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Brachial and Cerebrovascular Functions Are Enhanced in Postmenopausal Women after Ingestion of Chocolate with a High Concentration of Cocoa.

ENHANCED BRACHIAL AND CEREBROVASCULAR FUNCTION IN POSTMENOPAUSAL WOMEN FOLLOWING INGESTION OF HIGH CACOA CONCENTRADED CHOCOLATE

Channa E Marsh[1*], Howard H Carter[1*], Kym J Guelfi[1], Kurt J Smith[1], Kerryn E Pike[2], Louise H Naylor[1], Daniel J Green[1,3,4]

[1]School of Sport Science, Exercise and Health, University of Western Australia, Crawley, Western Australia
[2]School of Psychology & Public Health, La Trobe University, Melbourne, Victoria
[3]Research Institute for Sport and Exercise Science, Liverpool John Moores University, Liverpool, United Kingdom
[4]Principal Research Fellow, National Health and Medical Research Council, Australia
*These authors contributed equally and should be considered joint first authors

Marsh, Carter, Guelfi, Smith, Pike, Naylor, Green

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Corresponding Author
Winthrop Professor Daniel J Green, 35 Stirling Hwy, School of Sports Science, Exercise and Health, The University of Western Australia, Crawley, Western Australia, 6009
Phone: +61 (8) 6488 2378, Fax: +61 (8) 6488 1039, Email: danny.green@uwa.edu.au

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Abbreviations: WC, white chocolate; MC, milk chocolate; DC, dark chocolate; FMD, flow-mediated dilation; TCD, transcranial Doppler; CBF*v*, cerebral blood flow velocity; UWA, The University of Western Australia; MAP, mean arterial pressure; CVC, cerebrovascular conductance; NO, nitric oxide; eNOS, endothelial nitric oxide synthase

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Abstract

Background: Cocoa contains polyphenols that are thought to be beneficial to vascular health. **Objective:** We assessed the impact of chocolate containing distinct levels of cocoa on cerebrovascular function and cognition. **Methods:** Using a counterbalanced within-subject design, we compared the acute impact of consumption of energy-matched chocolate containing 80, 35 and 0% single-origin cacao on vascular endothelial function, cognition and cerebrovascular function in 12 healthy postmenopausal women (57.3±5.3 yr) who attended a familiarisation session, followed by 3 experimental trials, separated by 1 week each. Outcome measures included cerebral blood flow velocity responses, recorded before and during completion of a computerised cognitive assessment battery (CogState), brachial artery flow-mediated dilation (FMD) and hemodynamic responses (heart rate, blood pressure). **Results:** When pre versus post chocolate cerebral blood flow velocity (CBF*v*) data were compared between conditions using two-way ANOVA, an interaction effect ($P = 0.003$), and main effects for chocolate ($P=0.043$) and time ($P=0.001$) were evident. Post hoc analysis revealed that both milk chocolate (**MC;** P=0.02) and dark chocolate (**DC;** P=0.003) induced significantly lower cerebral blood flow responses during the cognitive tasks, after normalisation for changes in arterial pressure. DC (80% cocoa) consumption also increased brachial FMD compared with pre-chocolate baseline (P=0.002), while MC (35%) and white chocolate (WC; 0%) incurred no change (interaction between conditions P=0.034). **Conclusions:** Consumption of chocolate containing high concentrations of cocoa enhanced vascular endothelial function, reflected by improvements in FMD. Cognitive function outcomes did not differ between conditions, however cerebral blood flow responses during these cognitive tasks were lower in the MC and DC conditions. These findings suggest that chocolate containing high concentrations of cacoa may modify the relationship between cerebral metabolism and blood flow responses in postmenopausal women.

Keywords: Chocolate, cocoa, polyphenol, nitric oxide, cerebrovascular

Introduction

Chocolate is one of the world's most consumed foods. In the USA, approximately 5-6 kg of chocolate is consumed annually, per person (1). Despite this high consumption, chocolate is sometimes considered to be 'unhealthy' and has a reputation for contributing to weight gain (2) due to the high fat, sugar and caloric content of commercially manufactured products. However, there is growing evidence that some types of chocolate may provide health benefits attributable to the high polyphenol content, particularly flavanols, contained within the non-fat solids of cocoa liquor. These are found in greater concentrations (~5-fold) in dark chocolate (DC), compared with milk chocolate (MC) (3). By comparison, white chocolate (WC) contains limited polyphenols as it comprises butter extracted from cocoa liquor and is devoid of non-fat cocoa solids (4).

Importantly, flavanols have been associated with antioxidant and anti-inflammatory effects, along with reductions in platelet reactivity, aggregation and adhesion (5). These actions promote healthy vascular function (5) and potentially reduce the risk of cardiovascular mortality (6). Indeed, a number of systematic reviews have concluded that evidence from both laboratory studies and randomised trials indicate that chocolate and flavanols may confer cardiovascular benefit (7-10). Accordingly, some research on the potential health benefits of chocolate consumption has focussed on endothelial function, assessed via flow-mediated dilation (FMD). Systematic reviews have suggested that flavanol-rich cocoa and DC produce significant and favourable effects on brachial artery FMD (8), but this is not a universal finding (11). However, no studies have adopted a study design involving the acute impact of chocolate containing distinct concentrations of cocoa, using FMD to assay endothelial function.

Whilst enhanced FMD of the brachial artery is indicative of cardiovascular health (12-14), improvement in cerebrovascular endothelial function may reduce the risk of stroke and enhance cognitive function (15, 16). Only one previous study, to our knowledge, has assessed cerebrovascular perfusion in response to a flavanol-rich cocoa-based beverage using transcranial Doppler (TCD; 17). However, the results of this study are difficult to interpret, due to the variability in baseline measures, lack of dietary control and the absence of a control group. There was also no attempt to link cognition and cerebral perfusion.

The aim of the present study was to assess the acute effect of consuming differing types of chocolate (80% cocoa "DC", 35% cocao milk chocolate "MC" and a white chocolate "WC" containing only cocoa fats) on endothelial and cerebrovascular function in post-menopausal women. These formulations were manufactured from a single-source and single-batch of cacao bean and each condition was matched for energy content. We hypothesised that acute consumption of DC, high in cocoa solids and flavanols, would result in improved vascular function, including increased brachial artery FMD and cerebrovascular responses to a standardised cognitive challenge, compared to the consumption of MC. We did not hypothesise that changes would be apparent in any measures following consumption of WC, in which cocoa solids are absent.

Methods

Participants

Twelve apparently healthy, postmenopausal women (age: 57.3±5.3 yr, weight: 67.3±11.9 kg, and body mass index: 24.6±4.6 $kg.m^{-2}$) were recruited from The University of Western

Australia (UWA) and the local community. Those who smoked, were taking prescribed medication or had a previous diagnosis of any cardiovascular disease or cognitive disorder were excluded via a screening questionnaire. Prior to their inclusion in the study, each participant provided written informed consent and the study was approved by the UWA Human Research Ethics Committee.

Study Design and Chocolate Treatment

Using a repeated measures cross-over design, each participant was required to attend four separate laboratory sessions at the School of Sport Science, Exercise and Health, UWA at the same time of day. The first visit, a familiarisation session (including baseline assessment of resting cerebrovascular perfusion and neurovascular coupling with cognitive challenge), was followed by three experimental trials in which the order of trial administration was counterbalanced to control for any potential order effect, involving the consumption of (a) high concentration (80%) cocoa DC, (b) lower concentration (35%) cocoa MC and (c) a WC containing cocoa fats and no solids. All chocolate treatments were manufactured to our requirements by an artisanal chocolatier (*Gabriel* Chocolate Company, Margaret River, Western Australia) using the same batch of single-origin cacao bean from the Sambirano Valley, Madagascar, in the desired concentrations of 35% and 80% cocoa, with the WC condition consisting of the cocoa butter extracted from the same bean. The complete nutritional composition of each chocolate was analysed by the Australian National Nutritional Measurement Institute (Melbourne, Vic, 3207, Australia) and is summarised in Table 1. Based on the nutritional laboratory analysis, we matched the energy content of consumed chocolate between trials, by feeding participants 85 g of WC, 87 g of MC and 84 g of DC in a counterbalanced order to provide a total of 2099 kJ under each condition.

Familiarisation Session

Participants arrived at the laboratory in the morning after an overnight fast and were given an overview of the study protocol and requirements before providing informed signed consent. Participants were instructed to complete a food diary and abstain from caffeine, alcohol, chocolate and vigorous physical activity during the 24 h prior to each subsequent session. The food diary required them to record the type, portion size and timing of ingested food and beverage in detail, for the purpose of being replicated in the 24 h prior to each subsequent experimental session. This allowed prior energy intake to be matched within-subjects between trials, with mean total daily energy intake, together with the quantity of carbohydrate, fat, and protein consumed determined from these records using a commercially available software program (FoodWorks 7; Xyris Software, Queensland, Australia).

Body mass and height were recorded and participants were then fitted with a TCD to measure resting **cerebral blood flow velocity (CBF*v*)** for 5 min in the absence of any stimulus, with their eyes open. Neurovascular coupling of cerebral metabolism and blood flow was assessed by administering a standardised CogState test (details below) while CBF*v* was continuously recorded. Participants were also familiarised with the FMD equipment and procedures.

Experimental Trials

Participants were then required to visit the laboratory for three experimental testing sessions conducted over 3 h, on three separate occasions, approximately one week apart. These sessions were scheduled for the same time of the morning as the familiarisation session, following an overnight fast and replication of the participants 24 h food diary.

After arrival at the laboratory, on each occasion, participants underwent baseline measures of resting blood pressure, resting CBF*v* and endothelial function (FMD). The assigned chocolate treatment was then administered (treatment order was counterbalanced to control for any potential order effect), with a fixed time of 15 min allowed for consumption. The participants were blindfolded throughout the consumption phase to prevent visual recognition of the condition. Chocolate consumption was immediately followed by 30 min of passive rest in a temperature controlled laboratory environment. Following this, measures of blood pressure, CBF*v* and endothelial function were repeated along with the neurovascular coupling assessment (detailed below).

Outcome Measures

Assessment of vascular endothelial function

Brachial artery endothelial function was assessed using FMD at baseline and 80 min following chocolate consumption. Briefly, non-invasive high-resolution ultrasound (Terason, t3200, Burlington, MA 01803, USA) imaging of the brachial artery was performed on the non-dominant arm, as previously described in our papers. Details of our assessment and analysis techniques have been published in detail elsewhere (18, 19).

Assessment of resting cerebrovascular perfusion

CBF*v* was assessed using TCD (Spencer Technologies, Seattle, WA), described in detail elsewhere (20). Participants were instrumented with a headframe (Marc 600, Spencer Technologies) capable of bilaterally transfixing two 2-MHz ultrasound probes over the temporal window for the duration of both the familiarisation and experimental trials. Bilateral measures of each middle cerebral artery flow velocities were obtained for 5 min in a rested state in a standardised room devoid of stimulation. Participants were seated in front of a blank

whiteboard and told to focus on the screen. Measurements were obtained in this way prior to, and 60 min after chocolate consumption and exported in real time to a data acquisition system (PowerLab, LabChart 7; ADInstruments, Sydney, Australia) for post hoc analysis.

Assessment of neurovascular coupling and cognition

Neurovascular coupling was assessed as the responses of CBF*v* to increased neural activity induced by cognitive computer-based tasks (CogState test battery – see below). To minimise the impact of a learning effect within each trial, responses during these cognitive tasks were assessed during the familiarisation laboratory visit, which served as baseline data for the subsequent chocolate consumption experimental trial responses, collected 60 mins after chocolate consumption. In this way, approximately one week separated each repeat cognitive task performed in the counterbalanced conditions.

Cognitive function was assessed using a computer-based cognitive battery (CogState Research TM), a widely used and accepted academic research tool. In order to familiarise participants and standardise the administration of the CogState test, written instructions and three to five practice trials were completed prior to the commencement of each experimental trial, for each task. The set of assessments chosen for this study were based on other studies investigating the effect of cocoa ingestion on cognition (21) and included: a detection task assessing psychomotor function and speed of processing, an identification task assessing visual attention, the ‘one back’ and ‘two back’ tasks assessing attention and working memory, the ‘international shopping list learning’ and ‘recall’ tasks assessing verbal learning and memory, and the continuous paired association learning task assessing visual learning and memory. The stability and efficiency of the CogState battery for repeated assessment of cognitive function have been demonstrated (22).

Assessment of blood pressure

Beat-to-beat continuous arterial pressure and heart rate traces were recorded for the duration of all sessions using a Finometer PRO (Finapres Medical Systems, Amsterdam). Blood pressure and heart rate were continuously assessed with data exported in real time to a data acquisition system as above.

Statistical Analysis

Statistical analysis of the data was conducted using SPSS version 20.0 with statistical significance being accepted at a $P < 0.05$. The effect of the chocolate conditions on outcome measures (FMD, resting CBF*v* and responses to CogState testing), assessed before versus after consumption, were compared between the experimental conditions using two-way repeated measures ANOVA [3 x 2 way ANOVA: chocolate type (n=3) vs pre-post time (n=2)]. Changes in cerebrovascular velocity and conductance were also calculated by subtracting post administration values from their preceding baselines (see Figure 3). One way ANOVA was performed on these data. Post hoc paired *t*-tests were performed using Least Significant Difference analysis. All data are presented as mean±SD unless stated otherwise. Based on our published work (19), a sample size of 10 individuals would provide >90% power, assuming two-tailed alpha=0.05 (G*Power v3.1.2), to detect a change in FMD of 1.4%. A 1% difference in FMD is associated with clinically meaningful ~7% difference in cardiovascular events (14). Regarding cerebral measures: given very conservative assumptions, such as a group difference in volumetric CBF*v* of 3cms^{-1} (see Figure 3, WC vs familiarisation), SD=1.5 cms^{-1} and alpha=0.01, our study possessed 90% power.

Results

Cerebral blood flow responses before and after chocolate administration

Cerebral blood flow in the middle cerebral artery was successfully achieved in 10 participants; TCD equipment was unavailable in 1 subject and temporal bone thickness rendered Doppler signals unattainable in another. Baseline CBF*v* (pre-chocolate administration) did not differ between the conditions ($P = 0.166$; Figure 1A). When CBF*v* was normalised for mean arterial pressure (MAP, Figure 1B), cerebrovascular conductance (CVC = CBF*v* ÷ MAP) baseline values were similar ($P = 0.457$; Figure 1C).

When pre and post chocolate CBF*v* data were compared between conditions using two-way ANOVA, an interaction effect ($P = 0.003$), and main effects for chocolate ($P = 0.043$) and time (P 0.001; Figure 1A) were evident. Subsequent post hoc *t*-tests revealed no change between pre and post CBF*v* following WC administration, however significant decreases following MC ($P = 0.008$) and DC ($P = 0.001$). Similarly, there was a significant interaction ($P = 0.014$, Figure 1C) and time effect (P = 0.008) between pre and post chocolate CVC data. Subsequent post hoc *t*-tests revealed no difference between pre and post CBF*v* following WC administration ($P = 0.618$), however CVC was significantly decreased as a result of MC ($P = 0.018$) and DC consumption ($P = 0.001$).

Mean arterial pressure and heart rate responses before and after chocolate administration

MAP data for the ten participants that completed the assessment of CBF*v* before and after chocolate ingestion are presented in Figure 1B. These data indicate no significant difference in MAP between conditions at baseline ($P = 0.264$), and no change in MAP as a result of chocolate ingestion (conditions $P = 0.547$; time $P = 0.879$; interaction $P = 0.302$). Similarly, there was

no significant difference in heart rate between conditions at baseline ($P = 0.973$), and no impact of chocolate ingestion under any condition ($P > 0.05$)

Neurovascular coupling and chocolate administration: Cerebrovascular responses during cognitive tasking

When chocolate conditions were directly compared, there were no significant differences in any of the seven CogState measures. Cognitive test performances are shown in Table 2 (available online).

Cerebrovascular responses (CBF*v*, MAP and CVC) to the seven measures of cognitive performance conducted during the no-chocolate familiarisation condition, and 60 min following chocolate administration of each condition, are presented in figures 2A, 2B and 2C. These data are summarised in figures 3A and 3B which present change (from the familiarisation condition) in CBF*v* and CVC responses during completion of cognitive tasks, averaged across all 7 measures; analysis performed on these figures therefore assessed the overall effect of cognitive stimulation on cerebrovascular responses.

A one-way ANOVA revealed significant differences in CBF*v* between the conditions in response to the cognitive tasks ($P = 0.001$; Figure 3A). Post hoc *t*-tests revealed a significant decrease in CBF*v* during the cognitive battery following WC ($P = 0.029$), MC ($P = 0.001$) and DC ($P < 0.001$) ingestion, compared with the no-chocolate familiarisation session. CBF*v* also significantly decreased following MC ($P = 0.048$) and DC ($P < 0.001$) compared with WC consumption.

After accounting for blood pressure, the change in CVC was also significantly different between conditions (one-way ANOVA, P = 0.001; Figure 3B). Post hoc *t*-tests revealed a significant decrease in CVC during the cognitive battery following MC (P = 0.022) and DC (P = 0.003), but not WC (P = 0.728), compared with the no-chocolate familiarisation session. CVC also significantly decreased following MC (P = 0.006) and DC (P = 0.008) compared with WC consumption.

Vascular endothelial function: Brachial FMD responses to chocolate administration

FMD was recorded before and 80 min after administration of chocolate in all 12 participants. There was no difference in baseline (pre-chocolate administration) FMD measures between conditions (one-way ANOVA; P = 0.158; Figure 4). However, there was a significant interaction effect (two-way ANOVA; P = 0.034) between pre and post chocolate data between conditions. Post hoc tests (pre vs post) revealed no differences in FMD following WC or MC, however a significant increase following DC (P = 0.002). This finding was consistent across participants, with DC ingestion resulting in a higher FMD% than WC or MC chocolate in nine of the twelve participants.

Discussion

In this study we adopted a cacao concentration-response paradigm, using energy-matched and custom manufactured chocolate made from single-origin same-batch cacao bean, to examine impacts on vascular function and cognition in humans using state-of-the-art physiological and imaging techniques. Flow-mediated dilation (FMD), an endothelium-dependent response largely mediated by nitric oxide (NO; 23), increased following consumption of chocolate high in cocoa, but not MC or WC. Interestingly, despite no change in cognitive function across the

chocolate conditions, cerebral blood flow responses during the cognitive tasks were significantly lower following consumption of higher cocoa containing chocolate, but not WC. These findings indicate that consumption of chocolate containing high concentrations of cocoa can enhance vascular function and increase cerebrovascular efficiency in postmenopausal women.

Acute ingestion of DC increased FMD in our study by 2.4%, with no changes observed following WC or MC. We adopted optimal contemporary approaches to the assessment of FMD (18), using operator-independent edge detection and wall tracking software (19). An increase in FMD of this magnitude is potentially associated with clinically significant reductions in cardiovascular events (12, 13), although the impact of repeated acute treatment has yet to be established. Our results also correspond with previous acute studies that observed a 3-4% increase in FMD following DC and a 0-2% decrease following consumption of WC (24, 25). However, the chocolate consumed under comparator conditions in Faridi *et al.* was not matched for energy content (260 kJ difference), while Hermann *et al* did not disclose the composition of study comparator chocolate conditions. Neither study assessed a concentration-response of polyphenol consumption. A more recent study observed concentration-dependent improvements in FMD, pulse wave velocity and blood pressure after treatment with different concentrations of cocoa powder and flavanols (26). Of interest, it has been reported that a large proportion (62%) of previous published studies of the impact of chocolate on cardiovascular endpoints have been industry funded (7). Other previous concentration studies that have adopted a concentration-response approach have utilised beverages containing cocoa, long term ingestion, or other approaches that did not involve chocolate administration (27-31).

Our study used 3 isocaloric custom-manufactured, single-origin and single-batch cacao bean conditions (80% cocoa DC, 395 mg flavanols; 35% cocoa MC, 200 mg flavanols; 0% cocoa WC, 35 mg flavanols). Furthermore, our study is the first to specifically assess acute responses in postmenopausal women, thereby avoiding the confounding impact, in younger women, of the menstrual cycle on vascular endothelial responses (32). The improvement in endothelium-mediated vasodilation (FMD) we observed could potentially be due to elevated concentrations of plasma flavanols, prevalent in higher cocoa containing DC, that have been shown to activate endothelial NO synthase (eNOS) and increase NO production and bioavailability (33).

Another major finding relates to cerebral blood flow responses to cognitive demand. Whilst one previous study has investigated the acute effect of cocoa-based beverage consumption on cerebrovascular responses using TCD (17), to our knowledge this is the first study to specifically address the impact of differing cocoa concentrated chocolate on coupling between cognitive tasks and blood flow. In response to a comprehensive and standardised battery of tests designed to interrogate distinct cognitive domains (CogState), we observed consistent decreases in CBF*v* and conductance responses following ingestion of chocolate containing higher levels of cocoa. No such changes were observed following ingestion of WC. These results somewhat contradict those of Sorond *et al.*, who observed no change in cerebrovascular reactivity following acute consumption of a commercial cocoa beverage in elderly individuals (17). This disparity may relate to the different populations studied in each trial, or to methodological differences, as Sorond *et al.* did not include different concentrations of cocoa, or a control condition. In contrast, our 3 experimental chocolate conditions were matched for energy intake and principally differed by virtue of cocoa content, and hence flavanol concentration.

The decreases in CBF we observed in response to chocolate consumption persisted after normalisation for concurrent blood pressure change, so cannot be attributed to an impact on systemic hemodynamics. We also did not observe significant differences in cognitive performance, despite the blood flow requirement to sustain such performance being significantly diminished. These findings infer sustained performance in the face of diminished blood flow and, hence, oxygen delivery. Interestingly, Francis *et al* performed a study in which daily flavanol-rich cocoa consumption over 5 days increased blood oxygenation in active brain regions, assessed by fMRI, in the absence of any change in cognitive performance (34). These findings, and our FMD data in the current study, lead us to speculate that flavanol-mediated NO production in the presence of higher cocoa concentrated chocolate (33), may modify cerebral metabolism and consequently decrease oxygen demand in active brain regions. Further studies will be required to address this proposition pertaining to "neurovascular efficiency".

In terms of cognition, the current results conflict with those of Field *et al.* who found that DC acutely increased cognitive performance in domains similar to our visual attention and visual memory tasks, compared with WC in young adults (35). Like other studies, these researchers did not assess concentration-responses in regards to polyphenol consumption and used commercially available chocolate, likely made from differing cacao beans each containing distinct polyphenol breakdown. Another study of older individuals (mean 52 yr), which utilised differing concentrations of flavanols (0, 250 or 500 mg) in the form of commercial cocoa beverages consumed over 30 days (36), observed no effect on performance of a cognitive task similar to our visual memory task. This is consistent with our findings, across multiple CogState task domains (speed of processing, verbal memory and verbal memory recall). Our findings that differing cocoa concentrated chocolate did not modify cognitive performance is therefore broadly consistent with the extant literature.

This study possesses several strengths and some limitations. It is the first, to our knowledge, to strictly control the type, composition and energy content of the chocolate used, which we had specifically manufactured to our purpose of utilising a concentration-response approach, with conditions counterbalanced and blinded to the participant. The use of a single-origin cacao bean ensured the constituents, in particular flavonoid breakdown (catechin, epicatechin and proanthocyanidin concentrations) were consistent between conditions. All chocolate conditions were energy matched (2099 kJ) as participants consumed either 85 g of WC, 87 g of MC or 84 g of DC, in a counterbalanced order to control for any potential order effect. Additionally, participants were fasted the morning of testing and instructed to avoid caffeine, chocolate, alcohol and intense physical activity in the 24 h prior to experimental assessments. The completion and replication of a food diary allowed the 24 h prior to experimental testing to be assessed and this was similar between conditions. The techniques we adopted to assess peripheral and cerebral vascular responses are well validated and accepted in the literature (18, 20) and our approaches are state-of-the-art and largely operator independent (18, 19). We also adopted a standardised and well accepted psychometric tool (CogState) that provided information on a range of cognitive domains, after a thorough familiarisation. Although the chocolate we utilised was specifically manufactured and supplied for this study by an artisanal chocolatier, the product was purchased at the full commercial cost and no conflicts of interest existed in our study. The limitations of this study include the relatively small sample size, although our concentration-response findings are internally consistent (dark>milk>white) and the findings were statistically significant. Finally, it is an accepted limitation of the use of transcranial Doppler that diameter measures are not derived, and that velocity is used as a surrogate for flow (and in the calculation of conductance). Future research should focus on additional measurements including MRI-based CBF and arterial diameter measures.

In conclusion, this study suggests that higher concentrations of cocoa in chocolate induce favourable effects on endothelial function and neurovascular efficiency. Such effects may conceivably relate to the impact of flavanols on endothelial function and NO bioavailability, in both the peripheral and cerebral vasculature. If confirmed and extended in the context of chronic administration, our findings may have implications for arterial health in postmenopausal women at risk of cardiovascular disease, stroke and cognitive decline.

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The experimental design was developed by DG, LN, KG and HC, with CM responsible for participant recruitment. HC, CM and KS were responsible for the acquisition and analysis of vascular and cerebrovascular data while CM and KP were responsible for the acquisition and analysis of CogState. All authors were involved in the interpretation of data and drafting and revising the manuscript for publication.

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Figure 1. Impacts of chocolate containing different concentrations of cocoa on cerebral blood flow velocity and blood pressure at rest. A) Resting middle cerebral artery velocity ($cm.s^{-1}$), before and after consumption of white, milk and dark chocolate (WC, MC and DC respectively). B) Resting mean arterial pressure (mm Hg) before and after consumption of WC, MC and DC. C) Resting cerebrovascular conductance ($cm.s^{-1}$ mm Hg^{-1}), before and after consumption of WC, MC and DC. (n = 10; mean ± SE). * † ‡ Indicates significant difference from pre-chocolate consumption within condition (*P < 0.05; †P < 0.01; ‡P < 0.005).

Figure 2. Impacts of chocolate containing different concentrations of cocoa on cerebral blood flow velocity and blood pressure in response to individual cognitive tasks. A) Change (ie post-rest) in middle cerebral artery velocity ($cm.s^{-1}$) after consumption of white, milk and dark chocolate (WC, MC and DC respectively) or non-chocolate familiarisation during seven cognitive tasks B) Mean arterial pressure (mm Hg) at familiarisation (no chocolate treatment) and following consumption of WC, MC and DC during seven cognitive tasks C) Change in cerebrovascular conductance (cm^{-1} mm Hg^{-1}) following consumption of WC, MC and DC or non-chocolate familiarisation during seven cognitive tasks. (n = 10; mean ± SE). * † ‡ Indicates significant difference from familiarisation (*P < 0.05; †P < 0.01; ‡P < 0.005).

Figure 3. Impacts of chocolate containing different concentrations of cocoa on average cerebral blood flow velocity in response to all cognitive tasks. A) Change (ie post-rest) in middle cerebral artery velocity ($cm.s^{-1}$), following consumption of white, milk and dark chocolate (WC, MC and DC respectively) or a non-chocolate familiarisation, across the seven cognitive tests in Figure 2. B) Similarly calculated change in cerebrovascular conductance (cm^{-1} mm Hg^{-1}), as middle cerebral artery velocity normalised for blood pressure (n = 10; mean ± SE). * † ‡ Indicates significant difference from familiarisation (*$P < 0.05$; †$P < 0.01$; ‡$P < 0.005$).

Figure 4. Impacts of chocolate containing different concentrations of cocoa on brachial artery flow-mediated dilation. Flow-mediated dilation (%) of the brachial artery before and 80 min after chocolate consumption of white, milk and dark chocolate (WC, MC and DC respectively); n = 12; mean ± SE). ‡ Indicates significant difference from pre-chocolate consumption within trial (‡$P < 0.005$).

Table 1. Composition of different chocolate conditions

Nutritional components	**White chocolate (WC)**	**Milk chocolate (MC; 35% cocoa)**	**Dark chocolate (DC; 80% cocoa)**
Energy (kJ/100 g)	2470	2420	2490
Amount consumed (g)	85	87	84
Energy consumed (kJ)	2099	2099	2099
Carbohydrates (g)	44.2	42.6	36.1
Total sugars (g)	42.5	35.7	19.3
Fat (g)	34.1	34.0	36.3
Saturated fat (g)	21.3	21.1	22.1
Mono-unsaturated fats (g)	9.9	10.2	11.4
Poly-unsaturated fat (g)	1.1	1.0	1.0
Protein (g)	4.9	7.1	7.8
Total polyphenols (mg)	34.9	200.1	394.8
Total Flavonoids (mg/kg)	**370**	**980**	**3600**
Epicatechin (µg/g)	**Not detected**	**288.4**	**587.1**
Catechin (µg/g)	**38.4**	**770.1**	**1394.2**