



Dark Chocolate Elevates Resting Energy Expenditure in Postmenopausal Women

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Abstract

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<https://doi.org/10.70252/QRGN7992> Several recent reports have indicated positive health benefits of consuming (-)-epicatechin-rich cocoa products. Postmenopausal women are predisposed to reduced metabolism due to decreased levels and activity of the sex hormones estrogen, progesterone, and estradiol. The purpose of this study was to investigate the influence of dark chocolate consumption on resting and exercise metabolism in postmenopausal women. Using a randomized, double-blind design, 26 postmenopausal participants were assigned to a 30-day supplementation with 20-g per day of 72% dark chocolate (DC) or calorically matched white chocolate (WC). Before supplementation, participants underwent two control trials for assessments (PRE1, PRE2) of resting energy expenditure (REE) and exercise energy expenditure (EEE). Following the PRE2 assessment, participants were randomized and supplemented for 30 days, after which they repeated the assessments for REE and EEE. PRE1 and PRE2 REE and EEE were not significantly different within or between groups (REE: PRE1 DC 1215 ± 170, WC 1127 ± 174, $p=0.662$; PRE2 DC 1211 ± 174, WC 1145 ± 165 kcal/d, $p=0.720$; EEE: PRE1 DC 3.67 ± 0.72, WC 3.40 ± 0.81, $p=0.665$; PRE2 DC 3.41 ± 0.88, WC 3.39 ± 0.73 kcal/min, $p=0.373$). Post-supplementation REE was significantly increased by 3.2% in the DC group (Pre-Post change: DC 38.6 ± 49, WC -15 ± 31.2 kcal per day, $p=0.039$, Cohen's $d=0.724$ [95% CI: 0.078, 1.513]). These results indicate that DC supplementation in postmenopausal women was associated with a significant 3.2% increase in REE with no significant influence on EEE.

Keywords: Epicatechin, polyphenol, menopause

Introduction

Chocolate is widely recognized as a culinary treat. However, some have suggested that chocolate consumption, particularly in the form of dark chocolate, has the potential to reduce the risk of coronary heart disease, stroke, and diabetes, three of the leading causes of death and disability in the United States.¹⁻⁴ Recent reports have postulated that these health attributes are in some measure due to dark chocolate's antihypertensive, antiplatelet, antioxidant, and anti-inflammatory properties.^{1,5} Additional reported benefits of dark chocolate include

neuroprotection, decreased blood pressure, increased satiety, reduced intestinal absorption of carbohydrates and lipids, improved cholesterol profiles, improved antioxidant capacity, increased resting energy expenditure, improved mitochondrial efficiency, and increased aerobic capacity.^{2,6-10}

There are a considerable number of reports suggesting that the health benefits of dark chocolate consumption are due in large part to the high content of the flavonoid (-)-epicatechin, the most abundant flavonoid monomer in cocoa.^{9,11-14} Isolated (-)-epicatechin supplementation has been demonstrated to increase tissue mitochondrial content leading to improvements in mitochondrial function in both cardiac and skeletal muscle.¹⁵ Though the exact mechanisms are unclear, (-)-epicatechin is known as a potent stimulator of glucose oxidation and mitochondrial function.¹⁵

(-)-epicatechin has also been shown to mediate components of metabolic syndrome and improve the ability to partake in higher levels of aerobic exercise.¹⁶⁻¹⁸ In a high-fat diet and obesity study conducted by De Los Santos et al., a 15-day supplementation of twice-day 1 g/kg of body mass doses of (-)-epicatechin by oral gavage was shown to decrease weight gain, hyperglycemia, and hypertriglyceridemia in the male progeny of obese rats.¹⁸ In a recent randomized clinical trial of older individuals with peripheral artery disease, McDermott and colleagues found that six months of (-)-epicatechin supplementation administered daily through a cocoa beverage consisting of 15g of cocoa and 75mg of epicatechin demonstrated a significant improvement in the exercise endurance performance of adults 60 years and above.¹⁶

The mode of (-)-epicatechin supplementation in recent randomized-controlled trials varies based on the research questions. Studying the impact of (-)-epicatechin on metabolic health has been done through the provision of isolated (-)-epicatechin via pill or oral gavage.^{15,18} Additionally, cocoa is a food rich in (-)-epicatechin and has the potential to be used in randomized controlled trials as a means of (-)-epicatechin consumption.¹⁹ However, raw cocoa consumption is not palatable, being bitter in taste and gritty in texture.¹⁹ Because of this, there is little motivation for participants to consume cocoa in its unaltered form. Chocolate presents itself as a palatable combination of cocoa, butter, milk, and sugar, providing a beneficial dose of (-)-epicatechin and a motivation to consume it. Trials providing (-)-epicatechin through chocolate consumption have presented positive findings on metabolic health.⁹⁻¹¹

Menopause, the cessation of menstruation and reproductive function, is typically experienced during a woman's late 40s through early 50s and is characterized by decreased levels of the sex hormones estrogen, progesterone, and estradiol.²⁰⁻²³ Mitochondrial function is compromised as menopause takes place, and this is potentially due to reductions in estrogen.²⁴ In addition to this, the postmenopausal phase is associated with a decrease in fat oxidation and exercise energy expenditure, and an increase in appetite, all of which negatively contribute to postmenopausal changes in body composition.^{20-22,25,26}

The documented alterations in metabolism associated with post-menopause warrant investigation of possible measures to counter these negative effects. Considering the recent findings of improvements in metabolic function and resting energy expenditure associated with

dark chocolate consumption, this raises the logical question as to the metabolic effect of dark chocolate consumption by postmenopausal women.^{9,10} Consequently, the purpose of this study was to investigate the influence of dark chocolate consumption on resting and exercise metabolism in postmenopausal women.

Studies supporting this purpose include a randomized-controlled, single-blind study by Presler and Webster⁹ which evaluated the effects of consuming 20g of 72% dark chocolate for 30-d on resting energy expenditure (REE) and exercise energy expenditure (EEE) in premenopausal female athletes (18-30 years old). The results demonstrated that dark chocolate supplementation provided a 9.6% increase in REE ($p=0.017$) and no change in EEE ($p>0.05$). A study by Hodson et al. compared REE and total energy expenditure (TEE) between premenopausal (35-45 years old) and postmenopausal women (55-65 years old) and found that postmenopausal women had significantly lower resting metabolic rates than premenopausal women, despite adjusting resting metabolic rate for lean mass (1367 vs. 1484 kcal/day, $p=0.029$).²⁶ Additionally, they found that postmenopausal women performed less moderate physical activity each week than their premenopausal counterparts (140 vs. 183 min/wk, $p=0.03$).

With the significant improvements in REE demonstrated by Webster and Presler, and the reductions in EEE shown in Hodson et al., it was hypothesized that 30 days of dark chocolate consumption by postmenopausal women would increase their resting energy expenditure and have no effect on exercise energy expenditure.

Methods

Participants

The study employed a randomized, double-blind, placebo-controlled design and was approved by the Institutional Review Board for the use of Human Participants in Research (04214-2021). Additionally, this research was carried out fully in accordance to the ethical standards of the *International Journal of Exercise Science*.²⁷

Inclusion criteria were that participants be postmenopausal (defined as cessation of menstrual cycle for at least one year), over 40 years of age, have a body mass index (BMI) between 18.5-35 kg•m⁻², not regularly consuming chocolate, and not presently taking any nutritional supplements that might influence metabolic rate. The sample size was determined *a priori* in part by the results of a previous study conducted in our laboratory that achieved statistical significance with a sample size of $n = 18$.⁹ The goal of this study was to exceed the n of the previous study. The previous study focused on determining the benefits of consuming (-)-epicatechin-rich dark chocolate on resting and exercise energy expenditure in young female athletes.⁹ Menopause and the subsequent postmenopausal period are phenomena that all women will experience.²⁸ Identifying the effect of this supplementation protocol on postmenopausal women provides a comparison of changes in metabolism over time and across physical activity levels. Increasing the n of the current study was done with the hope of accounting for the attenuations in resting energy expenditure and exercise habits that have been cited to change with age.²⁶

While the previous study provided significant results, power analyses, and effect sizes were not provided.⁹ In light of this, *post hoc* analyses were conducted at the end of data collection to calculate the statistical power of the acquired sample size.

Participants were recruited from the University's Center for Exercise Medicine and Rehabilitation. This Center provides an environment for middle-aged to older adults to complete specialized exercise training programs alongside ACSM-certified exercise physiologists. Participants were also recruited through listserv postings with the University's Alumni Association.

Participants were instructed to maintain their normal diet and physical activity levels throughout the study. Compliance was monitored with a brief interview during the middle of the supplementation period. The interview included obtaining verbal assurance that the supplement was being consumed daily and reviewing potential complications in continuing the supplementation. When an in-person interview was not possible, an email was sent with the same purpose and structure as the interview.

Protocol

Participants participated in three laboratory visits over the course of 4-6 weeks. The participants were required to maintain physical activity and dietary habits throughout the duration of the study. The first laboratory visit (PRE 1) was a preliminary/familiarization session that required participants to arrive in the laboratory 4 hours postprandial and having not performed more than 20 minutes of low-intensity exercise during the previous 24-h. Upon arrival, participants completed an informed consent and physical activity and health history questionnaire and were assessed for resting measures of height, weight, and body composition via whole-body Dual-Energy X-ray Absorptiometry (DXA) scan (Hologic Horizon®, Marlborough, MA). Participants were also instructed on how to complete a 24-hour diet recall and were encouraged to replicate their recorded dietary intake as closely as possible in the 24-h prior to each subsequent laboratory session. In response to this instruction, each participant completed three 24-hr diet recalls. This process was done with the hope of controlling for the effect of changes in dietary intake on REE and EEE.

REE was assessed via open-circuit indirect calorimetry (Vmax Encore Metabolic Cart; Yorba Linda, CA). Flow volume and gas calibrations were performed prior to each testing session according to the manufacturer's instructions. 4% CO₂ /16% O₂ and 0% CO₂ /25% O₂ calibration gases were used for calibration and a 3-L calibration syringe was used for volume calibration.²⁹ Participants assumed a supine position on an examination table with a Plexiglas ventilated hood placed over their head. Expired gases were then assessed for ~30-min. The first 10-min allowed the participants to acclimate to the testing procedures and reach a metabolic steady state. The REE was subsequently determined from 10-min of steady state respiratory gas measurements assessed during min 10-30. During this time, the pump flow rate was manipulated to ensure a FECO₂ between 0.75-0.85. Energy expenditure was calculated as $[3.941 (\text{VO}_2) + 1.106 (\text{VCO}_2)]$, where VO₂ and VCO₂ were reported in L/min. Non-protein substrate utilization was calculated from respiratory quotient (RQ) and VO₂.³⁰

Upon completion of the assessment of REE, the metabolic cart was immediately calibrated to accommodate exercise testing, and the participants was fitted with a facemask, mass flow sensor, and polar heart rate monitor. The participants then performed 10-min of continuous exercise on a cycle ergometer (Monark, Ergomedic 894 E) at a workload chosen to elicit moderate intensity energy expenditure of 3-6 METS.³¹ To achieve the target intensity, the appropriate power output in Watts (W) was determined by each participants' physical activity level during the first laboratory visit and was replicated during subsequent sessions. Power output targets were 30, 40, and 50 W. Expired gases were analysed and used in the assessment of EEE and RQ. Heart rate was also recorded during minutes 8, 9, and 10 of cycling exercise.

The second laboratory visit (PRE 2) was scheduled ~7-d after the preliminary laboratory visit. During this visit, participants performed another assessment of REE and 10-min of cycling exercise in the same manner as the initial laboratory visit. Upon completion, each participant was provided with 30-d of the prescribed supplement. The third laboratory visit was performed 30 days after the onset of supplementation. The assessment consisted of a whole-body DXA scan, REE and EEE in the same fashion as described previously.

The intervention was a randomized, double-blind design.⁹ The experimental treatment consisted of 20 g•d⁻¹ of 72% Dark Chocolate (DC) (Organic Extra Dark Chocolate, Artisan Kettle, Madison, WI), or calorically matched (~100 kcal) volume of White Chocolate (WC) (White Chocolate Baking Chips, The Hershey Company, Hershey, PA). Supplementation was scheduled for a total of 30 days. The white chocolate was assumed to contain no (-)-epicatechin, which allowed for the comparison of the metabolic effects of DC to a similar cocoa product that has not been demonstrated to hold nutraceutical properties. An individual not affiliated with the project was responsible for the preparation of the supplement. Each day's chocolate was individually wrapped in foil and placed into a plastic bag with 5 days of chocolate in each plastic bag for a total of 6 bags for each participant. These were then placed in brown paper bags to disguise the contents from the investigators. Additionally, participants were informed that the purpose of the study was to investigate the effect of chocolate on resting and exercise metabolism; however, they were blinded to the fact that purported nutraceutical effects of DC specifically were under observation.

Upon receiving supplementation after their second assessment, participants were instructed to consume their prescribed chocolate each day and to return to the lab after 30 days of supplementation. During the supplementation period, participants were questioned regularly (~4-10 days) about their adherence to daily chocolate consumption.

Statistical Analysis

Statistical analyses were performed using Statistical Packaging for the Social Sciences (Version 29.0.2.0, SPSS). An independent t-test was used to compare REE and EEE data from PRE 1 and PRE 2. After determining that there were no significant differences between data from these visits, the data were averaged and compared to data from Visit 3. In this situation, paired one-tailed t-tests were used to evaluate for any significant increases in REE and EEE between conditions (WC vs. DC; pre-supplementation vs. post-supplementation). Paired t-tests were

used to evaluate any significant changes in substrate oxidation rates, oxygen uptake, and respiratory quotient (RQ) (WC vs. DC; pre-supplementation vs. post-supplementation). For significant results, Cohen's *d* was calculated to determine effect sizes and their confidence intervals. Interpretation of effect sizes were based on previously developed standards for large, medium, and small effect sizes.³² The significance level was set at $p < .05$. All data are presented as mean \pm SD.

Results

Inclusion criteria were postmenopausal (defined as cessation of menstrual cycle for at least one year), over 40 years of age, a body mass index between 18-35 kg \cdot m⁻², not regularly consuming chocolate, and not presently taking any nutritional supplements that might influence metabolic rate. Thirty-one participants met the inclusion criteria; however, five were eventually excluded due to a variety of factors including claustrophobia, incurring a physical injury over the course of the implementation period, an allergic reaction to white chocolate, and an inability to complete exercise testing. This left a final participants pool of $n=26$ (DC $n=13$, WC $n=13$). This participant pool exceeded the number of participants used in the study previously conducted in our lab that observed a significant increase in the REE of female college athletes consuming the same amount of 72% dark chocolate.⁹ *Post hoc* analyses revealed that the sample size analysed for independent t-tests ($n=26$) provided a power of 0.80, and the sample size analysed for paired one-tailed t test (DC $n=13$, WC $n=13$) yielded a power of 0.52.

While participants clearly understood that the study was investigating chocolate, they were not aware that DC was the experimental treatment being investigated. Participant characteristics are indicated in Table 1. The time since onset of menopause was significantly different for DC and WC groups (9.5 ± 6.4 vs. 13.0 ± 13.0 yrs, $p=0.032$).

Table 1. Participant characteristics.

	DC ($n=13$)	WC ($n=13$)	<i>p</i> -value
Age (yr)	59.0 ± 6.6	62.8 ± 10.0	.106
Time since onset of menopause (yr)	9.5 ± 6.4	13.0 ± 13.0	.032
Height (m)	1.64 ± 0.68	1.64 ± 0.04	.062
Weight (kg)	71.4 ± 15.1	69.7 ± 11.1	.794
BMI (kg \cdot m ²)	26.7 ± 5.0	26.2 ± 4.7	.769
Body fat (%)	42.4 ± 7.1	42.5 ± 4.4	.291

Values are expressed as mean \pm SD. BMI = Body Mass Index; DC = Dark Chocolate; WC = White Chocolate.

REE (kcal/day) and EEE (kcal/min) were not significantly changed prior to the intervention period from PRE 1 and PRE 2 (REE: PRE 1 DC = 1215 ± 170 , WC = 1127 ± 174 kcal \cdot d⁻¹, $p=0.662$; PRE 2 DC = 1211 ± 174 , WC = 1145 ± 165 , kcal \cdot d⁻¹ $p=0.720$; EEE: PRE 1 DC = 3.66 ± 0.73 , WC = 3.40 ± 0.81 kcal \cdot min⁻¹, $p=0.665$; PRE 2 DC = 3.41 ± 0.88 , WC = 3.39 ± 0.73 kcal \cdot min⁻¹, $p=0.373$). Post-supplementation REE values for the DC group were higher than REE for the WC group (DC = 1252 ± 147 , WC = 1120 ± 148 kcal \cdot d⁻¹). Post-supplementation REE increased by 3.2% in the DC group and decreased by 1.3% in the WC group, with a statistically significant difference between pre-post values (Pre-Post difference: DC = 38.6 ± 49 kcals, WC = -15 ± 31.2 kcals,

$p=0.039$) (Graph 1). The Cohen's d effect size for the changes in REE values between pre- and post-measures was 0.724 (95% CI: 0.078, 1.513).

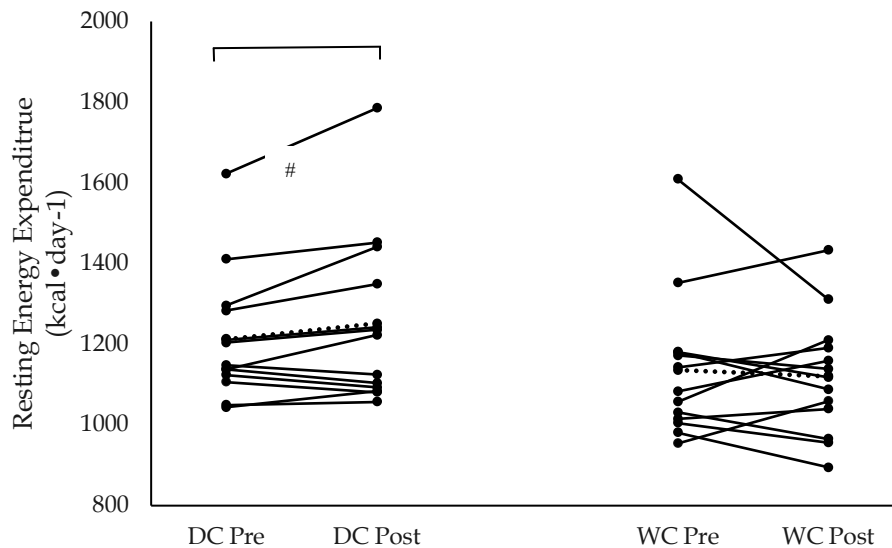


Figure 1. Individual participants REE pre- and post-supplementation. ... indicates mean data for each group. # indicates the pre-post change in REE is significantly greater in DC than in WC ($p=0.039$). DC = Dark Chocolate; WC = White Chocolate.

There were no significant differences in pre- and post-supplementation measures for substrate utilization during the assessment of REE for either group ($p>0.05$) (Table 2). Additionally, there were no significant differences between measures for EEE ($\text{kcal}\cdot\text{min}^{-1}$), exercise oxygen uptake (L/min), or RQ ($p>0.05$) (Table 3).

Table 2. Resting substrate utilization.

	DC PRE	DC POST	WC PRE	WC POST
Fat ($\text{g}\cdot\text{d}^{-1}$)	103.9 ± 21.1	103.4 ± 31.6	101.6 ± 16.9	93.6 ± 15.3
CHO ($\text{g}\cdot\text{d}^{-1}$)	54.5 ± 35.9	65.8 ± 44.7	41.1 ± 30.1	55.9 ± 32.8

Values are expressed as mean \pm SD. CHO = Carbohydrate; DC = Dark Chocolate; WC = White Chocolate; PRE = before supplementation; POST = after supplementation.

Table 3. Energy expenditure, oxygen uptake, and respiratory quotient during exercise.

	DC PRE	DC POST	WC PRE	WC POST
EE ($\text{kcal}\cdot\text{min}^{-1}$)	3.53 ± 0.72	3.58 ± 0.76	3.39 ± 0.73	3.47 ± 0.52
VO_2 ($\text{L}\cdot\text{min}^{-1}$)	0.71 ± 0.14	0.72 ± 0.15	0.68 ± 0.15	0.70 ± 0.11
RQ	0.95 ± 0.04	0.94 ± 0.06	0.94 ± 0.04	0.92 ± 0.05

Values are expressed as mean \pm SD. EEE = Energy Expenditure; VO_2 = Oxygen Uptake; RQ = Respiratory Quotient; DC = Dark Chocolate; WC = White Chocolate; PRE = before supplementation; POST = after supplementation.

Discussion

The purpose of this study was to investigate the influence of dark chocolate consumption on resting and exercise metabolism in postmenopausal women. The findings indicated significant insights, particularly in the realm of REE, where DC consumption led to a 3.2% increase in resting caloric expenditure ($38.6 \pm 49 \text{ kcal} \cdot \text{d}^{-1}$, $p = 0.039$). Conversely, no significant change was observed in EEE following DC supplementation ($+0.05 \text{ kcal} \cdot \text{min}^{-1}$, $p > 0.05$). In contrast, white chocolate (WC) consumption revealed no significant changes in either REE ($-15.31 \pm 31.2 \text{ kcal} \cdot \text{d}^{-1}$, $p > 0.05$) or EEE ($0.08 \pm 0.02 \text{ kcal} \cdot \text{min}^{-1}$, $p > 0.05$). These findings align with our hypothesis that DC supplementation would positively impact REE while leaving EEE unaffected. These results also provide a comparative response to DC supplementation between post-menopausal women and younger female athletes, indicating that the response was similar, yet attenuated in post-menopausal women.

The post-menopausal period brings with it deleterious physiological changes and a hallmark of this is compromised mitochondrial health and function. Compromised mitochondrial function can lead to a build-up of oxidative damage in tissues.²⁴ Consuming foods rich in antioxidants can counter this, as they can properly break down and dispose of the byproducts of oxidative activity.²⁴

In the present study, 30 days of consuming 20g of 72% dark chocolate yielded a $38.6 \pm 49 \text{ kcal} \cdot \text{d}^{-1}$ increase in REE. This increase in REE after dark chocolate consumption brings with it a small, yet significant implication, as this increase counters the expected reduction in REE after menopause. Due to its high concentration of (-)-epicatechin, dark chocolate can provide the antioxidants needed to counter oxidative damage and improve mitochondrial function.^{6,8} The improved REE may be a reflection of an improvement in mitochondrial function.

The findings of this study mirror those of Presler and Webster, where young female athletes underwent a similar experimental protocol.⁹ Notably, the previous study reported a significant 9.6% increase in REE, whereas the results of the present study revealed a more modest increase of 3.2%. Postmenopausal women commonly experience reductions in fat-free mass, a factor linked to diminished REE.^{26,28,33,34} So, this disparity may stem from demographic distinctions. Participants in the Presler and Webster study had a mean age of 21 years, markedly younger than the average age of 61 years among the postmenopausal participants of the present study.⁹ Additionally, the BMI of the participants of their study was $\sim 24.5 \text{ kg} \cdot \text{m}^{-2}$ (normal body weight) compared with $\sim 26.2 \text{ kg} \cdot \text{m}^{-2}$ (overweight) in the present study. While this measure is not that much different between studies, the percentage body fat of the participants in the present study was $\sim 42.5\%$ indicating they were obese. Percentage of body fat was not reported by Presler and Webster; however, the fact that they were all collegiate athletes would suggest that they were relatively lean, or at the very least, not obese. These age-related and body composition differences may shed some light on the response to dark chocolate supplementation observed in this study, warranting further exploration into the interplay between age, dietary interventions, energy expenditure dynamics.

Menopause typically occurs between the ages of 45 and 55 years and marks a universal transition in women's lives; ushering in a host of physiological changes that can impact health outcomes. Central to this transition are the associated hormonal fluctuations of estrogen, which have been purported as a cause for reductions in energy expenditure.^{30,34-36} Estrogen plays a pivotal role in regulating body composition, energy expenditure, and reproductive development in women. Specifically, the action of estrogen affects reproductive function and the process of menopause. The decline in estrogen levels during menopause precipitates many metabolic disturbances, as evidenced by studies implicating estrogen deficiency in mitochondrial dysfunction, alterations in body composition, insulin resistance, and diminished energy expenditure.^{28,30,33,35} The decline in estrogen levels has been linked to reductions in lean body mass and an increase in fat mass.³⁴ Increases in fat mass leads to a reliance on lipid and fatty metabolism.³³ Because of the increases in fat mass, post-menopausal women experience a reduced metabolic rate, as fat consumes less energy than lean mass ($4.5 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ and $13 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, respectively).^{33,37}

While estrogen deficiency contributes significantly to the decline in resting energy expenditure (REE) post-menopause, other factors may also play a role. Hodson et al. demonstrated lower REE levels in post-menopausal women even after accounting for differences in lean mass.²⁶ Despite the metabolic consequences of menopause, the findings of the present study suggest that habitual dark chocolate supplementation may hold promise in mitigating the decline in REE observed in postmenopausal women, thereby offering a potential avenue for improving overall energy expenditure. Dark chocolate supplementation's potential to improve energy expenditure is due to its rich concentration of (-)-epicatechin, as it has been proposed to improve mitochondrial structure and function and stimulate glucose oxidation.^{15,33}

Emerging research highlights the potential health benefits associated with the flavanol content found in dark chocolate, encompassing improvements in vascular function, glycemic control, and metabolism across various populations.^{12,15} This positive impact is often attributed to (-)-epicatechin, the primary flavanol present in dark chocolate. It reportedly has a rapid absorption in the small intestine and subsequent appearance in the circulatory system.^{15,38} Using a hyperglycemic mouse model, Ramírez-Sánchez et al. demonstrated the efficacy of (-)-epicatechin supplementation in restoring and enhancing the functionality of complexes I-IV within the electron transport chain, alongside notable improvements in nitric oxide function.³⁹ In addition, (-)-epicatechin's strong antioxidant properties serve to reduce oxidative damage induced by free radicals.²⁴ This results in an enhancement in mitochondrial function and content, which in turn can augment total energy expenditure (TEE). This notion is supported by the work of Taub et al.¹⁰ who investigated the effects of 20 g/day dark chocolate supplementation over six months on exercise capacity and mitochondrial function in sedentary individuals 40-75 years of age. Their findings revealed a notable trend towards increased maximal oxygen uptake (22.9 ± 1.9 to $25.7 \pm 1.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p=0.056$) among participants consuming dark chocolate, alongside a greater than 2-fold increase in citrate synthase activity within skeletal muscle, indicating enhanced mitochondrial function. The results of each study indicated improved mitochondrial function in response to (-)-epicatechin supplementation in isolation or within dark chocolate. The results of these studies were gathered using both mouse and human models within a broad age range and of both sexes, implying that (-)-epicatechin

supplementation has the potential to improve mitochondrial function in post-menopausal women.

(-)-epicatechin's potential ability to improve metabolic function was also demonstrated in a study employing a group of obese young adults ($\text{BMI} > 30 \text{ kg}\cdot\text{m}^{-2}$).⁷ Levya-Soto et al. investigated the effects of daily chocolate intake on symptoms and risk factors associated with cardiovascular disease and metabolic syndrome in participants aged 23-24 years.⁷ These participants consumed either $2 \text{ g}\cdot\text{d}^{-1}$ of 70% dark chocolate or $2 \text{ g}\cdot\text{d}^{-1}$ of milk chocolate for six months. The findings revealed statistically significant improvements in a variety of health parameters among those consuming 70% dark chocolate. This included reductions in waist circumference (99 to 90 cm), total cholesterol levels (221 to 201 $\text{mg}\cdot\text{d}^{-1}$), and fasting plasma glucose levels (114 to 91 $\text{mg}\cdot\text{d}^{-1}$).

The % cocoa concentration of chocolate in the previously mentioned study is similar to the % cocoa concentration of the chocolate used in the present study (70% and 72%, respectively).⁷ However, the present study required participants to consume a dosage of chocolate ten-fold greater than the previously mentioned study. The results provided in the study performed by Levaya-Soto et al. suggest that cocoa-rich chocolate can improve metabolic health in small amounts. The chosen dosage for the current study was developed based on the results of studies using larger doses^{9,10}. Taub et al. identified $20 \text{ g}\cdot\text{d}^{-1}$ of dark chocolate as the optimal dosage of dark chocolate; boasting the potential to provide the largest benefit possible.¹⁰ $20 \text{ g}\cdot\text{d}^{-1}$ of dark chocolate is comparable to half of a Hershey's chocolate bar. The dosage chosen for the present study is comprehensible and relevant to the participants who volunteered for the study.

While the absence of statistically significant changes in EEE following the 30-day DC supplementation period aligns with findings from Presler and Webster, it differs from that of Taub et al.^{9,10} They showed significant improvements in exercise capacity; however, this was after six months of dark chocolate supplementation. This disparity suggests that significant enhancements in EEE may necessitate a longer duration of supplementation.

While the present study's methodology yielded significant results, its limitations should be addressed. This study relied on previous data from younger, athletic women. While comparing the benefits of identical (-)-epicatechin supplementation protocols may prove insightful in determining benefits over time, the lack of support from studies using postmenopausal women reduces the ability to control for factors common to the postmenopausal period (i.e. hormonal status, physical activity, and dietary habits). Furthermore, the methodology used for the present study did not include the application of an in-depth dietary recall before each visit; and neither was physical activity tracking. Finally, the sample size analysed to determine the impact of dark chocolate supplementation on REE and EEE yielded a power of 0.52, making the results vulnerable to a type II error. While significant improvements in REE were found after 30-d of dark chocolate supplementation, there were no significant changes observed in EEE. This lack of change may be due in part to the level of statistical power used in this study.

In summary, this study investigated the impact of short-term DC supplementation on resting and exercise energy expenditure in post-menopausal women, revealing a significant increase in

REE but no notable change in EEE. While consistent with findings from previous studies, demographic differences between this cohort and previous participants suggest potential variations in response to supplementation. Building on these findings, long-term studies are warranted to assess the potential metabolic benefits of consuming dark chocolate.

Studying the variations in dosage within the postmenopausal population may yield results different from those cited in previous literature, as the hormonal changes experienced by postmenopausal women may intensify or attenuate the results seen in other studies with younger populations with different physical activity habits, dietary habits, and body composition.^{9,10} This phenomenon continues to be best observed when comparing the results of Presler and Webster's study to the findings of the present study, where it was found that the same dose of dark chocolate yielded an increase in the REE of young, athletic premenopausal women that where three times the increase of REE in postmenopausal women.⁹

The implementation of longer-term supplementation protocols are highly recommended. While only speculative, chronic supplementation may enhance mitochondrial function and impact EEE. These investigations would deepen the understanding of the metabolic effects of DC supplementation and its potential as a dietary strategy to mitigate some of the metabolic challenges associated with the development of chronic disease.

References

1. Caponio GR, Lorusso MP, Sorrenti GT, et al. Chemical characterization, gastrointestinal motility and sensory evaluation of dark chocolate: A nutraceutical boosting consumers' health. *Nutrients*. 2020;12(4):1-20. <https://doi.org/10.3390/nu12040939>
2. Petyaev IM, Bashmakov YK. Dark chocolate: Opportunity for an alliance between medical science and the food industry? *Front Nutr*. 2017;4(43):1-8. <https://doi.org/10.3389/fnut.2017.00043>
3. Raghupathi W, Raghupathi V. An empirical study of chronic diseases in the United States: A visual analytics approach. *Int J Environ Res Public Health*. 2018;15(3):1-24. <https://doi.org/10.3390/ijerph15030431>
4. Yuan S, Li X, Jin Y, Lu J. Chocolate consumption and risk of coronary heart disease, stroke, and diabetes: A meta-analysis of prospective studies. *Nutrients*. 2017;9(7):1-10. <https://doi.org/10.3390/nu9070688>
5. Latif R. Chocolate/cocoa and human health: a review. *Neth J Med*. 2013;71(2):63-68.
6. Katz DL, Doughty K, Ali A. Cocoa and chocolate in human health and disease. *Antioxid Redox Signal*. 2011;15(10):2779-2811. <https://doi.org/10.1089/ars.2010.3697>
7. Leyva-Soto A, Chavez-Santoscoy RA, Lara-Jacobo LR, Chavez-Santoscoy AV, Gonzalez-Cobian LN. Daily consumption of chocolate rich in flavonoids decreases cellular genotoxicity and improves biochemical parameters of lipid and glucose metabolism. *Molecules*. 2018;23(9):1-12. <https://doi.org/10.3390/molecules23092220>
8. Neilson AP, George JC, Janle EM, et al. Influence of chocolate matrix composition on cocoa flavan-3-ol bioaccessibility in vitro and bioavailability in humans. *J Agric Food Chem*. 2009;57(20):9418-9426. <https://doi.org/10.1021/jf902919k>

9. Presler KM, Webster MJ. Dark chocolate supplementation elevates resting energy expenditure in exercise-trained females. *Int J Exerc Sci*. 2021;14(2):250-259. <https://doi.org/10.70252/FODL5047>
10. Taub PR, Ramirez-Sanchez I, Patel M, et al. Beneficial effects of dark chocolate on exercise capacity in sedentary subjects: Underlying mechanisms. A double-blind, randomized, placebo-controlled trial. *Food Funct*. 2016;7(9):3686-3693. <https://doi.org/10.1039/C6FO00611F>
11. Decroix L, Tonoli C, Soares DD, et al. Acute cocoa flavanols intake has minimal effects on exercise-induced oxidative stress and nitric oxide production in healthy cyclists: A randomized controlled trial. *J Int Soc Sports Nutr*. 2017;14(1):1-11. <https://doi.org/10.1186/s12970-017-0186-7>
12. Rabadan-Chavez GM, Reyes-Maldonado E, Quevedo-Corona L, Paniagua-Castro N, Escalona-Cardoso G, Jaramillo-Flores ME. The prothrombotic state associated with obesity-induced hypertension is reduced by cocoa and its main flavanols. *Food Funct*. 2016;7(12):4880-4888. <https://doi.org/10.1039/C6FO01165A>
13. Strat KM, Rowley TJJ, Smithson AT, et al. Mechanisms by which cocoa flavanols improve metabolic syndrome and related disorders. *J Nutr Biochem*. 2016;35:1-21. <https://doi.org/10.1016/j.jnutbio.2015.12.008>
14. Shay J, Elbaz HA, Lee I, Zielske SP, Malek MH, Hüttemann M. Molecular mechanisms and therapeutic effects of (-)-epicatechin and other polyphenols in cancer, inflammation, diabetes, and neurodegeneration. *Oxid Med Cell Longev*. 2015;2015:1-13. <https://doi.org/10.1155/2015/181260>
15. Daussin FN, Heyman E, Burelle Y. Effects of (-)-epicatechin on mitochondria. *Nutr Rev*. 2021;79(1):25-41. <https://doi.org/10.1093/nutrit/nuaa094>
16. McDermott MM, Criqui MH, Domanchuk K, et al. Cocoa to improve walking performance in older people with peripheral artery disease. *Circ Res*. 2020;126(5):589-599. <https://doi.org/10.1161/CIRCRESAHA.119.315600>
17. Fahed G, Aoun L, Bou Zerdan M, et al. Metabolic syndrome: Updates on pathophysiology and management in 2021. *Int J Mol Sci*. 2022;23(2):1-38. <https://doi.org/10.3390/ijms23020786>
18. De Los Santos S, Reyes-Castro LA, Coral-Vázquez RM, et al. (-)-Epicatechin reduces adiposity in male offspring of obese rats. *J Dev Orig Health Dis*. 2020;11(1):37-43. <https://doi.org/10.1017/S2040174419000345>
19. Zugravu C, Otelea MR. Dark chocolate: To eat or not to eat? A review. *J AOAC Int*. 2019;102(5):1388-1396. <https://doi.org/10.5740/jaoacint.19-0132>
20. Górna I, Kowalówka M, Morawska A, Kosewski G, Bolesławska I, Przysławskia J. Influence of the frequency of consumption of foodstuffs on the risk of overweight and obesity in a group of post-menopausal women. *Prz Menopauzalny*. 2019;18(1):39-45. <https://doi.org/10.5114/pm.2019.84156>
21. Hansen M. Female hormones: Do they influence muscle and tendon protein metabolism? *Proc Nutr Soc*. 2018;77(1):32-41. <https://doi.org/10.1017/S0029665117001951>
22. Stachowiak G, Pertyński T, Pertyńska-Marczewska M. Metabolic disorders in menopause. *Prz Menopauzalny*. 2015;14(1):59-64. <https://doi.org/10.5114/pm.2015.50000>
23. Gold EB. The timing of the age at which natural menopause occurs. *Obstet Gynecol Clin North Am*. 2011;38(3):425-440. <https://doi.org/10.1016/j.ogc.2011.05.002>
24. Cervellati C, Bergamini CM. Oxidative damage and the pathogenesis of menopause related disturbances and diseases. *Clin Chem Lab Med*. 2016;54(5):739-53. <https://doi.org/10.1515/cclm-2015-0807>

25. Garcia-Yu IA, Garcia-Ortiz L, Gomez-Marcos MA, et al. Cocoa-rich chocolate and body composition in postmenopausal women: A randomized clinical trial. *Br J Nutr.* 2021;125(5):548-556. <https://doi.org/10.1017/S0007114520003086>
26. Hodson L, Harnden K, Banerjee R, et al. Lower resting and total energy expenditure in postmenopausal compared with premenopausal women matched for abdominal obesity. *J Nutr Sci.* 2014;3(3):1-9. <https://doi.org/10.1017/jns.2013.38>
27. Navalta JW, Stone WJ, Lyons TS. Ethical issues relating to scientific discovery in exercise science. *Int J Exerc Sci.* 2020;12(1):1-8. <https://doi.org/10.70252/EYCD6235>
28. Fenton A. Weight, Shape, and Body Composition Changes at Menopause. *J Midlife Health.* 2021;12(3):187-192. https://doi.org/10.4103/jmh.jmh_123_21
29. Technical reference manual. SensorMedics Corporation; 2005:9-10. Accessed January 15, 2025. <https://www.manualslib.com/manual/2599209/Viasys-Vmax-Encore.html>
30. McArdle W, Katch F, Katch V. Exercise physiology: Nutrition, energy, and human performance. Eighth ed. Wolters Kluwer Health; 2015.
31. ACSM's guidelines for exercise testing and prescription. 10th ed. Wolters Kluwer; 2018.
32. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Front Psychol.* 2013;4:1-12. <https://doi.org/10.3389/fpsyg.2013.00863>
33. Ko SH, Jung Y. Energy metabolism changes and dysregulated lipid metabolism in postmenopausal women. *Nutrients.* 2021;13(12):1-12. <https://doi.org/10.3390/nu13124556>
34. Kodoth V, Scaccia S, Aggarwal B. Adverse changes in body composition during the menopausal transition and relation to cardiovascular risk: A contemporary review. *Womens Health Rep (New Rochelle).* 2022;3(1):573-581. <https://doi.org/10.1089/whr.2021.0119>
35. Martin FP, Rezzi S, Peré-Trepát E, et al. Metabolic effects of dark chocolate consumption on energy, gut microbiota, and stress-related metabolism in free-living subjects. *J Proteome Res.* 2009;8(12):5568-5579. <https://doi.org/10.1021/pr900607v>
36. Fraga CG. Cocoa, diabetes, and hypertension: Should we eat more chocolate? *Am J Clin Nutr.* 2005;81(3):541-542. <https://doi.org/10.1093/ajcn/81.3.541>
37. Jaballah A, Soltani I, Bahia W, et al. The relationship between menopause and metabolic syndrome: Experimental and bioinformatics analysis. *Biochemical Genetics.* 2021;59(6):1558-1581. <https://doi.org/10.1007/s10528-021-10066-7>
38. Massaro M, Scoditti E, Carluccio M, Kaltsatou A, Cicchella A. Effect of cocoa products and its polyphenolic constituents on exercise performance and exercise-induced muscle damage and inflammation: A review of clinical trials. *Nutrients.* 2019;11(7):1-15. <https://doi.org/10.3390/nu11071471>
39. Ramírez-Sánchez I, Rodríguez A, Moreno-Ulloa A, Ceballos G, Villarreal F. (-)-Epicatechin-induced recovery of mitochondria from simulated diabetes: Potential role of endothelial nitric oxide synthase. *Diab Vasc Dis Res.* 2016;13(3):201-210. <https://doi.org/10.1177/1479164115620982>

