Article Title: The influence of different concentrations of flavanol chocolate bars under acute supplement conditions on exercise and performance.

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ABSTRACT

Objective: The purpose of this study was to assess the effects an acute dosage of different flavanol concentrations in a dark chocolate bar has on physiological parameters during steady state (SS) and incremental exercise.

Methods: In a double-blind, randomised, crossover study, fifteen healthy participants with a mean ± SD age of 30 ± 7 years; stature 176.8 ± 8.6 cm and body mass 80.3 ± 8.4 kg supplemented with high flavanol (HF) (1060mg), moderate flavanol (MF) (746mg), low flavanol (LF) (406mg) or a control (CON) (88mg) chocolate bar (~34 grams), 2 hours prior to 40 minutes of SS cycling (80% gas exchange threshold) followed by an incremental test to volitional fatigue. During the SS cycle oxygen consumption ($\dot{V}O_2$), respiratory exchange ratio (RER) and heart rate (HR) were continuously monitored. Plasma samples were collected prior to commencing exercise to determine nitrate (NO_3^-) and nitrite (NO_2^-) levels under each condition.

Results: There was no observed effect between flavanol concentrations on $\dot{V}O_2$, RER and HR during SS cycling (P>0.05). $\dot{V}O_{2\,peak}$, peak power, HR peak and RER peak also did not significantly differ between conditions (P>0.05). There was a small trend for higher plasma NO_2^- levels following higher flavanol concentration, however this did not reach statistical significance (P>0.05).

Conclusion: Acute supplementation with cocoa of differing flavanol concentrations does not appear to have any effect on exercise and performance. It is plausible that longer flavanol supplementation periods might have greater accumulative effects and thus may potentially elicit a larger effect.

Keywords: dark chocolate; nitric oxide; flavanol; nitrite; cycling.

ABBREVATIONS

BP	Blood pressure
CF	Cocoa flavanols
CON	Control
DC	Dark chocolate
EC	(-)-epicatechin
eNOS	Endothelial nitric oxide synthase
FMD	Flow mediated dilation
GET	Gas exchange threshold
HF	High flavanol
HR	Heart rate
LF	Low flavanol
MF	Moderate flavanol
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NOS	Nitric oxide synthase
RER	Respiratory exchange ratio
SS	Steady state
TT	Time trials
VCO_2	Volume of exhaled carbon dioxide
V_E	Ventilation
$\dot{V}O_2$	Volume of oxygen consumption
$\dot{V}O_{2\,peak}$	Peak oxygen consumption

INTRODUCTION

Regular consumption of cocoa particularly rich in flavanols has been associated with a reduction in the risk of cardiovascular morbidity, mortality and can also contribute towards the prevention of other chronic diseases (Al-Dashti et al. 2018; Lovegrove et al. 2017). The consumption of cocoa flavanols (CF) has been reported to induce acute and sustained improvements in endothelium-dependent vasodilation in healthy participants and especially in those with an increased cardiovascular risk (Balzer et al. 2008; Engler et al. 2004; Fisher et al. 2003; Fisher et al. 2012; Grassi et al. 2005a; Grassi et al. 2005b; Sudarma et al. 2011; Taubert et al. 2007). These effects have been ascribed, at least in part, to CFs capacity to enhance the bioavailability and bioactivity of nitric oxide (NO). The monomeric portion of CF, (-)-epicatechin (EC) and catechin and to a lesser extent the polymeric, procyanidins, are linked to blood pressure and vasoactive effects (Schroeter et al. 2006). Modern processing of cacao diminishes flavanol content and often negatively influences the EC/catechin ratio (Hurst et al. 2011). Moreover, the optimal quantity of chocolate required to elicit improvements in vasodilation has not been established, with studies supplementing between 6.3g/day to 100g/day of chocolate (Engler et al. 2004; Grassi et al. 2005a; Sudarma et al. 2011; Taubert et al. 2007). Thus, it is crucial to compare the concentrations of flavanol rather than simply the quantity of chocolate administered in clinical trials.

In a recent review investigating the effects of CF on blood pressure, flavanol enriched cocoa concentrations ranged between 30 to 1218mg (mean = 670mg), with trials supplementing for between two hours and eighteen weeks. Despite the vascular properties of CF being linked with the formation of endothelial NO, many studies consistently lack

reporting NO biomarkers, thus, making it challenging to determine the optimal CF concentration which elicits an optimal vascular effect.

Given that NO mediates blood flow and mitochondrial respiration during exercise, it has been postulated that enhanced NO production following CF supplementation may promote greater oxygen and nutrient delivery to active muscles, as well as improve mitochondrial efficiency, leading to improvement in the tolerance to steady state (SS) exercise. In mice, EC consumption has been reported to increase exercise performance and muscle fatigue following enhanced capillarity (Nogueira et al. 2011). However, the research within humans is limited; Decroix et al. (2017) found EC peaked two hours after a single acute dose of 900mg of CF and resulted in a significantly higher peak power during the first of two time trials (TT). Overall completion times tended to be quicker, although this did not reach statistical significance (P=0.09). Another study used acute supplementation of dark chocolate (DC) (100g containing 246.8mg of flavanol) and demonstrated a better maintenance of plasma glucose (Davison et al. 2012) over 2.5h of cycling ($\sim$60% $\dot{V}O_{2\,max}$, $\dot{V}O_{2\,max}$: 53.1 ± 1.9 ml/kg/min), however performance was not measured. Intriguingly chronic DC consumption of 40g (twice daily, total of 197.2mg of flavanol per day) for two weeks resulted in a 21% increase in plasma free fatty acids during prolonged exercise compared with an iso-fat–calorie–carbohydrate control (Allgrove et al. 2011), but these effects were not associated with enhanced exercise performance. More recently, we demonstrated two-weeks consumption of DC (259mg flavanol) led to a significant increase in gas exchange threshold (GET) and time trial performance (Patel et al. 2015), but the study design was limited by the lack of an appropriate placebo condition and our results should be viewed with caution.

To date, there is limited information on the optimal concentration of flavanol needed to elicit changes in NO bioavailability and whether CFs have an ergogenic effect on moderately trained participants during SS and incremental intensity exercise. Moreover, as most athletes would arguably prefer to use an acute supplementation strategy rather than chronic and EC peaks after two hours, the present study aimed to investigate whether acute (2 hours pre-exercise) supplementation with chocolate containing high to low concentrations of flavanol (88mg, 406mg, 746mg and 1060mg) could have an effect on exercise performance. We hypothesise that higher concentrations of flavanol will have an effect on the physiological variables measured during steady state, incremental and nitric oxide biomarkers.

METHODS

Participants

Fifteen healthy participants (10 males and 5 females; with a mean ± SD, age of 30 ± 7; stature 176.8 ± 8.6cm and body mass 80.3 ± 8.4 kg) were recruited to participate in the present study. All participants provided written informed consent, completed health screening and a physical activity questionnaire (PARQ). The group comprised recreationally active participants who regularly cycled for pleasure and the occasional sportive ($\dot{V}O_{2\,peak}$; Males 51.1 ± 3.5, Females 41.6 ± 5.5 ml/kg/min). Participants were advised to maintain the same level of physical activity throughout the duration of the study. Participants were excluded from the study if they met any of the following criteria: (1) <18 years or > 50years, (2) hypertension, (3) cardiovascular disease, (4) smokers or history of smoking and (5) habitual supplementation. The study commenced after approval by the

Ethics Committee at Kingston University (Ethics code 1415/005) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Procedure/Experimental Design

Dietary control

Participants were asked to maintain their normal diet and refrain from alcohol, vitamin supplements and anti-inflammatory products. Throughout the intervention period participants were asked to refrain from consuming any form of chocolate. At no given time were participants advised to reduce daily fruit and vegetable consumption between conditions. A list of high nitrate and polyphenolic fruits and vegetables was given to participants to avoid and replace with appropriate substitutes for the 24h prior to exercise testing. Participants were required to follow a similar diet throughout the intervention and replicate their diet the day prior to testing. All tests were carried out following a 12h overnight fast between 7-10 am. In most instances, participants consumed their DC condition on-site. For those participants that were unable to attend the laboratory 2hrs prior to testing, they were provided with the DC bar the night before for consumption at a precise time. Participants were then contacted at the time of consumption via telephone to ensure full compliance with the supplementation strategy. All participants self-reported adherence to the intake of all four conditions.

Blood pressure

Resting blood pressure (BP) was measured on all supplementation visits using an automated sphygmomanometer (Omron M4 Digital, Omron Healthcare, Wegelaan, Netherlands). Participants were rested supine for a minimum of 10minutes before three measurements were taken, each separated by 3 minutes, with the mean value being taken.

Plasma nitrite (NO_2^-) and nitrate (NO_3^-) analysis

Blood plasma samples were drawn using a 21g butterfly needle into EDTA BD vacutainer 2h following supplementation, immediately prior to exercise testing. Samples were centrifuged at 4000rcf at 4°C for 10 minutes within 3 minutes of collection. The plasma was subsequently extracted and immediately flash frozen at -80°C for later analysis. Analysis of NO_3^- and NO_2^- was performed via ozone-based chemiluminescence (Rogers et al. 2005)

Exercise protocols

Participants were asked not to perform intense exercise in the 48h prior to testing and refrain from any exercise in the 24h preceding the tests. The exercise protocols involved five separate visits to the laboratory. On the first visit participants performed an incremental test to failure to determine $\dot{V}O_{2\,peak}$ and gas exchange threshold (GET). The incremental test comprised a 5-minute warm up at 50W at a self-selected cadence (between 60 – 90 rpm) after which the work rate was increased at 30W min^{-1} until the participants reached volitional failure or the cadence fell below 50 rpm. Pulmonary gas exchange was collected breath-by-breath (Oxycon Pro, VIASYS GmbH, Eric Jaeger, Hoechberg, Germany) during the incremental tests and averaged over consecutive 15-s periods and used to establish gas

exchange threshold (GET). Gas exchange threshold was determined as the work rate corresponding at the precise cross over, at which V_E/VO_2 continually exceed V_E/VCO_2.

A double-blinded randomised crossover design was employed, which required four further visits to the laboratory, each separated by a minimum of a 7 day wash out period. Participants were randomly assigned acute (2hour) supplementation with one of four possible dark chocolate (DC) supplementation conditions. Bars of chocolate were specifically manufactured to the following specifications (~34g); high flavanol (HF, 1060mg), moderate flavanol (MF, 746mg), Low flavanol (LF, 406mg) or a control (CON, 88mg). The DC was calorifically matched and contained similar levels of theobromine, the only variation between conditions were the cocoa powder flavanol concentration used to make up each DC condition.

The full exercise protocol comprised a two-minute incremental warmup to a constant work rate corresponding to 80% of their GET and which was maintained for forty minutes. This was immediately followed by ten minutes of static seated rest (recovery phase), during which blood pressure was measured at the third and fifth minute. $\dot{V}O_2$, RER and HR were continuously measured and averaged over the SS cycle and the recovery phase. The exercise test was completed with an incremental test to failure, as conducted during visit one. $\dot{V}O_{2\,peak}$ was taken as the highest 30-s average value attained before the participant reached failure. The power output reached at the point of fatigue was recorded as the peak power output and HR max and RER max were taken as the highest values prior to the point of failure.

Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS v.21). To examine for difference between the four conditions, dependent variables were first analysed for normality. As all data met parametric assumptions, a within subject repeated measure analysis of variance (ANOVA) was utilised. Significance was accepted at $P<0.05$. When significance was found a post-hoc Tukey for within conditions was employed to identify the differences that occurred.

RESULTS

Steady state and incremental cycling

$\dot{V}O_2$, RER and HR values in response to steady state cycling after consumption of all conditions are reported in Table 1, there was no significant difference between conditions ($P>0.05$). Furthermore, during the incremental cycle test, $\dot{V}O_{2\,peak}$, peak power, HR peak and RER max did not significantly differ between all conditions ($P>0.05$).

Table 1. Pulmonary gas exchange measures during SS and incremental cycling after HF, MF, LF and CON intake.

	HF	MF	LF	CON	Main effect *p* value
			Steady state exercise		
$\dot{V}O_2$ (L•min^{-1})	1.84 ± 0.66	1.84 ± 0.68	1.85 ± 0.66	1.84 ± 0.64	>0.05
RER	0.89 ± 0.03	0.9 ± 0.03	0.89 ± 0.03	0.89 ± 0.02	>0.05
HR (BPM)	130 ± 4	132 ± 4	130 ± 5	131 ± 5	>0.05
			Incremental exercise		
$\dot{V}O_{2\ peak}$ (L•min^{-1})	3.95 ± 0.97	3.99 ± 1.12	4 ± 1.12	3.86 ± 1	>0.05
Peak power (W)	280 ± 43	278 ± 51	284 ± 52	274 ± 48	>0.05
RER max	1.22 ± 0.05	1.22 ± 0.04	1.21 ± 0.05	1.21 ± 0.03	>0.05
HR peak (BPM)	180 ± 4	180 ± 5	180 ± 5	180 ± 4	>0.05

Values are means ± SD *Note*. SS= steady state, RER = respiratory exchange ratio, HR = heart rate, HF = high flavanol, MF = moderate flavanol, LF = low flavanol and CON = control.

Post exercise blood pressure and cardiorespiratory measurements

Systolic and diastolic blood pressure was assessed at the third and fifth minute during the recovery phase immediately after SS cycling for each condition (Table 2). There were no significant differences between conditions for systolic or diastolic blood pressure in the third and fifth minute of the resting recovery phase (P>0.05). There was no significant difference between conditions for $\dot{V}O_2$, RER or HR values during the recovery phase (P>0.05, Table 2).

Table 2. $\dot{V}O_2$ and HR responses averaged over the ten-minute recovery phase. Mean changes in SBP and DBP assessed at the third and fifth minute during the recovery phase immediately after SS cycling.

	HF	MF	LF	CON	Main effect *p* value
$\dot{V}O_2$ (L•min^{-1})	0.70 ± 0.2	0.7 ± 0.21	0.71 ± 0.21	0.71 ± 0.19	>0.05
HR (BPM)	67 ± 5	66 ± 5	68 ± 5	67 ± 5	>0.05
SBP (mmHg)					
3rd min	143 ± 4	140 ± 4	140 ± 4	139 ± 5	>0.05
5th min	133 ± 3	132± 4	133 ± 5	131 ± 4	>0.05
DBP (mmHg)					
3rd min	86 ± 4	85 ± 4	86 ± 4	87 ± 4	>0.05
5th min	83 ± 5	82± 5	82 ± 5	83 ± 4	>0.05

Values are means ± SD. *Note*. RER = respiratory exchange ratio; DBP = diastolic blood pressure, SBP = systolic blood pressure, HR = heart rate, HF = high flavanol, MD = moderate flavanol, LF = low flavanol and CON = control.

Resting blood pressure

Systolic blood pressure following consumption of the control tended to be higher, although this was not necessarily in a dose dependent manner (P=0.1) (refer to Fig. 1). There were no clear trends observed between conditions for diastolic Blood Pressure (DBP) (P=0.19) (refer to Fig. 1). Mean arterial pressure (not presented) was not significantly different between conditions (P=0.35).

Pre-exercise plasma NO_2^- and NO_3^-

Pre-exercise plasma NO_3^- and NO_2^- levels were measured 2h after supplementation, with no significant difference between conditions (*P*>0.05), (Fig. 2).

DISCUSSION

The main finding of this study was acute supplementation with dark chocolate (DC) of differing flavanol concentrations 2h prior to exercise has no ergogenic effect on exercise performance in recreationally trained participants. Notably, plasma nitrite (NO_2^-) tended to be greater after higher flavanol dosages (refer to Fig. 2), although this did not reach the

statistical probability threshold. Similarly, systolic blood pressure tended to be lower after higher flavanol dosages, but also did not reach the threshold for significance.

Effects of dietary flavanol supplementation on the physiological responses to maximal and submaximal exercise.

In this study acute intake of different levels of CFs did not alter $\dot{V}O_2$, RER or HR during submaximal SS cycling. Furthermore, acute supplementation with CFs did not have an effect on $\dot{V}O_{2\,peak}$, peak power, HR peak and RER max during maximal cycling. This suggests that acute flavanol intake of concentrations between 88mg and 1060mg taken 2h prior to exercise has no ergogenic effect on performance in recreationally trained participants. These findings are in line with those of Davison et al. (2012) , who observed no change in HR, RER and $\dot{V}O_2$ during cycling for 2.5 h of cycling at 60% $\dot{V}O_{2\,max}$ after supplementation with 247mg flavanol. Similarly, Stellingwerff et al. (2014) reported no change in performance or recovery following 240mg of flavanols compared to a cocoa-depleted control. However, this was most likely because EC metabolites had appreciably decreased to 65% of peak concentrations on commence of the 15min TT, thus NO levels would likely be less than optimal (Stellingwerff et al. 2014). Similarly, in the present study, the incremental test commenced ~3h after supplementation and thus might have not corresponded to the optimal NO level.

Decroix et al. (2017) also reported no influence of acute intake of 900mg of flavanol compared to placebo on time to competition of repeated time trials. However, unlike Stellingwerff et al. (2014) the time trial commenced at 100 min after ingestion of CFs, which coincided with peak plasma EC. Furthermore, this was accompanied with no change

in NO biomarkers between conditions. The participants training status in the present study was considerably lower compared to Decroix et al. (2017) (Present study - Males 51.1 ± 3.5, Females 41.6 ± 5.5 Vs. 63 ± 3.5 ml/kg/min), indicating acute supplementation of CFs may also not appear to significantly improve performance or NO production in recreationally active participants. The beneficial effects on exercise following the enhancement via nitrate rich beetroot juice are typically observed after a 2-fold increase in plasma nitrite compared to baseline (Bailey et al. 2016; Wylie et al. 2013). Therefore, the lack of improvements in plasma nitrite observed in the present study and aforementioned may likely be insufficient to evoke any demonstrable physiological effects on performance (Decroix et al. 2017).

It is important to consider the population used within the present study. All regularly participated in physical activity and were considered healthy and moderately trained. Regular training has been associated with optimised NO production, vascular function, energy metabolism and antioxidant (Green et al. 2004). There is growing literature to support short-term/sub-chronic consumption of high flavanols doses daily, which may potentially accumulate and enhance measures of endothelial functioning, such as flow mediated dilation (FMD); an effect that has been shown to be dependent on local NO generation (Cooper et al. 2008; Heiss et al. 2007). Longer, 3-month intake of CFs (175mg) have been reported to increased $\dot{V}O_{2\,max}$ and max watts in sedentary participants compared to a placebo, which were attributed to enhanced mitochondrial function and reduced oxidative stress (Taub et al. 2016). It is likely that acute supplementation with CFs regardless of the concentration may be too small of an effect to actually improve exercise performance, particularly within healthy exercising populations.

Effects of dietary flavanol supplementation on blood plasma NO_3^-, NO_2^-.

The signalling molecule nitric oxide (NO) is produce by NO synthase (NOS) family of enzymes, which are responsible for catalysing the oxidation of L-arginine, yielding NO and L-citrulline (Jones 2014). The *in vivo* quantification of NO is technically challenging: therefore changes in the plasma concentration of the oxidation products of NO, namely NO_2^- and NO_3 are often measured to provide an indication of NO production (Bailey et al. 2010).

This study found a linear trend for higher NO_2^- with higher flavanol concentrations. Consumption of HF led to a 1, 9 and 13% increase compared to MF, LF and the CON chocolate respectively. However, no acute flavanol concentration corresponded with a significant increase in plasma NO_2^- or NO_3^-. Plasma NO_2^- has been reported to better reflect region endothelial nitric oxide synthase (eNOS) activity compared to NO_3^- (Lauer et al. 2001). This is of interest as the exact mechanistic pathway by which CFs exerts its effect is not fully understood but postulated to be the increase in eNOS-dependent NO production (Schewe et al. 2008). Most potential explanations for the increase in eNOS-derived NO levels, include the modulation in eNOS activity, a change in eNOS substrate availability or reduction in the elimination of NO levels (Schewe et al. 2008; Schnorr et al. 2008; Steffen et al. 2007). Several well controlled studies have shown acute dietary intervention with flavanols can provide beneficial cardiovascular and peripheral effects as a consequence of flavanol-induced increases in circulating NO pool as reflected by an improvement in endothelial function (Fisher et al. 2003; Heiss et al. 2003; Heiss et al. 2005; Schroeter et al. 2006). However, many of these studies have used populations with reduced

eNOS or endothelial functioning such as patients who are diabetic, overweight, obese, hypertensive, prehypertensive, have coronary artery disease, endothelial dysfunction or have higher cardiovascular risk factors (Balzer et al. 2008; Davison et al. 2008; Farouque et al. 2006; Grassi et al. 2005b; Lin et al. 2016). The mechanism currently purposed is likely to have a greater effect in patients with an increased cardiovascular risk, as eNOS activity is reduced compared to that of healthy participants. Moreover, studies have demonstrated that cocoa enriched with flavanols improved measures of endothelial function to a greater degree in healthy elderly populations than in younger population (Fisher and Hollenberg 2006; Heiss et al. 2005; Nehlig 2013). This may be as ageing is related to attenuation and in some circumstances the complete loss of endothelium-dependent vasodilation (Nehlig 2013).

Effects of dietary flavanol supplementation on blood pressure.

The blood pressure lowering effects of CFs have been reported by many studies (Hollenberg et al. 2009; Ried et al. 2017; Ried et al. 2010). Blood pressure was marginally influenced by various dosages of CFs concentrations; however, these effects were very limited, clearly indicating a single acute dosage of high CFs have minimal effects on attenuating blood pressure in healthy active participants. Blood pressure lowering effects are significantly stronger in diseased populations and within patients with higher baseline blood pressure (Desch et al. 2010; Lin et al. 2016; Ried et al. 2010). Several studies have failed to show significant blood pressure lowering effects of flavanols in healthy young participants (Desch et al. 2010; Lin et al. 2016; Ried et al. 2010). Some studies have demonstrated the capacity for flavanol to attenuate blood pressure in individuals who have

been deemed healthy, however these are often in older, overweight or smokers who are still likely to have a less than optimal endothelial function (Berry et al. 2010; Heiss and Kelm 2010).

In conclusion, given that CFs intake did not affect cardiorespiratory and blood plasma variables during SS and maximal cycling, this study failed to support our hypothesis. It appears that within healthy moderately trained participants, the acute addition of various concentrations of flavanol DC cannot aid in improving blood plasma NO_3^- and NO_2^- or enhance exercise capacity and performance. Many studies support the potential accumulation of flavanol metabolites if absorbed in high doses daily (Heiss et al. 2007). Thus, we propose that acute CF supplementation strategies are ineffective and do not provide an ergogenic effect, therefore, further research should focus on short term and chronic CF supplementation for enhancing NO bioavailability and exercise capacity in a healthy and active population.

Acknowledgments

This research was not supported by any external funding/grant. The authors would like to thank professor Roger Corder for his assistance with sourcing the flavanol conditions and Barry Callebaut for manufacturing all four chocolate conditions.

Authors Contribution

Conceptualization, R.K.P., J.B. and O.S; Methodology, Investigation, Writing – Original Draft Preparation, R.K.P; Writing – Review & Editing, J.B. and O.S.

Conflict of interest

There are not actual or potential conflicts of interest for any of the authors.

REFERENCES

Al-Dashti YA, Holt RR, Stebbins CL, Keen CL, Hackman RM (2018) Dietary Flavanols: A Review of Select Effects on Vascular Function, Blood Pressure, and Exercise Performance J Am Coll Nutr:1-15 doi:10.1080/07315724.2018.1451788

Allgrove J, Farrell E, Gleeson M, Williamson G, Cooper K (2011) Regular dark chocolate consumption's reduction of oxidative stress and increase of free-fatty-acid mobilization in response to prolonged cycling Int J Sport Nutr Exerc Metab 21:113-123

Bailey SJ, Blackwell JR, Williams E, Vanhatalo A, Wylie LJ, Winyard PG, Jones AM (2016) Two weeks of watermelon juice supplementation improves nitric oxide bioavailability but not endurance exercise performance in humans Nitric Oxide 59:10-20 doi:10.1016/j.niox.2016.06.008

Bailey SJ, Winyard PG, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Jones AM (2010) Acute L-arginine supplementation reduces the O2 cost of moderate-intensity exercise and enhances high-intensity exercise tolerance J Appl Physiol (1985) 109:1394-1403 doi:10.1152/japplphysiol.00503.2010

Balzer J et al. (2008) Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients a double-masked, randomized, controlled trial J Am Coll Cardiol 51:2141-2149 doi:10.1016/j.jacc.2008.01.059

Berry NM, Davison K, Coates AM, Buckley JD, Howe PR (2010) Impact of cocoa flavanol consumption on blood pressure responsiveness to exercise Br J Nutr 103:1480-1484 doi:10.1017/S0007114509993382

Cooper KA, Donovan JL, Waterhouse AL, Williamson G (2008) Cocoa and health: a decade of research Br J Nutr 99:1-11 doi:10.1017/S0007114507795296

Davison G, Callister R, Williamson G, Cooper KA, Gleeson M (2012) The effect of acute pre-exercise dark chocolate consumption on plasma antioxidant status, oxidative stress and immunoendocrine responses to prolonged exercise Eur J Nutr 51:69-79 doi:10.1007/s00394-011-0193-4

Davison K, Coates AM, Buckley JD, Howe PR (2008) Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects Int J Obes (Lond) 32:1289-1296 doi:10.1038/ijo.2008.66

Decroix L, Soares DD, Meeusen R, Heyman E, Tonoli C (2018) Cocoa Flavanol Supplementation and Exercise: A Systematic Review Sports Med 48:867-892 doi:10.1007/s40279-017-0849-1

Decroix L et al. (2017) Acute cocoa Flavanols intake has minimal effects on exercise-induced oxidative stress and nitric oxide production in healthy cyclists: a randomized controlled trial J Int Soc Sports Nutr 14:28 doi:10.1186/s12970-017-0186-7

Desch S et al. (2010) Effect of cocoa products on blood pressure: systematic review and meta-analysis Am J Hypertens 23:97-103 doi:10.1038/ajh.2009.213

Engler MB et al. (2004) Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults J Am Coll Nutr 23:197-204

Farouque HM, Leung M, Hope SA, Baldi M, Schechter C, Cameron JD, Meredith IT (2006) Acute and chronic effects of flavanol-rich cocoa on vascular function in subjects with coronary artery disease: a randomized double-blind placebo-controlled study Clin Sci (Lond) 111:71-80 doi:10.1042/CS20060048

Fisher ND, Hollenberg NK (2006) Aging and vascular responses to flavanol-rich cocoa J Hypertens 24:1575-1580 doi:10.1097/01.hjh.0000239293.40507.2a

Fisher ND, Hughes M, Gerhard-Herman M, Hollenberg NK (2003) Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans J Hypertens 21:2281-2286 doi:10.1097/01.hjh.0000084783.15238.eb

Fisher ND, Hurwitz S, Hollenberg NK (2012) Habitual flavonoid intake and endothelial function in healthy humans J Am Coll Nutr 31:275-279

Grassi D, Lippi C, Necozione S, Desideri G, Ferri C (2005a) Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons Am J Clin Nutr 81:611-614 doi:10.1093/ajcn/81.3.611

Grassi D et al. (2005b) Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives Hypertension 46:398-405 doi:10.1161/01.HYP.0000174990.46027.70

Green DJ, Maiorana A, O'Driscoll G, Taylor R (2004) Effect of exercise training on endothelium-derived nitric oxide function in humans J Physiol 561:1-25 doi:10.1113/jphysiol.2004.068197

Heiss C, Dejam A, Kleinbongard P, Schewe T, Sies H, Kelm M (2003) Vascular effects of cocoa rich in flavan-3-ols JAMA 290:1030-1031 doi:10.1001/jama.290.8.1030

Heiss C, Finis D, Kleinbongard P, Hoffmann A, Rassaf T, Kelm M, Sies H (2007) Sustained increase in flow-mediated dilation after daily intake of high-flavanol cocoa drink over 1 week J Cardiovasc Pharmacol 49:74-80 doi:10.1097/FJC.0b013e31802d0001

Heiss C, Kelm M (2010) Chocolate consumption, blood pressure, and cardiovascular risk Eur Heart J 31:1554-1556 doi:10.1093/eurheartj/ehq114

Heiss C, Kleinbongard P, Dejam A, Perre S, Schroeter H, Sies H, Kelm M (2005) Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers J Am Coll Cardiol 46:1276-1283 doi:10.1016/j.jacc.2005.06.055

Hollenberg NK, Fisher ND, McCullough ML (2009) Flavanols, the Kuna, cocoa consumption, and nitric oxide J Am Soc Hypertens 3:105-112 doi:10.1016/j.jash.2008.11.001

Hurst WJ, Krake SH, Bergmeier SC, Payne MJ, Miller KB, Stuart DA (2011) Impact of fermentation, drying, roasting and Dutch processing on flavan-3-ol stereochemistry in cacao beans and cocoa ingredients Chem Cent J 5:53 doi:10.1186/1752-153X-5-53

Jones AM (2014) Dietary nitrate supplementation and exercise performance Sports Med 44 Suppl 1:S35-45 doi:10.1007/s40279-014-0149-y

Lauer T, Preik M, Rassaf T, Strauer BE, Deussen A, Feelisch M, Kelm M (2001) Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action Proc Natl Acad Sci U S A 98:12814-12819 doi:10.1073/pnas.221381098

Lin X, Zhang I, Li A, Manson JE, Sesso HD, Wang L, Liu S (2016) Cocoa Flavanol Intake and Biomarkers for Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials J Nutr 146:2325-2333 doi:10.3945/jn.116.237644

Lovegrove JA, Stainer A, Hobbs DA (2017) Role of flavonoids and nitrates in cardiovascular health Proc Nutr Soc:1-13 doi:10.1017/S0029665116002871

Nehlig A (2013) The neuroprotective effects of cocoa flavanol and its influence on cognitive performance Br J Clin Pharmacol 75:716-727 doi:10.1111/j.1365-2125.2012.04378.x

Nogueira L et al. (2011) (-)-Epicatechin enhances fatigue resistance and oxidative capacity in mouse muscle J Physiol 589:4615-4631 doi:10.1113/jphysiol.2011.209924

Patel RK, Brouner J, Spendiff O (2015) Dark chocolate supplementation reduces the oxygen cost of moderate intensity cycling J Int Soc Sports Nutr 12:47 doi:10.1186/s12970-015-0106-7

Ried K, Fakler P, Stocks NP (2017) Effect of cocoa on blood pressure Cochrane Database Syst Rev 4:CD008893 doi:10.1002/14651858.CD008893.pub3

Ried K, Sullivan T, Fakler P, Frank OR, Stocks NP (2010) Does chocolate reduce blood pressure? A meta-analysis BMC Med 8:39 doi:10.1186/1741-7015-8-39

Rogers SC, Khalatbari A, Gapper PW, Frenneaux MP, James PE (2005) Detection of human red blood cell-bound nitric oxide J Biol Chem 280:26720-26728 doi:10.1074/jbc.M501179200

Schewe T, Steffen Y, Sies H (2008) How do dietary flavanols improve vascular function? A position paper Arch Biochem Biophys 476:102-106 doi:10.1016/j.abb.2008.03.004

Schnorr O, Brossette T, Momma TY, Kleinbongard P, Keen CL, Schroeter H, Sies H (2008) Cocoa flavanols lower vascular arginase activity in human endothelial cells in vitro and in erythrocytes in vivo Arch Biochem Biophys 476:211-215 doi:10.1016/j.abb.2008.02.040

Schroeter H et al. (2006) (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans Proc Natl Acad Sci U S A 103:1024-1029 doi:10.1073/pnas.0510168103

Steffen Y, Schewe T, Sies H (2007) (-)-Epicatechin elevates nitric oxide in endothelial cells via inhibition of NADPH oxidase Biochem Biophys Res Commun 359:828-833 doi:10.1016/j.bbrc.2007.05.200

Stellingwerff T et al. (2014) The effect of acute dark chocolate consumption on carbohydrate metabolism and performance during rest and exercise Appl Physiol Nutr Metab 39:173-182 doi:10.1139/apnm-2013-0152

Sudarma V, Sukmaniah S, Siregar P (2011) Effect of dark chocolate on nitric oxide serum levels and blood pressure in prehypertension subjects Acta Med Indones 43:224-228

Taub PR et al. (2016) Beneficial effects of dark chocolate on exercise capacity in sedentary subjects: underlying mechanisms. A double blind, randomized, placebo controlled trial Food Funct 7:3686-3693 doi:10.1039/c6fo00611f

Taubert D, Roesen R, Lehmann C, Jung N, Schomig E (2007) Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial JAMA 298:49-60 doi:10.1001/jama.298.1.49

Wylie LJ et al. (2013) Beetroot juice and exercise: pharmacodynamic and dose-response relationships J Appl Physiol (1985) 115:325-336 doi:10.1152/japplphysiol.00372.2013

FIGURE LEGENDS

Figure 1. Resting systolic blood pressure (SBP) (**A**) and diastolic blood pressure (DBP) (**B**) taken 2h after intake of high flavanol (HF), moderate flavanol (MF), low flavanol (LF) and control (CON). Values are means ± SD.

Figure 2. Plasma nitrite (NO_2^-)(**A**) and nitrate (NO_3^-) (**B**) taken 2h after intake of high flavanol (HF), moderate flavanol (MF), low flavanol (LF) and control (CON) for all participants; values are means ± SEM.

TABLE LEGENDS

Table 3. Pulmonary gas exchange measures during steady state (SS) and incremental cycling after high flavanol (HF), moderate flavanol (MF), low flavanol (LF) and control (CON) intake.

Table 4. $\dot{V}O_2$ and heart rate responses averaged over the ten-minute recovery phase. Mean changes in systolic blood pressure (SBP) and diastolic blood pressure (DBP) assessed at the third and fifth minute during the recovery phase immediately after steady state cycling.