ABSTRACT

Effects of Capsaicin and Evodiamine Ingestion on Energy Expenditure and Lipid Oxidation at Rest and After Moderately-Intense Exercise in Men

Christine Alemán

Director: Darryn Willoughby, PhD

Obesity has become a global epidemic within the last 25 years, with about 65% of the adult population in America being classified as either overweight or obese. In response to this epidemic, countless dietary supplements have emerged in recent years as an easy option to assist in the weight loss process. Capsaicin and evodiamine are two thermogenic agents each recognized for their ability to stimulate the sympathetic nervous system and are thus found in many dietary supplements. The exact effects of each agent, however, remain uncertain. In this randomized, cross-over experiment, seven healthy men were given either capsaicin, evodiamine, or a placebo supplement to ingest. Hemodynamics (heart rate, blood pressure, and core body temperature), energy expenditure, and markers of lipid oxidation (serum glycerol and free fatty acids) were measured at rest, after supplementation, after a single bout of moderately-intense exercise, and during recovery. Analyses of variance (ANOVA) were performed for each variable in order to determine whether between-group measurement differences were statistically significant, thus establishing measurable benefits, if any, that these agents offer to the body's metabolic system.

APPROVED BY DIRECTOR OF HONORS THESIS:

_____

Dr. Darryn Willoughby, Department of Health, Human Performance, and Recreation

APPROVED BY THE HONORS PROGRAM:

_____

Dr. Andrew Wisely, Director

DATE: _____

EFFECTS OF CAPSAICIN AND EVODIAMINE INGESTION ON ENERGY EXPENDITURE AND LIPID OXIDATION AT REST AND AFTER MODERATELY-INTENSE EXERCISE IN MEN

A Thesis Submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the

Honors Program

By

Christine Alemán

Waco, Texas

May 2012

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

First and foremost, I would like to extend my utmost gratitude to Dr. Darryn Willoughby, my thesis mentor, who made all this possible by accepting me as his student. Dr. Willoughby is perhaps the busiest man I know and, among being a father, professor, and Director of the Exercise and Biochemical Nutrition Laboratory, still offered me his time and patience throughout the conduction of this experiment. Without his dedication to his students, I certainly would not have learned this extraordinary breadth of knowledge that is otherwise unattainable through textbook lessons and classroom lectures. For this I will always be grateful.

I would also like to thank Neil Schwarz, my graduate student mentor, for his patience walking with me throughout this experiment as well. There were many times Neil sacrificed his own time to help me with my thesis amidst his own student life. His time and dedication for my study did not go unappreciated; I am very thankful for his service and provision throughout this entire study.

Many thanks are also owed to my dear friend, Constance Shing, who often saw my stressful, stubborn side, yet offered nothing but encouragements at all the right moments. I would also like to thank God for blessing me with this amazing opportunity to work in the HHPR lab and thus being able to truly marvel at His extraordinary creations in full light.

CHAPTER ONE

Introduction

Within the last 25 years, the dramatic increase of obesity has led to its recognition as a global epidemic. Unfortunately, America is one of the most obese countries, with about one third of all adults classified as clinically obese (Ogden & Carroll, 2010). The burden of obesity in America can clearly be seen through the rising cases of type 2 diabetes, stroke, hypertension, and various cardiovascular diseases. Thus, due to the health risks that obesity poses, individuals are ultimately at risk of premature death or severe chronic conditions that can limit overall quality of life.

The government is doing its best to educate individuals of the potentially fatal consequences of obesity. The United States Department of Health and Human Services (2008) published their first Physical Activities Guidelines for Americans. These guidelines provide professional recommendations for physical activity for persons between ages 6 and 65. The American College of Sports Medicine and American Heart Association openly supported these guidelines by recommending that Americans exercise 30 minutes a day for 5 days a week. Unfortunately, only about 30% of adults are doing this recommended amount of exercise (Ogden & Carroll, 2010).

In an effort to address the epidemic, countless exercise and weight loss programs have surfaced in recent decades; however, many Americans find it hard to fit 30 minutes of exercise into their busy lifestyle or are too tired to exercise after work. Whatever the reason, it can be difficult for any individual to begin exercising after a long period of

stagnant living. Thus, there have been numerous fad diets that have emerged in recent years, although the results seen are not always consistent, nor stable. Diet pills, on the other hand, are fairly recent fads that are growing in popularity in the U.S. The majority of diet pills are said to contain special agents that possess thermogenic capabilities which work to speed up the body's metabolism. Capsaicin and evodiamine are both agents that can be found in countless over-the-counter diet pills due to their recognized capabilities of increasing metabolic activity.

Capsaicin, which is the active, pungent component of chili peppers, is a vanilloid receptor agonist and works to speed up the body's metabolism by provoking the activity of the sympathetic nervous system. Due to its pungency, capsaicin has been found to stimulate nociceptors within the body, which ultimately leads to an increase in blood pressure, body temperature, and heart rate shortly after ingestion (Iida et al., 2003). Additionally, ingestion of capsaicin has also been shown to stimulate sustained fat oxidation for adults after weight loss when compared to a placebo group (Lejeune, Kovacs & Westerp-Plantenga, 2003). In rats, it has been observed that capsaicin increases swimming endurance through the increase of fatty acid utilization due to the adrenaline induced secretion caused by capsaicin (Kim et al., 1997).

The cause for the increase in metabolic rate that capsaicin evidently brings about is due to its activation of the vanilloid receptor, transient receptor potential channel vanilloid subfamily member 1 (TRPV1). The TRPV1 is a receptor located on primary sensory neurons, and is activated by various ligands as well as natural irritants. The sensitivity of TRPV1 to natural irritants, like capsaicin, leads to burning sensations to whatever it comes into contact with (Vennekens, Grzegorz & Bernd, 2008). Hence,

capsaicin is solely responsible for the spiciness that comes with eating chili peppers or even rubbing them on your skin. This characteristic of capsaicin can also explain the results obtained by Iida et al. (2003), who observed and concluded that, when ingested, capsaicin incites internal nociceptors which eventually increase metabolic rate. Ultimately, the enhancement of energy metabolism due to capsaicin's stimulation of the sympathetic nervous system leads to the increase of thermogenesis in adipose tissue. For these reasons, the anti-obese effects of capsaicin have been utilized by many weight loss advocates. The only limitation of capsaicin as a means to fight obesity is its peppery hot taste, which can ultimately cause microscopic blisters on skin and internal tissues when overexposed (Nolano et al., 1999). Because of this, there have been multiple studies to identify other vanilloids capable of activating TRPV1. It has been found that the agent, evodiamine, is an effective vanilloid receptor agonist comparable to capsaicin (Pearce et. al, 2004).

Evodiamine, regarded as a "hot nature" herb in Chinese and Japanese medicine, is extracted from the fruit of *Evodia rutaecarpa*. For centuries, fruits of *Evodia* were prescribed for treating headaches, digestive problems, and stomach pains. In recent studies, however, extracts of *Evodia* have been shown to have anti-obesity effects much like capsaicin. In rats, it was found that evodiamine induced heat loss and heat production while also stimulating the utilization of stored food energy at a rate like that of capsaicin (Kobayashi et al., 2000). Additionally, it has been observed that, if ingested daily at 3 grams for 8 weeks consecutively, evodiamine significantly reduces body mass index in premenopausal women (Kim et al., 2008). Although some research has been conducted in order to identify the effects of evodiamine, nothing has yet been empirically established.

In fact, compared to the numerous experiments conducted to analyze the effects of capsaicin, research regarding further investigation of evodiamine is rather limited. Thus, based on the increase in popularity that diet pills have experienced within the U.S., a study comparing the effects of capsaicin and evodiamine would be prolific in determining the exact measurable benefits these agents offer to the body's metabolic system.

Purpose of the Study

The purpose of this study was to observe the effects that capsaicin and evodiamine have on hemodynamics (heart rate and blood pressure), core body temperature, energy expenditure, and markers of lipid oxidation (serum glycerol and free fatty acids) while at rest and after a single bout of moderate intensity exercise.

General Study Overview

This study was conducted as a randomized, double-blind, cross over research experiment. Participants ingested one supplement containing either cellulose placebo, evodiamine, or capsaicin during each session. There were three sessions for each participant, each session 7-10 days apart, so that participants ingested each supplement once. The independent variables were the placebo, evodiamine, and capsaicin supplements. The dependent variables included: heart rate, blood pressure, core body temperature, energy expenditure, and serum levels of glucose, glycerol, triglycerides, and free fatty acids. All dependent variables were closely monitored before, during, and after a single bout of moderate intensity exercise.

Hypotheses

H_1: There would be an increase in heart rate, blood pressure, and core body temperature when participants ingest either the evodiamine or capsaicin supplement compared to placebo; however, evodiamine or capsaicin would not be different from the other.

H_2: There would be an increase in the rate of energy expenditure when participants ingest either the evodiamine or capsaicin supplement compared to placebo; however, evodiamine or capsaicin would not be different from the other.

H_3: There would be an increase in serum glycerol and free fatty acids within the blood after the ingestion of either the evodiamine or capsaicin supplement compared to placebo; however, evodiamine or capsaicin would not be different from the other.

Delimitations

1) Approximately 15 healthy and regularly active (not exercise-trained) men between the ages of 18-30 participated in this study.
2) All participants were required to come to 3 separate testing sessions, each session being 7-10 days apart.
3) Participants were instructed to refrain from exercise for 48 hours, fast for 8 hours, and record their dietary intake 4 days prior to their testing session for each of the three sessions.
4) Participants were randomly assigned a placebo, evodiamine, or capsaicin supplement to ingest 30 minutes after rest upon the start of each session.
5) Participants had their heart rate, blood pressure, and core body temperature assessed 4 times throughout the session.
6) Blood samples were collected from the antecubital vein.

7) All participants underwent a moderate intensity exercise protocol unique to their measured aerobic capabilities.
8) All testing was performed at Baylor University in the Exercise and Sport Nutrition Laband adhered to all policies and procedures designed for the laboratory.

Limitations

1) The number of participants that completed the study was dependent on their ability and desire to abide by the research protocol.
2) The dependence of equipment systems used to obtain quantifiable measurements of the tested variables.
3) The timing of sessions could affect the performance of participants if they were not accustomed to waking up early or exercising in the morning.

Assumptions

1) Participants had not exercise-trained on a regular, consistent schedule for at least one year prior to the conduction of this study.
2) Participants fasted 8 hours prior to each testing session.
3) Participants did not exercise at all 48 hours prior to each testing session.
4) Participants accurately recorded their dietary intake for 3 days prior to each testing session.
5) Participants performed to their best abilities during each exercise test.
6) All equipment used during the conduction of this experiment was reliable and consistent in the results they produced.

7) The overall protocol for this experiment was a reliable means for determining accurate measurements and results of the principle variables.

CHAPTER TWO

Review of Literature

Obesity has become a global epidemic within the last 25 years, with about 65% of the adult population in America being overweight or obese (National Center for Health Statistics, 2000). Although a balanced diet and exercise is vital for any healthy lifestyle, a recent fad that is growing in popularity is using dietary supplements as an extra and easy means to lose weight; however, history has shown that adequate empirical information is essential in order to establish whether such agents are safe and effective for human use.

Dietary Supplements

Because of the recent increase in obesity, dietary supplements are fairly new to the marketplace and are increasing in popularity within the United States. In order to protect consumers from the rising popularity of dietary supplements, Congress passed the Dietary Supplement Health and Education Act in 1994. In this Act, Congress officially defined a dietary supplement as a product containing vitamins, minerals, herbs, or an amino acid among other ingredients. Furthermore, a product is a dietary supplement if it is intended to enhance a diet and is to be ingested through capsule, powder, or liquid form. In order to ensure the safety of newer dietary supplements, the Act also requires that manufacturers provide Food and Drug Administration (FDA) with information on new ingredients if they are to be used in supplements. FDA will approve the ingredient only after reviewing the history of its usage and proof of its safety. Although this policy

requires proof of an ingredients efficiency and safety, it does not necessarily demand that this evidence be obtained through human studies. For this reason, as well as expense and convenience purposes, many newer ingredients are tested on lab animals and rarely on humans. Thus, the question that many ask is whether these diet pills are actually safe and effective for human use. Nevertheless, more and more people are purchasing diet pills as an extra means to enhance their diet, increase their energy expenditure, and ultimately lose weight. For the safety of the population that use dietary supplements, more research should be conducted, preferably on humans, in order to ensure quality and effectiveness of these dietary ingredients.

The unique characteristic that almost all dietary supplements possess is that they contain thermogenic agents that work to increase the body's metabolism. Through the process of thermogenesis, the body's core temperature increases and, thus, the metabolism is stimulated. The body then utilizes triglycerides within adipose tissues in order to provide for the additional energy – a process known as lipolysis. Thermogenic agents included in many diet pills that induce this process include ephedra, Citrus aurantium (bitter orange), caffeine, green tea (EGCG), geranium (DMHA), and ginseng.

Some of these thermogenic ingredients in dietary supplements have been found to be effective, yet unsafe for human consumption. Ephedra, for example, is a gymnosperm shrub and has been used in Chinese medicine for thousands of years. Ephedra acts like a stimulant in that it causes peripheral vasoconstriction which results in higher blood pressure and a faster heart rate. Ephedra is also shown to expand the bronchial tubes, which made it a popular treatment for asthma (Lee, 2011). Additionally, research shows that ephedra has thermogenic properties when used in combination with caffeine. In a 6

month, randomized, double blind, and placebo-controlled experiment, it was found that ephedra and caffeine results in a decrease of body weight and body fat, as well as improved blood lipids in overweight men and women (Boozer et al., 2002). Additionally, ephedra, by itself, was shown to have beneficial effects of short term weight loss (.9 kg per month more than placebo), but no evidence was acquired about long term benefits (Shekelle et al., 2003). Unfortunately, ephedra was banned by the FDA in 2004 due to adverse side effects that were reported in the U.S. Unfortunately, many deaths were reported due to using ephedra and autonomic side effects were also seen, such as irregular heart rhythms and increased blood pressure (Shekelle, 2003). Many researchers were disappointed about the banning of ephedra, stating that the herb had been used for thousands of years in Chinese culture and is most effective when used properly and not abused (Gordon, 2004). Today, few diet pills contain ephedra, and if manufacturers choose to use this ingredient, a warning must be clearly visible on the bottle for consumers.

Soon after the temporary banning of ephedra in 2004, Citrus aurantium, also known as bitter orange, appeared in the market. Bitter orange has for centuries been used in Chinese culture for several applications such as indigestion, diarrhea, insomnia, and anxiety (Stohs, Preuss & Shara, 2011). Like ephedra, bitter orange has chemical structures similar to epinephrine which causes it to act on adrenergic receptors, thus resulting in an increase in hemodynamics. In fact, in a randomized, double-blind, placebo-controlled, crossover study, it was found that both systolic and diastolic blood pressure, as well as heart rates of young healthy adults had significantly increased after a single dose of bitter orange (900 mg extract standardized to 6% synephrine) (Bui,

Nguyen & Ambrose, 2006). Another study was conducted to observe whether bitter orange extract and caffeine showed beneficial effects with improving body composition in overweight healthy adults. Based on the results to this study, it was found that bitter orange is safe and effective when promoting fat loss in overweight healthy individuals when combined with diet and exercise (Colker et al., 1999). Thus, bitter orange has been viewed as a safe alternative supplement that has similar effects to ephedra.

Caffeine is another common thermogenic agent used in many diet pills. Much like ephedra, caffeine is also considered a stimulant drug since it stimulates the central nervous system and, thus, promotes alertness and energy expenditure. Green tea extracts, on the other hand, are also stimulants found in dietary supplements that contain catechin-caffeine mixtures and work to activate the sympathoadrenal system. In other words, green tea stimulates the sympathetic nervous system via the adrenal glands. It was found in a meta-analysis that catechin-caffeine mixtures and caffeine-only supplements both increase energy expenditure; however, catechin-caffeine mixtures also stimulate fat oxidation as opposed to caffeine-only supplements which do not yield statistically significant results on fat oxidation (Hursel et al, 2011). Furthermore, green tea is also beneficial in that it contains anti-oxidants that work to modify the body's metabolism while detoxifying harmful chemicals, such as free radicals. The risk of cancers decreases when anti-oxidants neutralize free radicals within the cells in the body. The positive effects of anti-oxidants, however, are more constructive to participants subject to oxidative challenge (Ellinger et al, 2011).

Geranamine, also known as dimethylamylamine (DMAA), is another CNS stimulant, yet it is observed to have less of an effect than ephedra and other stimulants.

Like previous ingredients, geranamine has a chemical structure very similar to that of amphetamines and is commonly used as a nasal decongestant due to its effectiveness as a vasoconstrictor. Studies show that geranamine increases blood pressure and rate pressure product in a dose-dependent manner, yet heart rate is seemingly unaffected. Similar affects were also observed when it was taken in combination with caffeine (Bloomer et al., 2011). Ginseng is another ingredient that activates thermogenesis. It is one of the most popular herbal medicines used in Chinese culture and contains ginsenosides, which are active stimulants with antioxidant and immunostimulant properties. Ginsenosides are considered the major component within ginseng that increases metabolic activity. Research showed that an extract of wild ginseng prevented weight gain significantly in obese mice, while also decreasing blood glucose, triglycerides, and free fatty acid levels (Yin, Zhang & Ye, 2008). Nevertheless, most of the clinical trials that have been structured to learn more about the effects of ginseng have been of poor quality, to say the least. Research on ginseng and its effects after human consumption are still lacking. Thus, a broader understanding of the effects of ginseng is still needed in order to confidently state the exact implications of this drug on the human body (Xiang et al., 2008).

While there are presently a great variety of dietary supplements available over the counter, it is important that consumers realize the importance of knowing exactly what they are buying and the risks that come with each supplement they ingest. Individuals with diabetes, for example, should never consume supplements containing caffeine or any kind of stimulant. Doing so could exacerbate their diabetes by potentially worsening the body's insulin sensitivity. Thus, it is recommended that before using any dietary

supplement, an individual seek approval first from their physician. Many diet pills in the market are unsafe, such as past supplements containing ephedra. Because of an increasing amount of people presently demanding dietary supplements, it is only necessary to find ingredients that are both safe and effective for weight loss. For this reason, many researchers have turned to natural ingredients, such as herbs, as an option for finding an ideal thermogenic agent safe for human consumption.

Mechanisms of Lipolysis

Many dietary supplements claim to burn fat through activating the natural process of lipolysis. Adipose tissue is the largest tissue mass in humans and is also considered the largest energy depot. The energy within adipose tissue is stored as triglycerides, which are fat cells that the human body is capable of breaking down and using as energy. During high intensity exercise (>70% VO_2 max), the body for the most part utilizes carbohydrates for energy. On the other hand, fats are the primary fuel during low intensity exercise (<30% VO_2 max). Additionally, when the body needs extra fuel, for example, during prolonged aerobic exercise or periods of starvation, the body will work to breakdown lipids within the adipose tissue in a process known as lipolysis (Powers & Howley, 2011).

The process of lipolysis occurs when various hormones are released within the bloodstream and stimulate the fat cells within adipose tissue. Testosterone, glucagon, growth hormone, ACTH, and catelcholamines, such as epinephrine and norepinephrine, are all hormones that work to stimulate lipolysis. Catelcholamines are the most imperative regulators of lipolysis during prolonged physical activity. As one continues to

exercise, the sympathetic nervous system is stimulated and, thus, epinephrine and norepinephrine levels continue to increase within the blood stream. Research shows that after about 10 minutes of continued, low-intensity exercise, carbohydrate stores slowly deplete and the body calls on stored fat in order to provide for additional fuel in order to prolong exercise. It is during this time that the increased levels of catelcholamines within the blood stream come into play (Powers & Howley, 2011).

Epinephrine is capable of binding to adrenergic receptors on the plasma membrane of target cells. During low-intensity exercise, in order to incite lipolysis, the accumulation of epinephrine in the blood stream binds to β-adrenergic receptors, which are integral membrane proteins spanning the lipid bilayer of plasma membranes. The binding of epinephrine to the β-adrenergic receptors causes a conformational change, and the present hormone-receptor complex next comes into contact with a stimulatory GTP-binding protein G_s. This interaction causes a guanosine diphosphate (GDP) molecule to be exchanged for a guanosine triphosphate (GTP) molecule, which occurs at an allosteric site near the α subunit of the G_s protein. This binding causes the α subunit to dislodge from the β receptor and, in turn, activate adenylate cyclase. Adenylate cyclase works as an enzyme and catalyzes the formation of cyclic adenosine monophosphate (cAMP), which is an intracellular second messenger that next stimulates the cAMP dependent protein kinase, or protein kinase A. Protein kinase A works to stimulate lipases found within adipose tissue. Lipases are vital enzymes that regulate the breakdown of triglycerides into glycerol and free fatty acids (FFA). Although glycerol is not an important muscle fuel during exercise, free fatty acids are activated into fatty acyl-CoA and are transported through the mitochondrion (Stipanuk, 2006).

Within the mitochondrial matrix, a process known as beta oxidation occurs. After entering the mitochondrion, the fatty acyl-CoA is split into two carbon fragments thereby forming acetyl-CoA. It is then that acetyl-CoA is able to enter the Krebs Cycle and is used to create additional energy in the form of ATP. Ultimately, one acetyl-CoA will produce 12 ATP by means of the Krebs Cycle and the electron transport chain. Thus, starting from catecholamines provoking the breakdown of triglycerides to glycerol and free fatty acids, the final result is additional ATP that can be used for sustained energy when carbohydrate stores deplete. Although ultimately a very slow process, about 60% of energy expenditure comes from fat metabolism during prolonged, low-intensity exercises (<30% VO_2 max) (Powers & Howley, 2011).

There are countless dietary supplements that can be attained over the counter, although all are diverse and offer unique benefits. Dietary supplements that contain stimulants, such as caffeine and ephedra, affect energy expenditure by activating the sympathetic nervous system. As a result, catecholamines increase within the blood stream and, consequently, fat metabolism is stimulated through lipolysis. Thus, body fat is driven to break down in order to be used as an energy source, and various dietary supplements are said to activate this process. Although many dietary supplements claim they incite this metabolic process, it is questionable which are actually effective and show statistically significant results.

Capsaicin and Evodiamine

Capsaicin is an alkaloid compound best known for giving chili peppers their pungency taste and, thus, being an irritant to human tissues. When capsaicin comes in

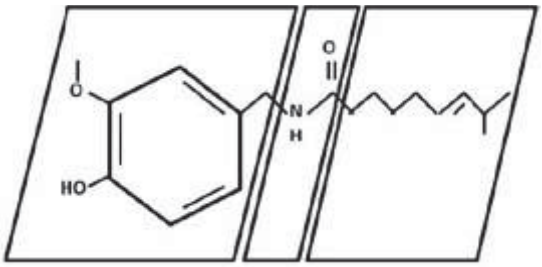
contact with sensitive skin areas or mucous membranes, it binds to a vanilloid receptor, TRPV1, which leads to a cascade of events that ultimately causes burning sensations. This characteristic of capsaicin has made it a popular compound to include in diet pills, since research shows that by capsaicin inciting internal nociceptors it ultimately heats up the body and increases metabolic rate (Iida et al., 2003).

Capsaicin is found in the fruit of the *Capsium* genus, which is the family belonging to chili peppers. In fact, capsaicin mostly dwells within the white ribs of chili peppers, although they can also be found within the inner membranes of peppers and the placental tissues which holds the seeds (Reyes-Escogido et al., 2011). It is believed that plants that rely on their dispersal of seeds in order to continue existence protect themselves by possessing chemicals that are unpleasant in taste to vertebrates. In fact, a study conducted by Tewksbury and Nabhan showed that capsaicin within chili peppers works to repel mammals that do not aid in the dispersal of their seeds (2001). Birds, on the other hand, ingest the seeds which possess capsaicin and are unaffected; the TRPV1 receptor is unresponsive to capsaicin, and, instead, capsaicin passes through birds' digestive tract and is thus able to germinate later. It is for this reason that the pungency characteristic of capsaicin is considered a defense mechanism for the various fruits it inhabits within the genus *Capsium*(Tewksbury & Nabhan, 2001).

Several capsaicinoids exist within peppers that contribute to its spiciness. There are six identified, natural capsaicinoids; however, capsaicin and dihydrocapsaicin constitute about 90% of the capsaicinoids found in peppers. Dihydrocapsaicin, alone, accounts for about 22% of capsaicinoids, and is an odorless, lipophilic compound. Its chemical structure is nearly identical to capsaicin. The only difference between the two

capsaicinoids is the presence of a carbon-carbon double bond in dihydrocapsaicin; however, if capsaicin were to go undergo hydrogenation, dihydrocapsaicin would be formed (Leete & Louden, 1968). Although very similar to capsaicin in appearance, the effects of dihydrocapsaicin are less powerful to human metabolism. A study conducted in 2010 showed that, although similar thermogenic effects are evident, dihydrocapsaicin on resting metabolic rate shows a smaller effect compared to those seen from capsaicin (Galgani & Ravussin, 2010). Various studies show that capsinoids in general increase resting oxygen consumption, body temperature, and lipid oxidation in humans, but there is little effect during exercise or recovery (Galgani, Ryan & Ravussin, 2010). Thus, although very similar in appearance, capsinoids have a smaller thermogenic impression on the human metabolism compared to capsaicin.

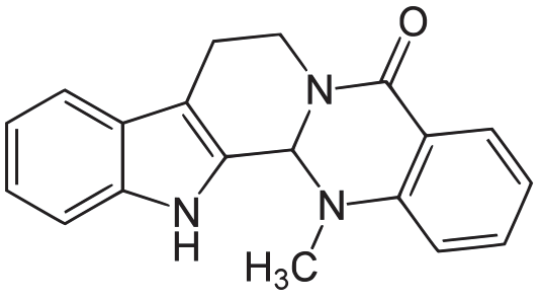
Figure 1: Three key regions of the alkaloid capsaicin: 1 (aromatic ring), 2 (amide bond), and 3 (hydrophobic side chain).



Capsaicin, like dihydrocapsaicin, is also a colorless, odorless, and lipophilic alkaloid. Its chemical makeup can be broken into three parts: an aromatic ring, an amide bond, and a hydrophobic side chain. The hydrophobic portion of capsaicin, which is the region to the far right in Figure 1, gives capsaicin its high potency. In fact, studies show that the lipophilicity of capsaicin is directly related to the activation of the vanilloid

receptor, thus, resulting in its pungency (Ursu et al., 2010). It is also the reason why capsaicin is fat, alcohol, and oil-soluble. The aromatic ring, which is the region to the far left, is important since research shows that the a-ring is essential for potent agonist activity when finding analogous molecules similar to capsaicin (Reyes-Escodigo et al., 2011). This region, along with the hydrophobic side chain, are key characteristics to consider when seeking vanilloid receptor agonists like capsaicin. The pungency of capsaicin limits its use as a dietary supplement, since extended use has been seen to irritate or damage internal tissues in humans (Nolano et al., 1999). For this reason, more research has begun to emerge in hopes to find a thermogenic agent similar to capsaicin but without a pungent kick.

Figure 2: Chemical Makeup of Evodiamine



Evodiamine, an alkaloidal extract from the fruits of *Evodia*, has been regarded as a possible alternate for capsaicin. It is regarded as a "hot nature" herb in Chinese and Japanese medicine, and has for centuries been used to treat headaches, stomach pains, and various other illnesses. The chemical makeup of evodiamine varies greatly to that of capsaicin. For instance, there is an absence of an aromatic ring in evodiamine. Instead, there are two benzene rings present on opposite sides, as opposed to capsaicin's one benzene ring. This indicates that both evodiamine and capsaicin are aromatic compounds.

In other words, within the benzene rings, the carbon atoms are constantly alternating between double and single bonds. Additionally, there is a methyl group present on evodiamine and, similar to capsaicin, there is also an amide bond. Unlike capsaicin whose lipophilicity is the main reason for its pungency taste, evodiamine has hydrophilic properties and has been characterized as having no perceptible taste whatsoever (Kobayashi et al., 2000).

Although the chemical makeup varies greatly, the actions that have been observed *in vitro* for evodiamine and capsaicin are quite similar; however, as previously stated, evodiamine has no perceptible, pungent taste like that of capsaicin. Research regarding the effects of evodiamine have been conducted on mainly rodents or isolated cell cultures. Unfortunately, research concerning the effects of evodiamine on the human metabolism is still lacking. It has been established, however, that the reason for evodiamine's stimulant and thermogenic properties come from the fact that it is a potent vanilloid receptor agonist like capsaicin. In other words, evodiamine binds to the vanilloid receptor, like capsaicin, and elicits a response.

Vanilloid Receptor Mechanism of Activation

The vanilloid receptor; transient receptor potential channel, vanilloid subfamily member 1 (TRPV1) is a protein belonging to the family of transient receptor potential (TRP) channels. Within this family of cation channels are six subfamilies: TRPA (Ankyrin), TRPC (Canoncial), TRPM (Melastatin), TRPML (Mucolipin), TRPP (Polycystin), and the TRPV (Vanilloid). The TRPV group is comprised of six additional

membrane channels, all of which are notably involved in nociception when stimulated (Szallasi & Blumberg, 1999).

TRPV1, which is the first member in the vanilloid subfamily, is a non-selective cation channel found on primary sensory neurons within the central and peripheral nervous systems. TRPV1 can be found mainly within the dorsal root ganglion, trigeminal ganglion, and nodose ganglion neurons of nociceptive afferent fibres. Thus, when stimulated, they carry their stimulus from the site of stimulation to the roots of the spinal cord. TRPV1 can also be found within various brain regions such as the hypothalamus, cerebellum, and medulla (Nilius, Vennekens & Owsianik, 2008). Additionally, TRPV1 can be found in non-neuronal tissues such as urinary bladder sensory fibers, in glial cells, and in the liver, although the function of TRPV1 in these tissues is still uncertain (Mittelstadt et al., 2012).

TRPV1 is activated by the binding of capsaicin and other noxious stimuli, which are commonly referred to as vanilloids. TRPV1 is characterized as being a heat-activated channel. Thus, in addition to the binding of capsaicin and other vanilloids, TRPV1 is capable of being activated by physical noxious stimuli such as heat (>48 °C) and low surrounding pH levels. Once TRPV1 is activated, the stimulus is carried from the binding site and into the roots of the spinal cord through nociceptive afferent fibers. These afferent fibers are specialized neurons that transmit information to the brain and spinal cord regarding tissue damage. Thus, when TRPV1 is activated, they transmit noxious stimuli to the central nervous system where it is then perceived as pain (Szallasi & Blumberg, 1999).

A release of inflammatory mediators as well as an increased permeability to cations by local plasma membranes also results from agonist binding. For this reason, TRPV1 is categorized as a non-selective cation channel; however, TRPV1 is relatively selective for calcium and magnesium ions versus sodium ions. In fact, when the channel is activated, calcium ions influx in and outnumber sodium ions 9 to 1. Magnesium ions, on the other hand, outnumber sodium ions 5 to 1 (Nilius, Vennekens & Owsianik, 2008). This unbalance of ions indicates a current-voltage is present when TRPV1 is stimulated, and the influx of cations usually results in membrane depolarization. It is when a large enough depolarization reaches threshold that an action potential occurs. This action potential, when generated, will run along the entire length of the vanilloid-sensitive neuron and be detected by the central nervous system as a pain, itch, or burning sensation. There is much that is still uncertain about this process; however, it is understood that ATP and various neuropeptides act in combination as a single transmitter during pain transduction (Szallasi & Blumberg, 1999).

After the action potential takes place, repolarization followed by a refractory period occurs. During the refractory period, the once vanilloid-sensitive neuron becomes unresponsive to vanilloids and other noxious stimuli that would otherwise be provocative. It is believed that this desensitization is due to a conformational change in the receptor protein that occurs when the nerve is initially aroused. This change in shape ultimately leads to the closing of the channel pore, which, furthermore, inhibits additional cations to enter. For this reason, desensitization is believed to be a calcium dependent process. The influx of calcium into cells activates a negative feedback signal. Although the initial influx of calcium into the nerve triggered an action potential, extended exposure to

calcium ultimately causes the fibers of the neuron to die. It is for this reason that capsaicin is commonly used as an analgesic agent when treating painful illnesses; through the continued influx of calcium caused by capsaicin, nociceptive afferent neurons are eventually destroyed and are thus non-responsive to the perception of pain that was once recognized (Szallasi & Blumberg, 1999).

Activation of TRPV1 is effective not only in the transmission of pain sensations, but it also plays a vital role in enhancing energy expenditure and thermogenesis when stimulated by a vanilloid. Capsaicin has been proven to increase energy expenditure and body temperature by means of first stimulating the sympathetic nervous system (SNS) (Iwasaki et al., 2008). The increase of catecholamines that results from the increase of the SNS not only increases alertness and energy expenditure, but it also plays a role in stimulating fat oxidation through the process of lipolysis. Thus, the stimulation of TRPV1 is vital in promoting the anti-obesity effects of capsaicin. Studies are currently being conducted with the hopes of finding another vanilloid much like capsaicin that exerts similar anti-obesity effects. Much is unknown about the molecular nature of TRPV1; however, the results it delivers when stimulated by vanilloids is definite and attractive for delivering to an obese society an effective tool for weight loss and weight gain prevention.

Research with Capsaicin and Evodiamine

Capsaicin comes from the fruit of the *Capsicum* genus and shows significant effects to the human metabolism when ingested. Numerous amounts of research have been conducted in order to empirically establish these results as significant, and what has

been found are consistent findings that verify capsaicin as a stimulant and thermogenic agent. Unlike with evodiamine, research concerning capsaicin has been conducted on humans as well as on rodents.

The majority of studies concerning the effects of capsaicin have been conducted mainly within Asian populations, where capsaicin is a popular ingredient included in the everyday diet. One study particularly added red pepper (capsaicin) to high-fat and high-carbohydrate meals and observed the effect it had on energy metabolism in Japanese female participants. More specifically, thirteen female subjects ingested a regulated dinner the evening prior to an experimental breakfast. The experimental breakfast was either high-fat, high-fat and red pepper, high-carbohydrate, or a high-carbohydrate and red pepper meal. Expired air was measured before and after the consumption of the experimental breakfast for each individual. What was found was a substantially lower level of carbohydrate oxidation and an increase of both lipid oxidation and diet-induced thermogenesis with the addition of red pepper to the experimental meals. Furthermore, these effects were more significant in subjects who ingested red peppers in high-fat meals (Yoshioka et. al, 1998). Another study was conducted in order to observe the effects of CH-19 Sweet, a non-pungent cultivar of capsaicin, on body temperature and oxygen consumption in humans. Eleven healthy volunteers participated in this study and were given either CH-19 Sweet or California Wander (containing neither capsaicin or capsiate) to ingest. Core body temperature, body surface temperature (obtained from the forehead and neck), and oxygen consumption were immediately measured after ingestion. It was found that the core body temperature in the group ingesting CH-19 sweet was significantly higher than that of the control groups. The body surface temperature of the

CH-19 sweet group was also higher than the control groups and continued to be elevated for about twenty minutes after ingestion. Oxygen consumption was also measured by indirect calorimetry, and was found to be significantly higher for the CH-19 Sweet group. These results suggest that CH-19 Sweet significantly effects body temperature and energy consumption in otherwise healthy humans (Onhuki et. al, 2001).

Numerous Asian studies have been conducted in order to better understand the effects of capsaicin on the human body; however, the majority of these studies are short-lasting and fail to demonstrate the long-term effects of capsaicin. The long term studies that have been conducted, most of which come from the United States, continue to verify the notion of capsaicin's anti-obesity effects to the human body. A twelve week, placebo-controlled, double-blind, and randomized study was conducted in 2008 to determine the safety and efficacy of ingesting capsinoids as well as to examine genetic variants that may be predictors for ideal capsinoid responses. Forty women and forty men were given either a capsinoid or placebo pill to ingest for twelve consecutive weeks and were given dietary advice. Participants were instructed to modify their diet for the duration of the study so that each day they attained an energy deficit of 300-600 kcal/day. The results of this experiment showed that 6 mg/day of capsinoids promoted fat loss particularly in abdominal fat in already overweight or obese humans. The adverse effects that occurred in this study posed no serious threat and, thus, promote the ingestion of capsinoids as otherwise safe. It was also found that genetic polymorphisms of the TRPV1 receptor are associated with intensified responses to the ingestion of capsinoids (Snitker et. al, 2009). Another relatively long, capsaicin-centered experiment was conducted in order to observe whether capsaicin assists in weight maintenance after weight loss in

humans. This study was double-blind, placebo-controlled, and consisted of ninety-one moderately overweight participants assigned to a four week low-energy diet followed by a three month weight maintenance period. During the weight maintenance period, participants blindly consumed either capsaicin or a placebo pill. The results to this study indicated that the ingestion of capsaicin led to higher fat oxidation, but, ultimately, capsaicin had no limited effect during this three month period after 5-10% weight loss (Lejeune, Kovacs & Westerp-Plantenga, 2003).

Capsaicin has been intensely studied for the past few decades only to obtain more information on its already understood and powerful effects. Unfortunately, as already stated, capsaicin's pungency has limited its use as a means to fight obesity, since the spiciness can be over-powerful for people to ingest while, as studies show, an over-exposure of capsaicin to internal tissues can ultimately damage them and cause microscopic blisters (Nolano et al., 1999). For this reason, synthesis of non-pungent analogues have been underway in order to find a more viable agent with similar anti-obesity effects like that of capsaicin. Evodiamine is a non-pungent, alkaloidal agent extracted from the fruits of *Evodia rutaecarpa* and has been shown to bind to the vanilloid receptor much like capsaicin. Not many studies have been conducted to show the effects of evodiamine, particularly, on the human metabolism; however, few studies show its effects on rodents. In a study conducted to specifically observe the capsaicin-like activities of evodiamine, male rats were fed a diet supplemented with evodiamine extracts. When evodiamine was supplemented in the diet at 0.03% for 12 consecutive days, the rats experienced a loss in perirenal fat weight and epididymal fat mass compared to the rats in the control group. Additionally, more significant results were

identified when evodiamine was supplemented in the rats' diet at 0.02% in the form of ethanol extract of *Evodia* fruits in addition to their already high-fat diet. What was observed was a significant reduction in body weight, perirenal fat weight, epididymal fat weight, free fatty acid levels within the serum, and total lipid count in the liver when compared to the control group. Thus, the results of this experiment verify evodiamine's anti-obesity effects to be fairly identical to those seen from capsaicin. Evodiamine ultimately induced heat loss and dissipated food energy in rats, thus preventing accumulation of fat and body weight (Kobayashi et. al, 2000a). In another experiment, the effects of evodiamine in isolated bronchus' of guinea-pigs were closely examined and compared to the bronchus' of guinea-pigs with capsaicin in their systems. Both evodiamine and capsaicin similarly evoked bronchial contraction. In other words, both agents caused the tightening of smooth muscles along the pathways of the lungs. The bronchial contractions were observed to be dose-dependent, thus, the higher the concentration of evodiamine or capsaicin that was entered into the system, the stronger the bronchoconstriction appeared to be. In order to determine whether the activation of TRPV1 played a role in this activity, capsazepine, a recognized antagonist of the vanilloid receptor, was injected into the system. Capsazepine appeared to competitively inhibit evodiamine by preventing bronchus constriction, thus suggesting that evodiamine works with the vanilloid receptors when activating bronchial constriction (Kobayashi et. al, 2000b). Other experiments have also been conducted in order to establish a relationship, if any, between evodiamine and TRPV1, most of which have only verified evodiamine as a vanilloid receptor agonist. A study conducted in 2004 also verified evodiamine's tendency to bind with the vanilloid receptor and have an antagonistic

relationship with capsazepine. Additionally, established through a molecular model depicting consistent overlap patterns between evodiamine and TRPV1, it was concluded that evodiamine represents a vanilloid receptor agonist for rats (Pearce et. al, 2004). As previously stated, the majority of information we have about the effects of evodiamine comes from the handful of experiments that have been conducted on rodents. Thus, in order to fully understand the effects of evodiamine on the human metabolism, it is necessary to conduct experiments centered on the administration of evodiamine on human subjects.

Summary

Past and present experiments continue to support the fact that capsaicin works as a strong stimulant and thermogenic agent when ingested by humans; however, little information is presently known about the effects that evodiamine has on human metabolism. Nevertheless, previous studies confirm evodiamine as a vanilloid receptor agonist capable of similar anti-obesity effects as those of capsaicin. More research is essential in order to empirically establish evodiamine as an agent both safe and effective for increasing the metabolic rate within humans.

CHAPTER THREE

Methods

Participants

Fifteen apparently healthy and active, but not exercise-trained, men between the ages of 18-30 were allowed to volunteer to participate in this study. Only participants who were considered as low to moderate risk for cardiovascular disease and had no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM), who had not consumed any nutritional supplements other than vitamins, and who had never been involved in any weight loss regimen in the last 6 months were allowed to participate.

Study Site

All testing sessions in this study were conducted in the Exercise & Sport Nutrition Laboratory at Baylor University. All sample analyses were completed in the Exercise and Biochemical Nutrition Laboratory at Baylor University.

Independent and Dependent Variables

The independent variables were the administered supplements (placebo, capsaicin, evodiamine). The dependent variables that were closely monitored while at rest pre-supplementation, at rest post-supplementation, during exercise, and during recovery

from the bout of exercise include: heart rate, blood pressure, core body temperature, energy expenditure, and serum levels of glucose, triglycerides, glycerol, and free fatty acids.

Testing Protocol

This study was conducted as a randomized, cross-over research experiment in which participants came in for 3 separate testing sessions, each session separated by 7-10 days. Each participant was instructed to fast 8 hours prior to each testing session as well as not exercise 48 hours prior. At the start of each session, participants were required to turn in a dietary analysis form, which was used to determine their average daily intake of fats, carbohydrates, and proteins in their diets. Their baseline heart rate, blood pressure, and core body temperature were then assessed. Additionally, a blood sample was taken from an intravenous catheter inserted into the participant's antecubital vein. Participants were then required to undergo a 30 minute rest period, during which their resting energy expenditure was assessed. At the end of their resting period, participants were required to ingest 500 mg of either a cellulose placebo, capsaicin, or evodiamine supplement. Another 30 minute rest period was administered and resting energy expenditure continued to be assessed. At the end of this period, heart rate, blood pressure, core body temperature, and a blood sample was obtained for a second time. Participants then began to perform a 3 minute warm up period on a treadmill at 2 mph with 2% grade incline. The speed was further adjusted to match their appropriate intensity level, which was determined from the VO_2 max test administered during the participant's entry visit. In order to maintain a steady intensity and energy expenditure level, heart rate and respiratory gas analysis were checked at 5 to 15 minute intervals throughout the exercise.

Treadmill speed and grade were adjusted, if necessary. Caloric expenditure and exercise duration were measured and recorded. At the end of the exercise, the participant's heart rate, blood pressure, core body temperature, and blood sample were assessed once again. Participants were then required to rest/recover for 30 minutes before heart rate, blood pressure, core body temperature, blood sample, and oxygen consumption were assessed for a final time. At the conclusion of each testing session, participants were required to complete a reported side effects from supplements questionnaire, which addressed whether they were able to tolerate the supplement and whether any symptoms arose during the conduction of the experiment.

Entry and Familiarization Session (Visit 1)

During the familiarization session, participants completed a medical history questionnaire and had a general physical examination in order to determine whether they meet the eligibility criteria of the study. Those who met criteria were then introduced to the experiments protocol by both a verbal and visual explanation of the study design. Assessments of body composition were taken and an incremental load exercise test was administered in order to determine peak oxygen consumption (VO_2peak) for each participant. At the conclusion of the familiarization session, participants arranged an appointment time to begin their first testing session.

Assessment of Body Composition (Visit 1)

Total body mass (kg) was determined with the use of a standard dual beam balance scale (Detecto). Body fat percent, fat mass, and fat-free mass were evaluated

using a DEXA. Assessment of body composition was only measured during the entry/familiarization session.

Assessment of Peak Oxygen Uptake (VO_2peak) (Visit 1)

During the entry/familiarization session, participants were instructed to perform an incremental load exercise test on a treadmill ergometer in order to determine their VO_2peak. After baseline measurements were recorded at rest, the administration of the test began with the treadmill starting at a velocity of 8 km/h at a 0% incline. Velocity then increased by an additional 2 km/h every 3 minutes until exhaustion. Oxygen uptake (VO_2) was measured during the conduction of this test every 20 seconds via an open circuit sampling system. The highest level of VO_2 reached by the participant was recorded as their VO_2peak.

Assessment of Heart Rate and Blood Pressure (Visits 1, 2, 3, & 4)

Heart rate and blood pressure was taken at the entry/familiarization session for each participant. Additionally, heart rate and blood pressure was again assessed at each of the three testing sessions 30 minutes prior to exercise, immediately prior to exercise, immediately after exercise, and 30 minutes after recovery period. Heart rate was measured by palpation of the radial artery. Blood pressure was measured after 5 minutes of resting in the supine position using a mercurial sphygmomanometer by means of standard procedures.

Assessment of Core Body Temperature (Visits 2, 3, & 4)

All participants ingested a silicon-coated tablet on the evening before each of the three testing sessions. The CorTemp® temperature pill is an ingestible sensor that is frequently used in laboratory settings to measure the internal body temperature of subjects. Using a hand-held wireless data recorder, researchers can accurately determine core temperatures during testing session. The pill has been designed to be easily ingested and voided through regular bowel movements within 48 hours. This pill was utilized for each participant in order to measure core body temperature in this study.

Assessment of Resting Energy Expenditure (Visits 2, 3, & 4)

At each of the three testing sessions for all participants, resting energy expenditure was measured 30 minutes prior to and after ingesting the supplement and 30 minutes post exercise. Measurements were obtained by way of open-circuit spirometry using the Parvo Medics 2400 TrueMax Metabolic Measurement System.

Blood Sampling

Venous blood samples were obtained and put into 10 ml vacutainer tubes from a 20 gauge intravenous catheter inserted into the participant's antecubital vein. The catheter was flushed with 10 IU/ml of sodium heparin prior to obtaining each blood sample. Blood samples were set aside at room temperature for 10 minutes before being centrifuged. The serum was then removed and frozen at -80° for later analysis. At each testing session, blood samples were obtained: 30 minutes prior to consumption of a supplement, immediately prior to exercise, immediately after exercise, and 30 minutes

after exercise. Thus, four blood samples were obtained during one session for a total of twelve blood samples during the course of the study.

Dietary Analysis (Visits 2, 3, & 4)

Participants were required to record their dietary intake for three days prior to each of their testing session. Participants were asked not to change their dietary habits for the duration of this study. Participant's dietary analysis were evaluated with the Food Processor dietary assessment software program to determine their average consumption of fat, carbohydrate, and protein in their diet during the course of this study.

Reported Side Effects from Supplements (Visits 2, 3, & 4)

At the conclusion of each of the three testing sessions, participants were required to fill out a questionnaire regarding their tolerance for the testing supplement. They were also to include any medical problems or symptoms that arose during the session, if any. However, if complications did arise prior to the completion of the questionnaire, participants were encouraged to report them as they occurred.

Assessment of Serum Glycerol and Free Fatty Acids

Serum glycerol and fatty acid content were determined spectrophotometrically using the free glycerol determination kit (Sigma Aldrich, St. Louis, MO) and glycerol standard (Sigma Aldrich, St. Louis, MO) at a wavelength of 490 nm. Serum free fatty acids were determined with a free fatty acid quantification kit (Bio Vision, Mountain View, CA) at a wavelength of 540 nm. Both assays were performed in duplicate with a

microplate reader (Wallac Victor 1420, Perkin Elmer, Boston MA). Data analysis was performed using MicroWin microplate data-reduction software (Mikrotek Laborsysteme, Germany).

Assessment of Serum Glucose

Serum glucose (Alpha Diagnostics, San Antonio, TX) was determined with competitive ELISA using a Wallac Victor-1420 micoplate reader (Perkin-Elmer Life Sciences, Boston, MA). The assays were performed at 450 nm wavelength, against a known standard curve.

Statistical Analyses

Statistical analyses was performed by utilizing separate 3 x 4 (supplement x time) factorial analyses of variance (ANOVA) with repeated measures for each criterion variable. Significant between-group differences were then determined involving the Neuman-Keuls Post Hoc Test. All statistical procedures were performed using SPSS 11.0 software and a probability level of < 0.05 will be adopted throughout. However, to protect against Type I error, the conservative Hunyh-Feldt Epsilon correction factor was used to evaluate observed within-group F-ratios.

CHAPTER FOUR

Results

Baseline Entry Data

A total of seven active, apparently health, but not exercise trained, men participated in this study. Table 1 illustrates the average baseline entry data for participants. The average (± standard deviation) age of our participants was 20 years, 176 cm tall, and 75 kg in weight. The average heart rate of participants at baseline was 59 bpm, while systolic and diastolic blood pressures were 112 and 68 mmHg, respectively. The peak VO2 max was 55.4 ml/kg/min and max RER was 1.25 (VCO2/O2).

Table 1. Baseline Participant Entry Data

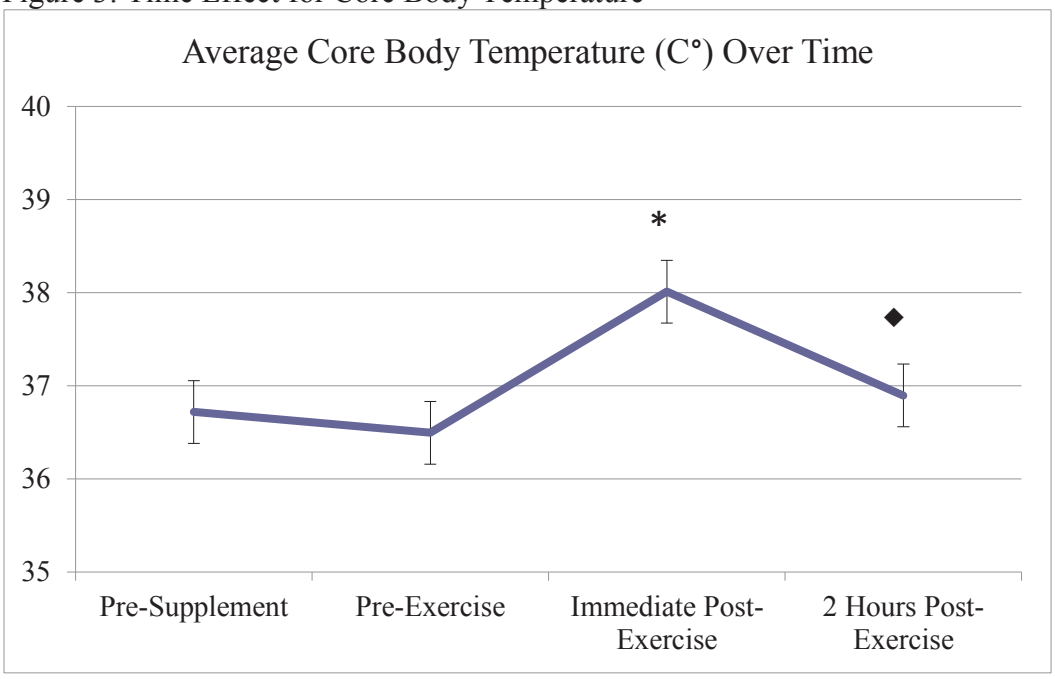
Variable	Mean	Standard Deviation
Age (years)	20.42	2.51
Height (cm)	176.42	5.06
Body weight (kg)	74.50	6.29
Systolic blood pressure (mmHg)	112.28	5.71
Diastolic blood pressure (mmHg)	68	7.30
Peak VO2 max (ml/kg/min)	55.4	5.26
Max RER (VO2/O2)	1.25	0.04
Body fat (%)	14.77	5.05
Lean mass (kg)	55.57	5.13
Fat mass (kg)	10.13	3.72
Total calories (kcal/day)	2584.71	1090.42
Fat intake (g/day)	90.66	37.14
Carbohydrate intake (g/day)	336.45	153.24
Protein intake (g/day)	93.96	34.44

Heart Rate, Blood Pressure, and Core Body Temperature

The data for hemodynamic and body temperature changes are shown in Figure 3 and Table 2. Statistical analysis did not reveal a significant time x supplement interaction for heart rate ($p = 0.895$), systolic blood pressure, ($p = 0.098$), diastolic blood pressure ($p = 0.424$), or core temperature ($p = 0.964$) indicating there to be no significant differences between the three supplements for these variables. There were, however, statistically significant time effects for heart rate ($p = 0.001$), systolic blood pressure ($p = 0.039$), diastolic blood pressure ($p = 0.005$), and core temperature ($p = 0.001$).

Post-hoc analysis indicated that heart rate was significantly greater immediately post-exercise, and that even 2 hours post-exercise, heart rate was still elevated compared to pre-supplement and pre-exercise time periods. Furthermore, systolic blood pressure during the pre-exercise time period was significantly greater than the pre-supplement period. Additionally, systolic blood pressure was significantly elevated immediately post exercise. At 2 hours post-exercise, systolic blood pressure dropped to a level even lower than pre-exercise systolic blood pressure. Diastolic blood pressure was significantly lower immediately post-exercise compared to the prior time period, pre-exercise. Core body temperature was lowest during pre-supplementation. It increased significantly immediately post-exercise. Two hours post exercise, a decrease in core body temperature was observed; however, it was still greater during this period than during pre-supplement and pre-exercise periods.

Figure 3: Time Effect for Core Body Temperature



* Significantly different ($p < 0.05$) from the pre-supplement, pre-exercise, and 2 hours post-exercise periods

◆ Significantly different ($p < 0.05$) from the pre-supplement, pre-exercise, and immediate post-exercise

Table 2. Data for heart rate, blood pressure, and core temperature levels.

Variable	Pre-Supplement (PS)	Pre-Exercise (PE)	Immediate Post-Exercise (IPE)	2 Hours Post-Exercise (2HPE)	Time $p < .05$	Time x Supplement $p < .05$
Heart Rate (bpm)					.001 IPE > PS; 2HPE > PS; IPE > PE; IPE > 2HPE	.895
Evodiamine	54.85 (5.1)	53.71 (6.47)	168.71 (7.80)	78.28 (13.43)		
Placebo	56.57 (6.70)	50.57 (4.11)	171.71 (11.68)	82.28 (12.40)		
Capsaicin	52.57 (4.27)	53.14 (5.98)	169.95 (5.51)	77.42 (14.17)		
Systolic Blood Pressure (mm/Hg)					.039 PE > PS; IPE> PS; IPE > PE; PE > 2HPE	.098
Evodiamine	106.28 (10.16)	111.14 (8.78)	129.42 (12.14)	109.42 (10.87)		
Placebo	109.42 (8.14)	112.00 (7.65)	128.85 (12.64)	109.71 (9.19)		
Capsaicin	112.14 (7.96)	115.14 (9.29)	124.57 (6.90)	105.71 (18.79)		
Diastolic Blood Pressure (mm/Hg)					.005 PE>IPE	.424
Evodiamine	70 (7.30)	74 (6.63)	71.14 (6.09)	69.71 (7.43)		
Placebo	69.14 (10.05)	70 (7.83)	70.57 (8.61)	68.57 (8.46)		
Capsaicin	69.14 (8.85)	74 (6.32)	68.28 (7.60)	67.71 (7.69)		
Core Temperature (degrees C)					.001 PE > PS; IPE > PS; 2HPE >PS; IPE > PE; 2HPE > PE IPE > 2HPE	.864
Evodiamine	36.65 (.384)	36.51 (.163)	38.07 (.388)	37.65 (.411)		
Placebo	36.75 (.343)	36.44 (.172)	38.08 (.505)	37.68 (.450)		
Capsaicin	36.74 (.201)	36.51 (.180)	37.86 (.817)	37.53 (.609)		

Data is expressed as means (standard deviations).

Respiratory Exchange Ration and Resting Energy Expenditure

Table 3 and Figures4 and 5 present the average (± standard deviation) data attained for the respiratory exchange ratio (RER) and resting energy expenditure (REE). Statistical analysis did not reveal a significant time x supplement interaction for RER ($p = 0.746$) or REE ($p = 0.114$) indicating there to be no differences between the three supplements for these variables. There was, however, statistically significant time effects for both RER ($p = 0.001$) and REE ($p = 0.001$). Post-hoc analysis further indicated that RER was significantly greater immediately post exercise compared to the other three time periods. Additionally, RER 2 hours post-exercise was significantly greater than during pre-supplement and pre-exercise periods. For REE, the level of energy expenditure at 2 hours post-exercise was significantly greater than at pre-supplementation and pre-exercise.

Table 3. Data for respiratory exchange ratio and resting energy expenditure.

Variable	Pre-Supplement (PS)	Pre-Exercise (PE)	Immediate Post-Exercise (IPE)	2 Hours Post-Exercise (2HPE)	Time $p < .05$	Time x Supplement $p < .05$
RER (VCO2/VO2)					.001 IPE > PS; 2HPE > PS; IPE > PE; IPE > 2HPE	.746
Evodiamine	.782 (.019)	.776 (.044)	.911 (.017)	.757 (.046)		
Placebo	.767 (.043)	.774 (.036)	.891 (.041)	.721 (.044)		
Capsaicin	.804 (.037)	.792 (.039)	.902 (.023)	.756 (.074)		
REE (kcals/day)					.001 2HPE > PS; 2HPE > PE	.114
Evodiamine	1652.42 (108.99)	1676.28 (115.99)		1885.39 (102.12)		
Placebo	1715.35 (168.59)	1699.85 (146.14)		1841.25 (101.84)		
Capsaicin	1593.14 (125.02)	1650.80.58		1854.21 (151.55)		

Data is expressed as means (standard deviations).

Figure 4: Time Effect for Respiratory Exchange Ratio

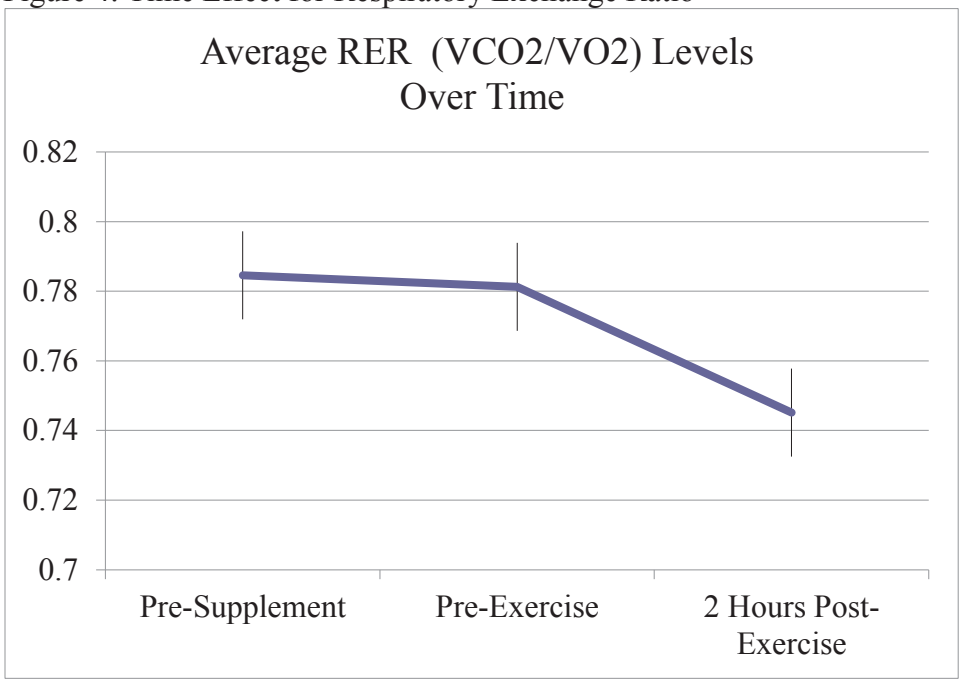
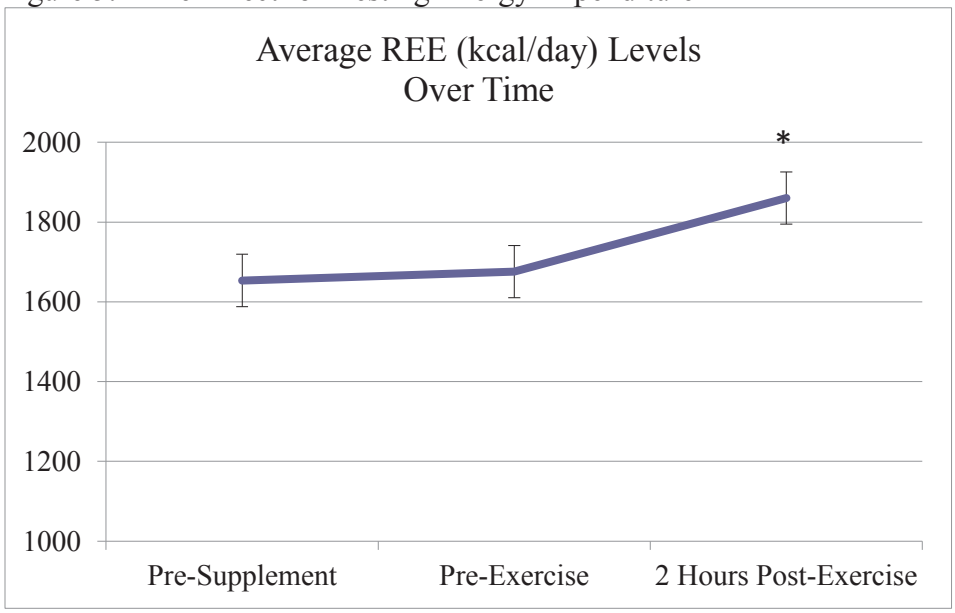


Figure 5: Time Effect for Resting Energy Expenditure



* Significantly different ($p < 0.05$) from the pre-supplement and pre-exercise periods

Serum Glucose, Triglycerides, Glycerol, and Free Fatty Acid Levels

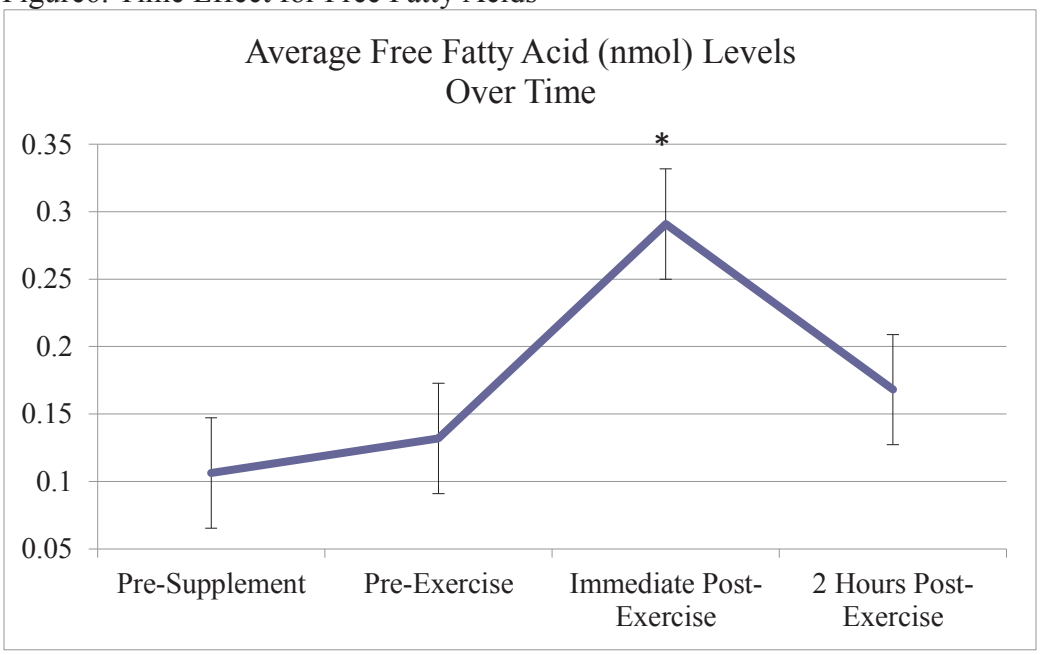
The average (± standard deviation) data for serum levels are shown in Table 4 and Figures 6 and 7. Statistical analysis did not show a significant time x supplement interaction for glucose ($p = 0.683$), triglycerides ($p = 0.135$), free fatty acids ($p = 0.605$), or glycerol ($p = 0.588$) indicating there to be no significant differences between the three supplements for any of the assessed serum variables. Additionally, analysis did not show a significant time effect for glucose ($p = 0.759$) and triglycerides ($p = 0.437$) indicating that neither of these variables changed significantly over the course of the four time points. There was, however, a significant time effect for free fatty acids ($p= 0.001$) and glycerol ($p = 0.001$). These substantial differences can be seen in Figure 4 and 5. Post-hoc analysis indicated that free fatty acids were significantly greater immediate post-exercise than at any other time point. Glycerol was significantly less at pre-supplementation compared to time pre-exercise, immediate post-exercise, and 2 hours post-exercise.

Table 4. Data for serum levels of glucose, triglycerides, free fatty acids, and glycerol.

Variable	Pre-Supplement (PS)	Pre-Exercise (PE)	Immediate Post-Exercise (IPE)	2 Hours Post-Exercise (2HPE)	Time $p < .05$	Time x Supplement $p < .05$
Glucose (mg/dl)					.759	.683
Evodiamine	91.03 (23.44)	91.78 (19.53)	103.64 (44.54)	87.63 (26.10)		
Placebo	87.25 (32.80)	93.67 (25.43)	93.03 (24.16)	97.90 (24.16)		
Capsaicin	100.74 (20.60)	105.92 (25.64)	102.32 (40.75)	97.70 (28.29)		
Triglycerides (mg/dl)					.437	.135
Evodiamine	53.12 (19.40)	57.02 (39.44)	84.69 (40.59)	69.18 (20.75)		
Placebo	67.73 (46.36)	47.17 (24.81)	81.44 (31.02)	66.71 (28.41)		
Capsaicin	67.93 (43.41)	83.37 (55.21)	74.69 (52.48)	54.52 (38.70)		
Free Fatty Acids (nmol)					.001	.605
Evodiamine	.108 (.067)	.153 (.087)	.246 (.087)	.176 (.163)		
Placebo	.097 (.084)	.086 (.082)	.342 (.137)	.167 (.073)		
Capsaicin	.109 (.077)	.156 (.093)	.282 (.131)	.161 (.082)		
Glycerol (µg/ml)					.001	.588
Evodiamine	9.22 (4.75)	15.15 (9.25)	20.32 (10.12)	16.85 (6.69)		
Placebo	10.50 (5.21)	14.07 (6.71)	18.85 (4.81)	14.56 (3.92)		
Capsaicin	8.28 (5.23)	15.88 (7.96)	19.24 (5.65)	17.64 (5.05)		

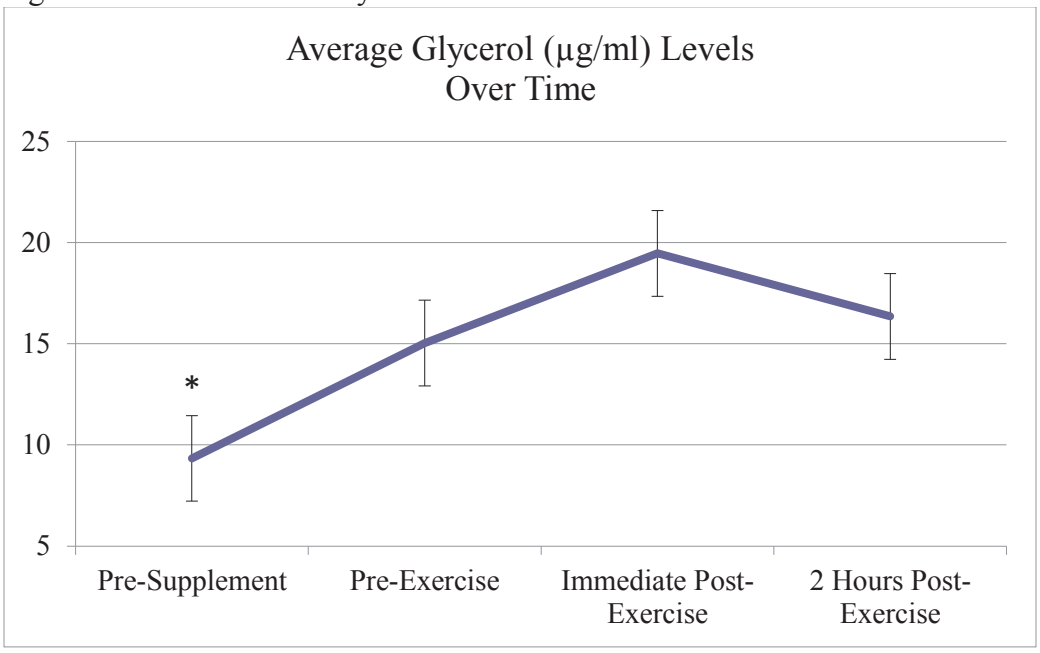
Data is expressed as means (standard deviations).

Figure6. Time Effect for Free Fatty Acids



* Significantly different ($p < 0.05$) from the pre-supplement, pre-exercise, and 2 hours post-exercise periods

Figure7. Time Effect for Glycerol



* Significantly different ($p < 0.05$) from the pre-exercise, immediate post-exercise, and 2 hours post-exercise periods

CHAPTER FIVE

Discussion

Introduction

In response to the obesity epidemic, countless dietary supplements have been emerging in the marketplace. Many of these supplements contain thermogenic ingredients that are said to increase human metabolism; however, some agents are still questionable in efficiency and safety for human consumption. Two thermogenic agents that are included in many dietary supplements are capsaicin and evodiamine. Although many experiments have been conducted in order to further evaluate capsaicin as an efficient ingredient, much is still unknown about evodiamine. The present study focused on identifying measurable benefits that capsaicin and evodiamine have on the human metabolism by comparing their effects to each other and to that of a placebo supplement. Hemodynamics, energy expenditure, and markers of lipid oxidation at rest, after supplementation, after a single bout of moderately intense exercise, and during recovery were measured and compared.

Heart Rate, Blood Pressure, and Core Temperature

A normal, resting heart rate can be anywhere between 60 to 100 beats per minute. A lower resting heart rate generally denotes physical fitness and cardiovascular health, since a lower resting heart rate indicates that the heart is able to pump blood more efficiently with less beats (Dimkpa, 2009). The average heart rate of our participants

during pre-supplement and pre-exercise periods ranged from 50 to 60 bpm indicating an otherwise healthy heart function. According to our data, heart rate increased substantially immediately post-exercise, as was expected. When one begins to exercise, the heart rate responds by increasing in order to supply more blood and thereby oxygen to the main working muscle groups. After exercise, the time it takes heart rate to return to its normal pattern depends on the intensity of the workout and the fitness of the individual. For our experiment, participants were required to undergo a moderately intense workout by achieving a steady RER of 0.9 (VCO2/VO2). The amount of time for heart rate to return to normal after a moderately intense exercise can take approximately four hours (Dimkpa, 2009). For this reason, as our data indicates our participants' heart rates two hours post-exercise were lower compared to heart rates immediately after exercise; however, they were still elevated compared to pre-supplement and pre-exercise periods. Thus, although the time effect is significant for heart rate, this is expected due to the exercise protocol administered.

As heart rate increased due to exercise, blood pressure underwent changes as well. Systolic blood pressure (SBP), which is the maximum arterial pressure during left ventricular contraction, should increase progressively with exercise (Kravitz, 2001). As muscles demand more oxygen in order to prolong exercise, the heart will contract faster and thus SBP will increase. Our data is consistent with this concept since a time effect was perceived immediately post exercise for SBP. Additionally, our data indicates a significant drop in DBP immediately post-exercise compared to the pre-exercise period; however, this too is expected due to the exercise bout that our participants underwent. Diastolic blood pressure (DBP) refers to the lowest pressure exerted on the walls of the

arteries when the heart relaxes between beats. During exercise, DBP should stay about the same or decrease due to the dilation of blood vessels that occurs in order to allow more blood to enter and heat to escape from working muscles (Kravitz, 2001). Thus, although changes in DBP were significant, they were also expected.

Core body temperature also underwent changes due to exercise. According to our data, core body temperature significantly increased immediately post exercise and dropped 2 hours post-exercise but still remained elevated compared to body temperature during resting periods. Although these changes were significant, they were also expected to change in this manner due to the exercise protocol administered to our participants. During exercise, extra blood is being sent to working muscles in order to provide oxygen and thereby energy. Humans are very inefficient in producing energy. In fact, muscles generate only 25% of energy in the form of ATP and the rest is lost as heat. This heat is transferred from muscles to the blood, which circulates throughout the body. For this reason, core body temperature increases during exercise which ultimately results in the hypothalamus sending signals to increase blood flow to the skin and thus activating sweat glands. After a moderately intense exercise bout, the heart rate slows down as previously discussed; however, this results in decreased amount of blood being sent to the skin in order to produce sweat. Because of this, core body temperature remains elevated in order for sweat to continue to be produced even after exercise is finished.

We had hypothesized that the consumption of capsaicin or evodiamine would increase heart rate, blood pressure, and core body temperature compared to the placebo supplement. Capsaicin is a well-established thermogenic agent that binds to the vanilloid receptor and stimulates the sympathetic nervous system (Vennekens, Grzegorz & Bernd,

2008). Through thermogenesis, core body temperature increases and metabolism is stimulated. Once the sympathetic nervous system is activated, heart rate and blood pressure are among several variables that increase. Evodiamine, on the other hand, has been shown through research to also elicit thermogenic responses by means of first binding to the vanilloid receptor (Pearce et. al, 2004). The present study was intended to further look at the effects of evodiamine, and compare these effects to those elicited by capsaicin and a placebo supplement. Analysis showed time effects in all four variables; however, as previously discussed, these differences over time were expected due to the exercise protocol that was administered. Unfortunately, there were no significant time x supplement interactions which would have presented us with differences between supplements among variables. We were thus forced to reject our hypothesis that heart rate, blood pressure, and core body temperature would be elevated when participants ingested either evodiamine or capsaicin supplements compared to placebo.

Respiratory Exchange Ratio and Resting Energy Expenditure

Respiratory exchange ratio (RER) is a measurement used to indicate what type of fuel your body is using to produce energy at a given time. RER takes the amount of carbon dioxide being exhaled and divides it by the amount of oxygen being inhaled in a single breath. An RER value should be between 0.7 and 1.0, with a lower value around 0.7 suggesting a fuel type of predominately fats and a higher value towards 1.0 suggesting that carbohydrates are the primary fuel (Mackenzie, 2011). During the resting periods of the experiment, our data shows that RER was about 0.7 to 0.8 for participants. This indicates that mostly fats were being used to provide for energy during rest. This is

accurate since almost 100% of ATP is produced by aerobic metabolism during rest (Powers & Howley, 2011). Table 3 indicates that RER was about 0.9 immediately post-exercise, which indicates that majority of fuel was coming from carbohydrates (about 65%) and the rest was coming from fats (about 35%) (Mackenzie, 2011). After a single bout of moderately intense exercise, it has been shown oxygen uptake remains elevated for a few hours afterwards in a phenomenon known as excess post-exercise oxygen consumption (EPOC). During this time, oxygen uptake is elevated in order to "repay" the oxygen deficit that occurred during exercise. Research shows, however, that only about 20% of elevated oxygen actually goes to replenishing the muscle and blood oxygen stores. The rest of the oxygen uptake is used to resynthesize creatine phosphate stores, convert lactic acid to glucose, and decrease heart rate and body temperature (Osterberg & Melby, 2000). Our data shows that RER was significantly lower 2 hours post-exercise than during both pre-supplement and pre-exercise periods. This is largely due to EPOC occurring and plasma free fatty acids being the main source of fuel during recovery. Research shows that EPOC can last up to 38 hours; however, this of course depends on the intensity of the exercise session and the physical fitness of the person (Schuenke, Mikat & McBride, 2001).

Resting energy expenditure (REE) refers to the amount of calories that would be consumed for energy by the body in a 24 hour period of only resting conditions. The REE for our participants ranged from about 1600 to 1700 kcals/day during pre-supplement and pre-exercise periods. REE was significantly elevated to about 1850 kcals/day at two hours post-exercise period. Like RER, this elevation in REE is consistent with the EPOC concept, since body temperature and heart rate are elevated after exercise and thus more

energy is being consumed in order for the body to achieve homeostasis. Thus, the significant increase in REE that was seen 2 hours post-exercise was expected due to the exercise bout that was previously administered.

We had hypothesized that energy expenditure levels would be higher with the ingestion of capsaicin or evodiamine compared to the levels attained when ingesting a placebo supplement. By inciting the sympathetic nervous system, capsaicin and evodiamine would also be increasing heart rate and blood pressure as previously mentioned. Energy expenditure is also increased since the metabolic rate is provoked. Thus, we expected to see a greater reliance on fat oxidation and increase in energy expenditure during post supplementation, exercise, and recovery periods with the ingestion of capsaicin or evodiamine. Unfortunately, our data did not provide any time x supplement interactions for REE. Additionally, we had hypothesized that the consumption of capsaicin or evodiamine would lower RER rates, since these agents would stimulate lipolysis and fat oxidation through the increase of catecholamines in the bloodstream. Like REE, there were no time x supplement interactions for RER. We were not able to assume any elevated energy expenditure rates between supplements and were thus forced to reject our hypothesis.

Serum Lipid Oxidation Markers

When lipids are being oxidized, there is an increase in glycerol, free fatty acids, and catecholamines within the blood stream. When fat is the main source of fuel, fat oxidation is increased. As a result, upon up-regulation of lipolysis in adipose tissue, triglycerides are degraded and the resultant fatty acids and glycerol released into the blood. The fatty acids and glycerol are able to be cleared from the blood by skeletal

muscle, and they are ultimately used to generate ATP by way of oxidative phosphorylation. Although triglycerides can be a vital fuel source for aerobic exercise, our data did not indicate any time effects for triglycerides in the serum of our participants. In other words, triglycerides did not show any significant change over the course of the four sampling periods. This is not unexpected as the participants were fasted and not ingesting any dietary fat, and the likelihood of low-density lipoprotein oxidation by lipoprotein lipase should have been minimal during only 30 minutes of exercise. Glycerol and free fatty acids, which are the products of triglyceride breakdown, both showed significant time effects ($p < 0.05$). Free fatty acids were significantly increased immediately post exercise, while glycerol showed significantly low levels pre-supplementation. Although these time effects were significant, they are appropriate due to the circumstances of our experiment. Both free fatty acids and glycerol levels start off at their lowest level during pre-supplementation, as can be seen from Figures 3 and 4. Both variables then increase very little during the pre-exercise period. It can be assumed that this small increase is due to participants ingesting either a capsaicin or evodiamine supplement; however, this increase in levels during pre-exercise period compared to the pre-supplementation period is not drastic enough to be considered as significant. For this reason, we are not permitted to make such an interpretation. Both free fatty acid and glycerol levels then experience a sharp increase immediately post exercise, and this is due to the exercise protocol that was administered. Participants were required to undergo a moderately intense exercise in which their RER was kept at about 0.9 (VCO2/VO2), which indicates about 35% of fuel coming from fat and 65% coming from carbohydrates. Since there was a fair amount of fat being oxidized, it can be expected that free fatty acid

and glycerol levels would increase substantially within the bloodstream compared to rest. Two hours post exercise, free fatty acid and glycerol levels decreased; however, they were still elevated compared to the levels that were measured before exercise.

Figures 6 and 7 illustrate what is expected to occur when a moderately intense exercise bout is administered; however, our results indicate that these changes in variable levels were not large enough to be labeled as significant. This could be due to the fact that our sample size was limited to only seven participants, or that the dose of capsaicin and evodiamine was not great enough to elicit such a response. Our data does demonstrate a slight, expected variation; therefore, a larger sample size might boost variables into significance and thus have a better, more accurate depiction of time x supplement interaction as well as additional time effects for variables.

We had hypothesized that the consumption of either evodiamine or capsaicin supplements would increase serum and free fatty acid levels within the blood stream compared to the levels elicited by a placebo supplement. Being thermogenic agents, evodiamine and capsaicin increase core body temperature through thermogenesis which leads to an increase in catecholamines within the blood stream thus inciting lipolysis to occur. Lipolysis is the breakdown of triglycerides into glycerol and free fatty acids in order to be used as energy. Thus, even during post-supplementation resting period, we did expect to see an increase in serum free fatty acid and glycerol levels with the assumption that capsaicin and evodiamine would incite lipolysis to occur even at rest. We expected to see an even more elevated level of serum free fatty acid and glycerol levels immediately post exercise compared to normal levels when given either evodiamine or capsaicin supplements. Our data, however, fails to show any time x supplement

interaction for all lipid oxidation markers, and we were forced to reject this hypothesis as well.

Past experiments show both capsaicin and evodiamine as being activators of lipolysis. There are countless experiments that have been conducted with capsaicin, especially, and its ability to provoke fat oxidation. An experiment in 2003, for example, found that capsaicin works especially well in stimulating sustained fat oxidation in adults who had just lost weight compared to a placebo group (Lejeune, Kovacs & Westerp-Plantenga, 2003). Additionally, an experiment that tested capsaicin's benefits within the Japanese diet was observed. What was found was that an increase in both lipid oxidation and diet-induced thermogenesis occurred with the addition of red peppers to meals (Yoshioka et. al, 1998). Although the effects of capsaicin are well established, researchers are still hesitant to claim evodiamine as a thermogenic agent with capabilities similar to capsaicin's. One main reason for this is that much of the research with evodiamine has been conducted on rodents. Kobayashi and colleagues found that evodiamine prompted heat loss and dissipated food energy in rats, which ultimately prevented the accumulation of fat and body weight (Kobayashi et. al, 2000a). Another experiment conducted in 2004 established evodiamine as a successful vanilloid receptor agonist, at least for rats (Pearce et. al, 2004). Thus, past research supports the theory of evodiamine's potential thermogenic nature, at least for rodents; however, a well-constructed experiment centered on human consumption of evodiamine is still necessary in order to empirically establish such benefits.

Summary and Conclusion

The main reason for the recent surfacing of dietary supplements and various "magic" pills is largely due to the increasing amount of people suffering from obesity in the United States. Dietary supplements can be easily accessible and are advertised as an extra, easy means to assist in the weight loss process; however, many of the ingredients included in these supplement have yet to be empirically established as efficient thermogenic agents. Evodiamine is said to be an agent capable of stimulating the vanilloid receptor much like capsaicin. The purpose of this study was to look at evodiamine's effect on the human metabolism and compare it to the effects of capsaicin and a placebo supplement.

Although we did not attain the results we had hypothesized, the limited participant size makes it difficult to see any significant changes within supplements, since differences would have to be very large in order to see any significance. A review of recent literature shows that capsaicin elicits beneficial results to the metabolic system when administered repeatedly or when administered for an extended period of time (Onuhuki et al., 2001; Snitker et. al, 2009). Thus, the reason we did not see the results expected could be largely due to our participant size, the size of the supplement doses, or the time frame for which they were administered. One-time consumption of the supplement might not be enough to reach the expected responses that have been seen in experiments where supplements are administered every day for a week. Additionally, capsaicin has been shown to increase in efficiency when coupled with caffeine (Tsi et. al, 2003). Capsiplex™, a fairly new and popular dietary supplement, takes this to their advantage by including in their ingredients capsaicin and caffeine among other agents.

Countless thermogenic agents, such as ephedra and bitter orange, show enhanced effects when coupled with caffeine (Boozer et al., 2002; Colker et al., 1999). When thermogenic ingredients are combined, they are able to work synergistically by operating different mechanisms and thus have the potential to reach greater metabolic effects (Diepvens et al., 2007). Further research should be conducted on evodiamine and caffeine intake in order to establish perhaps increased benefits for the human metabolic system.

Another potential flaw in our research design could have come from the fact that our exercise protocol was perhaps too intense. Capsinoids have been seen to have their greatest effect on the metabolic system at rest (Josse et al., 2010). As discussed earlier in this chapter, many of our variables such as RER, REE, glycerol, and free fatty acids displayed a fairly small increase from pre-supplement to pre-exercise periods. Perhaps if the resting period was prolonged, the levels of these variables would increase into significance; however, because our exercise bout was administered 30 minutes after supplementation, the natural adrenergic effect of exercise could have possibly over-powered any adrenergic effects from the supplements. Future research could either prolong the exercise period, change the exercise protocol to a lower, longer intensity bout, or take exercise out entirely. This would possibly give room for the supplements to take effect on the metabolism and thus see significant results.

As a result of the limitations of our study, and in light of our present results and data presented herein we can only conclude that at the doses provided and time points sampled, neither capsaicin or evodiamine are effective at inducing thermogenesis and increasing fat oxidation.

BIBLIOGRAPHY

Bloomer, R., Harvey, I., Farney, T., Bell, Z., & Canale, R. (2011). Effects of 1,3-dimenthylamylamine and caffeine alone or in combination on heart rate and blood pressure in healthy men and women. *The Physician and Sportsmedicine*, *39*(3).

Boozer, C., Daly, P., Homel, P., Solomon, J., Blanchard, D., Nasser, J., Strauss, R., & Meredith, T. (2002). Herbal ephedra/caffeine for weight loss: a 6 month randomized safety and efficacy trial. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity*, *26*(5), 593-604.

Bui, L., Nguyen, D., & Ambrose, P. (2006). Blood pressure and heart rate effects following a single dose of bitter orange. *The Annals of Pharmacotherapy*, *40*(1), 53-57.

Colker, C., Kaiman, D., Torina, G., Perlis, T., & Street, C. (1999). Effects of citrus aurantium extract, caffeine, and st. john's wort on body fat loss, lipid levels, and mood states in overweight healthy adults. *Cuurent Therapeutic Research*, *60*(3), 145-153.

Davidson, N., & Moreland, P. (2011, July 26). Know your blood glucose target range. Retrieved from www.mayoclinic.com

Diepvens, L., Westerterp, L., & Westerterp-Plantenga, M. (2007). Obesity and thermogenesis related to the consumption of caffeine, ephedrine, capsaicin, and green tea. *American Journal of Physiology: Regulatory, Integrative, and Comparative Physiology*, *292*(1), R77-R85. Dimkpa, U. (2009). Post-exercise heart rate recovery: An index of cardiovascular fitness. *Journal of Exercise Physiology*, *12*(1), 19-22.

Ellinger, S., Muller, N., Stehle, P., & Ulrich-Merzenich, G. (2011). Consumption of green tea of green tea products: Is there an evidence for antioxidant effects from controlled interventional studies?. *Phytomedicine*, *18*(11), 903-915.

Galgani, J., & Ravussin, E. (2010). Effect of dihydrocapsiate on resting metabolic rate in humans. *American Journal of Clinical Nutrition*, *92*(5), 1089-1093.

Galgani, J., Ryan, D., & Ravussin, E. (2010). Effect of capsinoids on energy metabolism in human subjects. *British Journal of Nutrition*, *103*(1), 38-42.

Gordon, J. (2004). Banning ephedra: good call, poor vision. *Alternative Therapies in Health and Medicine*, *10*(6), 18-19.

Hursel, R., Viechtbauer, W., Dulloo, A., Tremblay, A., Tappy, L., Rumpler, W., & Westerterp-Plantenga, M. (2011). The effects of catechin rich teas and caffeine on energy expenditure and fat oxidation: a meta-analysis. *Obesity Reviews*, *12*(7), e573-e581.

Iida, T., Moriyama, T., Kobata, K., Morita, A., Murayama, N., Hashizume, S., Fushiki, T., Yazawa, S., Watanabe, T., & Tominaga, M. (2003). TRPV1 activation and induction of nociceptive response by a non pungent capsaicin like compound, capsiate. *Neuropharmacology*, *44*(7), 958-967.

Iwasaki, Y., Saito, O., Tanabe, M., Inayoshi, K., Kobata, K., Uno, S., Morita, A., & Watanabe, T. (2008). Monoacylglycerols activate capsaicin receptor, trpv1. *Lipids*, *43*(6), 471-483.

Kim, H., Park, J., Kim, J., & Ko, B. (2008). Effect of herbal ephedra sinica and evodia rutaecarpa on body composition and resting metabolic rate: a randomized, double-blind clinical trial in korean premenopausal women. *Journal of Acupuncture and Meridian Studies*, *1*(2), 128-138.

Kim, K., Kawada, T., Ishihara, K., Inoue, K., & Fushiki, T. (1997). Increase in swimming endurance capacity of mice by capsaicin-induced adrenal catecholamine secretion. *Bioscience, Biotechnology, and Biochemistry*, *61*(10), 1718-1723.

Kravitz, L. (2001). Exercise and resting blood pressure. *IDEA Fitness Journal*.

Kobayashi, Y., Hoshikuma, K., Nakano, Y., Yokoo, Y., & Kamiya, T. (2000b). The bronchoconstrictive action of evodiamine, an indoloquinazoline alkaloid isolated from fruits of evodia rutaecarpa, on guinea-pig isolated bronchus: Possible involvement on vanilloid receptors. *Planta Med*, *66*(6), 526-530.

Kobayashi, Y., Nakano, Y., Kizaki, M., Hoshikuma, K., Yokoo, Y., & Kamiya, T. (2000a). Capsaicin-like anti-obese activities of evodiamine from fruits of evodia rutaecarpa, a vanilloid receptor agonist. *Planta Medica*, *67*(7), 628-633.

Lee, M. (2011). The history of ephedra (ma-huang). *The Journal of the Royal College of Physicians of Edinburgh*, *41*(1), 78-84.

Leete, E., & Louden, M. (1968). Biosynthesis of capsaicin and dihydrocapsaicin in capsicum frutescens. *Journal of the American Chemical Society*, *90*(24), 6837-6841.

Lejeune, M., Kovacs, E., & Westerp-Plantenga, M. (2003). Effects of capsaicin on substrate oxidation and weight maintenance after modest body-weight loss in human subjects. *British Journal of Nutrition*, *90*(3), 651-659.

Mackenzie, B. (2001) Exercise Intensity and Energy Source. Retrieved from http://www.brianmac.co.uk/esource.htm [Accessed 17/4/2012]

National Center for Health Statistics, Centers for Disease Control, (2000). Prevalence of overweight and obesity among adults: United states, 1999.

Nilius, B., Vennekens, R., & Owsianik, G. (2008). Vanilloid transient receptor potential cation channels: An overview. *Current Pharmaceutical Design*, *14*(1), 18-31.

Nolano, M., Simone, D., Wendelschafer-Crabb, G., Johnson, T., Hazen, E., & Kennedy, W. (1999). Topical capsaicin in humans: parallel loss of epidermal nerve fibers and pain sensation. *Pain*, *81*(1-2), 135-145.

Mittelstadt, S., Nelson, R., Daanen, J., King, A. . . . Lynch, J. (2012). Capsaicin-induced inhibition of platelet aggregation is not mediated by transient receptor potential vanilloid type 1. *Blood Coagulation & Fibrinolysis*, *23*(1), 94-97.

Ogden, C., & Carroll, M. (2010). Prevalence of obesity among children and adolescents: united states, trends 1963-1965 through 2007-2008.

Ohnuki, K., Niwa, S., Maeda, S., Inoue, N., Yazawa, S., & Fushiki, T. (2001). Ch-19 sweet, a non-pungent cultivar of red pepper, increased body temperature and oxygen consumption in humans. *Bioscience, Biotechnology, and Biochemistry*, *65*(9), 2033-2036.

Osterberg, K. L. & Melby, C. L., 2000. Effect of acute resistance exercise on postexercise oxygen consumption and resting metabolic rate in young women. International Journal of Sport Nutrition and Exercise Metabolism, 10 (1), 71-81.

Pearce, L., Petukhov, P., Szabo, T., Kedei, N., Bizik, F., Kozikowski, A., & Blumberg, P. (2004). Evodiamine functions as an agonist for the vanilloid receptor trpv1. *Organic and Biomolecular Chemistry*, *2*(16), 2281-2286.

Powers, S., & Howley, E. (2011). *Exercise physiology: Theory and application to fitness and performance*. (7 ed.). Humanities and Social Science.

Reyes-Escogido, M., Gonzalez-Mondragon, E., & Vazquez-Tzompantzi, E. (2011). Chemical and pharmacological aspects of capsaicin. *Molecules*, *16*(2), 1253-1270.

Schuenke, M., Mikat, R., & McBride, J. (2001). Effect of an acute period of resistance exercise on excess post-exercise oxygen consumption: Implications for body mass management . *European Journal of Applied Physiology*, *86*(5), 411-417.

Shekelle, P., Hardy, M., Morton, S., Maglione, M., Mojica, W., Suttorp, M., Rhodes, S., & Jungvig, L. (2003). Efficacy and safety of ephedra and ephedrine for weight loss and athletic performance . *The Journal of the American Medical Association*, *289*(12), 1537-1545.

Snitker, S., Fujishima, Y., Shen, H., Ott, S., Pi-Sunyer, X., Furuhata, Y., Sato, H., & Takahashi, M. (2008). Effects of novel capsinoid treatment on fatness and energy metabolism in humans: Possible pharmacogenetic implications. *The American Journal of Clinical Nutrition*, *89*(1), 45-50.

Stipanuk, M. (2006). *Biochemical, physiological, molecular aspects of human nutrition*. (2 ed., pp. 278-279). St. Louis, Missouri: Saunders Elsevier.

Stohs, S., Preuss, H., & Shara, M. (2011). The safety of citrus aurantium (bitter orange) and its primary protoalkaloid p-synephrine. *Phytotherapy Research*, *25*(10), 1421-1428.

Szallasi, A., & Blumberg, P. (1999). Vanilloid (capsaicin) receptors and mechanisms. *Pharmacological Reviews*, *51*(2), 159-212.

Tewksbury, J., & Nabhan, G. (2001). Seed dispersal. directed deterrence by capsaicin in chilies. *Nature*, *412*(6845), 403-404.

Tsi, D., Nah, A., Kiso, Y., Ono, H., & , (2003). Clinical study on the combined effect of capsaicin, green tea extract and essence of chicken on body fat content in human subjects. *Journal of Nutritional Science and Vitaminology*, *49*(6), 437-441.

Ursu, D., Knopp, K., Beattie, R., Liu, B., & Sher, E. (2010). Pungency of trpv1 agonists is directly correlated with kinetics of receptor activation and lipophilicity. *European Journal of Pharmacology*, *641*(2-3), 114-122.

U.S. Department of Health and Human Services, (2008). 2008 physical activity guidelines for americans.

U.S. Department of Health and Human Services, Office of Disease Prevention and Health Promotion. (1994). *Dietary supplement health and education act*

Vennekens, R., Grzegorz, O., & Bernd, N. (2008). Vanilloid transient receptor potential cation channels: an overview. *Current Pharmaceutical Design*, *14*(1), 18-31.

Xiang, Y., Shang, H., Gao, I., & Zhang, B. (2008). A comparison of the ancient use of ginseng in traditional chinese medicine with modern pharmacological experiments and clinical trials. *Phytotherapy Research*, *22*(7), 851-858.

Yin, J., Zhang, H., & Ye, J. (2008). Traditional chinese medicine in treatment of metabolic syndrome. *Endocrine, Metabolic * Immune Disorders-Drug Targets*, *8*(2), 99-111.

Yoshioka, M., St-Pierre, S., Suzuki, M., & Tremblay, A. (1998). Effects of red pepper added to high-fat and high-carbohydrate meals on energy metabolism and substrate utilization in japanese women. *British Journal of Nutrition*, *80*(6), 503-510.

For Mom and Dad