THE ACUTE EFFECTS OF CONSUMING CAPSAICIN-RICH FOODS ON RESPIRATORY QUOTIENT AND ENERGY EXPENDITURE IN HEALTHY ADULTS: A PILOT STUDY

By

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The Acute Effects of Consuming Capsaicin-rich Foods on Respiratory Quotient and Energy Expenditure in Healthy Adults: A Pilot Study.

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at George Mason University

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DEDICATION

This is dedicated to my loving husband, Farrakh, and my caring parents, Raheel and Ghazala. They have always been there to support me and make me laugh, so I can overcome exasperating milestones and be successful. Thank you!

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I would like to thank the many friends, relatives, and supporters who have made this happen. My caring parents, Raheel and Ghazala, who provided countless resources for me. My beloved husband, Farrakh, who assisted me with daily tasks, utilized his own personal time, and participated in the study. My supportive brother, Hamza, who provided transportation and participated in the study. Dr. De Jonge, who helped support me emotionally throughout the process and provided me with phenomenal feedback. Dr. Slavin, who helped me obtain the necessary resources to complete my research and helped with calculations. McCormick & Company, Inc who provided be with a supply of powdered cayenne pepper for my study. Finally, thanks go out to the George Mason University for providing a clean, quiet, and well-equipped repository in which to work.

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LIST OF ABBREVIATIONS

Basal Metabolic Rate ..... BMR
Blood Glucose ..... BG
Blood Pressure ..... BP
Body Mass Index ..... BMI
Caffeinated Beverage ..... CB
Carbohydrate ..... CHO
Diastolic Blood Pressure ..... DBP
Energy Expenditure ..... EE
Fat Mass ..... FM
Fat-Free Mass ..... FFM
Free Fat Mass Index ..... FFMI
High-performance liquid chromatography ..... HPLC
Lean Mass ..... LM
Moderate-Vigorous Physical Activity ..... M-V PA
Non-esterified fatty acids ..... NEFAs
Respiratory Quotient ..... RQ
Resting Energy Expenditure ..... REE
Resting Metabolic Rate ..... RMR
Scoville Heat Units ..... SHU
Systolic Blood Pressure ..... SBP
Total Energy Expenditure ..... TEE
Transient receptor potential vanilloid subtype 1 ..... TRPV1
Volume of Carbon Dioxide VCO2
Volume of Oxygen VO2
Waist Circumference ..... WC

ABSTRACT

THE ACUTE EFFECTS OF CONSUMING CAPSAICIN-RICH FOODS ON RESPIRATORY QUOTIENT AND ENERGY EXPENDITURE IN HEALTHY ADULTS: A PILOT STUDY.

Rafia Virk, M.S.

George Mason University, 2019

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The public and healthcare providers wish to modify diets by incorporating healthy bioactive foods to manage weight and combat some of the negative consequences of obesity. Research suggests that food containing capsaicin, a bioactive ingredient in peppers, may be used as a tool for anti-obesity therapy through thermogenic effects, macronutrient increased fat/CHO oxidation ratio, negative energy balance, appetite regulation, and improved insulin sensitivity. The research objectives of this study are to determine acute changes in substrate oxidation and energy expenditure (EE) after consumption of a capsaicin-rich meal. The hypothesis of this study is that ingestion of a capsaicin-rich meal will result in a increase in fat oxidation and EE than from ingestion of a meal without capsaicin. We tested 10 adults (ages 21-42, BMI 21-33 kg/m^2) for two days using the Metabolic Cart to test their substrate oxidation and EE before and after consuming a standard meal for two hours. During the first test day, participants consumed a meal without capsaicin, and on the second test day they consumed the same meal except with an addition of 4tsp (8.20g) cayenne pepper containing 20.4mg

capsaicin. Blood glucose, blood pressure, and anthropometric measurements were also monitored for all participants. There was no significant difference in baseline RMR and fasting blood glucose levels between the two test days. The respiratory quotient (RQ) was significantly lower (p-value 0.018) for the capsaicin test day, favoring fat oxidation. The average EE change over time was 19±8 kcal/2hr and 25±11 kcal/2hr for the non-capsaicin test day and capsaicin day respectively and was statistically significant (p-value: 0.000). The blood glucose control was not significant (p-value 0.538). The best estimate threshold level positively correlated with EE (p=0.024). The BP between the two test conditions was not significantly different. This study reports a lower RQ favoring fat oxidation and a higher EE after the consumption of a capsaicin-rich meal than without. More research needs to be done with a larger data set to present a significant outcome for blood glucose control.

CHAPTER ONE: LITERATURE REVIEW

Obesity and Energy Balance

Obesity is a major problem for the United States and has been increasing for the past 30 years. More than 60% of Americans are overweight or obese, while more than 30% Americans are obese. Obesity is a major contributor to four of the six leading causes of death, which include cardiovascular disease, cancer, stroke, and diabetes [1]. Obesity results from a positive energy balance over a prolonged time. Energy balance consists of energy intake, energy expenditure and energy storage [2]. Energy intake is due to the digestion and breakdown of food into proteins, carbohydrates, fats, and alcohol. Total energy expenditure (TEE) is a combination of energy expended from the body due to resting metabolic rate (RMR), thermic effect of food, and physical activity. RMR is a key player in determining how much energy is used for bodily functions at rest. The thermic effect of food determines how much energy is expended due to metabolization and absorption of food. The excess energy that is not expended gets stored to increase body mass (60-80% is fat) [2]. To prevent changes in body weight, energy intake should equal energy expenditure. If energy intake is greater than energy expenditure, then we have a positive energy balance and thus an increase in energy storage. Consequently, to lose weight a negative energy balance is required, where energy intake is less than energy expenditure, resulting in a decrease in energy stores.

The body also plays a role in maintaining energy balance through complex mechanisms which are not completely understood yet. The body influences energy intake and TEE due to the status of energy stores and to prevent changes in energy balance. The

energy homeostasis system in our body prevents drastic changes in body mass and is regulated by the central nervous system and through hormones. This physiological control on energy balance explains why body weight is relatively stable day to day [2]. Research indicates that the energy homeostasis mechanisms in the body work stronger to prevent weight loss rather than gain it [3,4]. This indicates it is easier to prevent weight gain than to sustain a loss in weight in obese individuals. When there is a disturbance in the energy homeostasis, the hormonal controls do not function normally, and therefore fat accumulation, inflammation, insulin resistance, and other factors relating to obesity result [5].

Obesity and Hormonal Balance

The disturbance in hormonal balance in obesity can lead to type II diabetes. There are many mechanisms at play for development for type II diabetes, but research suggests the development of insulin resistance in people who are overweight or obese increases the risk of diabetes. Normally, adipose tissue moderates metabolism through the release of non-esterified fatty acids (NEFAs), glycerol, hormones (leptin and adiponectin), and proinflammatory cytokines [6]. Whereas, due to obesity there is an increase in adipose tissue which results in the increased release of these products, which play a role in developing insulin resistance [6]. The insulin resistance and dysfunction of beta cells producing insulin, leads to poor control of blood glucose levels. It is important to make efforts in preventing or treating obesity to reduce the increased risk of diabetes and other hormonal dysfunctions.

Resting Metabolic Rate and Respiratory Quotient

Resting metabolic rate (RMR), also known as resting energy expenditure (REE) is 75-95% of TEE. Energy expended due to metabolism in brain, liver, heart, and kidney involved 60-70% of RMR and is relatively constant throughout the day. Variation in RMR is dependent on gender, body weight, age, sleep, illness, starvation, and many other factors [7]. Body composition is responsible for great variation in RMR as well. Research suggests that fat-free mass (FFM) or muscle mass more strongly predicts RMR than fat mass (FM) [8]. In spite of this strong correlation, about 25% can be explained by genetics.[8]

RMR can be measured using direct or indirect calorimetry. Direct calorimetry directly measures the total amount of heat generated by the body. This technique requires the subject be placed in confined environment, which is insulated for temperature, for a long period of time and tends to be very costly because of it. It also does not provide any information on what substrates are being oxidized to produce the energy. On the other hand, indirect calorimetry is a more inexpensive and accessible method. In indirect calorimetry, energy expenditure can be obtained by measuring the amount of oxygen consumed and carbon dioxide expired from the body [8].

This can be accomplished using a metabolic cart which involves a hood chamber that can be placed over the participant's head with connected ventilation tube. The metabolic cart can use the volume of oxygen (VO_2) and volume of carbon dioxide (VCO_2) to calculate energy expenditure (EE) using a modified version of the Weir equation ($EE = (3.94*VO_2) + (1.1*VCO_2)$ [7]. The respiratory quotient (RQ) can also be calculated by dividing VCO_2 that was produced by VO_2 that was consumed ($RQ = VCO_2/VO_2$). The respiratory quotient reflects what substrate is being oxidized (fat, protein, or

carbohydrate) during the procedure [9,10]. For carbohydrate (CHO) oxidation, every 1 mol of glucose requires 6 moles of oxygen to be consumed and 6 moles of carbon dioxide to be produced. Since the same amount of oxygen is consumed and carbon dioxide is produced, their ratio is 1:1 (RQ =1). For fat oxidation, more moles of oxygen are consumed and less moles of carbon dioxide is produced, therefore the ratio is lower and is generally close to RQ = 0.7. For protein oxidation, urine needs to be collected because the nitrogen in urine has trace amounts of oxygen and carbon dioxide. One gram of nitrogen in urine indicates consumption of 6 L of oxygen and production of 4.8 L which give san RQ ratio of 0.8 [10]. Using these RQ ratios, it can be established what substrate is mainly being oxidized.

When a urine sample is not obtained, protein RQ cannot be obtained, and therefore the RQ from the metabolic cart is a non-protein RQ (npRQ). Non-protein RQ is when protein oxidation is eliminated from the equation so the RQ represents the ratio for fat and CHO oxidation. This npRQ can be advantageous for weight loss strategies. For example, an npRQ of 0.85 indicates around 50% of both fat and carbohydrate oxidation and does not include any protein oxidation. Some factors that can increase RQ include CHO intake, hyperventilation, overfeeding, exercise, and metabolic acidosis. Some factors that can decrease RQ include fat oxidation, hypoventilation, starvation, ketoacidosis, and gluconeogenesis [7]. RMR and RQ are good indicators for health status as they provide information on the primary substrate being oxidized which can be useful in helping people dealing with obesity.

Bioactive Ingredients

The public and healthcare providers wish to modify diets by incorporating healthy bioactive foods to manage weight. Great attention has been placed on manipulating foods and bioactive ingredients, such as green tea, calcium, caffeine, and capsaicin, to reduce effects of a positive energy balance by changing energy expenditure, appetite, and substrate oxidation [11]. Green tea research suggests that green tea can increase energy expenditure and fat oxidation. The polyphenols present in green tea such as catechins and epigallocatechin gallate are responsible for the positive effect on weight loss and weight maintenance [12]. Many studies in humans and rodents suggest that consumption of calcium rich foods result in weight loss. This might be achieved through promotion of lipolysis in adipocytes and through a decrease in fat absorption by some calcium related mechanism [13]. Caffeine also has a positive effect on energy balance. A study where 100 mg of caffeine was administered found an increase of 3-4% in RMR, increase in energy expenditure and increase in thermic energy costs [14].

An abundance of research has been done on the bioactive ingredient capsaicin present in hot peppers and capsaicin analogs also known as capsinoids (capsiate, dihydrocapsiate, and nordihydrocapsiate) [15]. Research suggests many health effects of foods containing capsaicin and its analogs. Effects include thermogenic effects, increased fat/CHO oxidation ratio, negative energy balance, appetite regulation, and improved insulin sensitivity [11].

Background on Capsaicin

Capsaicin is the main pungent compound present in peppers which belong to the Capsicum genus. These peppers include more than 200 variations and range from green to hot red in color with differing pungencies [11]. The hot red peppers include many pungent compounds known as capsaicinoids which include but are not limited to capsaicin, dihydrocapsaicin, and norhydrocapsaicin. Capsaicin contributes 70% of the pungency burn in hot red peppers, which confirms its role as a key player. There is a new breed of non-pungent red peppers called CH-19 Sweet (Capsicum annuum L.) which contains capsinoids instead of capsaicinoids. Capsinoids are capsaicin analogs which consist of capsiate, dihydrocapsiate, and nordihydrocapsiate [11].

Some research suggests that capsiate is the main capsinoid in CH-19 peppers and serves to have similar affects in body as capsaicin [11]. Capsaicin and capsiate slightly differ in their chemical structures which results in differing pungency properties. Capsaicin chemical name is (E)-N-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide and capsiate chemical name is 4-hydroxy-3-methoxybenzyl (E)-8-methyl-6-nonenoate. The structures in Figure 1, only differ in the center where capsaicin has an amide bond and capsiate has an ester bond [11].

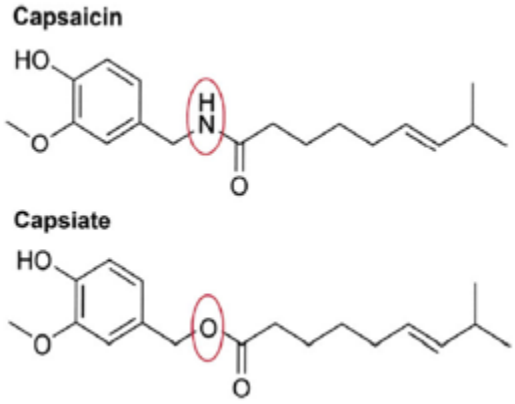

Figure 1 Capsaicin and Capsiate Chemical Structures
adapted from Ludy et al.[11]

The heat measurement of pepper is expressed in Scoville Heat Units (SHU). This unit represents the highest dilution of chili pepper extract where a human palette was originally used to detect heat. Now, instead of using a human taste panel, high-performance liquid chromatography (HPLC) is used to quantify the chemical entities which contribute to heat [16]. The number is then converted back to SHU. Peppers that are not pungent like bell peppers have an SHU of 0, whereas pungent peppers like Trinidad Moruga Scorpion can reach more than 2 million SHU [17].

Consumption of capsaicin is very different amongst different regions and cultures of the world. For example, the food culture in Mexico revolves around chili peppers. A study suggests that high capsaicin users within Mexican culture generally consumed around 9-25 jalapeño equivalents (90-250 mg capsaicin) per day whereas low consumers consumed <3 jalapeño equivalents (<30 mg capsaicin) per day. United States pepper

consumption is much lower than that of Mexican culture, where only 10.5% of US residents eat peppers on a daily basis [11].

The pungency of capsaicin is felt differently for users and non-users, since habitual users can get desensitized. When consumed, capsaicin will diffuse across the lingual epithelium in the oral cavity and bind to transient receptor potential vanilloid subtype 1 (TRPV1) receptors which are located on heat and pain sensory neurons. TRPV1 receptor is a non-sensitive calcium channel which will open upon capsaicin binding and result in an influx of calcium ions into the neuron leading to a release of neurotransmitter[11]. This action will also lead to feelings of warmth or pain depending on how much capsaicin is consumed. The presynaptic neurotransmitter, substance P, will become depleted after prolonged receptor activation and desensitization will result. So the next time capsaicin is consumed, the receptor won't respond nearly as much as it did the first time [11].

Capsaicin has a high binding affinity to TRPV1 receptors in the tongue and gut. It is absorbed passively with more than 80% efficiency in the stomach and duodenum. Capsaicin is more stable than capsiate and is metabolized by a variety of cytochrome P450 enzymes in the liver. Capsaicin binds to albumin for transportation to the adrenal glands to stimulate catecholamine release. Study suggests that the effects of capsaicin, like increasing EE, depend on simulation of β-adrenergic receptors [11]. Animal studies suggest that the catecholamine release results in an increase in substrate oxidation in muscles, glycogenolysis in liver, and lipolysis in adipocytes.[18–20] Some studies also report an increase in Sympathetic Nervous System (SNS) activity [11].

Both capsaicin and capsiate are responsible for the activation of sympathetic nervous system (SNS) and for increasing thermogenesis, but the heat loss in capsaicin is stronger. Unfortunately, capsaicin is responsible for elevating blood pressure and heart rate, whereas capsiate has no effect [21(p19)]. Since capsiate is less pungent than capsaicin and does not elevate blood pressure or heart rate, some studies like to use capsiate instead of capsaicin.

CHAPTER TWO: PAST RESEARCH ON CAPSAICIN AND ANALOGS

Energy Metabolism and Substrate Oxidation

Capsaicin and capsinoid research suggest that it has acute and long-term effects in increasing energy expenditure and decreasing respiratory quotient (RQ), implying a shift from carbohydrate oxidation to fat oxidation.[22–25] This can be explained due to the thermogenic effects and increased sympathetic activity (β-adrenergic stimulation) from capsaicin, as mentioned previously.[13] There are limited studies on long term effects of capsaicin consumption on resting metabolic rate and therefore it should be an area for further research.

A meta-analysis of nine studies on capsaicin and capsiate found EE to increase by 58 kcal/day and RQ to decrease by 0.216, suggesting an increase in fat oxidation.[26] A recent single-blind crossover study on obese young adults (n=10) found REE to increase after capsaicin (2mg) administration by 149kcal/day than by placebo.[27] 13-week Dutch study on obese individuals found a 119 kcal/day increase in EE compared to placebo after consumption of 135 mg/day capsaicin capsule with a meal [28]. However, a 4-week Australian study found no increase in EE in overweight individuals who consumed a 33 mg/day capsaicin blend.[29,30]

Other acute studies, performed shortly after consumption of a meal containing chili in lean adults, show an increase in energy expenditure (EE) as well as an increase in carbohydrate oxidation in males [23,31] and an increase in fat oxidation in females. [32] A more recent crossover study also found an increase in EE after administration of 2.56 mg of capsaicin and an increase in fat oxidation throughout the day. The study hypothesized

that increasing fat content of the meal with addition to capsaicin will also increase the amount of fat oxidation [24]. [27] A 4-week randomized double-blind study on ingestion of capsinoids found an increase in EE but also found an increase in fat oxidation that was positively correlated with BMI.[33]

However, there are some conflicting studies that report no effect on macronutrient oxidation [34–36] and EE [29,35,37]. A meta-analysis review on capsaicin suggests that a potential explanation for inconsistencies in studies might be due to thermogenic effects varying with differing body composition, which has been observed with other bioactive compounds [11]. This meta-analysis review on capsaicin also suggests that out of all the studies done on obese individuals, 4 show an increase in EE and 2 showed no effect [11].

The conflicting findings suggest further research needs to be done to determine effect of capsaicin and analogs on EE. Body composition is a major factor in determining risks for disease, therefore it needs to be included in studies relating to obesity. Crossover studies are the best predictors of capsaicin effect and takes body composition into account. Since most studies with inconsistent results were performed using capsaicin analogs, another explanation might be due to the degree of thermogenic effects between capsaicin and other capsinoids. Most studies that reported an increase in EE were done using lean subjects with no reference to body composition. The studies on overweight subjects provide inconsistent capsaicin effects on EE [11]. Additionally, there are few studies on obese individuals. More research needs to be conducted on a wide spectrum of BMI to elucidate the effect of capsaicin on EE and its correlation with bodyweight.

Glucose Metabolism

Other research has shown capsaicin to not only have a role in helping with weight loss, but also in helping with negating one of the chronic problems due to weight gain, diabetes. Inclusion of capsaicin-containing foods in the diet has been demonstrated to reduce effects of diabetes by improving insulin sensitivity in both animal models and humans. Studies on human subjects concluded that the habitual consumption of chili peppers may help alleviate effects of hyperinsulinemia [29]. A study on diabetic rats found capsaicin to reduce blood glucose levels and increase insulin levels after 28 days of 6 mg/kg by weight capsaicin administration. These results were compared to results for after administration by capsiate. The effect was greater from capsaicin administration than from capsiate and it was concluded that the pungent characteristic in capsaicin might be the cause [38].

In another study on rats, capsaicin and capsiate were both found to enhance insulin sensitivity and reduce visceral fat accumulation. There was also an improvement in glucose tolerance test as well, without changing energy intake. The β-cells responsible for insulin secretion also increased by mass due to enhanced proliferation and decreased apoptosis after capsaicin or capsiate administration [39]. There are limited studies pertaining to clinical trials on diabetic patients and how capsaicin might improve their blood glucose levels and enhance their insulin sensitivity. Further research should investigate how glucose metabolism and glucose tolerance is affected by capsaicin-containing foods and how it is relevant in preventing or treating onset of diabetes.

Weight Management

Capsaicin influences body weight due to an increase in EE and through appetite regulation. Self-reported data from individuals in various studies show decreased appetite for fatty foods, hot foods, sweet foods, salty foods, and food in general [11]. A cross-sectional study on "sensory and gastrointestinal (GI) effects of capsaicin on food intake" for 24 human subjects found similar results. The study reported a statistically significant increase in satiety for both men and women. Satiety was measured using energy intake area under the curve, which increased from 689 to 757 mmh for men and 712 to 806 mmh for women. They concluded that both oral and GI exposure of capsaicin enhanced satiety and decreased both fat and energy intake, but the oral capsaicin exposure had a stronger effect possibly due to pungency of capsaicin [40].

Another study suggests that the hunger hormone ghrelin is inhibited with capsaicin consumption while concentrations of glucagon-like peptide hormone is increased [36]. The feeling of fullness from capsaicin consumption with food has shown to decrease intake of total energy, fat, carbohydrates, proteins, and energy density in other studies as well [11]. A more recent study, found that replacing carbohydrates with proteins and adding capsaicin during energy restriction provided increased EE with addition to appetite fulfillment.[41] In conclusion, during energy restriction or balance, addition of capsaicin and analogs to food could help with satiety and fullness, and therefore aid in successful weight loss and weight management efforts.

Capsaicin Desensitization

Research is lacking whether the effect of capsaicin on EE will diminish after long-term capsaicin exposure. Every individual has a different response (threshold level) to capsaicin due to many factors such as the availability of capsaicin receptor TRPV1 present. Habitual users of capsaicin have been known to be desensitized to the pungency of capsaicin.[11] It is not clear if desensitization or level of sensitivity to capsaicin changes its effects on the body. There are few studies on humans where capsaicin action primarily takes place. A Japanese research study on males found that capsaicin action takes place in the gut instead of the mouth and is responsible for altering food behavior by decreasing fat and energy intake. [42]

Research suggests that the effects of capsaicin on satiety and hypermetabolism are due to capsaicin-sensitive abdominal afferent vagal nerve fibers.[43–45] A recent study on rats suggests the hypometabolic adaptation to fasting can be blocked by capsaicin administration due to desensitization.[46] This allows for enhanced energy expenditure and therefore more weight loss. Another study suggests that after long-lasting capsaicin desensitization, rats did not acquire age-associated obesity, implying enhancement of catabolic tone after desensitization.[47] Steiner et al. demonstrated that the TRPV1 receptor is responsible for inhibiting the cold-defense effector in the body.[43] It can be speculated that if the TRPV1 receptor is desensitized, than the cold-defense response will be enhanced, thereby increasing metabolic rate. Another study on rats suggests that TPRV1 may also be involved in fasting energy balance.[48] This implies, that the hypometabolism mechanism due to fasting may be attenuated in the absence/desensitization of TPRV1 channels.

In conclusion, long-term capsaicin exposure can desensitize hypometabolic mechanisms during periods of fasting or energy restriction. This suggests desensitization by capsaicin has an increased effect on EE and contributes to continued weight loss. Further research needs to be done on humans to see if this effect holds true.

CHAPTER THREE: SIGNIFICANCE AND HYPOTHESIS

Significance

This study explores the potential for a tool to help with weight management. This study aims to see if the inconsistent results from previous studies hold true. The results of this study can perpetuate further research that can resolve the inconsistencies from past research. Future research on capsaicin can provide public and health professionals with resources to aid in weight loss and improve health. If obesity can be prevented or reduced by consuming capsaicin-rich foods, then incidence of cardiovascular disease, cancer, stroke, and type II diabetes might decrease as well. Consequently, healthcare costs would be reduced, and public's efforts and resources can be directed elsewhere for other pressing matters.

Specific Aims and Hypothesis

The aims of this study will be:

Primary Aim: To investigate changes in respiratory quotient following consumption of capsaicin-rich foods compared to non-capsaicin foods.

- Hypothesis: Addition of capsaicin, to a meal versus a standard meal, will result in a decrease in respiratory quotient, suggesting an increase in fat oxidation.

Secondary Aim: To explore the potential of capsaicin-rich foods in increasing postprandial energy expenditure (EE) compared to non-capsaicin foods.

- Hypothesis: Ingestion of capsaicin-rich-foods will result in a higher EE than ingestion of foods without capsaicin.

Exploratory Aim 1: To determine the best estimate threshold of capsaicin and see if it is directly related to changes in postprandial EE.

Exploratory Aim 2: To determine the effect of capsaicin-rich foods by exploring changes in blood glucose after a standardized meal.

CHAPTER FOUR: MANUSCRIPT

Introduction

Obesity is a major contributor to four of the six leading causes of death in the U.S., which include cardiovascular disease, cancer, stroke, and diabetes. Research suggests that obesity can lead to higher risk for developing insulin resistance.[1] This high insulin resistance will lead to abnormal glucose metabolism, which if untreated can develop into type II diabetes mellitus (T2DM). The insulin resistance is increased due to the elevated adipose tissue in the body. Therefore, it is important to prevent or treat obesity.

Research suggests capsaicin, a bioactive ingredient in peppers, may combat some of the negative consequences of obesity through thermogenic effects, increased fat/CHO oxidation ratio, increased metabolic rate, appetite regulation, and improved insulin sensitivity.[11] Capsaicin is a compound that contributes 70% of the pungency in red hot peppers. The heat measurement of pepper is expressed in Scoville Heat Units (SHU). The heat from peppers is first quantified by high-performance liquid chromatography (HPLC) and then converted to SHU. [16] The SHU unit represents the highest dilution of chili pepper extract where a human palette is able to detect heat.

Past research suggests that capsaicin stimulates catecholamine release which is involved in substrate oxidation in muscles, glycogenolysis in liver, and lipolysis in adipocytes.[11] It also activates the sympathetic nervous system which leads to an increase in heart rate and blood pressure and thus an increase in energy expenditure.[11] Acute studies, done shortly after consumption of a meal containing chili in lean adults, show an

increase in energy expenditure (EE) as well as an increase in carbohydrate oxidation in males and an increase in fat oxidation in females.[23,31,32] A meta-analysis of nine studies on capsaicin and capsiate found EE to increase by 58 kcal/day and RQ to decrease by 0.216, suggesting an increase in fat oxidation.[26] A recent single-blind crossover study on obese young adults (n=10) found REE to increase after capsaicin (2mg) administration by 149kcal/day than by placebo.[27] A 13-week Netherlands study on obese individuals found a 119 kcal/day increase in EE compared to placebo after consumption of 135 mg/day capsaicin capsule with a meal.[28] However, a 4-week Australian study found no increase in EE in overweight individuals who consumed a 33 mg/day capsaicin blend.[29]

Previous research with capsaicin suggests a shift from carbohydrate oxidation to lipid oxidation.[24,28,32,36] This shift implies less fat is being stored and therefore decreases the chance of weight gain. One randomized double-blind 4-week study found that consuming 135 mg/day capsaicin with meals resulted in 1g/h increase in fat oxidation which is around 168 g of fat burned per week.[28]

Inclusion of capsaicin-containing foods in the diet has been demonstrated to reduce effects of diabetes by improving insulin sensitivity in both animal models and humans. Studies on human subjects concluded that the habitual consumption of chili peppers may help alleviate effects of hyperinsulinemia.[29] There are limited studies on humans but studies on rats show that capsaicin enhanced insulin sensitivity, reduced visceral fat, reduced blood glucose levels, and increased blood insulin levels [38,39]

Every individual has a different response (threshold level) to capsaicin due to many factors such as the availability of capsaicin receptor TRPV1 present. Habitual users

of capsaicin have been known to be desensitized to the pungency of capsaicin.[11] It is not clear if desensitization or level of sensitivity to capsaicin changes its effects on the body. There are few studies on humans where capsaicin action primarily takes place. A Japanese research study in men, found that capsaicin action takes place in the gut instead of the mouth and is responsible for altering food behavior by decreasing fat and energy intake. [42]

Research suggests that the effects of capsaicin on satiety and hypermetabolism are due to capsaicin-sensitive abdominal afferent vagal nerve fibers.[43–45] A recent study on rats suggests the hypometabolic adaptation to fasting can be blocked by capsaicin administration due to desensitization.[46] This allows for enhanced energy expenditure and therefore more weight loss. Another study suggests that after long-lasting capsaicin desensitization, rats did not acquire age-associated obesity, implying enhancement of catabolic tone after desensitization.[47] Steiner et al. demonstrated that the TRPV1 receptor is responsible for inhibiting the cold-defense effector in the body.[43] It can be speculated that if the TRPV1 receptor is desensitized, than the cold-defense response will be enhanced, thereby increasing metabolic rate. Another study on rats suggests that TPRV1 may also be involved in fasting energy balance.[48] This implies, that the hypometabolism mechanism due to fasting may be attenuated in the absence/desensitization of TPRV1 channels.

These studies suggest that long-term capsaicin exposure can desensitize hypometabolic mechanisms during periods of fasting or energy restriction. This suggests

desensitization by capsaicin has an increased effect on EE and contributes to continued weight loss. Further research needs to be done on humans to see if this effect holds true.

Most of the studies mentioned have mainly used capsules containing capsaicin instead of capsaicin-rich foods due to it being difficult to measure the exact amount present. This has led to some toxic effects such as gastric distress and therefore it might be beneficial for capsaicin to be consumed from food sources [11]. For this reason, our study tested the effects of capsaicin consumption from cayenne pepper powder. This study will contain two test days: one test day is when the participants consume a standardized meal without capsaicin and another test day is when they consume a meal rich in capsaicin. The aim of this research was to investigate the 2-hour effect of a meal containing capsaicin on respiratory quotient and energy expenditure. We hypothesized that respiratory quotient will decrease, and EE would increase after consumption of a meal containing capsaicin compared to the standard meal. We were also interested in how an individual's sensitivity (threshold level) to capsaicin is related to their postprandial maximum energy expenditure and if there are any acute effects of capsaicin on blood glucose control.

Methods

Participants

Ten healthy participants (21-42 years old) with a body mass index (BMI, kg/m^2) ranging from 21-33kg/m^2, were recruited for this study. Participants were recruited by posters distributed on George Mason University (GMU) campus bulletin boards and by

word of mouth. Interested individuals then contacted the investigator by e-mail or phone. Participants completed a screening questionnaire to determine eligibility. The survey inquired about height, weight, medical conditions, medications and inclusion/exclusion criteria and availability. If participants were determined eligible, they were contacted to set up a series of two study visits and pre-test instructions were provided. For all appointments, participants were asked to arrive to the test location after a 12-hour fast, and were asked to abstain from caffeine, alcohol, and have at least 8-hours of sleep the night before. Written and signed consent forms were obtained from all participants before any testing. The study was approved by the GMU institutional review board for ethical consideration and was registered at clinicaltrials.gov (NCT03859583).

Study Design

This study has a non-randomized crossover intervention study design. Participants arrived at the Nutrition Assessment Laboratory between 8:00 and 8:30am after a 12-hour fast. Details of the study were re-iterated and informed consent was obtained first on the morning of the first appointment. Eligibility was confirmed by measuring height and weight and BMI calculation, and an over the counter pregnancy test was given when applicable. After a 30-minute rest, baseline measurements fasting blood glucose, blood pressure, heart rate and RMR were obtained.

After obtaining baseline measures for RMR, participants were given 15 minutes to consume a standard meal in its entirety Following the meal, the postprandial energy expenditure (EE) was measured for 120 minutes. Participants were allowed a five-minute

break every 30 minutes during the 120 minutes of EE testing. During this time, glucose levels were measured. The blood pressure was measured after the first 30 minutes of postprandial EE testing and at the end. After completion of the measurements the subjects participated in the threshold test for capsaicin. This appointment lasted for a maximum of five hours.

The second test day was scheduled within the first week of the study. They consumed the same standardized meal as their first appointment but with the addition of capsaicin (4tsp cayenne pepper powder form) in their meal. After EE testing, for 2-hours, their body composition was determined. This appointment lasted around five hours. At the end of each test day, the participants completed a 24-hour recall of the meals they ate the day before.

Test Meals

For this study, participants were required to consume 4 teaspoons of powdered cayenne pepper (8.20 grams, 20.4 mg capsaicin; 26,000 SHUs from McCormick & Company, Inc.) in a standardized meal . The meal composition of the standardized meal is presented in Table 1. The standardized meal consisted of two large scrambled eggs, one butter pat, one bottle of orange juice, and two slices ofbread. The scrambled eggs were prepared with one teaspoon of olive oil and had a pinch of salt. For each participant, the meals were prepared the same way for both days, except for one of the days four teaspoons of cayenne pepper were added to their meal.

Table 1: Meal Composition

Food	Amount	Energy (kcal)	CHO (g)	Fat (g)	Protein (g)
Eggs	100 (g)	140	0	10	12
Juice	296 (mL)	140	33	0	2
Bread	52 (g)	140	26	2	6
Butter	5(g)	36	0	4.1	0
Olive oil	1 tsp	40	0	4.7	0
Total	n/a	496	59	20.8	20
Total (kcal)	n/a	n/a	236	187.2	80
% of Total Energy	n/a	n/a	48	38	16

Anthropometric measurements

The weight and height were measured for each participant on their first appointment by Weight scale and Height scale, a Healthometer 752KL scale (Perspective Enterprise, Portage MI). Waist circumference was obtained at the level of the umbilicus.

Respiratory Quotient and Energy Expenditure

Energy expenditure and RQ were assessed by indirect calorimetry using a QUARK RMR metabolic cart (Cosmed Inc. Rome Italy), Accuracy of the QUARK RMR was assessed monthly by complete ethanol combustion. Carbon dioxide recovery was 96.2+/- 0.1% over the past 16 months. Participants were asked to lay still and awake during both procedures. Participants could listen to music or watch videos on their electronic devices, if it did not interfere with the study. For each 30-minute measurement period, the first five minutes of the RMR data was deleted to eliminate random error and allow the participant's metabolism to stabilize. The baseline RMR was determined by averaging the data. The EE was determined by obtaining averages of postprandial EE data at 15, 30, 45, 60, 75, 90, 105, and 120 minutes. To calculate the meal response in EE

from the RMR after consuming a meal, the EE was subtracted from the RMR and converted to units of kcal/2hr. The area under the curve for the EE data was obtained to compare the thermic effect of the meals. The postprandial maximum EE change from baseline over the two-hours at a single time point was determined for each individual.

Threshold test

Participants' capsaicin threshold level was tested and compared with the maximum change in their EE due to the thermic effect of food. The threshold test experimental design was modified from the design presented by Schneider et al.[49] Because capsaicin cannot dissolve in water, a 1% polysorbate solution was used. The polysorbate solution was prepared by mixing 10 g of polysorbate powder (manufacturer: Milwaukee) with 1 liter of water. Pure 10 mg capsaicin powder (manufacturer: Allentown) was first added to 1 liter of 1% polysorbate to make 10 mg/L capsaicin solution. According to literature, the estimated threshold concentration for capsaicin in aqueous solutions is 0.310 ppm [50]. Therefore, serial dilutions were done to encompass the 0.310 ppm threshold and obtain the following concentrations of capsaicin solutions: 0.0195, 0.0391, 0.0781, 0.1562, 0.3125. 0.625, and 1.25 mg/L.

The study design was set up into seven rows and three columns. Each row consisted of three testing cups containing 5 mL of solution. One of the testing cups had the capsaicin solution (test sample) and the other two contained the 1% polysorbate solution (blank sample). The study design is displayed in Figure 1. The participants were asked to test the samples from left to right starting at the bottom row with the lowest

concentration. They were told to place at least half of the sample in their mouth, swirl it around and swallow it. They were asked to identify the sample that was different from the others. If they were uncertain, they were forced to make a choice. They also identified the localization of the burn: tongue, throat, or both. If the burn location could not be identified they were asked to mark unknown.

The individual best estimate threshold for each participant was determined by calculating the geometric mean of the last missed and first correct concentrations (starting at the point where there was two consecutive correct concentrations). The best estimate threshold for the group was calculated by taking the geometric means of the individual threshold estimates. These threshold estimates were plotted against the maximum EE for each participant to determine a relationship.

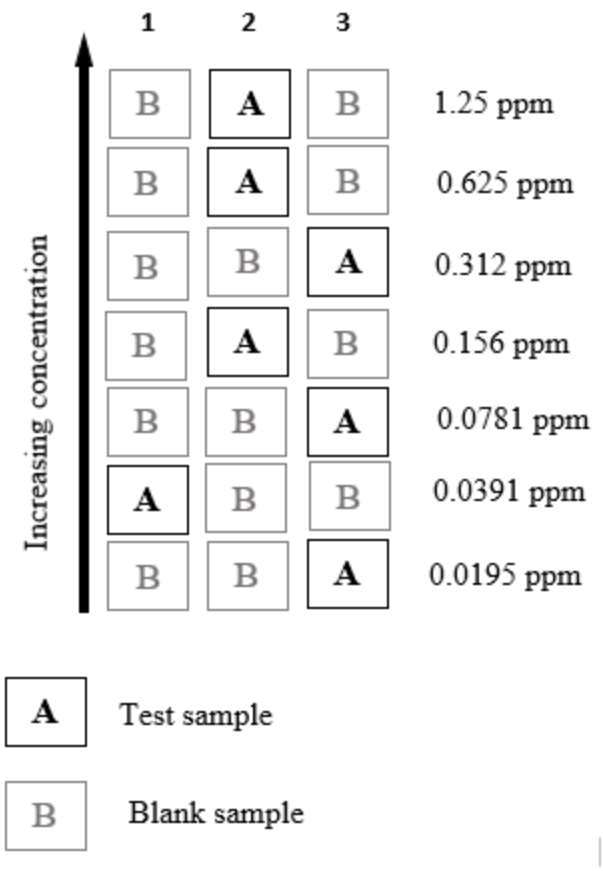

Figure 2: Threshold Test Experimental Design

Body composition

The body composition was determined by Dual energy x-ray absorptiometry (DXA, Hologic Horizon densitometer Hologic, Boston MA) scan once for each participant. This test involves lying on a table while wearing lose fitting, comfortable clothing. The scanner uses a low dose x-ray to determine the amount of fat, muscle and bone in your body.

Blood Glucose

Blood glucose was measured using a glucometer five times each day (contour NEXT EZ; Bayer Leverkusen Germany) The first measurement was in a fasted state as the participant came in. The other four measurements at 30min intervals after meal consumption.

Blood Pressure and Heart Rate

Blood pressure and heart rate were obtained, using a blood pressure automatic monitor (Omron 7 series; Omron Shimogyō-ku, Kyoto), three times each day. The first measurement was at the start of the study. The other two were 30 mins and 120 mins after meal consumption.

Statistical analysis

SPSS statistical analysis software was used in the analysis of all data (**IBM, version 23**). The normality test shows EE and RQ were normally distributed. One-way ANOVA for repeated measures was performed to evaluate the time effect and treatment effect. Paired sample t-test were used for treatment effect. Linear regression analysis (covariate) was used to predict the relationship between best-estimate threshold and maximum postprandial EE. The statistical tests for EE and EE(AUC) are one-sided and all other statistical tests are two-sided. The differences are considered statistically significant if $p < 0.05$.

Results

Participant characteristics

Descriptive characteristics of the participants are presented in Table 2. Out of these participants, two were normal weight, four were overweight, and four were obese. The group had a normal starting blood pressure (BP), with a systolic BP of 118±14 mmHg and diastolic BP of 74±9 mmHg.

The participants were primarily Asian (60%), followed by Caucasian (30%), and Hispanic (10%). The questionnaire reported that most of the participants consumed at least 0.5-1 teaspoon of pepper per week. Eighty percent of the participants drank three or less alcoholic drinks per week. Fifty percent drank one caffeinated beverage (CB) per day, followed by 20% who drank two CBs, 10% who drank three CBs, and 10% who drank 4 CBs per day.

Table 2: Descriptive Participant Characteristics

		Male (n=4)	Female (n=6)	Total (n=10)
Mean Values ± Standard Deviation				
	Age (years)	28±9	28±5	29±6
	Height (m)	1.80±0.06	1.66±0.08	1.72±0.10
	Body Weight (kg)	94.7±13.4	75.8±10.7	83.4±14.8
	BMI (kg/m^2)	29.1±3.6	27.6±3.7	28.2±3.5
	FFMI (kg/m^2)	20.3±1.2	17.0±0.5	18.3±1.9
	WC (m)	1.01±0.13	0.90±0.04	0.95±0.10
	LM (kg)	69.1±6.6	48.8±3.7	56.9±11.5
	FM (kg)	27.0±11.9	28.3±10.0	27.8±10.2
	Body fat (%)	27.2±9.9	35.8±9.3	32.4±10.0
	Systolic BP (mmHg)	127±18	112±6	118±14
	Diastolic BP (mmHg)	80±10	70±7	74±9
Ethnicity				
	Caucasian (%)	25	33	30
	Hispanic (%)	0	17	10
	Asian (%)	75	50	60
Weekly Average Pepper Consumption				
	0-0.5 tsp (%)	25	50	40
	0.5-1 tsp (%)	50	50	50
	1-1.5 tsp (%)	25	0	10
Weekly Alcoholic Drinks				
	0-3 drinks (%)	75	83	80
	4-7 drinks (%)	25	0	10
	8-11 drinks (%)	0	17	10
Daily Caffeinated Beverages				
	1 beverage (%)	75	33	50
	2 beverages (%)	0	50	30
	3 beverages (%)	25		10
	4 beverages (%)	0	17	10
Weekly Participation in M-V PA				

0-1 days (%)	25	50	40
2-3 days (%)	25	17	20
4-5 days (%)	25	17	20
6-7 days (%)	25	17	20

FFMI: Fat Free Mass Index; WC: Waist Circumference;
LM: Lean Mass; FM: Fat Mass; BP: Blood Pressure; M-V PA: Moderate-Vigorous Physical Activity.

Homogeneity between Appointments

There were no significant differences in baseline RMR, RQ, and BG between the two test-days. or between calories consumed prior to both appointment days.

Respiratory Quotient

The average baseline RQ for all participants for both test days was 0.741 After capsaicin containing food was consumed, the RQ value decreased below their baseline, which indicates metabolism shifted more from carbohydrate oxidation to lipid oxidation. The average postprandial RQ change from baseline RQ at certain time intervals is displayed in Figure 3. One-way ANOVA for repeated measures suggests the data at different time points are significantly different (p-value: 0.037). The average RQ on capsaicin test day for all participants was significantly lower (p-value: 0.018) than the average RQ on no-capsaicin test days, suggesting an increase in fat oxidation after capsaicin consumption. The RQ change from baseline RQ for each participant is displayed in Table 3 and was not statistically significant (p-value: 0.484).

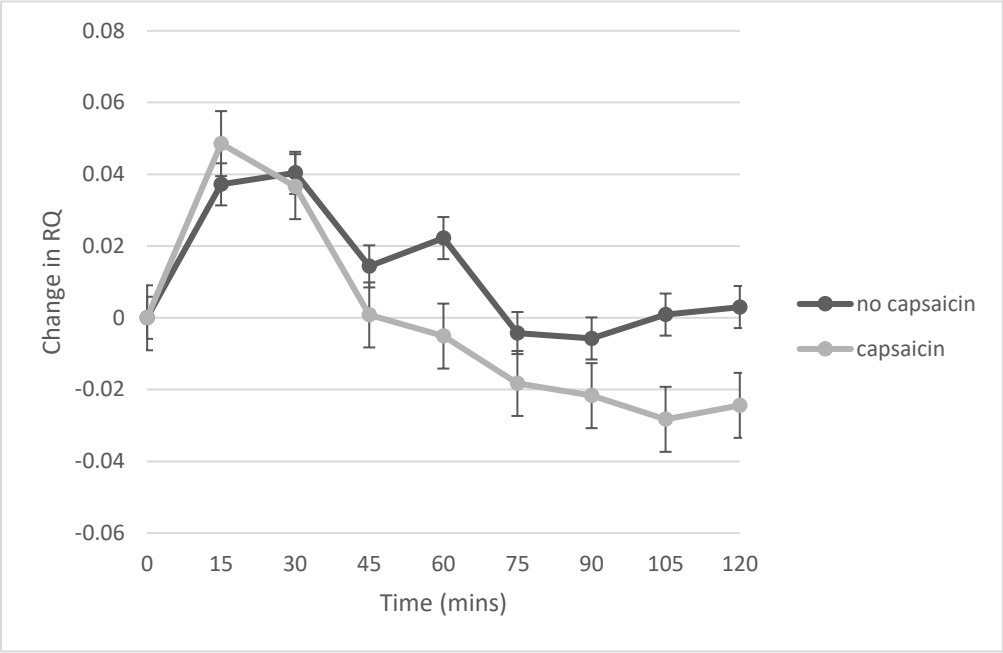

Figure 3: Average respiratory quotient (RQ) change over time for all participants; p-value: 0.018

Table 3: Change from baseline of Respiratory Quotient and Energy Expenditure and Blood Glucose

Participant ID	RQ	RQ-C	MAX Δ EE kcal/2hr	MAX Δ EE-C kcal/2hr	EE (AUC) kcal/2hr	EE-C (AUC) kcal/2hr	BG (AUC)	BG-C (AUC)
1	-0.007	0.084	12	25	96	248	1380	3270
2	-0.005	-0.019	40	48	294	370	6390	1110
3	0.013	-0.018	41	58	434	510	1140	120
4	0.0144	0.003	26	29	292	258	705	1770
5	0.027	0.008	31	29	362	115	2565	1980
6	0.027	0.011	20	28	84	307	765	-105
7	-0.002	0.021	32	60	283	694	-615	3090
8	0.007	-0.022	37	43	408	426	300	1575
9	0.022	0.019	41	34	225	290	1485	1335
10	0.054	-0.047	36	34	225	432	525	1890
Average	0.015±.018	0.004±0.033	31±10	39±13	270±118	365±161	1464±1921	1604±1089
p-value		0.484		0.025		0.060		0.858

RQ: Respiratory quotient; RQ-C: Energy expenditure from capsaicin group; EE: Energy expenditure; EE-C: Energy expenditure from capsaicin group; AUC: Area under the curve; BG: Blood glucose; BG-C: Blood glucose for capsaicin group

Postprandial Energy Expenditure

The postprandial maximum EE change from baseline for each participant is displayed in Figure 4 with a p-value of 0.025 from a paired t-test. The total group maximum EE change was 31±10 kcal/2hr for the non-capsaicin test days and 39±13 kcal/2hr for capsaicin test days (Table 3).

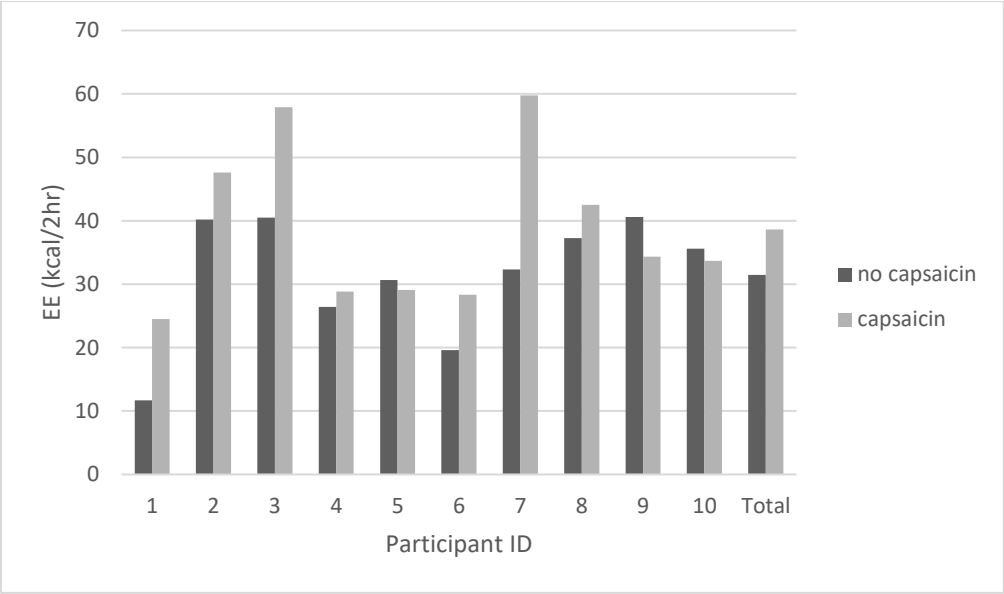

Figure 4: Maximum energy expenditure (EE) change from baseline for each participant; p-value: 0.025

Figure 5 shows the average EE at certain time intervals during the 2-hours of post-meal testing. From statistical analysis, it is evident that the average EE on capsaicin test day for all participants was significantly higher (p-value: 0.000 than the average EE on no-capsaicin test days. The average EE change was 19±8 kcal/2hr and 25±11 kcal/2hr for the non-capsaicin test day and capsaicin day respectively. The average difference in

non-capsaicin and capsaicin test day for EE for all participants is 0.77±0.31 kcal/15mins or 74±30 kcal/day.

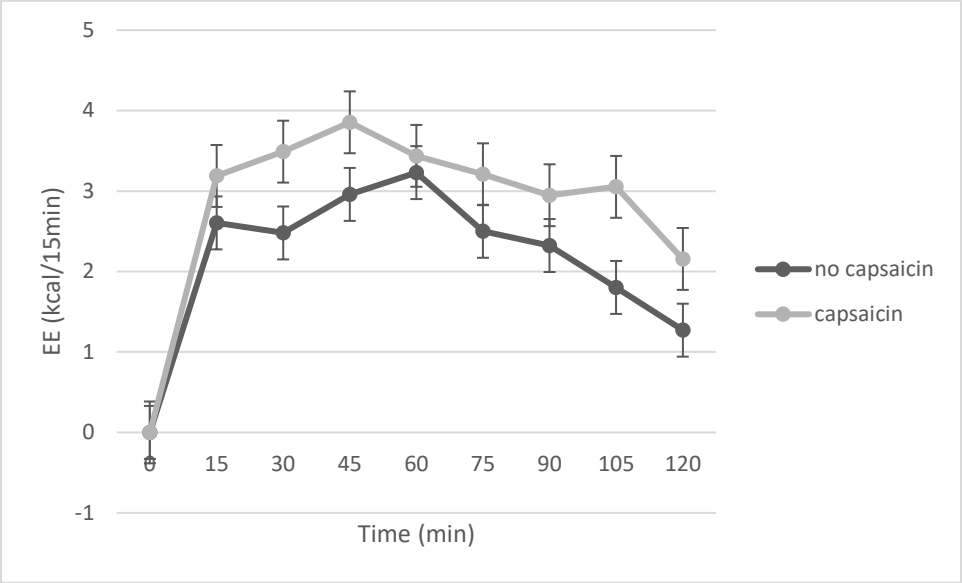

Figure 5: Average energy expenditure (EE) change over time for all participants; p-value: 0.000

The EE area under the curve (AUC) for each participant is displayed in Table 3 and Figure 6. The average AUC for non-capsaicin and capsaicin test days is 270±118 kcal/15min and 365±161 kcal/15min respectively. This EE(AUC) represents the thermic effect of food over 2 hours, which was not found to be significant (p-value: 0.060).

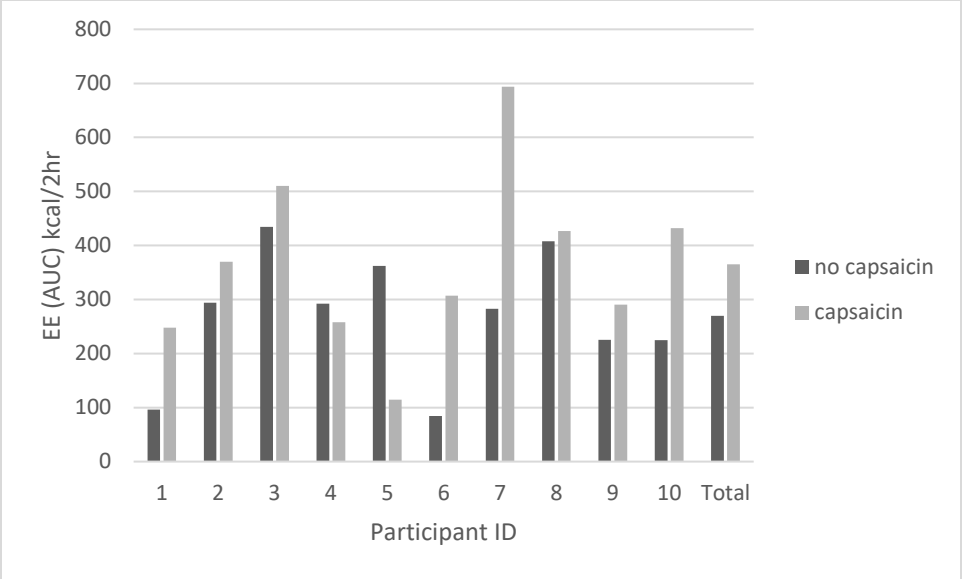

Figure 6: Thermic effect of food
represented by energy expenditure (EE) area under the curve (AUC); p-value: 0.060

Threshold Level

We determined the threshold level of capsaicin for each participant and determined if it is directly related to changes in postprandial EE. The average best estimate threshold for the group was 0.103 ppm. The percentage of correct response for each capsaicin concentration from all participants is represented in Figure 7. The percentage of correct responses at 0.312 ppm was 80%. This is the concentration at which a previous study determined was the average threshold level for capsaicin is at.[49,50] Most of the burn sensation was felt at the throat, which is consistent with previous capsaicin threshold test studies.[49,50] Figure 8 shows a statistically significant (p-value: 0.024) positive correlation ($R^2 = 0.491$) between the maximum EE and best estimate threshold

level for each participant. This relationship indicates that participants who detected capsaicin at higher concentration, had higher energy expenditure.

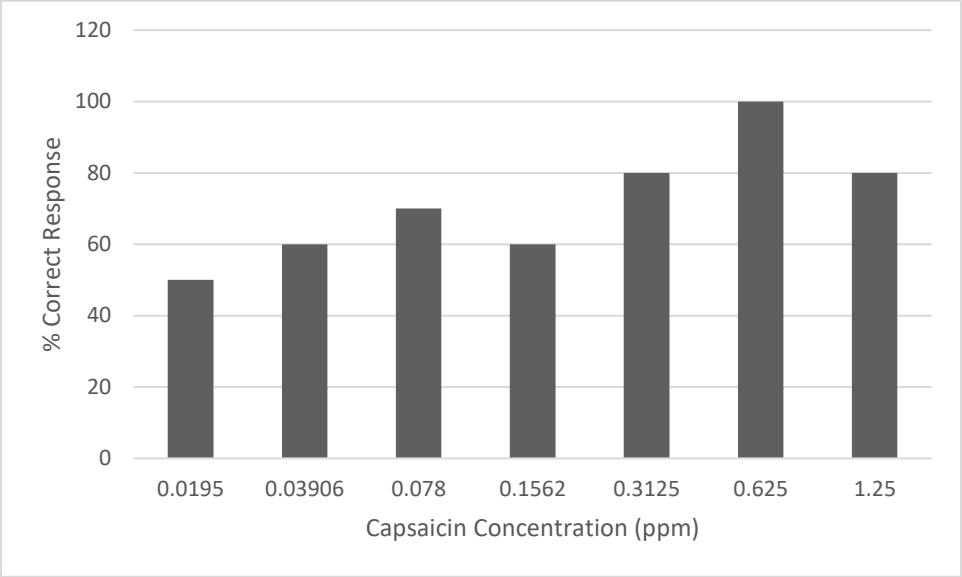

Figure 7: Percentage of correct response for each capsaicin concentration

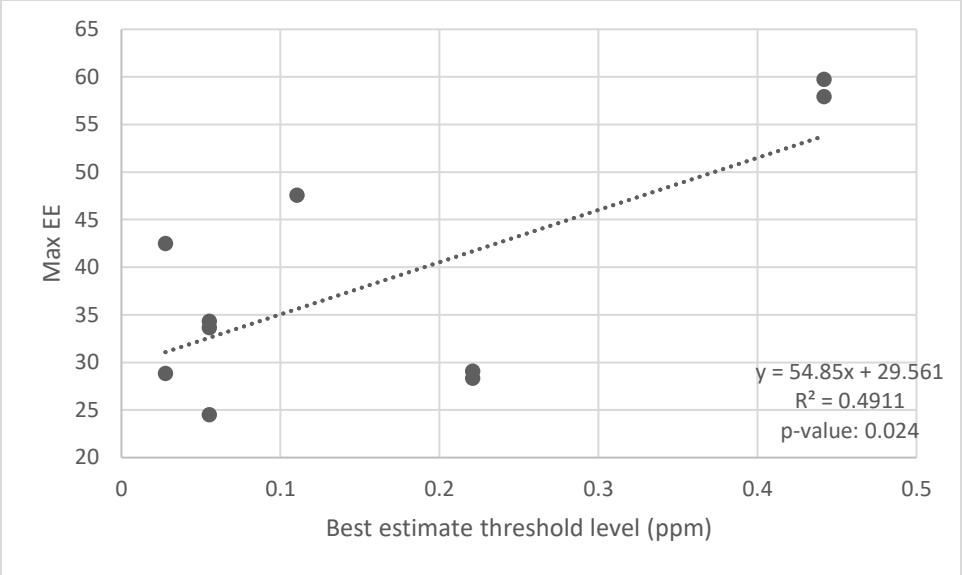

Figure 8: Regression analysis for best estimate threshold level against maximum energy expenditure (Max EE) of each participant

Blood Glucose

The average change in blood glucose from fasting blood glucose over the course of two-hours post meal was not statistically significant (p-value 0.538) between the two test days (Figure 9). Similarly, the BG (AUC) representing the blood glucose control for the two test days was also no significant (p-value: 0.838).

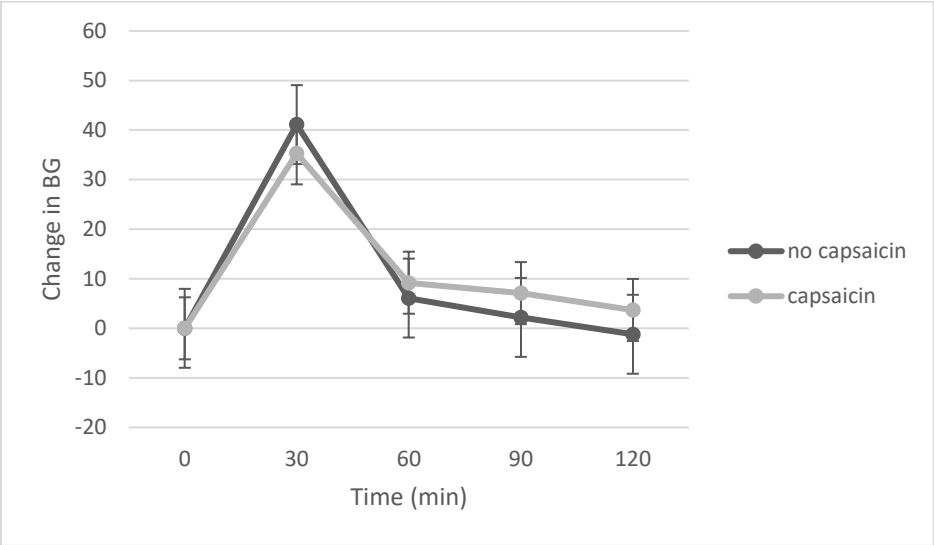

Figure 9: Average blood glucose (BG) change over time for all participants; p-value: 0.538

Blood Pressure

The change in systolic BP from baseline was not statistically significant between the two test days (p-value: 0.606). The change in diastolic BP from baseline was also not statistically significant between the two test days (p-value: 0.076).

Discussion and Conclusion

The primary aim of this study was to investigate changes in respiratory quotient (RQ) that could implicate changes in substrate oxidation. The RQ is the ratio of the amount of carbon dioxide produced over the amount of oxygen consumed. The RQ value provides information on what substrate (protein, fat, or carbohydrate) is mainly being oxidized. This study found that the overall RQ from baseline for all participants was

significantly lower for the capsaicin test days. This result is consistent with our hypothesis and with previous studies.[28,28,31,32,51] The larger decrease suggests that consumption of capsaicin-containing food may increase the amount of fat that is burned compared to an identical meal not containing capsaicin. This effect of capsaicin is expected to be beneficial on body composition since it can promote a decrease in FM.[25] Research suggests that an increase in fat oxidation after capsaicin consumption is generally seen in a meal with a high fat content. Yoshioka et al. observed an increase in lipid oxidation when their energy% content of protein/fat/CHO was 15/45/40.[32] Our study also had a high fat energy percentage (protein/fat/CHO: 16/38/48) but because we did not have a low fat meal as comparison to elaborate on whether the response would have been different if a lower fat meal would have been consumed

This study also found that the postprandial EE was elevated with capsaicin. Several studies have indicated that capsaicin, when consumed with a meal or as, has a higher thermic effect and therefore has the potential to lead to an increase in total energy expenditure.[11,13,15,24] Lejeune et al. found EE to increase by around 119 kcal/day from capsaicin consumption and our study found an increase of about 75 kcal/day.[28] Lejeune's study provided participants with a capsaicin capsule (135mg/day) for 3-months whereas we only provided participants with 20.4 mg capsaicin in the form of cayenne pepper added to a meal for one day. We still managed to get an increase in EE, even though we gave the participants less than half the amount of capsaicin.[28] The EE effect size of 74±30 kcal/day is based on many assumptions. First, we did not measure the full effect from meal consumption. If we assume the meal effect does not change over time and

capsaicin is consumed throughout the day (every 2-3hrs), then we should expect to burn an additional 74 kcals per day. That equates to 26,640 calories and a total decrease in fat accumulation of 8 pounds per year The increase in EE can be explained due to the thermogenic effects and increased sympathetic activity (β-adrenergic stimulation) from capsaicin and analogs.[13,21] Additionally, in a single-blind crossover study, capsaicin analogs, capsinoids, were shown to increase EE by activating brown adipose tissue in humans, without increasing temperature.[15] It is important to note that the postprandial effect of capsaicin seems to be longer than 2-hours and more research needs to be done to determine the full effect of capsaicin consumption on RQ and EE.

An exploratory aim was to determine the threshold level of capsaicin for each participant and see if it is directly related to changes in postprandial EE. The maximum postprandial EE was used to relate with participant's best estimate threshold because it provided with the biggest potential treatment effect. We found that EE is positively correlated with a higher threshold level for capsaicin. This result is consistent with what other studies suggest.[43–47] Participants who had a higher threshold level were less sensitive to capsaicin and that could be due to many factors. One reason could be they have less TRPV1 (transient receptor potential vanilloid) receptor which detects capsaicin.[11] Another reason could be they regularly eat spicy foods, which in turn desensitized them to capsaicin over time.[11] It be possible that some individuals had more receptors for capsaicin in the stomach than their month, since this test was only testing the mouth receptors. A Japanese research study found that capsaicin action takes place in the gut instead of the mouth and is responsible for altering food behavior by decreasing

fat and energy intake. [42] This study mainly included Asians (60%) who because of their cultural background habitually consume spicy food and could be desensitized to capsaicin. Although these individuals, who had a higher threshold for capsaicin, only reported consuming around 1 to 2 teaspoons of pepper per week, it is not clear how much capsaicin was in the peppers they consumed.

We also explored the effect of capsaicin-rich foods on postprandial blood glucose, but we did not find a significant effect This can be due to a small study population but likely a better method other than a glucometer is needed. Due to budgetary restraints this was not possible but will be considered in future studies.

Research suggests that capsaicin increases blood pressure (BP).[40] Most studies have included capsaicin in pill form and that could have had a greater impact on BP. Our study found that there was no statistically significant difference in BP between the two test days. This finding suggests that we obtained an increase in EE without increasing the BP, potentially due to delivery of capsaicin distributed in food rather than concentrated in supplement form. This is a potential advantage over other nutraceuticals used for weight control.

There were a few limitations and weaknesses in the study. The study population was small but despite this, we were able to find a significant effect of capsaicin added to a meal on substrate oxidation and energy expenditure. We did not find a significant effect on blood glucose levels and blood pressure. The study only shows acute effects of capsaicin, but research suggests capsaicin could possibly have long-term effects. There were some missing data points for EE and RQ for two participants (participant ID 4 and

10) at different times for one or the other test day due to unforeseen circumstances that interrupted testing (i.e. fire alarm).

In summary our work suggests that capsaicin has the potential to have a positive effect on substrate oxidation and EE. More research needs to be done on a wider population for a longer duration of time to establish possible long-term beneficial effects of capsaicin on the body. It is also advisable to obtain a larger and more diverse ethnic sample to see if the positive relationship between EE and capsaicin threshold level still hold true. The results of this study show a potential for capsaicin rich foods to be used as a tool for weight management and as a consequence, the development of co-morbidities such as possibly negating some of the consequences of obesity like diabetes and hypertension.

APPENDIX

Data graphs for participant 1

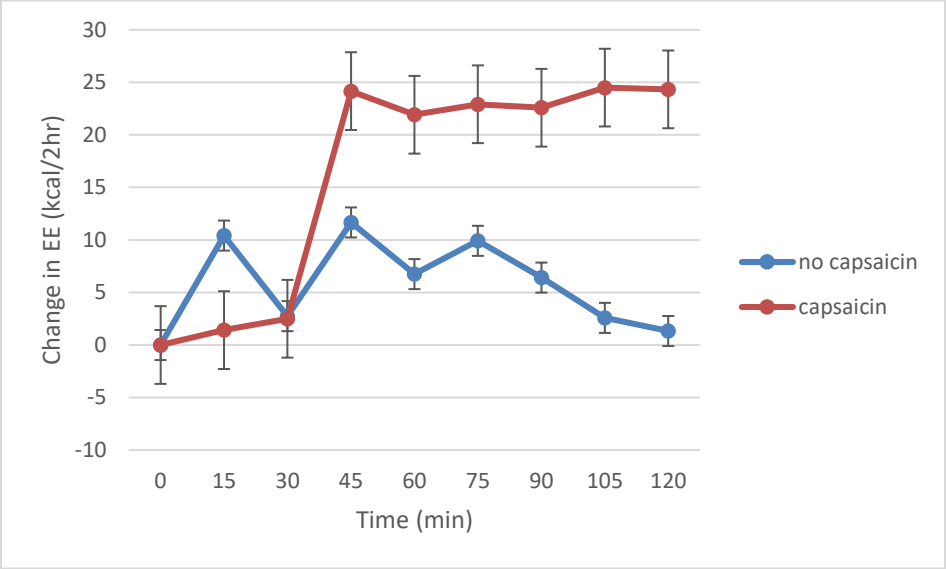

Figure 10: Change in energy expenditure (EE) over time for participant 1

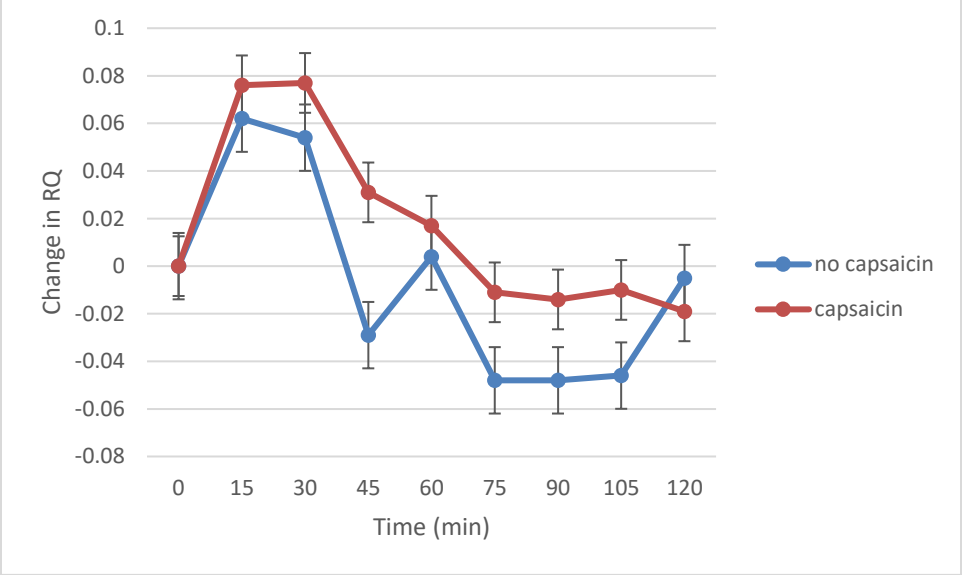

Figure 11: Change in respiratory quotient (RQ) over time for participant 1

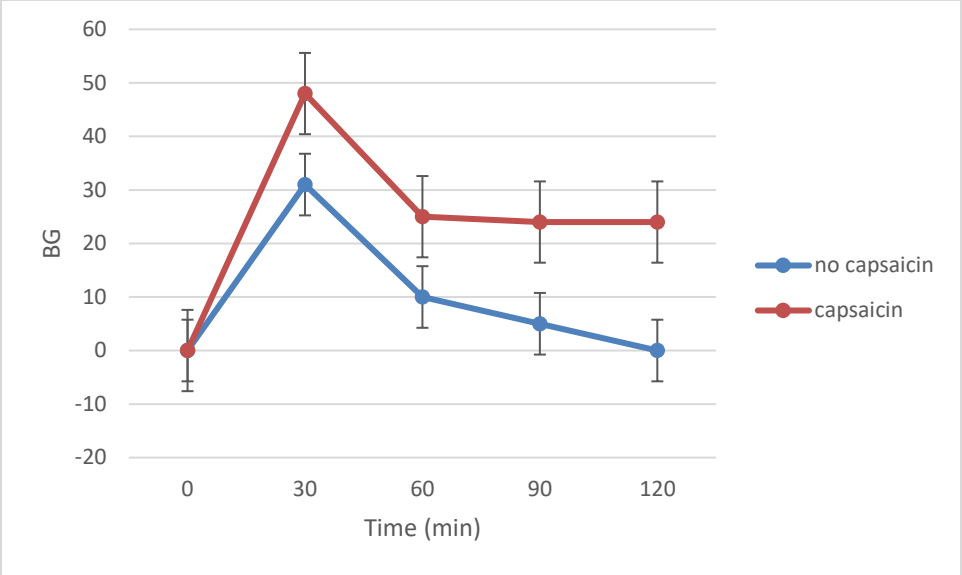

Figure 12: Change in blood glucose (BG) over time for participant 1

Data graphs for participant 2

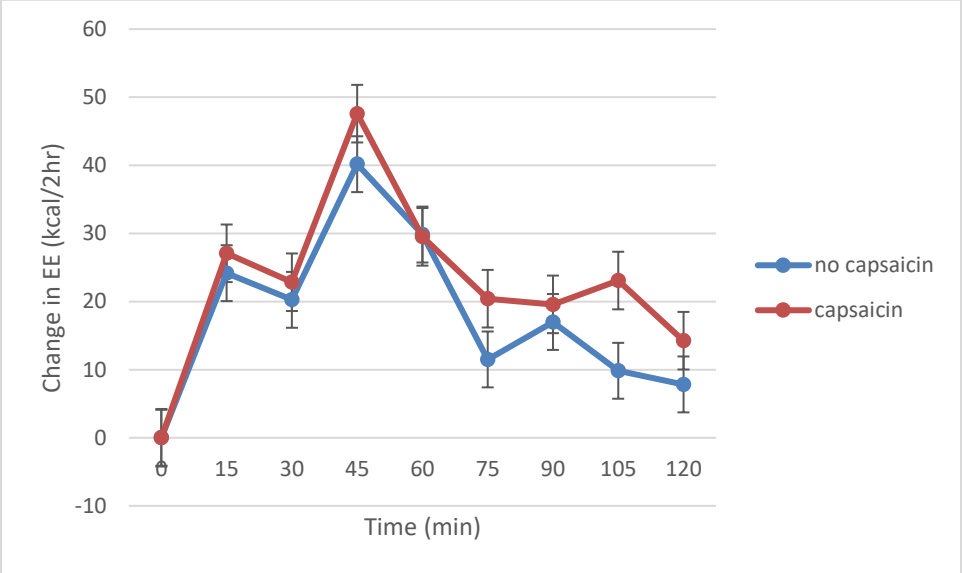

Figure 13: Change in energy expenditure (EE) over time for participant 2

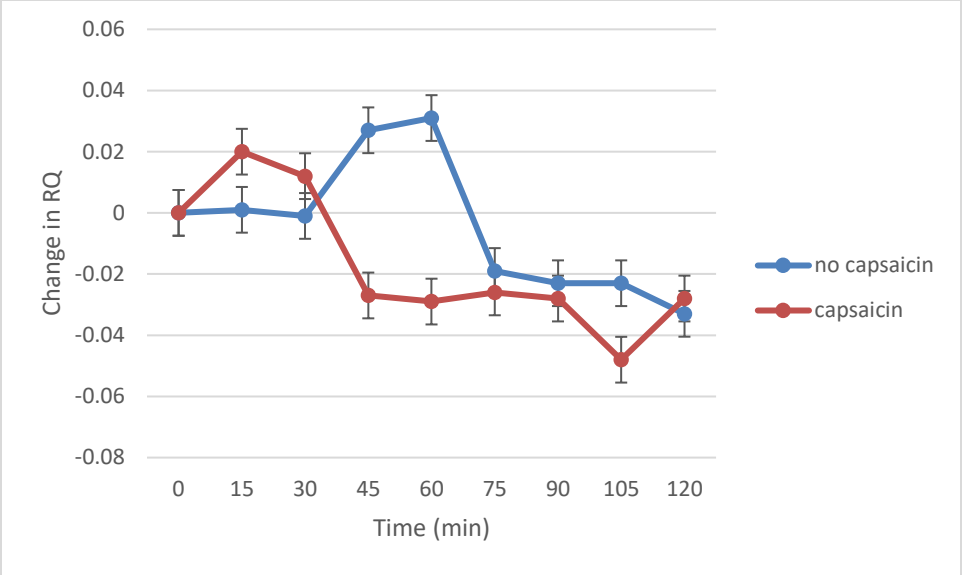

Figure 14: Change in respiratory quotient (RQ) over time for participant 2

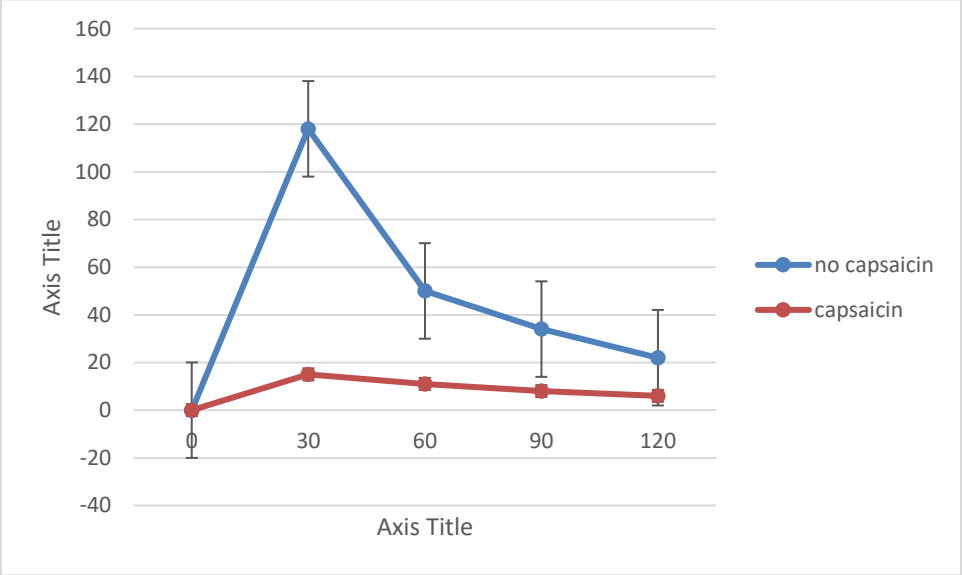

Figure 15: Change in blood glucose (BG) over time for participant 2

Data graphs for participant 3

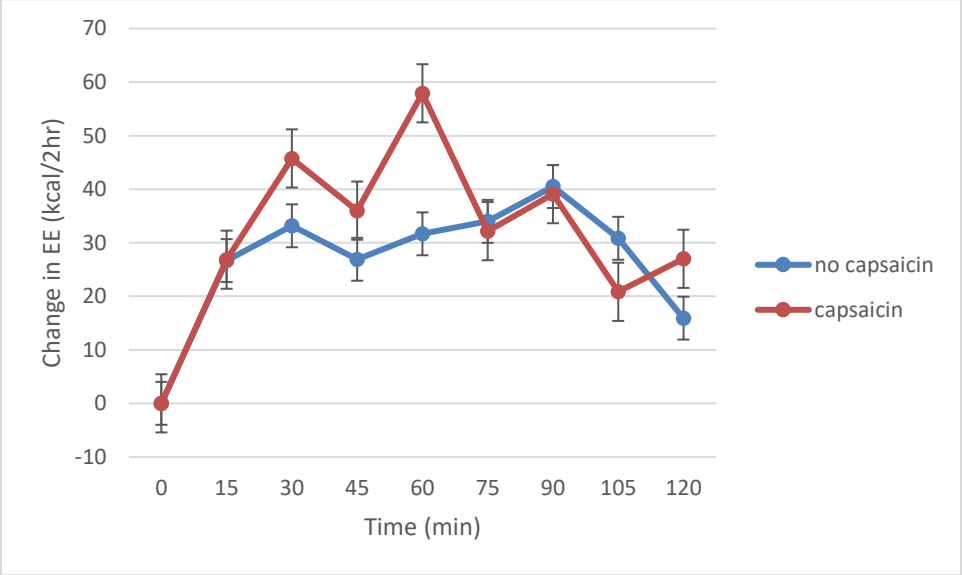

Figure 16: Change in energy expenditure (EE) over time for participant 3

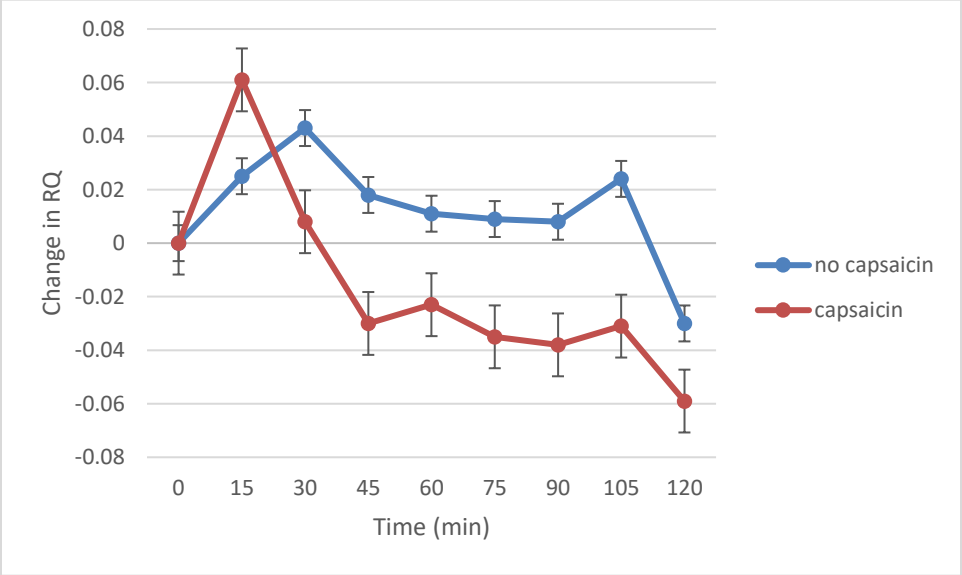

Figure 17: Change in respiratory quotient (RQ) over time for participant 3

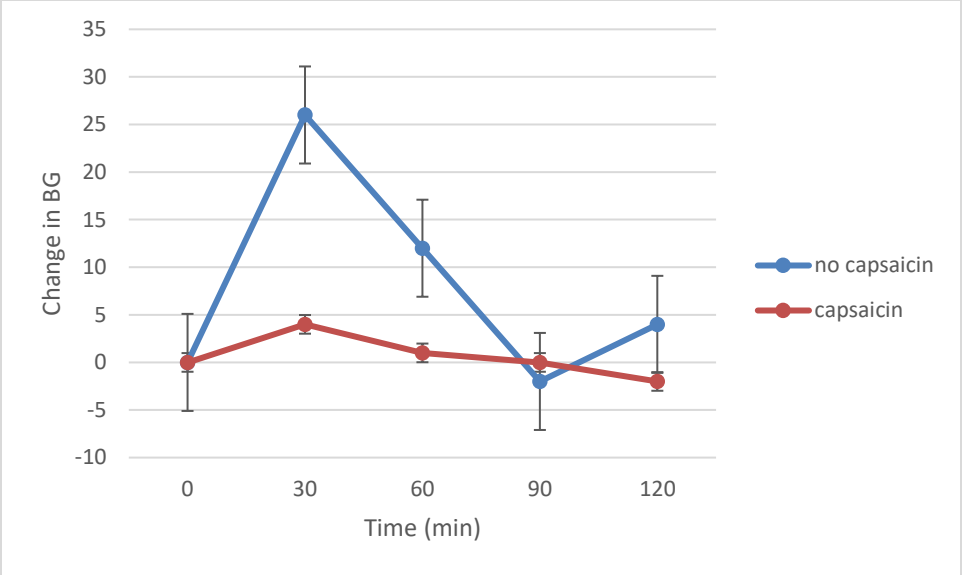

Figure 18: Change in blood glucose (BG) over time for participant 3

Data graphs for participant 4

Missing some data points for EE and RQ graphs.

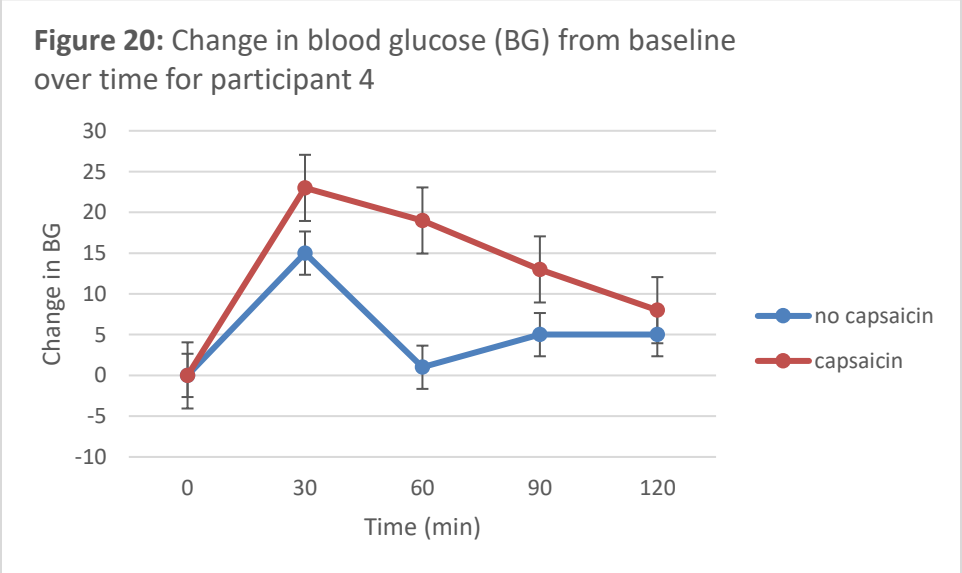

Figure 19: Change in blood glucose (BG) over time for participant 4

Data graphs for participant 5

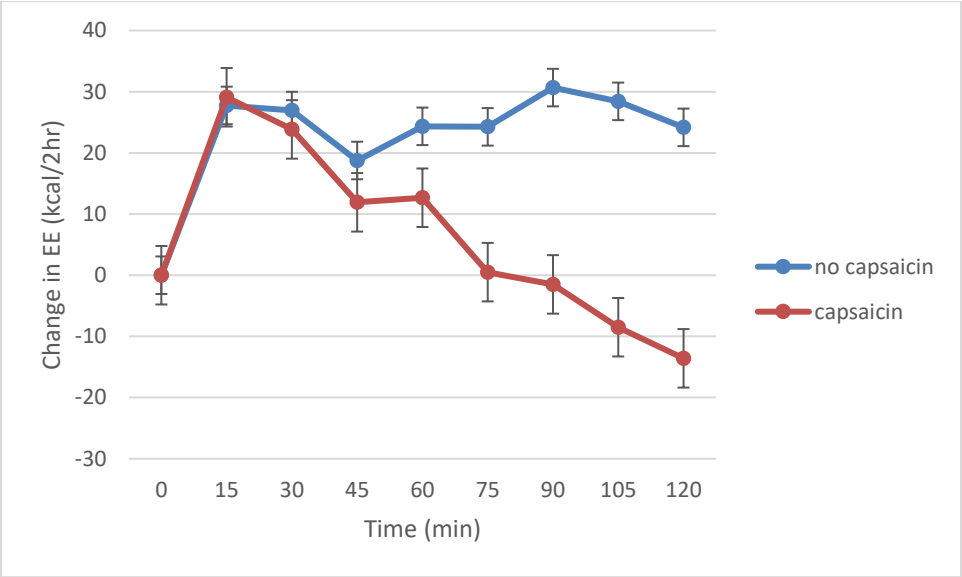

Figure 20: Change in energy expenditure (EE) over time for participant 5

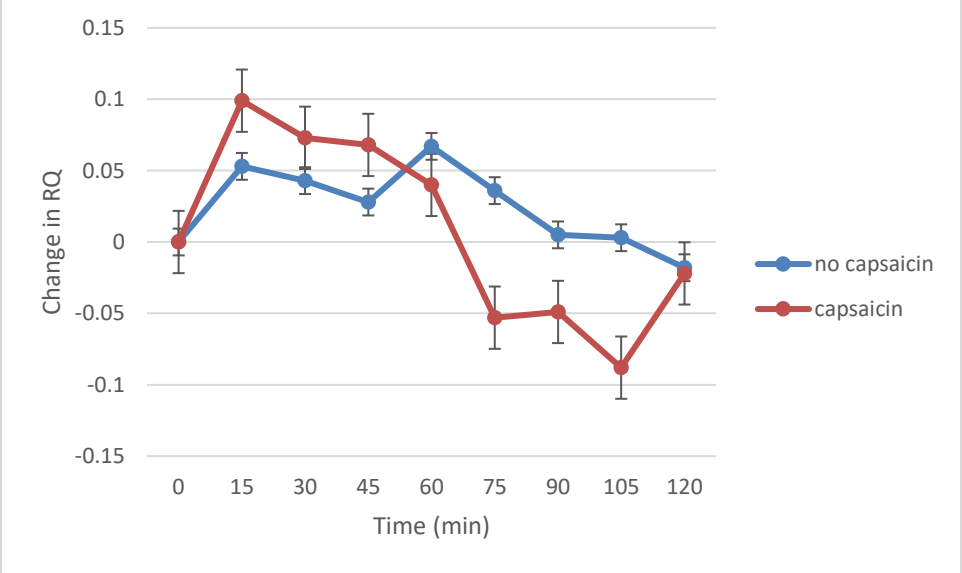

Figure 21: Change in respiratory quotient (RQ) over time for participant 5

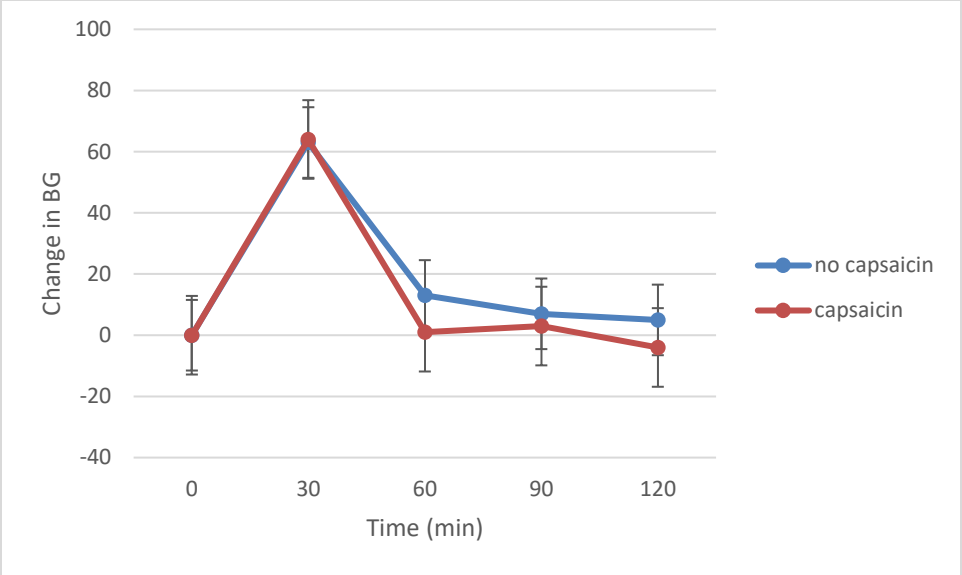

Figure 22: Change in respiratory quotient (RQ) over time for participant 5

Data graphs for participant 6

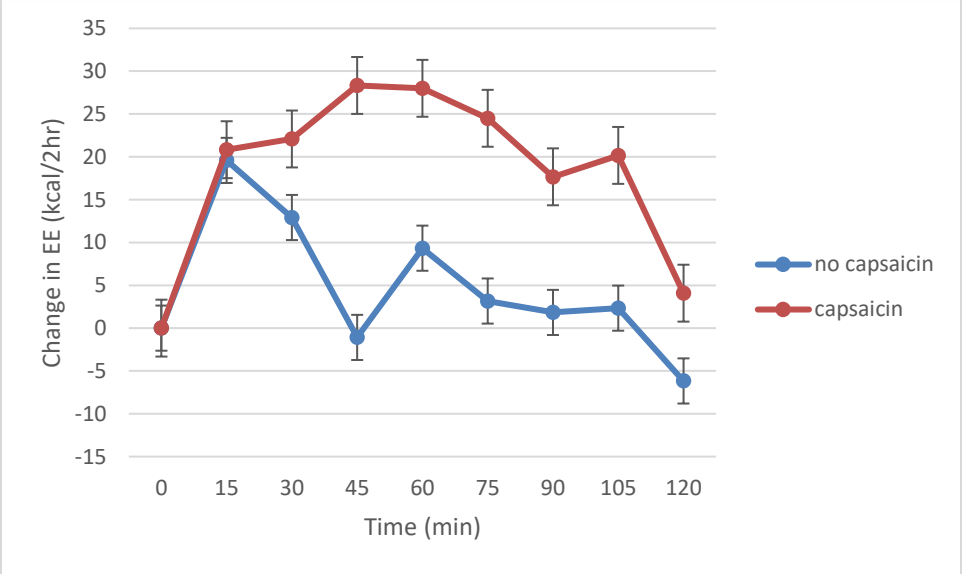

Figure 23: Change in energy expenditure (EE) over time for participant 6

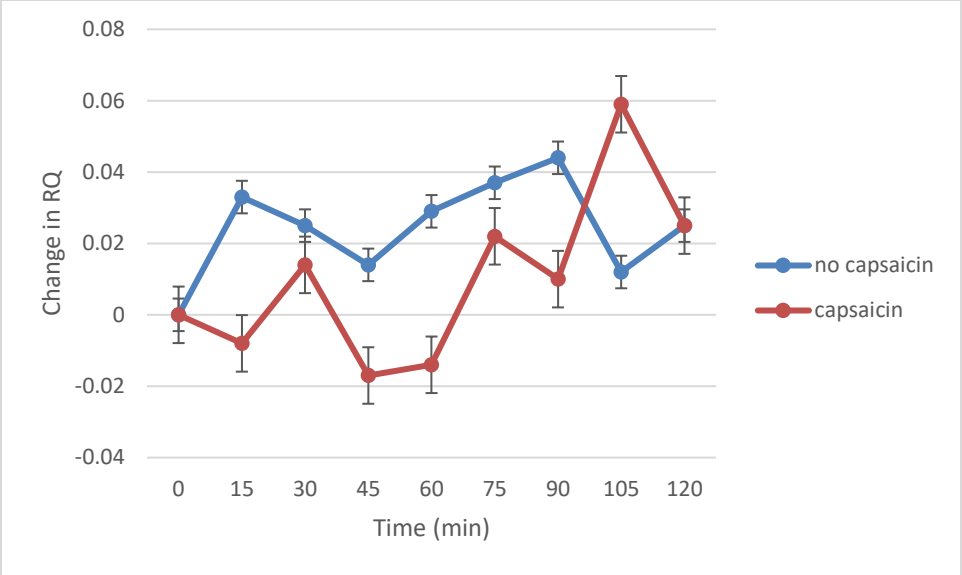

Figure 24: Change in respiratory quotient (RQ) over time for participant 6

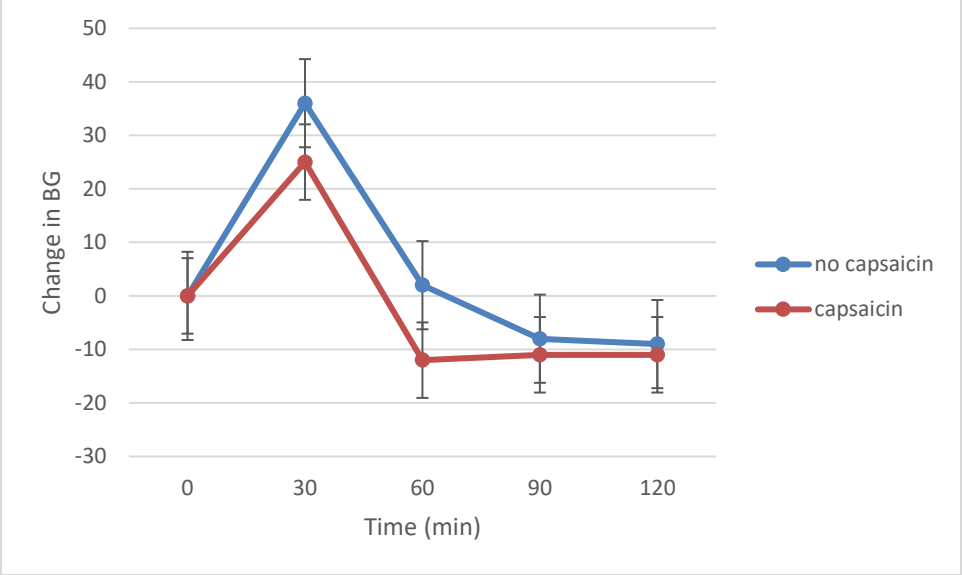

Figure 25: Change in blood glucose (BG) over time for participant 6

Data graphs for participant 7

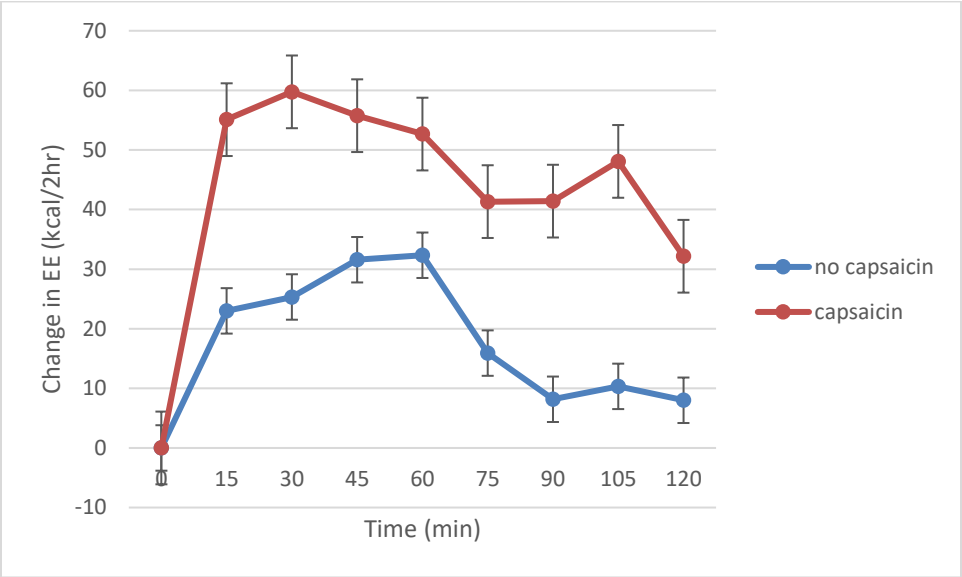

Figure 26: Change in energy expenditure (EE) over time for participant 7

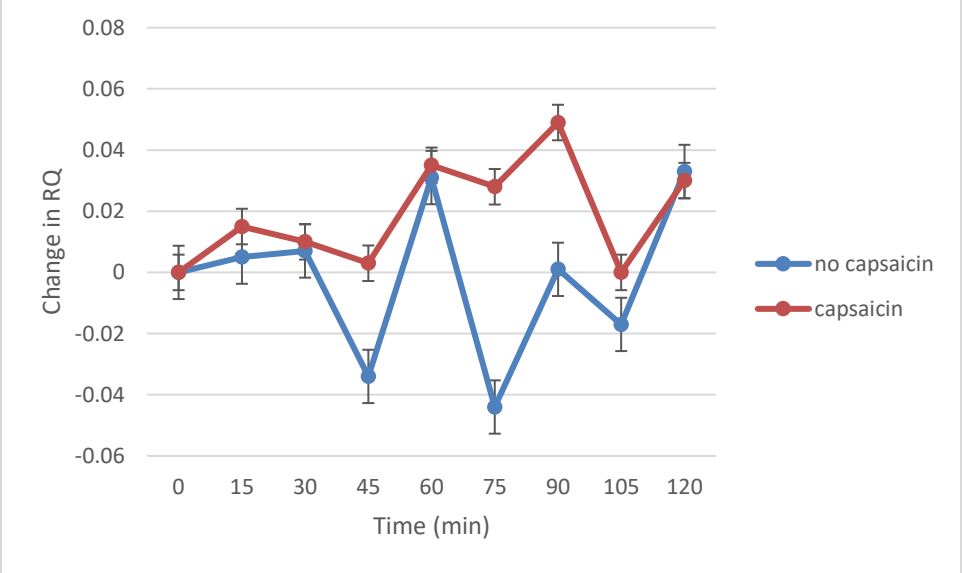

Figure 27: Change in respiratory quotient (RQ) over time for participant 7

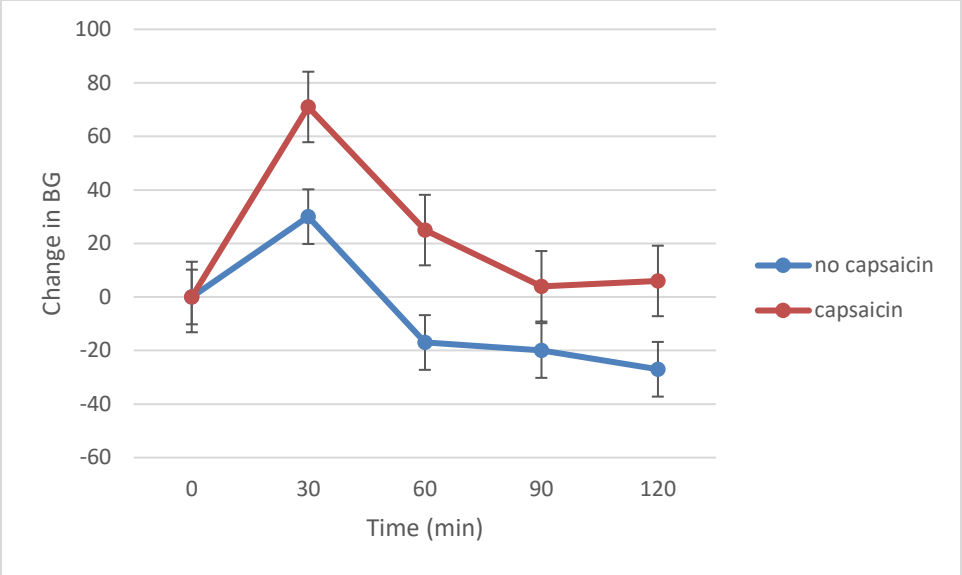

Figure 28: Change in blood glucose (BG) over time for participant 7

Data graphs for participant 8

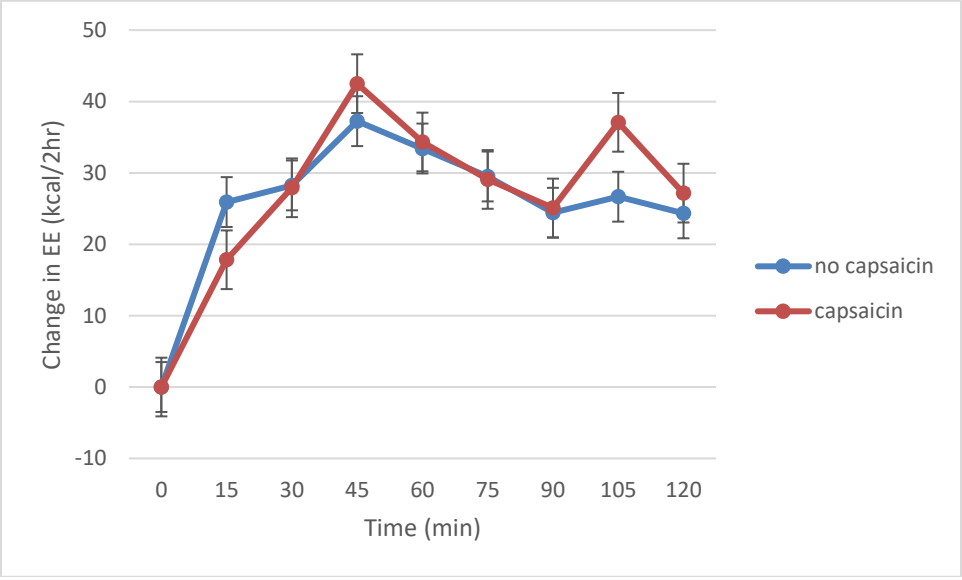

Figure 29: Change in energy expenditure (EE) over time for participant 8

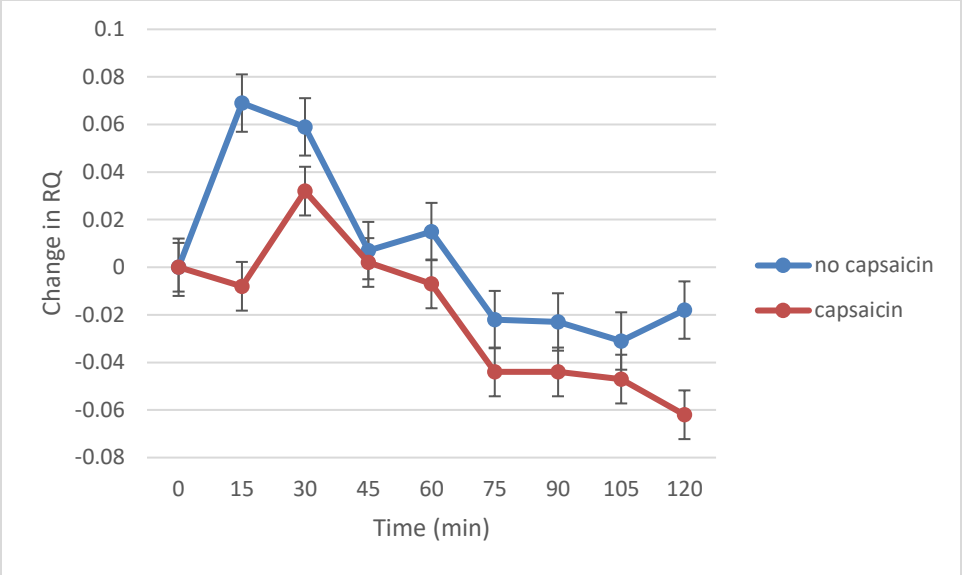

Figure 30: Change in respiratory quotient (RQ) over time for participant 8

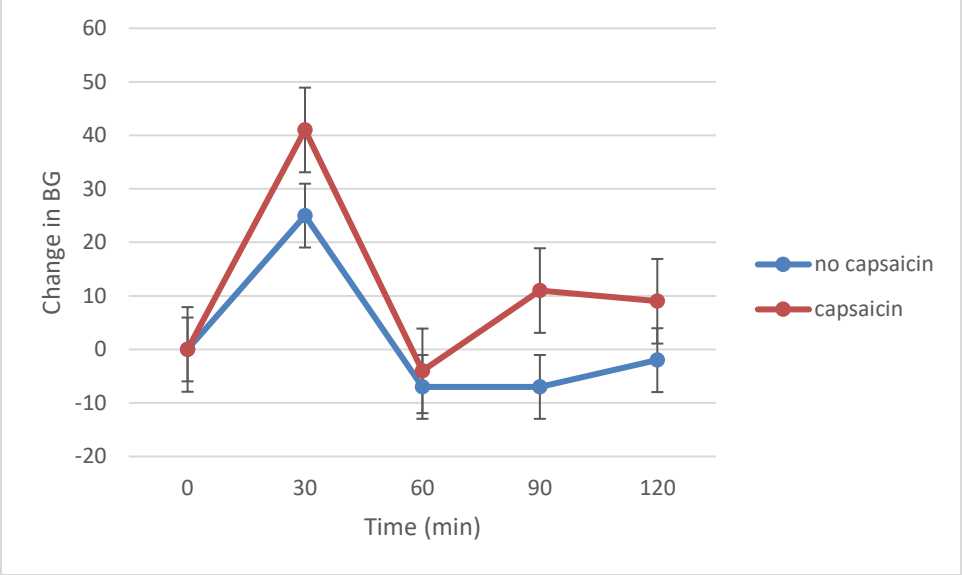

Figure 31: Change in blood glucose (BG) over time for participant 8

Data graphs for participant 9

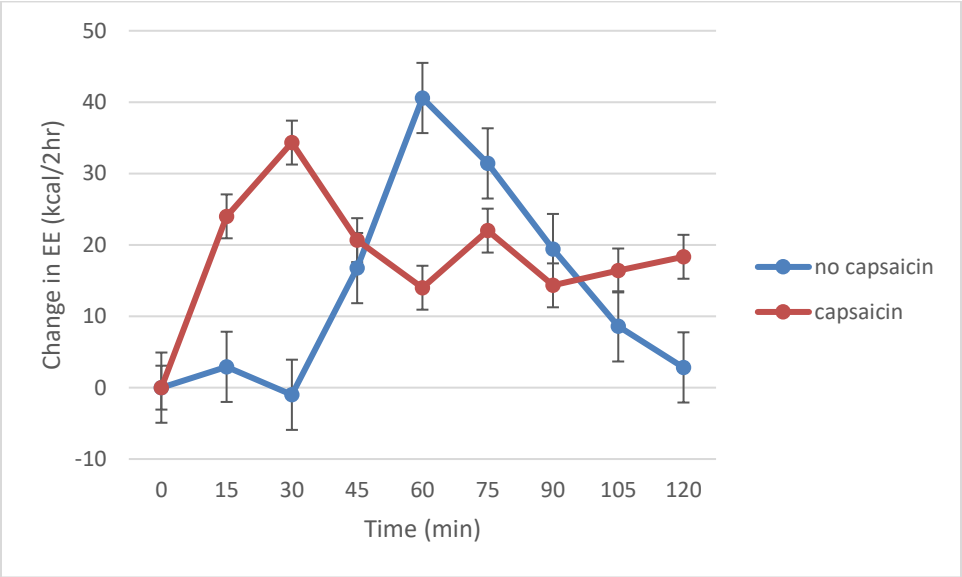

Figure 32: Change in energy expenditure (EE) over time for participant 9

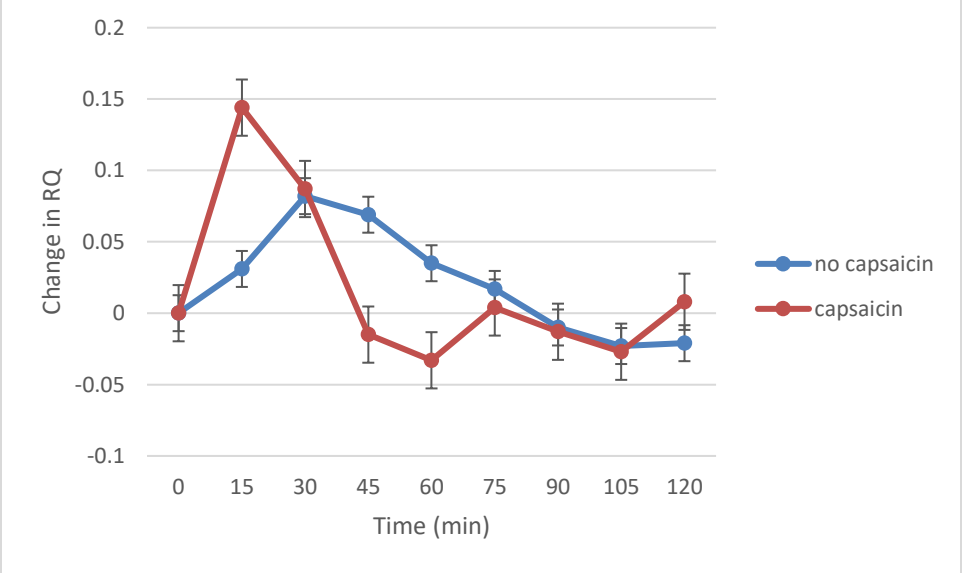

Figure 33: Change in respiratory quotient (RQ) over time for participant 9

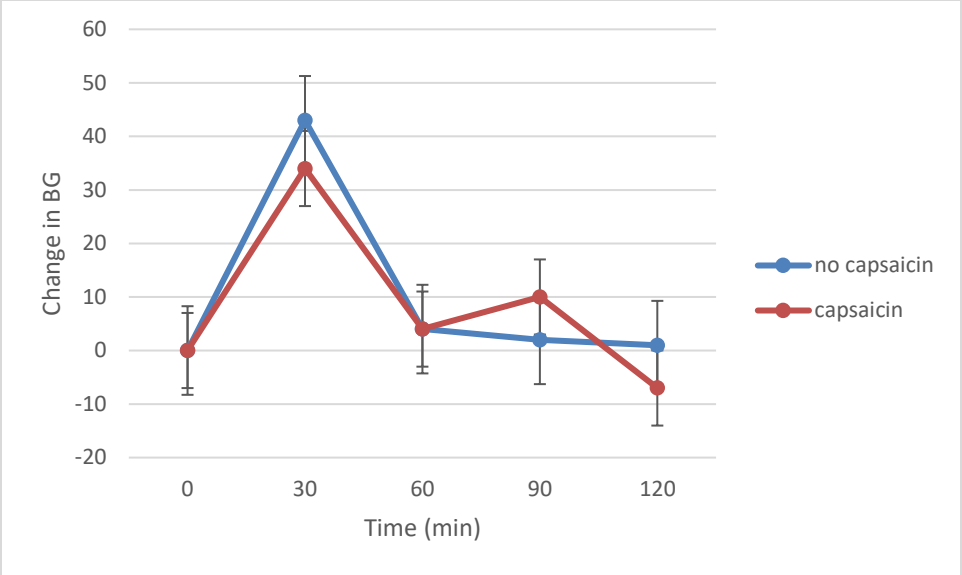

Figure 34: Change in blood glucose (BG) over time for participant 9

Data graphs for participant 10

Missing some data on EE and RQ

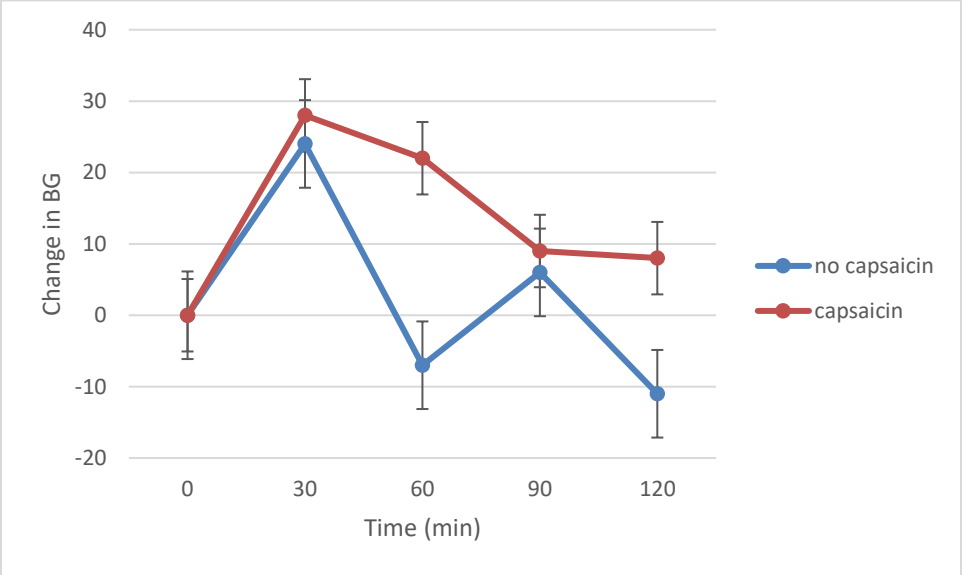

Figure 35: Change in blood glucose (BG) over time for participant 9

REFERENCES

1. Wallinga D, Schoonover H, Muller M. Considering the Contribution of US Agricultural Policy to the Obesity Epidemic: Overview and Opportunities. *J Hunger Environ Nutr*. 2009;4(1):3-19. doi:10.1080/19320240802706817

2. Hill JO, Wyatt HR, Peters JC. Energy Balance and Obesity. *Circulation*. 2012;126(1):126-132. doi:10.1161/CIRCULATIONAHA.111.087213

3. Jebb SA, Prentice AM, Goldberg GR, Murgatroyd PR, Black AE, Coward WA. Changes in macronutrient balance during over- and underfeeding assessed by 12-d continuous whole-body calorimetry. *Am J Clin Nutr*. 1996;64(3):259-266.

4. Dulloo AG, Jacquet J. Adaptive reduction in basal metabolic rate in response to food deprivation in humans: A role for feedback signals from fat stores. *Am J Clin Nutr*. 1998;68(3):599-606.

5. Richard D. Cognitive and autonomic determinants of energy homeostasis in obesity. *Nat Rev Endocrinol Lond*. 2015;11(8):489-501. doi:http://dx.doi.org.mutex.gmu.edu/10.1038/nrendo.2015.103

6. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 2006;444(7121):840-846. doi:10.1038/nature05482

7. Fujii TK, Phillips BJ. Quick Review: The Metabolic Cart. *Internet J Intern Med*. 2002;3(2). http://ispub.com/IJIM/3/2/4212. Accessed July 1, 2019.

8. Johnson RK, COWARD-McKENZIE D. CHAPTER 2â€ ” Energy Requirement Methodology. In: Coulston AM, Rock CL, Monsen ER, eds. *Nutrition in the Prevention and Treatment of Disease*. San Diego: Academic Press; 2001:31-42. doi:10.1016/B978-012193155-1/50004-0

9. Penicaud L, Benani A, Datiche F, Fioramonti X, Leloup C, Lienard F. Chapter 24â€ ” Animal Models and Methods to Study the Relationships Between Brain and Tissues in Metabolic Regulation. In: Conn PM, ed. *Animal Models for the Study of Human Disease*. Boston: Academic Press; 2013:569-593. doi:10.1016/B978-0-12-415894-8.00024-5

10. Ayling RM. CHAPTER 10â€ ” Clinical biochemistry of nutrition. In: Marshall WJ, Lapsley M, Day AP, Ayling RM, eds. *Clinical Biochemistry: Metabolic and Clinical Aspects (Third Edition)*. Churchill Livingstone; 2014:180-199. doi:10.1016/B978-0-7020-5140-1.00010-9

11. Ludy M-J, Moore GE, Mattes RD. The Effects of Capsaicin and Capsiate on Energy Balance: Critical Review and Meta-analyses of Studies in Humans. *Chem Senses*. 2012;37(2):103-121. doi:10.1093/chemse/bjr100

12. Hursel R, Viechtbauer W, Westerterp-plantenga MS. The effects of green tea on weight loss and weight maintenance: A meta-analysis. *Int J Obes Lond*. 2009;33(9):956-961. doi:http://dx.doi.org.mutex.gmu.edu/10.1038/ijo.2009.135

13. Belza A, Jessen AB. Bioactive food stimulants of sympathetic activity: Effect on 24-h energy expenditure and fat oxidation. *Eur J Clin Nutr*. 2005;59(6):733-741. doi:10.1038/sj.ejcn.1602121

14. Dulloo AG, Geissler CA, Horton T, Collins A, Miller DS. Normal caffeine consumption: Influence on thermogenesis and daily energy expenditure in lean and postobese human volunteers. *Am J Clin Nutr*. 1989;49(1):44-50.

15. Yoneshiro T, Aita S, Kawai Y, Iwanaga T, Saito M. Nonpungent capsaicin analogs (capsinoids) increase energy expenditure through the activation of brown adipose tissue in humans. *Am J Clin Nutr*. 2012;95(4):845-850. doi:10.3945/ajcn.111.018606

16. Popelka P, Jenvinva P, Smejkal K, Roba P. Determination of Capsaicin Content and Pungency Level of Different Fresh and Dried Chilli Peppers. *Folia Vet*. 2017;61(2). doi:https://doi.org/10.1515/fv-2017-0012

17. Lillywhite JM, Simonsen JE, Uchanski ME. Spicy Pepper Consumption and Preferences in the United States. *HortTechnology*. 2013;23(6):868-876.

18. Kawada T. Physical activity, obesity and insulin resistance. *Int J Cardiol*. 2012;159(3):237-238. doi:10.1016/j.ijcard.2012.05.092

19. Watanabe T, Kawada T, Kato T, Harada T, Iwai K. Effects of capsaicin analogs on adrenal catecholamine secretion in rats. *Life Sci*. 1994;54(5):369-374. doi:10.1016/0024-3205(94)00793-4

20. Reinbach HC. Hot spices: Effects on appetite, energy intake and sensory properties of a meal : Ph.D. thesis. 2008. https://vbn.elsevierpure.com/en/publications/hot-spices-effects-on-appetite-energy-intake-and-sensory-properti. Accessed July 19, 2019.

21. Hachiya S, Kawabata F, Ohnuki K, et al. Effects of CH-19 Sweet, a non-pungent cultivar of red pepper, on sympathetic nervous activity, body temperature, heart rate, and blood pressure in humans. *Biosci Biotechnol Biochem*. 2007;71(3):671-676. doi:10.1271/bbb.60359

22. Yoshioka M, Doucet E, Drapeau V, Dionne I, Tremblay A. Combined effects of red pepper and caffeine consumption on 24 h energy balance in subjects given free access to foods. *Br J Nutr*. 2001;85(2):203-211. doi:10.1079/BJN2000224

23. Yoshioka M, Lim K, Kikuzato S, et al. Effects of red-pepper diet on the energy metabolism in men. *J Nutr Sci Vitaminol (Tokyo)*. 1995;41(6):647-656.

24. Jansenns P, Hursel R, Martens E, Westerterp-Plantenga MS. Acute effects of capsaicin on energy expenditure and fat oxidation in negat... *PLoS ONE*. 2013;8(7). doi:e67786. doi:10.1371/journal.pone.0067786

25. Schutz Y, Tremblay A, Weinsier RL, Nelson KM. Role of fat oxidation in the long-term stabilization of body weight in obese women. *Am J Clin Nutr*. 1992;55(3):670-674. doi:10.1093/ajcn/55.3.670

26. Zsiborás C, Mátics R, Hegyi P, et al. Capsaicin and capsiate could be appropriate agents for treatment of obesity: A meta-analysis of human studies. *Crit Rev Food Sci Nutr*. June 2017:1-9. doi:10.1080/10408398.2016.1262324

27. Rigamonti AE, Casnici C, Marelli O, et al. Acute administration of capsaicin increases resting energy expenditure in young obese subjects without affecting energy intake, appetite, and circulating levels of orexigenic/anorexigenic peptides. *Nutr Res*. 2018;52:71-79. doi:10.1016/j.nutres.2018.02.002

28. Lejeune M, Kovacs E, Westerterp-Plantenga M. Effect of capsaicin on substrate oxidation and weight maintenance after modest body-weight loss in human subjects. *Br J Nutr*. 2003;90:651-659.

29. Ahuja KD, Robertson IK, Geraghty DP, Ball MJ. Effects of chili consumption on postprandial glucose, insulin, and energy metabolism. *Am J Clin Nutr*. 2006;84(1):63-69.

30. Huang J, Mabury SA, Sagebiel JC. Hot chili peppers: Extraction, cleanup, and measurement of Capsaicin. *J Chem Educ Easton*. 2000;77(12):1630-1631.

31. Lim K, Yoshioka M, Kikuzato S, et al. Dietary red pepper ingestion increases carbohydrate oxidation at rest and during exercise in runners. *Med Sci Sports Exerc*. 1997;29(3):355-361.

32. Yoshioka M, St-Pierre S, Suzuki M, Tremblay A. Effects of red pepper added to high-fat and high-carbohydrate meals on energy metabolism and substrate utilization in Japanese women. *Br J Nutr*. 1998;80(6):503-510. doi:10.1017/S0007114598001597

33. Inoue N, Matsunaga Y, Satoh H, Takahashi M. Enhanced Energy Expenditure and Fat Oxidation in Humans with High BMI Scores by the Ingestion of Novel and Non-Pungent Capsaicin Analogues (Capsinoids). *Biosci Biotechnol Biochem*. 2007;71(2):380-389. doi:10.1271/bbb.60341

34. Ahuja KDK, Robertson IK, Geraghty DP, Ball MJ. The effect of 4-week chilli supplementation on metabolic and arterial function in humans. *Eur J Clin Nutr*. 2007;61(3):326-333. doi:10.1038/sj.ejcn.1602517

35. Galgani JE, Ryan DH, Ravussin E. Effect of capsinoids on energy metabolism in human subjects. *Br J Nutr*. 2010;103(1):38-42. doi:10.1017/S0007114509991358

36. Smeets AJ, Westerterp-Plantenga MS. The acute effects of a lunch containing capsaicin on energy and substrate utilisation, hormones, and satiety. *Eur J Nutr*. 2009;48(4):229-234. doi:10.1007/s00394-009-0006-1

37. Snitker S, Fujishima Y, Shen H, et al. Effects of novel capsinoid treatment on fatness and energy metabolism in humans: Possible pharmacogenetic implications. *Am J Clin Nutr*. 2009;89(1):45-50. doi:10.3945/ajcn.2008.26561

38. Zhang S, Ma X, Zhang L, Sun H, Liu X. Capsaicin Reduces Blood Glucose by Increasing Insulin Levels and Glycogen Content Better than Capsiate in Streptozotocin-Induced Diabetic Rats. *J Agric Food Chem*. 2017;65(11):2323-2330. doi:10.1021/acs.jafc.7b00132

39. Kwon DY, Kim YS, Ryu SY, et al. Capsiate improves glucose metabolism by improving insulin sensitivity better than capsaicin in diabetic rats. *J Nutr Biochem*. 2013;24(6):1078-1085. doi:10.1016/j.jnutbio.2012.08.006

40. Westerterp-Plantenga MS, Smeets A, Lejeune MPG. Sensory and gastrointestinal satiety effects of capsaicin on food intake. *Int J Obes 2005*. 2005;29(6):682-688.

41. Smeets AJ, Janssens PLHR, Westerterp-Plantenga MS. Addition of Capsaicin and Exchange of Carbohydrate with Protein Counteract Energy Intake Restriction Effects on Fullness and Energy Expenditure. *J Nutr*. 2013;143(4):442-447. doi:10.3945/jn.112.170613

42. Yoshioka M, Imanaga M, Ueyama H, et al. Maximum tolerable dose of red pepper decreases fat intake independently of spicy sensation in the mouth. *Br J Nutr Camb*. 2004;91(6):991-995. doi:http://dx.doi.org.mutex.gmu.edu/10.1079/BJN20041148

43. Steiner AA, Turek VF, Almeida MC, et al. Nonthermal Activation of Transient Receptor Potential Vanilloid-1 Channels in Abdominal Viscera Tonically Inhibits Autonomic Cold-Defense Effectors. *J Neurosci*. 2007;27(28):7459-7468. doi:10.1523/JNEUROSCI.1483-07.2007

44. Székely M, Balaskó M, Kulchitsky VA, Simons CT, Ivanov AI, Romanovsky AA. Multiple neural mechanisms of fever. *Auton Neurosci*. 2000;85(1):78-82. doi:10.1016/S1566-0702(00)00223-X

45. Dogan MD, Patel S, Rudaya AY, Steiner AA, Székely M, Romanovsky AA. Lipopolysaccharide fever is initiated via a capsaicin-sensitive mechanism independent of the subtype-1 vanilloid receptor. *Br J Pharmacol*. 2004;143(8):1023-1032. doi:10.1038/sj.bjp.0705977

46. Garami A, Balaskó M, Székely M, Solymár M, Pétervári E. Fasting hypometabolism and refeeding hyperphagia in rats: Effects of capsaicin desensitization of the abdominal vagus. *Eur J Pharmacol*. 2010;644(1-3):61-66. doi:10.1016/j.ejphar.2010.07.002

47. Melnyk A, Himms-Hagen J. Resistance to Aging-Associated Obesity in Capsaicin-Desensitized Rats One Year after Treatment. *Obes Res*. 1995;3(4):337-344. doi:10.1002/j.1550-8528.1995.tb00159.x

48. Kanizsai P, Garami A, Solymár M, Szolcsányi J, Szelényi Z. Energetics of fasting heterothermia in TRPV1-KO and wild type mice. *Physiol Behav*. 2009;96(1):149-154. doi:10.1016/j.physbeh.2008.09.023

49. Schneider D, SeuB-Baum I, Schlich E. Determination of Thresholds for Capsaicin in Aqueous and Oil-Based. *Am Chem Soc*. June 2015:171-182. doi:10.102/bk-2015-1191.ch013

50. Lawless HT, Hartono C, Hernandez S. Thresholds and Suprathreshold Intensity Functions for Capsaicin in Oil and Aqueous Based Carriers. *J Sens Stud*. 2000;15(4):437-477. doi:10.1111/j.1745-459X.2000.tb00281.x

51. Blaak EE, Saris WHM. Substrate oxidation, obesity and exercise training. *Best Pract Res Clin Endocrinol Metab*. 2002;16(4):667-678. doi:10.1053/beem.2002.0226

BIOGRAPHY

Rafia Virk graduated from Westfield High School, Chantilly Virginia, in 2009. She received her Bachelor of Science from University of Mary Washington in Chemistry (2013) and from George Mason University in Biology (2015). She later started working on her Masters of Science in Nutrition and Food Studies at George Mason University in 2017.