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Dark Chocolate Supplementation Elevates Resting Energy Expenditure in Exercise Trained Females

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ABSTRACT

International Journal of Exercise Science 14(2): 250-259, 2021. Several recent reports have indicated positive health and exercise benefits of (-)-epicatechin-rich cocoa products. This study investigated the influence of dark chocolate (DC) supplementation on resting and steady state exercise metabolism in a group of athletically fit females. Using a randomized, single-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). Pre-supplementation (PRE), subjects underwent indirect calorimetry assessment for resting energy expenditure (REE) and exercise energy expenditure (EEE) consisting of steady state cycling for 20 min, 10 min each at 50 W (EEE-50) and 100 W (EEE-100). Upon completion of the 30-d supplementation (POST), subjects repeated the assessment for REE, EEE-50, and EEE-100. Post supplementation REE was significantly increased by ~9.6% in the DC group (Δ REE: DC 140 ± 132, WC -3 ± 92 kcal · d^{-1}, p = .017). Post supplementation, neither EEE-50 (DC 4.51 ± 0.59, WC 4.51 ± 0.32 kcal · min^{-1}) nor EEE-100 (DC 6.56 ± 0.60, WC 6.69 ± 0.42 kcal · min^{-1}) were significantly different between groups ($p \geq .05$). There were no significant within or between group time effects for substrate utilization at rest or during EEE-50 or EEE-100 ($p \geq .05$). To our knowledge this is the first study to demonstrate that a relatively small daily dosage of DC can significantly elevate REE. However, it does not impact steady state EEE or substrate utilization in a group of athletically fit females.

KEY WORDS: epicatechin, cacao, polyphenol

INTRODUCTION

Moderate dark chocolate (DC) consumption has been reported to promote arterial vasodilation and cerebral blood flow, enhance oxygen and glucose delivery, and improve cognitive function (21). It has also been shown to decrease fat deposition in adolescents (21), low density lipoprotein (LDL) cholesterol, high sensitivity C-reactive proteins, and inflammation and platelet aggregation (6, 12, 27). Contrary to contemporary thought, moderate DC consumption has not been shown to be associated with obesity (10, 21).

Flavonoids, naturally occurring polyphenolic plant compounds, are an active component of cocoa to which many of its health benefits are attributed (5). Most recently these health benefits have specifically been attributed to its antioxidant properties stemming from the bioflavonoid

(-)-epicatechin (8). Using a mouse model, (-)-epicatechin administration was observed to reduce skeletal muscle fatigue, improve treadmill performance, and increase muscle capillary density (18). These positive findings were supported by the recent work of Taub et al. (26) indicating that sedentary middle-age subjects consuming 20 g · d^{-1} of DC (% not reported) for three months demonstrated a significant reduction in markers of oxidative stress, improvement in mitochondrial function, maximal oxygen uptake, and cycling power output. Likewise, Cavarretta et al. (3) while employing a slightly different DC dosing strategy (85% DC, 40 g · d^{-1}, 30 d) reported significant improvements in antioxidant power and markers of muscle damage in elite soccer athletes.

These positive findings are intriguing and raise a question of optimal DC dosing. There is accumulating evidence suggesting that the consumption/administration of flavonoids with high total antioxidant capacity (TAC) may actually inhibit, rather than enhance, skeletal muscle adaptations to exercise (7, 20). Consequently, it is quite plausible that a larger dose of DC may actually exhibit a negative impact on physiological function, whereas a smaller dosage would not.

While these recent reports indicate positive health and physiological exercise performance benefits with the administration of (-)-epicatechin and (-)-epicatechin-rich cocoa products, no studies have investigated the impact of DC consumption on resting and exercise metabolism. Consequently, the purpose of this study was to investigate the influence of 70% DC supplementation on energy metabolism at rest and also during two different intensities of steady state exercise, in a group of recreationally fit/athletic females. In an effort to minimize any potential inhibitory effect of DC consumption associated with an increase in TAC, we chose to administer DC in the same 20 g · d^{-1} dosage as Taub et al. (26) but for a significantly shorter duration of 30 d. It was hypothesized that 30 d of DC consumption would stimulate significant increases in both resting and exercise energy expenditure.

METHODS

Participants

The study employed a blind, placebo-controlled design and was approved by the Institutional Review Board for the use of Human Subjects in Research (IRB 03572-2017) and is fully in accordance to the ethical standards of the International Journal of Exercise Science (17). Inclusion criteria were non-smoking, normal weight females (body mass index 18.5-25 kg · m^2), 18-30 years of age, performing a minimum of 5 h · wk^{-1} of moderate- to high-intensity exercise. 18 subjects completed the study and there were no reported adverse events. Subjects were instructed to maintain their normal physical activity levels throughout the duration of the study.

Protocol

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, 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). Flow volume and gas calibrations were performed prior to each testing session according to the manufacturer's instructions. Subjects removed their shoes, assumed 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 0.75-0.85. Energy expenditure was calculated as [3.941(VO_2) + 1.106(VCO_2)], where VO_2 and VCO_2 were reported in L/min (29). Non-protein substrate utilization was calculated from respiratory quotient (RQ) and VO_2 (13).

Upon completion of the assessment of REE, the metabolic cart was immediately calibrated to accommodate exercise testing, and the subject was fitted with a facemask and mass flow sensor. The subject then performed 20-min of continuous exercise on a Velotron cycle (Racermate, Seattle, WA) 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.

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 (24). 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.

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 REE and EEE in the same fashion as described in the pre-supplementation assessment.

Supplementation was assigned using a randomized, 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 (~100 kcal) 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 (24). 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 resting and exercise metabolism; however, they were blinded to the fact that DC

was the treatment of interest and that the WC was the 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 not to consume their prescribed chocolate within1-h before or after consumption of any dairy product (i.e., yogurt, sour cream, milk, cottage cheese, cheese, and butter/margarine). 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 (11, 22).

Statistical Analysis

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 ± SD.

RESULTS

Inclusion criteria were non-smoking, normal weight females (body mass index 18.5-25 $kg \cdot m^2$), 18-30 years of age, performing a minimum of 5 $h \cdot wk^{-1}$ of moderate- to high-intensity exercise. Twenty-five subjects met the inclusion criteria; however, seven were eventually excluded due to a variety factors such as non-compliance with supplementation/exercise, incurring a physical injury during the course of the study that limited their ability to continue, and surprisingly a dislike for chocolate. While subjects clearly understood that the study was investigating chocolate, they were not aware that DC was the experimental treatment being investigated. Subsequent to initiating the study, one subject expressed a dislike for the taste of the 70% DC and voluntarily discontinued. This left a final subject pool of $n = 18$, (DC $n = 9$, WC $n = 9$). Subjects reported no adverse health 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 and there were no significant differences between groups.

Neither Pre-supplementation REE (DC 1448 ± 174, WC 1560 ± 155 $kcal \cdot d^{-1}$) or Post-supplementation REE (DC 1588 ± 248, WC 1557 ± 169 $kcal \cdot d^{-1}$) were significantly different between groups ($p \geq .05$). However, there was a significant treatment by time effect, with the post supplementation mean REE significantly increased by 9.6% in DC (Δ REE: DC 140 ± 132,

WC -3 ± 93 kcal · d^{-1}, p = .017) with 7 of 9 subjects demonstrating an increase in REE. Group effects and individual subject responses are shown in Figure 1.

Table 1. Subjects' characteristics.

	WC (n = 9)	DC (n = 9)	p-value
Body weight (kg)	69.4 ± 11.7	71.4 ± 11.8	.72
Height (m)	1.71 ± 0.07	1.69 ± 0.7	.83
BMI (kg · m^2)	23.4 ± 2.2	25.0 ± 3.3	.24
Age (yr)	21.1 ± 1.7	21.3 ± 1.9	.80

Note. Values are expressed as mean ± SD. WC = White Chocolate; DC = Dark Chocolate.

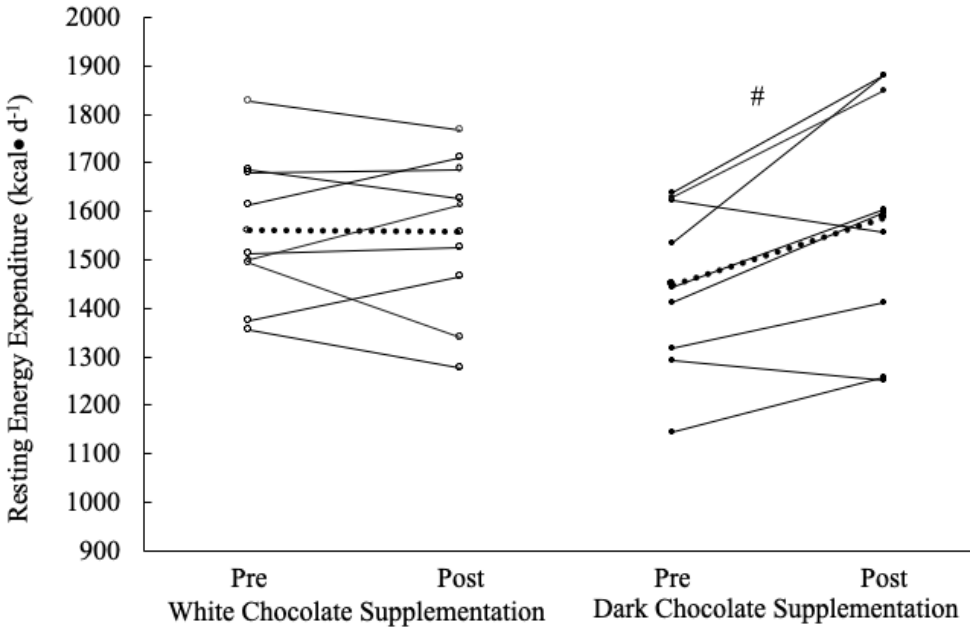

Figure 1. Individual subjects REE pre- and post-supplementation with white chocolate or dark chocolate (•). ••• indicates mean data for each group. # indicates a between groups time effect with the dark chocolate pre-post significantly greater than white chocolate pre-post (p = .017).

There were no significant interaction, time, or group effects for resting fat or carbohydrate (CHO) substrate utilization during the assessment of REE (p > .05) (Table 2).

Table 2. Resting substrate utilization.

	WC PRE	WC POST	DC PRE	DC POST
Fat (g · d^{-1})	108.7 ± 32.5	99.6 ± 42.0	125.0 ± 36.6	107.1 ± 29.4
CHO (g · d^{-1})	128.2 ± 76.0	148.6 ± 107.1	63.7 ± 52.1	138.7 ± 74.3

Note. Values are expressed as mean ± SD. WC = White Chocolate; DC = Dark Chocolate; PRE = before supplementation; POST = after supplementation.

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 > .05), VO_2 at cycling power outputs of both 50W (VO_2-50) and 100W (VO_2 -100) (p > .05), or RQ determined at cycling power outputs of both 50W (RQ-50) and 100W (RQ-100) (p > .05) (Table 3).

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

	WC PRE	WC POST	DC PRE	DC POST
Exercise energy expenditure (kcal · min^{-1})				
EEE-50	4.65 ± 0.56	4.51 ± 0.32	4.90 ± 0.33	4.52 ± 0.59
EEE-100	6.82 ± 0.53	6.69 ± 0.42	7.13 ± 0.45	6.56 ± 0.60
Exercise oxygen uptake (L · min^{-1})				
VO_2-50	0.94 ± 0.11	0.91 ± 0.06	0.99 ± 0.07	0.91 ± 0.12
VO_2-100	1.36 ± 0.11	1.34 ± 0.08	1.43 ± 0.09	1.31 ± 0.12
Respiratory Quotient (RQ)				
RQ-50	0.91 ± 0.05	0.92 ± 0.05	0.91 ± 0.04	0.93 ± 0.04
RQ-100	0.97 ± 0.05	0.97 ± 0.05	0.96 ± 0.05	0.97 ± 0.04

Note. Values are expressed as mean ± SD. WC = White Chocolate; DC = Dark Chocolate; PRE = before supplementation; POST = after supplementation; Exercise energy expenditure at cycling power outputs of 50W, (EEE-50); and 100W, (EEE-100). Oxygen uptake at cycling power outputs of 50W (VO_2-50) and 100W (VO_2-100). Respiratory quotient (RQ) at cycling power output of 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 · d^{-1}; ~100 kcal) of short duration (~30-d) on REE and EEE at two different intensities of steady state exercise in a group of recreationally fit/athletic females. The most notable finding was that DC consumption increased daily REE by ~9.6% (+140 kcal · d^{-1}); however, this change was not observed in EEE at either of the two exercise intensities.

The specific mechanisms by which DC impacts physiological function have not been clearly elucidated. Being that nutritional supplements are not closely regulated (28), 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 (7). However, accumulating evidence suggests that flavonoids with high total antioxidant capacity (TAC) may actually inhibit, rather than enhance, skeletal muscle adaptations to exercise (7, 20). Taub et al. (26) chose to supplement subjects with 20 g · d^{-1} of DC based on their previous work indicating that this provided blood (-)-epicatechin levels that elicited peak effects in cultured cells. 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}) over a short time duration (30 d).

Whereas, an increase in TAC associated with DC consumption may potentially inhibit physiological function, recent evidence suggests a mechanism whereby (-)-epicatechins can significantly alter mitochondrial structure and stimulate mitochondrial biogenesis (14, 25). It has been posited that (-)-epicatechins may bind to cell surface G protein-coupled estrogen receptors, positively impacting metabolic control and also stimulating mitochondrial biogenesis (15, 16).

Taub et. al. (26) recently demonstrated that a 3-month administration of 20 $g \cdot d^{-1}$ of (-)-epicatechin-rich cocoa increased maximal oxygen uptake and cycling power output in a group of sedentary, middle-aged (~50 yrs.) 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 (26). Earlier work in isolated mouse muscle indicated that (-)-epicatechin by itself, or combined with exercise, can also induce structural and metabolic changes in skeletal and cardiac muscle (18). In light of these previous reports, while only speculative, our findings of a 9.6% increase in REE would suggest significant changes in mitochondrial function likely did occur with the administration of a relatively small ($20\ g \cdot d^{-1}$) and brief (30 d) supplementation of 70% DC.

Previous studies of both high intensity interval training (9) and aerobic exercise training (23) 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 (26). In addition, positive markers of oxidative stress were significantly improved with DC supplementation (26) in a magnitude quite similar to that observed with chronic physical conditioning (19). 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 (18). In contrast, Copp et al. (4) 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. (18) were in response to an (-)-epicatechin administration of $2\ mg \cdot kg^{-1} \cdot d^{-1}$ for 15 d, whereas the absence of positive findings reported by Copp et al. (4) were in response to an (-)-epicatechin administration of $4\ mg \cdot kg^{-1} \cdot d^{-1}$ for 24 d. Considering the accumulating evidence suggesting that a high TAC may actually inhibit skeletal muscle adaptations (7, 20), 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. (26) 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. (26) were middle aged

(~49 years) and sedentary. Due to the difference between the exercise protocols, as well as the difference between subject characteristics, the conflicting findings are not surprising.

One potential confounding factor with the interpretation of the findings of this study relates to the differences in the caffeine content of the DC and the placebo (no caffeine). Caffeine supplementation has been reported to increase REE by as much as 13% (1) which is in the range observed in the present study. However, the dosage of caffeine that elicited this response far exceeds that which would have been consumed with the DC in the present study. It is estimated that 20 g of 70% DC contains a total of only 8.5 mg of caffeine which is dramatically less than 10 mg/kg that elicited the 13% change in REE. While it is possible that the effects reported may be partly attributable to the action of caffeine, it is quite unlikely that this played a significant role. Never-the-less, it is suggested that future investigations attempt to control for this variable.

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 aged individuals with sarcopenia.

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