International Journal of Sport Nutrition and Exercise Metabolism, 2020, 30, 420-426
https://doi.org/10.1123/ijsnem.2020-0051

Human Kinetics
ORIGINAL RESEARCH

Effect of Dark Chocolate Supplementation on Tissue Oxygenation, Metabolism, and Performance in Trained Cyclists at Altitude

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Dark chocolate (DC) is high in flavonoids and has been shown to increase nitric oxide in the blood. Increased nitric oxide has the potential to improve delivery of oxygen to muscle, especially in hypoxic conditions, such as altitude. Our aim was to assess the impact of DC supplementation on cycling performance at altitude. Twelve healthy, trained cyclists ($n = 2$ females, $n = 10$ males; age = 35 [12] years; height = 177 [7] cm; mass = 75.2 [11.0] kg; VO_2max = 55 [6] ml·kg^{-1}·min^{-1}) were randomized to supplement with 60 g of DC or placebo twice per day for 14 days in a double-blind crossover study. After the 2 weeks of supplementation, the participants attended a laboratory session in which they consumed 120 g of DC or placebo and then cycled for 90 min at 50% peak power output, followed immediately by a 10-km time trial (TT) at simulated altitude (15% O_2). The plasma concentration of blood glucose and lactate were measured before and at 15, 30, 60, and 90 min during the steady-state exercise and post TT, while muscular and prefrontal cortex oxygenation was measured continuously throughout exercise using near-infrared spectroscopy. DC resulted in a higher concentration of blood glucose (5.5 [0.5] vs. 5.3 [0.9] mmol/L) throughout the trial and lower blood lactate concentration following the TT (7.7 [1.92] vs. 10.0 [4.6] mmol/L) compared with the placebo. DC had no effect on the TT performance (19.04 [2.16] vs. 19.21 ± 1.96 min) or oxygenation status in either the prefrontal cortex or muscle. The authors conclude that, although it provided some metabolic benefit, DC is not effective as an ergogenic aid during TT cycling at simulated altitude.

Keywords: cycling, muscle metabolism, nitric oxide, polyphenols, prefrontal cortex

Many sports involve athletes travelling to areas of high altitude for competition or training, which leads to decreases in both maximal and submaximal exercise performance. For example, athletes can expect a 7–9% decrease in their maximal aerobic capacity for every 1,000 m above 1,000 m altitude that they travel (Balke et al., 1965). A drop in atmospheric PO_2 leads to decreased arterial PO_2 due to a decrease in pressure gradient, resulting in a proportional decrease in oxygen delivery to the muscle and brain during exercise (Decroix et al., 2018; Stenberg et al., 1966). This decrease in O_2 delivery leads to the development of reactive oxygen species, contributing to the development of both central and peripheral fatigue (Decroix et al., 2018).

To maximize their potential, dietary supplementation with exogenous forms of nitrate (i.e., nitrate-rich beetroot juice or pure sodium nitrate) has become increasingly popular among endurance athletes due to its effects in reducing the oxygen cost of moderate-intensity exercise and improving performance (Cermak et al., 2012; Domínguez et al., 2017).

Nitrate supplementation may enhance exercise performance through the actions of plasma nitric oxide (NO). Domínguez et al. (2017) reviewed the effects of beetroot juice on endurance exercise performance and concluded that chronic supplementation with beetroot juice may lead to a decreased oxygen consumption (VO_2) at an intensity of 70% VO_2max, improved time to exhaustion, and an enhanced metabolic efficiency at the ventilatory threshold. This type of supplementation is effective for mitigating some of the negative effects on performance that occur at altitude by decreasing oxygen consumption, improving time trial (TT) performance, and increasing muscular oxygenation (Masschelein et al., 2012; Muggeridge et al., 2014). The interested reader is referred to recent reviews by Shannon et al. (2017) and Senefeld et al. (2020) for the effects of nitrate at hypoxia and on exercise performance, respectively.

Evidence suggests that dark chocolate (DC) may provide similar effects to nitrate supplementation due to its high polyphenol content. Polyphenols found in DC (epicatechin and procyanidins) increase the bioavailability of NO and decrease the reuptake of NO, while managing reactive oxygen species that are created through the oxidative stress caused by both exercise and a hypoxic environment (i.e., altitude; Bayat et al., 2014; Faridi et al., 2008; Patel et al., 2015; Schewe et al., 2008; Sudarma et al., 2011). Epicatechin, found in high quantities in DC, increases nitrite concentration, an indirect marker of endothelial NO synthase-dependent NO production (Brossette et al., 2011). Increased endothelial NO has vasodilatory effects, increasing the efficiency by which nutrients are delivered and waste products removed, which enhances muscle metabolism. Evidence also supports the use of 2 weeks of DC supplementation as an ergogenic aid for exercise. Patel et al. (2015) observed an increase in 2-min TT cycling performance following supplementation for 14 days of DC, while a review by Somerville et al. (2017) suggests that chronic polyphenol supplementation (≥7 days) improves power output in a healthy adult population in

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events lasting >10 min. Increased NO through DC supplementation may increase blood flow and delivery of O_2, helping to offset the negative impact on performance that occurs at altitude. Decroix et al. (2018) observed an increase in prefrontal cortex oxygenation after 6 days of cocoa flavanol supplementation, which may assist with decision making during exercise and decrease central fatigue (Thomas & Stephane, 2008). Research also suggests that a reduction in cerebral oxygenation may limit exercise performance by inhibiting cortical activation of motor neurons (Subudhi et al., 2007), while limitations in muscular oxygenation may inhibit exercise performance through changes in intracellular metabolism, which may lead to the accumulation of metabolites and inhibiting excitation–contraction coupling (Amann & Calbet, 2008). Additionally, the theobromine and caffeine content of DC may have ergogenic effects through increasing lipolysis, leading to the sparing of blood glucose and glycogen and maintaining aerobic metabolism, which could reduce lactate levels, thus improving endurance performance (Allgrove et al., 2011).

Elite cycling races take athletes through various altitudes, from sea level to almost 2,800 m (~15% O_2). Although the current literature is scarce, DC may assist in enhancing work at a given oxygen uptake during exercise and maximize blood flow to and from active muscles, leading to enhanced TT performance at altitude. The purpose of this study was to investigate the effects of 2 weeks of DC supplementation on cycling TT performance and metabolic parameters of exercise at a simulated (normobaric hypoxia) altitude of 2,500 m (15% O_2), as well as VO_2 and oxygenation of the prefrontal cortex and vastus lateralis muscle (VL). It was hypothesized that DC would improve the oxygenation status in the VL and the prefrontal cortex during exercise at simulated (normobaric) altitude while enhancing energy metabolism (evidenced by enhanced blood glucose stability and decreased blood lactate). It was hypothesized that these beneficial effects will result in improved TT performance in trained cyclists.

Methods

Participants

Following ethical approval from the University of Saskatchewan Research Ethics Board, 12 trained cyclists volunteered to take part in this double-blind, counterbalanced crossover study and provided written informed consent ($n = 2$ females; age = 35 [12] years; height = 177 [7] cm; body mass = 75.2 [11.0] kg; VO_2max = 55 [6] ml·kg^{-1}·min^{-1}). The sample size was calculated using G*Power app (version 3.1.9.7; Heinrich Heine Universität Dusseldorf, Düsseldorf, Germany), based on an effect size of 0.67 for our primary outcome, TT performance (Muggeridge et al., 2014), a 95% confidence interval (CI), and a power of 80%. Participants were included if they were ≥18 years of age and engaged in a training regime of at least 1 hr three times per week of cycling. Individuals were excluded if they did not have above-average aerobic fitness (>50 ml·kg^{-1}·min^{-1} for men; >45 ml·kg^{-1}·min^{-1} for women; Kaminsky et al., 2015; Shvartz, & Reibold, 1990) if they consumed more than 50 g DC per day, 5 days per week, or they were currently supplementing with exogenous nitrate. None of the participants reported being habitual smokers, which could impact the endothelial function.

Supplement

The participants consumed a DC with 72% cocoa solids (Belcolade Premium Belgium Chocolate, Erembodegem, Belgium) or a closely matched nonchocolate placebo (Merckens, Cumberland, RI; Table 1). The DC is estimated to contain approximately 14.9 GAE polyphenols, 0.312 mg/g epicatechin, and 0.107 mg/g catechin, based on the assessment of a similar product by Miller et al. (2009), and approximately 73 mg of caffeine and 883 mg of theobromine per 100-g serving, based on an assessment by Harland (2000) and Meng et al. (2009), respectively. This particular DC was used for the high percentage of cocoa solids and the availability to consumers. The placebo was a "DC flavored molding wafer" and lacked the active ingredients found in the DC, while containing a similar appearance, taste, and caloric load. Seven out of 12 participants (58%) correctly guessed the order in which they received the supplements. The participants consumed 60 g twice per day (morning and evening at the same time) for 2 weeks prior to performance testing. A dose of 120 g per day for 2 weeks was used in the current study, based on previous work (Patel et al., 2015; Stellingwerff et al., 2014). The participants had a 2-week washout period before supplementing for the opposite condition. With measures 4 weeks apart, the female participants were assessed at approximately the same point of their menstrual cycle to avoid any performance/metabolism alterations due to changes in hormonal status (Oosthuyse & Bosch, 2010). All exercise testing was performed under the environmental conditions at 577 m and 712 mmHg (elevation and barometric pressure, respectively).

Visit 1

On the first visit, the participants provided their written informed consent, and body mass, height, and age were recorded. The participants completed the Get Active Questionnaire (Canadian Society for Exercise Physiology, 2017) to ensure no contraindications and that all participants were healthy and safe to exercise. The participants completed an aerobic power test (VO_2max) to determine their cardiovascular fitness. The participants completed a 10-min self-paced warm-up on a cycle ergometer (Velotron Racer Mate, Seattle, WA) or their own bicycle affixed to a "smart" bicycle trainer (Wahoo Fitness, Atlanta, GA), following which, a mask was affixed to cover the nose and mouth. This mask was connected to a computerized metabolic cart to collect and analyze expired gases (TrueOne 2400; Parvo Medics, Salt Lake City, UT). The power output was increased by 30 W every 3 min until volitional fatigue or when participants could no longer maintain >60 revolutions per minute. Any participant who did not reach the cutoff value for aerobic fitness level were excluded from the trial. The VO_2max was determined as the highest VO_2 in a 30-s collection interval until volitional fatigue. A true VO_2max was said to be reached if VO_2 plateaued, evidenced by an increase smaller than 0.2 ml·kg^{-1}·min^{-1}, or decreased in the last minute of the test, and if RER exceeded levels of 1.1. The participants who failed to reach their true maximum ($n = 1$) were still included the VO_2peak was above the designated cutoff values.

Table 1 Nutritional Information of Supplements

Composition	DC per 100 g	Placebo per 100 g
Kilocalories	560	540
Carbohydrates (g)	44	61
Fat (g)	41	34
Protein (g)	8	4

Note. DC = dark chocolate.

Visit 2

The participants returned to the lab a week later and completed an aerobic power test at a simulated altitude of 2,500 m (15% O_2; Muggeridge et al., 2014) using a 2.4-m^3 normobaric hypoxic chamber (Altitudetech Inc., Kingston, Ontario, Canada). The principle of operation for the chamber is that O_2 is removed, which then increases the N_2 content, leaving $FICO_2$ at approximately 0.04%. An identical exercise protocol as used in Visit 1 was followed.

Visit 3

A week later, the participants returned to the lab and completed a familiarization trial of the experimental protocol at normobaric hypoxia (712 mmHg, 15% O_2). This protocol consisted of 90-min cycling at 50% peak power reached in Visit 2, followed immediately by a 10 km TT. The participants were given a 5-s countdown to signify that they were transitioning from steady-state exercise to the TT. During the TT, consistent verbal encouragement was provided, and the participants were permitted to know the distance covered, but not the time elapsed. Following the familiarization trial, the participants were randomized to consume either DC or a placebo for 14 days.

Visits 4 and 5

The morning following completion of the 14-day supplementation period, the participants returned to the lab between 7 a.m. and 10 a.m., at the same time of day for both conditions, after a ≥10 hr fast. The participants were instructed to avoid caffeine for 2 hr, alcohol for 12 hr, and exercise for 12 hr prior to the experimental trials. Fasted finger prick measures of blood glucose (Accu-chek; Roche Diabetes Care, Basel, Switzerland) and lactate (Lactate Scout; EKF Diagnostics, Wales, United Kingdom) were taken prior to the participants' consuming 120 g of DC or placebo. Finger prick blood samples were taken from the second, third, or fourth finger, as per the participants' preference. A small finger prick was created using a spring-loaded lancet (Roche Diabetes Care, Switzerland). Blood was collected on strips designed for the aforementioned equipment, and values were produced within 30 s. Oxygenation was measured via near-infrared spectroscopy (NIRS; Artinis Medical, Elst, The Netherlands) during exercise at the VL muscle (PortaMon) and at the right prefrontal cortex (PortaLite). The participants were fitted with NIRS probes on the VL of their dominant leg, as well over the right side of the forehead (see description below). The participants completed the same protocol as Visit 3. Water ingestion was permitted ad libitum. Blood glucose and lactate were measured via finger prick at 15, 30, 60, and 90 min and within 1 min following the TT. Expired gases were collected for 1 min every 15 min during the 90 min of steady-state cycling, but not during the TT, to ensure a maximal effort unaffected by altered breathing due to the gas collection apparatus. Oxygenation of the VL muscle and prefrontal cortex were measured continuously throughout the entirety of the trial.

Near-Infrared Spectroscopy

The NIRS system was used to measure oxygenated hemoglobin, deoxygenated hemoglobin, and total hemoglobin in the VL and right prefrontal cortex. These measures were used to infer relative changes in oxygenation and hemodynamics. NIRS detects changes in light absorption of chromophores hemoglobin and myoglobin using the modified Beer Lambert Law (Ferrari & Quaresima, 2012). The PortaMon NIRS was applied to the midthigh over the VL muscle, approximately 14 cm above the patella, and held in place using an elastic bandage (Neary et al., 2002). The exact location was recorded for each participant to ensure consistent location between trials. The PortaLite NIRS cerebral probe was positioned over the prefrontal cortex region on the right side, approximately 1.5 cm above the eyebrow, as laterally as possible to avoid sinus cavities. All probes were held in place using a dark-colored generic elastic sleeve to minimize probe movement and block ambient light. Prior to applying the probes, the skin was cleansed using an alcohol wipe, and excess hair on the quadriceps was shaved to eliminate the signal interference. The NIRS probes were wrapped in "cling wrap" cellophane to prevent sweat from forming on the LED sensors, which would distort the signal. Prior to exercise, a baseline measure of oxygenation was recorded after the participants had been resting for a minimum of 5 min, which all measurements during exercise would be compared to. Once the exercise trial started, both the muscle and brain data were collected continuously throughout the duration.

Gas Analysis

The expired gases were continuously monitored for oxygen and carbon dioxide concentration, using a computerized metabolic cart (TrueOne 2400; Parvo Medics), which is a non-breath-by-breath gas analyzer that uses a 4-L high-efficiency mixing chamber. The values obtained were averaged over 30 s during exercise. The values for O_2 consumed and CO_2 were applied to equations described by Jeukendrup and Wallis (2005) to determine the primary substrate being metabolized and to calculate the respiratory exchange ratio to estimate substrate metabolism. Expired gas data collected in hypoxia (Visits 2, 4, and 5) was transformed using the Haldane equation to reflect the effect of altitude. The gas analyzer was calibrated as per the manufacturer's instructions at the beginning of each day, using primary standard gases (4.00% CO_2, 16.00% O_2, remainder N_2) for tests done at normoxia and between each participant for tests done at hypoxia to account for changes in temperature and humidity in the normobaric hypoxic chamber. A 3-L Hans *Rudolph* syringe (Hans Rudolph, Inc., Shawnee, OK) was used to calibrate for ventilation. During each calibration, the temperature, humidity, and barometric pressure were recorded to reflect any fluctuations of these parameters.

Dietary and Exercise Control

For the duration of the study, the participants were asked to maintain their regular dietary, training, and supplementation regime and to avoid consuming any antioxidant supplements. The participants were asked to refrain from eating DC, except for that supplied by the research team, for the duration of the trial. In the 2 days leading up to Visit 4, the participants were asked to keep a detailed food log. The participants were then to replicate their intake in the 2 days prior to Visit 5 and to limit their intake of polyphenol-rich foods, such as coffees, teas, berries, and dark leafy greens. The participants were informed of the caloric load of the supplements and asked to replace a dessert or snack with the supplement in order to maintain a stable caloric intake.

Statistical Analysis

All data were analyzed using JASP statistical software (version 0.10.2; 2013–2019, University of Amsterdam). TT performance was analyzed using a dependent (paired) *t* test. Blood lactate, blood glucose, expired gasses, and prefrontal cortex and muscular

oxygenation were analyzed using a two-factor (Treatment × Time) repeated-measures analysis of variance. The significant findings were subsequently followed by a Bonferroni post hoc test. All data were normally distributed, as assessed using the Schapiro–Wilk's test. The results were considered significant at $p < .05$. The results are reported as mean (*SD*), and also as effect sizes and 95% CIs for our main outcomes.

Results

TT Performance

The TT performance did not differ between the DC and placebo conditions (19.04 [2.16] min, 95% CI [17.8, 20.3] vs. 19.21 [1.96] min, 95% CI [18.1, 20.3]; effect size = –0.09; $p = .77$).

Blood Analysis

There was a main effect of supplement on blood glucose. The DC condition resulted in a higher mean blood glucose concentration (5.5 [0.5] mmol/L, 95% CI [5.2, 5.8] vs. 5.3 [0.9] mmol/L, 95% CI [4.8, 5.8]; effect size = 0.22; $p = .013$; Figure 1). A main effect for time was found with glucose concentrations increasing over time, with post TT values being significantly higher than fasted, 15 min, and 30 min values and 60 min values being significantly higher than fasted and 15 min values ($p < .05$). No Supplement × Time interaction was found for blood glucose. A significant main effect of time for lactate was found, with fasted levels of blood lactate being significantly lower than all other time points ($p < .05$). A Supplement × Time interaction was also found for plasma lactate concentration, with DC resulting in lower lactate concentration following the TT compared with placebo (7.7 [1.9] mmol/L, 95% CI [6.6, 8.8] vs. 10.0 [4.6] mmol/L, 95% CI [7.4, 12.6]; effect size = –0.5; $p = .009$; Figure 2).

Expired Gases

No effect of supplement, time, or an interaction was found for oxygen uptake between the two conditions ($p > .05$). Likewise, no effect of supplement, time, or interaction was seen for RER or carbohydrate or fat oxidation ($p > .05$; Table 2).

Tissue Oxygenation

There were no effects of supplement or an interaction on oxygenated hemoglobin, deoxygenated hemoglobin, or total hemoglobin in the prefrontal cortex or the VL ($p > .05$). There was an effect of time on all measures at both the VL and prefrontal cortex, indicating an increase in oxygen extraction, as would be expected during prolonged exercise ($p < .05$; Table 3).

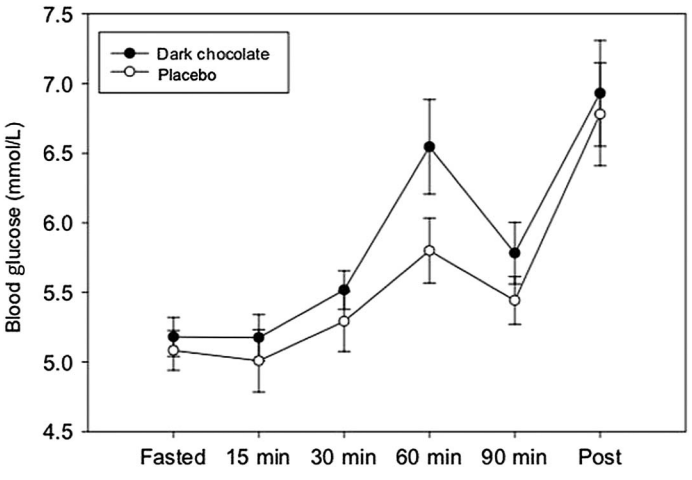

Figure 1 — Blood glucose values observed at different time points during submaximal cycling exercise and after a cycling TT. Dark chocolate resulted in significantly higher blood glucose levels throughout the duration of exercise compared with placebo ($p = .013$). Data are displayed as mean ±*SE* of the mean to avoid figure distortion. TT = time trial.

Discussion

The results of the current study suggest that, although DC provides some small metabolic benefit during exercise at altitude (i.e., higher blood glucose and lower lactate concentrations), it had no ergogenic effect on TT performance.

Our findings with DC supplementation were consistent with other studies assessing the effects of DC supplementation on longer, endurance-based TT of any modality at normoxia (running, cycling, and swimming; Peschek et al., 2014; Stellingwerff et al., 2014). Other research indicates that DC may produce increased performance in a shorter TT, such as a 2-min cycling TT at normoxia (Patel et al., 2015). These differences could be due to the different energy systems at work during longer (>6 min) efforts compared with shorter (<4 min) efforts, as shorter efforts place a greater reliance on anaerobic metabolism, in which lactate

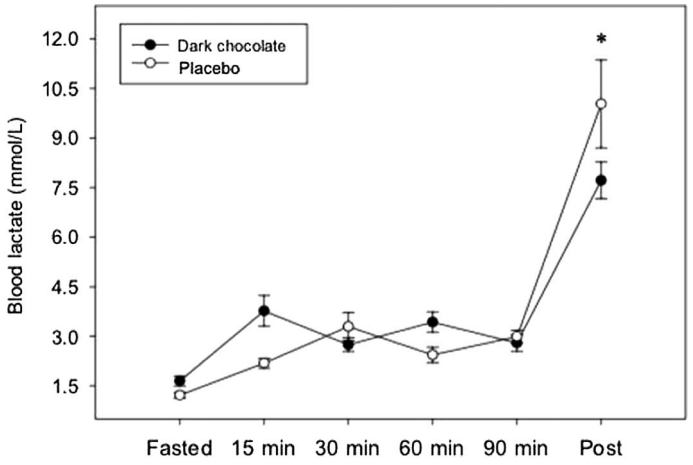

Figure 2 — Blood lactate values observed at different time points during submaximal cycling exercise and after a cycling TT. Data are displayed as mean ± *SE* of the mean to avoid figure distortion. TT = time trial. *Signifies significantly different from placebo ($p = .009$) in a Supplement × Time interaction.

Table 2 Gas Analysis Observed During Submaximal Cycling Exercise

Condition	VO_2 (L/min)	VCO_2 (L/min)	RER
DC	1.76 (0.07)	1.60 (0.05)	0.84 (0.02)
Placebo	1.85 (0.04)	1.62 (0.04)	0.88 (0.02)

Note. Values are expressed as mean (*SD*). DC = dark chocolate; VO_2 = oxygen consumed; VCO_2 = expired carbon dioxide; RER = respiratory exchange ratio.

Table 3 Oxygenated, Deoxygenated, and Total Hb in the VL and Prefrontal Cortex Averaged Throughout Exercise

	VL				Prefrontal cortex			
	Submaximal exercise		TT		Submaximal exercise		TT	
Parameter	DC	Placebo	DC	Placebo	DC	Placebo	DC	Placebo
Oxygenated Hb	–0.84 (3.56)	–5.67 (5.34)	–1.02 (3.41)	–5.50 (5.31)	3.99 (8.34)	10.65 (5.06)	10.84 (17.63)	24.43 (20.92)
Deoxygenated Hb	2.17 (1.64)	4.50 (5.52)	2.81 (2.32)	6.24 (5.23)	3.84 (5.02)	5.26 (3.51)	8.84 (5.59)	14.14 (15.10)
Total Hb	1.26 (4.07)	–1.20 (7.69)	0.98 (2.72)	0.74 (34.05)	8.21 (12.32)	16.38 (6.85)	19.68 (26.61)	38.56 (35.69)

Note. Values are expressed as relative to a baseline resting measure, arbitrarily recorded as 0.0 µmol. Positive values indicate greater than baseline, and negatives indicate less than baseline. Data are expressed as mean (*SD*). VL = vastus lateralis; DC = dark chocolate; Hb = hemoglobin; TT = time trial.

accumulation could be a limiting factor as opposed to longer efforts, which rely primarily on aerobic metabolism.

The current study found no differences in lactate accumulation during 90 min of steady state, submaximal exercise, which is in accordance with Patel et al. (2015), as well as Davison et al. (2012). Both studies found no differences in lactate concentration during 20 min or 2.5 hr of steady-state exercise, respectively. However, the current study did find significantly decreased lactate concentration following a 10-km TT race effort in the DC condition compared with the placebo. Although the mechanisms responsible for the lower lactate concentration remain unclear, others have suggested that polyphenols in DC might have an effect on anaerobic metabolism, leading to a reduced production of lactate (Ostertag et al., 2017). Such decreases in lactate following DC consumption suggest that DC supplementation may have beneficial effects on performance for activities that traditionally produce high amounts of lactate, where blood acidity may be a limiting factor. The current study also observed a main effect of glucose, with the DC condition eliciting higher glucose concentration compared with the placebo. This is in agreement with Stellingwerff et al. (2014), who observed that the consumption of DC augmented blood glucose concentrations during low-intensity exercise compared with a placebo. The increased plasma glucose may be associated with high concentrations of polyphenol and theobromine found in DC, which could potentially attenuate muscle glucose uptake (Stellingwerff et al., 2014). This is important when considering exercise in hypoxic environments, as carbohydrate has been shown to be the preferred fuel due to its high ATP yield per unit of oxygen (Hochachka et al., 1991); therefore, maintaining higher levels of blood glucose might relate to increased performance. The results of the current study, however, should be interpreted with caution, as there were significant differences in macronutrients between the DC and placebo supplement, with the DC containing higher amounts of both fat and protein, which may have affected concentrations of blood glucose compared with the placebo (Hollenbeck et al., 1986). In addition, although the difference of 0.2 mmol/L was a statistically significant result, the difference may be irrelevant in a practical setting during steady-state exercise. No differences were found for oxygen uptake during steady-state exercise or substrate utilization. This is in agreement with the findings by Patel et al. (2015), who observed no differences in VO_2 or RER after 14 days of DC supplementation, but did observe a difference in the gas exchange (ventilation) threshold. These results are in contrast to those found by Allgrove et al. (2011), who observed a lower RER during prolonged exercise following 2 weeks of DC supplementation and an acute dose of DC 2 hr prior to exercise, suggesting a greater proportion of energy coming from fatty acid oxidation. Differences in the observed results could be accounted for by differing amounts of caffeine, theobromine, and polyphenols in the supplements used, all of which may independently impact lipolysis (Castro-Barquero et al., 2018; Duncan et al., 2007). The supplements used in the current study reported lower levels of epicatechin, but higher levels of caffeine and theobromine compared with the Allgrove et al. (2011) trial. A detailed composition of the supplements used in the study by Patel et al. (2015) was not provided, so a comparison of the supplements used is not possible.

The DC does not affect the oxygenation status of the right prefrontal cortex or the VL muscle during exercise, as measured by NIRS. The results showed no effect of the supplement on oxygenated, deoxygenated, or total hemoglobin at either of the sites measured. There was an effect of time on all measures at both the VL and prefrontal cortex, indicating an increase in oxygen extraction, as would be expected during a prolonged exercise test. These results are in agreement with Decroix et al. (2016), who found acute supplementation with cocoa flavanols to increase brain oxygenation at rest, but not during cycling exercise. Masschelein et al. (2012) also found that a chronic (6 days) supplementation with nitrate (in the form of beetroot juice) did not affect the prefrontal cortex oxygenation status during maximal or submaximal cycling. However, the same group observed increased oxygenation in the VL muscle at both submaximal and maximal exercise at a simulated altitude (11% O_2), along with increased exercise performance, which is contrary to the results of the current study.

Limitations

The current study has a number of limitations. Although our study included females, we did not have enough female participants to do a well-powered subgroup analysis. We did not monitor the female participants' phase of menstrual cycle; however, measurements across the DC and placebo conditions were performed a month apart, which most likely minimized any differences due to the menstrual cycle phase. The researchers cannot confirm whether all chocolate was from the same batch, and Andres-Lacueva et al. (2008) reported that the flavanol concentration between commercial batches can vary. Future researchers can also build on the current research by measuring plasma NO, epicatechin, and NO bioavailability.

Conclusion and Future Recommendations

Although DC decreased lactate following a high-intensity TT and maintained higher blood glucose compared with a placebo during cycling in hypoxia, it did not result in enhanced performance or oxygenation in either the VL or prefrontal cortex. It is recommended that future researchers investigate the effects of DC using a

more closely matched placebo substance and also investigate the effects in short-term, power-based activities, such as track cycling, bobsleigh, or ice hockey. Future research could also investigate the effects of DC at hyperbaric hypoxia, as there is potential for altered physiological responses compared with normobaric hypoxia (Coppel et al., 2015).

Acknowledgments

The authors wish to thank all of the voluntary participants for their dedication and time in this study. The study was designed by K. Shaw, P.D. Chilibeck, and J.P. Neary; the data were collected by K. Shaw, L. Sirant, and J. Singh; the data were analyzed by K. Shaw and P.D. Chilibeck; the data interpretation and manuscript preparation were undertaken by K. Shaw and P.D. Chilibeck. All authors approved the final version of the paper. The authors declare no conflict of interest.

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