EFFECT OF LIFEWAVE PATCHES ON FAT METABOLISM IN MODERATELY ACTIVE WOMEN

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To the Dean of the Graduate School:

I am submitting herewith a thesis written by John Witt entitled "Effect of Lifewave Patches on Fat Metabolism in Moderately Active Women." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Exercise and Sports Nutrition.

Nancy DiMarco, Major Professor

We have read this thesis and recommend its acceptance:

Chandan Prasad, Department Chair

Accepted:

Dean of the Graduate School

ABSTRACT

JOHN WITT

EFFECT OF LIFEWAVE PATCHES ON FAT METABOLISM IN MODERATELY ACTIVE WOMEN

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Lifewave patches are purported to restore the body's electromagnetic field, resulting in increased energy and fat metabolism. PURPOSE: To determine the effects of wearing Lifewave patches on body mass (BM), percent body fat (%BF), VO_2 Peak, and mean exercise respiratory exchange ratio (RER) over 2 weeks. METHODS: Moderately active, nonsmoking, not pregnant or postpartum women were randomly assigned to an active or placebo group. Participants' BM, %BF, VO_2 Peak, and mean RER at 60-70% VO_2 peak were assessed on two separate sessions scheduled 2 weeks apart. Between sessions, participants wore one set of patches in different locations (wrists, shoulders, knees, ankles) every other day, and recorded daily food intake and daily exercise. RESULTS: There was no significant change in any of the variables over the intervention ($p > .05$). CONCLUSION: Lifewave patches do not improve BM, %BF, VO_2 Peak, or fat utilization during submaximal exercise in moderately active women.

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CHAPTER I

INTRODUCTION

Overweight and obesity are the result of overconsumption of calories compared to calories expended (Centers for Disease Control and Prevention, 2009). Increased body weight is positively correlated with increased morbidity and mortality through metabolic and cardiovascular disease (Mokdad et al., 2003). Although the exact causes of the rise are debatable, Americans are clearly consuming too many calories and not participating enough in physical activity. Reducing caloric intake through diet can be effective at controlling obesity. However, restrictive diets may result in a deficiency of necessary vitamins and minerals and also promote a decrease in lean body mass (LBM). Restrictive diets may also not be maintainable for a long period of time, resulting in weight re-gain. Increased energy expenditure through increased physical activity can expend calories, but the intensity, frequency, and duration needed to cause a meaningful change in body mass may exceed an individual's willingness to participate. In addition to diets and physical activity, over-the-counter supplements have been utilized to reduce body fat. However, these alternatives have risks of their own and are at best only part of a solution to reduce body fat. Since there exists a minimum caloric intake needed to survive, strategies to increase energy expenditure and fat oxidation while limiting energy intake would be more effective at controlling overweight and obesity than simply restricting caloric intake.

The Lifewave (Lifewave, San Diego CA) energy patches are transdermal patches that are purported to alter the body's electromagnetic field to be more conducive to burn fat. The patches are made from sterioisomers (L/D) of amino acids which interact with the body's normal electromagnetic field. Through electromagnetic frequency modulation at the cellular level, the Lifewave patches trigger more Ca^{2+} release during muscular contraction and optimize carnitine function, resulting in improved strength endurance and fat metabolism (Haltiwanger, 2005). As the patches are most effective when physical activity is performed, the endurance and fat burning effect of the patches may encourage an individual to perform increased physical activity and thus increase energy expenditure.

Purpose of the Study

The purpose of this study was to analyze the effects of Lifewave energy patches on fat metabolism in moderately active women. Participants wore the patches over a 2 week period, varying the locations of the patches as recommended by the manufacturer. Maximal oxygen uptake (VO_2 peak) and percent fat oxidized during sub maximal exercise were measured at the start and completion of the study. A placebo group wearing identical patches but with an inactive patch compound (saline solution) served as the control group. The goal of the study is to determine if these patches improve fat metabolism during exercise and potentially contribute to additional fat loss in this population.

Research Questions

The study addressed the following research questions:

1. Does wearing Lifewave energy patches over a 2-week period contribute to body mass loss over a 2 week period in moderately active women?

2. Does wearing Lifewave energy patches over a 2-week period contribute to decreased percent body fat (% BF) over a 2-week period in moderately active women?

3. Does wearing Lifewave energy patches improve VO_2 peak in moderately active women?

4. Does wearing Lifewave energy patches increase fat metabolism during submaximal exercise in moderately active women?

Null Hypotheses

Null hypotheses are as follows:

1. Wearing Lifewave energy patches over a 2 week period will have no effect on body weight in moderately active women.
2. Wearing Lifewave energy patches over a 2 week period will have no effect on percent body fat in moderately active women.
3. Lifewave energy patches will have no effect on VO_2 peak in moderately active women.
4. Lifewave energy patches will have no effect on fat metabolism during submaximal exercise in moderately active women.

Definitions

ATP – adenosine triphosphate, high energy phosphate consisting of an adenosine molecule and three phosphate groups utilized to provide energy for cellular chemical reactions (McArdle, Katch, & Katch, 2001)

Beta Oxidation – mitochondrial cellular process of oxidizing long chain fatty acids into acetyl CoA, $NADH_2$ ($NADH^+ + H^+$), and FADH (McArdle, Katch, & Katch, 2001)

Body mass index (BMI) - a ratio calculated from an individual's weight and height. Calculations are used for assessments of underweight, normal, overweight, and obese populations. Can be calculated with the following formula: weight (kg) / height $(m)^2$. Using BMI individuals are classified into the following categories:

Underweight: BMI < 18.5 k^1g / m^2

Normal Weight: BMI 18.6 kg / m^2 to 24.9 kg / m^2

Overweight: BMI 25.0 kg / m^2 to 29.9 kg / m^2

Obese: BMI >30.0 kg / m^2

(American College of Sports Medicine, 2006, p. 58)

Duration – the amount of time in which an exercise is completed (American College of Sports Medicine, 2006)

Exercise - a structured form of physical activity with the intent to improve or maintain one or more components of physical fitness (American College of Sports Medicine, 2006)

Frequency – how often exercise in performed, usually defined as number of sessions per week (American College of Sports Medicine, 2006)

Glycolysis – anaerobic cellular process converting 1 mole of glucose into 2 moles of pyruvate, 2 moles of ATP, 2 moles of water, and 2 moles of $NADH_2$ (McArdle, Katch, & Katch, 2001)

HR – heart rate, as measured in beats per minute (bpm)

Intensity – how "hard" the exercise is performed, usually defined as a percentage of maximal heart rate or VO_2 max or metabolic equivalents (American College of Sports Medicine, 2006)

Lipolysis – the process of breaking down triglycerides into glycerol and three fatty acids

MET – metabolic equivalent, refers to a percentage of basal metabolic rate (American College of Sports Medicine, 2006)

Mitochondria – cellular organelle responsible for the oxidation of NADH and $FADH_2$ into ATP and H_2O during the electron transport chain (McArdle, Katch, & Katch, 2001)

Physical Activity - any bodily movement produced through muscle contractions that significantly increase energy expenditure (American College of Sports Medicine, 2006)

Physical Fitness – ability to perform physical activity. Comprised of skill related, health related, or physiologic related components (American College of Sports Medicine, 2006)

Recreationally Active – for this study defined as a non-athletic population participating in at least 60 minutes of exercise per week

RER – respiratory exchange ratio, ratio of carbon dioxide produced to oxygen consumed (McArdle, Katch, & Katch, 2001)

RPE – rate of perceived exertion, typically measured via the Borg scale (i.e. 6 = rest, 20 = maximal intensity) (American College of Sports Medicine, 2006)

Sarcolema – the cytoplasm of the muscle cell (McArdle, Katch, & Katch, 2001)

Sub Maximal Exercise – exercise performed at an intensity below VO_2 max, for this study defined as an intensity of 60 – 70% VO_2 peak

Triglyceride - a compound consisting of three molecules of fatty acid esterified with glycerol (McArdle, Katch, & Katch, 2001)

VDAC - voltage dependent anion channels are protein gradients located on the cell membrane, between the cytosol and intermembrane space, controlling ion transfer into and out of the cell (Colombini, 2004)

VO_2 Max – the maximal amount of oxygen an individual can utilize during exercise. Should include at least two of the following 1) failure of HR or oxygen uptake to increase after successive increases in intensity, 2) RER > 1.1, and 3) a lactic acid concentration of > 8 mmol (American College of Sports Medicine, 2006)

VO_2 Peak - indicates the highest value of recorded oxygen consumption during a maximal exercise test – may not represent VO_2 max (i.e. participant voluntarily terminated the exercise test prior to achieving VO_2 max) (McArdle, Katch, & Katch, 2001)

Assumptions

All participants could read and write English. All participants participated in exercise at least 1 hr / week and were deemed fit enough to complete the test procedures. All participants were competent enough to understand the risks. All participants were females over the age of 18 and under 55. All participants were nonsmoking and not lactating. Participants did not possess any physical or mental ailments to prevent them from completing test procedures. All equipment utilized was accepted as reliable. All data obtained from the participants (i.e. medical history, compliance) was accepted as accurate. Participants did not change their diet or exercise habits as a result of the intervention. There was no significant difference in exercise RER between groups at the start of the intervention. Any change in RER for the final session was a result of the patches.

Limitations

The study consisted of only women between the ages of 18 and 55 years. As maximal fat oxidation rate is affected by gender, men were excluded. The study excluded diseased, obese, and sedentary women as well as lactating and smoking women. Recruiting was done throughout Denton, but primarily targeted at students attending Texas Woman's University (TWU). The mode of exercise testing used in the study was treadmill exercise and results may not be applicable to other exercise modes. Duration of the study was limited to 2 weeks. The sample consisted of two, independent groups of women with a target sample size of 30.

Delimitations

The study sample consisted exclusively of the population of women aged 18-55 years who did not smoke, were not lactating, and were recreationally active. The control and experimental groups were randomized and not predetermined based on any study variables (i.e. body mass), although both participants and researchers were blinded to group membership. Oral contraceptive use was not recorded.

Significance of the Study

If indeed Lifewave patches increase fat metabolism and improve endurance during exercise, then Lifewave patches should cause more weight to be lost and encourage this population to continue their diet and exercise regimen, thus reducing obesity-related risk factors, increasing self-esteem, and decreasing obesity related health care costs (Donnelly, 2004). In addition, the results of this study should encourage more research into the specific mechanisms demonstrated by the Lifewave patches.

CHAPTER II

REVIEW OF THE LITERATURE

The following topics were reviewed in the literature: energy expenditure, physical activity and exercise, ATP production, exercise metabolism, fat oxidation in women, fatty acid oxidation and regulation, measuring energy expenditure and fat oxidation, supplements and fat loss, and Lifewave energy patches

Energy Expenditure

Humans convert energy from food ultimately to adenosine triphosphate (ATP) to fuel living cells. Resting metabolic rate (RMR) is defined as the minimum amount of energy needed to maintain bodily functions in the resting state (Whitney & Rolfes, 2002). This is essentially the amount of calories that is expended while resting in a supine position. For non-active individuals this represents the largest amount of daily energy expenditure. Energy contributions towards RMR vary by organ. For example, the liver accounts for 27% of daily RMR with the brain accounting for 19%. (Elia, 1992). Skeletal muscle accounts for 18% of daily RMR, and therefore changes in muscle mass may cause small changes in RMR. However, changes in RMR from lean body mass changes (LBM) are equivocal. Dolezal & Potteiger (1998) measured RMR changes in 30 healthy men following 10 weeks of strength training, endurance training, or concurrent training. Participants in the strength and concurrent training groups gained lean body mass and increased RMR (~96 kcal per day), while those in the endurance group lost LBM and

decreased RMR (50 kcal per day). The authors observed a strong correlation ($r = .74$) between LBM gains and increases in RMR, concluding that muscle mass positively affects RMR. Byrne & Wilmore (2001) measured RMR changes in 36 sedentary, obese women following 20 weeks of strength training or strength training and walking. The women increased LBM and RMR in both groups; however, the increase in RMR was approximately 40 kcal per day, which was only half the increase compared to the Dolezal & Potteiger study even though LBM gains were similar (~2 kg). Geliebter et al. (1997) measured LBM and RMR changes in 65 obese men and women after performing 8 weeks of strength (ST) or endurance (ET) training on a hypocaloric diet (70% RMR). Both groups lost LBM significantly, although LBM losses were less for ST than ET (-1.1 vs. -2.3 kg). Both groups significantly decreased RMR, but there was no significant difference between groups ($p > .05$). While LBM changes do affect RMR, the magnitude of the changes cannot accurately predict RMR changes. It is important for the dieter or athlete to realize that changes in LBM will have only a small effect on RMR, and therefore overall energy expenditure.

Thermogenesis refers to the body's generation of heat as a result of metabolic reactions (Whitney and Rolfe, 2002). Under resting conditions thermogenesis is regulated through external body temperature and hormonal control. However, thermogenesis, and therefore RMR, can be increased due to a variety of metabolic activities (Whitney and Rolfe, 2002). Haman (2006) summarized that shivering due to exposure to cold temperatures increases RMR anywhere from 150 – 400%. Although this represents a

significant amount of energy, it is an impractical and dangerous method to increase RMR. The active thyroid hormones, triiodothyronine (T3) and thyroxin (T4), regulate overall metabolism as well as thermogenesis. Thyroid hormones (TH) increase thermogenesis through increased ATP utilization, decreased ATP production in the electron transport chain, and a synergistic relationship with sympathetic nervous system (SNS) catecholamines. Thyroid hormones stimulate ATP utilization by promoting futile cycles that concurrently activate ATP consuming and ATP producing cycles, such as lipolysis with lipogenesis, glycolysis with gluconeogenesis, and proteolysis with protein synthesis (Silva, 2006). Thyroid hormones also increase the permeability of ion gradients such as Na^+, K^+, and Ca^{2+} across the cell membrane. Leakage of ions across the membranes requires ATP expenditure to maintain the gradient (Haber, Ismail-Beigi, & Loeb, 1988). In addition to affecting ATP utilization, TH also decreases ATP production during the electron transport chain through uncoupling proteins, and increased reliance on the glycerol-3-phosphate (G3P) pathway (Silva, 2006). During the electron transport chain process of oxidative phosphorylation, electrons travel down the mitochondrial matrix while pumping protons across the membrane to create a gradient. The energy of this gradient, when the protons return to the matrix, is used to synthesize ATP. However, it is believed that uncoupling proteins such as uncoupling protein-3 (UCP3) offer the protons a path of lower impedance back to the matrix, bypassing the pathway for ATP synthase and preventing ATP synthesis (Gong, He, Karas, & Reitman, 1997). During the G3P shuttle, dihydroxyacetone phosphate (DHAP) and NADH in the cytosol are reduced

to G3P and NAD^+ in the cytosol. On the outer mitochondrial membrane, G3P can be reconverted back to DHAP, but the redox reaction results in $FADH_2$ in the mitochondria instead of NADH in the mitochondria. As NADH produces 3 ATP in the electron transport chain and $FADH_2$ only 2 ATP, increased utilization of the G3P shuttle towards G3P production during lipid and glucose metabolism as compared to full oxidation to pyruvate will also reduce ATP production. Thyroid hormones stimulate activation of the mitochondrial enzyme mGPD, which catalyzes the rate limiting step of the G3P shuttle (Lee & Lardy, 1965).

Catecholamines, particularly norepinephrine and epinephrine, are released by the SNS in times of stress when immediate energy is required. These neurotransmitters increase cardiac output by increasing heart rate, vasoconstriction (most organs), vasodilation (working muscles), glycolysis, and lipolysis, resulting in an increase in ATP utilization from glucose and fatty acids, and heat generation (Sival, 2005). Increased TH enhances the cells to the actions of catecholamines, as one example increasing the lipolytic response of catecholamines (Wahrenberg, Wennlund, & Arner, 1994). In addition, catecholamines increase conversion of T4 to T3 in cells, exhibiting a synergistic relationship where catecholamines can increase the concentration of T3 and TH can increases catecholamine response through increased sensitivity or suppression of the SNS during hyperthyroidism (Silva & Larsen, 1986). Finally, catecholamines increase the amount of substrate cycling such as the Cori and fatty acid-triglyceride cycles (Newsholme, 1982). During substrate cycling, the substrates and products are repeatedly

interconverted resulting in excess heat generation and no increase in ATP or other products. No increase in ATP combined with an increase in wasteful heat production results in excess calories expended and can contribute to fat loss. In conclusion, RMR is primarily determined by organ energy metabolism although changes in LBM can have a small effect on RMR. TH and catecholamines can increase RMR through increased fat metabolism and thermogenesis.

Physical Activity and Exercise

Although RMR can be considered for the most part static, individuals can increase their daily energy expenditure through increased physical activity. Physical activity is defined as any bodily movement produced through muscle contractions that significantly increase energy expenditure (American College of Sports Medicine, 2006). Increased physical activity can be utilized to prevent or deter obesity, and studies have shown an inverse relationship between obesity and the amount of physical activity performed and this same relationship was seen for obesity comorbidities such as hypertension and type 2 diabetes (Bouchard & Blair, 1999). Despite the benefits, total physical activity has been declining in the US (Brownson, Boehmer, & Luke, 2005). Although the causes of less physical activity are debatable, the result is that Americans are not performing as much physical activity as they should.

Physical activity can be quantified into a percentage of basal metabolic rate, known as a MET, or metabolic equivalent. Metabolic equivalents can then approximate the caloric expenditure of the physical activity using the equation kcal / min = (METS *

3.5 * weight in kg) / 200 (American College of Sports Medicine, 2006). While physical activity of high METs will expend the most calories, this intensity is typically too high for a sedentary population and a lower MET intensity is utilized, depending on the subject's fitness level. However, this level of intensity may not produce the amount of weight loss the individual desires. For example, a sedentary 220 lb man may only be able to perform 4 METs during 30 mins of walking, and this will expend approximately 210 kcal. Noting that it requires a deficit of 3500 kcal to lose one pound of fat, the small amount of calories expended through walking may demotivate the individual to see increased physical activity as an effective way to lose weight.

Exercise is a structured form of physical activity with the intent to improve or maintain one or more components of physical fitness (American College of Sports Medicine, 2006). With Americans living a more sedentary lifestyle with less day to day physical activity, a structured exercise program may be the primary physical activity most individuals perform. Exercise can be further defined as having a mode, frequency, intensity, and duration component, as in physical activity. The mode refers to the type of physical activity being performed. Exercise which includes larger muscle groups expends more energy than that utilizing smaller muscle groups. In addition, exercise which targets multiple muscle groups will expend more energy than those targeting one group. The frequency of the exercise is how often the activity is performed in a given time, usually in number of times per week. The duration of the exercise defines how long the exercise is performed. Finally, the intensity of the exercise is how hard the exercise is performed.

Intensity is typically measured as a percentage of maximal heart rate or maximal oxygen consumption rate. The four components of exercise determine the amount of energy the exercise will expend (American College of Sports Medicine, 2006).

Maximal oxygen consumption, or VO_2 max, defines the maximal amount of oxygen that muscles can utilize to perform work, and is the standard to define maximal aerobic capability (American College of Sports Medicine, 2006). Although VO_2 max can be estimated through various formulas, the gold standard is to use a metabolic cart which measures the amount of oxygen consumed during exercise. To determine VO_2 max, the subject performs a graded exercise test until the subject cannot continue the test. The Bruce protocol is a common treadmill maximal exercise test utilizing 3-min stages of increased speed and grade which is suitable for a younger and overall fit population (American College of Sports Medicine, 2006). The criteria to determine whether a true VO_2 max has been reached should include at least two of the following 1) failure of oxygen uptake to increase after successive increases in intensity, 2) RER > 1.1, and 3) a lactic acid concentration > 8 mmol / L (American College of Sports Medicine, 2006). Since many individuals are not accustomed to exercising at such a high intensity (especially non-athletes), they may conclude the exercise session before reaching true VO_2 max. This criterion, referred to as VO_2 peak, indicates the highest value of recorded oxygen consumption during the test (McArdle, Katch, & Katch, 2001). Exercise prescriptions are typically determined from percentages of VO_2 max or VO_2 peak.

ATP Production

Exercise requires ATP to supply energy needed for repeated muscle contractions. ATP is available in muscle sarcolema (cytoplasm), but its stores are limited and exercise will deplete the supply quickly. If the intensity of the exercise is high and ATP is depleted, the cell uses creatine phosphate to replenish ATP and creatine to continue energy production though the creatine phosphate cycle. This supply is also limited and will exhaust after about 10 s of high intensity exercise (McArdle, Katch, & Katch, 2001). Should exercise continue at this intensity the cell will begin converting carbohydrate sources into ATP through glycolysis. Glycolysis initially produces 2 ATP from 1 mole of glucose through substrate level phosphorylation, resulting in 2 moles pyruvate and NADH. If sufficient oxygen and NAD^+ are present the pyruvate and NADH can be further converted to carbon dioxide and water, resulting in approximately 36 additional ATP molecules in muscle (McArdle, Katch, & Katch, 2001). However, if sufficient oxygen is not available and pyruvate builds up, the pyruvate is converted to lactate. Lactate can be converted back to pyruvate in neighboring muscle cells or converted back to glucose in liver via the Cori cycle. As lactate builds up, heart rate and oxygen consumption increase in a negative feedback mechanism to reduce lactate. Lactate is buffered via bicarbonate to preserve blood pH. However, if the cardiovascular system cannot clear lactate faster than it is being generated, the bicarbonate will be overwhelmed and blood pH will decrease, causing the exercise intensity to decrease or cease altogether.

The above example describes exercise under high intensity. Under resting conditions and low intensity exercise, fat stores provide the primary fuel for energy expenditure through lipolysis and beta oxidation. Fats are stored primarily as triglycerides, which consists of 3 fatty acids esterified to 1 glycerol molecule. Triglycerides are broken down to glycerol and fatty acids through lipolysis by the hormone sensitive lipase. While glycerol can enter glycolysis, beta oxidation in the mitochondria converts fatty acids into carbon dioxide, water, and ATP. The amount of ATP generated from beta oxidation varies depending on the type of fatty acid being degraded but generates far more ATP than from glucose. For example, while one mole of glucose can produce 36 – 38 moles ATP, one mole of a triglyceride containing three linoleic fatty acids can produce 130 moles of linoleic acid or 390 ATP. However, beta oxidation is slow and cannot meet the immediate needs of high intensity exercise. As intensity increases the cell must rely more on creatine phosphate and glycolysis while increasing oxygen usage and fatty acid degradation to help meet the energy needs of the cell.

Exercise Metabolism

Higher intensity exercise relies more on glycolysis from carbohydrates and stored glycogen, with lower intensity exercise relying more on beta oxidation from lipids. However, high intensity exercise cannot be maintained indefinitely as glycogen stores are limited when compared to fat stores (McArdle, Katch, & Katch, 2001). Therefore exercise duration is dependent on the exercise intensity and the amount of glycogen

stores or exogenous carbohydrate available. A higher utilization of fat for energy during exercise can improve exercise performance by sparing carbohydrates and delaying fatigue. One benefit of increasing the frequency of exercise is that the body adapts by utilizing more fat during the same absolute level of intensity (Messonnier, Denis, Prieur, & Lacour, 2005). Exercise increases TH and SNS catecholamines, which results in an increase in fat metabolism (McArdle, Katch, & Katch, 2001). TH levels are controlled by the hypothalamus hormone thyroid releasing hormone (TRH). This triggers the pituitary to secrete thyroid stimulating hormone, which triggers the thyroid to release TH. Weiss et al. (2008) reported higher T3 concentrations among those who lost weight with exercise compared with those who only dieted. Ciloglu et al. (2005) reported higher T3, T4, and thyroid stimulating hormone (TSH) as a result of acute exercise. Catecholamines increase in response to exercise intensity and contribute to fat oxidation through lipolysis (Mora-Rodriguez & Coyle, 2000). However, there is no evidence that aerobic exercise training results in an increase in resting levels of catecholamines (Péronnet et al., 1981).

Carbohydrate sparing results in more fat utilized for energy at the same absolute level of intensity of exercise. Talanian, Galloway, Heigenhauser, Bonen, and Spriet (2007) investigated fat oxidation changes during 2 weeks (seven exercise sessions totaling 3 hrs over 2 weeks) of high intensity interval exercise (HIIT) in moderately active women. Participants performed 60 min cycling time trial at 60% initial VO_2 peak at the pre and post intervention. The results showed a significant increase in fat oxidation during the final exercise bout compared to the first, an effect seen in all participants (n =

8). Messonnier, Denis, Prieur, & Lacour (2005) exercised eight sedentary volunteers 2 hrs per session on a cycle ergometer between 60-80% VO_2 peak for 6 days per week for 4 weeks. The authors noted an increase of 8% in VO_2 peak and a significant reduction in RER ($p < .05$) during intensities of > 65% relative VO_2 peak. These studies demonstrate that adherence to an exercise regimen increases fat oxidation at both absolute and relative levels of VO_2 peak.

In addition to exercise, fat oxidation is associated with obesity in a sedentary population. Westerterp, Smeets, Lejeune, Wouters-Adriaens, & Westerterp-Plantenga (2008) measured fat oxidation from a palmitic acid infusion in normal weight, overweight, and obese participants. The authors noted a significant ($p < .001$) negative correlation ($r = -.65$) between fat oxidation and percent fat, indicating that lower fat oxidation is associated with higher body fat. Kim, Hickner, Cortright, Dohm, & Houmard (2000) measured fat oxidation across the vastus lateralis of sedentary lean and obese women. The authors noted palmitate oxidation, palmitoyl carnitine oxidation, and palmitoyl carnitine oxidation were all significantly reduced in the obese group, and all three variables were significantly negatively correlated with obesity ($p < .05$). Cortright et al. (2006) measured fat oxidation changes in sedentary, lean women over a period of 10 days of exercise at 75% VO_2 Peak for 60 mins daily. The authors observed a significant increase ($p < .05$) in fat oxidation by the 10^{th} day as measured by increased palmitate oxidation.

Fat Oxidation in Women

Fat oxidation during exercise correlates with fat loss, but is not universal among all populations. Most studies confirm that women utilize more fat during exercise than men, perhaps due to the fat oxidative properties of estrogen (Tarnopolsky, 2008). Venables, Achten, & Jeukendrup (2005) studied substrate utilization during a graded, maximal exercise test of 300 participants consisting of men and women. The authors discovered the men achieved a maximal fat oxidation point at a significantly less relative VO_2 peak (45%) than the female participants (53% - $p < .01$). Devries, Hamadeh, Graham, and Tarnopolsky (2005) investigated gender differences in substrate utilization and reported that 17-estradiol administration in men significantly increased fat oxidation, likely due to a carbohydrate and amino acid sparing effect. D'Eon et al. (2002) measured substrate oxidation in women using varying levels of estrogen (E) and (P) progesterone during exercise at 60% VO_2 max for 60 minutes. The authors utilized a crossover design consisting of a baseline group of low estrogen and progesterone, a group with supplemented estrogen (high progesterone with low progesterone), and a final group of both supplemented estrogen and progesterone (high estrogen and high progesterone). The authors determined that only the high estrogen with low progesterone group experienced significantly lower carbohydrate (CHO) oxidation and increase fatty acid oxidation, indicating that 1) estrogen spared CHO usage and 2) progesterone works to somewhat oppose estrogen in this role. As estrogen (as estradiol) affects fat oxidation, changes in estrogen during the various stages of the menstrual cycle on fat oxidation have been

investigated but remain equivocal. Zderic, Coggan, and Ruby (2001) measured glucose oxidation following the follicular and luteal phase of the menstrual cycle during a 25 min 70% lactate threshold (LT) cycle ride followed by a 25 min 90% lactate threshold ride. The authors demonstrated no change at 70% LT but a significant decrease in CHO oxidation at 90% LT during the luteal phase. However, the sample size for this study consisted of only 6 women, with 3 women exercising in each phase. Horton, Miller, Glueck, and Tench (2002) measured substrate oxidation during 90 mins of moderate intensity exercise (50% VO_2 max) during three phases of the menstrual cycle (early follicular, mid follicular, and mid luteal). The women (n = 13, crossover) were athletic (exercise > 90 min per week), but not competitive athletes. No significant changes in RER, glucose kinetics, or free fatty acids during rest or exercise were observed. The authors concluded that at moderate intensity exercise the phase of the menstrual cycle had no effect on substrate oxidation. Devries et al. (2005) investigated RER in women during the follicular and luteal phases of the menstrual cycle during 90 mins of 65 VO_2 peak. There was no significant difference in RER between the two groups during the 90 mins of exercise. Devries, Hamadeh, Philips, and Tarnopolsky (2006) investigated RER changes during 65% VO_2 max exercise in women during follicular and luteal phase. No differences in RER were observed during 90 mins of exercise. These studies indicate that at moderate intensity exercise the menstrual phase does not have a significant impact on fat metabolism.

In addition to menstrual cycle phase, oral contraceptives affect the concentration of estrogen and may impact fat oxidation. Current oral contraceptives are available in the form of estrogen plus progesterone or progesterone only. However, several studies have shown no effect of oral contraceptives on fat metabolism during moderate intensity exercise (Jensen & Levine, 1998; Suh, Casazza, & Horning, 2003; Jacobs, Casazza, Suh, & Brooks, 2005). Suh et al. (2003) studied eight healthy women during the follicular and luteal phase of the menstrual cycle, both while taking high dose of triphasic oral contraceptives for 4 months and without. During the study the concentration of estrogen (ethinyl estradiol) was held constant at 0.035 mg / day while progesterone (norgestimate) varied weekly between 0.18 and 0.25 mg / day. Substrate oxidation was measured at rest for 90 mins and during 60 mins of leg cycling at 45% and 60% VO_2 max. No significant differences were observed for any of the variables, indicating that exercise state, menstrual phase, and oral contraceptive usage had no effect on substrate oxidation. Jensen and Levine (1998) also reported no differences in substrate oxidation in oral contraceptive users; however, an infusion of epinephrine was used to simulate lipolysis as compared to exercise itself. Their sample consisted of women currently taking oral contraceptives (OC) and those who were not. The OC sample consumed pills containing estrogen (ethinyl estradiol) at a concentration between 0.030 - 0.035 mg / day while progesterone stayed constant (the concentration varied between subjects but was not reported). Jacobs, Casazza, Suh, and Brooks (2005) measured the effect of OC on FFA oxidation during 60 mins of exercise at both 45 and 60% of VO_2 max. The OC contained

the same concentrations of estrogen and progesterone as Suh et al., 2003. There was no difference in FFA oxidation as a result of OC usage. The authors concluded oral contraceptives had no effect on substrate usage during exercise.

As there are differences in substrate oxidation gender must be considered when determining the optimal exercise intensity to maximize fat oxidation. In addition to gender, the trained state of the population will also affect the intensity range. Stisen (2006) determined the maximal fat oxidation intensity rate of 53% and 56% VO_2 max for untrained and trained women respectively using cycle ergometry, though some women showed maximal oxidation rates as high as 65% VO2 max. Romijn, Coyle, Sidossis, Rosenblatt, and Wolfe (2000) measured substrate oxidation in eight endurance trained women at intensities of 25% (60 mins), 65% (60 mins), and 85% (30 mins) VO_2 max using a cycle ergometer. The authors determined the maximal rate of fat oxidation for the trained women was 65% VO_2 max. These studies demonstrate that maximal fat oxidation rates for trained women are between 55% - 65% of VO_2 max.

Fatty Acid Oxidation and Regulation

Fat is metabolized both at rest and exercise primarily through beta oxidation. Fat in the form of triglycerides are stored in adipose tissue and muscle. Lipase breaks down triglycerides into one glycerol molecule and three fatty acids through lipolysis. The glycerol molecule can be metabolized in glycolysis, while fatty acids are metabolized in the mitochondria. The mitochondria contains both an outer membrane and inner membrane. However; the outer mitochondrial membrane is not permeable to fatty acids

and requires active transport for metabolism. Carnitine is required to transport fatty acids from the cytosol through the outer mitochondrial membrane, and eventually the inner mitochondrial membrane, leading ultimately to the mitochondrial matrix. The fatty acid is first activated with ATP to form a fatty acyl adenylate, which then reacts with coenzyme A through the enzyme fatty acyl-CoA synthetase. The acyl-CoA is attached to carnitine through the enzyme carnitine acyltransferase I, and with the enzyme carnitine-acylcarnitine translocase the acyl-carnitine is transported across the outer mitochondrial membrane. Finally, carnitine acyltransferase II transfers the acyl-carnitine across the inner mitochondrial membrane, liberating the carnitine to return to the cytosol and leaving the acyl-CoA in the mitochondrial matrix to undergo beta oxidation. Each beta oxidation iteration of the acyl-CoA yields one molecule of acetyl-CoA, $FADH_2$, and NADH. Beta oxidation continues until the acyl-CoA has been fully converted to acetyl CoA. For odd numbered acyl-CoAs, the remaining three carbon propionyl CoA is converted to succinyl CoA. Both acetyl CoA and succinyl CoA are metabolized by the Krebs cycle in the mitochondrial matrix. In the Krebs cycle, acetyl CoA is combined with oxaloacetate (OAA) to yield one mole of OAA, 3 moles of NADH, and one mole of GTP. Succinyl CoA is a Krebs cycle intermediate and yields OAA, one mole of NADH, and one mole of GTP. The NADH and $FADH_2$ from beta oxidation and the Krebs cycle are metabolized back to NAD and $FADH_2$ in the electron transport chain across the inner mitochondrial membrane. Both NADH and $FADH_2$ transfer electrons across the complexes of the ETC, resulting in a proton pump (three protons for NADH, two for

$FADH_2$) across the inner mitochondrial membrane into the intermembrane space. The transfer of electrons combined with protons in the matrix result in the conversion of oxygen to water. This reaction creates an electrochemical gradient between the intermembrane space and the matrix, allowing the protons to pass though back to the matrix, but the only path is through the ATP synthase pathway. This transfer liberates energy, and this energy is harnessed to create ATP from ADP. Each proton transfer results in one mole of ATP (McArdle, Katch, & Katch, 2001).

As the end product of beta oxidation is acetyl CoA, fatty acid metabolism is highly dependent on the Krebs cycle and ETC to generate ATP. The outer mitochondrial membrane regulates ion passage and ultimately the rate of beta oxidation through voltage dependent anion channels (VDAC). Mitochondrial function can then be regulated through the gradual opening or closing of the VDACs (Colombini, 2004). VDACs are protein gradients located on the outer mitochondrial membrane, between the cytosol and intermembrane space. The high conducting or open state allows for passage of anions through the membrane. As most metabolites are anions this state allows the highest mitochondrial activity. However, some metabolites, such as short chain fatty acids and acetaldehyde can still cross through the membrane regardless of the VDAC state. As the VDAC begins to close the pore diameter decreases and the channel takes on a positive voltage, resulting in the low conductance state. The change in pore diameter coupled with the voltage change decreases the anion flux across the membrane (including ADP and ATP) while increasing flux of small cations. Ethanol and reactive oxygen species (ROS)

promote the closing of the VDACs (Lemasters, 2007). In the extreme case the VDAC completely closes to the outer membrane while the inner membrane, typically highly selective to metabolites, becomes highly porous. The result is no ADP or ATP exchange, uncoupling of oxidative phosphorylation, and eventual apoptosis (Lemasters, 2007; Rostovtseva, Tan, & Colombini, 2005). Available pharmacological inhibitors of VDAC closure remain undiscovered.

The regulation of the state of the VDAC is not fully understood, but is believed to be a result of the charge of macromolecules and mitochondrial metabolites in the cytosol and intermembrane space (Columbini, 2004). These molecules can exert a charge gradient across the membrane that then regulates the VDAC. For example, the pore is typically in a high conductance state at voltages below 30 mv and begins to close at higher voltages. In the presence of the macromolecule detran, the pore begins to close at only 10 mv (Vyssokikh & Brdiczka, 2003). As well, although NADH does not affect the voltage at which opening and closure occur, it does increase the charge across the gradient, increasing the sensitivity of small voltage changes relative to their effect on the VDAC (Zizi, Forte, Blachly-Dyson, & Colombini, 1994). In addition to its role as an ion selector, VDACs also provide the binding sites for several enzymes specifically hexokinase. Hexokinase phosphorylates glucose to glucose-6-phosphate, the first step in glycolysis in most tissues. Hexokinase binds preferentially to VDAC when it is in the low conductance configuration and is released through excess glucose-6-phosphate, and therefore glycolysis will be promoted when the VDAC is in this state (Vyssokikh &

Brdiczka, 2003). High concentrations of hexokinase resulting in increased aerobic glycolysis with decreased oxidative phosphorylation is common in tumor cells and referred to as the Warburg effect (Lemasters & Holmuhamedov, 2006). As hexokinase binding occurs only when VDAC is in the low conductance state, the closing of the VDAC channel is a contributor to the high glycolysis seen in cancer patients. The cause of the closure of the VDAC is unknown, but one theory is that toxins can disrupt the cell membrane electromagnetic gradient and ultimately the mitochondrial membranes, resulting in more glycolysis (Haltiwanger, 2003; Seeger & Woltz, 1990). The disruption of the electromagnetic gradient at the cell membrane will permit metabolites of various charges to flow across the cell membrane, where the charge around the outer mitochondrial membrane could theoretically affect the permeability of the VDAC. Tumor cells exhibit a highly porous cell membrane and inner mitochondrial membrane, which is consistent with the VDAC closed or low conductance state (Haltiwanger, 2003). This causes more sodium and water to enter the cell and more potassium, magnesium, and calcium to leave the cell (Seeger & Wolz, 1990). The result is less utilization on oxidative phosphorylation and a higher reliance on anaerobic glycolysis for energy, and this effect has been demonstrated not only in tumor cells (Haltiwanger, 2003). Therefore, a possible therapeutic agent would be to correct the cell membrane electromagnetic field to balance the charges around the cell membrane and ultimately mitochondrial membranes to restore energy homeostasis. In the case of cancer, applying a positive current into the tumor and a negative current outside the tumor will force electrons into

the tumor and disrupt the cell membrane potential. Yu-Ling (1997) has reported over 7000 cases of treating malignant tumors with this technique. In addition, since the cell membrane is essentially a capacitor (stores electrical charge), cell membranes exhibiting low conductance can be "charged" through the application of a low power, high frequency electromagnetic field to restore the cell membrane's natural conductance (Haltiwanger, 2003). Although there is currently no research of this technique being used in healthy cells, it is conceivable that healthy cell membranes may be partially damaged due to reactive oxygen species (ROS), toxins, or poor nutrition and that restoring the correct conductance to the cell membrane may improve the electrochemical properties of the cell, allowing the mitochondrion to function optimally. In this state the mitochondrion would maximize oxidative phosphorylation over glycolysis, which should ultimately result in more fat oxidized.

In addition to mitochondria, muscle cells contain VDAC, specifically in the sarcoplasmic reticulum (Shoshan – Barmatz et al., 1996). Shoshnan – Barmatz and Israelson (2005) have summarized the role of VDAC in the sarcoplasmic reticulum (SR) as ATP transport, exchange of metabolites between the cytoplasm and SR lumen, and regulation of uptake and release of Ca^{2+} ions. As such, techniques to alter the cell's electromagnetic field may alter conductance in the membrane and promote opening of the VDAC, contributing to more oxidative phosphorylation and Ca^{2+} release.

Measuring Energy Expenditure and Fat Oxidation

Strategies to reduce focus primarily on reducing caloric intake and increasing caloric expenditure. However, how does the individual know how many calories are consumed and expended, and also how much fat is actually being oxidized? The gold standard is to utilize a metabolic cart to measure both inspired and expired air to determine calories expended, as 1L of utilized oxygen represents an energy expenditure of approximately 4.6 – 5.1 kcal (Lusk, 1928). It has been determined that a subject's age, height, weight, and mode are the strongest predictors of energy expenditure and as such several equations have been devised to predict resting and energy expenditure. The Mifflin-St. Jeor equation is a popular equation for predicting RMR in a healthy population (Mifflin et al., 1990). Ainsworth et al. (2000) collected MET data for a variety of physical activities, which can be used to estimate energy expenditure. For measuring caloric intake, the most accurate procedure would be to provide pre-measured amounts of food and monitor the participants for compliance. As it is not feasible for participants to remain in clinics for long periods of time to monitor intake, a more practical approach is for the participant to track all food and drink consumed, and recorded throughout the day (Thompson & Byers, 1994). There are on-line tools such as mypyramid.gov that can assist with this tracking. While this is approach is practical, it requires significant instruction from the researcher to ensure the participant knows how to accurately track amounts and type of food and drinks consumed. Even with this approach, there is a potential of underreporting calories consumed. Scagliusi, Polacow, Artioli, Benatti, and

Lancha (2003) reported 49% of women underreported their caloric intake during a 7 day intervention using dietary records, reflecting a deficit of 21%. Additional training and coaching reduced the underreporting rate from 49% to 33%. These results are in line with Hoidrup et al. (2002) who reported an underreporting deficit in calories of 20% in 175 men and 173 women. These studies demonstrate that even with proper coaching, dietary records may underreport the total amount of calories consumed.

Measuring fat oxidation can be determined through measurements of inspired and expired air. Carbohydrate oxidation generates an equal amount of carbon dioxide for each oxygen molecule utilized. While fatty acids vary in structure, it is generally accepted that for every 10 oxygen molecules consumed only seven carbon dioxide molecules are expired. Dividing the amount of carbon dioxide expended by the amount of oxygen utilized determines the respiratory exchange ratio (RER) and reflects the percentage of fat oxidized by utilizing the formula (1 - RER) / (1 - 0.7) * 100 (McArdle, Katch, & Katch, 2001). For example, a subject's RER of 0.90, would correspond to 33% fat utilization. It has already been determined that fat oxidation, and therefore RER, is affected by obesity and exercise. A high carbohydrate diet or meal prior to test can also cause a substantially higher RER measurement than normal. Kirwan et al. (2001) compared glucose and fatty acid response to exercise either with or without a 75 g carbohydrate meal 45 mins prior to exercise at 60% of maximal VO_2. The authors observed that RER was significantly elevated following the meal vs. placebo (water) up to 120 mins of exercise. Goedecke et al. (2000) determined that type 1 muscle fiber type ($r = .53$), type 2a muscle fiber type (r

= -.46), and percentage of carbohydrate in the diet (r = .42) correlated the most with resting RER, while at 70% work peak only plasma lactate correlated with RER (r = .63). At an exercise intensity of 70% work peak, the variables lactate, percent fat in the diet, training volume, muscle glycogen content, and blood free fatty acids combined accounted for 56% of the variance seen between participants (note that there was no significant correlation between percent body fat and RER). However, there were significant differences in resting and exercise RERs between subjects, with only 56% of the 70% peak work accounted for it is likely there are other unknown variables affecting exercise RER.

While RER will show the current ratio of fat to CHO oxidation, it does not accurately reflect changes in actual fat stores over time. Body weight changes over time may reflect muscle and fluid changes, therefore not accurately reflecting true fat loss. Skinfold measurements, when performed by a trained technician, represent a convenient and reliable tool for assessing body fat, with correlations of r = .7 to .9 to hydrostatic weighing (American College of Sports Medicine, 2006). The assessment requires a caliper that measures skinfold thickness to the nearest millimeter at various skinfold sites. The sum of the skinfolds along with age are entered into equations derived by Jackson and Pollock (1978) below to determine body density. Body density can then be converted to body composition. Equation 1 below shows a common 3 site formula (triceps, suprailiac, and thigh) to be used for women:

Density = 1.099421 - 0.0009929(sum of 3 sites) + 0.0000023(sum of 3 sites)^2 - 0.0001392(age)

Body composition can be derived from body density by using equation 2:

% Fat = (457 / Body Density) – 414.2

Percent body fat changes over time can be used to show progress in the client and also show how much fat has been lost over the intervention.

Supplements and Fat Loss

Several over the counter agents are purported to increase fat oxidation both at rest and during exercise. Caffeine's effect on fat loss and exercise performance has been studied extensively in the literature. Caffeine is theorized to exert its fat loss effects primarily by inhibiting the effect of phosphodiesterases, which degrade cyclic AMP (cAMP). cAMP is a secondary messenger which is synthesized as a result of catecholamines and low energy levels causing a high concentration of AMP. Since cAMP amplifies the signal of the catecholamines, cAMP potentiates their effect on fat oxidation. This amplification of the effect of catachelomines results in an increase in fatty acid turnover and oxidation, increasing energy expenditure (Acheson et al., 2004). Acheson, Zahorska-Markiewicz, Pittet, Anantharaman, and Jéquier, (1980) measured changes in metabolic rate over 3 hrs of rest following 4 mg / kg caffeine, 8 mg / kg caffeine, or placebo in both normal weight (BMI < 25) and obese (BMI > 30) subjects. Using the normal weight sample, the 8 mg / kg dose significantly ($p < .02$) increased energy expenditure by 16% and significantly ($p < .02$) increased fat oxidation. The 4 mg / kg

dose significantly increased energy expenditure by 12% ($p < .01$) significantly ($p < .02$) increased fat oxidation. In the obese group, 4 mg / kg significantly ($p < .05$) increased energy expenditure by 10%, but there was no significant ($p > .05$) increase in fat oxidation. While the results support that caffeine ingestion can increase fat oxidation in a normal weight population, the authors caution that there was a wide variation in response between subjects, and it may not be as beneficial for the obese.

Caffeine has been shown to consistently increase plasma FFAs during exercise; however, several studies have not observed a change in RER during exercise, indicating that there was no additional overall fat oxidation during exercise (Greer, Friars, & Graham, 2000; Tarnopolsky, Atkinson, Macdougall, Sale, & Sutton, 1989). Graham, Helge, MacLean, Kiens, and Richter (2000) investigated whether caffeine's effect may be specific to the working muscle's metabolism as opposed to whole body metabolism. The study involved 10 men performing cycle ergometry at 70% VO_2max for 60 mins supplemented with 6 mg/kg caffeine or placebo. A catheter was inserted into the femoral artery and muscle biopsies performed to measure muscle metabolites during exercise. Caffeine ingestion did not affect RER, but fatty acid and glucose concentrations at the muscle increased significantly ($p < .05$). No differences were found in glycogen usage, cAMP, or glucose uptake by the muscle. The authors conclude that caffeine does not have a pronounced effect on working muscle and any stimulation of the sympathetic nervous system involves non-exercising tissues. Davis et al. (2003) suggest that caffeine's ergogenic effect during exercise is primarily due to blocking adenosine

receptors. Adenosine inhibits many of the brain's excitability neurotransmitters, and exercise increases adenosine concentrations through ATP degradation. By blocking adenosine receptors the fatigue resulting from exercise can be delayed, resulting in improved performance. The authors investigated whether blocking brain adenosine receptors through intracerebroventricle caffeine injections would affect treadmill run time to exhaustion in rats compared with the adenosine agonist 5-N-ethylcarboxamidoadenosine (NECA). Caffeine significantly increased run time to exhaustion compared to NECA and a placebo injection. NECA significantly ($p < .05$) decreased run time to exhaustion compared to placebo, and this decrease was abated when the NECA injection was combined with caffeine. The authors concluded that the ergogenic effect was due to caffeine blocking adenosine receptors. While caffeine may not directly increase fat oxidation and contribute to weight loss, the delayed onset of fatigue may allow the user to exercise longer which would expend additional energy and fat. Although caffeine does not adversely affect blood pressure or hydration, caffeine's effect on gastrointestinal disturbance, palpitations, headaches, and anxiety may overcome any individual benefit in fat loss during rest or performance increases during exercise (Astrup et al., 1990). In addition, competitive athletes taking caffeine must be aware that the International Olympic Committee bans caffeine consumption exceeding urinary caffeine of 12 ug / mL corresponding to an intake greater than 9 mg / kg (Kovacs, Stegen, & Brouns, 1998). In conclusion, caffeine is a safe supplement that increases fat oxidation at rest and time to exhaustion during exercise.

Ginseng, a common herb, refers to any of the plants of the species Araliaceae. There are several forms of ginseng including Chinese (panax), Japanese, American, and Siberian (ciwujia). Ginseng is purported to increase fat utilization and spare glycogen during exercise; however, the actual mechanism by which ginseng increases fat utilization during exercise is unknown, and exercise studies on ginseng are equivocal. Wu et al. (1996) reported a 43% increase in fat utilization during incremental cycling exercise following 14 days of ciwujia supplementation. The sample consisted of 8 men, who were given a placebo for 3 days, at which time an incremental cycle test was performed. For the next 14 days the subjects consumed 800 mg / day of ciwujia, then repeated the tests. This study has obvious design limitations: it was not double blind, there was no true placebo since the subjects served as their own controls, diet was not controlled, and it is impossible to determine if the ciwujia or learning effect was the cause of the benefit. Liang, Podolka, and Chuang (2005) studied the effects of Panax Ginseng on mean arterial pressure (MAP), VO_2 during submaximal exercise, and exercise time to exhaustion. The sample consisted of 29 untrained men and women who were divided into a placebo and Panax group. Both groups performed an incremental cycling test to exhaustion at the start of the study. Both groups consumed 1350 mg of either a placebo (starch) or Panax ginseng daily for 30 days, then repeated the maximal cycle test. Results showed significantly lower submaximal VO_2 values and increased time to exhaustion compared to placebo, with no change in VO_2 max. The authors concluded that studies showing no benefit may not have had accurate ginseng in the supplement and that future studies

should attempt to confirm. Pieralisi, Ripari, and Vecchiet (1991) demonstrated increases in VO_2 max and workload in treadmill exercise from 6 weeks of ginseng supplementation. The increases were not significant in subjects with a VO_2 max greater than 60 ml / kg / min, and the authors suggested that the ergogenic benefits of ginseng may mimic the benefits of exercise, such that benefits are not seen in a fit population. Engels, Kolokouri, Cieslak, and Wirth (2001) measured VO_2 response after 8 weeks of supplementing with a placebo, low dose (1000 mg), or high dose (2000 mg) of panax ginseng. The sample consisted of 36 healthy men, who performed maximal cycling exercise test at baseline and after 8 weeks of supplementation. There were no significant main or interaction effects on VO_2 max, lactate, fat utilization, or VO_2 response at any point as a result of the intervention. No significant differences were found in the 3 day diet records of the subjects prior to the test. None of the three groups had a mean VO_2 max greater than 43 ml O_2 / kg / min, which is in disagreement with the results of Pieralisi, Ripari, and Vecchiet. However, the Pieralisi study included ingredients other than ginseng which may have affected the response. Cheuvront et al. (1999) measured VO_2 response to cycle ergometry following 7 days of 800 mg / day ciwujia supplementation or placebo. The sample consisted of 10 male subjects, who performed two cycling tests consisting of 30 mins at 25% VO_2 max followed by 10 mins of 65% VO_2 max. Prior to each test the subjects supplemented for 7 days with either the placebo or ciwujia, then following a 1 week washout period repeated the procedure with the other supplement. There were no significant changes in submaximal VO_2, fat utilization, or

lactate when supplementing with the ciwujia. The authors concluded that the supplement was ineffective, but acknowledge there was no way to determine if each supplement contained the expected amount of ciwujia. These studies demonstrate that ginseng does not equivocally increase fat utilization or lower lactate during exercise. There is, therefore, no recommendation for those exercising to consume ginseng in an effort to increase fat oxidation.

Ephedra or ma huang is an herb that contains ephedrine and norephedrine alkoids. Ephedra stimulates the sympathetic nervous system and can induce thermogenesis similar to the catechelamine epinephrine, and as such ephedra has been marketed as both a weight loss and ergogenic aid. Pasquali et al. (1987) investigated ephedrine's effect on weight loss in 10 overweight women. The crossover study combined a low calorie diet with either 50 mg ephedrine three times per day or placebo for 2 months for each treatment. The women lost significantly more weight ($p < .05$) during the ephedrine phase than the placebo phase. The authors concluded that ephedrine was effective at promoting weight loss in overweight women. Ephedrine is commonly combined with caffeine in an effort in increase the overall thermogenetic effect. Boozer et al. (2002) investigated ephedrine (ma huang) and caffeine's (kola nut) combined effect on body weight and body fat over 6 months compared with placebo. Participants consisted of 167 men and women with BMI between 25 and 40 kg / m^2. At the conclusion of the study there was a significant decrease in body weight ($p < .01$) and body fat ($p = .02$) in the ephedrine and caffeine group compared with placebo. Dry mouth, heartburn, and

insomnia were reported in the ephedrine and caffeine group, but the retention rate between groups was not different. The authors concluded that an herbal supplement containing ma huang and kola bean can contribute to body weight and fat loss. Similar to caffeine, ephedrine may improve exercise time to exhaustion, especially when combined with caffeine, but does not seem to affect energy expenditure or fat oxidation (Powers, 2001). Bell, Jacobs, and Zamecnik (1998) investigated time to exhaustion when treated with caffeine, ephedrine, or a combination of ephedrine and caffeine in eight male cyclists. There were no significant changes in VO_2 (energy expenditure) or RER during the time trials for any group, and plasma FFA levels only increased in the caffeine group. However, the ephedrine and caffeine group had a significantly greater time to exhaustion ($p < .05$) compared to the other groups. The authors also investigated the effects of caffeine, ephedrine, or caffeine and ephedrine on 10 km performance in 12 trained men. Ephedrine and the combination of ephedrine and caffeine resulted in significantly lower run times compared to the caffeine and placebo trials. There were no significant changes in VO_2 across any of the trials. While ephedra may increase fat loss during rest and exercise performance, it is not without risks. Haller and Benowitz (2000) reviewed 140 reports of adverse cardiovascular effects as a result of taking ephedra, concluding that ephedra poses a health risk to certain individuals. The death of Baltimore Orioles pitcher Steve Bechler raised awareness of the risks of ephedra, and in 2004 the FDA banned the sale of ephedra based supplements (Rados, 2004). Finally, ephedra is also considered a banned substance by the USOC and NCAA. Although ephedra and ephedra with caffeine

may have some weight loss benefits the risks do not justify their use as a means to decrease body fat.

The rate limiting step in fatty acid oxidation is the entry of the fatty acid into the mitochondria. While TH and catecholamines can increase fat oxidation through lipolysis and thermogenesis, they do not affect the entry of fatty acids into the mitochondria. Supplemental carnitine has been suggested to increase fatty acid oxidation by allowing more fatty acids to enter the mitochondria. The theory is that the amount of carnitine available limits the amount of fatty acids that can be transported through the mitochondrial membrane, and that supplementing with carnitine will allow fatty acids to be oxidized. Villani, Gannon, Self, and Rich (2000) investigated the effects of 4 g carntine supplementation daily over an 8 week period on fat mass and resting lipid oxidation. The authors matched overweight women by BMI to either the carnitine group or placebo (lactose) group. In addition to the supplement, the women walked for 30 mins at an intensity of 60 - 70% maximum heart rate 4 days / week. After the 8 week period there were no significant differences between groups for body mass, fat mass, fat free mass, or lipid oxidation at rest. The authors concluded that supplementation with carnitine provided no fat loss benefit. Broad, Maughan, and Galloway (2008) measured the effects of 2 g / day supplementation of carnitine during exercise. The sample of 20 males were matched to the carnitine or placebo group. Subjects followed a prescribed diet for 2 days at which time they completed a 90 min exercise test at 70% VO_2max. The subjects followed their usual diet and exercise program for 2 weeks while supplementing

with carnitine or placebo. The subjects then repeated the 2 day prescribed diet and exercise test. There was no significant difference in carbohydrate or fat metabolism during exercise within or between the groups. The authors concluded that carnitine supplementation had no effect on fat utilization during exercise. Therefore, supplemental carnitine does not appear to be effective at improving fat utilization.

Lifewave Energy Patches

The Lifewave Energy Enhancer product consists of pairs of patches purported to increase fat oxidation, primarily during physical activity. The transdermal patches do not introduce any chemicals into the body; rather they are designed to work with the body's own electromagnetic field to maximize cellular processes involved in fat oxidation. The pairs of patches are placed on known acupuncture points (wrists, chest, knees, and ankles). The patches are made from sterioisomers (L/D) of amino acids which interact with the body's normal electromagnetic field. The interaction of the body's electromagnetic field with the patches induces several electromagnetic frequencies that are designed to match the resonant frequency of specific biological processes, including Ca^{2+} release and fat oxidation (Haltiwanger, 2005). It is believed that these frequencies maximize the cell's biological processes by increasing electron and ion flow across the cell membrane, resulting in an increase in strength, endurance, and fat oxidation (Haltiwanger, 2005). Regarding fat oxidation, the creators of Lifewave claim that the patches enhance the delivery of long chain fatty acids into the mitochondria and enhance mitochondrial ATP production through matching the resonant frequencies of these

biological processes, though the specific mechanisms are not reported (Haltiwanger, 2003). While the specifics of what frequencies the body is tuned at, and how the Lifewave patches affect this are not disclosed, the likely mechanism would be manipulation of the VDAC channels located on the mitochondria and sarcolplasmic retriculum.

Although the Lifewave website lists numerous studies validating their results, a Pubmed search only listed two studies. Nazeran, Chatlapalli, and Krishnam (2005) measured heart rate variability (HRV) in 10 young, healthy volunteers under the following conditions: rest with no patches, rest with Lifewave placebo patches, rest with Lifewave active patches, 5 mins of exercise with no patches, exercise with Lifewave placebo patches, and exercise with active patches. The participants were told the patches were set 1 and set 2, but it is unknown if the participants or researchers were blinded. HRV refers to the beat-to-beat change in heart rate and is regulated by the sympathetic and parasympathetic systems. Low frequency (LF) fluctuations at 0.05 - 0.15 Hz are indicative of sympathetic control (i.e. exercise, standing up), whereas high frequency (HF) fluctuations at 0.15 - 0.4 Hz are indicative of parasympathetic control (i.e. rest, controlled respiration). The authors presented data on two representative (male and female) participants and reported that wearing the active energy patches resulted in less LF fluctuations and more HF fluctuations in both rest and exercise compared against no patches or the placebo patches. The authors concluded that the active patches reduced sympathetic control and increased parasympathetic control, inducing a more relaxed

state, during both the relaxed and exercise state. Jacobson et al. (2008) investigated the effect of Lifewave patches on strength parameters including: standing vertical jump, dominant hand grip strength, 225 lb bench press repetition maximum, peak torque, torque to body weight ratio, total work, average power, and average peak torque as measured by 50 isokinetic repetitions of knee extensions at 180° / s. The sample of 33 varsity collegiate football players was randomly assigned to the placebo or experimental patch group. Measurements were taken 1 week apart, with the second measurements taken while wearing the patches. ANCOVAs using pretest means as the covariate showed a significant increase in peak torque ($p < .04$) with the experimental patches. There were no significant differences for any of the other dependent variables. The authors concluded that many of the dependent variables approached significance and that it is possible the placement of the patches affects the outcome. Future studies should then attempt to limit the effect of placement.

CHAPTER III

METHODS

The study was approved by the TWU IRB to be compliant with institutional and federal guidelines for research on human participants.

Participants

Eligibility to participate in the study was limited to women aged 18-55 who regularly exercise over 1 hr / wk and were apparently healthy with low exercise risk. As maximal fat oxidation rate is affected by gender, men were excluded. Women who were pregnant, lactating, smokers, and taking drugs or supplements which increased heart rate were not eligible. Participants were recruited via flyers (Appendix B) posted at Texas Woman's University (TWU) and local fitness facilities in the Denton, TX area, via selected nutrition and kinesiology classes at TWU, and posting on the TWU Institute for Women's Health (IWH) website. Participants who called to inquire about their eligibility were screened via a checklist (Appendix C). Final screening of eligible participants were determined via the interview, identification of no contraindications to performing vigorous exercise, and no more than one risk factor for performing vigorous exercise as defined by the AHA / ACSM Health and Fitness Facility Preparticipation Screening Questionnaire (Appendix D). Participants completed the study as recruited. Control and experimental participants were not predetermined prior to participation. In addition to the Informed Consent, a summary of the procedures, expectations, and researcher contact

information was provided to all participants (Appendix E). A neutral party uninvolved in the research assigned a participant ID to each sealed envelope containing the active or placebo patches for each participant. Two sealed copies of the list containing participant ID and whether they are active or placebo were stored in a locked file in the IWH.

Summary of Procedures

Following the completion of the Informed Consent, participants arrived at the Exercise and Sport Nutrition clinic at the mutually agreed time. Anthropometric measurements as well as diet and exercise history for the day were recorded. Participants then began a maximal exercise test to determine the 60 - 70% maximal VO_2 intensity. Following a short rest period, participants completed a 20 min submaximal exercise protocol between 60 and 70% maximal VO_2 intensity. At the completion of the protocol participants were briefed on how to complete the diet and exercise records (Appendix F), patch placement (Appendix G), and given the patches to wear. Both the researchers and participants were blinded as to which patches were placebo and active. The participants arrived at the clinic 2 weeks later (within ± 1 day) and repeated the same procedures while wearing the patches.

Pre-Study Meal

Participants were reminded to consume identical meals prior to both tests approximately 3 hrs prior to the start of the sessions. To assist in standardizing the effect of this pre meal on exercise tests, participants were provided access to a sandwich by a local establishment to be consumed 3 hrs prior to the exercise protocol. Participants were

given a limited choice of options including a vegetarian option. Participants were encouraged to consume a drink with no calories and not consume any sides such as chips.

Pre-Test Measurements

The participant had the option of requesting a female researcher to perform and record all measurements. Participants were asked to void and remove shoes prior to all measurements. Age was recorded as the year reported. Body mass was recorded to the nearest 0.1 kg (Tanita Corp., Arlington Heights, IL) with the participant wearing exercise attire. Participants were reminded to wear similar if not identical exercise attire for both sessions. Height was recorded using a stadiometer to the nearest 0.5 cm, also without wearing shoes (Perspective Enterprises, Kalamazoo, MI). BMI was calculated by dividing body weight in kilograms by height in meters squared. Percent body fat was measured using a skin caliper (Beta Technologies, Santa Cruz, CA). Skinfolds were measured to the nearest 1.0 mm. Measurements were taken at the triceps, suprailiac, and thigh on the right side of the body in triplicate (ACSM, 2006). Site locations were marked to ensure precise measurement at each site. If both measurements were within 1 mm, the mean of the measurements was recorded, otherwise subsequent measurements were taken until two readings were within 1.0 mm difference, and the mean of those two recorded. The sum of skinfolds was used to determine density, and from density, percent body fat determined as per the following equations (Jackson and Pollock, 1978):

Body Density = 1.099421 - 0.0009929(sum of skinfolds) + 0.0000023(sum of skinfolds)^2 - 0.0001392(age)

Percent Body Fat = (457 / Body Density) – 414.2

Exercise Protocol

A Medgraphics CPX/D (Medgraphics, St. Paul, MN) metabolic cart was powered on for at least 1 hr prior to the start of calibration and was calibrated (volume and gas) as per manufacturer's recommendation within 30 mins prior to the start of the maximal exercise test. If multiple tests were performed on the same day, the cart remained powered up, and a subsequent calibration performed within 30 mins prior to each subsequent maximal exercise test. Following anthropometric measurements, participants were instructed how to affix the Polar heart rate (HR) cheststrap and were asked to affix as per manufacturer's guidelines (Polar, Lake Success, NY). Participants were instructed to sit for 5 mins in a comfortable position, at which time resting HR and blood pressure (BP) were recorded. HR was recorded via the Polar HR monitor, and BP recorded using a stethoscope and aneroid sphygmomanometer.

The participant then stood on the treadmill (Cardiac Science, Bothell, WA) and was again familiarized with the procedure. The participant was affixed with a noseclip and mouthpiece to measure VO_2 and VCO_2 (Medgraphics, St. Paul, Minnesota). The treadmill was initially be set at a speed of 1.6 mph and grade of 10%. The Bruce protocol, consisting of 3-min stages of increasing speed and grade, was utilized to determine VO_2 peak (Bruce, Blackman, Jones, and Strait, 1963). Blood pressure was taken at the 2 min

mark of each stage, and HR and RPE (as per a visual aide using the Borg 6-20 scale) were recorded after the BP was recorded, but prior to the completion of the stage (Borg, 1998). The participant was routinely asked if she would like to continue, including at the end of each stage. The participant voluntarily ended the test by grabbing the front handrail, shaking her head side-to-side "No" when asked if she would like to continue, or placing her feet outside the width of the treadmill belt. The test was terminated if the participant voluntarily ended the test, age predicted maximal heart rate was exceeded (due to absence of ECG monitoring), or systolic blood pressure dropped 10 mm / Hg below resting. Following termination of the test the mouthpiece and noseclip were removed, the grade reduced to 0%, and the speed reduced to 3.0 mph. HR and BP were recorded, and the recovery on the treadmill was at least 5 mins and terminated when the participant felt comfortable. Maximal VO_2 recorded was the maximum reported VO_2 during any 30-s period.

The participant then sat in a chair and rested in preparation for the submaximal exercise test. The participant was allowed to consume water ab libitum, but no food or other drink was permitted. The participant rested for at least 15 mins and began the submaximal exercise test when ready. The participant was again fitted with the noseclip and mouthpiece, and the treadmill speed and grade initially set at a speed of 3 mph and grade of 2%. Speed and grade were manipulated every 2 mins until the current VO_2 stabilized between at least 60% and less than 70% of peak VO_2. Once achieved, the speed and grade were fixed, and the participant instructed to continue the exercise for 20 mins.

HR, BP, and RPE were recorded at the start, and every 5 mins thereafter. Following termination of the test, recording on the cart was stopped, the mouthpiece and noseclip were removed, the grade reduced to 0%, and the speed reduced to 3.0 mph to allow the participant to recover. The recovery was at least 5 mins and terminated when the participant felt comfortable. Mean RER, VO_2, and VCO_2 during the test were recorded. Final speed and grade were also recorded to assist in determining speed and grade for the second visit.

Diet and Exercise Records

Participants were instructed to record their daily food and drink intake, physical activity performed, and any menstrual changes. They were also reminded to continue their typical daily diet and activity regimen, and not to make any specific changes as a result of the study. Participants were coached how to record portion sizes and encouraged to list as much detail as possible. Following participant completion, caloric intake was determined via Nutritionist Pro (Axxya, Stafford, TX). From the 14-day diet logs, a sample of 3-days of intake was included for analysis and then averaged to determine daily caloric intake. The 3-day record included at least one week day and one weekend day (i.e. 2 weekdays with 1 weekend day). To determine calories expended, first RMR was estimated using the Mifflin – St. Jeor equation and a factor of 1.2 applied for sedentary (Mifflin et al., 1990). Calories expended for all physical activity listed were calculated via the data from Ainsworth et al. (2000). All exercise recorded in the 14 day logs were used to determine total expenditure. The average of the total physical activity

over the 14-day period was then added to the RMR plus sedentary calculation to estimate daily caloric expenditure. The estimated daily caloric expenditure was subtracted from the estimated daily caloric intake to determine net daily caloric surplus or deficit. The result was used to estimate the amount of body mass gained or lost over the 14-day period to compare with actual body mass changes.

An example of the Diet and Activity Log used to analyze caloric intake and expenditure can be found below in Figure 1.

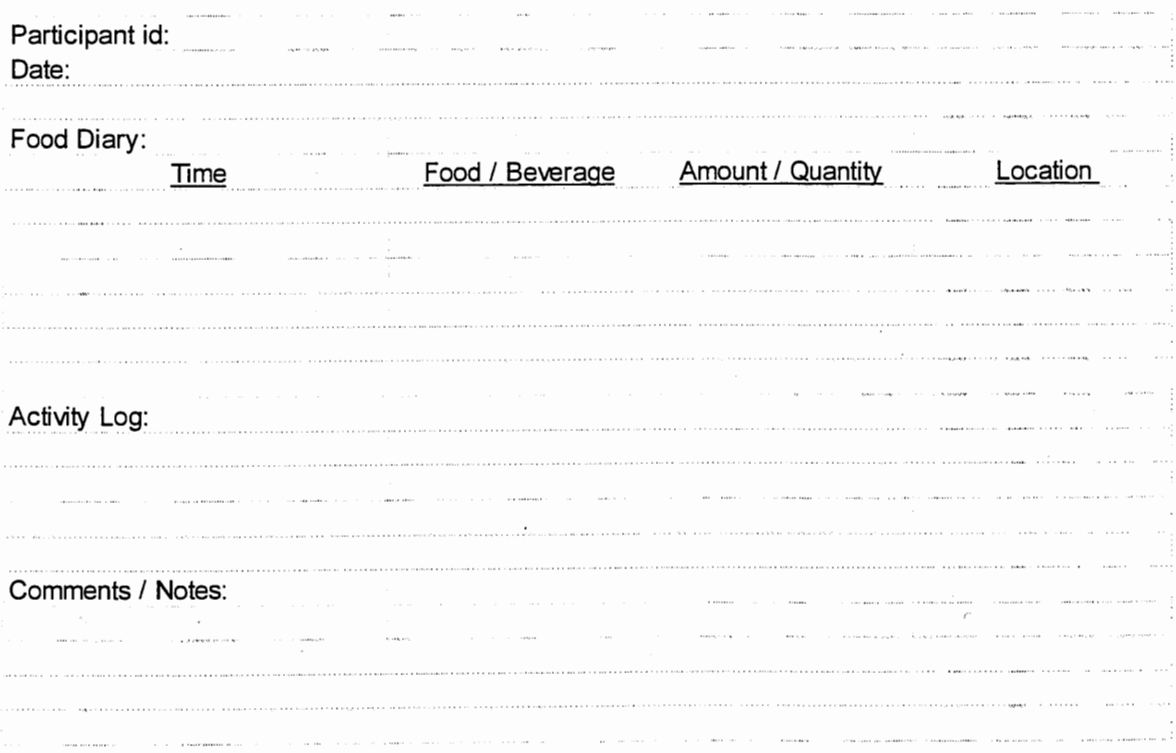

Figure 1. Sample Diet and Activity Log

Patch Placement

Participants were given 14 sets of patches to wear during the 2 week period between visits. The manufacturer states that there are four locations (wrist, chest, ankles, knees) where the patches should be worn. The manufacturer recommends to rotate the placement of the patches, as the maximal effect of placement per participant or activity (i.e. Running vs. cycling) has not been determined. The manufacturer also does not specifically mention how often patches should be worn, recommending either every other day or cycling 4 days on with 3 days off. As such the participants were instructed to wear the patches every other day, rotating the placement among each of the four sites. On the day of the second test, the participants wore the final set of patches on the knees throughout the session.

Statistical Analyses

The variable of interest (power variable) was determined to be RER, as this variable would most accurately determine if there was increased fat oxidation during exercise. Sample size was estimated through a review of the literature and power calculator. As there are no previous studies involving Lifewave, RER changes (i.e. what the expected SD should be) or fat oxidation studies on similar treatments (i.e. magnets, bracelets), analysis included studies showing a significant change in RER both between and within groups of women, during an exercise intervention of 2 to 12 weeks at an intensity between 40-70% VO_2 max, with the assumption that the current study's sample exercise RER would not be significantly different between groups initially ($p > .05$). It is

currently unknown what effect Lifewave patches have on overall fat metabolism via a change in RER, but for the purpose of this study it is hypothesized that wearing the patches would have a similar effect to changes resulting from an exercise intervention (in the current study, participants were instructed not to change their current diet and activity habits). Table 1 is a summary of the included studies that were utilized for sample size estimation. The lowest standard deviation per group that resulted in a detectable difference was 0.01; however, most studies analyzed could detect a difference in means at 0.02, therefore, this SD level was used for the SD portion of the effect. The mean change in RER in the analysis was 0.06, with 0.04 as the smallest detectable mean as well as the mean from a study most closely resembling the current study (Talanian et al., 2007). The calculated effect size (delta mean / SD) from these studies is 2 (0.04 / 0.02), which is a large effect size. The "effect" of most of the studies was exercise's effect on exercise RER over a duration between 2 and 12 weeks. The current study also assumes that there would be no change in exercise RER in the control group after 2 weeks, so any effect seen would be the direct result of the patches. Of the six studies analyzed, there were an average of 10 ($n = 9.4$) subjects per group.

Table 1

Intervention Studies Affecting RER

Sample	Duration / Intervention	Test	SD Pre	SD Post	RER Pre	RER Post	Source
8 trained women	2 weeks, 7 HIIT sessions	cycling 60% VO_2 max	0.02	0.02	0.88	0.84	Talanian, 2007
9 healthy women	12 weeks, 5day/wk @ 75%	cycling 65% VO_2 max	0.01	0.02	0.81	0.8	Friedlander ,1998
8 trained women	one session	cycling 65% VO_2 max	0.01		0.81		Romijn, 2000
8 post-op gastric bypass / 8 wt matched control	one session	cycling 65% VO_2 max	0.01	0.02	0.96	0.89	Guesbeck, 2001
13 obese women	12 weeks, 3day/wk @ 40%	cycling 40% VO_2 max	0.02	0.02	0.85	0.81	Van Aggel-Leijssen, 2001
10 post-men women	12 weeks, 5day/wk @ 65%	cycling 65% VO_2 max	0.02	0.01	0.96	0.91	Zarins, 2008

An effect can be seen (per group) in as small as eight participants. In the one study comparing independent groups, the total sample was 16 participants. Using charts from Cohen (1972) a 2x2 Repeated Measures power calculation with a significance of .05 and strong correlation (.6) yielded only three participants needed for a power of .8. Using an online power calculation with a mean of 0.04, SD of 0.02, and alpha of 0.05 yielded a sample size of 12 participants (Sheonfeld, 2011), which is similar to the average number of participants (16) for the studies analyzed. To account for dropout rate and possible outliers, a sample size of at least 30 participants was targeted.

All data were entered and analyzed with Statistical Package for the Social Sciences (SPSS, Chicago, IL, version 15.0) and presented as means ± SD. Descriptive statistics utilized were age, BMI, calories consumed, calories expended, body mass, percent body fat, maximal VO_2, and RER. A one-way analysis of variance (ANOVA) was used to determine if there were any significant differences in age, height, weight, BMI, calorie intake, calories expended, VO_2 peak during the first session, and submaximal exercise RER during the first sessions between the two groups. A 2x2 Mixed Repeated Measures ANOVA was used to determine group (placebo vs. active) by time (initial vs. 2 weeks later) interactions for body mass, percent body fat, VO_2 peak, and RER. The criterion for significance was set at $p < .05$.

CHAPTER IV

RESULTS

Participant Characteristics

The target sample size for this study was set at 30 women as described in the Methods section. At the conclusion of the study, 53 women had signed Informed Consent forms while 41 actually participated in the study. Nine participants could not complete the study and three were removed due to failing to complete the second VO_2 peak test, resulting in a total sample size of 29 women with 14 women in the placebo group and 15 women in the active group. However, anthropometric data on the three women who did not complete the VO_2 peak test were included in the analysis for body mass and percent body fat changes over the 2 week period. Of the nine women who did not complete the study, four could not attend the second session due to scheduling, three could not attend the second session due to illness or injury, one lost interest in the study and did not wear the patches during the second week, and one failed to show up to the second session, but she did not give a reason as to why. Table 2 describes the initial characteristics of the placebo and active groups. There were no significant differences on any initial variables between groups, as determined by a one-way ANOVA ($p > .05$).

Table 2

Descriptive Characteristics of Participants

	Placebo		Active		
	N	Mean ± SD	N	Mean ± SD	*p*
Age (years)	16	26.9 ± 10.0	16	32.3 ± 13.9	0.21
Height (cm)	16	164.5 ± 6.6	16	161.8 ± 6.2	0.24
Weight (kg)	16	69.0 ± 12.7	16	63.9 ± 8.9	0.19
BMI (kg / m^2)	16	25.6 ± 4.9	16	24.4 ± 3.3	0.44
Initial %body fat	16	23.6 ± 5.5	16	23.1 ± 5.3	0.79
Initial peak VO_2 (ml/kg min)	14	33.5 ± 6.8	15	33.9 ± 8.6	0.91
Initial submax RER	14	0.943 ± 0.05	15	0.923 ± 0.05	0.27

Diet and Activity Logs

Not all women completed the 2 week diet and exercise log for the study. In addition, many of the returned logs had incomplete (i.e. missing meals or days, physical activity not recorded) or unusable data (i.e. poor description of food or portion size, quantity, unreadable entries). A total of 18 logs were used for analysis, equally split between groups. Table 3 summarizes the daily caloric differences between groups. The placebo group had a significantly greater daily caloric intake ($p = .021$) compared to the active group (1925 ± 474 vs. 1467 ± 305 kcal). However, daily caloric expenditure was not significantly different between groups. Subtracting the caloric expenditure from caloric intake per participant yielded the net surplus or deficit of calories. There were no

significant differences between groups when comparing net caloric surplus or deficit. A one-way ANOVA was used to analyze any differences between groups.

Table 3

Summary of Daily Caloric Intake and Activity Logs

Measure	Placebo (N=9)	Active (N=9)	*p*
Intake (kcal / day)	1925 ± 474	1467 ± 305	0.02*
Expenditure (kcal / day)	1968 ± 319	1724 ± 108	0.12
Surplus / Deficit (kcal / day)	-42 ± 469	-257 ± 383	0.14

* $p < .05$

Anthropometric Changes

Body mass and percent body fat changes over the 2 week period are shown in Figures 1 and 2 respectively. There were no significant changes in body mass (69.0 ± 12.7 kg vs. 69.2 ± 13.1 kg for the placebo group and 63.9 ± 8.9 kg vs. 63.9 ± 8.6 kg for the active group) or percent body fat (23.6 ± 5.5% vs. 23.5 ± 4.3% for the placebo group and 23.1 ± 5.3% vs. 23.8 ± 5.1% for the active group), as determined by a 2 x 2 (group x time) mixed repeated measures ANOVA. ($p > .05$).

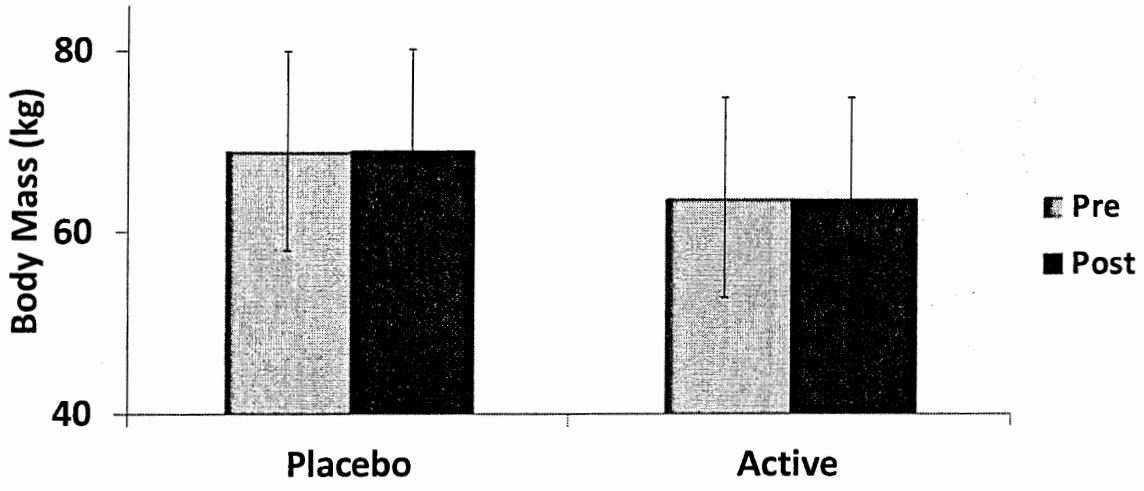

Figure 2. Body mass changes between groups

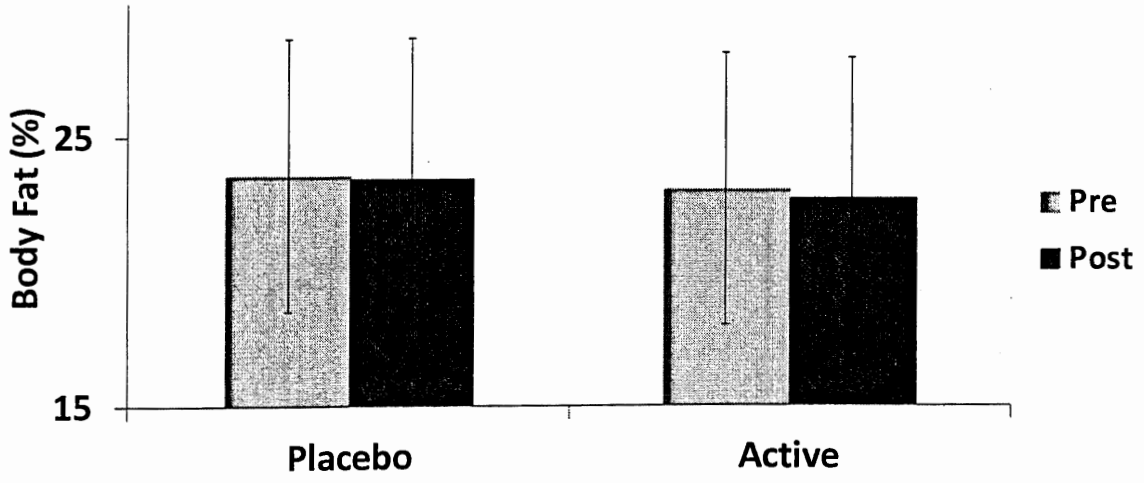

Figure 3. Percent body fat changes between groups

VO_2 Peak

Changes in VO_2 Peak between the first and second testing session are shown in Figure 3. There were no significant changes between sessions (33.5 ± 6.8 ml O_2 / kg / min vs. 33.4 ± 5.3 ml O_2 / kg / min for the placebo group and 33.9 ± 8.6 ml O_2 / kg / min vs. 34.8 ± 8.9 ml O_2 / kg / min for the active group), as determined by a 2 x 2 (group x time) mixed repeated measures ANOVA, ($p > .05$).

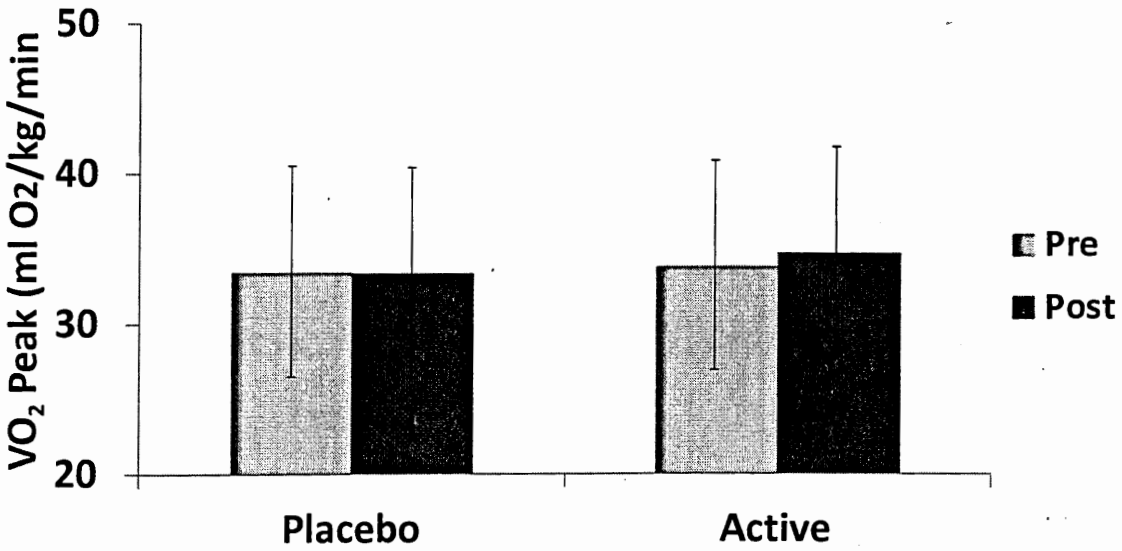

Figure 4. VO_2 peak changes between groups

Respiratory Exchange Ratio

Respiratory exchange ratio (RER) changes between the first and second testing session are shown in Figure 4. There were no significant changes between sessions (0.94 ± 0.05 vs. 0.93 ± 0.04 for the placebo group and 0.92 ± 0.05 vs. 0.91 ± 0.04 for the active

group), as determined by a 2 x 2 (group x time) mixed repeated measures ANOVA, ($p > .05$). Observed power for the interaction effect between groups and time was 0.089.

A summary of all dependent variables can be found in Table 4. There were no significant changes within groups or between groups ($p > .05$).

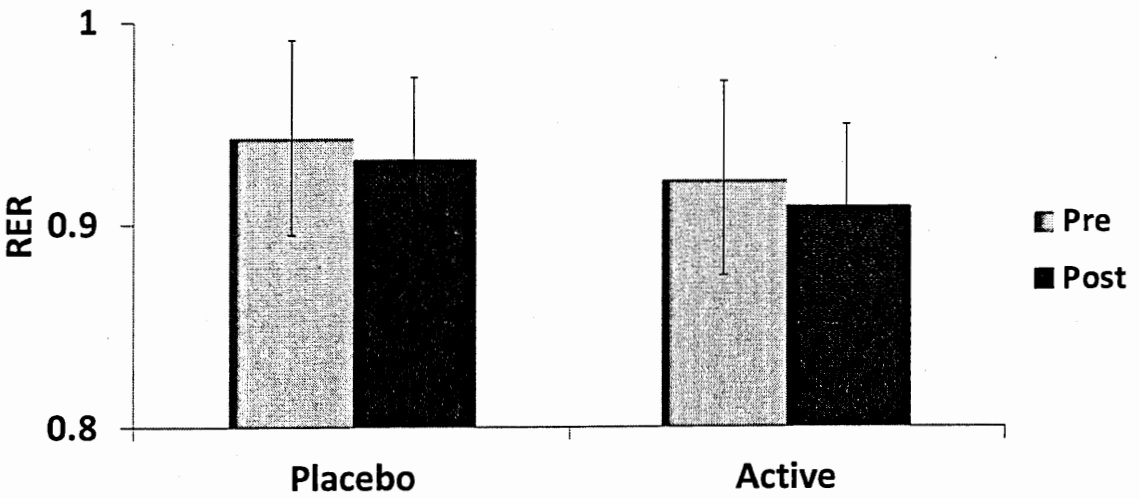

Figure 5. Respiratory exchange ratio changes between groups

Table 4

Summary of all Dependent Variable Changes

	Placebo		Active	
Variable	Pre	Post	Pre	Post
Weight (kg)	69.0 ± 12.7	69.2 ± 13.1	63.9 ± 8.9	63.9 ± 8.6
% Body Fat	23.6 ± 5.5	23.5 ± 4.3	23.1 ± 5.3	22.8 ± 5.1
Peak VO_2 (ml/kg/ min)	33.5 ± 6.8	33.4 ± 5.3	33.9 ± 8.6	34.8 ± 8.9
RER	0.94 ± .05	0.93 ± .04	0.92 ± .05	.091 ± .04

Though not dependent variables, heart rate (HR) and rating of perceived exertion (RPE) were recorded every 5 mins during the submaximal exercise test. There were no significant changes ($p > .05$) in HR or RPE between or within groups during the last 5 mins of the 20 min submaximal test ($p > .05$). There were also no differences between or within groups as a percentage of age predicted maximum heart rate ($p > .05$). In addition, average VO_2 relative to VO_2 peak during the submaximal test was also recorded. Table 5 shows a summary of the RPE, HR, and average VO_2 values.

The raw data for all data analyzed can be found in Appendix H.

Table 5

Summary of HR, RPE, and % VO_2 Peak Changes during the Submaximal Test

	Placebo		Active	
Variable	Pre	Post	Pre	Post
RPE	11.0 ± 1.4	11.5 ± 2.9	10.8 ± 2.4	10.5 ± 2.2
HR (bpm)	162.4 ± 15.6	163.8 ± 17.0	157.8 ± 20.8	157.7 ± 20.7
% Max HR	84.0 ± 5.8	84.7 ± 6.1	83.8 ± 6.5	83.7 ± 5.9
Average				
%VO_2 peak	65.0 ± 0.06	65.2 ± 0.07	65.2 ± 0.03	65.1 ± 0.04

CHAPTER V

DISCUSSION

Anthropometrics

Over the 2 week intervention period there were no significant changes in either body mass or percent body fat whether between or within groups, and as a result both null hypotheses for body mass and percent body fat were accepted. Average BMI for the entire sample was 25.0 ± 1.1 kg / m^2, with three participants classified as obese (BMI > 30.0 kg / m2). Exclusion criterion was determined via the AHA / ACSM Health and Fitness Facility Preparticipation Screening Questionnaire (Appendix D), a subjective measurement. Participants were not directly asked if they were obese, and were not excluded during the initial session if it was determined they were. Removing these three participants from the analysis post-hoc did not result in any change in dependent variables. Using an ANCOVA adjusting for initial body mass and initial percent body fat also did not result in any significant differences. The initial anthropometric measurements per participant did not impact the effect of the Lifewave patches, so they were not accounted for in the Results.

Assessing percent body fat changes using a skinfold caliper has been determined to be a reliable measurement of percent body fat (American College of Sports Medicine, 2006). This requires measurements to the nearest 1 mm per site, and the sum of measurements used to determine body density and eventually percent body fat. All

researchers were trained in taking skinfold measurements, and the same researcher was used for both pre and post-assessments to eliminate inter-researcher variability. The delta between pre and post-percent body fat were -0.13 and -0.36% percent for each group, or an averaged difference of -0.25%. Using the average sample of 23.5% percent body fat and age of 30 years, even a 1.0 cm difference in the sum of skinfolds (within measurement reliability) would result in a change ±0.30%. Therefore, while the change in percent body fat was insignificant between and within groups, the actual change is within the acceptable reliability range of measurement, further indicating no effect. Expected changes in percent body fat based on measured body mass changes would also fall within this expected variance, and there was no significant correlation between percent body fat and body mass changes ($p > .05$).

It is possible that the 14 day intervention was not long enough to measure significant anthropometric changes. This period of time is half the time that the manufacturer packages the product (sold as a 30 day supply). It was also unknown what the effect size of wearing the patches would be, as there are no previous studies on this product. It was assumed the patches would have an effect similar to a diet or exercise intervention, which have resulted in significant body mass changes within 2 weeks (Zachwieja et al., 2001). It is possible that wearing the patches for a longer period would have increased any potential effect of the patches. However, the intervention required significant interaction from the participants in the form of daily records and remembering to apply the patches every other day. The dropout rate for the current 2 week intervention

was 22% (40% if including those who signed consents but never started). Most of the 12 women who did not begin the study, but signed Informed Consent, did not specify a reason, but those who did respond mentioned they reconsidered due to the length of time of the intervention and the work involved (i.e. daily reporting). Of the nine participants who could not complete both sessions, seven participants did not complete both sessions due to illness or scheduling issues. A significant number of the participants were university students whose schedules and commitments may be unpredictable and vary week to week. It is possible that semester changes such as employment, exams, and semester breaks may have introduced additional variance to caloric intake and expenditure, accurate recording of logs, or contribute to higher dropout. In addition, participants may not have noticed significant changes after 2 weeks and determined it was not worth continuing the study, increasing the dropout rate.

Recording caloric intake and expenditure would help substantiate the true effect of the Lifewave patch intervention on body mass and percent body fat. Participants were instructed to record their daily intake, expenditure, and menstrual status while not making any significant lifestyle changes (i.e. diet and physical activity) during the 2 week study intervention. Caloric intake was assessed via a consecutive 3 day food record. Expenditure was assessed by estimating RMR, applying a factor 1.2 for sedentary, and averaging all physical activity recorded over the 14 day period. Compliance with recording menstrual status was low (only four recorded), so it was not included in any analysis. Participants were given example detailed diet and exercise records, and

instructed how to properly record. However, six participants never submitted records, and of the remaining 26 participants, their records were difficult to accurately assess due to illegible entries, poor descriptions of what food or quantity was consumed, poor description of activity performed, or missing days. As a result, not all diet and activity logs could be accurately assessed. Inclusion criteria for assessment included significant daily food detail including item, quantity, and description, full 14 days of recording, detail of what physical activity was performed, and consistency in recording, resulting in a total of 18 records for analysis, representing nine from each group. Intake was measured using a continuous 3 day record including at least one week day and one weekend day. All physical activity recorded in the 14 days of records was analyzed and averaged over 14 days to determine average daily physical activity. Both intake and expenditure were analyzed as daily caloric amounts, and the difference between these amounts analyzed and reported as caloric surplus or deficit. Extrapolating these results over 14 days predicted a body mass change of -0.07 kg for the placebo group and 0.45 kg for the active group for these 18 participants. However, actual body mass changes over the 14 days for these 18 participants were -0.05 kg for the placebo group and 0.44 kg for the active group. In addition, there was no correlation between the predicted body mass changes from the diet and exercise records and actual body mass changes for these 18 participants ($p > .05$).

The placebo group had a significantly higher estimated caloric intake (458 ± 164 kcal / day) between groups ($p = .021$). However, there was no significant difference

between estimated caloric expenditure between groups. In addition, there was no significant difference between the difference of caloric expenditure and intake. Part of this difference may be accounted for in the differences between groups. RMR estimations from the Mifflin – St. Jeor equation indicated a difference approaching significance ($p = .053$) of 233 ± 103 kcal between groups. In addition, variances in reporting intake can be between 20-33%, even with coaching, and it is impossible to account for this variance with the data provided (Hoidrup et al., 2002; Scagliusi et al., 2003). It is also possible some participants adjusted their lifestyle as a result of the intervention (i.e. one participant confessed she did not consume a candy bar as she did not want to write it down). Finally, as this was a subjective method, it cannot be ruled out that there was bias in the assessment of intake and expenditure. Future studies including daily recording may want to include a period of one-on-one coaching during the intervention to reduce inaccuracies during reporting.

VO_2 Peak

There were no significant differences between or within groups for VO_2 Peak, and as a result the null hypothesis for VO_2 peak is accepted. Average VO_2 Peak in the second session for both groups was 34.1 ± 7.3 ml O_2 / kg / min, which is between the 30th and 40th percentile of VO_2 Max for women aged 20-29 years. This is lower than the expected 50th percentile (37.8 ml O_2 / kg / min). Since the VO_2 peak was used, it is likely many of the participants did not achieve true VO_2 max. As per the Informed Consent, the maximal test concluded if the age predicted maximal heart rate was exceeded, which occurred in

five of the participants. Although exceeding maximal heart rate is not an absolute criterion for maximal test termination, this was done to ensure additional safety of the participants since electrocardiograms were not performed. Also, while the VO_2 peak was recorded, the HR corresponding to the VO_2 peak was not. Finally, study criteria included participants who were moderately active, but this status was assessed using a subjective measure by the participant (i.e. asked what type of physical activity they performed). However, five of the participants had VO_2 Peaks that were less than the 20^{th} percentile, indicating a sedentary lifestyle or perhaps inability to complete the test to exhaustion. Of those five, two were obese, two were overweight, and one was normal weight as determined by BMI. Excluding these participants from VO_2 analysis did not affect the results. In addition, running an ANCOVA with percent body fat as a covariate did not affect VO_2 peak results.

It is possible that a learning effect may have been seen in some of the participants who had never performed the test previously. Katch, Sady, and Freedson (1982) determined that VO_2 max between sessions may vary 5.6% (68% of the time), and as much as 11.2% (98% of the time). As an additional analysis, eight participants had a final VO_2 Peak that was at least 5.6% less than initial VO_2 Peak, and four participants had a final VO_2 Peak that was at least 11.2% less than initial VO_2 Peak, possibly indicating a maximal effort was not achieved on the second test. In comparison, seven participants had a final VO_2 Peak that was at least 5.6% more than initial VO_2 Peak, and three participants had a final VO_2 Peak that was at least 11.2% more than initial VO_2 Peak,

possibly indicating the a true maximal effort was not achieved on the first session, and the second session was perhaps higher due to a learning effect. While these results suggest maximal effort may not have been attained, it is also not possible to eliminate any equipment malfunction as the cause for the differences. Future studies may want to consider an additional maximal session as the learning session to better exclude this effect from results. As well, final RPE and maximal HR should be recorded in addition to the VO_2 peak.

Respiratory Exchange Ratio

As Lifewave energy patches are purported to increase fat oxidation during exercise, it was expected they would show the pronounced effect through RER during the submaximal exercise test. As such, RER was the variable used for a-priori power analysis. A review of previous studies indicated a large effect size through an RER difference of 0.04 with SD of 0.02. In the current study, the RER difference of the active sample was 0.02 with an averaged standard deviation (between both active sessions) of 0.04. The effect size from partial eta squared was 0.01, while manually calculating effect size from eta squared (by utilizing the sum of squares) also resulted in an effect size of 0.01. The observed power was .89. As there were no significant changes between groups for RER, the null hypothesis is accepted. In addition, while the low power indicates a probability of type 2 error, the low effect size indicates there was likely no treatment effect.

It was assumed that the Lifewave patches would have a large effect on fat oxidation similar to exercise intervention studies (i.e. 13% representing a mean RER change of .04). Wu et al. (1996) reported a decrease of 0.08 (0.96 to 0.88) while supplementing with ginseng, another supplement promoted to increase fat oxidation. The results of the current study determined that the largest, potential mean change is less than 0.01 or 3% change. It is possible that patch placement among the four locations may have affected the change in RER. Unfortunately, the manufacturer makes no recommendations on which populations would benefit per location. As the intervention involved running, it was felt this would be the best location out of the four for the RER analysis during the second session. Analyzing the RER changes, only four participants in the active group experienced an RER decrease of the expected mean 0.04 or more; however, three participants in the placebo group also experienced decreases of 0.04 or more. From the data collected, there is nothing recorded to indicate why these participants would see an effect while others did not. Acute RER values are sensitive to recently consumed carbohydrates (Kirwan et al., 2001). In an effort to minimize the impact of this variable, participants were supplied a sandwich at a local sandwich shop to be consumed approximately 3 hrs prior to the exercise intervention. Including the anthropometric measurement, VO_2 peak test, time between exercise tests, and the time to achieve steady state RER, it is hypothesized that by the time submaximal RER was recorded the variance of the carbohydrate oxidization of the previous meal would be minimized. Not all participants consumed the sandwich as the meal prior to the first session. These

participants were reminded to consume the same meal at the same time prior to the exercise session. While no participant consumed food within 2 hrs prior and compliance to consume prior to the first and second sessions was high, it is not possible to completely rule out the effect of the previous meal on the current test's RER value. Future studies may want to consider a more rigid standardized meal at an exact time prior or perform the tests fasted.

The current study attempted to measure RER at an intensity just above maximal fat oxidation intensity, determined between 60-70% VO_2 peak. The value obtained during the current session's VO_2 peak was used to determine the intensity for the subsequent submaximal test. This means that changes in VO_2 peak between sessions could have an impact on the current session's submaximal intensity (i.e. a higher VO_2 peak on the second session would mean a higher intensity for the submaximal exercise test when compared to the first session). This ensures the participant is working at the prescribed intensity for that day. No correlation was seen between VO_2 peak changes and RER changes between sessions, indicating this was not a contributor. In addition, HR and RPE values for the final 5 mins of the submaximal test were recorded and no significant differences were found within groups. The RPE mean of about 11 indicates the participants felt the submaximal was a fairly light intensity at approximately 50% of maximal intensity. However, when adjusting for percent of maximum heart rate (220-age), both groups were almost identical at 84% of maximal heart rate. Differences in percent of maximal heart rate did not correlate with RER changes ($p > .05$). In this study,

a range of 60-70% maximal VO_2 peak corresponded to an almost 20% difference with percent maximal heart rate, which is higher than expected (Swain, Abernathy, Smith, Lee, & Bunn, 1994). It may indicate a sympathetic response (epinephrine) due to perceived stress of the test, or that this sample can achieve higher maximal heart rates than predicted; however, neither max heart nor epinephrine concentrations were measured during this study.

Standard deviation within groups was expected to be 0.02; however, in the current study it ranged between 0.04 and 0.05. The studies that were analyzed for power analysis all involved female participants exercising at intensities between 40-65% VO_2 max (most were between 60 and 65%). Participant characteristics ranged between obese, healthy, and fit, with standard deviation unaffected by each. No specific dietary interventions were listed. The primary difference between studies is that all used cycling whereas the current study utilized running. None of the studies mentioned that participants were trained in cycling, so there is no reason to suspect the mode of exercise would affect RER. Goedecke et al. (2000) reported that at 70% peak power only lactate correlated with RER, and in addition muscle type distribution and glycogen status accounted for most of the individual differences between participants, with training status and fat intake having a small negative correlation, although these only represented 56% of the variability. At this intensity, RER variability was between 0.88 and 1.04. The authors used both men and women, and used cycling as the mode of exercise. They too found no correlation between body mass and exercise RER. The current study did not measure lactate, muscle

distribution, or glycogen status. Training status was also not measured; however, measured VO_2 peak would indicate overall fitness. RER analysis with final VO_2 peak as the covariate in the ANCOVA did not result in any significant differences, and there was no significant correlation between final VO_2 peak and final submaximal RER ($p > .05$ for both). Future studies measuring RER changes should attempt to analyze how to minimize RER standard deviations and account for them in the research.

The mean RER of the current study was between 0.91 to 0.94 at an intensity of between 60-70% VO_2 Peak. However, in the analysis of previous studies on fit women the mean RER was between 0.81 to 0.88 for an intensity of 60-65% VO_2 Max, indicating the RER of the current study is higher than expected. The current study utilized the Medgraphics CPX/D, which uses a breath-by-breath analysis with both gas and volume calibration prior to each session. Recent studies have brought into question the validity of the CPX/D system. Gore et al. (2003) noted that VO_2 and VCO_2 were significantly lower than equivalent Douglas Bag assessments for submaximal exercise. Further investigation showed that many of the CPX/D systems have C:\breeze3\cfg\cal.cfg files that are incorrectly configured, resulting in an underestimate of VO_2 up to 15% and increased RER (Gore, 2005). While the exact configuration cannot be currently validated, current RER values were higher compared to equivalent NewLeaf measurements (also a Medgraphics cart) even with replacements of the O_2 sensor, drying cartridge, gas module, and flow module.

Conclusion

The current study did not show any effect from wearing the Lifewave patches for 2 weeks on body mass, percent body fat, VO_2 peak, or RER. In addition, the effect of the patches was small at 0.01. As a result, all four null hypotheses were accepted, though the low power indicates the possibility of type 2 error. Future studies on this product should focus on determining optimal placement, minimizing standard deviation between participants, and the actual mechanism of action to maximize the results of the intervention. In addition, studies utilizing the Medgraphics CPX/D should ensure the configuration files are correct, as the calibration procedure will not detect errors in the file.

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APPENDIX A

IRB APPROVAL LETTER

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
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e-mail: IRB@twu.edu

March 28, 2006

Mr. John Witt
Department of Nutrition and Food Sciences
Old Main Building 311A

Dear Mr. Witt:

Re: Effect of Lifewave™ Patches on Fat Metabolism in Recreationally Trained Women

The above referenced study has been reviewed by the TWU Institutional Review Board (IRB) and appears to meet our requirements for the protection of individuals' rights.

If applicable, agency approval letters must be submitted to the IRB upon receipt PRIOR to any data collection at that agency. A copy of the approved consent form with the IRB approval stamp and a copy of the annual/final report are enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. The signed consent forms and final report must be filed with the Institutional Review Board at the completion of the study.

This approval is valid one year from March 3, 2006. According to regulations from the Department of Health and Human Services, another review by the IRB is required if your project changes in any way, and the IRB must be notified immediately regarding any adverse events. If you have any questions, feel free to call the TWU Institutional Review Board.

Sincerely,

David L. Nichols

Dr. David Nichols, Chair
Institutional Review Board - Denton

enc.

cc. Dr. Carolyn Bednar, Department of Nutrition & Food Sciences
✓Dr. Nancy DiMarco, Department of Nutrition & Food Sciences
Graduate School

APPENDIX B

ANNOUNCEMENT FOR THE STUDY FLYER

<u>Female Volunteers Needed to Participate in Exercise Study</u>

The purpose of this study is to determine whether wearing transdermal patches worn over a two week period causes the body to burn more fat. Participants will be required to keep accurate food/exercise logs during the study and participate in two separate maximal / sub-maximal exercise tests (beginning and end of study). The total expected time commitment for this study is approximately 13 hours (1-2 hours for the consent and explanation of procedures, 30 minutes per day or 7 hours total to record the food/activity log, and 3-4 hours to complete both testing periods).

<u>Requirements for Participation</u>:

- Females aged 18-55
- Regular exercise over 1hr / wk
- Apparently healthy – low exercise risk
- Not pregnant, non-lactating, non-smoker
- Not taking drugs / supplements which increase heart rate

Volunteers completing the study will not receive compensation but will have their cardiovascular fitness (maximal VO2) and body composition (percent body fat) determined. They will also receive two free sandwich meals. Potential benefits include increased fat loss and increased exercise performance.

<u>Interested Persons Please Contact</u>:
John Witt - TWU Nutrition and Food Sciences
(940) 898-2785
<u>jwitt72@mail.twu.edu</u>
<u>http://www.twu.edu/womenshealth/index.htm</u>

This research study has been approved by the Institutional Review Board, under Federal regulations, at Texas Woman's Universi

Patch / exercise study John Witt Jwitt72@mail.twu.edu (940) 898.2785	Patch / exercise study John Witt Jwitt72@mail.twu.edu (940) 898.2785	Patch / exercise study John Witt Jwitt72@mail.twu.edu (940) 898.2785	Patch / exercise study John Witt Jwitt72@mail.twu.edu (940) 898.2785	Patch / exercise study John Witt Jwitt72@mail.twu.edu (940) 898.2785	Patch / exercise study John Witt Jwitt72@mail.twu.edu (940) 898.2785	Patch / exercise study John Witt Jwitt72@mail.twu.edu (940) 898.2785	Patch / exercise study John Witt Jwitt72@mail.twu.edu (940) 898.2785

APPENDIX C

ANNOUNCEMENT FOR THE STUDY CHECKLIST

Lifewave Interview Checklist

Only candidates who meet the following criteria will be permitted to participate in the study:

- **Females between 18-55 years old**
- **Non-smoking, not pregnant, and non-lactating**
- **Free from disease or other health risks including heart disease, diabetes, high blood pressure, high cholesterol, or more than 20lbs overweight**
- **Not taking drugs or supplements which increase heart rate**
- **Perform regular exercise, outside of normal day to day activities such as walking to class, for at least 1 hour per week**
- **Are willing to record and submit diet, menstrual, and exercise information to the investigators**
- **Are willing to allow the investigators to measure your height, weight, and body fat %**
- **Are willing to wear patches in sometimes visible locations (ie. wrists, feet) throughout the day**
- **Are willing to complete a medical history questionnaire**

"Based on the above conditions for participation, would you like to continue and schedule a meeting to complete the consent procedure and the medical history questionnaire in order to participate in the study?"

APPENDIX D

SCREENING QUESTIONNAIRE

AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire

Assess your health needs by marking all *true* statements.

History

You have had:

___ A heart attack
___ Heart surgery
___ Cardiac catheterization
___ Coronary angioplasty (PTCA)
___ Pacemaker/implantable cardiac defibrillator/rhythm disturbance
___ Heart valve disease
___ Heart failure
___ Heart transplantation
___ Congenital heart disease

Symptoms

___ You experience chest discomfort with exertion.
___ You experience unreasonable breathlessness.
___ You experience dizziness, fainting, blackouts.
___ You take heart medications.

*If you marked any of the statements in this section, consult your physician or other appropriate healthcare provider before engaging in exercise. You may need to use a facility with a **medically qualified staff**.*

Other health issues

___ You have diabetes
___ You have or asthma other lung disease.
___ You have burning or cramping in your lower legs when walking short distances.
___ You have musculoskeletal problems that limit your physical activity.
___ You have concerns about the safety of exercise.
___ You take prescription medication(s).
___ You are pregnant.

Cardiovascular risk factors

___ You are a man older than 45 years.
___ You are a woman older than 55 years, you have had a hysterectomy, or you are postmenopausal.
___ You smoke, or quite within the previous 6 mo.
___ Your BP is greater than 140/90.
___ You don't know your BP.
___ You take BP medication.
___ Your blood cholesterol level is >200 mg/dL.
___ You don't know your cholesterol level.
___ You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).
___ You are physically inactive (i.e., you get less than 30 min. of physical activity on at least 3 days per week).
___ You are more than 20 pounds overweight.

*If you marked two or more of the statements in this section, you should consult your physician or other appropriate healthcare provider before engaging in exercise. You might benefit by using a facility with a **professionally qualified exercise staff** to guide your exercise program.*

___ None of the above is true.

You should be able to exercise safely without consulting your physician or other healthcare provider in a self-guided program or almost any facility that meets your

APPENDIX E

SUMMARY OF PROCEDURES

Participant Notes for Lifewave Study

Before Test Sessions:

- Eat and exercise as you normally would.
- Write down your daily food intake and exercise using the forms given to you. Record any menstrual changes in the comments section.
- Drink plenty of water.
- Try to get as much sleep as possible the day before the test session.
- Avoid strenuous exercise the day before the session.
- Don't forget to pick-up your sandwich the day before your test session. You should consume the sandwich as your last meal before the session.
    - If your session is first thing in the morning, then you should eat the sandwich as your last meal the night before the session.
    - If your session is during the day, then you should eat the sandwich approximately 3 hours before the test session.
- If you have an emergency situation and cannot attend the test session, please notify the researchers immediately (214.364.0153).

Exercise Sessions:

- Avoid caffeine or alcohol the day of your session.
- Avoid strenuous exercise the day of your session.
- Drink plenty of water the day of the session.
- Avoid food 3 hours prior to the start of your exercise session.
- Please report to the test session approximately 20 minutes prior to your scheduled time. The test session will be held in the Institute for Women's Health located in the ground floor (bottom floor) of the Human Development Building, room 011A.
- Notify the researchers if you are ill or have any complications (ie. muscle pain) that may affect your exercise.
- Wear comfortable clothes and shoes.
- Make sure your shoelaces are tied tightly or double-tie them. Remove any jewelry that my affect your exercise performance.
- The researchers will review your food/exercise log and measure your height, weight, and percent body fat. Please let the research team know if you prefer a female researcher for this. The exercise lab has two rooms, one of which can be used privately for taking these measurements.
- You will have a Polar heart rate monitor affixed, which consists of a chest strap. The researcher will hold the watch.
- Inform the researchers when your last meal was.

- Your blood pressure will be taken while you are in a seated position prior to the exercise session. You will also begin breathing into the mouthpiece to obtain baseline measurements.
- Once baseline measurements are obtained you will begin walking on the treadmill at a slow (1.5 mph) pace to familiarize yourself with the treadmill. The researcher will point out the various handrails to use if you should lose your balance.
- There will be two researchers present during the session – one next to you who will adjust the treadmill and take your blood pressure and another behind you who will monitor the metabolic cart and assist you if you should lose your balance on the treadmill.
- The exercise session will consist of two parts – a maximal exercise session and a sub-maximal exercise session. For the maximal exercise session you will exercise on the treadmill until you can no longer exercise anymore or if the researchers determine you are at your maximum. The sub-maximal will be at 65% of your maximum for a time of 30 minutes.
- For the maximal session, the researcher will increase the speed and grade (incline) every 3 minutes. Just prior to increasing, the researcher will take your blood pressure on your left arm during exercise. This will require you to wear the blood pressure cuff during the session and will also limit your arm movement while your blood pressure is being taken. During this session the researcher will ask you periodically "Are you doing OK? Do you want to continue?", at which time you should shake your head no (left and right) if you want to stop. At any time during the session you feel nauseous, dizzy / lightheaded, extreme pain, or you need to stop then alert the researcher by raising your right hand so the researcher will end the protocol. The researcher will slowly decrease the speed and grade to allow you to cool down gradually.
- After the maximal session you will continue to walk at a slow speed for approximately 5 minutes. The researcher will ask you if you need to sit down or if you need a drink.
- The researcher will inform you when you are can begin the sub-maximal test. You may being the session at this time or wait a few more minutes until you are comfortable. The researcher will adjust the treadmill speed / grade until you are at 65% of your maximum. Once this level is reached you will exercise at this pace for 30 minutes. The researcher will adjust the treadmill every 3 minutes to ensure you remain at your 65% maximum. At any time during the session you feel nauseous, dizzy / lightheaded, extreme pain, or you need to stop then alert the researcher by raising your right hand, at which time the researcher will end the protocol. You will continue to walk at a slow speed for approximately 5 minutes, at which time the exercise session is complete.
- At the conclusion of the first exercise session, you will be given a package containing 1) patches for the 2 weeks, 2) picture showing the correct locations of

the patches, 3) schedule of when to apply the patches and to which location, and 4) additional food/exercise logs.

Wearing Patches between Sessions

- Eat and exercise as you normally would.
- Patches are not to be worn on consecutive days.
- Ensure you follow the proper schedule / locations for the patches.
- Wear the patches for 10-14 hours each day.
- Do not re-use the patches after the full day. However, if the patches slip off during the day or if you need to take them off momentarily then they can be re-affixed to the same location. Document in your food/exercise log any time when you have to remove the patches for an extended period of time during the day.
- Drink plenty of water.
- You may experience mild-to-severe fatigue, headaches, sleepiness, joint pain, aches, foggy thinking, poor concentration, and mild blood pressure and pulse fluctuations as toxins leave the body. If you experience any of the above symptoms while wearing the patches, you should discontinue using the patches for 4 hours or until symptoms subside. If the symptoms return, you should select another location for patch application and document the occurrence in the food / activity log.
- Do not remove the patches while exercising, as this is when they provide the most benefit.
- Do not place the patches near any electronic devices such as radios, microwaves, or TVs. Avoid exposing the patches to extreme heat or water.
- Contact the research team at (214.364.0153) if you experience any discomfort not mentioned above or if you need additional patches.

APPENDIX F

DIET AND EXERCISE LOG

Participant id:			
Date:			
Food Diary:			
Time	Food / Beverage	Amount / Quantity	Location
Activity Log:			
Comments / Notes:			

APPENDIX G

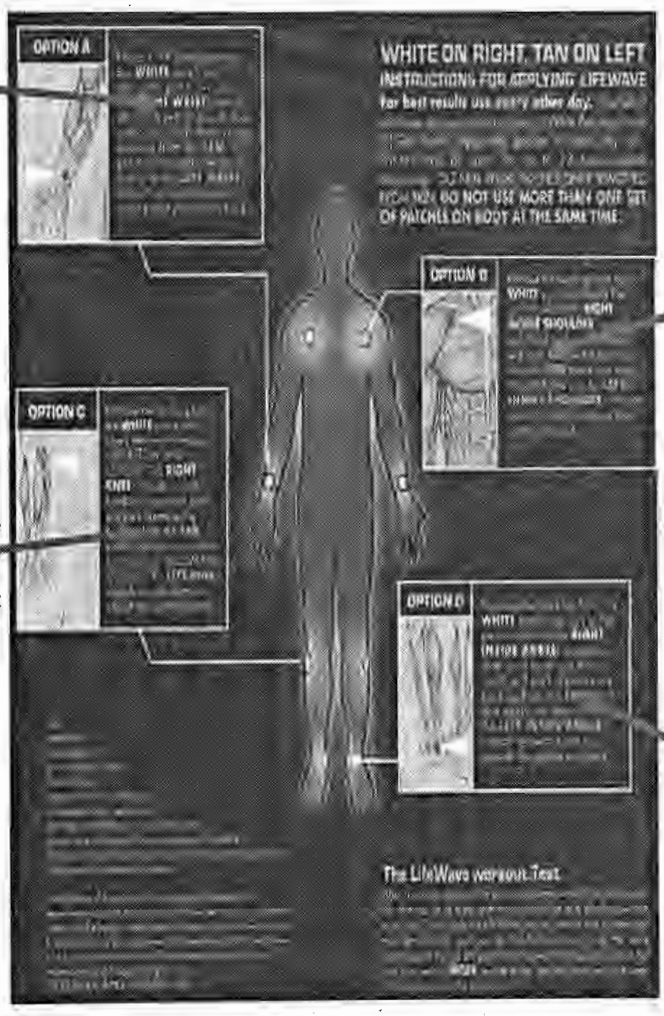
PATCH PLACEMENT

White on Right, Green on left. Instructions for applying Lifewave Energy Patches

Day 1 is the day after the 1st test. Test 2 day is 14 – last 2 days wear consecutive

Days 1,9
Thoroughly clean the area of application.Remove the backing from the **white** patch and apply it firmly to the **right wrist**; smooth on with hand to assure that the patch will stick. Remove the backing from the **green** patch and apply firmly to the **left wrist**; smooth on with hand to assure that the patch will stick.

Days 7, Test 2:
Thoroughly clean the area of application. Remove the backing from the **white** patch and apply it firmly to **just below and outside the right knee**; smooth on with hand to assure that the patch will stick. Remove the backing from the **green** patch and apply firmly to **just below and outside the left knee**; smooth on with hand to assure that the patch will stick.



Days 3,11
Thoroughly clean the area of application. Remove the backing from the **white** patch and apply it firmly to the **right inside shoulder**; smooth on with hand to assure that the patch will stick. Remove the backing from the **green** patch and apply firmly to the **left inside shoulder**; smooth on with hand to assure that the patch will stick.

Days 5, 13:
Thoughly clean the area of application. Remove the backing from the **white** patch and apply it firmly to the **right inside ankle**; smooth on with hand to assure that the patch will stick. Remove the backing from the **green** patch and apply firmly to the **left inside ankle**; smooth on with hand to assure that the patch

APPENDIX H

RAW DATA

Data for all Dependent Variables

			1st session				2nd session			
ID	Age	Ht (cm)	Wt (kg)	%BF	Peak VO_2 (ml O_2 / min / kg)	RER	Wt (kg)	%BF	Peak VO_2 (ml O_2 / min / kg)	RER
Placebo										
1	20	164	84.6	18.5	33.7	0.96	84.7	20.2	32.5	0.96
2	23	160	60.5	20.7	37.9	1.02	60.4	21.4	38	0.99
9	25	170	73.6	22.5	33.6	0.93	73.2	21.9	42.1	0.88
13	18	162	98.8	34	24.6	0.88	100.6	33.6	29.5	0.93
15	50	155	66.6	27	28.3	0.89	66.7	26	29.1	0.87
18	32	167	65.4	19.9	47.3	0.94	65.7	21.1	44.6	0.91
21	28	163	74.6	35.1	26.5	0.97	74.4	30.6	27.3	0.92
25	26	160	62.5	23.9	40.7	0.84	62.7	23.1	36.3	0.9
29	33	160	53.6	17.6	31.1	0.94	54.3	19.8	33.8	0.96
30	18	155	61.2	24.2			59.9	24.2		
33	49	172	71.9	25	39.3	0.99	72	24	33.9	0.91
35	19	170	58.7	26.5	22.7	1	58.4	24.8	30	0.93
36	20	176	76.4	20.4	31.8	0.96	76.1	20.8	35.3	0.96
37	18	175	53.4	14.6	33.1	0.93	54	15.7	31.4	0.9
38	23	160	57.4	22.2	38.9	0.92	56.9	22.2	40.7	0.96
39	28	167	84.8	26			87.5	26.6		
Active										
3	24	157	53.6	13.5	33.3	0.91	55.2	14	35.6	0.92
5	40	164	63.7	18.1	26	0.98	64.2	20.4	25.4	0.94
8	54	158	71.2	33.8	17.3	0.89	69.4	32.3	20.9	0.85
10	49	156	56	22.7	34.5	0.86	55.8	23.2	33.5	0.88
14	18	158	66.2	20.5			66.3	21		
16	55	157	82.2	33	22.1	0.92	82.6	32.8	20.8	0.9
20	23	164	71	24	41.2	0.87	71.6	26.4	39	0.84
23	23	153	57.6	21	45	0.96	58.1	19.2	50	0.95
24	23	170	56.5	20.6	48.1	0.83	56.5	20.9	44.2	0.9
19	20	165	59.4	21.2	43.1	1	59.9	17.4	45.1	0.91
26	43	170	65.7	25.3	34	0.95	64.4	24.3	34.6	0.92
27	22	176	82.8	27.7	34.8	0.91	81.5	25.7	38.1	0.89
31	53	165	59.6	25.2	29.5	0.96	59.1	25	27.8	0.97
32	23	160	57.7	17.1	40.3	0.97	57.5	16.9	43.9	0.88
41	26	158	59.6	22.4	50.2	0.9	59.6	20.6	48	0.9
42	21	161	59.7	24	32.7	0.94	60	24.2	34.3	0.92

Data for Submaximal HR, RPE, and Average VO_2

	1st Session				2nd Session			
ID	RPE	HR (bpm)	% Max	Avg VO_2 (ml O_2 / min / kg)	RPE	HR (bpm)	% Max	Avg VO_2 (ml O_2 / min / kg)
Placebo								
1	9	173	86.5%	65.0%	7	166	83.0%	63.1%
2	14	164	83.2%	63.7%	18	177	89.8%	61.8%
9	12	163	83.6%	67.6%	13	158	81.0%	64.6%
13	10	165	81.7%	61.3%	13	172	85.1%	69.5%
15	11	132	77.6%	61.2%	11	130	76.5%	59.5%
18	11	166	88.3%	64.9%	12	163	86.7%	62.8%
21	10	170	88.5%	64.0%	7	167	87.0%	64.8%
25	12	160	82.5%	66.6%	11	145	74.7%	59.2%
33	10	138	80.7%	60.7%	10	142	83.0%	82.3%
35	11	152	75.6%	58.8%	13	174	86.6%	59.3%
36	12	180	90.0%	81.8%	12	180	90.0%	74.2%
37	9	158	78.2%	64.1%	9	162	80.2%	60.5%
38	12	190	96.4%	64.7%	13	193	98.0%	65.4%
Active								
3	12	166	84.7%	62.8%	12	154	78.6%	65.4%
5	12	147	81.7%	64.6%	11	144	80.0%	64.6%
8	6	120	72.3%	66.5%	6	124	74.7%	62.7%
10	9	144	84.2%	64.9%	8	129	75.4%	62.0%
14	12	168	83.2%	75.1%	9	173	85.6%	81.5%
16	10	127	77.0%	68.2%	9	124	75.2%	71.6%
20	11	180	91.4%	64.1%	12	172	87.3%	67.9%
23	12	172	87.3%	67.6%	12	180	91.4%	62.0%
24	12	160	81.2%	62.0%	12	176	89.3%	61.4%
19	12	190	95.0%	69.4%	12	181	90.5%	69.4%
26	7	137	77.4%	61.5%	7	143	80.8%	63.5%
27	9	149	75.3%	65.2%	10	169	85.4%	63.5%
31	13	141	84.4%	67.1%	12	144	86.2%	71.6%
32	8	169	85.8%	62.3%	10	164	83.2%	61.3%
41	14	167	86.1%	62.4%	13	161	83.0%	64.6%
42	14	188	94.5%	70.0%	13	185	93.0%	65.6%

Data for Diet and Activity Logs

ID	Intake (kcal)	Expenditure (kcal)
Placebo		
1	1511	2160
2	1777	1790
9	1360	2072
13	2065	2252
15	2000	1550
36	2494	2350
37	2284	1720
38	1285	1556
39	2553	2160
Active		
3	1625	1596
5	1368	1705
8	1144	1814
10	1735	1558
14	1573	1711
16	1120	1908
31	1212	1800
41	1387	1690
26	2043	1737