The Effect of Dark Chocolate on Metabolism and Performance in Trained Cyclists at Simulated Altitude

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Abstract

Dark chocolate (DC) is high in flavonoids, a bioactive micronutrient that increases the synthesis of nitric oxide (NO) and reduces the rate at which NO is removed from the blood. Nitric oxide is a potent vasodilator and its increase has potential to improve blood flow, delivery of oxygen to muscle, and endurance exercise performance, especially in conditions such as altitude, where hypoxia compromises delivery of oxygen to muscle. The purpose of this research was to investigate the effects of DC on cycling performance, metabolism, and blood oxygenation status in trained cyclists at altitude. We hypothesized that DC would result in enhanced muscular oxygenation, more efficient exercise metabolism, and improved performance. Twelve trained cyclists (n=2 females, average VO_2 peak=54.6±6.2 ml/kg/min) were randomized to supplement with 60g of DC or an isocaloric placebo twice per day for 14 days in a cross-over study. After the 2-weeks of supplementation, participants attended a lab session in which they cycled 90 minutes at 60% VO_{2max} followed immediately by a 10km time trial (TT) at a simulated altitude of 2500m (15% O_2). Plasma levels of blood glucose and lactate were measured before, throughout, and after exercise while muscular and pre-frontal cortex oxygenation were measured continuously throughout exercise by near infrared spectroscopy. DC resulted in improved maintenance of blood glucose throughout the experimental trial (5.9±0.5 vs. 5.6±0.5 mmol/L; p=0.03) and decreased blood lactate following the time trial (7.7 mmol/L vs. 10.0 mmol/L, *p*=.027). DC had no effect on time trial performance (1142±129 vs. 1152±118 s for placebo) or oxygenation status in either the brain or muscle. There was an increase in total hemoglobin and deoxygenated hemoglobin in the pre-frontal cortex, and an increase in total hemoglobin in the vastus lateralis over time. Consumption of DC for two weeks prior to a bout of cycling at

simulated altitude allows for maintenance of blood glucose during exercise and decreased lactate production following intense and prolonged TT but does not improve TT performance. Dark chocolate had no effect on oxygenation status of the pre-frontal cortex or working muscles.

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Chapter 1: Introduction and Background

1.1 Introduction

In elite sporting competitions, 0.5%-1.5% changes in performance are considered critical (Dominguez et al. 2017). Performance is especially challenging when athletes are competing at altitude (e.g. Tour de France mountain stages). Ascent to higher altitude leads to decreases in both maximal and submaximal exercise performance due to a decreased partial pressure of oxygen (PO_2) (Beidleman et al., 2008). 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 during exercise (Stenburg et al., 1966).

To maximize their potential, athletes often turn to nutritional supplementation as a way to further enhance their performance. 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; Dominguez et al. 2017). When ingested, exogenous nitrate is converted to nitrite by facultative anaerobic bacteria in the oral cavity. The nitrite is then acted upon by reducing compounds such as polyphenols, hemoglobin, myoglobin, and protons to produce nitric oxide (NO) (Lundberg et al. 2008) (see figure 1.1). NO can also be synthesized in the body through Nitric Oxide Synthase (NOS), an enzyme which catalyzes the production of NO from L-arginine (Delker et al. 2010). NO has various beneficial physiological effects such as improving oxygen uptake, increasing blood flow through vasodilation, improving muscle contractility, regulating myocyte differentiation, assisting in calcium and glucose homeostasis, and mitochondrial biogenesis (Cermak et al. 2012; Dominguez et al. 2017).

Nitrate supplementation may enhance performance through the actions of plasma NO. Dominguez et al. (2017) reviewed the effects of beetroot juice, the most popular form of nitrate

supplementation, on endurance exercise performance and concluded that acute supplementation with beetroot juice may lead to a decreased oxygen consumption (VO_2) at intensities up to maximal oxygen consumption (VO_2max) and may increase aerobic capacity while decreasing energy expenditure at the anaerobic threshold, suggesting an increase in metabolic efficiency. Supplementation with nitrate in the form of beetroot juice is effective in enhancing performance and mitigating some of the detrimental effects of altitude by decreasing oxygen consumption, improving time trial (TT) performance and increasing muscular oxygenation (Masschelein et al. 2012; Muggeridge et al. 2014).

Evidence is emerging that suggests that dark chocolate (DC) may provide similar benefits. DC has been traditionally been seen as a food of high palatability and indulgence, which, when paired with potential performance-enhancing effects, makes it a food of great interest to endurance athletes. Various compounds making up DC increase the bioavailability of NO for the body and decrease the reuptake of NO while decreasing the amount of damage sustained by the muscle and increasing the rate at which a muscle recovers (Bayat et al. 2014; Faridi et al. 2008; Patel et al. 2015; Schewe et al. 2008; Sudarma et al. 2011). Thus, DC not only increases the amount of NO available for use by tissues but also decreases the rate at which NO is removed from the bloodstream. An increase in serum NO may increase blood flow, helping to partially offset the detrimental effects of altitude.

With these prospective benefits to endurance performance, DC has the potential to become a very useful and enjoyable sport supplement for endurance athletes. With 85% of athletes reporting the use of supplements (Maughan et al., 2007), DC has the potential to aid thousands of elite and amateur athletes alike. Elite cycling races such as the Tour de France and the Giro d'Italia take athletes through various altitudes, ranging from nearly sea level to 4800 m, an

attitude corresponding to ~11.5% O_2, almost half the amount of O_2 found at sea level. The purpose of this study is to investigate the effects of 2 weeks of dark chocolate supplementation on performance and metabolic parameters of exercise at simulated altitude as well as oxygen uptake (VO_2) and cerebral and muscular oxygenation.

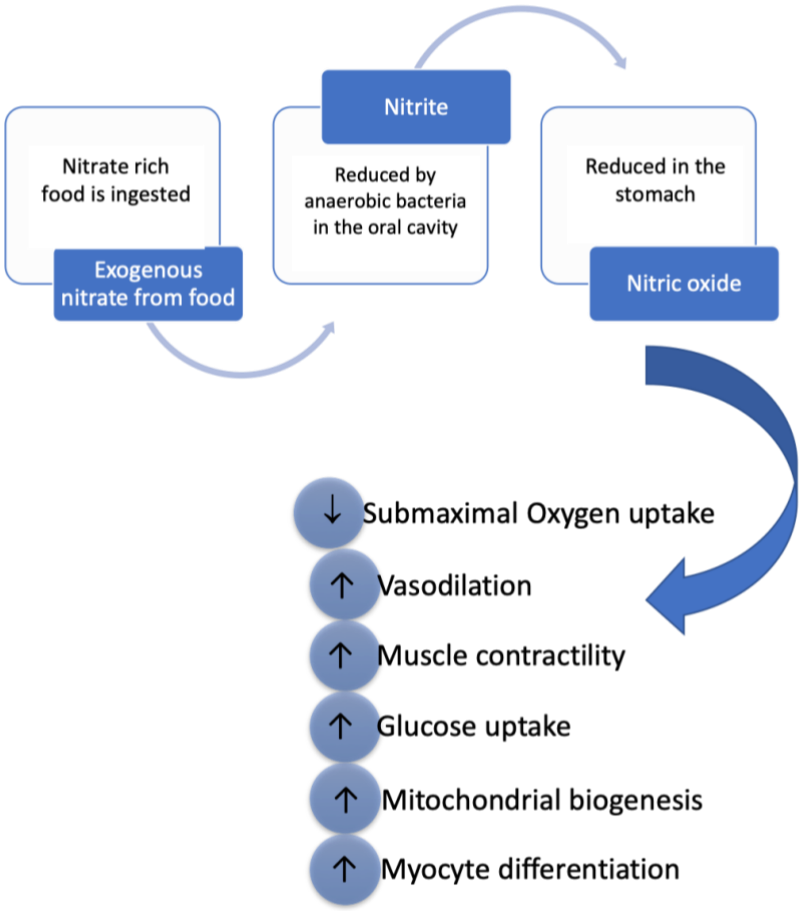

Figure 1.1. The pathway of nitric oxide (NO) production from the ingestion of exogenous nitrate. Nitrate is reduced to nitrite in the oral cavity by anaerobic bacteria and further reduced to NO in the stomach through various pathways including hemoglobin, myoglobin, polyphenols, and protons. NO leads to various performance enhancing effects such as improving glucose uptake, myocyte differentiation, mitochondrial biogenesis, muscle contractility, vasodilation, and oxygen uptake.

1.2 Review of the Literature

1.2.1 Role of Nitric Oxide During Exercise

Nitric Oxide is the by-product of the breakdown of other nitrogenous compounds, nitrate, and nitrite. When exogenous nitrate (NO_3^-) is ingested, it is reduced to nitrite (NO_2^-) by facultative anaerobic bacteria in the oral cavity. As the nitrite passes through the digestive system, it is further reduced to nitric oxide (NO) by reducing compounds such as polyphenols, hemoglobin, and myoglobin (Lundberg et al. 2008). NO is synthesized intracellularly through the action of a family of nitric oxide synthase (NOS) enzymes. These enzymes catalyze the NADPH and O_2-dependent oxidation of L- arginine to L-citrulline and NO. NO is a signaling molecule for the cardiovascular system and is considered to be a critical modulator of cell and tissue function (Hill et al., 2010) and acts by activating guanylyl cyclase in vascular smooth muscle. This results in an increase in cyclic guanosine monophosphate which in turn activates myosin light-chain phosphatase, inducing relaxation (Pappano & Wier, 2012). NO is crucial for its control of blood pressure, blood flow, and other vital bodily functions including regulation of intermediate metabolism and cellular energy production (Levett et al., 2011). It is classified as a free radical scavenger, combating the oxidative stress that the body undergoes due to oxidative metabolism.

Nitric oxide has various ergogenic effects on human performance. One pertinent benefit is its role as a vasodilator. When the endothelium undergoes shear stress due to increased blood flow caused by physical exertion, endothelial nitric oxide (eNO) is released from the vascular endothelium (Hill et al., 2010). This nitric oxide acts on the smooth muscle of the local tissues to cause relaxation by hyperpolarizing the plasma membrane of the smooth muscle through activation of large-conductance KCa channels (Mughal et al., 2018). Other notable effects of NO

include improved muscle contractility, regulation of myocyte differentiation, assistance in calcium and glucose homeostasis, and enhancing mitochondrial biogenesis (Cermak et al. 2012; Dominguez et al. 2017). When taken before bouts of exercise, consumption of nitrate-rich beetroot juice decreases oxygen consumption (VO_2) at intensities up to VO_2max, which may increase aerobic capacity while decreasing energy expenditure at the anaerobic threshold (Dominguez et al. 2017). This increase in efficiency increases time to exhaustion at submaximal intensities and may improve performance at the aerobic threshold (Dominguez et al. (2017) while directly improving time trial performance in cyclists (Lansley et al., 2011).

1.2.2 Effects of High Altitude on Human Performance

The effects of altitude on human performance are well documented (Beidleman et al., 2007; Drust & Waterhouse., 2010). As one reaches higher levels of altitude, barometric pressure decreases, decreasing the partial pressure of O_2 (PO_2) (McArdle et al., 2015). This decreased PO_2 leads to a decreased PO_2 in the arteries (aPO_2), bringing it down from 100 mmHg at sea level to ~60 mmHg at 2400 m elevation. The decreased aPO_2 causes a decreased pressure gradient to occur between the arteries and skeletal muscle, decreasing the efficiency of oxygen diffusion to the muscles, decreasing the amount of O_2 available for aerobic metabolism and forcing the body to undertake anaerobic metabolism, thus increasing the acidity in the blood, leading to increased measures of fatigue (Linnarsson et al., 1974). The decreased PO_2 gradient also leads to increases in minute ventilation, causing hyperventilation and hypocapnia, leading to a decrease in cerebral blood flow and increased central fatigue (Huang et al., 1984; Siebenmann et al., 2013). Even for those not attempting to pursue athletic endeavors, exposure to altitude can lead to various symptoms such as decreased motor skills, mood, and memory, a decrease in hearing and sight, lack of appetite, nausea, fatigue, headaches, insomnia, and, in extreme cases, high altitude

pulmonary or cerebral edema (McArdle et al., 2015). The body's decreased ability to bring in and transport O_2 also impacts the fuel of choice, both at rest and during exercise at any intensity. At altitude, carbohydrate metabolism has historically been seen as the metabolic pathway of choice due to its high level of ATP production per unit of O_2 consumed (McClelland et al., 1998). However, in endurance sporting events such as road cycling decreased levels of glycogen are known to cause fatigue and thus a decrease in performance (Snyder, 1998). Also, when metabolizing higher proportions of carbohydrates lactic acid is produced at a greater rate, which can be detrimental to performance (Cairns, 2006). Thus, the ability to utilize fatty acids and spare muscle glycogen during exercise is seen as a preferential trait. O_2 transport to bodily tissues is compromised during physical exertion involving large amounts of muscle mass (Billaut & Smith., 2010), potentially compromising cerebral oxygenation status, especially at the end of exhaustive exercise (Subudhi et al., 2007). This decrease in cerebral oxygenation status results in a decreased mitochondrial O_2 to levels below what is needed to maintain motor function (Nybo et al., 2007). Exercise at altitude is also known to lower VO_2max scores of athletes due to compromised transfer of O_2 to the working muscles (Beidleman et al., 2008). The VO_2 required for a given workload remains the same as at lower altitudes, but because the workload is now a greater percentage of maximal, it feels more difficult than if completed at lower altitudes.

It has been suggested that NO is an important signaling molecule for adaptation to altitude/hypoxia, as those who live in communities at altitude exhale higher concentrations of NO (Levett et al., 2011) and others have demonstrated that lower levels of expired NO coincide with increased incidence of altitude sickness (Duplain et al., 2000). Dietary nitrate supplementation also reduces muscle metabolic perturbation during exercise in hypoxia (Masschelein et al., 2012) and improves the diffusion of O_2 to tissues further away from

capillaries, creating a more precise matching of O_2 delivery to metabolic demand (Vanhatalo et al., 2011).

Tissue oxygenation can be measured non-invasively using near-infrared spectroscopy (NIRS) (Artinis®, Einsteinweg, The Netherlands). The device uses 6 light-emitting diodes (3 pairs of LEDs) and one receiver. External light must be blocked so as to not contaminate the reading with infrared light from external sources (Bishop et al., 2018). A quality control factor (QCF) is used to indirectly monitor external light to ensure a valid measurement. NIRS works on the principle that the absorbance of light by oxygenated and deoxygenated hemoglobin and myoglobin differs at different near-infrared lengths (MacDonald et al., 1999). This method of measuring oxygenation status has been validated for use in humans by Mancini and colleagues (1994), who also demonstrated that the NIRS signal was not influenced by skin blood flow or by the change in body and skin temperature with exercise

1.2.3 Energy Metabolism During Exercise

To create useable energy from the food we ingest, the body must degrade macronutrients to the useable form of adenosine triphosphate (ATP). During exercise, ATP is primarily created through glucose metabolism using glycolysis or in the electron transport chain after metabolism in the Krebs Cycle or through fatty acid metabolism using Beta-oxidation, the Krebs Cycle, and electron transport chain. Production of ATP from a glucose molecule through glycolysis results not only in ATP but also in pyruvate, which either is taken up by the mitochondria (where it enters the Krebs Cycle before the electron transport chain) to create more ATP or is converted to lactate. Accumulation of lactate and its associated hydrogen ion leads to a decrease in pH, causing muscular pain and nausea as well as inhibition of muscular contraction, fatty acid oxidation, and the binding of O_2 to hemoglobin, thus inhibiting the transfer of O_2 to working

muscle (McKenna et al., 1997). Thus, an accumulation of lactate is seen to be a contributor to fatigue of endurance performance. Fatigue during endurance performance is also due to a decrease in stored glycogen and blood glucose. During exercise, active muscle breaks down stored glycogen from within the muscle itself as well as draws glucose from the blood. When blood glucose is diminished, stored glycogen in the liver is broken down to maintain stable blood glucose levels, ensuring the brain has a constant supply. Thus, to spare glycogen stores and decrease the production of lactate, the ability to metabolize fatty acids at higher intensities of exercise is preferential.

Although a useful insight into bodily processes, measuring substrate usage during exercise has its complications. To estimate the percentage of carbohydrate and fatty acid usage, we use the O_2 consumed and CO_2 expired during exercise and apply them to equations (described by Jeukendrup and Wallis, 2005) to determine the primary macronutrient being metabolized. Carbohydrate and fat oxidation (in g/min) can be calculated using the following respective equations:

$$CHO_{ox} = 4.210 * VCO_2 - 2.962 * VO_2$$

$$FAT_{ox} = 1.695 * VO_2 - 1.701 * VCO_2$$

Knowledge of the primary substrate being utilized lends itself to inferring metabolic efficiency, as a more efficient metabolic system will give preference to fatty acid metabolism. By decreasing the body's reliance on carbohydrate, muscle glycogen will be spared, allowing more glycogen to be accessible when intensity increases, such as in a final sprint at the end of a race.

1.2.4 Dark Chocolate on Human Physiology

Dark chocolate (DC) has been emerging as the subject of many endurance athletes' interest, as it has been suggested to increase endurance performance while providing a portion of the energy

endurance athletes need for optimal performance. DC is a food of plant origin (Theobroma cacao) and thus a rich source of flavonoids (Engler et al., 2004). Flavonoids have different subclasses, the most prominent being epicatechin (Nogueira et al., 2011). Epicatechin activates endothelial NOS and enhances endothelium-dependent relaxation (Karim et al., 2000) while diminishing the removal of NO in the bloodstream through the inhibition of NADPH Oxidase, which decreases the production of O_2-, a known scavenger of NO (Schewe et al., 2008). DC is also a rich source of the flavonoid catechin, which increases the rate of lipolysis and has favorable effects on body composition (Maki et al., 2008), as well as procyanidins (polymers of catechins and epicatechins). Procyanidins modulate various cytokines in the body into reduce inflammatory effects (Ding et al., 2006).

In addition to increasing levels of serum NO in the body, DC is an energy-dense food and traditionally seen as being highly palatable, which could provide endurance athletes with a portion of the energy they require for performance. Road cycling is a highly aerobic sport with athletes traveling hundreds of kilometers at a time at high intensities. Tour de France riders have been found to expend a mean of 6,071 kcal per day (Saris et al. 1989), and their ability to properly fuel before, during, and after exercise is a large predictor of performance, as the ability of a muscle to produce ATP and complete long- term heavy exercise is dependent on muscle glycogen which is constantly being degraded during exercise (Bergström et al. 1967; Ørtenblad et al. 2013). DC contains approximately 560 kcal per 100 g of chocolate, lending it to be an easy method to maintain and restore glycogen levels during and after exercise.

DC is also a source of caffeine and theobromine, both of which have individual performance enhancing effects (Dodd et al., 1993). DC contains approximately 73 mg of caffeine and 883 mg of theobromine per 100g serving (Harland. 2000; Meng et al. 2009). Caffeine is one of the

world's most widely used drugs amongst athletes and non-athletes a like (Dodd et al., 1993). It has both physical and mental performance enhancing effects. Caffeine acts at the cellular level by increasing the affinity of the myofilaments for calcium and increasing calcium release from the sarcoplasmic reticulum while inhibiting adenosine receptors (Graham, 2001), increasing lipolysis. Caffeine acts on the adenosine receptors of the brain as well as peripheral receptors. By blocking adenosine receptors of the brain, caffeine inhibits the behavioral depression that activation of adenosine receptors cause, thus improving overall mood (Dodd et al., 1993). Theobromine, found primarily in chocolate (Mitchell et al., 2011), also blocks adenosine receptors, increasing muscular contractility and blocking adenosine receptors in both the brain and the periphery. Unlike caffeine, theobromine also acts to lower blood pressure and acts as a smooth muscle relaxant, further increasing the vasodilatory effects of NO.

1.2.5 Caffeine and Theobromine on Exercise Performance

Caffeine is well known for its many ergogenic effects on endurance exercise (Tarnopolsky, 1994). Some of these effects include, but are not limited to, increased time to fatigue in both running and cycling (Costill et al., 1978; Graham & Spriet, 1991), an increase in power output during 2-hour cycling (Costill et al., 1978), faster performance times in Nordic skiers at low and high altitudes(Berglund & Hemmingsson, 1982), increased force production (Westerblad & Allen, 1991), augmentation of plasma norepinephrine and plasma epinephrine to large-muscle, continuous exercise (Anderson & Hickey, 1994), and increased free fatty acid metabolism during exercise (Powers & Dodd, 1985). Caffeine has a half-life of 4-6 hours (Graham, (2001), and Quinlan et al. (1997) reported that peak plasma levels of caffeine are observed between 30-75 minutes after ingestion when taken orally. However, it has been suspected that caffeine consumption 3 hours prior to long distance exercise is optimal, as this is when free fatty acid

levels are the highest due to the resultant lipolysis induced by caffeine ingestion (Nehlig & Debry, 1994). A review by Sökmen and colleagues (2008) suggests taking caffeine, at the latest, 3 hours prior to power or short endurance events and 1 hour prior to longer endurance events. Graham (2001) has reported doses ranging from 3-9 mg/kg body weight to be effective for increasing endurance performance. Kovacs et al. (1998) have found that doses as low as 2.1 g/kg were effective, but doses ranging from 3.2-4.5 mg/kg had a greater effect. Graham (2001) reports that doses of 3-6 mg/kg result in the greatest improvements in performance. Given that caffeine is both water and fat soluble, normalizing intake relative to fat-free mass is not necessary (McCall et al., 1982).

Theobromine is part of the drug class methylxanthines, along with caffeine. Stellingwerff et al. (2013) observed an increase in plasma glucose levels during exercise that correlated with increased serum levels of theobromine, but not caffeine and theobromine is a phosphodiesterase inhibitor and adenosine receptor antagonist (Daly et al. 1987). Neufingerl and colleagues (2013) speculated that theobromine may be the component in DC that increases HDL cholesterol. Because theobromine is often found in conjunction with caffeine, timing and dosing recommendations have not been reported for theobromine independently.

1.2.6 Effect of Dark Chocolate on Exercise Performance

Dark chocolate as an ergogenic aid for athletic performance remains a relatively new area in the world of sport nutrition and exercise physiology. To date, there are a number of studies done on DC in a clinical population for improving clinical measures such as cardiovascular health, endothelial function, lipid profiles, among others (Araujo et al., 2016). However, the research in an athletic population remains scarce. There are conflicting views on the benefits of using DC to improve endurance performance.

When looking at performance measures in endurance-based sport, time trials (TTs), time to exhaustion (TTE), and power output are often the outcome variables measured. Patel and colleagues (2015) observed improvements in a 2-minute cycling TT performance, while Stellingwerff et al. (2013) and Peschek et al. (2014) found no change in cycling or running TTs, respectively. This discrepancy could be due to the lengths of the TT the athletes underwent. The Patel group had athletes complete a 2-minute time trial while the other studies mentioned assessed performance over a 5km running TT (lasting ~20 minutes) and a cycling TT lasting approximately 15 minutes. The difference in the duration of the effort is likely a large contributor to the different findings of the studies. It has been demonstrated that an effort of ~2 minutes is fueled by approximately equal parts anaerobic and aerobic energy systems (Astrand & Rodahl, 2003; Spencer & Gastin, 2001; Hill, 1999), while efforts of 15-20 minutes are fueled primarily by the aerobic system (Ward-Smith, 1985; Peronnet et al., 1989; Di Prampero et al., 1993). Thus, the differences in energy system contributions could be a large factor in the differences in finding between these studies.

Other measures of performance such as TTE, power output, and VO_2max show similarly contradictory results. Sanguigni et al. (2017) assessed double product (a measure of myocardial oxygen uptake) and saw improvements in physical performance following ingestion of 100g of homemade cocoa antioxidant ice cream acutely, while Patel et al. (2015) and Fraga et al., (2005) saw no difference in VO_2max scores following 2 week or acute ingestion of DC, respectively. However, Taub et al., (2016) observed an increase in VO_2max scores following consumption of 20g of DC daily for 3 months. These results suggest that consumption for greater than 2 weeks is necessary to elicit an increase in VO_2max. Taub et al. (2016) also found an increase in power output following chronic consumption, which agrees with a systematic review by Somerville et

al. (2017), who converted all measures to power output in order to make a direct comparison and found an increase in power output following chronic supplementation (for >7 days). Patel et al. (2015) also analyzed gas exchange threshold (GET), an indicator of anaerobic threshold (Magalang & Grant, 1995), and found that acute consumption of DC led to an increased power output at the GET in moderately trained males (VO_2max: 41.89 ± 5.4 ml/ kg/min). Due to the inconsistency in the literature regarding the effects of DC on exercise performance in endurance sports, it is clear that further research is needed to strengthen the evidence for or against DC as an ergogenic aid in endurance sport. See table 1.1 below for a summary of the current literature on the topic.

1.3 Conclusion

Altitude can be detrimental to endurance exercise performance due to a decreased PO_2 resulting in a decreased transfer of O_2 to the brain and muscles, causing fatigue. Athletes often seek out nutritional supplementation to decrease detrimental effects and allow them to perform at their greatest potential while staying within anti-doping regulations. Dark chocolate may be a beneficial supplement to assist exercise at altitude by increasing the bioavailability of NO, leading to increased vasodilation, decreased oxygen consumption at submaximal intensities, and increased metabolic efficiency. Current research in the area remains scarce and inconsistent. Although some research has been done with chocolate at low altitudes, no research has been done at high altitudes to date and findings are conflicting, demonstrating the need for more research in the area to fill gaps in the literature.

Table 1.1. A review of the current literature looking at the effects of dark chocolate on exercise performance

Reference	Study Design	Participants	Exercise Protocol	Chocolate intervention	Performance Measure	Outcome
Patel et al., 2015	single blinded, randomized, cross-over design	9 moderately trained males	-Incremental exercise test - 20 min cycling @ 80% GET - 2 min maximal sprint	40g of dark or white chocolate daily for 2 weeks	-2 min TT -Gas exchange threshold (GET) -VO_2	- ↑ TT - ↑ GET - ↔ VO_2
Stellingwerff et al., 2013	single blinded, randomized, cross-over design	16 healthy male cyclists	-2.5 hours cycling at ~45% VO_2peak - ~15 min TT	561kcal DC or 544 kcal control chocolate 2 hours prior to exercise	-~15 min TT	- ↔ TT
Sanguigni et al., 2016	single blinded, randomized, cross-over design	14 healthy individuals, n=7 females	- stress test on a cycle ergometer with a scalar profile and increasing loads of 25 watts/2 min	Acute ingestion of 100g of either natural antioxidant ice cream or milk chocolate ice cream (control)	- Incremental stress test	- ↑ myocardial O_2 consumption
Allgrove et al., 2011	single blinded, randomized, cross-over design	20 healthy men who engaged in regular physical activity for a minimum of 2 hr, three times per week	- steady-state cycling at 60% VO2max for 1.5 hr; every 10 min the exercise intensity was increased to 90% VO2max for 30 s - time to exhaustion	-acute ingestion of 40 g DC or 30.4 g isocaloric CON	- Time to exhaustion	-↔ TTE

Table 1.1 continued

Peschek et al., 2014	single blinded, randomized, cross-over design	8 well-trained runners and triathletes	-steady state downhill running @ 70% VO_2max -5k TT 48h later	- carbohydrate protein beverage plus natural cocoa or control within 1 h and at 2 h post downhill running - 1.0 g/kg/h beverage	- 5k running TT	-↔ TT
Fraga et al., 2005	single blinded, randomized, cross-over design	28 healthy male soccer players	- multistage 20 min shuttle run test	- 105g flavanol-containing milk chocolate or white chocolate	-shuttle run test (VO_2)	-↔ VO_2
Taub et al., 2016	double blind, randomized, placebo controlled trial	20 sedentary, overweight	-VO_2max test	-20g (~100kcal) DC or placebo/day for 3 months	-VO_2max -power output	-↑ VO_2max -↑ power output
Somerville et al., 2017	Systematic Review and Meta analysis	Healthy participants between the ages of 18 and 70		Polyphenol supplementation for ≥7 days	-Power output	-↑ performance

Abbreviations: CON = control; DC = dark chocolate; GET = gas exchange threshold; TT = time trial; TTE = time to exhaustion; VO_2 = oxygen consumption

1.4 Purpose Statement and Hypothesis

1.4.1 Purpose Statement

Dark chocolate is rich in polyphenols and flavonoids which may increase the amount of nitrite that is converted to NO, enhancing vasodilation and exercise performance at altitude. Therefore, the primary purpose of this research was to determine the effects of 60g of dark chocolate twice daily for 14 days on time trial performance in trained cyclists at a simulated altitude of 2500 m (15% O_2). The secondary purpose was to investigate the effects of two weeks of dark chocolate supplementation on exercise metabolism, as assessed by measures of plasma glucose and lactate, oxygen consumption, and substrate (i.e. fat and carbohydrate) oxidation.

1.4.2 Hypotheses

The primary hypothesis was that supplementation with 60g of dark chocolate twice daily for two weeks prior to a 10 km time trial at a simulated altitude of 2500m would lead to improved cycling TT performance.

The secondary hypotheses of this study are fourfold:

Two weeks of dark chocolate supplementation before a bout of exercise at simulated altitude would:

1) Enhance the metabolic efficiency of the body, as indicated by decreased plasma lactate concentrations and improved plasma glucose homeostasis,

2) Decrease oxygen consumption,

3) Increase reliance on fatty acids for ATP generation during constant-load, submaximal exercise performed at simulated altitude, and

4) Increase oxygenation status of the cerebral lobe of the brain and vastus lateralis.

Chapter 2: Methods

2.1 Study Design

The study design was a double-blind counter-balanced cross-over study where dark chocolate was compared to a placebo condition. Twelve athletes were recruited and received both conditions. Participants and researchers involved in data collection and outcome assessment were blinded to whether dark chocolate or placebo were given and to the order in which participants received the conditions. This was accomplished by having separate researchers generate the randomization schedule (using a computerized random number generator) and prepare the dark chocolate or placebo. The allocation schedule was concealed from other researchers by the researcher who generated the randomized allocation schedule. When a participant entered the study, the condition assignment for that participant was revealed by the researcher who generated the allocation schedule to the researcher in charge of preparing the dark chocolate and placebo. The trial was registered at www.clinicaltrials.gov, NCT number NCT03945916.

2.2 Participants

Trained cyclists, triathletes, and duathletes were recruited to take part in this study. The target sample size was 12 participants. 16 participants were recruited and provided consent, with 4 participants failing to reach the required aerobic fitness scores in order to be classified as "trained" (see figure 2.1). Participants were considered to be "trained" if their maximal oxygen consumption (VO_{2max}) was above average, ≥45 ml/kg/min for females and ≥50 ml/kg/min for males and if they cycled for 60 minutes or more at least 3 times per week. Recruits who were habitual consumers of dark chocolate (>40g/day, 5x/week) and those who supplemented with beetroot juice or other exogenous forms of nitrate were excluded. Participant characteristics are shown in Table 3.1 of the results.

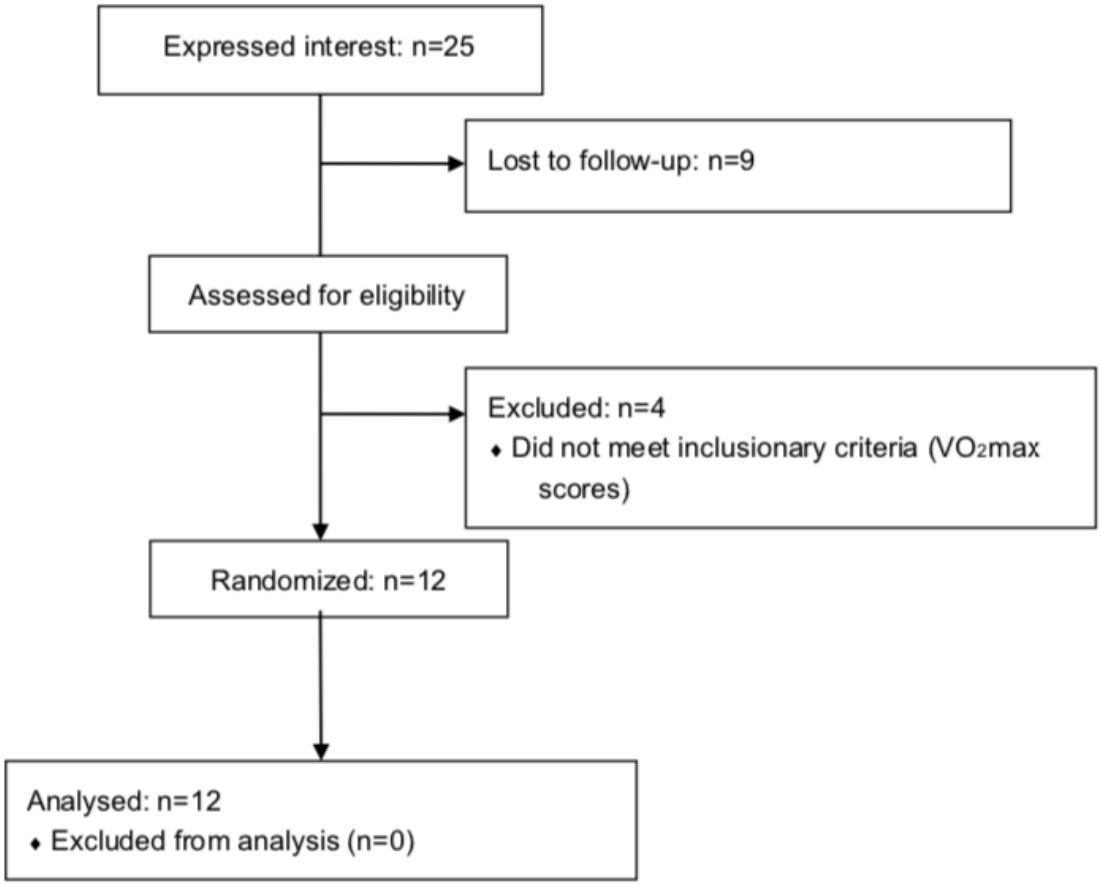

Figure 2.1. Diagram of participant recruitment

2.3 General Methodology

This study was approved by the University of Saskatchewan Biomedical Research Ethics Board (BIO ID 189). Participants made a total of 5 visits to the exercise physiology lab at the University of Regina School of Kinesiology and Health Sciences. During the first lab visit, informed consent was obtained and participants filled out the Get Active Questionnaire (Appendix A) (Canadian Society for Exercise Physiology, 2017) to ensure they could safely participate in physical activity. Participant demographic information (height, weight, age) was then collected. Briefly, the protocols for height and weight were as follows. Height was measured using a stadiometer (Tanita®). Participants stood in sock feet with their heels together

and against the wall. The flat measuring ledge of the stadiometer was then lowered to the participants head, depressing the hair to lie flat on the head. Participants were instructed to take a deep breath and height was measured to the nearest 0.1cm. Weight was measured using a digital scale (Health O Meter®). Participants stood in sock feet and minimal clothing. Weight was measured to the nearest 0.1kg. Following the collection of consent and demographic information, participants underwent an incremental exercise test on a stationary bike (either their own affixed to a trainer or a Velotron®**)** in order to determine their maximal oxygen uptake in normoxia (VO_{2max}) and eligibility for the study. Participants were instructed to maintain their normal training regime over the course of the study.

Participants returned a minimum of one week later for a second incremental exercise test to determine VO_{2max} at a simulated altitude of 2500 m (15% O_2), achieved using a normobaric altitude chamber. A power output corresponding to 50% maximal power output achieved during the incremental test completed at simulated altitude (approximating about 65% VO_{2max}) was deemed the workload for testing sessions. This power output was chosen to replicate the intensity that amateur athletes might cycle at during a road race and to ensure excessive fatigue did not impact the participant's ability to complete the TT (Lucia et al., 2000; Vogt et al., 2006). On the third visit, participants were familiarized with the exercise protocol used in the testing sessions (i.e. 90 minutes at 50% power output achieved in visit 2 followed by a 10 km time trial) at hypoxia (15% O_2). After visit 3, participants were randomly assigned to consume either DC or a similarly flavored control substance (CON) Participants supplemented with 60 g twice per day (morning and evening) for 14 days of either DC or CON. On the 15^{th} day, participants arrived to the lab in the morning after an overnight (10 hour) fast. Fasted measures of plasma lactate and glucose were measured via finger prick blood samples. Following fasted measure,

120 g of either DC or CON chocolate were consumed. This dose of chocolate was used to maximize polyphenol levels in the body prior to testing session. A dose of 120g of dark chocolate per day is greater than used by Patel et al. (2015), Sudarma et al. (2011), and Faridi et al. (2008), all of whom found favorable results on performance, serum nitric oxide, and endothelium function, respectively. The participants cycled at a simulated altitude of 2500m (15% O_2), achieved using a normobaric hypoxic chamber, at an intensity of 50% of workload achieved in visit 2 for 90 minutes. Blood lactate and glucose were collected via finger prick at 15 and 30 minutes and every 30 minutes thereafter. Expired air was collected for 1 minute every 15 minutes for analysis of VO_2, VCO_2, and respiratory exchange ratio (RER). Immediately following completion of the 90 minutes at steady state, participants began a 10 km time trial (TT), which they were instructed to complete as quickly as possible. Oxygen levels of the chamber were monitored using a built-in O_2 monitor and levels were maintained between 14.7% and 15.1% O_2. Oxygenation status was measured via near-infrared spectroscopy (Artinis®) during exercise at the vastus lateralis (Portamon®) and at the cerebral lobe (Portalite®). These sites were chosen to be measured in order to be able to compare to the current body of literature, as the vastus lateralis is the most common site of NIRS measurement. Additionally, the muscle belly of the vastus lateralis is easily visible on a trained individual, allowing for greater precision of probe placement. Immediately following the TT, blood glucose and lactate were measured again while the participant engaged in a self-selected active recovery. Following a two-week washout period, participants began 14 days of supplementation under the opposite treatment (DC or CON) and the same exercise performance testing for a double-blind, counterbalanced, crossover design. See figure 2.2 for a pictorial representation of the study design.

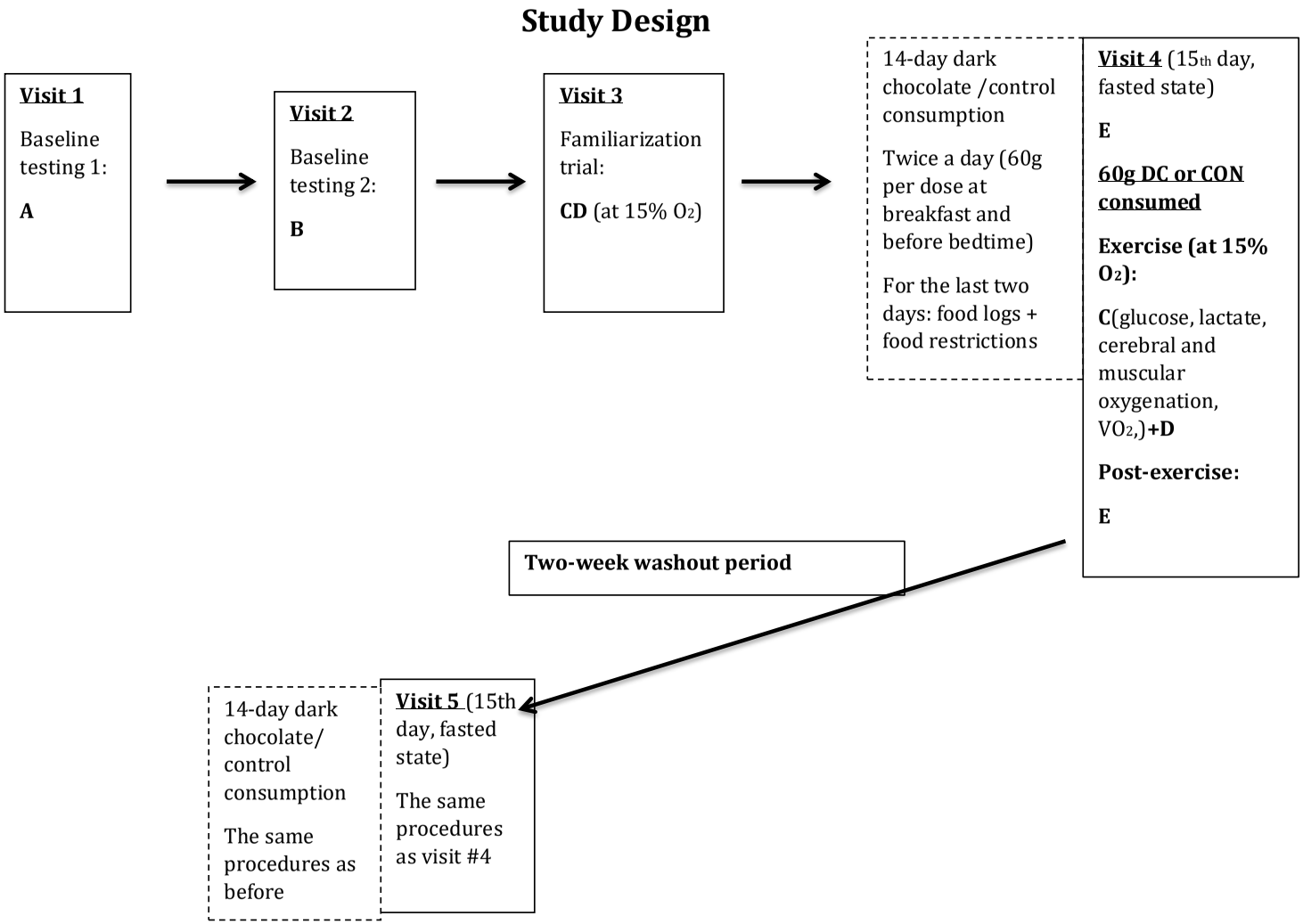

Figure 2.2: Study design. A: VO_2max test at normoxia; B: VO_2max test at hypoxia (11% O2); C: 90 min steady state cycling at hypoxia; D: time trial at hypoxia; E: fingerprick blood sample for plasma glucose and lactate.

2.4 Supplement

Participants consumed 60g of either 72% dark chocolate (Belcolade Premium Belgium Chocolate, Erembodegem, Belgium) or a similarly flavored control (Merckens, Cumberland USA) twice per day for 14 days prior to all experimental testing. The control chocolate contained no cocoa butter or cocoa liquor but used alkalized cocoa for coloring. This alkalization process reduces the antioxidant potential and polyphenol content, leaving the control essentially void of polyphenols (Miller et al., 2008). The DC condition contained 107mg epicatechin, 30mg catechin, and 84mg procyanidin per daily serving . See figure 2.3 for detailed nutritional breakdown.

Dark Chocolate

Nutrition Facts

Serving size	(100g)
Amount Per Serving	
Calories	**560**
	% Daily Value*
Total Fat 41g	**53%**
Saturated Fat 25g	**125%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 0mg	**0%**
Total Carbohydrate 44g	**16%**
Dietary Fiber 11g	**39%**
Total Sugars 28g	
Includes 0g Added Sugars	**0%**
Protein 8g	**16%**
Vitamin D 0mcg	0%
Calcium 52mg	4%
Iron 16.2mg	90%
Potassium 0mg	0%

*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Ingredients: cocoa mass, sugar, cocoa butter, soy lecithin, natural vanilla flavoring

Placebo

Nutrition Facts

Serving size	(100g)
Amount Per Serving	
Calories	**540**
	% Daily Value*
Total Fat 34g	**44%**
Saturated Fat 30g	**150%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 0mg	**0%**
Total Carbohydrate 61g	**22%**
Dietary Fiber 3g	**11%**
Total Sugars 56g	
Includes 0g Added Sugars	**0%**
Protein 4g	**8%**
Vitamin D 0mcg	0%
Calcium 104mg	8%
Iron 8.1mg	45%
Potassium 0mg	0%

*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Ingredients: (sugar, vegetable oils, skim milk powder, alkalized cocoa, cocoa, glyceryl lacto esters of fatty acids, soy lecithin

Figure 2.3. Nutritional information of supplement.

2.5 Procedures

2.5.1 Incremental Exercise Test

In order to assess the aerobic capacity of participants and determine their eligibility of the study, participants underwent an incremental exercise test (VO_{2max}) at normoxia (20.93% O_2) on their first visit to the lab. Participants pedaled on a stationary bike (either their own affixed to a trainer or a Velotron®). 10 minutes of self-selected warm-up was provided, and after 8 minutes a mask to collected expired gases was fitted to their head. After the 10 minutes of warm up, resistance was increased by 20 watts from their self-selected warm up power. Resistance was increased by 30 watts every 3 minutes thereafter until participants could no longer maintain cadence above 70 RPM or volitional fatigue. Five participants did not reach the required VO_{2max} (50 ml/kg/min for males; 45ml/kg/min for females) and thus were excluded from the study. On the second visit to the lab, participants underwent the same test at a simulated altitude of 2500m (15% O_2). Subsequent exercise tests were completed at 50% power output achieved in the incremental exercise test done at hypoxia.

2.5.2 Measures of physical performance

A 10km TT was used as a measure of physical performance. The TT was completed on a stationary cycle (either their own bike affixed to a trainer or a Velotron®) immediately after 90 minutes of submaximal cycling. Participants were permitted to manipulate gearing and cadence as required throughout the TT. Participants were permitted to see the distance covered throughout but were blinded to the time elapsed.

2.5.3 Metabolic measures

Plasma glucose and lactate were assessed via finger prick blood samples upon fasted arrival to the lab and at 15, 30, 60, and 90 minutes during steady state, as well as immediately following

completion of the TT. Prior to data collection, the metabolic cart (Parvo Medics) was calibrated according to manufacturer's specifications. Expired air was collected at for 1 minute at 15, 30, 45, 60, 75, and 90 minutes during steady state exercise.

The measurement of expired air was used to determine the O_2 cost of exercise as well as the primary substrate being metabolized. The values for O_2 consumed and CO_2 produced were applied to equations described by Jeukendrup and Wallis (2005) to determine the primary substrate being metabolized. Carbohydrate and fat oxidation (in g/min) were calculated using the following respective equations:

$$CHO_{ox} = 4.210 * VCO_2 - 2.962 * VO_2$$

$$FAT_{ox} = 1.695 * VO_2 - 1.701 * VCO_2$$

The knowledge of the primary substrate being metabolized also lends itself to inferring the metabolic efficiency of exercise, as a more efficient metabolic system will metabolize a greater percentage of lipids and thus spare stored glycogen, delaying fatigue (Patel et al., 2015). This is especially important in endurance athletes because depletion of glycogen is large contributor to fatigue (Ørtenblad et al., 2013), so glycogen sparing by way of lipid metabolism will allow the athlete to maintain a given exercise intensity for longer and preserve glycogen stores to produce a strong sprint in the final minutes of exercise.

2.5.4 Tissue Oxygenation

The use of the NIRS system to measure tissue oxygenation is based on the Beer-Lambert Law, which describes the relationship between light absorption and chromophore concentration (Simonson & Piantadosi, 1996). NIRS detects changes in light absorption in hemoglobin, myoglobin, and cytochrome oxidase. Although NIRS measures changes in all three of the aforementioned chromophores, the light attenuation due to myoglobin and cytochrome oxidase is

minimal compared to that of hemoglobin (Kennedy et al., 2006). Oxygenated hemoglobin absorbs light at 850nm while deoxygenated hemoglobin absorbs light at 760nm. Thus, by measuring the difference in the amount of light emitted into a tissue at 850 and 760nm compared to that which is absorbed provides information on the concentration of oxygenated and deoxygenated hemoglobin, expressed as optical density units (Kennedy et al., 2006). Tissue oxygenation of the cerebral cortex (Portalite) and the knee extensors (Portamon) was measured via NIRS. Participants were instructed to maximally extend the knee of their dominant leg without any load to allow palpation of the muscle belly of the vastus lateralis. One probe was placed on the belly of the vastus lateralis, approximately 15 cm above the proximal border of the patella and 5 cm lateral to the midline of the thigh, parallel to the long axis of the muscle in accordance with Subudhi et al. (2007). Kennedy et al. (2006) described differences in muscle oxygenation within the vastus lateralis depending on probe placement (i.e. greater muscle oxygenation at proximal areas of the vastus lateralis relative to the distal portion) therefore, probes were placed within 2 cm on visit 5 relative to placement on visit 4, ensuring any changes measured were due to physiologic changes rather than probe placement. Probes were secured to the leg using a dark coloured generic elastic sleeve to ensure the probe did not move and to shield the device from ambient light. A second probe was positioned on the prefrontal cortex on the right side, ~2 cm above the eyebrow as laterally as possible. The cerebral probe was held in place using a dark coloured elastic headband to minimize probe movement and block ambient light. In the case of excessive hair growth impeding the signal on the quadriceps, the skin was shaved to eliminate the signal interference. Micromolar changes in tissue oxygenation and deoxygenation were measured throughout the duration of exercise in visits 4 and 5. Total hemoglobin was calculated as the sum of oxygenated and deoxygenated hemoglobin and used as

a measure of site-specific blood flow. Prior to exercise, a measurement was taken while the participant sat quietly on the bicycle to provide a baseline measurement, which all measurements during exercise would be compared to (arbitrarily defined as 0μM). A leg-cuff induced ischemia post exercise to obtain a low-oxygen reference point was not used, as Chance et al. (1992) showed minimal additional desaturation during supra-systolic cuff occlusion of the vastus lateralis muscle immediately following a maximal exercise test. As discrepancies in muscle oxygenation of the vastus lateralis have been observed based on diode placement (Kennedy et al., 2006), great care was taken to ensure analogous placement of the probe on repeat visits.

2.6 Sample Size and Analysis

A sample size of 12 was chosen based on an effect size of 0.67 for our primary outcome, time trial performance. Sample size was calculated using G*Power app (© Franz Faul, Edgar Erdfelder, Albert-Georg Lang, and Axel Buchner, 2006, 2009) based on a beetroot study by Muggeridge and colleagues (2013) (Baseline: 1716 ± 17 s; BR: 1664 ± 14 s, P = 0.006, CI 15.3 – 66 s; effect size d=0.67), a 95% confidence interval, and a power of 80%. Similar studies (Masschelein et al., 2012; Muggeridge et al., 2014; Patel et al. 2015) have found sample sizes of 9-15 participants to be effective in detecting a significant difference between groups. By using a cross-over design, inter-individual variability can be eliminated, increasing the power and precision of the study and protecting against type II error. By including a familiarization trial prior to experimental trials, any learning effect can be reduced and the validity of the measures is increased (Laursen et al., 2003). Experimental trials under opposite conditions occurred one month apart, ensuring that any female participants were in a similar phase of their menstrual cycle to eliminate any hormonal effects of performance. This timeline means that a two-week

washout period between conditions was respected. The half-life of flavonoids ranges from 2-28 hours (Manach & Donovan, 2004), so a 2-week washout period is sufficient. All tests were conducted in the morning following a minimum 10-hour fast to eliminate diet and circadian rhythms as confounding factors.

The primary hypothesis was tested using a one-way repeated measures Analysis of Variance to assess TT performance. The secondary hypotheses were assessed using two-factor (2x6) repeated measures Analysis of Variance investigating the interaction of supplement and time to determine if there were differences in plasma glucose, plasma lactate, oxygen consumption, carbon dioxide production, and substrate oxidation. A 2x7 design was used to assess cerebral and muscular oxygenation at different time points. In the case that sphericity was violated while running the interactions, a Greenhouse-Geisser adjustment was used to correct the degrees of freedom. By using this correction factor, we are able to protect against the increased risk of Type I error that would arise if sphericity was violated. Box plots were used to determine whether there were any outliers in the data. NIRS data showed many outliers (~50-60%) while other measures showed less (~15-25%) If an outlier was present, the datum was removed. In the case that removal of outliers decreased the sample size significantly, the outlying data points were removed and replaced with the mean of the group. Measures of plasma glucose and lactate were broken into 6 different time points: pre-exercise, 15, 30, 60, and 90 minutes during steady state, and immediately following the time trial. Gas analysis (VO_2 carbon dioxide production, and substrate oxidation) was also broken into 6 different collection points: 15, 30, 45, 60, 75, and 90 minutes during steady state exercise. When main effects of time or interactions between condition and time were detected from the two-way ANOVA, a Tukey's HSD post hoc test was used to assess differences between pairs of means. An alpha of 0.05 was considered statistically

significant. Results were analyzed using JASP statistical software, version 0.10.2 (2013-2019, University of Amsterdam). Results are expressed as means and standard deviations, except in figures, where standard error of the mean was used for clarity (i.e. to prevent distortion of the figures).

2.7 Dietary and Training Control

During the experimental trial, participants were instructed to keep their training and dietary habits constant. However, in the two days leading up to visit 4 (i.e. days 13 and 14 of supplementation), participants were asked to refrain from foods that are high in polyphenols, such as berries, dark leafy greens, coffee, and tea. Participants were asked to keep a food log during these two days and were instructed to keep the food log in order to replicate it in the two days leading up to visit 5.

Chapter 3: Results

Seventeen athletes consented to take part in the study. Of these, 5 did not meet the inclusionary criteria for VO_2max, leaving 12 athletes (n=2 females) to participate in the study. All athletes came from a background of cycling, triathlon, or duathlon. Participants were trained athletes who cycled at least 3 times per week in bouts of 60 minutes or more, had above average VO_2max scores (>50ml/kg/min for males and >45 ml/kg/min for females), and were not regular consumers of dark chocolate (<50g/day, 5 days/week). Data is presented as mean ± standard deviation unless specified as otherwise, in which case standard error of the mean was used to preserve figure quality. Demographic information is shown in table 3.1 below.

Age	Height	Weight	VO_2max
34.9±11.9 years	177±9 cm	75.2±11.0kg	55±6ml/kg/min

Table 3.1. Participant Characteristics. Participant characteristics are displayed in means ± standard deviations. VO_2 max = maximum oxygen consumption.

3.1 Time Trial

There was no statistical difference in time trial performance between the two conditions, $F(1, 10)=0.91$, $p=0.77$. The mean time to complete the time trial under the dark chocolate condition was 19.04 (±2.16) minutes and the mean time to completion under the placebo condition was 19.21 (±1.96) minutes.

3.2 Blood Parameters

Glucose: The assumption of sphericity was not met ($p=.009$) for time effects so a Greenhouse-Geiser epsilon was used to adjust the degrees of freedom. There was a significant main effect of

supplement on plasma glucose $F(1, 10)=6.020$, $p=.034$, with the DC maintaining blood glucose at a higher level than the placebo (5.5±0.5 mmol vs 5.3± 0.9 mmol/L for dark chocolate versus placebo, respectively). There were also significant main effects of time on plasma glucose, $F(2.482, 24.816)=14.714$, $p<.001$. The 60-minute time point was significantly higher than fasted ($p=.013$) and 15 minute ($p=.028$) measurements, while the post TT measurement was significantly higher from fasted ($p=.004$), 15 minute ($p=.011$), and 30 minute ($p=.013$) time points. No statistically significant interaction between chocolate and time on blood glucose was found, $F(2.764, 27.642)= 1.416$, $p=.260$. Results are displayed in figure 3.1 below.

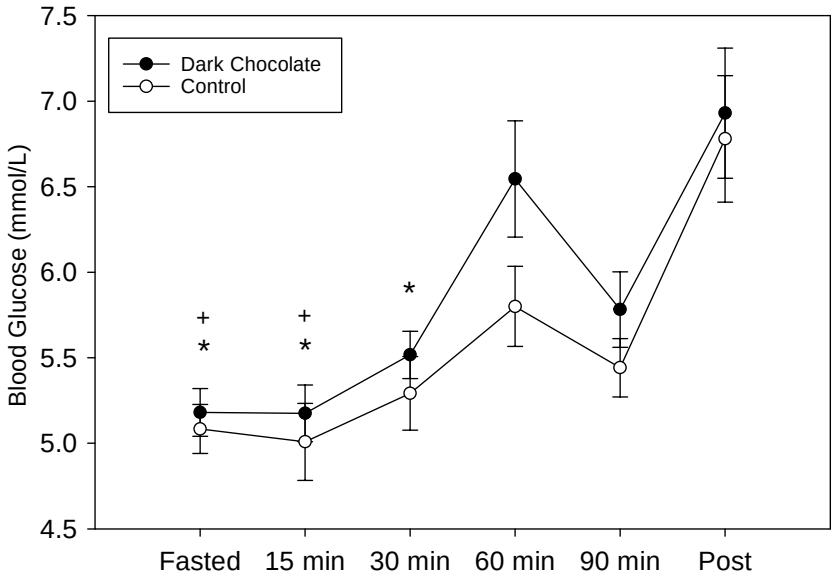

Figure 3.1. Blood glucose values observed at different time points. * denotes significantly different from post TT measurement, $p<.05$. + denotes different from the 60 min measurement, $p<.05$. Data are displayed as mean ± standard error of the mean.

Lactate: The assumption of sphericity was not met ($p<.001$) for supplement x time interaction so a Greenhouse-Geiser epsilon was used to adjust the degrees of freedom. There were no

significant main effects of supplement on blood lactate, $F(1, 11) = .393$, $p = .544$. A significant main effect of time on blood lactate was found, $F(1.450, 15.974) = 30.649$, $p < .001$. Fasted measures were significantly lower than all other time points ($p < .05$). Post TT measurements were significantly higher than all other time points, except at the 90-minute time point ($p < .01$). A significant interaction between supplement and time was also observed, $F(1.528, 16.811) = 4.955$, $p = .027$. The DC condition resulted in significantly lower lactate levels compared to the placebo following the TT, $p = .009$ (7.7 mmol/L vs. 10.0 mmol/L).

Results are depicted in figure 3.2.

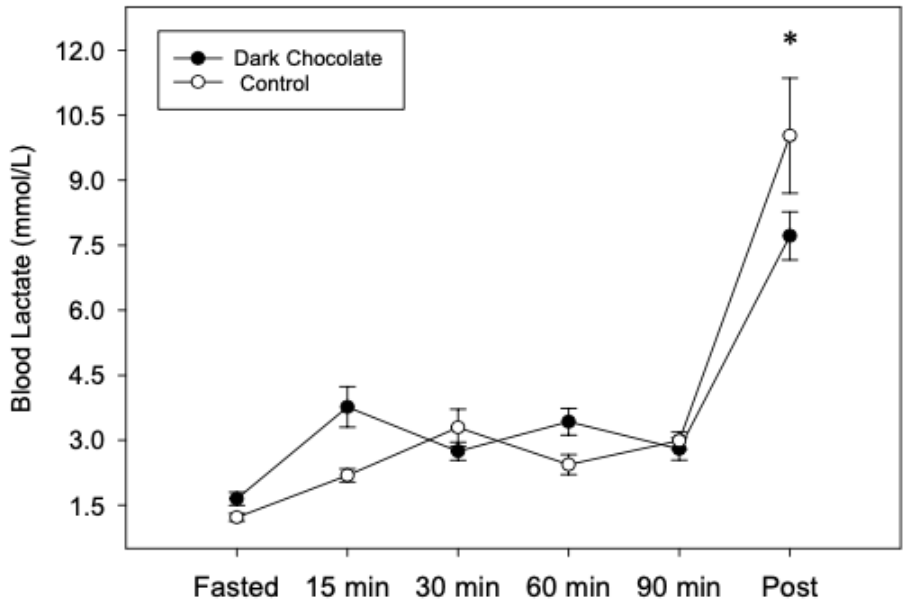

Figure 3.2. Supplement x time interaction on blood lactate. * signifies significantly different from placebo, $p < .05$. Data are displayed as mean ± standard error of the mean.

3.3 Gas analysis

In analyses where assumption of sphericity was violated based on Mauchly's test of sphericity, a Greenhouse-Geiser epsilon was used to adjust the degrees of freedom.

VO2: There was no significant main effect of supplement on VO2, (F(1, 9)=.936, p=.359). No significant main effect was found for time (F(5, 45)=6.439, p=.454). There was no supplement x time interaction (F(5, 45)=7.324, p=.387). Results are depicted in figure 3.3.

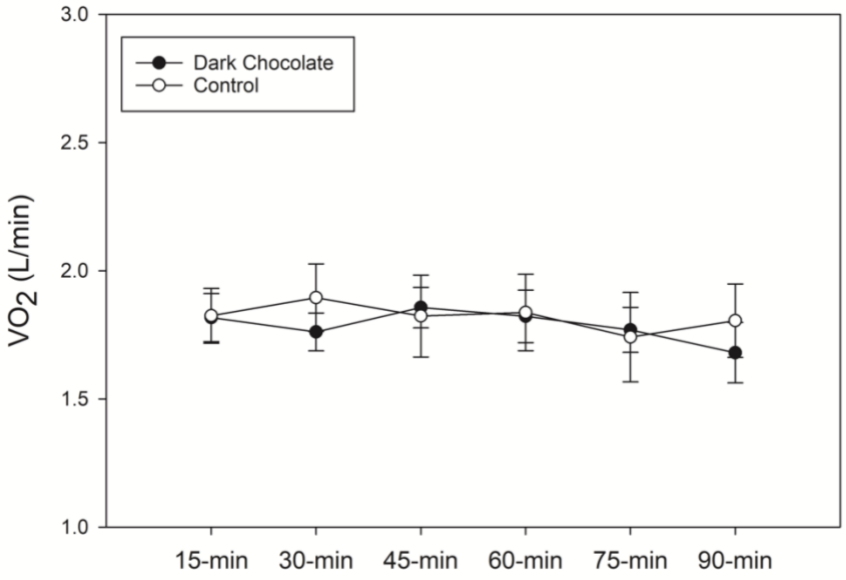

Figure 3.3. Oxygen consumption (VO2) observed at different time points. Data are displayed as mean ± standard error of the mean. No significant differences were found across the analysis.

RER: There was no significant main effect of supplement on RER, (F(1, 8)=2.513, p=.152). No significant main effect was found for time (F(1.980, 15.839)=.372, p=.639). There was no supplement x time interaction (F(5, 40)=1.293, p=.286).

Carbohydrate and Fat Oxidation: No significant effects of supplement on carbohydrate or fat oxidation were found, (F (1, 9) =2.071, p=.184 and F (1, 9) =.577, p=.467, respectively). There were no significant effects of time on carbohydrate or fat oxidation, (F (5, 45) =.071, p=.661 and F (2.380, 21.417) =.470, p=.664, respectively). There was also no significant interaction of

supplement and time on carbohydrate or fat oxidation, (F (5, 45) =.804, p=.553 and F(5, 45)=.774, p=.573, respectively). Results are depicted in figures 3.4 and 3.5 respectively.

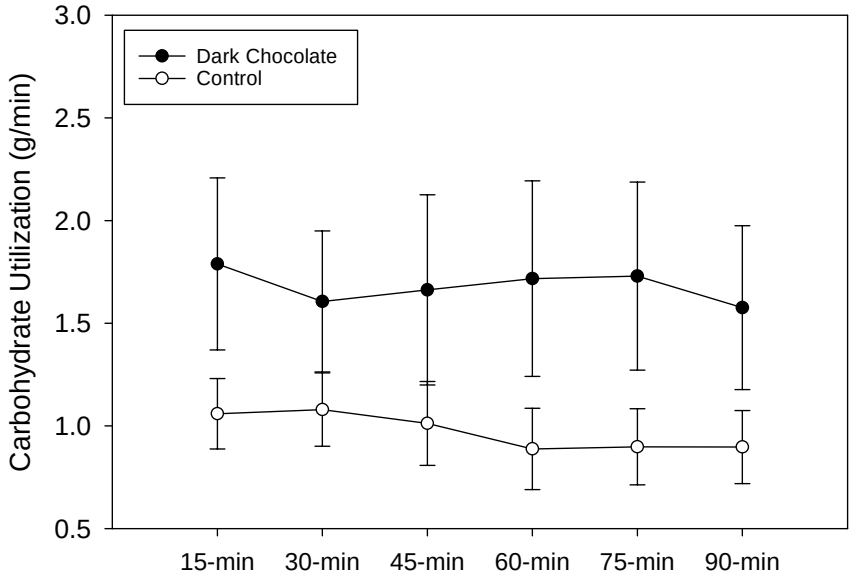

Figure 3.4. Carbohydrate oxidation at different time points in g/min. Data are displayed as mean ± standard error of the mean. No significant differences were found.

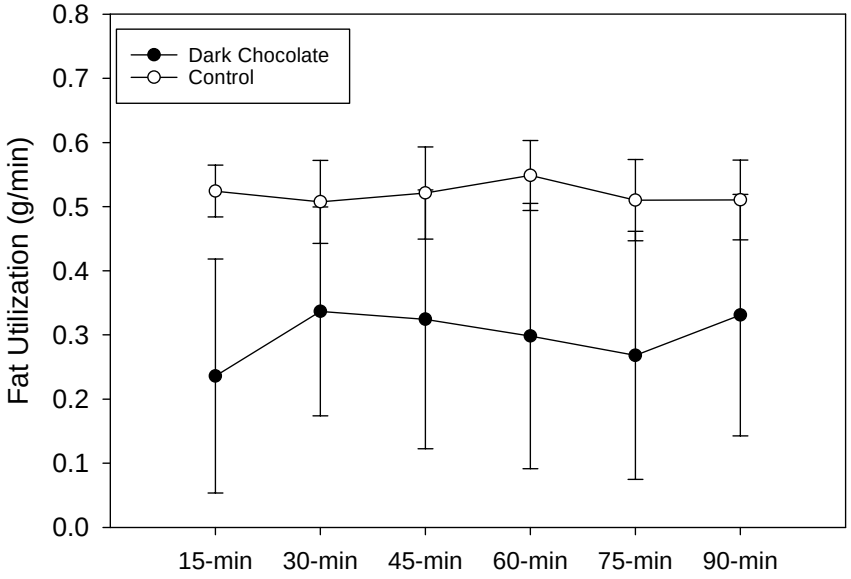

Figure 3.5. Fat oxidation at different time points in g/min. Data are displayed as mean ± standard error of the mean. No significant differences were found.

3.4 Near Infrared Spectroscopy

3.4.1 Vastus Lateralis

Oxygenated hemoglobin: There was no significant effect of supplement on the amount of oxygenated hemoglobin, (F (1, 11) =3.389, p=.093), nor was there a significant interaction of supplement and time, (F (1.828, 20.112) =.409, p=.652). There was a significant main effect of time on oxygenated hemoglobin, (F (2.127, 21.272) =8.451, p=.002). The 15-minute measurement was significantly lower than at 90 minutes (p=0.020). Measurements at 30 minutes were significantly lower than at 60 minutes (p=.018), 75 minutes (p=.007), and 90 minutes (p=.011). Measurements at 45 minutes were significantly lower from those at 60 minutes (p=.024), 75 minutes (p=.022) and 90 minutes (p=.011). Results are depicted in figure 3.6.

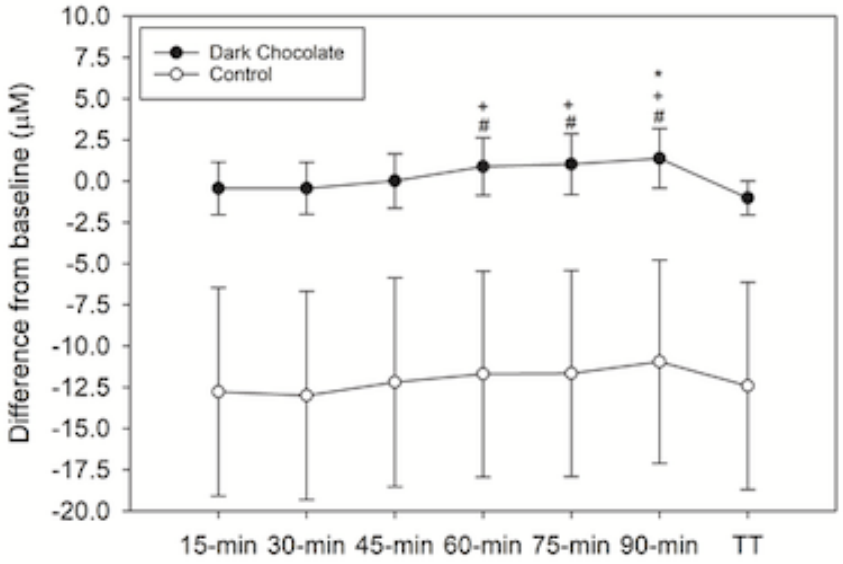

Figure 3.6. The amount of oxygenated hemoglobin in the vastus lateralis of the dominant leg compared to a baseline measure, as measured by near infrared spectroscopy. * signifies significantly different from 15 minute, + signifies significantly different from 30 minute, and # signifies significantly different from 45 minute ($p<.05$). Data are presented as mean ± standard error of the mean

Deoxygenated hemoglobin There was no effect of supplement or interaction of supplement and time on deoxygenated hemoglobin, (F (1, 10) =1.510, p=.247 and F(3.014, 30.139)=1.764, p=.175, respectively). There was an effect of time on deoxygenated hemoglobin, F(2.753, 27.528)=9.069 p<.001. 15-minute measurements were significantly lower than 30 (p=.001), 45 (p=.005), 60 (p=.002), 90 (p=.031), and during the TT (p=.009). Results are depicted in figure 3.7

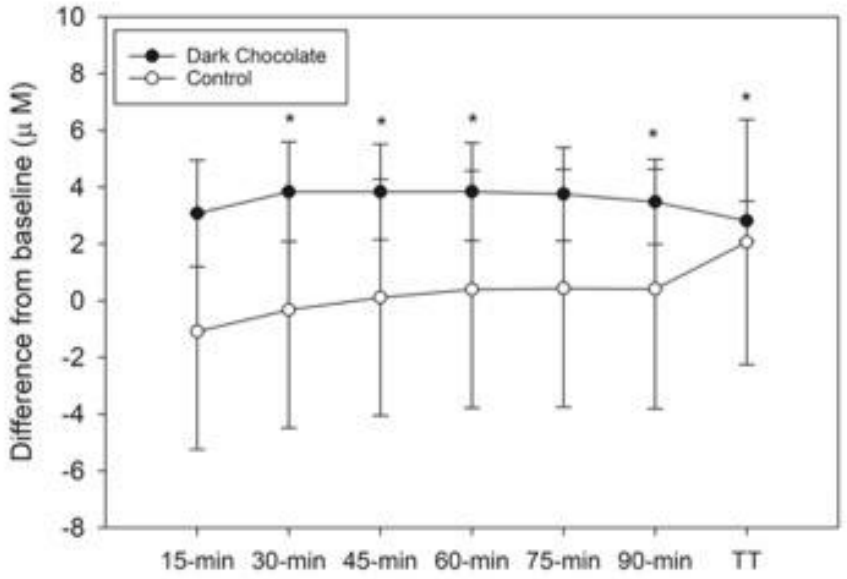

Figure 3.7. The amount of deoxygenated hemoglobin in the vastus lateralis of the dominant leg compared to a baseline measure, as measured by near infrared spectroscopy. * signifies significantly different from 15-minute, p<.05. Data are presented as mean ± standard error of the mean.

Total hemoglobin: There was no effect of supplement or interaction of supplement and time on total hemoglobin, (F (1, 7) =0.967, p=.358 and F (2.173, 15.213) =.345, p=.731, respectively). There was a significant effect of time on total hemoglobin in the vastus lateralis, (F(1.718, 12.025)=16.421, p<.001). 15-minute measurements were significantly lower than 45 (p=.008), 60 (p=.007),75 (p=.007), and 90 minutes (p=.020). 30-minute measurements were significantly lower than time points 45 (p=.038), 60 (p=.017), 75 (p=.017), and 90 minutes (p=.040). Results are displayed in figure 3.8.

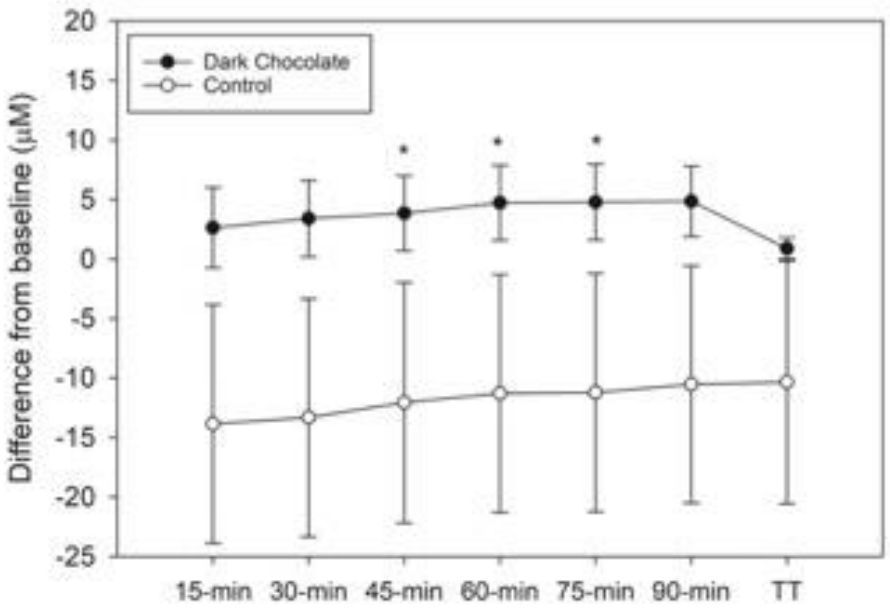

Figure 3.8. The total amount of hemoglobin in the vastus lateralis of the dominant leg compared to a baseline measure, as measured by near infrared spectroscopy. * signifies significantly different from 15 minute and + signifies significantly different from 30-minute, $p<.05$. Data are presented as mean ± standard error of the mean.

3.4.2 Cerebral Lobe

Oxygenated hemoglobin: There was no significant effect of supplement on the amount of oxygenated hemoglobin, (F (1, 8)= 4.404, p=.069), nor was there a significant interaction of supplement and time, (F(1.423, 11.383)=.68, p=.482) in the cerebral lobe. There was however a significant effect of time on the amount of oxygenated hemoglobin (F(1.345, 10.757)=10.167, p=.006). 15-minute measurements were lower than 45 (p=.016), 60 (p<.001), 75 (p=.019), and 90 minutes (p=.043). Results are depicted in figure 3.9.

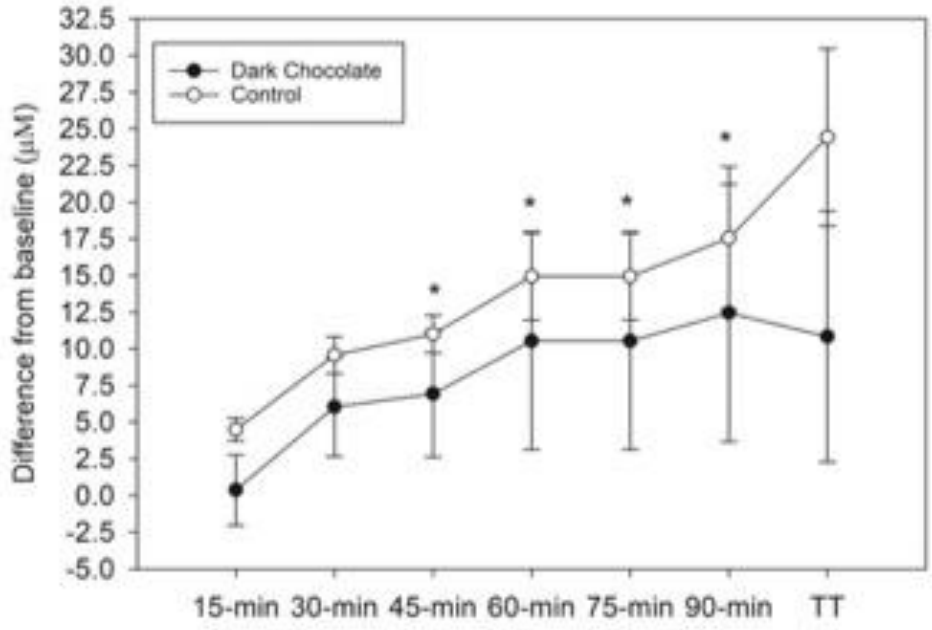

Figure 3.9. The amount of oxygenated hemoglobin in the right cerebral lobe relative to baseline measures, as measured by near infrared spectroscopy. * signifies significantly different from 15 minute for oxygenated hemoglobin, p<.05. Data are presented as mean ± standard error of the mean.

Deoxygenated hemoglobin: There was no significant effect of supplement on the amount of deoxygenated hemoglobin, (F (1, 9) =.262, p=.621), nor was there a significant interaction of supplement and time, (F(1.797, 16.169)=.351, p=.687). There was a significant main effect of time on deoxygenated hemoglobin, (F (1.465, 11.723) =10.765, p=.004). The amount of deoxygenated hemoglobin was significantly lower at 15 minutes than 45 minutes (p=.017). Results are depicted in figure 3.10.

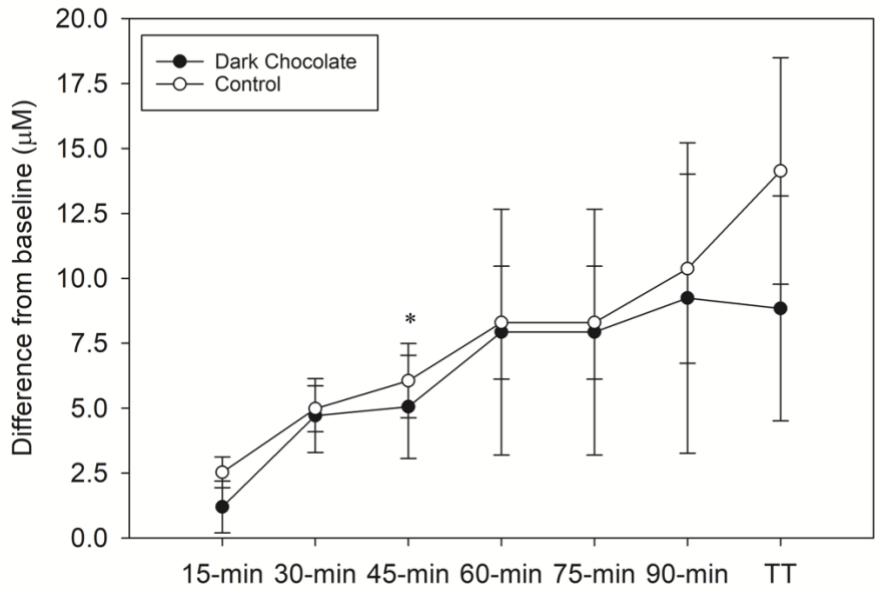

Figure 3.10. The amount of deoxygenated hemoglobin in the right cerebral lobe relative to baseline measures, as measured by near infrared spectroscopy. * Signifies significantly different from 15 minutes, $p<.05$. Data are presented as mean ± standard error of the mean.

Total hemoglobin: There was no significant effect of supplement on the total amount of hemoglobin, (F (1, 9) =.960, p=.353), nor was there a significant interaction of supplement and time, (F(1.714, 15.430)=.467, p=.606). There was a significant main effect of time on total hemoglobin, (F (1.289, 10.314) =8.366, p=.012). 15-minute measurements were significantly lower than 45 (p=.019), 60 (p=.004), and 75 minutes (p=.032). Results are displayed in figure 3.11.

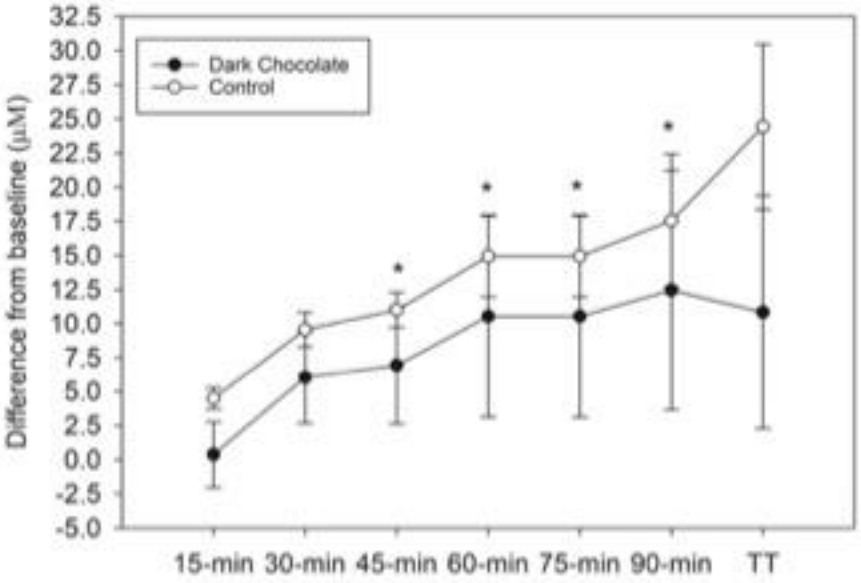

Figure 3.11. The total amount of hemoglobin in the right cerebral lobe relative to baseline measures, as measured by near infrared spectroscopy. * signifies significantly different from 15-minute measurements, $p<.05$. Data are presented as mean ± standard error of the mean.

3.5 Adverse Effects

Two participants reported GI distress while consuming DC during the 2-week supplementation period. These symptoms included abdominal pain, diarrhea, and general "upset stomach". One reported mild symptoms that did not interfere with consumption of the supplement or with daily activities. The second participant, however, reported more severe symptoms that limited the amount of the supplement that could be consumed as well as interfered with daily activities. This participant consumed approximately 50% of the required dose and data from this participant was treated as per usual, without any outlying data being removed. No adverse effects were reported for the placebo condition.

3.6 Compliance

Eleven participants reported a compliance of 100% for the combined 4 weeks of supplementation. One participant reported 60% compliance rate for the DC condition due to GI distress and 100% compliance for the placebo.

3.7 Sub Analyses

Past research has demonstrated an increase in cerebrovascular function in males while lesser effects were observed in females (Fan et al., 2019). To assess potential sex differences in the current study, a sub analysis was run on the male participants only (n=10). No difference was found in TT performance between conditions, ($F(1,8)=.014$, $p=.908$). Similarly, no differences were found between conditions for any other measurement when females were removed from analysis.

Chapter 4: Discussion

The results of the current study demonstrate that two weeks of DC supplementation have no effect on TT performance at simulated altitude in trained cyclists. DC did, however, improve the maintenance of blood glucose throughout exercise and decrease the accumulation of lactate following the TT. To the author's knowledge, this is the first study to examine the effects of dark chocolate on performance measures at simulated altitude. This research is important, as many cycling races such as the Tour de France and Giro d'Italia have mountain stages that reach as high as 2600m. Many riders do not have the resources to acclimatize prior to a race at high altitude, and thus must rely on other methods to properly prepare for the reduced oxygen partial pressure at altitude. We hypothesized that 2 weeks of dark chocolate supplementation with 60g twice daily would improve 10km time trial performance, enhance oxygenation of the vastus lateralis and cerebral lobe, improve glucose and lactate handling, decrease the oxygen cost of submaximal exercise, and decrease the reliance on carbohydrate metabolism during cycling at a simulated altitude of 2500m.

4.1 Time Trial Performance

The results of the current study suggest that dark chocolate does not affect 10km TT performance at a simulated altitude of 2500m in trained cyclists, which is in contrast to our research hypothesis. This is, in agreement with the findings of Stellingwerff and colleagues (2013), who found no improvement in 15-minute time trial performance completed after a 2.5-hour bout of steady-state exercise completed at 43.5% VO_{2peak} at normoxia following consumption of dark chocolate. The authors speculated that a greater exercise intensity before the time trial may have elicited greater performance enhancements due to an increased reliance on carbohydrates at higher intensities. Likewise, Peschek and colleagues (2013) observed no

increase in running time trial performance the day after a damage-inducing bout of downhill running after consuming a beverage fortified with additional cocoa. Other studies have suggested an improvement in exercise performance or metabolic markers when dark chocolate is consumed before exercise. For example, Patel et al. (2015) found that 40g of dark chocolate for 14 days before exercise testing resulted in increased performance in a 2-minute time trial as well as an increased gas exchange threshold. Allgrove et al. (2011) found that two weeks of supplementation with 40g of dark chocolate twice daily did not improve time to exhaustion but resulted in lower resting and post-exercise plasma levels of the oxidative stress markers F_2-isoprostanes and oxidized LDL. Differences in findings in the literature could be related to the duration of the TT performed. Patel et al (2015) found that DC enhanced TT performance for a 2-minute TT, while Peschek et al. (2013) and Stellingwerff et al. (2013) used protocols that lasted 15-20 minutes. Allgrove et al. (2011) used a TTE protocol which lasted a mean of 6.6 minutes. Thus, differences in findings could be related to the duration of the trial and the relative contribution of the aerobic and anaerobic energy systems. A second potential explanation could be due to differences in control substances used. The current study used a chocolate flavored molding wafer containing no cocoa liquor or cocoa butter, while Patel and colleagues (2015) used a white chocolate. Other researchers (Allgrove et al., 2011; Stellingwerff et al., 2013; Pescheck et al., 2013) used a control chocolate that had similar appearance but lacked polyphenols.

Our results are in contrast to other studies using different nutritional supplements that are promoted for increasing nitric oxide production and therefore blood flow during exercise. For example, Masschelein et al. (2012) found that supplementation with ~500ml of nitrate-rich beetroot juice for 2 weeks increased cycling time to exhaustion completed at 11% O_2.

Muggeridge et al. (2013) also found an improvement in 16.1km TT performance when athletes supplemented with 70 ml of concentrated nitrate-rich beetroot juice three hours before exercise testing completed at a simulated altitude of 2500m (15% O_2). This study may have resulted in differing results compared to those that utilized beetroot juice due to the mechanism by which each of the supplements work. Beetroot juice acts as an exogenous source of nitrate for the body to break down to NO. DC acts as a source of polyphenols, increasing the production of NO as well as the reuptake. However, if individuals do not have the necessary influx of dietary nitrates, these mechanisms may not be effective.

4.2 Metabolic Analyses

We hypothesized that dark chocolate supplementation would result in superior exercise metabolism, evidenced by improved glucose and lactate handling, decreased VO_2 for a given workload, and an increased reliance on fats for energy. A significant interaction between supplement condition and time was observed for blood lactate concentration. When post hoc testing was conducted, decreased blood lactate following the time trial in the dark chocolate condition (8.6±3.8 vs. 10.0±4.6 mmol/L p=0.009) was observed. We found no differences in lactate levels between conditions before the time trial (i.e. during the constant-load cycling). This is similar to Patel and colleagues (2015) who reported no differences in blood lactate concentration during a 20-minute moderate intensity cycle following 2-weeks of 40g/day dark chocolate supplementation. Similar results were found by Davison et al. (2012), who reported no effect of an acute dose of 100g of 70% dark chocolate on blood lactate at rest, immediately post exercise, or one-hour post exercise. Interestingly, Fraga et al. (2005) observed an increase in resting lactate measures in healthy, young soccer players following the consumption of dark

chocolate compared to white chocolate consumption. The authors did not provide speculation into the reasons that this may have occurred.

Main effects of both supplement and time were observed for blood glucose concentration, showing increased blood glucose values throughout the exercise (time effect) and showing improved maintenance of blood glucose throughout the experimental trial under the dark chocolate condition (5.9±0.5 vs. 5.6±0.5 mmol/L; p=0.03). These results are consistent with those found by Stellingwerff et al. (2013) as well as Davison et al. (2012). Stellingwerff and colleagues (2013) observed that the consumption of DC augmented blood glucose concentrations during low-intensity exercise compared with a nearly iso-caloric and iso- carbohydrate cocoa-deplete control. The increased plasma glucose coincided with high concentrations of polyphenol and theobromine found in DC that could potentially attenuate muscle glucose uptake. It was suggested that the augmented plasma glucose was due primarily to a decreased rate of glucose uptake paired with an unaltered rate of appearance of glucose. Animal studies have shown a competitive inhibition of insulin-mediated GLUT-4 transporters by some classes of polyphenols (Strobel et al., 2005). However, the authors noted that exercise-mediated GLUT-4 translocation mechanisms would be at work during this trial rather than insulin-mediated effects; therefore, the mechanisms behind differences in glucose uptake are unknown.

Davison et al. (2012) found that DC consumption 2 h before an acute bout of prolonged exercise (2.5 h at 60% VO_{2max}) resulted in significantly higher pre-exercise plasma insulin concentrations as well as smaller perturbations in plasma glucose concentration compared with the consumption of a closely matched control. It remains unclear whether alterations in the insulin and glucose responses are due to alterations in the insulin response, glucose handling, interference of glucose uptake, the action of insulinogenic amino acids in the cocoa, or some

other mechanisms. Differences in the current study are possibly due to the differences in macronutrients between the conditions. The dark chocolate condition contains higher levels of fat and protein which could lead to better maintenance of blood glucose, while the control contained higher levels of carbohydrate.

There were no effects of either supplement or time on gas analysis, including oxygen uptake during steady-state exercise and carbohydrate or fat oxidation (calculated using VO_2 and VCO_2 values). This is in agreement with findings by Patel et al. (2015), who observed no differences in VO_2 or RER after supplementation with dark chocolate but did observe a difference in the gas exchange threshold. These results are in contrast to those found by Allgrove et al. (2011), who observed a lower RER during prolonged exercise following two weeks of dark chocolate supplementation suggesting a greater proportion of energy coming from fatty acid oxidation. The differing results could be due to the time frame in which the DC was consumed prior to exercise. The Allgrove lab (2011) had their participants consume the chocolate two hours prior to exercise, while in the current study the DC was consumed about 30 minutes prior to exercise. This difference in time could result in differential absorption rates and thus actions of the caffeine and theobromine in the chocolate, both of which can independently increase lipolysis (Durham et al., 2000). Epicatechin acts synergistically with caffeine and theobromine to further enhance fat metabolism (Dulloo et al., 2000). Differences could also be due to the exercise protocol, as Allgrove et al. (2011) had participants sprint at 90% VO_2max for 10 seconds every 10 minutes during the 90-minute ride at 60% VO_2max, while the current maintained a steady state of 60% VO_2max for the full duration (90 minutes).

In contrast to our results with dark chocolate, supplementation with other nutrients that are reported to enhance nitric oxide production and blood flow (i.e. beetroot juice) was effective for

decreasing VO_2 at submaximal intensities at altitude (Muggeridge et al. 2013), whereas the current study found no change in oxygen uptake at submaximal intensities. These discrepancies in findings could be due to the mechanisms by which each of DC and beetroot juice act, as beetroot juice directly provides exogenous nitrates to be converted to nitrites and then to NO, while DC increases the rate at which the body can convert nitrites to NO. Therefore, if the body is already producing the maximal amount of NO relative to the amount of nitrates and nitrites available, the effects of the DC would be irrelevant.

The results for blood glucose and lactate partially support our hypotheses that the dark chocolate would improve glucose homeostasis and lactate handling. This is an important finding, as a maintenance of blood glucose and decreased lactate accumulation during prolonged exercise may reduce fatigue by decreasing ratings of perceived exertion, low blood glucose levels, and acidosis, permitting the athlete to maintain a higher intensity for longer. However, gas analyses refuted our hypothesis that dark chocolate would lead to decreased oxygen consumption as well as an increased reliance on fatty acids for ATP generation and thus glycogen sparing.

4.3 Oxygenation of the Brain and Vastus Lateralis

Dark chocolate does not affect the oxygenation status of the cerebral lobe or the vastus lateralis muscle during exercise, as measured by near-infrared spectroscopy. Results showed no effect of the supplement on oxygenated, deoxygenated, or total hemoglobin at either of the sites measured. However, there was an effect of time on all measures at both the vastus lateralis and cerebral lobe, indicating an increase in oxygen extraction as well as blood flow, as would be expected during a prolonged exercise test. These results indicate the tool utilized to measure oxygenation was valid. These results are in agreement with Decroix et al. (2016), who found supplementation with cocoa flavanols to increase brain oxygenation at rest but not during

exercise. The researchers found that cocoa flavanols had no additive effect on the exercise-induced cognitive enhancement and the associated increased cerebral oxygenation and perfusion. Masschelein and colleagues (2011) also found that supplementation with nitrate (in the form of beetroot juice) did not affect cerebral oxygenation status or acute mountain sickness. However, the same group observed increased oxygenation in the vastus lateralis at both submaximal and maximal exercise at simulated altitude (11% O_2), which is contrary to the results of the current study. Once again, differences in findings could be due to the body optimally converting all of the accessible nitrites to NO, and thus increasing the bioavailability further through the use of DC does not show an effect, whereas by directly ingesting exogenous nitrates, the body is provided additional substrate to convert to NO, thus inducing endothelium-dependent vasodilation.

An interesting finding in the current study is that of the oxygenation status of the brain during the time trial. Often we see an increase in blood flow to the brain directly related to arterial CO_2 tension (Ide & Secher, 2000). However, the current study showed a decrease in cerebral oxygenation during the 10km TT, although this did not reach significance. One speculation as to the mechanisms behind this has to do with our significant lactate findings. As the muscle increases lactate production with increasing exercise intensity, bicarbonate enters the cell in order to buffer the increased lactate. For each mmol of lactic acid buffered, 1mmol of additional CO_2 is generated (Péronnet & Aguilaniu, 2006). Due to the rapid rate of diffusion of CO_2 across the blood brain barrier, one might expect this CO_2 to alter the pH of the cerebral extracellular fluid and thus moderate cerebral vascular resistance to allow for enhanced blood flow. Due to our results showing significantly decreased lactate following the TT in the DC condition

compared to the placebo, this decreased lactate could result in decreased CO_2 crossing the blood-brain barrier, leading to a decrease in cerebral vascular vasodilation.

4.4 Strengths

To our knowledge, this is the first study to investigate the effects of dark chocolate on exercise performance at altitude. Before this, dark chocolate had only been investigated at normoxia, often investigating the effects on blood pressure in a clinical population. Studies have also been done researching the effect of exogenous nitrate (beetroot juice) on exercise performance at altitude. This study is novel in that it combines these two areas, looking at dark chocolate on exercise at altitude, measuring not only performance outcomes but also blood flow, oxygen extraction, and exercise metabolism. The use of the NIRS system allowed for real-time information of the oxygenation status of, and blood flow to both the cerebral lobe and working muscles, both sites which are known to contribute to fatigue (Secher et al, 2008; Subudhi et al., (2007).

The research investigating dark chocolate on exercise often does not differentiate between trained and recreational athletes. Through training, the athlete becomes more efficient both biomechanically and physiologically (Woo et al., 2006). As such, trained and untrained individuals may respond to the same exercise and nutritional strategies very differently. It is important to differentiate between trained and untrained to make the most accurate recommendations for every level of training and competition. Using trained athletes is also a strength because those who are more trained have less variation in day-to-day exercise performance, increasing the validity of the study.

4.5 Limitations

A large limitation of the current study was the conditions present inside of the altitude chamber. Being an airtight box to maintain oxygen levels, the limited airflow leads to an increase in both ambient temperature and relative humidity levels. The temperature increased by as much as 3 degrees Celsius and humidity increased as much as 45% over the course of the ~2 hours that participants spent in the chamber. Due to such high temperature and humidity readings, excessive sweat measurements impacted the ability of the NIRS probes to read the oxygenation status and in some cases caused complete malfunction of the probe. This decreased the number of participants we were able to get data from; therefore, increasing the possibility of a Type II error, and potentially missing significant differences in cerebral oxygenation status. Although this impacted our ability to collect all NIRS data, temperature and humidity were monitored every 15 minutes for each participant and environmental conditions for each participant were similar for both visits 4 and 5.

Participants reported that the amount of chocolate they were required to consume was very significant and the palatability of the chocolate decreased significantly over the two weeks of supplementation. Some participants reported GI distress after consuming large amounts of the dark chocolate consisting of cramping and abdominal pain.

Because the hormonal differences between men and women vary significantly, a larger proportion of female participants would be required to look at subgroup difference between men and women and potential differences within females in different phases of their menstrual cycle. Another potential limitation of the current study is that we used RER to infer what was happening at the cellular level. However, this could lead to inaccurate interpretations, as a

decrease in pH caused by an increase in lactate may result in heightened CO_2 levels, confounding the RER measurement (Bar et al., 1965).

4.6 Recommendations for future research

To determine if dark chocolate could be a viable sport supplement in endurance athletes, it is recommended that further research investigate the effects of dark chocolate on performance in recreational athletes as well as endurance athletes from different sports and athletes from power-based sports such as track cycling, bobsleigh, and powerlifting. Current literature investigating the effects of exogenous nitrates (i.e. beetroot juice) shows benefits in performance of recreation athletes, but not in a trained population (Jonvik et al., 2015). This may be due to the benefits on NO for optimizing endothelial function, as the endothelium of highly trained athletes is likely already functioning close to optimally. Also, the impact of NO on mitochondrial biogenesis could also explain the differences in impact between trained and untrained/recreational individuals, as those who are well trained may see a ceiling effect for mitochondrial biogenesis and thus may not see performance benefits due to further increases. Implications of dark chocolate on the anaerobic energy systems will also provide greater knowledge on the benefits of dark chocolate across different sports. Due to the high antioxidant content of dark chocolate, future research should investigate the effects of dark chocolate on recovery and multi-day performances. Assessment of oxygen intake and macronutrient metabolism may also be better understood by collecting gasses in a more realistic setting such as on the road using a portable indirect calorimetry system.

Dark chocolate activates nitric oxide synthase, catalyzing the conversion of nitrite to NO for the body to use. Future research should investigate a combination of exogenous nitrate (beetroot juice, sodium nitrate) along with dark chocolate or other flavonoid-rich foods as a method to

increase endothelial modulated vasodilation and blood flow. This could be a beneficial combination due to their potentially complimentary mechanisms. Ingestion of exogenous nitrates as beetroot juice could lead a greater supply of nitrates and thus a greater concentration of nitrite, which DC may then convert to NO in order to see additive effects of the supplements.

4.7 Conclusion

Although dark chocolate improved glucose handling and decreased lactate levels after a high-intensity TT completed at a simulated altitude of 2500m, it did not result in improved TT performance in trained cyclists. By maintaining blood glucose and improving lactate handling, athletes may find performance benefits in longer, glycogen depleting exercise and routes that include variations in power production such as climbing.

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APPENDICIES

Appendix A- Get Active Questionnaire

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

Get Active Questionnaire

CANADIAN SOCIETY FOR EXERCISE PHYSIOLOGY –
PHYSICAL ACTIVITY TRAINING FOR HEALTH (CSEP-PATH®)

Physical activity improves your physical and mental health. Even small amounts of physical activity are good, and more is better.

For almost everyone, the benefits of physical activity far outweigh any risks. For some individuals, specific advice from a Qualified Exercise Professional (QEP – has post-secondary education in exercise sciences and an advanced certification in the area – see csep.ca/certifications) or health care provider is advisable. This questionnaire is intended for all ages – to help move you along the path to becoming more physically active.

☐ I am completing this questionnaire for myself.

☐ I am completing this questionnaire for my child/dependent as parent/guardian.

PREPARE TO BECOME MORE ACTIVE

The following questions will help to ensure that you have a safe physical activity experience. Please answer **YES** or **NO** to each question before you become more physically active. If you are unsure about any question, answer **YES**.

	YES	NO
1	Have you experienced **ANY** of the following (A to F) **within the past six months**?	
A	A diagnosis of/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity?	○
B	A diagnosis of/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher?	○
C	Dizziness or lightheadedness during physical activity?	○
D	Shortness of breath at rest?	○
E	Loss of consciousness/fainting for any reason?	○
F	Concussion?	○
2	Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active?	○
3	Has a health care provider told you that you should avoid or modify certain types of physical activity?	○
4	Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active?	○

NO to all questions: go to Page 2 – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

YES to any question: go to Reference Document – ADVICE ON WHAT TO DO IF YOU HAVE A YES RESPONSE

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

Get Active Questionnaire

ASSESS YOUR CURRENT PHYSICAL ACTIVITY

Answer the following questions to assess how active you are now.

1 During a typical week, on how many days do you do moderate- to vigorous-intensity aerobic physical activity (such as brisk walking, cycling or jogging)? ☐ DAYS/WEEK

2 On days that you do at least moderate-intensity aerobic physical activity (e.g., brisk walking), for how many minutes do you do this activity? ☐ MINUTES/DAY

For adults, please multiply your average number of days/week by the average number of minutes/day: ☐ MINUTES/WEEK

Canadian Physical Activity Guidelines recommend that adults accumulate at least 150 minutes of moderate- to vigorous-intensity physical activity per week. For children and youth, at least 60 minutes daily is recommended. Strengthening muscles and bones at least two times per week for adults, and three times per week for children and youth, is also recommended (see csep.ca/guidelines).

GENERAL ADVICE FOR BECOMING MORE ACTIVE

Increase your physical activity gradually so that you have a positive experience. Build physical activities that you enjoy into your day (e.g., take a walk with a friend, ride your bike to school or work) and reduce your sedentary behaviour (e.g., prolonged sitting).

If you want to do **vigorous-intensity physical activity** (i.e., physical activity at an intensity that makes it hard to carry on a conversation), and you do not meet minimum physical activity recommendations noted above, consult a Qualified Exercise Professional (QEP) beforehand. This can help ensure that your physical activity is safe and suitable for your circumstances.

Physical activity is also an important part of a healthy pregnancy.

Delay becoming more active if you are not feeling well because of a temporary illness.

DECLARATION

To the best of my knowledge, all of the information I have supplied on this questionnaire is correct.
If my health changes, I will complete this questionnaire again.

I answered NO to all questions on Page 1

Sign and date the Declaration below

I answered YES to any question on Page 1

Check the box below that applies to you:

☐ I have consulted a health care provider or Qualified Exercise Professional (QEP) who has recommended that I become more physically active.

☐ I am comfortable with becoming more physically active on my own without consulting a health care provider or QEP.

Name (+ Name of Parent/Guardian if applicable) [Please print] | Signature (or Signature of Parent/Guardian if applicable) | Date of Birth

Date | Email (optional) | Telephone (optional)

With planning and support you can enjoy the benefits of becoming more physically active. A QEP can help.

☐ Check this box if you would like to consult a QEP about becoming more physically active.
(This completed questionnaire will help the QEP get to know you and understand your needs.)

Appendix B- Consent Form

Participant Information and Consent Form

Title: The effect of dark chocolate on metabolism and performance in trained cyclists at simulated altitude
Principal Investigator: Philip D. Chilibeck, Ph.D., Professor, College of Kinesiology, University of Saskatchewan, phone: 306-966-1072
Co-Investigator: Patrick Neary, Ph.D. Professor, Faculty of Kinesiology and Health Studies, University of Regina, phone: 306-585-4844:
Student Researchers: Keely Shaw, B.Sc. (graduate student supervised by Philip Chilibeck), College of Kinesiology, University of Saskatchewan, phone: 306-966-1082
Emergency Telephone Number (Philip Chilibeck): 306-230-3849

Introduction

You are invited to take part in this research study because you are an experienced cyclist and we want to determine the effects of a dark chocolate compared to a dark chocolate flavored placebo (i.e. an artificial chocolate that simulates dark chocolate, but lacks cocoa liquor) on your cycling performance at a simulated altitude of 2500m and recovery from exercise.

Your participation is voluntary. It is up to you to decide whether or not you wish to take part. If you decide to participate, you are still free to withdraw at any time and without giving any reasons for your decision. If you do not wish to participate, you will not affect your relationship with the researchers or the university.

Please take time to read the following information carefully. You can ask the study staff to explain any information that you do not clearly understand. You may ask as many questions as you need. Please feel free to discuss this with your family, friends or family physician before you decide.

Who is conducting the study?

Dr. Chilibeck of the College of Kinesiology at the University of Saskatchewan and Patrick Neary of the Faculty of Kinesiology and Health Studies at the University of Regina are conducting this study.

Why is this study being done?

This study is being done because the research team wants to determine the effectiveness of dark chocolate for improving performance during exercise at simulated altitude and for improving recovery from exercise. This will inform coaches and athletes if dark chocolate is an effective nutritional supplement for endurance performance at altitude.

Who can participate in this study?

You are eligible to participate in this study if you are male or female 18 years of age or older and are an experienced cyclist (i.e. bicycle exercise at a vigorous intensity on a regular basis and have above average aerobic fitness. A "vigorous intensity" means that you bicycle at an intensity that elicits heavy breathing. "Regular" is defined as at least three times per week for 60 minutes). The researchers will determine whether exercise testing is safe for you by having you fill out a brief questionnaire (the Get Active Questionnaire) after you have signed this consent form but before you do any exercise testing. If there is doubt about the safety of exercise participation for you based on this questionnaire, the researchers will require that you get clearance from a health care professional before you participate. You should not participate if you have any allergies to chocolate.

What does the study involve?

There will be 7 study visits to our lab. About half of the testing (study visits) will be done to evaluate one of the treatments (dark chocolate or the dark chocolate flavoured placebo). A month later, the other visits will be done to evaluate the opposite treatment.

Visit #1 will consist of an aerobic capacity test on a cycle ergometer (i.e. a stationary bike) to determine fitness level and eligibility for the study. This involves cycling at a progressively increasing workload until volitional fatigue. During the test, we will measure respiratory gases by having you breathe into a mouthpiece. The test will last approximately 10-15 minutes, depending on your fitness level.

Visit #2 will consist of assessing the reactivity of your blood vessels, testing for maximal knee extension strength, determination of fatigue of the knee extensors through repeated contractions, a second aerobic capacity test at a simulated altitude of 2500m, and a 10km time trial at simulated altitude. The assessment of your blood vessels includes reducing the blood flow in your arm for 5 minutes by inflating a blood pressure cuff, releasing it, and assessing the reaction of the blood vessels using ultrasound. The knee extension strength test will require that you exert as much force as possible on a machine with the front of your leg while in a seated position (i.e. knee extension). You will be asked to exert force for 5 seconds. You will take a 1-minute rest and then we will test the rate at which your quadriceps fatigue through 10 repeated maximal contractions. EMG stickers will be applied to your thigh in order to assess the electrical activity of your muscle.. The purpose of this test is to evaluate the amount of muscle fatigue you have from previous exercise. You will then do an aerobic cycling test at simulated altitude. The aerobic capacity test involves pedaling on a stationary bike with resistance increasing every minute until volitional fatigue insde a chamber that has reduced oxygen content (to simulate conditions at about 2,500 m altitude). The test takes about 10-15 minutes, depending on your fitness level. During the test, oxygen consumption is measured through a mouthpiece and the level of oxygen consumption is used as a measure of aerobic fitness. Visit #2 will also include a practice of a 10km time trial on a stationary bike, which will be the performance indicator in the chocolate testing. During the 10 km time trial, you will cover 10 km as fast as possible on a stationary bike, again inside the chamber with reduced oxygen content to simulate altitude. You will be able to see how much distance you have covered, but you will not know how much time has elapsed. This entire lab visit will last about 2 hours.
Visit #3 will consist of a familiarization trial using the cycling test used in the study. You will be asked to cycle for 90 minutes at a simulated altitude of 2500m on a stationary bike at an intensity corresponding to 60% of the maximal oxygen uptake reached during the aerobic capacity test done at simulated altitude (visit #2) followed by a 10km time trial, where you will cycle as fast as possible until you cover the equivalent of 10 km. You will be able to see the distance covered on the bike display, but you will not be able to see the speed you are cycling at. The familiarization trial is necessary to reduce the amount of variability across subsequent testing sessions (i.e. the subsequent testing sessions which compare dark chocolate to the control placebo chocolate). This lab visit will take about 105 minutes.
After visit #3, before you leave the lab after performing the exercises, you will be randomized (i.e. assigned by chance by a computer) to receive either dark chocolate or a dark chocolate flavoured placebo (i.e. a substance that looks and tastes like dark chocolate, but which contains none of the active ingredients found in dark chocolate). You will not know which chocolate you are receiving. The chocolate will be supplied to you in individual portions for each serving. This will be consumed twice a day (60g per per dose at breakfast and before going to bed) for 14 days. Two days before visit #4 (i.e. on the 13th and 14th day of the chocolate supplementation) we want you to try to minimize the amount of fruits, vegetables, tea, coffee, alcohol, chocolate, cereals, whole meal bread, and grains you consume because these foods contain some of the same beneficial ingredients as the dark chocolate. You will be given a food diary to record all foods and drinks you consume for these two days. These diaries will be photocopied and given back for the testing of the opposite condition (i.e. dark chocolate or chocolate flavoured placebo) in the next phase.
Visit #4 will occur on the 15th day of chocolate supplementation.

- You will come into the lab after a 10 hour fast.
- You will have your blood tested for lactate and glucose by finger prick sample
- You will be tested again for reactivity of your blood vessels
- You will be tested again for maximal isometric voluntary contraction and fatigue through repeated contractions
- You will then be given 60 grams of either dark chocolate or the dark chocolate flavored placebo 60 minutes before exercise testing. Neither you nor the researcher will know which chocolate you are receiving. The chocolate will be prepared by a different research assistant who is not involved with administering the exercise test.
- The exercise testing will involve 90 minutes of cycling at a simulated altitude of 2500m at 60% of the workload reached on the aerobic capacity test in visit #2, followed by a 10 km time trial where you will cover 10 km as fast as you can.

- After the time trial, you will engage in a 10 minute cool down followed by another test of maximal voluntary contraction and fatigue through repeated contractions
- The total amount of time for this study visit will be about 3 hours.
- Visit #5 to the lab will occur the next morning, again in a fasted state (at least 10 hours fasted). You will be given 60g of dark chocolate or dark chocolate flavored placebo to consume. Thirty minutes after consumption the chocolate, maximal voluntary contraction, and fatigue through repeated contractions will be determined.

Visits #6 and 7 will occur a month later after 14 days of dark chocolate or dark chocolate flavoured placebo supplementation (i.e. the opposite condition to what you received prior to visit #3). All testing will be identical to tests in visits #4 and 5
There will be a total of 12 participants in the study.
The total time for study visits is about 12 hours.

What are the benefits of participating in this study?

You will receive an assessment of your aerobic fitness. You will receive this after completion of visit 1. The results of the study will help athletes and coaches decide whether dark chocolate is beneficial for sport performance and recovery. These benefits are not guaranteed.

What are alternatives to the study?

You do not have to participate in this study to receive these benefits. You can receive an assessment of your aerobic fitness through appointments with a personal trainer through the Faculty of Kinesiology and Health Studies.

What are the possible risks and discomforts?

The exercise testing may cause some discomfort and there is a small risk of injury. Trained personnel will administer all testing to minimize the risks.
There may be risk of feeling faint or nauseous when exercising in a fasted state.
There may be risk of feeling faint or nauseous when exposed to simulated altitude.

What happens if I decide to withdraw?

Your participation in this research is voluntary. You may withdraw from this study at any time. You do not have to provide a reason. Your relationships with the researchers or the university will not be affected.
If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment will be retained for analysis.

What happens if something goes wrong?

If an adverse event related to the study occurs, trained staff will be available throughout the conduct of the study who can respond immediately. Necessary medical treatment will be made available at no additional cost to you. As soon as possible, notify the research team. By signing this document, you do not waive any of your legal rights.

What happens after completion of the study?

The researchers will inform you of the overall study results, as well as your individual results, after they have analyzed all data (approximately April 2019) by sending you an email. You will receive results of your test of maximal aerobic capacity the same day of the test.

What will the study cost me?

You will not be charged for the chocolate or any research-related procedures.

Will my Study Data be Kept Confidential?

In Saskatchewan, the Health Information Protection Act (HIPA) defines how the privacy of your personal health information must be maintained so that your privacy will be respected. The study data will be stored securely (in a locked cabinet contained within a locked office under the supervision of the PI) by the study team for a minimum of 5 years after the final results are published. Research records identifying you may be inspected by the University of Saskatchewan Biomedical Research Ethics Board for quality assurance and monitoring purposes.
It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be disclosed.

Who do I contact if I have questions about the study?

If you have any questions or desire further information about this study before or during participation, you can contact Philip Chilibeck at 306-966-1072 or phil.chilibeck@usask.ca
If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, contact the Chair of the University of Saskatchewan Research Ethics Board, at 306-*966-2975 (out of town calls 1-888-966-2975).* The Research Ethics Board is a group of individuals (scientists, physicians, ethicists, lawyers and members of the community) that provide an independent review of human research studies. This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Research Ethics Board.

CONSENT TO PARTICIPATE

- I have read the information in this consent form.
- I understand the purpose and procedures and the possible risks and benefits of the study.
- I have been informed of the alternatives to the study.
- I was given sufficient time to think about it.
- I had the opportunity to ask questions and have received satisfactory answers.
- I am free to withdraw from this study at any time for any reason and the decision to stop taking part will not affect my future relationships at the university.
- I agree to follow the principal investigator's instructions and will tell the principal investigator at once if I feel I have had any unexpected or unusual symptoms.
- I have been informed there is no guarantee that this study will provide any benefits to me.
- I understand that by signing this document I do not waive any of my legal rights.
- I will be given a signed and dated copy of this consent form.

Printed name of participant: _____

Signature _____ Date_____

Printed name of person obtaining consent: _____
Signature _____ Date___