Dark Chocolate Supplementation Elevates Resting Energy Expenditure in Exercise Trained Females

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ABSTRACT

PURPOSE: To investigate the influence of dark chocolate (DC) supplementation on resting and steady state exercise metabolism in a group of recreationally fit/athletic females. METHODS: Using a randomized, double-blind design, 18 exercise trained female subjects were assigned to a 30-d supplementation with either 20g•d^{-1} of 70% DC (n = 9) or a calorically matched white chocolate (WC) (n = 9). Prior to supplementation (PRE), subjects underwent indirect calorimetry assessment for resting energy expenditure (REE) followed by an assessment of exercise energy expenditure (EEE) consisting of steady state cycling for 20 min, 10 min at 50 watts (EEE-50) and 10 min at 100 watts (EEE-100). Upon completion of the 30-d supplementation (POST), subjects repeated the assessment for REE-0, EEE-50, and EEE-100. All data are presented as mean (SE). RESULTS: Pre-supplementation REE (DC 1455 ± 49, WC 1566 ± 49 kcal•d^{-1}, $p \geq .05$), EEE-50 (DC 4.85 ± 0.15, WC 4.60 ± 0.15 kcal • min^{-1}), and EEE-100 (DC 7.06 ± 0.16, WC 6.76 ± 0.16 kcal•min^{-1}) were not significantly different between groups ($p \geq .05$). Post supplementation REE was significantly increased by 9.5% in the DC group (Δ REE: DC 138 ± 21, WC -30 ± 21 kcal•d^{-1}, $p = .001$). Neither EEE-50 (DC 4.48 ± 0.16, WC 4.47 ± 0.16 kcal•min^{-1}) nor EEE-100 (DC 6.50 ± 0.17, WC 6.63 ± 0.17 kcal•min^{-1}) were significantly different between groups ($p \geq .05$). There was no significant difference within or between group time effects for substrate utilization at rest or during EEE-50 or EEE-100 ($p \geq .05$). CONCLUSION: To our knowledge this is the first study to demonstrate that a relatively small daily dosage of DC can significantly elevate REE but does not impact steady state EEE or substrate utilization in a group of athletically fit females.

* Product was provided by The Hershey Company, Hershey, Pennsylvania.

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ACKNOWLEDGEMENTS

I would like to recognize a few important people in my life. Without them, I would not have survived graduate school. First and foremost, I am nothing without Christ. His mercy, kindness, and love sustained me and gave me courage, forgiveness, and strength when I was incapable of these attributes. Second, Dr. Bryan Presler is the best husband a lady could ask for. In the days and evenings that I wanted to give up, he told me, "You can't give up. You have come too far, and you are not a quitter." He was there to give me constant encouragement in various ways, but my favorite was a playlist that was played at the beginning of my days that consisted of the Rocky, Top Gun, and LOTR theme songs. He was there to give me hugs when I needed them and expertise in editing my thesis. There were countless hours that he would stay up with me to make sure that the formatting was correct on data and tables. I am one blessed woman to have such a man standing by my side. Third, my parents, Dr. Richard and Dorothy Henderson, have always pushed me to my limits. They knew what I was capable of and made sure that I had the best education possible to be able to live up to that potential. Thank you, Dad and Mom. Fourth, the athletic department and all of their staff and student athletes were a huge support and help through this process. Thank you for all of the encouragement and opportunities that I was blessed to earn. It was truly an honor working with and for all of you. Go BLAZERS! Last, to my family, friends, and cohort mates. These past couple years would have been filled with more stress and tears, and less laughs and smiles without you. Thank you for supporting and keeping me sane through some of the toughest years of my life. The movie Balto says it perfectly, "I would be lost without you."

DEDICATION

This is dedicated to all the little girls out there who have a dream, but the doors are closed. The only way to open those doors is by tearing them open and persevering through the process. Life is not just about the dream, but is made up of life fulfilling moments that are lost if we close our eyes and open them only at the end.

Chapter I

INTRODUCTION

Chocolate is considered to be a delicacy by many cultures today, and its popularity is increasing exponentially. Chocolate provides the income for more than 40 million individuals yielding approximately 4.2 million tons of cocoa worth $11.8 billion (Beg, Ahmad, Jan, & Bashir, 2017). This literature review will discuss the origins of chocolate and how it became the dessert that the world enjoys today in the 21st century; how chocolate is processed from a bitter nut to a sweet delicacy; the different types of chocolate and their nutritional content (Latif, 2013), and epicatechin health effects (Hurst et al., 2011). In the 16th-19th centuries, people believed that chocolate could heal various disease or emotional states such as cancer, nervousness, and flatulence (Dillinger et al., 2000). As the years progressed, DC was suggested to assist with heart disease, Alzheimer's disease, and central adiposity (Petyaev & Bashmaokv, 2017). With the increased interest in athletics, research has started to test DC for performance enhancing effects. Research has suggested that DC reduces oxidative stress (Morillas-Ruiz et al., 2005; Morillas-Ruiz, Garcia, Lopez, Vidal-Guevara, & Zafrilla, 2006), resting blood pressure (Grassi, Lippi, Necozione, Desideri, & Ferri, 2005), and time to fatigue (Nogueira et al., 2011). Dark chocolate has also been shown to stimulate lipid catabolism (Venables, Hulston, Cox, & Jeukendrup, 2008), insulin sensitivity (Grassi, Lippi,

Necozione, Desideri, & Ferri, 2005), maximal oxygen uptake and exercise power output (Taub et al., 2016), and muscle anabolism (Gutierrez-Salmean et al., 2014).

Chapter II

REVIEW OF LITERATURE

History

Chocolate has been an item of reverence and worship by some Latino cultures for quite some time. Cacao was first discovered by the Mayas, Incas, and Aztecs in Central America (Dillinger et al., 2000). Before cacao was consumed as candy, it was originally drunk as a hot, bitter beverage with cocoa powder, cinnamon, and pepper (Verna, 2013). This was only consumed by royal or important adult men such as priests, government officials, military officers, and decorated warriors. Cacao was known to be intoxicating, valuable, and significant, thus, women and children were not allowed to partake. However, there was one other group who was allowed to consume cacao and that was prisoners before they faced their death (Dillinger et al., 2000). According to the Aztec's creation story, the god Quetzalcoatl (Feather Serpent) found cacao in a mountain. Because of this divine discovery, priests offered it to many other deities by pouring their own blood from their earlobes onto the cacao and this was deemed as a worthy sacrifice for the gods. In keeping with this practice, they fed their prisoners cacao before they sacrificed them to the gods, who would deem it as an acceptable sacrifice. Thus, cacao was known to be the food of the gods (Coe & Coe, 1996).

In 1502, Columbus was the first European to discover cacao, and explorers from other countries soon followed after him in his discovery (Dillinger et al., 2000).

Columbus found that cacao was roasted into an almond like bean and used as currency. While Cortez, a Spaniard, found that Montezuma drank cocoa before he would go meet with his wives. By 1544, the cocoa beverage was introduced into the Spanish courts and within a century, all of Western Europe was experiencing chocolate in high demand. For instance, France founded plantations in the Caribbean, and Spain settled their plantations in the Philippines so they could meet the demand of cocoa consumption in their home lands. (Dillinger et al., 2000)

In the 16th-19th centuries, cocoa was not only consumed because of its delectable aroma and flavor, but because of its purported medicinal functions. In the early 16th century in Mesoamerica, medicinal treatment was based off of a hot/cold system called allopathy. Diseases that are perceived as hot were treated using cold foods or medicines and vice versa. It was observed that foods such as chili peppers, rhubarb, and vanilla were used by Mexicans as a purgative and cocoa was used as a laxative. Cocoa was also used to treat stomach and intestinal discomfort. For the Mexicans who had phlegm, they would mix a drink of opossum tail and follow it with a hot beverage of cocoa which had been mixed with three herbs: piper sanctum, chiranthodendron pentadactylon, and vanilla planifolia. In addition, cocoa was added as a flavoring agent for medicines. Some other medical conditions that were treated with cocoa were angina, constipation, tartar removal, dysentery, gout, heart attack, hemorrhoids, and lactation difficulties. (Dillinger et al., 2000)

The Western Europeans used chocolate to treat angina, belching, and flatulence in the same fashion as the Mexican culture, and additionally used cocoa to treat liver disease, menstrual irregularity, strengthening of the heart, heart palpitations,

hypochondria, inflammation, insomnia, cancer, probability of conception, hangovers, vaginal irritation, toothaches, scurvy, antidote against poison, nervousness, old age, and the list goes on (Dillinger et al., 2000). However, Hernández (1577) first recorded that even though there were a lot of medicinal uses for cocoa, it could cause obesity because of the sugar and the fatty oils that were used in the preparation of the drink. Conversely, if someone were underweight, chocolate was considered to be a viable option to promote weight gain (Dillinger et al., 2000). In the 20th century, cocoa was still used for some of the same disease states or discomforts that were used during the previous century, but also included treatment for hemorrhoids, cracked skin, sores, and bronchitis. It was advised that caution be used when treating children and anxious individuals because it was believed to cause excitation (Dillinger et al., 2000).

The Cacao Tree and Bean

The cacao tree has long and wide leaves with oblong fruit, starts bearing fruit about 5 years of age, and reaches maximal reproduction in approximately 10 years. It grows the best at 10-20 degrees north and south of the equator, an area which is commonly known as the cocoa belt. The fruit on the cacao tree is called a pod or a cabosside, shaped like a melon, with a variety of colors ranging from brown/yellow to purple. Each pod contains approximately 20-40 cacao beans and each plant produces 20-50 cabossides each year with 10 cabossides yielding one kilogram of cocoa. The taste of the cacao bean depends on the type of tree, soil, temperature, sunlight, and amount of rain (Verna, 2013).

Varieties of Chocolate

Before the seeds are harvested, they are known as cacao beans; however, after they are roasted and grounded, they are then known as cocoa. Cocoa and chocolate are two distinctive food products. Cocoa is comprised of non-fat, finely ground cacao beans. Chocolate is cocoa powder combined with cocoa butter and sugar (Lippi et al., 2008). There are three different types of chocolates: white, milk, and dark. White chocolate has cocoa butter, milk, and sugar with no cocoa solids. Milk chocolate is high in milk fat with sugar and up to 10% cocoa bean solids. Dark chocolate contains cocoa butter and up to 80% cocoa bean solids (Petyaev & Bashmakov, 2017).

Nutrition Content and Bioavailability

The fat content in DC is known as cocoa butter. It is 33% oleic acid (monounsaturated), 33% stearic acid (saturated), and 25% palmitic acid (saturated). Oleic acid has been shown to have a positive effect on blood lipid levels; whereas stearic acid, most commonly associated with the saturated fat found in animal products, has been demonstrated to increase plasma LDL-cholesterol, triglyceride levels, blood pressure, and the risk for coronary vascular disease (CVD) (Latif, 2013). However, there is not enough research to suggest that the stearic acid from the cacao bean impacts plasma lipids in the same manner as it does from animal fat (Latif, 2013). Palmitic acid, which is a saturated fat, has been thought to be associated with heart disease and cancer. However, these suggestions are unclaimed and not conclusive (Fattore & Fanelli, 2013).

Polyphenols have been widely used to help reduce oxidative stress especially in the field of athletics. Cocoa flavonoids have been suggested to have the highest concentration of polyphenols suggesting that cocoa and DC could provide the greatest

health benefits, specifically with regards to the vasodilator and anti-inflammatory properties (Decroix et al., 2017). Cocoa has a significantly greater flavonoid content than tea, wine, and milk chocolate (Latif, 2013). Other foods that contain flavonoids include apples, berries (Rodriguez-Mateos, et al., 2015), green and black tea, grapes, olives, DC, hazelnuts, and pecans (Decroix et al., 2017). Dark chocolate is high in flavonoids which include epicatechins and catechins. The bioavailability (absorption rate) is the greatest in (-)-epicatechins versus catechins, and a lipid environment promotes absorption rate in the small intestine within 2-4 hours of ingestion (Petyaev & Bashmakov, 2017). However, the more processed (drying, roasting, and fermentation) the DC, the less (-)-epicatechin content; however, the processing is necessary to reduce the bitterness and improve the palatability of the final product (Hurst et al., 2011; Petyaev & Bashmakov, 2017).

Medicinal Uses

Dark chocolate ingestion has been shown to promote vasodilation, stimulate cerebral blood flow, improve oxygen and glucose delivery, and decrease some of the symptoms associated with Alzheimer's disease (Petyaev & Bashmaokv, 2017). Cocoa has also been reported to play an important role in synaptic function, neuronal growth, memory, anti-inflammatory, mood, and cognitive function (Petyaev & Bashmaokv, 2017). Varela et al. (2017) suggests that the (-)-epicatechin, found in cocoa, can promote the browning of fat which can increase fatty acid metabolism via the formation of new mitochondria. G-protein coupled estrogen receptors (GPER) have been shown to have a significant effect in muscle cells thereby stimulating the effects of (-)-epicatechins on mitochondrial biogenesis and skeletal muscle anabolism (Moreno-Ulloa et al., 2015). Cocoa flavonoids have, also, been shown to decrease high sensitivity C-reactive proteins

which reduce inflammation and reduce aggregatory effects of platelets (Hamed et al., 2008).

There have been concerns from the lay population with chocolate consumption and weight gain. However, research suggests that moderate DC consumption has been shown to decrease adiposity in European adolescents (Petyaev & Bashmaokv, 2017) and has not been associated with obesity (Petyaev & Bashmakov, 2017). Dark chocolate has also been shown to reduce the absorption of lipids and carbohydrates thereby improving insulin sensitivity, hypoglycemia (Petyaev & Bashmaokv, 2017), and plasma LDL cholesterol levels (Tokede, Gaziano & Diousse, 2011). Despite the evidence, some individuals are unwilling to consume DC, for reasons such as a dislike for taste, flavor, texture, and aroma, or the belief that it is fattening and unhealthy (Dillinger et al., 2000).

Athletic Application

The polyphenols found in DC have been shown to reduce exercise induced oxidative stress (Morillas-Ruiz et al., 2005; Morillas-Ruiz, Garcia, Lopez, Vidal-Guevara, & Zafrilla, 2006; Lyall et al., 2009; Peake, Suzuki, & Coombes, 2007; Powers, Quindry, & Hamilton, 2004), inflammatory response (Lyall et al., 2009; Peake, Suzuki, & Coombes, 2007; Powers, Quindry, & Hamilton, 2004), and muscle fatigue and immunosuppression (Peake, Suzuki, & Coombes, 2007; Powers, Quindry, & Hamilton, 2004). (-)-Epicatechins have also been shown to stimulate lipid catabolism and fat oxidation (Venables et al., 2008), and endurance capacity via increased mitochondrial biogenesis (Davis, Carlstedt, Chen, Carmichael, & Murphy, 2010). Dark chocolate was demonstrated to positively impact arterial and endothelial function (Vlachopoulos et al., 2005), resting blood pressure and insulin sensitivity (Grassi, Lippi, Necozione, Desideri,

& Ferri, 2005), high-density lipoprotein (HDL) concentration (Mursu et al., 2004), resting and post exercise plasma levels of oxidative stress markers (Davison, Callister, Williamson, Cooper & Gleeson, 2011; Allgrove, Farrell, Gleeson, Williamson, & Cooper, 2011), plasma free fatty acids during exercise and a lower respiratory exchange ratio (RER) (Allgrove et al., 2011). Gutierrez-Salmean et al. (2014) suggested that the consumption of (-)-epicatechin twice a day increased follistatin (promoter of muscle growth) and decreased myostatin (suppressor of muscle growth). Nogueira et al. (2011) suggested (-)-epicatechins reduced muscle fatigue, improved treadmill performance, and increased muscle capillarity. Findings of Taub et al. (2016) indicated that three months of DC consumption increased VO_{2max}, cycling power output, mitochondrial function, plasma HDL cholesterol concentration, and decreased oxidative stress.

Summary

The effects of DC consumption indicate association with improvements in cardiovascular health, maximal oxygen consumption, cycling power output, mitochondrial function, and decrease in oxidative stress. Even though it has a greater energy content, a moderate DC consumption has not been associated with weight gain or central adiposity. However, it has been associated with an elevation of mood, a decrease in depression, and improvement in cognitive function due to its vasodilatory effect.

Chapter III

MANUSCRIPT

Introduction

Chocolate is considered a delicacy by many cultures and provides income for more than 40 million individuals annually yielding approximately 4.2 million tons of cocoa worth $11.8 billion (Beg, Ahmad, Jan, & Bashir, 2017). It has been enjoyed for centuries because of its rich and delectable flavor and its purported health benefits such as a reduction in oxidative stress, inflammation, and physical fatigue (Decroix et al., 2017). Flavonoids, naturally occurring polyphenolic plant compounds, are an active component of cocoa to which many of its health benefits are often attributed (Decroix et al., 2017). Flavonoid rich foods include wine, milk chocolate (Latif, 2013), apples, berries (Rodriguez-Mateos, et al., 2015), green and black teas, grapes, olives, DC, hazelnuts, and pecans (Decroix et al., 2017).

Moderate DC consumption has been reported to promote arterial vasodilation and cerebral blood flow, enhance oxygen and glucose delivery, and improve cognitive function (Petyaev & Bashmaokv, 2017). It has also been shown to decrease fat deposition in adolescents (Petyaev & Bashmaokv, 2017), low density lipoprotein (LDL) cholesterol, high sensitivity C-reactive proteins, and inflammation and platelet aggregation (Hamed et al., 2008; Lyall et al., 2009; Tokede, Gaziano, and Diousse, 2011). Dark chocolate has been shown to stimulate insulin sensitivity and hypoglycemic

control (Petyaev & Bashmaokv, 2017), and lipid oxidation (Venables, Hulston, Cox, & Jeukendrup, 2008). Contrary to contemporary thought, moderate DC consumption has not been shown to be associated with obesity (Petyaev & Bashmakov, 2017).

The flavanol (-)-epicatechin, found in high concentration in cocoa, has been shown, in a mouse model, to reduce skeletal muscle fatigue, improve treadmill performance, and increase muscle capillary density (Noguiera et al., 2011). These findings are supported by Taub et al. (2016) who indicated that three months of DC consumption by humans may decrease oxidative stress and improve maximal oxygen uptake, cycling power output, mitochondrial function, and high-density lipoprotein (HDL) concentration.

Considering the recent health and physiological exercise performance benefits attributed to the administration of (-)-epicatechin and supplementation of (-)-epicatechin-rich cocoa products, the purpose of this study was to investigate the influence of 70% DC supplementation on REE and steady state EEE. We investigated a relatively small dosage of DC ($20g \bullet d^{-1}$) for a short duration (~30 d) in a group of recreationally fit/athletic females.

Methods

The study employed a randomized double blind, placebo-controlled design and was approved by the Institutional Review Board for the use of Human Subjects in Research (IRB 03572-2017) (Appendix A & B). Inclusion criteria were non-smoking, normal weight females (body mass index 18.5-25 $kg \bullet m^2$), 18-30 years of age, performing a minimum of 5 $h \bullet wk^{-1}$ of moderate- to high-intensity exercise. Twenty-five subjects met the inclusion criteria; however, seven were eventually excluded due to factors such

as dislike of chocolate, non-compliance with supplementation, or incurring a physical injury during the course of the study that limited their ability to continue. This left a final subject pool of n = 18. There were no reported adverse events associated with the study. Subjects were instructed to maintain their normal physical activity levels throughout the duration of the study.

Each subject was required to arrive in the laboratory on three separate occasions over ~35 days. The first visit was a preliminary/familiarization assessment, the second session was a pre-supplementation assessment, and the final session was a post-supplementation assessment.

Preliminary/familiarization assessment. The preliminary/familiarization assessment required subjects to arrive to the laboratory 3-4 hours post-prandial and having refrained from intense exercise for 48-h prior. Upon arrival, subjects completed an informed consent and physical activity and health history questionnaire (Appendix D), and were assessed for resting measures of body weight, height, heart-rate, and blood pressure. Resting energy expenditure (REE) was assessed via open-circuit indirect calorimetry (Vmax Encore Metabolic Cart; Yorba Linda, CA) (Appendix E). Flow volume and gas calibrations were performed prior to each testing session according to the manufacturer's instructions. Subjects were asked to remove their shoes, assume a supine position on an examination table, and a Plexiglas ventilated hood was placed over their head. Expired gases were then assessed for ~30-min. The first 10-min allowed the subject to acclimate to the test and reach a metabolic steady state. The REE was determined from 10-min of steady state respiratory gas measurements assessed during min 10-30. During this time, the flow pump rate was manipulated to insure a $FECO_2$ between .75-.85. Energy

expenditure was calculated from an abbreviated Weir equation [3.9(VO_2) + 1.1(VCO_2) x 1.44] (Weir, 1949).

Upon completion of the assessment of REE, the metabolic cart was immediately recalibrated to accommodate exercise testing, and the subject was fitted with a facemask and mask flow sensor (Appendix F & H). The subject then performed 20-min of continuous exercise on a Velotron cycle (Racermate, Seattle, WA) (Appendix G) with the first 10-min performed at 50 watts and the last 10-min performed at 100 watts. Expired gas volumes and content were analyzed and used in the assessment of EEE and substrate utilization. Cycling cadence was selected by each subject and then replicated during subsequent sessions.

Pre-supplementation assessment. The pre-supplementation assessment was scheduled ~7-d after the preliminary/familiarization assessment and ~14-d after the onset of their last menstrual cycle to control for fluctuations in metabolic rate (Solomon, Kurzer, & Calloway, 1982). Subjects then performed another assessment for REE and 20-min of cycling with 10-min each at 50 and 100 watts. Upon completion of the second visit, each subject was provided 14-d of supplementation.

Post-supplementation assessment. The post-supplementation assessment was performed, as closely as possible, to the same day of their menstrual cycle as performed during the pre-supplementation assessment. The post-supplementation assessment consisted of resting and exercise energy expenditure in the same fashion as described in the pre-supplementation assessment.

Supplementation. Supplementation was assigned using a randomized, double-blind design. The experimental treatment consisted of 20 g of 70% DC (Scharffen Berger

Baking Chunks or Squares, The Hershey Company, Hershey, PA) and the placebo consisted of a calorically matched volume of WC (White Chocolate Baking Chips, The Hershey Company, Hershey, PA). Supplementation duration was initially scheduled for a total of 28 days; however, in an effort to minimize the influence of the menstrual cycle on metabolic rate, the duration was individualized for each subject (Solomon et al., 1982). An individual not affiliated with the project was responsible for the preparation of the supplementation. Each day's chocolate was individually wrapped in foil and placed into a plastic bag with 7 days in each plastic bag for a total of 4-5 bags for each participant. These were then placed in single brown paper bags to disguise the content from the investigators. Additionally, subjects were informed that the purpose of the study was to investigate the effect of chocolate on metabolic rate; however, they were not aware that the intent of the study was to investigate the effect of DC on metabolic rate and that the WC was a placebo. To our knowledge, there was no communication between subjects.

Subjects were instructed to consume their prescribed chocolate and to return in 14-d to receive the other 14-d of assigned supplementation. At this time subjects were questioned regarding compliance with the supplement ingestion protocol and those unable, or unwilling, to comply were excluded from further participation in the study. During the supplementation period, subjects were instructed to refrain from the consumption of high flavonoid containing foods such as extra chocolate, blueberry, cherry, strawberry, blackberry, raspberry, apple, pomegranate fruits and/or products, chestnuts, hazelnuts, black tea, green tea, and red wine. Additionally, subjects were instructed to not consume their prescribed chocolate with any dairy product (i.e. yogurt,

sour cream, milk, cottage cheese, cheese, and butter/margarine) 1-h before and after consumption of supplementation since dairy. There are conflicting reports on the influence of dairy on the bioavailability of flavonoids, so we chose a conservative approach to restrict dairy use with the consumption of the chocolate (Lippi et al., 2008; Roura et al., 2007).

Statistical analyses were performed using Statistical Packaging for the Social Sciences (SPSS). A 2 X 2 mixed design ANOVA (Treatment: WC vs. DC; Time: pre-supplementation vs. post-supplementation) was used to assess differences within and between conditions for REE, EEE, and substrate oxidation rates. The significance level was set at $p < .05$. All data are presented as mean ± SE.

Results

Demographics. Inclusion criteria were non-smoking, normal weight females (body mass index 18.5-25 $kg \bullet m^2$), 18-30 years of age, performing a minimum of 5 $h \bullet wk^{-1}$ of moderate- to high-intensity exercise. Twenty-five subjects met the inclusion criteria; however, seven were eventually excluded due to factors such as dislike of chocolate, non-compliance with supplementation, incurring a physical injury during the course of the study that limited their ability to continue, or data was an outlier. This left a final subject pool of n = 18. Subjects reported no adverse effects with supplementation. The duration of supplementation for each group was not significantly different (DC 30.8 ± 2.6, WC 30.6 ± 4.2 d). Subject characteristics are indicated in Table 1.

TABLE 1. Subjects' Characteristics.

	WC (n = 9)	DC (n = 9)
Body weight (kg)	69.5 ± 11.7	71.6 ± 11.8
Height (m)	1.7 ± 0.1	1.7 ± 0.1
BMI (kg•m^2)	23.4 ± 2.2	25.0 ± 3.3
Age (yr)	21.0 ± 2.0	21.0 ± 2.0

Values are expressed as mean ± SE. WC, White Chocolate; DC, Dark Chocolate.

Resting Energy Expenditure (REE). Pre-supplementation REE (DC 1455 ± 49, WC 1566 ± 49 kcal•d^{-1}) were not significantly different between groups ($p \geq .05$). Post supplementation REE was significantly increased by 9.5% in DC (Δ REE: DC 138 ± 21, WC -30 ± 21 kcal•d^{-1}, $p = .001$) (Fig. 1 and Fig 2).

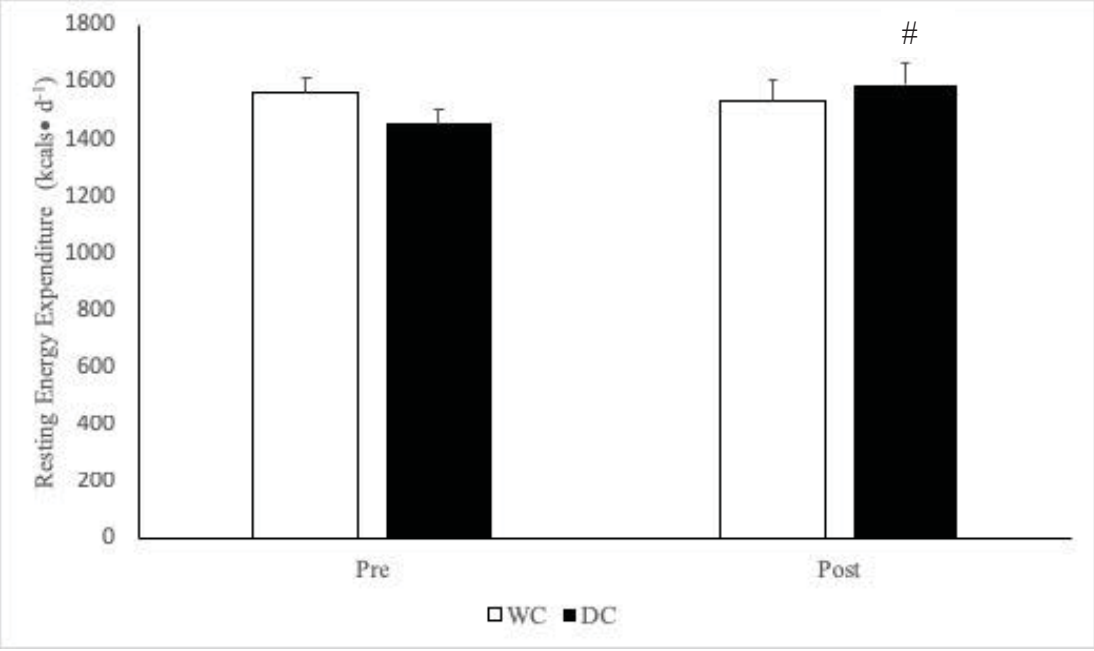

Fig. 1 – REE before (Pre) and after (Post) dark chocolate (DC) or white chocolate (WC) supplementation. Values are expressed as means ± SE. # indicates a between groups time effect with the DC pre-post significantly greater than WC ($p = .001$).

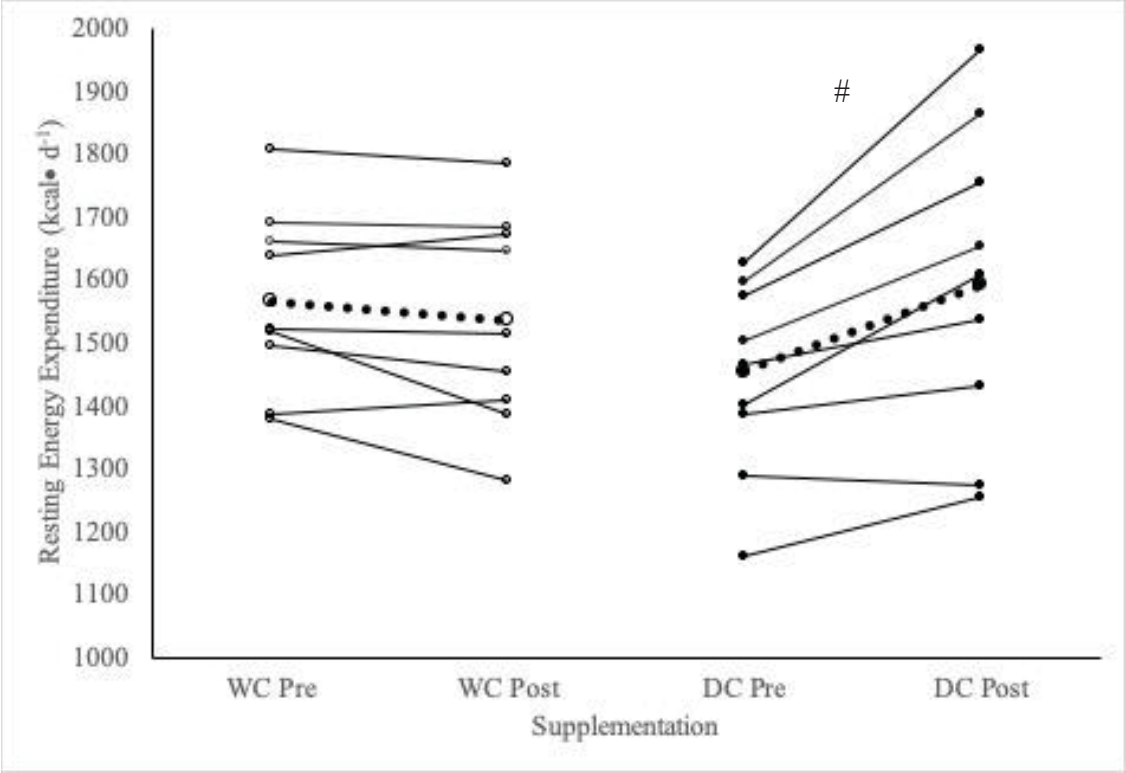

Fig. 2 – Individual subjects REE pre- and post-supplementation with WC (o) or DC (•). ••• indicates mean data for each group. # indicates a between groups time effect with the DC significantly greater than WC (p = .001).

Substrate Utilization. There were no significant interaction, time, or group effects for resting fat or carbohydrate (CHO) substrate utilization during the assessment of REE (Table 2).

Table 2. Substrate Utilization

	WC PRE	WC POST	DC PRE	DC POST
Fat ($g \bullet d^{-1}$)	108.7 ± 11.5	100.6 ± 12.1	125.1 ± 11.5	107.2 ± 12.1
CHO ($g \bullet d^{-1}$)	128.4 ± 21.7	147.3 ± 30.9	63.7 ± 21.7	138.8 ± 30.9

Values are expressed as mean ± SE. WC, White Chocolate; DC, Dark Chocolate; PRE, before supplementation; POST, after supplementation. There were no significant treatment or time effects ($p \geq .05$).

Exercise Energy Expenditure (EEE). There were no significant interaction, time, or group effects during the assessment of EEE at cycling power outputs of both 50W (EEE-50) and 100W (EEE-100) ($p \geq .05$) (Table 3).

Table 3. Exercise Energy Expenditure ($kcal \bullet min^{-1}$)

	WC PRE	WC POST	DC PRE	DC POST
EEE-50 ($kcal \bullet min^{-1}$)	4.61 ± 0.15	4.48 ± 0.16	4.86 ± 0.15	4.44 ± 0.16
EEE-100 ($kcal \bullet min^{-1}$)	6.77 ± 0.16	6.65 ± 0.17	7.07 ± 0.16	6.51 ± 0.17

Values are expressed as mean ± SE. WC, White Chocolate; DC, Dark Chocolate; PRE, before supplementation; POST, after supplementation; EEE at cycling power outputs of both 50W, (EEE-50); and 100W, (EEE-100).

Oxygen Uptake (VO_2). There were no significant interaction, time, or group effects during the assessment of VO_2 at cycling power outputs of both 50W (VO_2-50) and 100W (VO_2 -100) ($p \geq .05$) (Table 4).

Table 4. Absolute VO_2 ($L \bullet min^{-1}$)

	WC PRE	WC POST	DC PRE	DC POST
VO_2-50 ($L \bullet min^{-1}$)	.939 ± .032	.910 ± .031	.990 ± .032	.907 ± .031
VO_2-100 ($L \bullet min^{-1}$)	1.362 ± 0.033	1.336 ± 0.034	1.425 ± 0.033	1.307 ± 0.034

Values are expressed as mean ± SE. WC, White Chocolate; DC, Dark Chocolate; PRE, before supplementation; POST, after supplementation; cycling power output 50W (VO_2-50) and 100W (VO_2-100).

Respiratory Quotient (RQ). There were no significant interaction, time, or group effects during the assessment of RQ was determined at rest (RQ-0) and at cycling power outputs of both 50W (RQ-50) and 100W (RQ-100) (Table 5).

Table 5. Respiratory Quotient

	WC PRE	WC POST	DC PRE	DC POST
RQ-0	.81 ± .02	.82 ± .02	.76 ± .02	.81 ± .02
RQ-50	.91 ± .02	.92 ± .02	.91 ± .02	.93 ± .02
RQ-100	.97 ± .02	.97 ± .02	.96 ± .02	.97 ± .02

Values are expressed as mean ± SE. WC, White Chocolate; DC, Dark Chocolate; PRE, before supplementation; POST, after supplementation; resting (RQ-0) cycling power output 50W (RQ-50) and 100W (RQ-100).

Discussion

The purpose of this study was to investigate the influence of a relatively small dosage of 70% DC (20 $g \bullet d^{-1}$) of short duration (~30-d) on REE and steady state EEE in a group of recreationally fit/athletic females. The notable findings indicated that DC

consumption increased daily REE by ~9.5% (+138 kcal•d^{-1}); however, this change was not observed in EEE.

Chocolate has a very rich and storied history, having its beginnings attributed to the divine discovery of cacao by the Aztecan god Quetzalcoatl (Feather Serpent). Blood mixed with cacao was deemed as a worthy sacrifice for the gods, and Aztecan human sacrifices (prisoners) were fed roasted and ground cacao beans so the gods would find their sacrifice most worthy (Dillinger et al., 2000). Thus, cacao was originally referred to as the food of the gods (Coe & Coe, 1996).

Over the past 500 years, cacao, and its processed form chocolate, have been used as a treatment for a plethora of diseases and medical conditions. These include menstrual irregularity, angina, strengthening of the heart, heart palpitations, hypochondria, inflammation, insomnia, cancer, conception, hangovers, vaginal irritation, toothaches, scurvy, antidote against poison, belching, flatulence, nervousness, and old age. It was also noted that cocoa consumption was sometimes associated with obesity, consequently, if someone were underweight, chocolate might provide a tasty option to promote weight gain. (Dillinger et al., 2000)

The specific mechanisms by which DC impacts physiological function have not been clearly elucidated. Being that nutritional supplements are not closely regulated (U.S. Food and Drug Administration, 1994), the recommended dosages are often at the discretion of the manufacturer and/or consumer, leading some to the assumption that "if a little is good, more is better". Thus, a major confounding factor in the interpretation of the literature is that there is little consistency in the dosing used in nutritional supplementation studies. A positive health benefit attributed to DC consumption is the

strong polyphenol qualities of the flavonoid (-)-epicatechin (Hurst et al., 2011). However, there is accumulating evidence suggesting that flavonoids with high total anti-oxidant capacity (TAC) may actually inhibit, rather than enhance, skeletal muscle adaptations to exercise (Hollman et al., 2011; Paulsen et al., 2014). Thus, it is unlikely that the increase in TAC associated with DC supplementation utilized by Taub et al. (2016) was responsible for the improvement in maximal oxygen uptake. It is quite plausible that a larger dose of DC may actually exhibit a negative effect on physiological function, whereas a smaller dosage would not. With this thought in consideration, we wanted to minimize any potential inhibitory effect of DC associated with an increase in TAC and consequently chose to employ a relatively small DC supplementation dosage (20 g•d^{-1}) and short duration (30 d).

Whereas, an increase in TAC associated with DC consumption may potentially inhibit physiological function, recent evidence suggests a mechanism whereby (-)-epicatechins may bind to cell surface G protein-coupled estrogen receptors thereby positively impacting metabolic control (Moreno-Ulloa et al., 2015). Taub et. al. (2016) recently demonstrated that a 3-month administration of 20g/d^{-1} of (-)-epicatechin-rich cocoa in human adults increased maximal oxygen uptake and cycling power output in a group of sedentary, middle-aged males and females. It was suggested that this positive effect might be due to an improvement in mitochondrial efficiency in response to the flavonoid, (-)-epicatechin, and indeed this notion was supported by their observed 140% increase in citrate synthase activity, a marker of mitochondrial function (Taub et al., 2016). Earlier work in isolated mouse muscle indicating that (-)-epicatechin by itself, or combined with exercise, also can induce structural and metabolic changes in skeletal and

cardiac muscles (Nogueira et al., 2011). While speculative, our findings of a 9.5% increase in REE would suggest significant changes in mitochondrial function likely did occur with the administration of a relatively small and brief supplementation of DC.

Previous studies of both high intensity interval training (Jacobs et al., 2013) and aerobic exercise training (Short et. al., 2003) demonstrated 20% and 45% increases in skeletal muscle citrate synthase, and an approximate 10% increase in maximal oxygen uptake, respectively. Both of these exercise-induced measures are significantly less than that demonstrated in sedentary adults after 3 months of DC supplementation (Taub et. al., 2016). In addition, positive markers of oxidative stress were significantly improved with DC supplementation (Taub et. al., 2016) in a magnitude quite similar to that observed with chronic physical conditioning (Olesen et. al., 2014). Collectively, these data suggest that (-)-epicatechin rich DC may influence bioenergetics in a magnitude similar to, or possibly even greater than, that observed with physical conditioning.

As indicated previously, (-)-epicatechin alone, and in combination with exercise, promotes structural and metabolic changes in mouse skeletal and cardiac muscles and endurance capacity (Nogueira et al., 2011). In contrast, Copp et al. (2013) reported that (-)-epicatechin administration had no impact on resting oxygen uptake or exercise performance. While any explanation for the contrary findings is just speculative, two thoughts come to mind: 1) Is there a species difference (mice vs. rats) in the response to (-)-epicatechin administration? 2) Was the difference in (-)-epicatechin dosage responsible for the different findings? The positive findings of Nogueira et al. (2011) were in response to an (-)-epicatechin administration of 2 $mg \bullet kg^{-1} \bullet d^{-1}$ for 15 d, whereas the absence of positive findings reported by Copp et al. (2013) were in response to an (-)-

epicatechin administration of 4 mg•kg^{-1}•d^{-1} for 24 d. Considering the accumulating evidence suggesting that a high TAC may actually inhibit skeletal muscle adaptations (Hollman et al., 2011; Paulsen et al., 2014), the conflicting exercise performance findings are not surprising.

With regards to steady state EEE, we had conditionally hypothesized that if changes in REE were observed in response to DC supplementation, similar changes would be observed during steady state EEE. Surprisingly, this was not the case. While Taub et al. (2016) employed a similar DC dosage for 90 d and reported a 17% increase in maximal oxygen uptake and improved cycling endurance to exhaustion, our protocol supplementation duration was only 30 d and evaluated the metabolic response during steady state exercise, not maximal exercise performance. In addition, the subjects assessed in our study were relatively young (~21 years), recreationally fit/competitive females, whereas the subjects of Taub et al. (2013) were middle aged (~49 years) and sedentary. Due to the difference between the exercise protocols, as well as the difference between subject characteristics, our findings are not surprising.

In summary, to our knowledge this is the first study to demonstrate that a relatively small daily dosage of DC can significantly elevate REE but does not impact steady state EEE. Future DC supplementation studies are warranted investigating: 1) energy balance, weight control, and body composition, 2) energy metabolism in sedentary vs. exercise trained males and females, 3) energy metabolism of post-menopausal and aged females, and 4) exercise metabolism and skeletal muscle performance in individuals with sarcopenia.

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APPENDIX A:

Institutional Review Board Approval

Valdosta State University

APPLICATION FOR USE OF HUMAN PARTICIPANTS IN RESEARCH

EXPEDITED APPLICATION

INSTRUCTIONS: Complete this form by checking all appropriate boxes. Attach all CITI training documents, and obtain all necessary signatures before submitting to the Office of Sponsored Programs & Research Administration.

Project Title: Chocolate Consumption: Effect on Resting and Exercise Metabolism Project Dates: Jan 2018 to Dec 2018

MM/DD/YYYY MM/DD/YY

Responsible Researcher: Katie Presler
Mailing Address: HSBA building office 2137
Department: Health Science Exercise Physiology
☒ No
E-mail: KPresler@Valdosta.edu
Telephone: 951-533-3693

Minimum # of Participants: 40
Maximum # of Participants: 50
External Funding: ☐ Yes

If Yes, Sponsor:

(Note: If the research is or will be externally funded, include a copy of the portion of the proposal or award that describes use of human participants.)

Supervising Faculty: Michael Webster
Supervising Faculty Email: MJWebster@Valdosta.edu

VSU Status:

☐FT/PT Faculty
☐Adjunct Faculty
☐Research Associate
☐ Administrator/Staff Member
X Graduate Student
☐Doctoral Dissertation
X Master's Thesis
☐Undergraduate Student
☐Senior Project

Co-investigator	Institutional Affiliation	Email Address	*IRB FWA #

☐*Unaffiliated Investigator

***Note: Unaffiliated Investigators must fill out the last column "IRB FWA#" and complete an unaffiliated agreement form found at: http://www.valdosta.edu/academics/graduate-school/research/office-of-sponsored-programs-research-administration/institutional-review-board-irb-for-the-protection-of-human-research-participants.php**

1. ☒ YES ☐ NO Does your proposed study (a) meet the Valdosta State University Institutional Review Board definition of research (as cited below) or (b) does it involve a condition for IRB oversight as listed below?

VSU IRB Definition of Research: Valdosta State University describes research as a systematic investigation, including research development, testing and evaluation designed to develop or contribute to generalizable knowledge.

Conditions: The following conditions may not meet the definition of "research" as provided above, but will cause your research to be subject to IRB oversight:

- Intent to produce results that will be submitted for peer-reviewed publication or presentation
- Include minors (e.g. those under the age of 18)
- Target potentially vulnerable individuals
- May place pregnant women and/or fetuses at risk of physical harm
- Deal with a topic of sensitive nature in a way which anonymity cannot be sustained

- Involve any activity that places the participants at more than minimal risk (see Question 9 for definition of "minimal risk")

2. ☐ YES ☒ NO Are the human participants in your study living individuals or are you collecting information about deceased persons that may put third parties (i.e., surviving spouses and/or living descendants) at more than minimal risk of harm?

3. ☒ YES ☐ NO Will you obtain data through intervention or interaction with living or third-party individuals?

"Intervention" includes both physical procedures by which data are gathered (e.g. measurement of heart rate of venipuncture)

"Interaction" includes communication or interpersonal contact between the investigator and participant (e.g. surveying or interviewing)

4. ☐ YES ☒ NO Will you obtain identifiable private information about these individuals?

Private information includes information about behavior that occurs in a context in which an individual can reasonably expect that no observation or recording is taking place. Identifiable means that the identity of the participant maybe ascertained by the investigator.

(1) Does your work involve human blood, body fluids, cells, or tissue components?

(2) Does your work involve recombinant DNA or a biohazardous agent?

Note: If you answered **NO** to Question 1 or 2, no IRB review is required. If you answered **NO** to Question 3 **AND** 4, no IRB review is required. If you have any questions on whether you are conducting research that needs IRB oversight please review the IRB Exempt Flowchart on our website.

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5. EDUCATIONAL REQUIREMENTS: In accordance with federal regulations, the VSU IRB requires all responsible researchers, co-investigators, key personnel, including unaffiliated investigators, and faculty advising student researchers to complete the CITI educational program. Co-investigators from other institutions are not required to complete this if they have a certificate of completion from their own federally assured IRB.

Please visit: http://ww.citiprogram.org to complete all of the following mandatory trainings:

1. Introduction
2. History and Ethical Principles
3. Defining Research with Human Subjects
4. The Regulations and the Social and Behavioral sciences
5. Basic Institutional Review Board (IRB) Regulations and Review Process
6. Assessing Risk in Social and Behavioral Sciences
7. Informed Consent
8. Privacy and Confidentiality
9. Valdosta State University Module

Additional modules may be required for specific types of research. Please check all that apply and complete the corresponding modules:

Study population targets	**Additional CITI Modules Required**
☐ a. Minors (under the age of 18)	Research with Children
☐ b. Public school Children	Research in Public Elementary and Secondary Schools
☐ c. Pregnant Women	Vulnerable Subjects
☐ d. Prisoners	Research with Prisoners
☐ e. Potentially vulnerable individuals (those whose consent maybe compromised due to socio-economic, educational or linguistic disadvantage.)	Research with Protected Populations
☐ f. Individuals in foreign countries	International Research
☐ g. Individuals from different cultures or individuals from a particular racial/ethnic group	Group Harms: Research with Culturally or Medically Vulnerable groups
☐ h. Individuals about whom data will be collected from records (e.g., educational, health, or employment records)	Records-Based Research
☒ i. Individuals from or about whom Private Health Information (PHI) subject to HIPAA compliance will be collected	HIPAA and Human Subjects
☐ j. Individuals from whom information will be collected via Internet	Internet Research
☒ k. VSU Employees	Workers as Research Subjects

6. ☐ YES ☒ NO ***Does the primary researcher, co-investigator, or any other key person, have a potential or actual significant financial conflict of interest in performance of the research?***

If YES, please complete the CITI module "Conflicts on Interest in Research Involving Human Subjects" AND complete the VSU Conflict of Interest form available at http://www.valdosta.edu/grants/forms.

7. As a researcher you are expected to follow VSU's code of ethics. Will there be an additional code of ethics followed?

Include organization's name & Web address:

NO

8. Name and location of external organization(s) providing research participants (attach letter(s) of cooperation):

9. ☐ YES ☒ NO ☐ UNCERTAIN Does the study present more than minimal risk to the participants?

"Minimal Risk" means that the risks of harm or discomfort anticipated in the proposed research are not greater, considering probability and magnitude, than those ordinarily encountered in daily life or during performance of routine physical or psychological examinations or tests. Note that the concept of risk includes psychological, emotional, or behavioral risks to employability, economic well-being, social standing, and risk of civil criminal liability.

10. If the research project can be described by one or more of the categories listed below, please check all that apply:

☐ **Category 1** - Clinical studies of drugs and medical devices only when

(a) the research is on drugs for which an investigational new drug application (21 CFR 312) is not required or

(b) the research is on medical devices for which

(i) an investigational device exemption application (21 CFR 812) is not required or

(ii) the medical device is cleared/approved for marketing and the medical device is being used in accordance with its cleared/approved labeling.

☐ **Category 2 -** Collection of blood samples by finger stick, heal stick, ear stick, or venipuncture from

(a) healthy, non-pregnant adults who weigh at least 110 pounds for whom

(i) the amounts drawn do not exceed 550 ml in an 8-week period and

(ii) collection does not occur more frequently than 2 times per week or

(b) other adults and children, for whom, considering the age, weight, and health of the participants, and the collection procedures,

(i) the amount of blood to be collected does not exceed the lesser of 50 ml or 3 ml per kg in an 8-week period and

(ii) collection does not occur more frequently than 2 times per week.

(NOTE: Children are defined as "persons who have not attained the legal age for consent to treatments or procedures involved in the research, under the applicable law of the jurisdiction in which the research will be conducted.")

☐ **Category 3 -** Prospective collection of biological specimens for research purposes by noninvasive means, including:

(a) hair and nail clippings, in a non-disfiguring manner;

(b) deciduous teeth at time of exfoliation or if routine patient care indicates a need for extraction;

(c) permanent teeth if routine patient care indicates a need for extraction;

(d) excreta and external secretions (including sweat);

(e) uncannulated saliva collected either in an unstimulated fashion or stimulated by chewing gum base or wax or by applying a dilute citric solution to the tongue;

(f) placenta removed at delivery;
(g) amniotic fluid obtained at the time of rupture of the membrane prior to or during labor;
(h) supra- and subgingival dental plaque and calculus, provided the collection procedure is not more invasive than routine prophylactic scaling of the teeth and the process is accomplished in accordance with accepted prophylactic techniques;
(i) mucosal and skin cells collected by buccal scraping or swab, skin swab, or mouth washings; and
(j) sputum collected after saline mist nebulization.

☒ **Category 4 -** Collection of data through non-invasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving x-rays or microwaves. Such procedures include:
(a) physical sensors that are applied either to the surface of the body or at a distance and do not involve input of significant amounts of energy into the participant or an invasion of the participant's privacy;
(b) weighing or testing sensory acuity;
(c) magnetic resonance imaging;
(d) electrocardiography, electroencephalography, thermography, detection of naturally occurring radioactivity, electroretinography, ultrasound, diagnostic infrared imaging, Doppler blood flow, and echocardiography; and
(e) moderate exercise, muscular strength testing, body composition assessment, and flexibility testing where appropriate given the age, weight, and health of the individual.
(NOTE: Where medical devices are employed, they must be cleared/approved for marketing.)

☐ **Category 5 -** Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected, solely for non-research purposes (such as medical treatment or diagnosis).

☐ **Category 6 -** Collection of data from voice, video, digital, or image recordings made for research purposes.

☐ **Category 7 -** Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies.

11. Selection of Participants and Voluntariness:

a) Participant Population:

40-50 females will be recruited for participation in the study. Half (N = 20-25) of these individuals will be 18-30 years of age whereas the other half (N = 20-25) will be post-menopausal 50-70 years of age. Inclusion in the study requires that they have a body mass index (BMI) (weight in kg / height in meters^2) of greater than 18.5 or less than 30.0, be non-smokers, not regularly (2-3 times per week) consuming chocolate and not presently taking any nutritional supplements that might possibly alter metabolic rate. They must also indicate that they are free of any physical condition(s) that would prevent them from completing the physical demands of participation, or put them at more than the expected physical risk, when performing the required exercise. Individuals that are knowingly pregnant, think they might be pregnant, or actively trying to conceive, will be excluded from participation in the study. All participants will be required to complete a physical activity and pre-participation health history questionnaire to screen-out any individuals that present exclusionary criteria.

Inclusion:

- 18-30 years of age (N = 20-25)

- BMI of 18.5 - 30.0
- Free of any physical condition(s) that would prevent them from completing the physical demands of participation, or put them at more than the expected physical risk, when performing the required exercise protocol.

Exclusion:
- Males
- Not 18-30 years of age
- BMI of < 18.5 or > 30.0
- Presently taking, or have taken in the past 30 days, any nutritional supplement or medication that might possibly alter metabolic rate
- Individuals that are knowingly pregnant, think they might be pregnant, or actively trying to conceive
- Presenting a pre-participation health history questionnaire indicating any health conditions, or any potential medication/supplement interactions that would warrant exclusion from participation. All responses to the questionnaire will be evaluated by a qualified, licensed medical professional and final approval for participation will be provided by this individual. If exclusion is warranted, the subject will be informed via an in-person meeting with the investigator.
- Smoker
- Regular consumer of dark chocolate

b) Methods for Selecting Participants:
- E-mail blast to the VSU faculty and student population
- Fliers will be posted on bulletin boards around the HSBA building and in the VSU Recreation Center (see appendix 9)
- Word-of-mouth
- Any individual that meets the requirements for participation is welcome

c) Assurance that Participation is Voluntary:
- Participation in the study is 100% voluntary
- All participants are free to withdraw from the study at any time without penalty
- For students, there are no academic incentives (e.g., extra credit) for participation and likewise there are no academic penalties for not participating

12. Informed Consent

(See Appendix – 1)

13. Compensation:
- Participation is completely voluntary and there are no costs to the participant
- Upon completion of the project subjects will be provided with information about their:
    - Body composition
    - Resting energy expenditure
    - Exercise energy expenditure
- If requested, subjects will also be provided with any presentation abstracts, publication abstracts, or manuscripts resulting from their participation

14. Deception:

The research protocol is a randomized, matched-pairs, double-blind design; however, there is no deception involved.

15. Research Protocol:

There are two groups of subjects for this study, each of which will be randomly assigned to one of two experimental conditions:

- 18- to 30-year-old females. N = 20-25
    - **Experimental condition A** = Dark chocolate – 20 grams of dark chocolate powder per day for 30-days
    - **Experimental condition B** = White Chocolate – 20 grams of a white chocolate placebo per day for 30-days

Both the dark chocolate and white chocolate are provided by the Hershey. White chocolate is serving as the placebo due to the fact that it does not contain any (-)-epicatechin, which is the active component of dark chocolate that is being evaluated in this study. Subjects will not be aware that the specific intent of the study is to investigate dark chocolate.

Laboratory Visit #1 (~90-minutes):

Laboratory Visit #1 will require the subject to read the informed consent and then they will also have the opportunity to ask any questions related to their participation in the study. At this visit they will also complete a physical activity and health history questionnaire (see appendix 3). Initial resting measurements of body weight, height, heart-rate, and blood pressure will be obtained. Body composition (body fat and lean tissue) will be determined via DEXA (see appendix 7).

A preliminary/familiarization 20-minute submaximal cycling exercise test will be performed on a laboratory cycle (see appendix 6). This test will require the subject to be fitted with a heart rate monitor chest strap (see appendix 8) and also a facemask that covers both the nose and mouth (see appendix 5). This facemask will allow the subject to breathe freely so that during the exercise test breathing rate, air volume, and expired concentrations of oxygen and carbon dioxide can be determined. Once the heart rate monitor and facemask are in place, the subject will perform a 20-minute bout of exercise on the cycle. The first 10 minutes will be performed at a relatively low workload and the second 10 minutes will be performed at a moderate workload.

- 10 minutes cycling at 50 watts
- 10 minutes of cycling at 100 watts

Upon completion of the 20-minutes of cycling, subjects will be given a 3-minute recovery before commencing with a maximal exercise performance test as follows:

- **Exercise Performance Test**
    - 1 km cycling time-trial. The goal is to complete the 1 km as quickly as possible (~2-3 minutes)

Summary of the Laboratory Visit #1 (~90 minutes):

Participation documents

- Informed Consent
- Physical activity and health history questionnaires

Resting Measurements

- body weight
- height
- heart-rate
- blood pressure
- body composition via DEXA

Cycling Exercise Test

- 20 minutes test on the cycle

Exercise Performance Test

  - 1 km cycling time-trial

Assignment of date for next visit

- Schedule a day and time for their return to the laboratory within the following week.

Laboratory Visit #2 (~60-90 minutes):

During this visit the subject will be required to arrive in the laboratory in an 8-hour fasted state (no food or drink other than water). Additionally, they must not have consumed any caffeine or alcohol, nor performed more than 20 minutes of low intensity exercise during the previous 24-hours.

Upon arrival to the laboratory, the subject will be requested to void their bladder and then their resting energy expenditure (REE) will be determined by analysis of the gases expired by them while resting in a supine position (Vyasis Vmax Encore) on an examination table. This process requires the subject to remain relatively motionless, yet remain awake, on an exam table for approximately 30-min. To obtain the metabolic measurements, a transparent canopy will be placed over the subject's neck and head and this is connected by a tube to an expired air mixing chamber for the analysis of expired gases (oxygen and carbon dioxide) (see appendix 4).

After completion of the test for REE, a 20-minute submaximal cycling exercise test will be performed in the same manner indicated previously, with the first 10 minutes performed at a relatively low workload and the second 10 minutes performed at a moderate workload. Upon completion of the 20-minutes of cycling, subjects will be given a 3-minute recovery before commencing with an exercise performance test, again, in the same fashion described previously.

After completion of the exercise performance test, subjects will be provided with either the dark chocolate or the placebo - white chocolate using a randomized, double-blind, matched pairs research design. Before departing the laboratory, the day and time for the subject's next visit to the laboratory in 30-days will be scheduled.

Summary of Laboratory Visit #2 (~60-90 min):

Resting Measurements

- Resting Energy Expenditure

Cycling Exercise Test

- 20-minutes test on the cycle

Exercise Performance Test

  - 1 km cycling time-trial

Assignment of experimental supplement (randomized, double-blind, matched pairs research design)

- Dark chocolate
- White chocolate - Placebo

Assignment of date for next visit

- Schedule a day and time for their return to the laboratory in 30-days.

Laboratory Visit #3 (~60-90 minutes):
During this visit, the subject will replicate all testing as performed in laboratory visit #2.

Summary of **Laboratory Visit #3 (~60-90 min):**

Resting Measurements

- Resting Energy Expenditure

Cycling Exercise Test

- 20-minute test on the cycle

Exercise Performance Test

- 1 km cycling time-trial

Data collector's qualifications and Training
Primary Investigator – Masters' graduate student, Katie Presler, is trained in all aspects of this study. She is experienced with the use and programming of the laboratory cycle, the calibration and use of metabolic measurement system for the assessment of resting and exercise energy expenditure, the calibration and use of the DEXA for the assessment of body composition, and she is adult CPR/First Aid certified. She has completed CITI training for "Conflicts of Interest" and "Human Research – IRB" (see appendix 2).

Major Professor/Co Investigator - Dr. Michael Webster, Associate Professor of Exercise Physiology, has been conducting human performance laboratory exercise studies in higher education for 30 years. He has completed CITI training for "Conflicts of Interest" and "Human Research – IRB" (see appendix 2). He has American Red Cross Adult/Infant CPR and AED certification and has also been certified with the American College of Sports Medicine as a Clinical Exercise Physiologist since 1988. Part of this certification is the demonstration of appropriately responding to medical emergencies.

Arrangements for referral of participants to support services or assistance that may be needed as a result of their participation in the research

If a subject is injured as a result of their participation in this study, they are instructed to contact Dr. Michael Webster at 601-543-8926 (cell) or 229-333-7191 (office). They are also informed that neither the researcher nor Valdosta State University has made special provision for services required to treat any injury/psychological distress that may result from participation in this research study beyond those normally provided to VSU students. VSU student participants will be informed that they may request health care services from Student Health Services at 229-333-5886 or from their own private health care provider under their selected health insurance plan. By agreeing to participate in this research project, subjects are informed that they are not waiving any rights that they may have against Valdosta State University for injury resulting from negligence of the University or its researchers.

16. Privacy and Confidentiality:

Upon agreement to participate in the study, each subject will be assigned a letter/number identification known only by the researchers and kept in a file secured in the PI office. Individual data collected in the course of the study will only be divulged upon completion of the study to the individual participant from which the data are collected. Only group data will be reported for presentation and publication purposes. In the event of a medical emergency an individual's data will be provided to their physician upon request by the physician and/or the subject. This will necessitate exclusion from further participation in the study. All data collected during the course of the study will be kept in a file secured in the PI office and/or computer. All data will be retained for a period of 3 years after completion of the study and then all paper data shredded and electronic data deleted.

17. Risks:
Risk of participation is minimal; however, no risk can be completely eliminated. The following are aspects of risk associated with participation:

- **Exercise** - Exercise of any kind puts an individual at an elevated, yet still rare, risk of dizziness, fainting, nausea, vomiting, acute myocardial infarction (heart attack), stroke, and/or sudden death. While there is a risk, the likelihood of any of these events occurring is minimal due to the fact that you have no known personal health risk history, and/or signs and symptoms of cardiovascular disease.

- **Supplementation** – Commercially available dark chocolate is being assessed in this study. There are no reported side-effects/adverse events associated with the consumption of dark chocolate in the quantity (20 grams = <2/3 of an ounce) consumed in this study.

- **DEXA (dual energy x-ray absorptiometry)** - is a commonly used procedure to assess an individual's body composition (percentage fat and lean tissue) as well as bone density. This requires the participant to lay in a supine position (on their back) with their arms at their sides for 5-minutes while a very low dose x-ray (0.04 - 0.08 mrem) is passed across the body to determine the density of their tissues (fat, lean, bone). While there is X-ray exposure, this is very minimal, as a 5-minute scan provides approximately the same x-ray exposure as 2 hours under normal room lighting. There have been no reported side-effects/adverse events associated with multiple DEXA measurements.

18. Benefits:
Subjects will not benefit academically from participation in this research project.

- Upon completion of the project subjects will be provided with information about their:
    - body composition
    - resting energy expenditure
    - exercise energy expenditure
    - exercise performance
    - If requested, subjects will also be provided with any presentation abstracts, publication abstracts, or manuscripts resulting from their participation

19. Prior Research:
Katie Presler – Primary Investigator, is the PI for this study and is trained in all aspects of this study. She is experienced with the use and programming of the laboratory cycle, the calibration and use of metabolic measurement system for the assessment of resting and exercise energy expenditure, and she is adult CPR/First Aid certified. She has completed CITI training for

"Conflicts of Interest" and "Human Research – IRB" (see appendix 2).

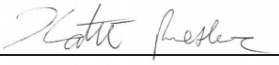
Dr. Michael Webster, Associate Professor of Exercise Physiology, is the supervising faculty of this project. He has been conducting human performance laboratory exercise studies in higher education for >30 years. He has completed CITI training for "Conflicts of Interest" and "Human Research – IRB" (see appendix 2). He has American Red Cross Adult/Infant CPR and AED certification and has also been certified with the American College of Sports Medicine as a Clinical Exercise Physiologist since 1988. Part of this certification is the demonstration of appropriately responding to medical emergencies.

CERTIFICATIONS AND REQUIRED SIGNATURES

(Note: Please print this page to sign. Applications without all required signatures will be not be reviewed.)

Statement of Responsible Researcher:

I certify that I have completed required training regarding human participant research ethics and am familiar with the ethical guidelines and regulations regarding the protection of human participants from research risks. I will adhere to the policies and procedures of the Valdosta State University Institutional Review Board (IRB). I will not initiate this research project until I receive written exemption or approval from the IRB. I will not involve any participant in the research until I have obtained and documented his/her informed consent as required by the IRB. I agree to (a) report to the IRB any unanticipated problems or adverse events which become apparent during the course or as a result of the research and the actions taken as a result, (b) cooperate with the IRB in the continuing review of this project; (c) obtain prior approval from the IRB before amending or altering the scope of the project or the research protocol, and (d) maintain documentation of consent and research data and reports for a minimum of three years and in accordance with approved data retention and procedures and confidentiality requirements after completion of the final report or longer if required by the sponsor or the institution. I understand that my department chair/unit director/faculty advisor (if I am a student) will receive a copy of my IRB exemption or approval report.

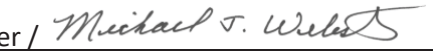
SIGNATURE: Katie Presler / 

Date: 12/7/2017

Responsible Researcher

Statement of Supervising Faculty if Responsible Researcher is a Student:

I certify that I am familiar with the ethical guidelines and regulations regarding the protection of human participants from research risks and have completed training required by the VSU IRB. I agree to provide guidance and oversight as necessary to the above-named student regarding the conduct of his/her research. I will ensure the student's timely requests for protocol modifications and/or continuing reviews, compliance with the ethical conduct of human participant research, and the submission of the final report. I understand that an IRB protocol cannot be closed until final report is submitted, and I agree that, if the student fails to complete a final report, I will be responsible for timely completion and submission of the report.

SIGNATURE: Michael Webster /  Date: 12/7/2017

Supervising Faculty

VALDOSTA STATE UNIVERSITY
Consent to Participate in Research

You are being asked to participate in a research project entitled "Dark Chocolate Consumption: Effect on Resting and Exercise Metabolism". This research project is being conducted by Katie Presler, a graduate student in the School of Health Sciences at Valdosta State University. The researcher has explained to you in detail the purpose of the project, the procedures to be used, and the potential benefits and possible risks of participation. You may ask the researcher any questions you have to help you understand this project and your possible participation in it. A basic explanation of the research is given below. Please read this carefully and discuss with the researcher any questions you may have. The University asks that you give your signed agreement if you wish to participate in this research project.

Purpose of the Research:
This study involves research. Nutritional ingredients impact a variety of physiological functions and can impact weight loss, weight gain, alter energy levels, improve mood, and improve alertness. The purpose of the study is to investigate the effect of dark chocolate on resting and exercise energy expenditure and exercise performance.

Procedures: I am being invited to participate in a laboratory research study investigating the effect of the ingestion of a nutritional supplement on resting and exercise metabolism. For inclusion in the study, I must be female, 18-30 years of age or post-menopausal 50-70 years of age and a body mass index between 18.5-30 (determined by my weight and height), not presently taking, or have taken in the past 30 days, any nutritional supplements that may significantly impact metabolism. I must be free of any physical condition that would prevent me from completing the physical demands of participation, or put me at more than the normal expected physical risk, when performing the required exercise task. I cannot participate if I am knowingly pregnant, think I might be pregnant, or actively trying to conceive. As a participant, I will be required to complete a pre-participation health history questionnaire to screen for the presence of any of these, other health conditions, or any potential medication/supplement interactions that would prevent me from participation. All my responses to this questionnaire will be evaluated by a qualified, licensed medical professional and final approval for participation will be provided by this individual. In the event that exclusion from the study is warranted, I will be informed during an in-person meeting with the primary investigator. There are no alternatives to the experimental procedures in this study. Consequently, the only alternative is to choose not to participate at all.

If I choose to participate in the study, I will be asked to come to the Human Performance Laboratory on several occasions (one visit being separated by 3–7 days, and another by 30 days). The visits will be as follows:

Laboratory Visit #1 (approximately 90-minutes):
Laboratory Visit #1 will require me to read the informed consent and then I will also have the opportunity to ask any questions related to my participation in the study. At this visit, I will complete a physical activity and health history questionnaire and initial resting measurements of body weight, height, heart-rate, and blood pressure will be obtained. Body composition will be determined via DEXA.

A preliminary 20-minute submaximal exercise test will be performed on a laboratory cycle. This test will require me to be fitted with a heart rate monitor chest strap and also a facemask that covers both my nose and mouth. This facemask will allow me to breathe freely during the exercise test and will allow for the determination of my breathing rate, air volume, and expired concentrations of oxygen and carbon dioxide. Once the heart rate monitor and facemask are in

place, I will then begin pedaling for 10-minutes on the cycle at a relatively low workload. At the end of 10-minutes the cycling, I will continue to pedal for another 10 minutes; however, the workload will be increased to a moderate workload which is as follows:

- **Group 1:**
  - 10 minutes cycling at 50 watts (relatively easy effort)
  - 10 minutes of cycling at 100 watts (relatively moderate effort)
- **Group 2:**
  - 10 minutes of cycling at 40 watts (relatively easy effort)
  - 10 minutes of cycling at 80 watts (relatively moderate effort)

I will then be given a 3-minute recovery before commencing with an exercise performance test as follows:

- **Group 1 (age 18-30): Exercise Performance Test**
  - 1 km cycling time-trial. The goal is to complete the 1 km as quickly as possible (~2-3 minutes)
- **Group 2 (post-menopausal): Exercise Performance Test**
  - 6-minute walk test. The goal is to walk the greatest distance possible in 6 minutes

Summary of the **Laboratory Visit #1**:

Participation documents

- Informed Consent
- Physical activity and health history questionnaires

Resting Measurements

- body weight
- height
- heart-rate
- blood pressure
- body composition via DEXA

Exercise Test

- 20-minute test on the cycle
- Exercise performance

Assignment of date for next visit

- Schedule a day and time for my return to the laboratory within the following three to seven days.

Laboratory Visits #2 - #3 (~60-90 minutes):

At each of these visits I will be required to arrive in the laboratory in the morning after being fasted for at least 8 hours, having not consumed caffeine or alcohol for 24 hours, nor performed more than 20 minutes of low intensity exercise within the past 24 hours. Fasted means to have not consumed any food or drink (other than water) in the previous 8 hours.

Upon arrival to the laboratory, I will be requested to void my bladder and then my resting energy expenditure (REE) will be determined by analysis of the gases expired by me while resting in a supine position on an examination table. This process requires me to remain relatively motionless, yet remain awake, on an exam table for approximately 30-min. To obtain the metabolic measurements, a transparent canopy will be placed over my neck and head and this is connected by a tube to an expired air mixing chamber for the analysis of expired gases (oxygen and carbon dioxide).

Immediately following completion of the assessment for REE, the assessment of my exercise energy expenditure (EEE) will be determined in the same fashion as described in laboratory visit #1. Upon completion of the 20-minutes of cycling, I will be given a 3-minute recovery before commencing with an exercise performance test, again, in the same fashion described previously.

After completion of the exercise performance test, I will be provided with either the experimental dark chocolate or the placebo dark chocolate. Before departing the laboratory, the day and time for my next visit to the laboratory in 30-days will be scheduled.

Summary of **Laboratory Visit #2 (~60-90 min):**

Resting Measurements

- Resting Energy Expenditure

Cycling Exercise Test

- 20-minute test on the cycle
- Exercise Performance
  - Group 1 (age 18-30): 1 km cycling time-trial
  - Group 2 (post-menopausal): 6-minute walk test

Assignment of experimental supplement (randomized, double-blind, matched pairs research design).

- Experimental Dark chocolate
  or
- Placebo Dark Chocolate

The chocolate will be provided in individual 20-gram (2/3 of an ounce) packages for each day. I will need to consume one package each morning for 30 consecutive days.

Assignment of date for next visit

- Schedule a day and time for their return to the laboratory in 30 days.

Summary of **Laboratory Visit #3 (~60-90 min):**

Resting Measurements

- Resting Energy Expenditure

Cycling Exercise Test

- 20-minute test on the cycle

Exercise Performance Test

- Group 1 (age 18-30): 1 km cycling time-trial
- Group 2 (post-menopausal): 6-minute walk test

Possible Risks or Discomfort:

Risk of participation is minimal due to my age, health status, and exercise history. However, no risk can be completely eliminated. There are three primary aspects of risk associated with my participation in this study:

The following are aspects of risk associated with my participation:

1) **Exercise** - Exercise of any kind puts me at an elevated, yet still rare, risk of dizziness, fainting, nausea, vomiting, acute myocardial infarction (heart attack), stroke, and/or sudden death. While there is a risk, the likelihood of any of these events occurring to me is minimal due to the fact that I have no known personal health risk history, and/or signs and symptoms of cardiovascular disease, and the exercise is of a low and moderate intensity.

2) **Dark chocolate** consumption is being assessed in this study. The chocolate and placebo used in this study are specifically prepared for research by the clinical research division of the Hershey Company. There are no reported side-effects/adverse events associated with the consumption of dark chocolate in the quantity (20 grams/day = 2/3 of an ounce) consumed in this study.

3) **DEXA** (dual energy x-ray absorptiometry) is a commonly used procedure to assess an individual's body composition (percentage fat and lean tissue) as well as bone density. This requires me to lay on my back on

an exam table with my arms at my sides for ~5-minutes while being exposed to a very low dose of x-ray energy (0.003 mrem). The amount of x-ray exposure during the 5-minute scan is approximately equal to the exposure I would get sitting for 2 hours in a room with normal lighting.

It is not possible to identify all potential risks of my participating in a research study of this fashion. However, the University has taken reasonable safeguards to minimize potential, but unknown, risks.

If I am injured as a result of my participation in this study, I should contact Dr. Michael Webster at 601-543-8926 (cell) or 229-333-7191 (office). Neither the researcher nor Valdosta State University has made special provision for services required to treat any injury/psychological distress that may result from my participation in this research study beyond those normally provided to VSU students. As appropriate, VSU student participants should be informed that they may request health care services from Student Health Services at 229-333-5886 or from their own private health care provider under their selected health insurance plan. By agreeing to participate in this research project, I am not waiving any rights that I may have against Valdosta State University for injury resulting from negligence of the University or its researchers.

Potential Benefits:
I will not benefit academically, and there is not monetary compensation, from participation in this research project. However, upon completion of the project I will be provided information regarding my body composition (% fat and % muscle), resting metabolic rate, and exercise metabolic rate. Also, I can request copies of future presentation abstracts or published manuscripts that might arise from my participation in this study.

Assurance of Confidentiality: Valdosta State University and the researcher will keep my information confidential to the extent allowed by law. Members of the Institutional Review Board (IRB), and a university committee charged with reviewing research to ensure the rights and welfare of research participants, may be given access to your confidential information.

Upon agreement to participate in the study, I will be assigned a letter/number identification known only by the researchers and kept in a file secured in the PI office. My individual data collected in the course of the study will only be divulged upon completion of the study to myself. Only group data will be reported for presentation and publication purposes. In the event of medical emergency my personal data will be provided to my physician upon request by the physician and/or by myself. This will necessitate my exclusion from further participation in the study. All data collected during the course of the study will be kept in a file secured in the PI office and/or computer. All data will be retained for a period of 3 years after completion of the study and then all paper data shredded and electronic data deleted.

Voluntary Participation:
My decision to participate in this research project is entirely voluntary. If I agree now to participate and change my mind later, I am free to leave the study. My decision not to participate at all, or to stop participating at any time in the future, will not have any effect on any rights I have or any services I am otherwise entitled to from Valdosta State University. If I am a VSU student, my decision not to participate will not affect my grade or standing in any courses or program of study at the university.

Information Contacts:
Questions regarding the purpose or procedures of the research should be directed to Dr. Michael Webster at 601-543-8926 (cell) 229-333-7191 (office) or MJWebster@Valdosta.edu. This study has been approved by the Valdosta State University Institutional Review Board (IRB) for the Protection of Human Research Participants. The IRB, a university committee established by Federal law, is responsible for protecting the rights and welfare of research participants. If you have concerns or questions about your rights as a research participant, you may contact the IRB Administrator at 229-333-7837 or irb@valdosta.edu.

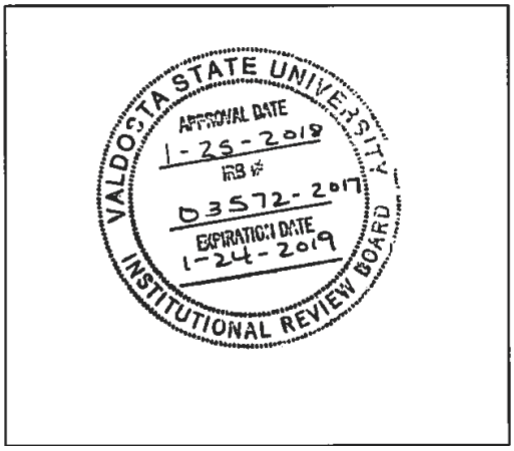
Agreement to Participate: The research project and my role in it have been explained to me, and my questions have been answered to my satisfaction. I agree to participate in this study. By signing this form, I am indicating that I am 18 years of age or older. I have received a copy of this consent form.

I would like to receive a copy of the results of this study: _____ Yes _____ No

Mailing Address: _____

E-mail Address:

This research project has been approved by the Valdosta State University Institutional Review Board for the Protection of Human Research Participants through the date noted below:



APPENDIX B:

Institutional Review Board Expedited Protocol Approval

Institutional Review Board (IRB) for the Protection of Human Research Participants

EXPEDITED PROTOCOL APPROVAL

PROTOCOL NUMBER: IRB-03572-2017		**RESPONSIBLE RESEARCHER:**	**Ms. Katie Presler**
		SUPERVISING FACULTY:	**Dr. Michael Webster**
PROJECT TITLE:	***Dark Chocolate Consumption: Effect on Resting and Exercise Metabolism.***		
APPROVAL DATE:	**01.25.2018**	**EXPIRATION DATE:**	**01.24.2019**

LEVEL OF RISK: ☒ Minimal ☐ More than Minimal

TYPE OF REVIEW: ☒ Expedited Under Category **4** ☐ Convened (Full Board)

CONSENT REQUIREMENTS:

☒ Adult Participants – Written informed consent with documentation (signature)
☐ Adult Participants – Written informed consent with waiver of documentation (signature)
☐ Adult Participants – Verbal informed consent
☐ Adult Participants – Waiver of informed consent
☐ Minor Participants – Written parent/guardian permission with documentation (signature)
☐ Minor Participants – Written parent/guardian permission with waiver of documentation (signature)
☐ Minor Participants – Verbal parent/guardian permission
☐ Minor Participants – Waiver of parent/guardian permission
☐ Minor Participants – Written assent with documentation (signature)
☐ Minor Participants – Written assent with waiver of documentation (signature)
☐ Minor Participants – Verbal assent
☐ Minor Participants – Waiver of assent
☐ Waiver of some elements of consent/permission/assent

APPROVAL: This research protocol is **approved** as presented. If applicable, your approved consent form(s), bearing the IRB approval stamp and protocol expiration date, will be mailed to you via campus mail or U.S. Postal Service unless you have made other arrangements with the IRB Administrator. Please use the stamped consent document(s) as your copy master(s). Once you duplicate the consent form(s), you may begin participant recruitment. **Please see Attachment 1 for additional important information for researchers.**

COMMENTS:

Elizabeth Ann Olphie *01.12.2018* ***Thank you for submitting an IRB application.***

Elizabeth Ann Olphie, IRB Administrator Date ***Please direct questions to irb@valdosta.edu or 229-259-5045.***

Form Revised: 06.02.16

EXPEDITED PROTOCOL APPROVAL REPORT
Attachment 1

ADDITIONAL INFORMATION FOR RESEARCHERS:

If your protocol received expedited approval, it was reviewed by a two-member team, or, in extraordinary circumstances, the Chair or the Vice-Chair of the IRB. Although the expediters may approve protocols, they are required by federal regulation to report expedited approvals at the next IRB meeting. At that time, other IRB members may express any concerns and may occasionally request minor modifications to the protocol. In rare instances, the IRB may request that research activities involving participants be halted until such modifications are implemented. Should this situation arise, you will receive an explanatory communiqué from the IRB.

Protocol approvals are generally valid for one year. In rare instances, when a protocol is determined to place participants at more than minimal risk, the IRB may shorten the approval period so that protocols are reviewed more frequently, allowing the IRB to reassess the potential risks and benefits to participants. The expiration date of your protocol approval is noted on the approval form. You will be contacted no less than one month before this expiration date and will be asked to either submit a final report if the research is concluded or to apply for a continuation of approval. It is your responsibility to submit a continuation request in sufficient time for IRB review before the expiration date. If you do not secure a protocol approval extension prior to the expiration date, you must stop all activities involving participants (including interaction, intervention, data collection, and data analysis) until approval is reinstated.

Please be reminded that you are required to seek approval of the IRB before amending or altering the scope of the project or the research protocol or implementing changes in the approved consent process/forms. You are also required to report to the IRB,

through the Office of Sponsored Programs & Research Administration, any unanticipated problems or adverse events which become apparent during the course or as a result of the research and the actions you have taken.

Please refer to the IRB website (http://www.valdosta.edu/ospra/HumanResearchParticipants.shtml) for additional information about Valdosta State University's human protection program and your responsibilities as a researcher.

APPENDIX C:

Investigators Citi Documents

CITI PROGRAM

Completion Date 22-Aug-2017
Expiration Date 21-Aug-2020
Record ID 24267593

This is to certify that:

Katie Presler

Has completed the following CITI Program course:

CITI Conflicts of Interest (Curriculum Group)
Conflicts of Interest (Course Learner Group)
1 - Stage 1 (Stage)

Under requirements set by:

Valdosta State University

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/?w86fada28-c407-4bd9-870c-527778752b41-24267593

CITI PROGRAM

Completion Date 26-Jan-2016
Expiration Date 26-Jan-2019
Record ID 18508062

This is to certify that:

Michael Webster

Has completed the following CITI Program course:

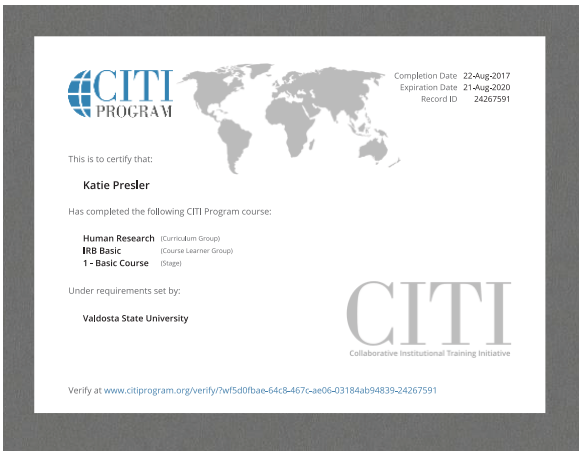
Human Research (Curriculum Group)
IRB Basic (Course Learner Group)
1 - Basic Course (Stage)

Under requirements set by:

Valdosta State University

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/?w542a5327-c207-4f8b-8909-255473c1a8b9-18508062



CITI PROGRAM

Completion Date 22-Aug-2017
Expiration Date 21-Aug-2020
Record ID 24267591

This is to certify that:

Katie Presler

Has completed the following CITI Program course:

Human Research (Curriculum Group)
IRB Basic (Course Learner Group)
1 - Basic Course (Stage)

Under requirements set by:

Valdosta State University

CITI
Collaborative Institutional Training Initiative

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CITI PROGRAM

Completion Date 26-Jan-2016
Expiration Date 25-Jan-2020
Record ID 18508063

This is to certify that:

Michael Webster

Has completed the following CITI Program course:

CITI Conflicts of Interest (Curriculum Group)
Conflicts of Interest (Course Learner Group)
1 - Stage 1 (Stage)

Under requirements set by:

Valdosta State University

CITI
Collaborative Institutional Training Initiative

Verify at www.citiprogram.org/verify/?we3c30038-0318-45a1-b539-31da6fe5ae5d-18508063

APPENDIX D:

Valdosta State University Health History and Physical Activity Questionnaire

VALDOSTA STATE UNIVERSITY HUMAN PERFORMANCE LAB – HEALTH HISTORY QUESTIONNAIRE

Name:						**Date:**				
Address:		**City:**		**State:**		**Zip:**				
Cell Phone:		**Home Phone:**								
E-mail:		**Preferred contact method:**		**Phone**		**E-mail**				
Employer:		**Highest level of education:**		**HS**	**BS**	**MS**	**Professional**			
Sex:	**M**	**F**	**Date of Birth:**		**Age:**		**Marital Status:**	**Single**	**Married**	
Race:	**White**	**Black**	**Asian**	**Hispanic**	**Other (specify):**					
Emergency Contact:					**Phone:**	**Relationship:**				

Personal Physician:		**Location:**	**Phone:**		
Date/Reason for last visit:		**Have you ever had a stress test:**	**Yes**	**No**	**Date:**
Date/Result of Last Cholesterol Test:		**Date/Result of Last Blood Glucose/Sugar Test:**			

PERSONAL HEALTH HISTORY PRESENT SYMPTOMS FAMILY HEALTH HISTORY *Have you ever had or do YOU currently have or have any immediate family been told that you have...*			**PRESENT SYMPTOMS** *Do you currently have or have had in the last 3 months…*			**FAMILY HEALTH HISTORY** *Have any immediate family or grandparent had…*		
	NO	**YES**		**NO**	**YES**		**NO**	**YES**
Anemia			Blood in urine/stool			Angina/chest pain		
Angina/chest pain			Chest pain/discomfort			Any heart surgery		
Any heart surgery			Common cold/sinusitis			Diabetes		
Any heart trouble			Coughing of blood			Heart attack		
Arthritis/joint pain			Coughing of excretion			Heart disease		
Asthma/bronchitis			Frequent headaches			High blood pressure		
Back pain/injury			Indigestion/heartburn			High cholesterol		
Cancer			Lightheaded/dizzy			Stroke		
Diabetes			Rapid heart beat			Sudden death		
Depression			Skipped heart beats					
Disease of the arteries			Shortness of breath			**TOBACCO HISTORY**		
Eating disorder			Pain in jay/neck/arm/shoulder			Do you currently smoke		
Emboli(us)/phlebitis						**What?** Cigarettes		
Emphysema						Cigars		
Heart attack			**ALCOHOL**			Pipe		
Heart murmur			Do you drink alcohol How much in one week			Chewing tobacco		
High blood pressure			Beer (can/bottle)			E-cigarettes		
High cholesterol			Wine (glasses)			Are you trying to quit?		
Hospitalizations			Hard liquor (drinks)			How much per day?		
Joint/muscle swelling						Have you ever quit?		
Osteoporosis			**CAFFEINE**			When?		
Stroke			How much per day?			How many years & how much?		
Varicose veins			Coffee (cups)			Regularly exposed to smoke		

			Tea (cups)				
			Soft drinks (can/bottle)		NUTRITION/DIETARY PRACTICES		
			Energy drinks (can/bottle)		Do you refrain from eating beef, pork, poultry, and/or fish?		
			ALLERGIES				
			Pollen/grass				
			Medicines				
			specify				
			Foods				
			specify				
			Other				

CURRENT MEDICATIONS (PRESCRIPTION, NON-PRESCRIPTION, VITAMINS, SUPPLEMENTS)

NAME	REASON	STARTED TAKING?	HOW OFTEN?	DOSAGE?

FEMALES ONLY:

Are you: Premenopausal_____ Perimenopausal_____ Post-menopausal_____ at age_____

Hormone therapy? Currently _____ Past _____ For how long? _____

HOSPITALIZATIONS IN THE LAST 5 YEARS (EXCEPT NORMAL PREGNANCIES)

DATE	REASON

LIFESTYLE AND PHYSICAL ACTIVITY

How STRESSED do you feel in your daily life? (circle one)	Very little	Fairly	Somewhat	Most of the time	All of the time

Why do you want to start an exercise program/routine? (circle all that apply)	Doctor recommendation	Lose weight	Enjoyment	Reduce stress	All of the time
	Improve appearance	Better health	Other:		

Are you currently following a weight reductions program?	YES	NO
If yes, for how long and what type of program?		

How would you rate your occupational activity level (how much activity /how hard is the activity level of your job)?			
	Sedentary (computer/office work)		Light (non-sedentary office work, cashier, teacher, nursing)
	Moderate (mail carrier, restaurant server)		Heavy/vigorous (construction, fire service, fitness instructor)

OVER THE LAST 3 MONTHS, HAVE YOU REGULARLY… (Indicate those that apply and complete the corresponding questions)

	Performed aerobic exercise (walking, jogging, swimming, cycling aerobics class, Zumba, etc.)					
	What type?					
	Days per week?		Minutes per week?			
	If you walk/jog/run, how many miles do you usually do?		What is your average time/pace per mile?			

	Played strenuous sports (basketball, tennis, racquetball, soccer, martial arts, etc.)			
	What type?			
	Days per week?		Minutes per week?	

	Participated in resistance/strength/weight training (lift weights, body pump class, TRX, etc.)			
	What type?			
	Days per week?		Minutes per week?	

	Participated in flexibility, core strengthening, neuromotor fitness activities (yoga, Pilates, boot camp classes, etc.)			
	What type?			
	Days per week?		Minutes per week?	

During exercise, do you ever experience any of the following (check all that apply):						
	Uncomfortable shortness of breath		Chest pain/discomfort (does it go away with rest?	YES	NO	
	Lightheaded/dizzy		Bone or joint discomfort/swelling			
	Muscle pain/swelling		Other problems:			
Do you have any other medical concerns/problems not already identified? Please list:						

APPENDIX E:

Assessment of Resting Energy Expenditure

APPENDIX F:

Measurement of Oxygen Uptake and Exercise Metabolism

APPENDIX G:

Velotron Laboratory Cycle

APPENDIX H:

Polar Heart Rate Monitor


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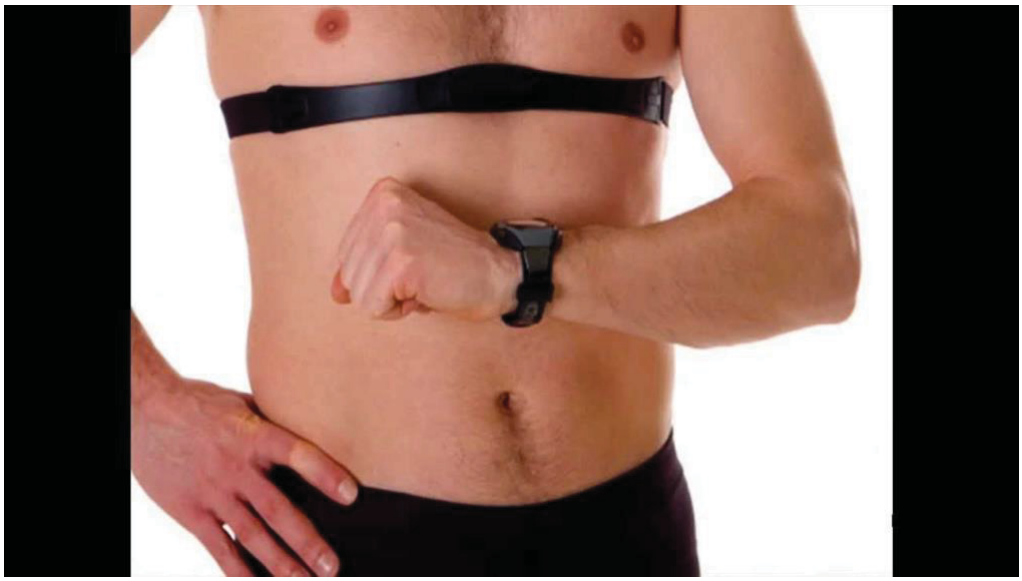

APPENDIX I:

Recruitment Poster


FREE CHOCOLATE!!!
Participants Needed - "Chocolate: Effect on metabolic rate"
HERSHEY'S
NEEDED: Females/Non-smoker/Age: 18-30 & 50-70
Contact: kpresler@valdosta.edu
Questions regarding the purpose or procedures of the research should be directed to Katie Presler at kpresler@valdosta.edu. This study has been exempted from Institutional Review Board (IRB) review in accordance with Federal regulations. The IRB, a university committee established by Federal law, is responsible for protecting the rights and welfare of research participants. If you have concerns or questions about your rights as a research participant, you may contact the IRB Administrator at 229-259-5045 or irb@valdosta.edu.