.4 ± 4.7
% BF	45.2 ± 4.5	43.7 ± 5.2	48.5 ± 3.6	47.3 ± 4.2	47.0 ± 4.2	45.7 ± 5.2*
Waist circumference (inches)	34.9 ± 1.6	33.9 ± 1.7*	38.1 ± 3.6	37.4 ± 4.2	36.6 ± 3.2	35.8 ± 3.7*

Abbreviations: FM = Fat Mass, FFM, fat Free Mass, BF= Body Fat.
Values are means ± SD, * =Difference Between pre and post treatment (p= 0.05)

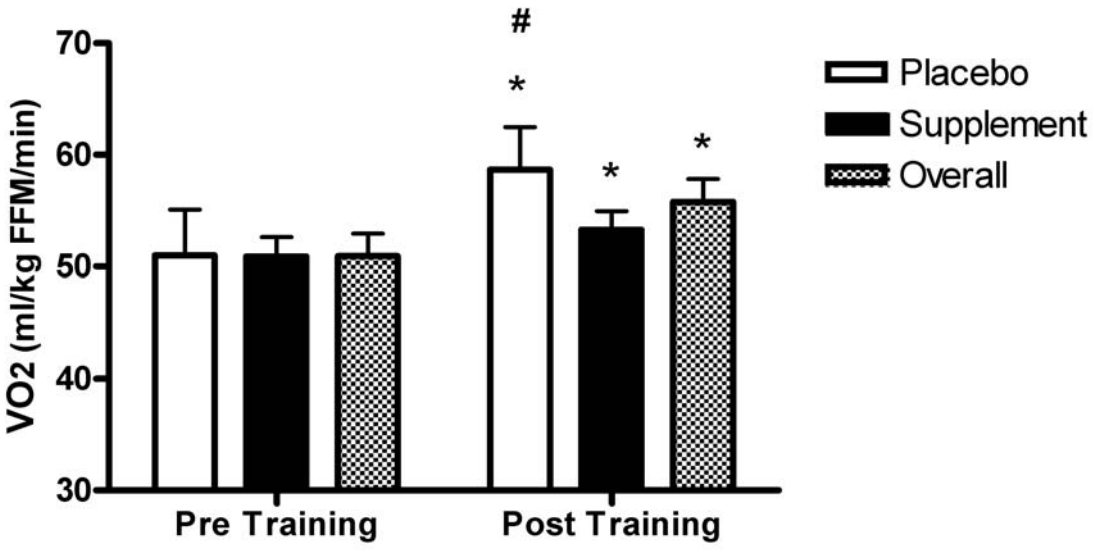


Abbreviations: Kcals = Calories Expended
The plot displays changes in waist circumference and calories expended per week.

Figure 3. Correlation Between Waist Circumference and Weekly Calories

4.1.4 Physical Fitness Response to Intervention

Both groups had a significant improvement in physical fitness (Figure 4). The mean improvement in VO_2max as the gold standard of the change in physical fitness was 11% in all subjects combined. The Placebo group exhibited slightly greater improvements in VO_2 max compared to the Supplement group. The amount of weekly exercise did not predict the degree in improvement in physical fitness.



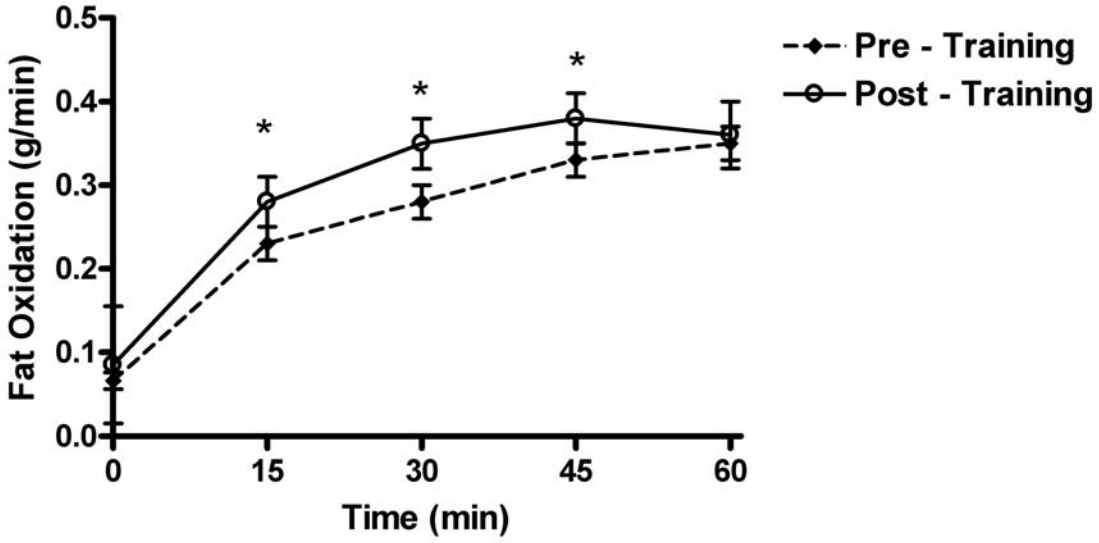
Values are means ± SEM, * =Difference Between pre and post treatment ($p<0.05$)
#= Difference between Placebo and Supplement Group ($p < 0.05$)

Figure 4. Changes in Maximal Oxygen Consumption

4.1.5 Fatty Acid Oxidation During Submaximal Exercise

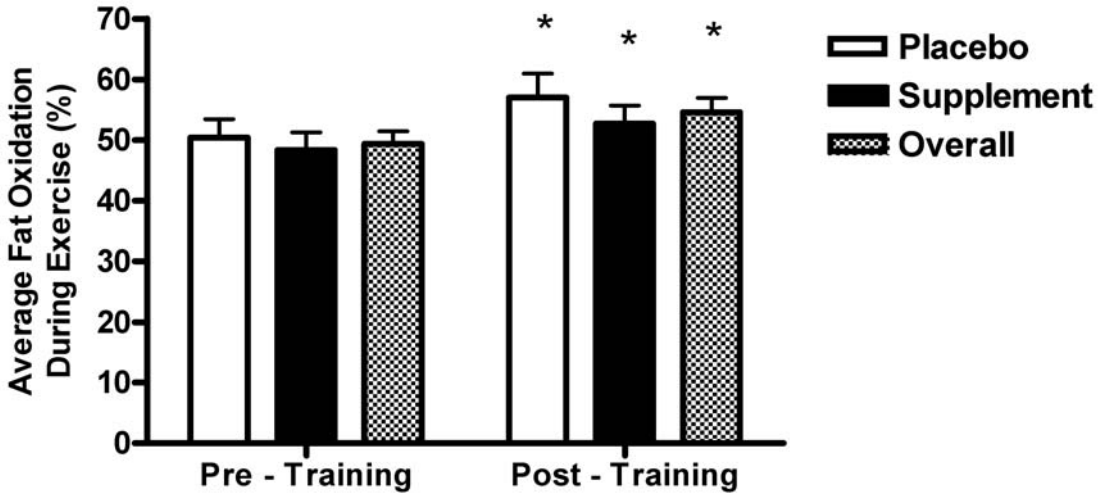
The rate of fatty acid oxidation increased over the course of 60 minutes of exercise performed at 55% of VO_2 max both before and after the 16-week intervention. This increase in fat oxidation over the 60 minutes of exercise was not different between Supplement and Placebo group. Thus the results are depicted for both of these groups combined (Figure 5).

The proportion of energy derived from fat during acute sub-maximal exercise increased in both groups as a result of the exercise intervention (Figure 6). However, there were no significant differences in the degree of improvement in exercise-induced fat oxidation between groups. This improvement translated into an increase in the contribution of fat calories from 49% to 55% of total energy expenditure following the exercise intervention when all subjects were combined (Figure 7).



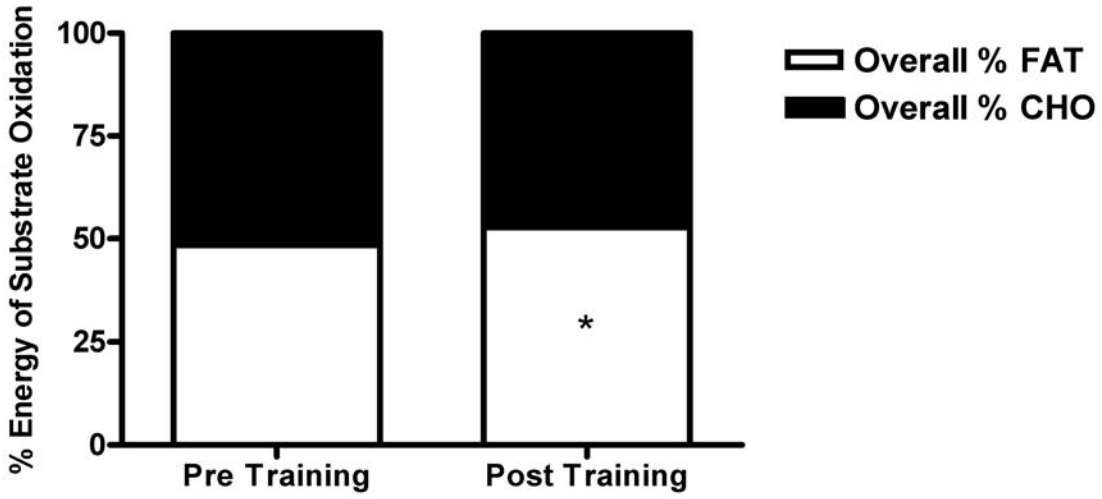
Values are means ± SEM, * =Difference Between pre and post treatment ($p < 0.05$)

Figure 5. Changes in Pre and Post Training in Fat Oxidation During



Values are means ± SEM, * =Difference Between pre and post treatment ($p < 0.05$)

Figure 6. Changes in Average Fat Oxidation During Submaximal Exercise



Values are means ± SEM, * =Difference Between pre and post treatment ($p < 0.05$)

Figure 7. Changes in Percent Energy of Substrate Oxidation During Submaximal Exercise In All Participants

4.2 SECONDARY ANALYSIS

4.2.1 Blood Lipid Profile in Response to Intervention

Total, LDL and HDL cholesterol decreased in the combined groups as a whole. This decrease was completely due to the decrease in both total and LDL cholesterol in Supplement Group, as cholesterol did not change in Placebo Group. Thus, the Supplement Group had a significant decrease in total and LDL cholesterol, an effect not observed in Placebo Group (Table 5).

Table 5. Changes in Blood Lipid Profile

	Placebo (n=7)		Supplement (n=8)		Overall (n=15)	
	Pre	Post	Pre	Post	Pre	Post
Cholesterol (mg/dl)	158.4 ± 22.3	162.3 ± 30.6	190 ± 34.7	162.5 ± 34.9**	175.3 ± 32.9	162.4 ±31.8*
LDL (mg/dl)	97.6 ± 23.3	103.3 ± 30.9	117.9 ± 28.0	101.3 ± 30.2**	108.4 ± 27.1	102.2 ± 29.5
HDL (mg/dl)	50.6 ± 8.5	49.1 ± 7.6	57.5 ± 10.4	47.3 ± 7.7 *	54.3 ± 9.9	48.1 ± 7.4*
Triglycerides (mg/dl)	69.0 ± 21.6	62.7 ± 25.5	92.1 ± 30.3	81.3 ± 28.3	81.3 ± 28.3	75.8 ± 29.8

Abbreviations: LDL- Low Density Lipids, HDL- High Density Lipid.
Values are means ± SD, * =Difference Between pre and post treatment ($p < 0.05$)
**= Difference between Placebo and Supplement group ($p < 0.05$).

5.0 CHAPTER 5

5.1 DISCUSSION

5.1.1 Introduction

Chronic exercise training induces changes in fuel metabolism. Prior research has largely been limited to examining the effects of exercise on fatty acid oxidation in normal weight individuals (Friedlander et al., 1999 and Phillips et al., 1996). Relatively little is known about the effects of chronic exercise on fatty acid metabolism in previously sedentary obese women. Moreover, although many dietary supplements are purported to enhance fatty acid metabolism, there are few objective clinical trials that exist. Therefore, the current investigation aimed to fill a void in the existing literature by examining the effects chronic exercise training, as well as the effectiveness of a "fat burner" dietary supplement, on fat metabolism and body composition in previously sedentary, obese women.

In comparing the effectiveness of the dietary supplement, two groups were compared: Dietary supplement plus exercise training group, and a Placebo plus exercise-training group. To our knowledge, previous research has yet to examine whether popular dietary supplementation elicits further improvements in fat metabolism and body composition when added to an exercise -training regime. It was hypothesized that obese women consuming a current popular dietary

supplement, when combined with regular exercise, would have additional improvements in fat metabolism and body composition compared to those who exercise with Placebo.

5.1.2 Exercise and Supplement Intervention

Seven women who were randomized into the Placebo group and eight who were randomized into the Supplement group completed the study. There was no difference in the average number of days that subjects exercised or their caloric expenditure during exercise. However, there was a wide variance in weekly exercise energy expenditure among all participants (321 to 2206 Kcal per week). On average participants reported compliance on taking the pill 5 days per week twice a day.

Unfortunately, 8 individuals dropped out of the study during the course of the intervention. This was an unexpectedly high rate of participant drop out. Six of these participants were withdrawn due to the lack of compliance with the intervention. One participant did not complete follow – up testing due to cold virus- induced illness and one participant withdrew due to family relocation. Attrition rate did not differ between treatment groups (4 Placebo, 4 Supplement). Furthermore, there were no significant differences in characteristics between completers and non-completers. Thus the supplement treatment did not affect whether or not participants were more likely to drop out.

Participants were equally compliant with the supplement or placebo. There were few reported side effects of either Placebo or Supplement. Two subjects on Placebo and one on supplement) complained of mild nausea upon consumption, and this prevented only one subject from consuming the pill as directed. Otherwise, subjects stated having no other problems with the pills they ingested. We had no adverse events to report. We withdrew product from one of

the subjects because after beginning the study, it came to our attention that she was enrolled in another research study, and her participation in that study could have been confounded by the dietary supplement

5.1.3 Resting Metabolic Rate

The intervention had no effect on resting metabolic rate in either the supplement or placebo group. However, when both groups were combined in the analysis, the amount of weekly exercise was positively associated with an increase in resting metabolic rate. It has been well established that dietary restriction results in a decreased resting metabolic rate (Thompson, 1996). However, previous studies are inconclusive regarding reductions in metabolic rate following an exercise intervention. Some have shown increases (Sjodin et al., 1996), while others have shown no change (Wilmore et al., 1998 and Wilmore et al., 1999) or decreases (Horton et al., 1994, Westerterp et al., 1994) in resting metabolic rate. It has been suggested that FFM represents a key determinant of the magnitude of resting metabolic rate (Stiegler and Cunliffe, 2006). In this study there were no changes in FFM. It is possible that prior studies failed to observe an effect on resting metabolic rate if there is indeed a dose-response association between the amount of exercise performed and the degree of change in resting metabolic rate as our data suggests.

5.1.4 Chronic Exercise and Fat Oxidation

A primary objective of this study was to determine whether chronic exercise has an effect on fat metabolism and body composition in obese women. As we hypothesized, fat oxidation induced

by sub-maximal exercise increased with a 16- week exercise intervention in these premenopausal sedentary obese women. There was no significant difference between groups in this improvement; both groups had an improved fatty acid oxidation during acute sub-maximal exercise. Thus, these results do not support the secondary hypothesis that the supplement would have an additive effect on exercise training. The intervention did not improve rates of post-absorptive fatty acid oxidation at rest, although those who performed more weekly exercise appeared to have greater fatty acid oxidation at rest in a dose-response manner.

Alterations in skeletal muscle fat metabolism during postabsorptive state and during exercise have been suggested to be important in the etiology and pathophysiology of obesity (Blaak, 2000). Unfortunately, few studies have examined the effects of exercise on fatty acid metabolism in obesity. In normal weight individuals, chronic exercise stimulates the mobilization and oxidation of fatty acids from endogenous triglycerides (Horowitz and Klein, 2000). It has been shown in normal weight individuals that exercise training increases lipolytic activity (Horowitz and Klein, 2000). Aerobic training also increases blood flow and oxygen delivery to the muscle. This provides key elements for increases in lipid metabolism. Within the muscle, there is an increase in mitochondria size, content and increase in fatty acid transport proteins (e.g. caritine palmitoyltransferase) thus, enhanced ability to use fat for energy (McArdle, 1996).

The greater use of fat substrate during submaximal exercise after a period of increased physical activity could be vital in delaying glycogen depletion as well as in a reducing lactate production during exercise. Therefore, increasing reliance on fatty acid oxidation may help to improve physical fitness by delaying muscle fatigue.

The improvement in fat metabolism during acute exercise in this investigation is an apparent contrast to a previous study by Kanaley et al (2000) who reported no alteration in exercise-induced fat oxidation in obese women following 16 weeks of exercise training. These investigators stated that 16-weeks might not be long enough to see an alteration in fat oxidation. Given the likely dose-response association between the amount of physical activity performed and the improvement in fat oxidation, it is possible that subjects in their study did not perform enough weekly exercise to have an improvement. Similar to our study, Kanaley et al. (2000) showed an unchanged resting fat oxidation in obese women with acute exercise. However, the amount of weekly exercise performed tended to be associated with an improvement in the rate of post-absorptive resting fatty acid oxidation.

All subjects were tested during the same phase of their menstrual cycle. Research has been shown that fluctuations in ovarian hormones between the follicular phase and luteral phase of the menstrual cycle do not influence glycerol turnover or whole body fatty acid oxidation at rest or during prolonged exercise (Jacobs, 2005). Previous research has also shown that plasma FFA turnover at rest is similar between the follicular phase and luteral phase. However, within-subject variability exists in ovarian hormones, thus potentially accounting for some within-subject variability in substrate metabolism (Jacobs, 2005).

5.1.5 Chronic Exercise and Body Composition

The exercise intervention had a significant effect on changes in body composition. On average participants in both groups lost 2.0kg during the intervention ($p<0.05$). There was a significant change in percent body fat (-3%, $p<0.05$) and a decrease in fat mass (5 % loss, $p<0.05$) in both groups. The differences in whole body composition changes between the supplement and

placebo groups were subtle. Total body lean mass was preserved, an effect that is usually not observed when women lose weight with caloric restriction without increased physical activity.

Waist circumference as a marker of abdominal adiposity was decreased as a result of the exercise intervention. This loss of abdominal fat was not different between groups. Interestingly, the amount of weekly exercise was strongly associated with the reduction in abdominal adiposity. This is in accord with Ross et al. who found that exercise reduces abdominal adiposity independent of weight loss in obese men (2000). Thus these results suggest a more selective depletion of abdominal fat with exercise with relatively little total weight loss.

5.1.6 Chronic Exercise and Physical Fitness

An average improvement of 11% in maximal oxygen consumption (VO_2max) was observed after the intervention in both groups. This is similar to changes observed in previous studies for sedentary individuals (Hoppeler, 1985). This demonstrates that the exercise intervention was successful in eliciting improvements in physical fitness. The Placebo group had a slightly greater increase in physical fitness compared to the Supplement group. It is not clear why this differential response occurred, although one must question the physiological significance of this statistically significant difference. There were no apparent outliers in either of the groups with respect to changes in VO_2max.

5.1.7 Chronic Exercise and a Dietary Supplement

This supplement containing calcium did not obviously result in further improvements in fat oxidation when combined with regular exercise in these obese women. Moreover, there was

no significant difference between the Placebo group and dietary supplement group in regards to body composition changes. An increase in dietary calcium has been showed to significantly improve weight and fat loss secondary to caloric restriction (Zemel, 2004). This apparent discrepancy could be due to the fact that in this study, this was a dietary supplement containing relatively little calcium compared to other studies examining the effects of increasing calcium in the diet via increased dairy products (e.g. cheese, milk). A study conducted by White et al. (2005) showed that acute dietary calcium did not alter fat utilization and actually decreased endurance performance in trained female runners.

Previous research has also demonstrated that green tea extract stimulates fat oxidation in mice in combination with exercise training (Shimotoyodome, 2005). Again, this particular supplement containing green tea extract did not elicit this improvement when combined with exercise. Another study that by Diepvens et al. (2005) assessed the effects of ingestion of green tea extract along with a low – energy diet on resting energy expenditure, substrate oxidation and body weight. Similar to our results with chronic exercise training, they concluded that there was no difference between the green tea group versus the Placebo group in regards to any body weight or body composition measurements or significant decreases in resting energy expenditure.

5.1.8 Chronic Exercise and a Dietary Supplement on Blood Lipoprotein Levels

A somewhat surprising result was a 15% decrease in total and LDL cholesterol in Supplement Group, an effect that was not observed in the Placebo Group. It is possible that this effect could be attributed to the higher baseline values for total, LDL and HDL cholesterol levels in the supplement group. In other words, it is possible that this improvement is only observed in those

who had higher values at baseline irrespective of treatment group. However, the baseline cholesterol levels were not associated with the degree of change when all subjects were combined in the analysis. Therefore, it appears that there may have been some effect of the dietary supplement on blood lipoprotein levels independent of the exercise intervention.

5.1.9 Limitations

A limitation to this study is that there were no men in this study. Therefore we cannot examine a gender effect. A second limitation is the high-unexpected drop out rate in this study. A total of 23 subjects were enrolled but only 15 subjects completed this study. This was a 35% drop out rate for this exercise intervention study. Another limitation was that independent effects of supplement without exercise training were not determined.

5.1.10 Conclusion

In conclusion, exercise training increases the reliance on fatty acids for energy during physical activity in obese women. This improvement was not affected by the dietary supplement. The improvements in fatty acid oxidation were not associated with the initial level of obesity or physical fitness. Exercise training also improved body composition. Women lost body fat and had a decrease in waist circumference, but no loss of lean mass. Although this study was not set up to specifically examine dose-response effects of the amount of exercise and degree of improvement in fuel metabolism or body composition, it appears that those obese women who perform more weekly exercise are more likely to experience beneficial changes in fat

metabolism and body composition. In summary exercise training increases the reliance on fat for energy in obese women, concomitant with improved body composition

5.1.11 Future Research Recommendations

The results suggest a potential dose-response relationship between the amount of exercise and degree of improvement in fuel metabolism and body composition. Therefore a prospective study set – up specifically to examining a dose- response in weekly exercise in obese women needs to be performed to properly examine this relationship. This exercise training study was limited to obese women. Therefore, a similar study on obese men to investigate whether they can also experience potential benefits from regular exercise, as well as examining potential gender differences in improvements should also be performed.

Another future study that should be performed is to determine an optimal exercise prescription to elicit the greatest changes in fat metabolism in obesity. Finally, due to the fact that this investigation reports only on whole body fat oxidation by indirect calorimetry, further examination of plasma and intramuscular fatty acid oxidation using stable isotope data will be analyzed as part of the larger parent study.

APPENDIX A

INFORMED CONSENT DOCUMENT

University of Pittsburgh

School of Medicine
Department of Medicine
Division of Endocrinology & Metabolism

CONSENT TO ACT AS A SUBJECT IN AN EXPERIMENTAL STUDY

Protocol Title: THE EFFECTS OF EXERCISE TRAINING AND DIETARY SUPPLEMENATION ON FAT METABOLISM AND BODY COMPOSITION IN OBESE WOMEN

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SOURCES OF SUPPORT: Experimental and Applied Sciences (EAS)

Why is this research being done?
You are being asked to participate in this research study to determine how your body burns fat for energy, which may be important for the understanding of fat metabolism and prescribing exercise prescriptions for women. In addition, there are several dietary Supplements on the market that claim to increase fat metabolism. We will also examine whether one of these Supplements will actually result in additional improvements in fat metabolism and body composition (decreased body fat) when combined with exercise.
Who is being asked to take part in this research study?
You are being asked to participate in this research study because you are an overweight, premenopausal female between the ages of 18 and 45. You will have a medical examination to determine if you are in good health for this study and these results will be given to you for your records. We will screen women upon entering this study; if you qualify, you will be one of 80 women in this study. If you ingest more than three (12 ounces) of caffeinated beverages or food daily you are excluded from the study due to the fact that the dietary Supplement contains caffeine. If you have a history of peptic or duodenal ulcer disease you will be excluded due to the caffeine content of the Supplement.

What procedures will be performed for research purposes?
If you participate in this study, you will be asked to participate in the following procedures: (1) Screening procedures consisting of reading and signing this consent form, a blood draw, physical, and an exercise test to determine your fitness level; (2) Study procedures consisting of (2a) a scan (called a DEXA scan which is similar to an x-ray) to measure your total body fat; (2b) and two exercise tests lasting one hour which will determine how your body burns fat; (2c) a one-repetition max (1RM) where you will be lifting the heaviest weight you possibly can at one time; (2d) and you will be placed under a clear canopy (plastic hood) where your resting metabolic rate (RMR) will be assessed. Resting metabolic rate (RMR) is how many calories an individual burns while at rest. Following these tests, you will be randomized (like a flip of coin) into one of four groups 1) Aerobic Training only receiving a dietary Supplement 2) Resistive and aerobic training group receiving a dietary Supplement 3) Aerobic training only receiving a Placebo (sugar pill) 4) Resistive and aerobic training only receiving a Placebo. Once you have been assigned to a group, you will participate in a 16-week exercise program to determine if exercise and/or a dietary Supplement change your body's ability to use fat.

Screening Procedures:
On your screening visit, you will arrive at the General Clinical Research Center (GCRC), on the 8th floor Montefiore University Hospital, at approximately 8:00 a.m. to see if you qualify for this study. This visit will take approximately 2 hours. You will be asked to come to this visit fasting from food and drinks, except water, for 12 hours prior. You are able to take your routine medications. After signing informed consent, you will undergo a physical exam, a blood draw,

and a urine analysis to determine if you are pregnant. The physical exam will be conducted by a Cardiologist Fellow to determine if you are in good health for participating in this study. The following tests will also be done: measurement of your temperature, heart rate, blood pressure, baseline weight, and height; and laboratory blood and urine tests. Approximately 2 tablespoons of blood will be drawn from your vein for laboratory studies that will measure your blood count (hemoglobin and hematocrit), electrolytes (potassium, sodium, calcium, phosphorus, chloride and bicarbonate), kidney function tests (BUN, total protein, creatinine, uric acid, and urinalysis), liver function tests (AST, ALT, total bilirubin, and alkaline phosphatase), blood glucose (sugar), plasma caffeine, glycosolated HbA1c (3 month blood glucose average) and a lipid (blood fat) panel that will measure your cholesterol and triglycerides. If your low-density lipoprotein (LDL), or "bad" cholesterol, is less than 160 and your blood pressure is 150/95 or less, you will be asked to perform a graded exercise test to assess your physical fitness. Prior to performing the physical fitness test on the treadmill, your heart's electrical activity will be evaluated by adhesive pads attached to your chest that are connected to an electrocardiogram (EKG). During the exercise test your heart rhythm will also be monitored. You will be asked to breathe through a mouthpiece in order to measure how much oxygen your body is using as you walk on the treadmill. An armband will be placed on your arm to measure how much energy you use when you exercise. Every minute the grade (elevation) of the treadmill will get higher with the speed staying constant; you will continue to keep walking until you are too tired to continue. If you develop any pain or discomfort in your chest or develop any particular patterns on the heart rate monitor, you will be asked to stop walking. This screening visit will be approximately two hours long. If an abnormal lab or screening results are obtained you will be notified and be recommended referral to your PCP for follow-up. If illness or injury occurs during a study procedure you will be transported to the Emergency Room if needed.

Study Procedures: Prior to visit #1 (within first week of screening procedures), you will be instructed to eat 200 g of carbohydrates per day for three days and will not have performed any type of rigorous physical activity the night prior. Also, you will have written down your food intake three days prior to this second visit. You will arrive after an overnight fast at approximately 8:00 a.m. (you will not have eaten after 10 o'clock that night except water) to the GCRC where a RMR (resting metabolic rate) will be assessed to determine the amount of oxygen your body uses. Also, a DEXA scan will be performed to measure your body's fat, and a one hour exercise test will be performed to measure how your body uses fat. This visit will take approximately 2 hours.

The RMR will be performed at 8:00 a.m. at the GCRC. You will lie on a bed and a clear plastic canopy (hood) will be placed over your face and neck to collect your exhaled air. This will be done to measure the amount of oxygen your body uses. An armband will also be placed on your arm to estimate how much energy you are burning at rest. This test will last approximately 30 minutes.

Prior to the DEXA scan you will be screened for pregnancy prior to any testing unless you have a history of proven surgical contraception. If you have a positive pregnancy test then you will be excluded from the study due to safety of the fetus. The DEXA scan will be performed at 8:30 a.m. and is similar to an x-ray. You will lie on an examination table in a room with a DEXA scanner for approximately 30 minutes. The test is painless except for any discomfort you may experience because of lying on your back on the firm examination table.

Participants will then come for the next outpatient visit (Visit #2), which will be approximately one week after visit #1. Prior to visit #2, you will be instructed to avoid strenuous physical

activity for two days prior to this study and to eat at least 200 g of carbohydrate per day for the three days preceding the study to make sure that your energy levels are high for the exercise bout. You will be given standard dinner the evening prior and then not eating until after the exercise study is completed the following day, although you can have water. You are allowed to take all your routine medications. The next morning you will perform the exercise test. At about 6:30 A.M., you will be come to the Obesity Nutrition Research Center Laboratory on the 8th floor Montefiore University Hospital where an intravenous catheter (plastic needle in your vein) will be placed in your arm to introduce glucose and fatty acid metabolic tracers into your blood. These tracers, containing absolutely no radioactivity, are used to measure your body's use of glucose and fat. Another catheter will be placed in the vein of your hand to periodically sample blood. For this part of the study including rest and exercise, you will have 8 blood samples taken, each being about 15 ml or about 3 teaspoons total.

After starting the tracers at about 7:30 A.M., you will rest in a chair for approximately two hours before you get on a treadmill at ~ 9:30 A.M. for exactly one hour of walking. The walk on the treadmill will be relatively easy in the beginning but may become more difficult as your muscles tire. During the exercise, you will be wearing a belt around your chest, which contains a transmitter to measure your heart rate. You will be asked to breathe through a mouthpiece connected to a breathing valve (hose) for 5 minutes at four different times during the exercise test in order to collect your expired air (air you breathe out) for the measurement of your exercising metabolism. During this period you will be breathing in normal room air. A nurse will take a blood sample (less than one teaspoon) from you every 15 minutes during exercise. You will be allowed to drink water at any time during exercise and cooled by a fan if you request.

You will next be scheduled for three visits at the Exercise Intervention suite of the Obesity Nutrition Research Center Laboratory on the 8th floor Montefiore University Hospital. An exercise physiologist will supervise all exercise sessions and water will be provided to ensure safety and adequate hydration. The Visit #4 (2-3 days after visit #3) and visit #5 (approximately 3 days after visit #4) will help you become familiar with the weight machines so you can lift with proper form and good technique. You will be shown and instructed by an exercise physiologist to stretch before and after each exercise session to ensure proper warm-up and safety. For visit #6 (2-3 days after visit #5), you will perform a one repetition maximum (1RM) where you will lift the heaviest weight you can. You will lift this heavy weight once for each exercise.

After all six visits, you will be randomized (flip of a coin to determine what group you are going to be in) into one of the four study groups outlined above: 1) aerobic training only receiving a dietary Supplement, Thermo Dynamx®; 2) resistance plus aerobic training receiving the same dietary Supplement; 3) aerobic training only receiving Placebo, or 4) resistance plus aerobic training receiving Placebo.

This dietary supplement contains caffeine and green tea extract, which have been reported to increase your body's ability to burn fat for energy. Neither you nor the investigators will know whether you are given the Supplement or Placebo, and you will be required to stay in your respective group for the remainder of the study. You will be asked to consume either product or Placebo exactly five days per week and no more. You will be instructed to take the dietary supplement twice daily, at least 4 hours between servings and 1 hour before exercise. You will begin the supplement program with one capsule twice daily for two weeks, then 2 capsules twice daily for two weeks, then 3 capsules twice daily for 3 weeks. This will be the halfway point (Visit #7) in the 16-week program at which time you will stop the supplement for two week

(You will continue, however, with the exercise component of the intervention). After this two-week hiatus (pause) you will restart the dietary supplement, consuming 3 capsules twice daily for the remaining 7 weeks of the intervention (study). You will be asked to discontinue the use of the supplement immediately if you experience any nervousness, insomnia (can not sleep), rapid heartbeat, elevated blood pressure, dizziness, severe headache, and shortness of breath or other symptoms. After 16 weeks of dietary supplementation, you will come to the GCRC within one week for assessments of the outcome parameters/ repeat of initial measures (Visit #8). This will allow us to ask you questions concerning any adverse (unpleasant) reactions to stopping the supplement such as headache, agitation, etc that may be associated with acute caffeine withdraw. If you ingest more than three (12 ounces) of caffeinated beverages or food daily you are excluded from the study due to the fact that the dietary supplement contains caffeine. You are also instructed not to consume more than one additional caffeinated beverage per day during this study. You can continue with your current use of vitamin supplements if you do so unless they contain any ingredients in the dietary supplement (Green tea, caffeine). If you have a history of peptic or duodenal ulcer disease you will be excluded due to the caffeine content of the supplement.

Either group you are in will be encouraged to exercise daily, with a minimum being 4 sessions weekly. The two main choices for exercise will be cycling on a stationary bicycle or walking/jogging. You can choose the type of exercise they will participate in and can alternate the type of exercise that you perform. You will be given instructions on proper stretching and warm-up. All sessions will start with 5 minutes of stretching. In at least one exercise session per week, a graduate student in exercise physiology, trained in the protocol, will join you. The exercise physiologist will also measure your blood pressure and your heart rate to monitor changes that may occur from the dietary supplement. Aerobic and resistive exercise training will be conducted at the Exercise Facility of the Obesity Nutrition Research Center Laboratory (ONRC) on the 8th floor of Montefiore Hospital. You will report at least once a week for supervised exercise session for the aerobic groups and at least twice a week for the resistance group to the Exercise Intervention Suite for the 16-week intervention (program). Either group you are in will be encouraged to exercise daily, with a minimum being 4 aerobic sessions weekly. All your sessions will be approximately one hour in length. You are advised to wear comfortable clothes and shoes to exercise (i.e. tennis shoes, T-shirt and shorts). For all your unsupervised exercise sessions, you will be instructed to use a stationary cycle ergometer or a treadmill at home, at a fitness facility or at the newly available ONRC Exercise Facility. This ONRC Exercise Facility will offer unsupervised weekday hours from approximately 8am to 4pm. You should be aware that the risks of unsupervised exercise at the ONRC Exercise Facility are comparable to those of unsupervised exercise

After the 16-week exercise program you will be asked to perform all three exercise tests, DEXA, and 1RM in the same series and in the same manner as prior to exercise training. This will determine how you may have improved during the exercise program.

The focus of the study is to examine the effects of exercise and dietary supplementation on body composition and fat metabolism; therefore, we will not set goals for weight loss or restrict total energy to encourage weight loss. At the same time, we will not attempt to prevent weight loss. All women will meet with a registered dietitian one time per week for approximately one hour in length to assess consistency of dietary habits and offer nutritional guidance on making healthful choices.

What are the possible risks, side effects, and discomforts of this research study?
There are potential risks associated with this research study. These risks for overweight women such as yourself, with out symptoms of heart disease such as chest pain or shortness of breath, have not been shown to be different than those for the general population. Information on the likelihood that these may occur are as follows: Likely – occurs in more than 25% of people (more than 25 out of 100 people); Common – occurs in 1% to 25% of people (1 to 25 out of 100 people); Rare – occurs in less than 1% of people (less than 1 out of 100 people). As with any investigational study, there may be adverse affects that are currently unknown and it is possible that certain of these unknown risks could be permanent, serious, or life threatening.

These potential risks are:
1) Exercise Test/Training: There is a possibility that certain changes could occur during the treadmill test. Any time you exercise, you have a risk of injuring a joint or muscle (common, occurs in 1% to 25% of people). The test will start with slow walking to help your muscles warm up and prevent injury. When participating in exercise testing or exercise training there is rare (occurs in less than 1% of people) risk of falling. Other changes include shortness of breath, abnormal blood pressure, fainting (common; occurs in 1% to 25% of people), disorders of heart rhythm are rare (occurs in less than 1% of people), and heart attack, stroke, or sudden death are rare (occurs in less than 1% of people). According to American College of Sports Medicine (ACSM), the risk of a myocardial infarction (MI) in symptomatic (categorized with males and females having ventricular arrhythmias) and asymptomatic (without any signs or symptoms indicative of ischemic heart disease) individuals during exercise is less than 0.04%. The risk of death in the same population is less than or equal to 0.01% and the risk of needing hospitalization (including acute heart attack and/or serious arrhythmia) is less than or equal to 0.2%. The risk of a cardiovascular complications occurring during exercise is rare (occurs in less than 1% of people). A retrospective survey by the YMCA revealed 1 death and 1 cardiac arrest per 2,897,057 and 2,253,267 person-hours. The EKG electrodes that will be placed on your chest may cause some skin irritation and redness (rare; occurs in less than 1% of people). Every effort will be made to minimize these risks by reviewing information about your health and fitness before the test and by watching you closely during the treadmill test. Emergency equipment and trained staff are available to deal with unusual situations that may arise. In the event of a home medical emergency, you would have to call 911.
2) Dietary Supplement: This dietary supplement does have some risks. You will be asked to stay well hydrated, drink at least 8 glasses of 8oz. of water daily. You will be specifically instructed on using this supplement according to the product's label and as described above. The two main ingredients in the dietary supplement are green tea and caffeine. Caffeine is one of the main ingredients in the dietary supplement. The maximum amount of caffeine from this dietary supplement would be 400 mg. One 8 ounce cup of coffee contains ~150mg (National Soft Drink Association). Thus the maximum amount of caffeine is ~2-3 cups per day. Green tea leaf can cause irritability, nervousness and/or palpitations (sensation of a rapidly or irregularly beating heart). Less frequently reported adverse effects associated with green tea are the following; appetite decrease, constipation, gastric (stomach) irritation, headache, hyperacidity (heartburn), vertigo (dizziness) and vomiting. Individuals are at risk of a drug interaction (interference) if taking an anticoagulant (blood thinners) medication and using green tea. Therefore if you are on any of the medications Acenocoumarol, Anisindione, Dicumarol, and Wararfin, you are not to participate in the study due to possible drug interactions Ingestion of caffeine can be associated

with the following side effects; nausea, stomach upset, insomnia, restlessness, nervousness, tremor, headache, and lightheadedness. Large amounts of caffeine may worsen ulcers, cause frequent urination, flushing, muscle twitch or irritability. The following side effects are less frequently reported when ingesting caffeine: dizziness, depression, rapid breathing, chest pain, confusion, fatigue. There are several drug interactions (interferences) when ingesting caffeine and taking any of the following; Dipyridamole (Aggrenox/ Persantine), Theophylline (Theo-24/ Uniphyl), Cimetidine (Tagment) and Fluvoxamine.

Green Tea has the potential to increase your body's metabolism via its high caffeine and catechin polyphenol content. The catechin that occurs in the largest percentage is known as epigallocatechin (ECGC), which has been shown to inhibit an enzyme that degrades prolonging the bodies able to metabolize. Green tea appears to be safe for most adults. You may be sensitive to the high caffeine content. The extract seems to be safe for most adults short-term; long-term data is not available. Green Mate is used orally as a diuretic (any substance that increases the lost of body water in the urine) for modifying mood or emotional disorders, as a mild pain reliever for headache and aching pain, and as a laxative in large amounts. It is also used orally for weight loss. It contains 0.2-2% caffeine (compared to 1-2% in coffee), which acts as a central nervous system stimulant. The typical dose of green mate is 2-4 grams of the dried leaf (recommended serving of Thermo DynamX provides 500 mg). If you have an allergy to artichokes, you will be excluded due to the fact that it is a contraindication for taking green mate.

If you become aware of any of the above side effects during the course of your participation, you must, contact the primary investigator listed on the first page of this form as soon as possible. During the course of the study if your physician prescribes you on any of the above medications you must, discontinue immediately the dietary supplement and contact, as soon as possible, the primary investigator listed on the first page of this form.

3) DEXA: Participation in this study involves a minimal amount of radiation exposure from the DEXA. The amount of radiation exposure you will come in contact with from this procedure is about 0.06 mRAD to your whole body. For comparison, this is a small fraction of the annual whole body radiation exposure (300 mRAD) received by all members of the general public from natural, background radiation sources.

There is no minimum amount of radiation exposure that is recognized as being totally free of the risk of causing genetic mutations (abnormal cells) or cancer. However, the amount of radiation that you will come in contact with is thought to be low and comparable to everyday risks. DEXA scanning has certain conditions that would exclude you from participating in this study. Pregnancy will also cause you to be excluded due to radiation exposure to the fetus.

4) Blood sampling: The risks of blood sampling are common (occurs in 1% to 25% of people) and may include bleeding, bruising, hematoma (blood bruise) soreness and infection. Infection from the blood sampling is rare (less than 1% of people). In our experience using similar protocols, subjects have not experienced adverse effects from these procedures other than a small amount of residual localized soreness at the blood sampling area.

5) Pregnancy during study: For the DEXA scan you will undergo a pregnancy test prior to the enrollment in the study and at post testing. If you become aware that you are pregnant during the course of your participation in the study, you must, discontinue immediately the dietary supplement and contact, as soon as possible, the primary investigator listed on the first page of this form.

What are possible benefits from taking part in this study?

You will not benefit personally from participating in this study. However, you may benefit in this study as an extent of health benefits associated with regular exercise. Information on exercise capacity, fitness and energy metabolism during exercise will be shared with you if you desire. This will include a copy of the maximal exercise test and body composition results. You should inform the investigators if you have participated in any other research study during the previous year. Prior participation in any other research study during the previous year does not necessarily exclude you from this study. However, this information may be used to determine whether or not any prior participation in research studies may affect the results of this study. This study is a research study and may not be of direct benefit to you. If requested, a report will be generated for your medical record that will include any information important for your medical care.

What treatments or procedures are available if I decide not to take part in this research study?

If you decide not to take part in this study there are a wide variety of both weight loss programs (Weight Watchers®, LA Weight Loss®, etc.) and exercise facilities available outside of this study.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?

If any new information, good or bad, about this study comes to the attention of the investigator during the course of this study, which may relate to your willingness to participate, it will be provided to you or your representative.

Will my insurance provider be charged for the costs of any procedures performed as part of this research study?

Neither you, nor your insurance provider will be required to pay for any procedures associated with the research study. There will be no charge to you or your insurance provider for the screening evaluation, the screening labwork, the ONRC visits, laboratory tests, the EKGs or the study supplement. The study will cover the costs of all research services. You and/or your insurance provider will be responsible for any routine care costs, including any applicable copays, coinsurances and deductibles.

Will I be paid if I take part in this research study?

To help defray the costs of your participation, you will be compensated a total of $100 if you complete the entire study. The amount of $50 will be given to you for the completion of the pre-intervention testing. Another $50 if post-intervention testing is completed will be given to you for time spent away from work, travel, childcare or related costs. There will be no financial reimbursement for the screening procedures (i.e. lab work, exercise testing) however, transportation/parking will be reimbursed up to $10 and you will receive a copy of all results.

COMPENSATION FOR INJURY

University of Pittsburgh researchers and their associates, who provide services at the UPMC, recognize the importance of your voluntary participation in their research study. These individuals and their staff will make reasonable efforts to minimize, control and treat any injuries that may arise as a result of this research. If you believe that you are injured as a the result of the research procedures being performed, please contact immediately the Principal Investigator listed on the cover sheet of this form or the University of Pittsburgh Institutional Review Board at (412) 383-1480.

Emergency medical treatment for injuries solely and directly relating to your participation in this research will be provided to you by hospitals of the UPMC. It is possible that the UPMC may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the cost of this follow-up unless otherwise specifically stated below. You will not receive monetary payment from UPMC for, or associated with, any injury that you suffer in relation to this research. The U.S. Department of Health and Human Services or any agency funding this study in which you are taking part will not provide payment or medical treatment if the study results in adverse effects. If you experience an injury, as a result of the research procedure, the study's doctors will help you find the correct treatment. The study will not provide any money for the treatment, nor will the study's doctors be able to provide you with ongoing treatment at no cost to you.

CONFIDENTIALITY

Any information about you will be handled in a confidential (private) manner consistent with other medical records. Information obtained about you from this research project will be part of your research record. Research records will be kept in locked files in the office of Dr. Goodpaster. All data obtained will be maintained by Dr. Goodpaster's laboratory for a period of at least five years upon termination of this study.

All information about you and your answers to questions for this study will be kept confidential to the extent the law allows. Records about you will be put under a code number. In order to ensure that we can keep in contact with you, we will record some personal information and this will be stored in a locked file cabinet at the clinic and in a secured, password-protected database at the Data Center. The results of the study may be published, but your identity will not be given, and the results will be given only for groups of people, not individuals. Any medical information about you will be kept in computer records for analysis with such information from all other individuals in the study, but these records will not contain your name, or Social Security or Medicare number, or any other information that could identify you.

The University of Pittsburgh Institutional Review Board (IRB) may request access to your records as part of routine monitoring to ensure that your rights as a research participant are being properly maintained. Any such requests made by the IRB will be honored.

Any information about you obtained from this research study will be kept as confidential and private as possible. You will not be identified by name in any publication of research results unless you sign a separate form giving your permission. In unusual cases, your research records may be released in response to an order from a court of law. It is also possible that authorized representatives of the Food and Drug Administration, the NIH and of the University Research Conduct and Compliance Office may inspect your research records. If the researchers learn that you or someone with whom you are involved is in serious danger or harm, they will need to

inform the appropriate agencies as required by Pennsylvania law. The fact that you are participating in a research study and that you are undergoing certain tests (but not the results of the tests) may also be made known to individuals involved in insurance billing and or other administrative activities associated with the conduct of the study. After the study is completed these records will be kept for a period of five years.

RIGHT TO PARTICIPATE OR WITHDRAW FROM PARTICIPATION

You have the right to ask questions concerning any aspects of this study at any time. Your participation in this research study is completely voluntary. You do not have to take part in this research study and, should you change your mind, you can withdraw from the study at any time. If you withdraw from the study during this screening process, there will be no risk to you other than not having a chance to participate in this study. Your current and future care at a UPMC facility and any other benefits for which you qualify will be the same whether you participate in this research study or not. If any medical condition develops that would put you in danger, if you would continue to participate in the study, the researchers have the right to remove you from the study without your permission. You may also be removed from the study if you fail to follow their instructions. Every effort would be made to ensure your safety and assist you in finding appropriate care, if you would be removed from the study. Your doctor may be an investigator in this research study, and as an investigator, he is interested both in your medical care and in the conduct of this research. Before entering this study, or at any time during the research, you may discuss your care with another doctor who is in no way associated with this research project. You are not under any obligation to participate in any research study offered by your doctor.

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VOLUNTARY CONSENT:

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions which I have about my rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668).

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____ _____

Participant's Signature Date

CERTIFICATION of INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise."

_____ _____

Printed Name of Person Obtaining Consent Role in Research Study

_____ _____

Signature of Person Obtaining Consent Date

APPENDIX B

EAS STUDY GENERAL HEALTH HISTORY

EAS

IRB # 0402069

The Effects of Exercise Training and Dietary Supplementation on Fat Metabolism and Body Composition in Obese Women

General Health History

Please fill out this questionnaire carefully:

Date:_____

Street Address:_____ Home Phone (___)_____

City:_____ State_____ Zip Code_____ Work Phone (___)_____

1. Sex M____ F_____ 2. Age:____ 3. Birthdate_____/___/___
month day year

4. Current Weight_____lbs. 5. Height_____ft. _____in.

6. Race
   1) Caucasian
   2) African American
   3) Hispanic
   4) Asian
   5) Other (please specify)

7. Name and phone number of friends or family we can contact in case of an emergency.

Name:_____ Phone Number (____)_____

8. Please list <u>all</u> medications that you are currently taking:

	Medication	Dose	Reason for taking
a.			
b.			
c.			
d.			
e.			
f.			

General Health History

9. Do you have or have you ever had any of the following medical conditions?

Condition	Check if yes	Date of Diagnosis (Approximately)	Describe the Problem
a. Heart attack			
b. Angina (chest pain on exertion)			
c. Irregular heart rhythm			
d. Heart failure			
e. Fainting spells			
f. Other heart problems			
g. Liver disease			
h. Kidney disease			
i. Chronic diarrhea			
j. Stroke			
k. Substance abuse			
l. Thyroid problems			
m. Kidney stones			
n. Gallstones			
o. Gout			
p. Emotional/psychiatric problems			
q. Diabetes			
r. Cancer			
s. High blood pressure			
t. Circulation problems			
u. Any other medical problems			

ALCOHOL INTAKE

1. On how many weekdays (Monday through Friday) do you usually drink wine, beer, or liquor.

Never	_____(0)	Two days/week	_____(4)
Less than one/month	_____(1)	Three days/week	_____(5)
1-2 times/month	_____(2)	Four days/week	_____(6)
One day/week	_____(3)	Five days/week	_____(7)

2. On those weekdays you drink wine, beer, or liquor how many drinks do you have?

3. On how many weekend days (Saturday & Sunday) do you usually drink wine, beer, or liquir?

Never	_____(0)	Two days/week	_____(4)
Less than one/month	_____(1)	Three days/week	_____(5)
1-2 times/month	_____(2)		

4. On those weekend days you drink wine, beer, or liquor how many drinks do you have?

For Women

1. Are you currently pregnant? Yes_____ No_____

2. Do you plan to become pregnant in the next two years? Yes_____ No_____

3. Have you gone through menopause or the change of life? Yes_____ No_____

4. Have you had a hysterectomy? Yes_____ No_____

5. If yes, was the hysterectomy partial (uterus only) or complete (uterus & ovaries)?
Partial_____ Complete_____

6. When was your last menstrual period? _____/_____/_____
month day year

7. Do you take: Birth control pills? Yes____ No_____
Estrogens (e.g., premarin)? Yes____ No_____
Progesterone (e.g., provera)? Yes____ No_____

Current physician:

Name:_____

Address:_____
_____

City_____ State_____ Zip_____

Phone (____)_____

APPENDIX C

EAS STUDY MAXIMAL EXERCISE TEST DOCUMENTATION FORM

EAS STUDY EXERCISE TEST

Name_____ ID#_____ Date_____

Wt(lbs)_____(Kg)_____ Ht.(ft)_____(cm)_____

APM_____ Age_____

Resting HR_____ Resting BP_____

Speed:_____

Grade	Time	HR	BP	RPE
0%	0-2			
2%	2-4			
4%	4-6			
6%	6-8			
8%	8-10			
10%	10-12			
12%	12-14			
14%	14-16			
16%	16-18			

Termination time_____

Reason for Termination_____

Peak: HR_____ BP_____

Immediate Post BP_____

Recovery:

__2___min HR_____ BP_____

__4___min HR_____ BP_____

__6___min HR_____ BP_____

__8 __ min HR_____ BP_____

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