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Original Research

Acute Ingestion of Dark Chocolate Fails to Affect Running Economy in Recreationally Trained Female Runners

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ABSTRACT

Topics in Exercise Science and Kinesiology Volume 5: Issue 1, Article 1, 2024. Ingestion of dark chocolate (DC), a dietary source high in flavanols, may increase nitric oxide bioavailability. Elevating blood nitric oxide concentrations may augment metabolic efficiency by reducing the amount of oxygen or energy needed to perform a given task. Utilizing a crossover design, the purpose of this study was to investigate the effect of acute ingestion of DC on running economy (RE). Nineteen recreationally trained females (age: 20±1 years) volunteered for this investigation, with 16 completing all procedures (n = 16). Two-hours before RE assessment, participants consumed either 42.5 g of DC or an isocaloric amount of white chocolate (WC) (37.2 g) with a 34 mg caffeine pill. Participants ran on the treadmill at 2.68 m/s for 10-minutes to assess RE. However, only the last 5-minutes of the test were used for oxygen utilization (VO2), energy expenditure (EE), and respiratory exchange ratio (RER) determination via indirect calorimetry. Identical testing procedures were utilized for DC and WC treatments with a seven-day washout period separating trials. A repeated measure paired t-test was used to determine differences between dependent variables with statistical significance set at $p < 0.05$. There were no significant mean differences (ps > 0.05) between trials for VO2, EE, or RER. In conclusion, supplementation of DC 2-hours prior to steady state running had no effect on RE or fuel utilization compared to an isocaloric serving of WC in recreational female runners.

KEY WORDS: Ergogenic aid, dietary supplement, flavanols

INTRODUCTION

An ergogenic aid is any substance, mechanical aid, energy enhancer, or training method that enhances athletic performance (Haff et al., 2015). Edible ergogenic aids are often derived from food sources that are high in flavanols, such as anthocyanins. Anthocyanin-rich supplements and foods have demonstrated acute improvements in exercise performance in healthy young populations (Pekas et al., 2020), thus making them an attractive method for supporting sport performance.

One potential ergogenic food of interest for use in endurance sports is dark chocolate (DC). Dark chocolate is high in flavanols, with the most abundant flavanol being anthocyanins (Zugravu & Otelea, 2019). Although not entirely clear, it has been proposed that the flavanols present in DC may mediate improvements in exercise performance by way of several mechanisms, such as improvements in the bioactivity and bioavailability of nitric oxide, which is a potent vasodilator (Shaw et al., 2020). Improved nitric oxide bioavailability has been reported to support improvements in vascular function and reduced peripheral vascular resistance, thus resulting in greater oxygen transport to the working skeletal muscle during exercise (Domínguez et al., 2017). Additionally, it has been proposed that nitric oxide may support metabolic efficiency and ultimately reduce the ATP cost of exercise (Anselm et al., 2009; Bailey et al., 2012; Brixius et al., 2006; Clerc et al., 2007; Pekas et al., 2020). Overall, there are various ways nitric oxide can improve physiological function and thus result in improved physical performance.

Previous literature has demonstrated several exercise performance benefits from DC intake, both chronically and acutely. Taub et al. (2016) investigated 3-months of DC intake in sedentary adults (20 g/day) and noted improvements in VO_{2MAX}, markers of mitochondrial biogenesis and content, and blood lipids (Taub et al., 2016). Similarly, Patel et al. (2015) investigated the effects of consuming DC chronically (14 days). Results revealed DC consumption led to a 6% improvement in VO_{2MAX} as well as improvements in gas exchange threshold and time trial performance (Patel et al., 2015).

On the contrary to chronic intake, acute intake has demonstrated mixed results. Patel and colleagues (2020) investigated the impacts of acute DC supplementation in a healthy adult population with different flavanol concentrations (high: 1060 mg, moderate: 746 mg, and low: 406 mg, control: 88 mg) 2-hours prior to steady state cycling in recreationally active adults. Dark chocolate at all doses failed to show beneficial effects on indicators of exercise performance (i.e., respiratory exchange ratio (RER), heart rate (HR), VO2 peak, peak power, or HR peak) despite a trend for improved nitric oxide bioavailability.

Moreover, Stellingwerff et al. (2013) demonstrated a reduction in plasma glucose oxidation while elevating glycogen utilization during steady state cycling following DC intake in healthy males, but time trial performance did not improve. However, it must be noted that Patel et al. (2015) and Stellingwerff et al. (2013) recruited solely male participants. Therefore, investigating the impacts of DC intake, particularly acute DC intake, on exercise economy and efficiency in recreationally trained females remains relatively under-studied.

Another factor to consider within the literature is that there is no clear-cut optimal dosage for eliciting an ergogenic effect. Further, studies with supplementation contain an extensive range of chocolate consumption (6.3□100.0 g/day) (Engler et al., 2004; Grassi et al., 2005; Patel et al., 2020; Sudarma et al., 2011; Taubert et al., 2007). In addition, studies within the literature have investigated both acute and chronic supplementation prior to exercise. Unfortunately, the recommended timing before exercise to improve performance is unknown.

Therefore, the purpose of the current study was to investigate the effects of acute DC supplementation in the form of baking chocolate on running economy (RE) in recreationally active females. The use of recreationally trained (4□20 h/week of training) (Stellingwerff et al., 2013) participants was due to better odds of eliciting a measurement effect in exercise economy via a presumable nitric oxide-dependent mechanism than may be seen in better-trained participants (Macuh & Knap, 2021). Further, there will be more of an increase in endothelial nitric oxide synthase activity and nitric oxide production within the vasculature in less-trained individuals compared to more-trained.

It was hypothesized that acute supplemental DC consumption would improve running economy compared to an isocaloric amount of white chocolate (WC) with similar caffeine content but void of the anthocyanins that are otherwise present in DC.

METHODS

Participants

An *a priori* power analysis conducted with G*Power (version 3.1) determined that 15 participants were needed in the present study to achieve a power and effect size of 0.80 ($\alpha = 0.05$) (Faul et al., 2007). In addition, an aim of 15 recreationally trained participants was used based on a previous investigation (Patel et al., 2020). Nineteen recreationally trained females participated in this investigation upon providing written informed consent approved by a University located in the Northeast Institutional Review Board. This research was carried out thoroughly in accordance with the ethical standards of the Declaration of Helsinki and the Belmont Report (Navalta et al., 2020). Demographic data for participants who completed all procedures can be found in Table 1.

Table 1. Descriptive Statistics of Demographic Information for Recreationally Trained Females.

Variables	Mean	SD	n
Age (years)	21.2	1.6	16
Height (cm)	164.8	7.3	16
Weight (kg)	60.1	7.0	16
BMI (kg/m2)	22.2	2.2	16
Est. Body Comp (%)	19.1	3.7	16
Systolic (mmHg)	112.0	10.7	16
Diastolic (mmHg)	76.4	8.9	16
RHR (bpm)	68.7	9.9	16

Note. BMI = Body Mass Index, Diastolic = Diastolic Blood Pressure, Est. Body Comp = Estimated Body Composition (% fat mass), RHR = Resting Heart Rate, Systolic = Systolic Blood Pressure.

The inclusion criteria for participation in this study were (a) female; (b) 18 years or older; (c) free of any history of major medical problems including metabolic, cardiovascular, endocrine, thermoregulatory, musculoskeletal issues, or bone pathologies, (d) able to continuously run a minimum of 5 km determined via self-report, and (e) no medicine or nutritional supplement other than a multivitamin or mineral supplement within 3-months prior to beginning the study determined via self-report (Casado et al., 2021). Exclusion criteria include participants with a surgical history of lower body orthopedic dysfunction/injury within the past 6-months due to

potential altered running mechanics. Participants who had any allergy or intolerance to chocolate (or cocoa) were also excluded. Finally, due to the possible cross-contamination of peanuts or tree nuts when manufacturing chocolate products, any individuals with a peanut or tree nut allergy/intolerance were excluded.

Protocol

Participants were asked to report to the Laboratory for a total of two sessions under the same ambient temperatures (20^{o} C) at approximately the same time of day. A coin was flipped for the first participant to determine if they were receiving the control or the experimental treatment. A block randomization methodology was utilized to place participants into groups of equal sizes and thus, ensure a balance within the study. Further, each subsequent participant alternated from the treatment of the prior participant. Current studies' hypotheses were blinded, and participants were informed that the effects of two types of chocolate on running performance were being investigated. The study participant allocation and analysis are displayed in Figure 1.

In the 24-hours preceding the first session, participants recorded their food intake and were asked to replicate their diet the 24-hours before their second laboratory visit.

Participants were instructed to avoid strenuous exercise and alcohol 24-hours prior to each session (Lansley et al., 2011). In addition, refrain from caffeine for 6-hours and food/water for 4-hours prior to each session. Basic demographic and anthropometric data (e.g., age, height, weight, current weekly mileage, and training history) were collected. The Jackson-Pollock Three-Site method for skinfolds (triceps brachii, thigh, suprailiac) was utilized to determine body composition (Boye et al., 2002). In addition, resting heart rate (radial pulse, 60 seconds) and blood pressure using a manual sphygmomanometer were recorded prior to exercise.

Next, participants consumed either 42.5 g of Ghirardelli Unsweetened baking chocolate or an isocaloric amount (approximately 213 kcals) of Ghirardelli WC (37.2 g) containing no flavanols. While specific flavanol concentrations were not measured, baking chocolate has been shown to contain approximately double the polyphenol content of commercially available "dark chocolate" (Miller et al., 2006).

Additionally, when participants consumed WC, 34 mg of caffeine was ingested via gelatin capsules. Caffeine content naturally present in baking chocolate was matched based on published averages (i.e., 33.4 mg caffeine/42 g chocolate) (Temple et al., 2017). Eight ounces of water were provided to all participants during feeding. Following the standardized water consumption, participants were prohibited from consuming additional fluids. As blood flavanol concentration peaks approximately 2-hours post-ingestion (Schramm et al., 2003), participants rested for 2-hours in a seated position post-food and drink consumption.

After the 2-hour rest, the participant ran on the treadmill at 2.68 m/s (6.0 miles/hour) for approximately 5-minutes as a warmup. During this time, stride frequency was determined in the first trial's warm-up via metronome (bpm), played during both conditions to promote consistent running and, thus, eliminate confounding variables. Since participants were not all at

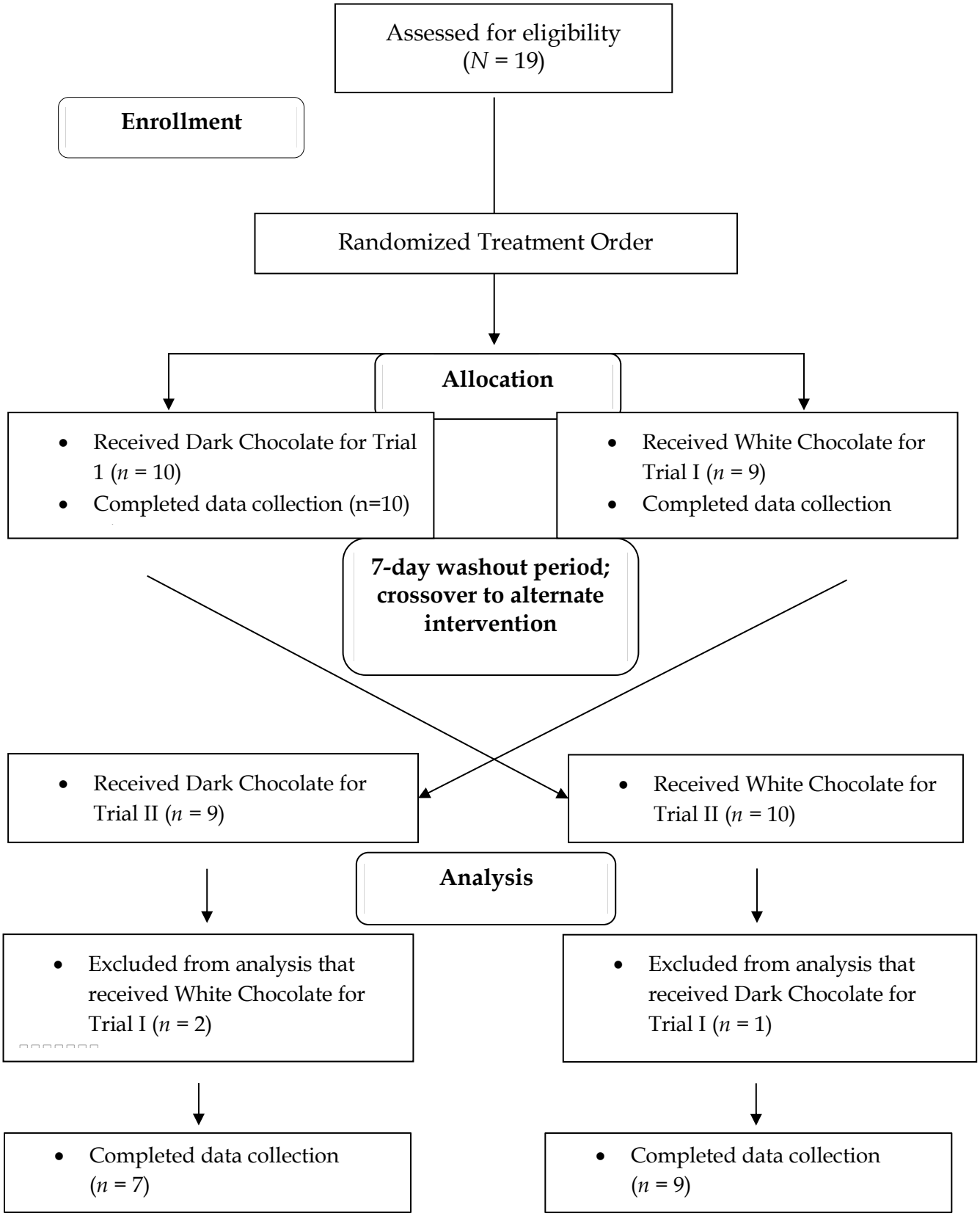
Assessed for eligibility
(N = 19)
Enrollment
Randomized Treatment Order
Allocation
Received Dark Chocolate for Trial 1 (n = 10)
Completed data collection (n=10)
Received White Chocolate for Trial I (n = 9)
Completed data collection
7-day washout period; crossover to alternate intervention
Received Dark Chocolate for Trial II (n = 9)
Received White Chocolate for Trial II (n = 10)
Analysis
Excluded from analysis that received White Chocolate for Trial I (n = 2)
Excluded from analysis that received Dark Chocolate for Trial I (n = 1)
Completed data collection (n = 7)
Completed data collection (n = 9)

Figure 1.

the same fitness level, only the last 5-minutes of the 10-minute trial were analyzed to ensure steady state was achieved. In other words, the more fit you are, the quicker you reach steady state and vice versa.

Running economy (VO_2) is operationally defined as the energy demands at a fixed velocity (steady state exercise) and is determined by the consumption of oxygen, expressed as VO_2 (ml/kg/min) and the RER (Saunders et al., 2004). Further, according to Saunders et al. (2004), RE is a stronger predictor of distance running performance when compared to maximal oxygen uptake, also known as $VO_{2MAX.}$ The current protocol was based on a previous investigation assessing dark chocolate consumption and its impact on enhancing running efficiency, also known as increasing RE (Perl et al., 2012).

A gas analysis using indirect calorimetry known as Parvo Medics' TrueOne® 2400 (Salt Lake City, UT) was used to determine metabolic function (i.e., VO_2, RER, kcals expended/time) during exercise. Steady state conditions were verified by ensuring less than a 10% change in VO_2 per minute within the collection period (Reeves et al., 2004). Additionally, RERs were required to be less than 1.0 for all analyses. Energy expenditure (EE) was determined with the use of an updated nonprotein respiratory quotients (Péronnet & Massicotte, 1991). Participants were asked to record their food intake 24-hours before their first session and replicate it 24-hours before their second session. In addition, they were able to follow their regular dietary and water intake. There was a one-week wash-out period in between sessions. Then, participants returned to the lab following the same procedures except with the alternate dietary treatment (DC or WC). Participants were not restricted from their normal training regimen but were asked to avoid strenuous exercise 24-hours prior to trials.

Statistical Analysis

Data were assessed for normality using a Shapiro-Wilks test. A repeated measure paired t-test was run for each dependent variable (VO_2, EE, and RER). If statistical significance were obtained, a Bonferroni correction would be applied for multiple comparisons. Additionally, Cohen's *d* effect sizes were calculated for each metric and interpreted as trivial (0.0-0.2), small (0.2-0.6), moderate (0.6-1.2), large (1.2-2.0), and very large (≥ 2.0) respectively (Hopkins et al., 2009). Statistical analyses were performed using SPSS® Version 29.0 (IBM, Armonk, NY, USA). The *a priori* alpha level was set at 0.05.

RESULTS

Three participants were removed from the study because they were unable to consume all the DC due to reported gastrointestinal distress/nausea. Further, data was collected and analyzed from 16 recreationally trained individuals. A complete summary of descriptive statistics is displayed in Table 1.

Skewness was calculated to ensure data was normally distributed (DC VO_2 γ = -0.81; WC VO_2 γ = 0.35; DC EE γ = 0.65; WC EE γ = -0.33; DC RER γ = -0.23; WC RER γ = 0.005).

There was no significant mean difference in VO_2 when consuming DC (35.17±4.58) or WC (34.81±5.98), $t(15) = 0.276$, $p = 0.786$, $d = 0.06$, 95CI: -2.42-3.14. In addition, there was no statistically significant difference in EE after consuming DC (10.74±2.04) or WC (10.64±2.27), $t(15) = 0.230$, $p = 0.821$, $d = 0.05$, 95CI: -0.82-1.01. Finally, there was a lack of significant mean disparity in RER when consuming DC (0.89±0.07) or WC (0.90±0.07), $t(15) = -1.041$, $p = 0.314$, $d = -0.26$, 95CI: -0.04-0.01 (Figure 2).

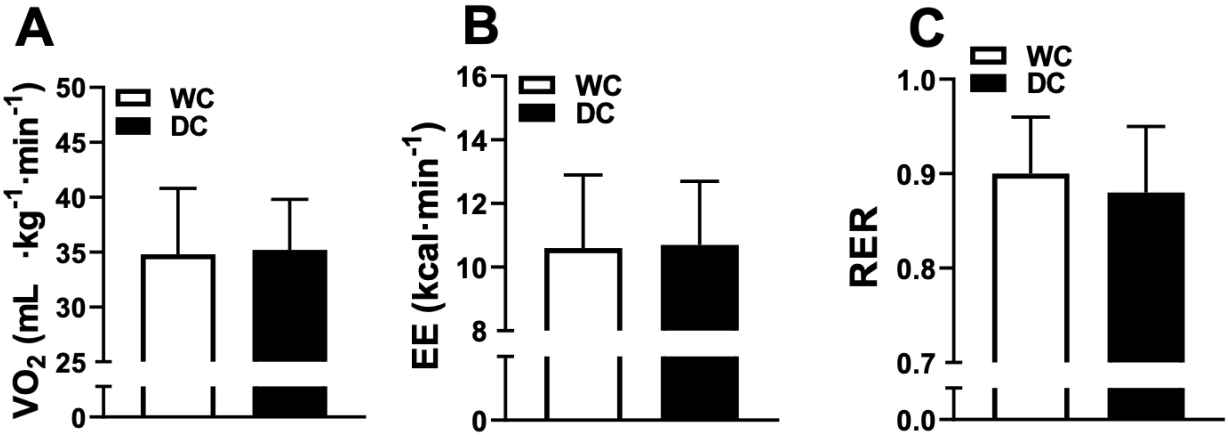

Figure 2. The volume of oxygen consumption (VO_2), energy expenditure (EE), and respiratory exchange ratio (RER) during the last 5-minutes of submaximal exercise testing. **(A)** No changes in VO_2 were noted between conditions ($p = 0.786$). **(B)** No changes in EE were noted between conditions ($p = 0.821$). **(C)** No differences in RER were noted between conditions ($p = 0.314$).
Values are Mean ± SD.

DISCUSSION

The purpose of the current study was to investigate the effects of acute DC supplementation in the form of baking chocolate on RE during a steady state run. In addition, the study utilized recreationally trained females to aid in bridging the gap within the literature. It was hypothesized that consuming DC 2-hours prior to steady state exercise would improve RE compared to an isocaloric amount of WC with similar caffeine content.

Dark chocolate contains anthocyanins, one of the most abundant flavonoids (Zugravu & Otelea, 2019). After acute consumption (2-hours prior to exercise), it is hypothesized that the antioxidant properties of the anthocyanins will support nitric oxide bioavailability, thus supporting vascular function and oxygen delivery to the working skeletal muscle. However, findings contradict the current hypothesis and reveal no difference exists between treatment conditions for VO_2, EE, or RER. Overall, acute supplementation may not be enough time to elicit tissue perfusion via nitric oxide.

Running economy (VO_2) is defined as oxygen cost at a given velocity and can strongly predict endurance performance (Saunders et al., 2004). Energy expenditure is inversely related to RE. Further, a more efficient distance performance will result in a higher RE and a lower EE (Saunders et al., 2004). Another physiological metric relating to distance performance is RER. For an individual to be considered in steady state, RER has to be less than a value of one (Conley

& Krahenbuhl, 1980; Saunders et al., 2004). Overall, RE, EE, and RER are physiological metrics that assess running performance efficiency.

The primary finding of this investigation is that acute supplementation of DC prior to the start of steady state exercise had no significant influence on VO_2, EE, or RER. This is consistent with previous research reporting no differences in cycling economy or performance following acute cocoa ingestion with varying flavanol concentrations (Decroix et al., 2017; Patel et al., 2020). In addition, current study findings in females are similar to the literature when assessed in male participants (Decroix et al., 2017).
To our knowledge, the only data suggesting any significant metabolic influences of acute (i.e., a single dose) DC ingestion are related to carbohydrate fuel utilization (i.e., the use of glucose or glycogen) (Davison et al., 2012; Stellingwerff et al., 2013).

The results of the present study indirectly support the contention that chronic supplementation of DC may be the more viable route to influencing aerobic performance, as has been previously suggested by Patel et al. (2020). Male cyclists supplementing for 14 days with DC (40 g) saw benefits in both their gas exchange threshold and distance covered during the 2-minute time trial as compared to baseline measures (Patel et al., 2015). These improvements occurred with a similar serving size of chocolate despite a much lower cacao percentage (approximately 53-60%) as compared to the baking chocolate (100% cacao) used in the present study.

A counterbalancing study design was utilized for DC/WC treatments to minimize a potential order effect. Interestingly, most participants reported that the second trial was easier, regardless of their condition. Further, a dependent paired t-test was conducted to assess whether physiological metrics differed from trial one to trial two. A dependent paired t-test showed a non-significant trend ($p = 0.05$) towards lower EE in trial 2 (10.3±2.0 kcal/min) when compared to trial 1 (11.1±2.1 kcal/min).

Another limitation of the current study is the dosage of DC used, which was 42.5 g of Ghirardelli Unsweetened or an isocaloric amount (approximately 213 kcals) of Ghirardelli WC (37.2 g) containing no flavanols. Further, the specific concentration of flavanol in the DC is unknown as it is not said on the packaging. Baking chocolate (100% cacao) has been shown to contain double the polyphenol content compared to other DCs (Miller et al., 2006). However, the concentration of flavanol and polyphenols cannot be quantified.

Within the literature, there is an extensive range of chocolate consumption (6.3–100.0 g/day) (Engler et al., 2004; Grassi et al., 2005; Patel et al., 2020; Sudarma et al., 2011; Taubert et al., 2007). Therefore, the proper dosage of acute and chronic DC is unknown to elicit an ergogenic effect.

Participants performed two conditions (DC vs. WC) with a one-week washout period in between. They were instructed to complete a 24-hour food journal before the first session and follow it 24-hours prior to the second session. Participants were also informed to maintain their normal diet, and there were no food restrictions throughout the course of the study. Pekas and colleagues (2020) investigated the effects of berry extract on physiological biomarkers (e.g.,

antioxidant capacity, endothelial function, resting heart rate). Participants were asked to avoid foods that were considered antioxidant-rich, as well as antioxidant supplements. Future research investigating the effects of DC on RE should have participants avoid antioxidant-rich foods throughout the washout period.

On a final note, another limitation is that the current study did not consider birth control/hormonal contraception or controlling for menstrual cycle phasing. When conducting research on female participants, there will be a fluctuation in hormones over the course of the menstrual cycle. Further, it is important to monitor the menstrual cycle as sex hormones can impact vascular function on the molecular and functional level (Miller & Duckles, 2008; Stanhewicz et al., 2018; Usselman et al., 2016; Wenner, 2020).

The consumption of DC 2-hours prior to exercise did not affect running economy in recreationally trained females (4–20 h/week of training) due to a similar VO_2 and EE between conditions. In addition, substrate utilization was consistent since both conditions had a similar RER of ~1.0, which indicates a primary utilization of carbohydrates. It cannot currently be recommended to use DC acutely for the augmentation of running performance. Due to a non-significant trend in order effect and the dropout rate due to self-reported gastrointestinal distress/nausea, future investigations related to flavanols and running economy should include familiarization trials as well as the use of concentrated supplements that may be better tolerated.

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