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The effect of acute consumption of a flavonol-rich cocoa drink on cerebral vasomotor reactivity in African Americans

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Abstract

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African Americans (AA) are at great risk of cardiovascular diseases (CVD) which can lead to brain damage, dementia, and endothelial dysfunction. Decreased nitric oxide (NO) bioavailability contributes cardiovascular disease in AA population. Flavonols of the subclass known as flavonoids that have several beneficial effects on cerebral blood flow and cerebral vasomotor reactivity(CVMR). This study investigated the effects of the acute consumption of a flavanol-rich cocoa drink on CVMR. Ten non-smoking African American (6 males and 3 females) participants were randomly recruited. The subjects participated in two experimental sessions which were separated before and after the consumption of cocoa drink. For the pre-session, baseline CVMR was measured by the hypercapnia rebreathing (CVMR test) prior to the consumption of the cocoa drink and the again at 2h after consumption of one serving of the cocoa drink (45g of cocoa mixed with 8oz of cold water). Cerebral vascular conductance (CVC) was significantly

increased in the post-study during hypercapnia rebreathing compared with the pre-study(post-study: 3.649 ± 1.833 CVC % of baseline/mmHg, pre-study: 2.483 ± 1.418 CVC % of baseline/mmHg vs. $P < 0.05$) Thus, CVMR was significantly increased in the post-study after the acute consumption of a flavonol-rich cocoa drink compared to the pre-study in AA.

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CHAPTER 1

INTRODUCTION

1.1 Background

Cardiovascular disease (CVD) is the leading cause of death in the United States (3). It is well known that African Americans are at greater risk for CVD (7). The relationship between cardiovascular disease in African Americans (AA) and major risk factors is complex; however, Anita et al. (7) suggested that hypertension, diabetes, and hypercholesterolemia are the primary risk factors for CVD which can lead to brain damage, dementia, and endothelial dysfunction in AA (10,15,16). Though the underlying mechanisms remain unclear, it has been recently suggested that decreased nitric oxide (NO) bioavailability contributes to cardiovascular disease in African American population (35,55,69).

NO, which acts as a potent vasodilator, is a significant cellular signaling molecule included in vascular functioning, and NO induced from endothelial cells regulates cerebral blood flow (CBF) and vascular smooth muscle tone (36). Melikian et al., (55) have suggested that NO bioavailability is decreased in relatively young AA men, which likely leads to impaired endothelium-dependent vasodilation in AA (69). In addition, a previous study (48) has demonstrated that, structural rarefaction which is structural loss in microcirculation was induced by impaired endothelium-dependent vasodilation, resulting in an elevation in peripheral vascular resistance, and a subsequent elevation in arterial blood pressure. Endothelial dysfunction is a critical factor in the pathogenesis of cardiovascular diseases such as hypertension and stroke which can contribute to cognitive

impairment and Alzheimer's disease by impairing vasodilation in the cerebral vasculature and damaging vascular endothelial cells in the brain (24,25,28,91).

Cerebral vasomotor reactivity (CVMR) indicates the compensatory constrictive or dilatory ability of distal cerebral arteries to a vasoactive stimulus (29). In clinical practice, impaired CVMR, indicating a decreased cerebrovascular reserve, forecasts enhanced risk of ischaemic stroke (30, 31). It has been well described that a change of cerebral blood flow and of CVMR in systemic disease states such as hypertention, and diabetes (30-32).

African Americans, therefore, may have impaired cerebral vasomotor reactivity. This is also supported by our unpublished study which shows that African Americans have impaired cerebral vascular response to hypercapnia, stiffer arteries, and lower Vitamin D levels than Caucasian Americans.

It is well known that flavonoid-rich foods can strongly affect the incidence and onset of cardiovascular and neurodegenerative diseases, and thus flavonoids-rich foods have been promising, particularly in the area of cardiovascular and cerebrovascular function. Cocoa powder includes a large percentage of flavonols of the subclass known as flavonoids that have several beneficial effects on the brain, vascular system and cerebral blood flow (22). Flavonoids in cocoa activate NO synthesis in healthy humans (21) by stimulating endothelial cells to provide NO via elevation of intracellular calcium level (26, 27). It has also been found that flavonoids interact with signalization cascades including protein and lipid kinases that contribute to the inhibition of neuronal death by apoptosis caused by neurotoxicants such as oxygen radicals, and support neuronal survival and synaptic plasticity. (22)

According to these findings, it is reasonable that the intake of flavonoids may have a positive effect with a particular emphasis on cerebrovascular system in African Americans. Our hypothesis is, therefore, that acute consumption of flavonol-rich cocoa drink may improve cerebrovascular vasomotor reactivity in African Americans.

1.2 Statement of purpose

The purpose of the present investigation was to determine whether there are effects on cerebral vasomotor reactivity in healthy young African Americans. The specific objectives of the study were to;

1. Determine whether the acute flavonoid consumption has a positive effect on cerebral vasomotor reactivity in African Americans

1.3 Hypothesis

In the current study, we tested the following hypotheses;

1. The acute flavonoid consumption would improve the cerebral vasomotor reactivity in African Americans

CHAPTER 2

METHODOLOGY

2.1 Subjects

Ten non-smoking African American (6 males and 3 females) participants completed the study. Subject characteristics were (mean ± SD) 23.4 ± 0.8 years, 169.5 ± 8.2 cm, 72.84 ± 12.1 kg, 25.3 ± 4.0 kg/m^2 for age, height, weight, and BMI, respectively (Table 1). The African American subjects (ages 18-30; both genders) were recruited from the University of Texas at Austin and the large Austin area to participate in the study. All subjects completed a health questionnaire Subjects were excluded from the study following criteria: cardiovascular, neurological, metabolic, orthopedic, or cognitive diseases; currently taking medications to influence the autonomic nervous system and pregnant women and children (i.e. younger than 18). Subjects were asked to avoid vigorous exercise and alcoholic beverages for 24 hours and to avoid caffeine and food for 12 hours before the protocol. Temperature and relative humidity were maintained at ~24℃ and 40% while conducting all experiments and procedures. The Institutional Review Board at The University of Texas at Austin approved all techniques and protocols used in the current study and written informed consent was obtained from all participants before testing.

2.2 Instrumentation and Measurements

An electrocardiogram (ECG)(HP Patient Monitor, Agilent, Santa Clara, CA) interfaced with a cardiotachometer (CWE, Ardmore, PA) were continuously used to monitor heart rate and cardiac rhythms. A Penaz method (CNAP, Monitor 500, Austria) was used to record continuous finger arterial blood pressure. In addition, electrosphygmomanometry (Tango+; SunTech, Raleigh, NC) was used to measure intermittent blood pressure with auscultation of the brachial artery. Mean arterial blood pressure (MAP) was calculated as one-third pulse pressure plus diastolic blood pressure. A capnograph (VitalCap Capnograph Monitor, Oridion, Needham, MA) with a mouthpiece was continuously used to collect end-tidal carbon dioxide concentration ($PETCO_2$) during all data collection periods and was used as an index of arterial carbon dioxide concentration.

Transcranial Doppler ultrasonography was used to measure cerebral blood flow, which was indexed from the velocity (MCA V_{mean}) of blood flowing through the middle cerebral artery. The middle cerebral artery was figured through a 2-MHz Doppler probe (Multi-flow, DWL Elektronische Systeme, Singen, Germany) modified over the temporal window of the right or left middle cerebral artery until an optimal signal was clarified. A head strap was used to stabilize the Doppler probe for the duration of the study. An index of cerebral vascular conductance (CVCi) was calculated from the ratio of the middle cerebral artery blood velocity (MCA V_{mean}) to MAP acquired from the beat-to-beat arterial pressure measurement. Cerebral vasomotor reactivity (CVMR) was estimated based on changes in cerebral vascular conductance (CVCi; MCA V_{mean}/ MAP) in response to rebreathing-induced hypercapnia. The Delta raise in $PETCO_2$ and the % change in CVCi were used to acquire its slope of regression line. To prevent hypoxic

events (i.e. cerebral hypoxia), oxygen (calculated by height and weight) was continuously provided during the CVMR test.

2.3 Experimental Protocol

The subjects were required to participate in two experimental sessions. These sessions were separated before and after the consumption of cocoa drink. For the pre-session, baseline CVMR was measured prior to the consumption of the cocoa drink and the again at 2h after consumption (48g of cocoa mixed with 8oz of cold water). The cocoa powder (Hershey's) used in this study consisted of sucralose, carrageenan, maltodextrin, salt, acesulfame potassium and sulfur dioxide. The nutrient content of the test drink is listed in table 1.

Nutritional Composition Per serving	Flavanol Test Drink
Calories	149
Fat, g (calculated)	2
Sat fat, g	1
Trans fat, g	0
Cholesterol, mg	11
Sodium, mg	364
Carbohydrates, g	25
Dietary fiber, g	5
Sugar, g	17
Protein, g	14
Vitamin A, IU	10
Vitamin C, mg	2
Calcium, mg	429
Iron, mg	1
Magnesium, mg	113
Potassium, mg	780
Proanthocyanidins 1-10, mg	247.2
PACs 1 mers	64.8
*Catechin, mg	17.8
*Epicatechin, mg	47.0
PACs 2 mers	39.8
PACs 3 mers	25.4
PACs 4 mers	26.4
PACs 5 mers	22.1
PACs 6 mers	20.6
PACs 7 mers	18.7
PACs 8 mers	12.0
PACs 9 mers	9.6
PACs 10 mers	7.7
Total Proanthocyanidins (DMAC), mg	528.0

Table 1. Nutritional Composition Per serving

Upon arriving at the laboratory, height and weight were measured, which were then used to calculate the rate of oxygen that was continuously provided during the CVMR test. Subjects were asked to take a rest quietly in a supine position on a patient bed. ECG electrodes were attached to trial participants to monitor heart rate, and electrosphygomanometer was used to obtain intermittent blood pressure. MAP was calculated as one-third of pulse pressure plus diastolic blood pressure. Finger cuffs were attached to two fingers to measure beat-by-beat arterial blood pressure throughout the trial (Penaz method). This instrumentation took approximately 15 minutes and was followed by a 6-minute period of baseline data collection with blood pressure measured via auscultation of the brachial artery in the final minute of this baseline period.

A 2-MHz Doppler probe was then instrumented using a head strap for stabilizing and modified over the temporal window until the optimal signal was acquired. This modification was followed by the CVMR test. Subjects remained in a supine position and an adjusted mouthpiece was put into the mouth. Subjects went from breathing atmospheric air to rebreathing their own breathed-out air from a specialized rubber bag though mouthpiece, that has the ability to switch a valve. In addition, a tube connected to a capnograph was used to observe $PETCO_2$ during the test. Before the CVMR test six-minute baseline data were acquired and averaged to express baseline values for MCA V_{mean}, CVCi, and $PETCO_2$. Participants went through a rebreathing procedure (CVMR test) after the baseline data collection. Subjects then began to rebreathe their own air by closing the valve to complete this procedure. This lead to an increase in $PaCO_2$ (as indexed by $PETCO_2$) and breath-by-breath data were collected until the delta increase in $PETCO_2$ was achieved (see below), approximately 2-3 minutes. The rebreathing procedure ceased once subjects attained the target increase in $PETCO_2$, which was delta

15 mmHg or if subjects felt dizziness, shortness of breath, and/or tingling or numbing sensations.

2.4 Data Analysis

MAP, MCA V_{mean}, subsequent calculation of CVCi and $PETCO_2$ were estimated on a breath-by-breath basis and were sampled at 125 Hz via a data-acquisition system (Biopac System, Santa Barbara, CA).

The last minute of the 6-minute baseline period was used for the baseline data analysis (MAP, $PETCO_2$, and MCA V_{mean}, CVCi). The percent changes in CVC from the baseline value during hypercapnic rebreathing period were determined while absolute changes in $PETCO_2$ from the baseline value were used. Pulse wave velocity was calculated as addressed above (see 3.1 Instrument and measurements).

2.5 Statistical Analysis

Descriptive analysis was carried out to represent the characteristics of the subjects. The means and standard deviation (SD) were used for continuous variables (e.g. age, height, weight, and BMI), and the numbers of subjects were appreaed for categorical variables (sex). The means and standard deviation were also appreared for continuous variables (HR, MAP, $PETCO_2$, MCA V_{mean}, CVCi) at the 6-minute baseline before hypercapnic rebreathing period. Statistical significance between the first test and the second test was evaluated using paired t-test for hemodynamic variables at baseline (HR, MAP, $PETCO_2$, MCA V_{mean}, CVCi). The linear regression was applied for calculating the slope of the percent changes in CVCi from baseline thru progressive hypercapnia with

respect to the delta changes in $PETCO_2$ from the baseline. One-way repeated measures ANOVA was used to analyze differences in CVMR between the first test and the second test. The alpha level for statistical significance was used at 0.05. IBM SPSS statistics (Systat Software, Inc., Chicago, Illinois) was used for statistical analysis.

CHAPTER 3

RESULTS

3.1 Subjects

Nine African Americans participated in this study(six male and three female). Table 2 displays participant characteristics. Average age of the participants was 23.4 ± 0.8 yrs. The participants' average of height and weight were 169.5 ± 8.2 cm and 72.84 ± 12.1 kg. Average body mass index was 25.3 ± 4.0 kg/m^2.

Variables	Subjects
Age(yrs)	23.4 ± 0.8
Sex(m/f)	6/3
BMI(kg/$m^{2)}$	25.3 ± 4.0
Height(cm)	169.5 ± 8.2
Weight(kg)	72.84 ± 12.1

Table 2. Subject Characteristics

3.2 Cerebral Vasomotor Reactivity

Hemodynamic information during eucapnic period (baseline) is shown in table 3. There were no significant differences in HR (pre-study : 67.6 ± 4.1 beats × min^{-1} vs. post-study: 64.5 ± 6.0 beats × min^{-1}, P = 0.07), MAP (pre-study: 97.5 ± 16.1mm Hg vs. post-study: 95.1 ± 14.8 mmHg, P = 0.40), MCA V_{mean} (pre-study: 64.2 ± 19.0 cm × sec^{-1} vs. post-study: 60.0 ± 14.0 cm × sec^{-1}, P = 0.22), and $PETCO_2$ (pre-study: 43.5 ± 3.7 mm Hg vs. post-study: 44.0 ± 2.6 mm Hg, P = 0.72). cerebral vascular conductance (CVC) (pre-study: 1.49 ± 0.5 cm × sec^{-1} × $mmHg^{-1}$ vs. post-study: 1.37 ± 0.4 cm × sec^{-1} × $mmHg^{-1}$, P = 0.05).

Variables	Pre-study (n=9)	Post-study (n=9)	*P*-value
HR (beats×min^{-1})	67.6 ± 4.1	64.5 ± 6.0	0.07
MAP (mmHg)	97.5 ± 16.1	95.1 ± 14.8	0.40
MCA V_{mean} (cm×sec^{-1})	64.2 ± 19.0	60.0 ± 14.0	0.22
$PETCO_2$ (mmHg)	43.5 ± 3.7	44.0 ± 2.6	0.72
CVC (cm × sec^{-1} × $mmHg^{-1}$)	1.49 ± 0.5	1.37 ± 0.4	0.05

Notes:
BMI, body mass index, MAP, mean arterial pressure. CVC, cerebral vascular conductance. MCA V_{mean}, middle cerebral artery mean velocity. $PETCO_2$, partial pressure of end-tidal carbon dioxide.
Values are means ± SD.

Table 3. Hemodynamic state during eucapnia

The slopes of the percent increase in CVC per mmHg increase in $PaCO_2$ indexed by $PETCO_2$ were significantly increased in the post-study relative to the pre-study (post-study: 3.649 ± 1.833 CVC % of rebreathing/mmHg, pre-study: 2.483 ± 1.418 CVC % of rebreathing/mmHg vs. $P < 0.05$) (Figure 1).

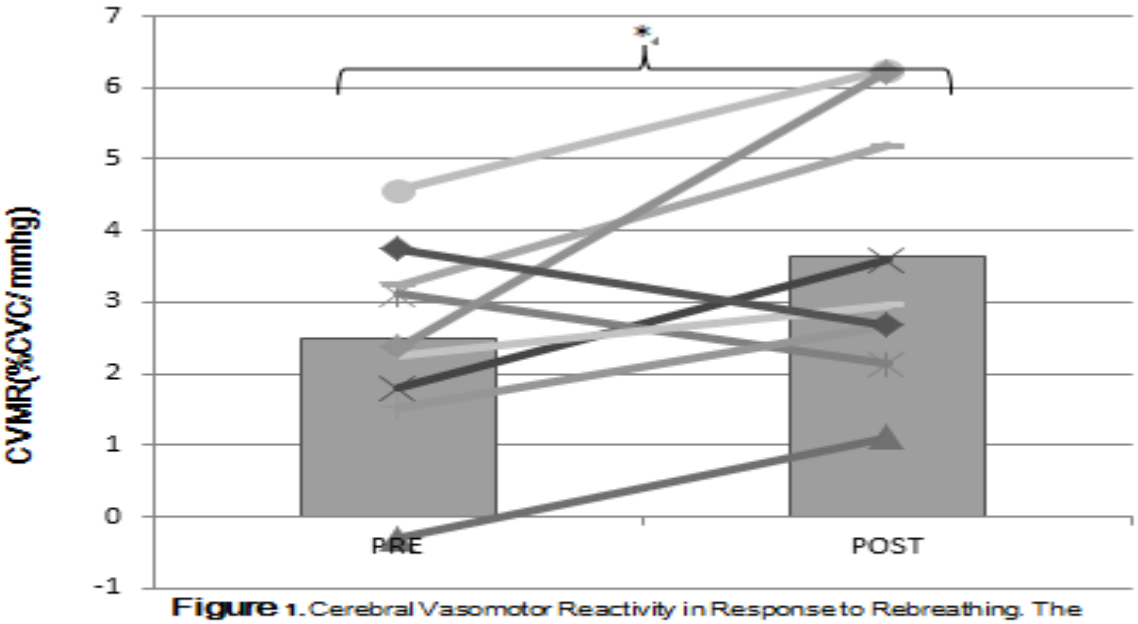

Figure 1. Cerebral Vasomotor Reactivity in Response to Rebreathing. The percentage change of CVC from its rebreathing as a function of $PETCO_2$ after the consumption of 8oz flavonol-rich cocoa drink (48g Cocoa Powder) increased CVMR (n = 9 AA). Lines = individual values; Bars = pre and post-study averaged values. *P < 0.05

CHAPTER 4

DISCUSSION

In this study, a hypercapnia intervention was used to induce an increased need for blood flow in the brain, caused by the vasodilatory effects of NO. This hypercapnia intervention measures possible changes of blood flow related to the effect of flavanol-rich cocoa drink on the endothelial NO system. This study found that the CVMR increased in AA after the acute consumption of flavanol-rich cocoa drink. CVC, which is an index of cerebral blood flow (CBF), was significantly higher in the post-study than in the pre-study.

Some previous animal and epidemiologic studies have demonstrated that flavonoids may act to reduce the risk of dementia, which is related to declined CBF (38,40). Fruit and vegetable polyphynolds play a role in decreasing the sensibility of the rat brain to damage from oxidative stress cauased by aging (39). Consuming flavonoids resulted in decreased risk of incident dementia in a cohort of over 1300 French elderly (18). One contributing factor is that the benefits of flavonoids include increased vascular function and improved CBF. Hollenberg et al (33) found that flavanol-rich cocoa-induced NO production displays a marked effect on blood vessels that play an essential role in brain activity in healthy individuals. In addition, Fisher et al (34) provided first evidence using the transcranial Doppler ultrasound (TCD) that flavanol-rich cocoa could induce increased mean flow velocity in the middle cerebral artery in healthy subjects. This likely contributes to the improvement of CVMR induced by the consumption of flavanol-rich cocoa drink.

Epidemiogical studies have found that African Americans are at higher risk of cardiovascular disease than Caucasian Americans (35). The prevalence of high blood pressure and type 2 diabetes in AA in the United States is particularly high (41, 42). Some intervention studies suggested that hypertension is related with lowering CBF, and that lowering BP causes an incline in CBF (45, 46). In humans, long-standing hypertension contributes to reductions in CBF, metabolism and cognitive function. (9) Type 2 diabetes-induced hyperglycemia and hyperinsulinemia surfeit free fatty acids, prothrombotic state contribute to endothelial dysfunction which may decrease CBF (14) (15). In addition, AA have a tendency to have the lower NO bioavilability which may be induced by high plasma asymmetrical dimethyl arginine. NO induced from endothelial cells controls cerebral blood flow (CBF) and vascular smooth muscle tone (36). Increased consumption of flavonoid-rich foods may decrease the risk of hypertension and stroke(43, 44). Some studies suggested that flavanol compounds have the ability to activate endothelial NO synthesis and contribute to activation of NO (37). Therefore, it is possible that the consumption of a flavonol-rich cocoa drink may increase cerebral blood flow through NO-induced vasodilation in the brain in the AA population as our study showed.

The current study is the first to investigate the influence of flavanol-rich cocoa on CVMR in AA. The primary finding in this study is the improvement of CVMR in African Americans after the acute consumption of flavanol-rich cocoa drink. This finding is of special interest in the area of between CVMR and flavanols. To our knowledge, the interactions between flavonol-induced improvement in CVMR and ethnicity remains unclear, but the effect of the acute consumption of flavonol-rich cocoa drink on CVMR in African Americans could be a key step toward explaining the action of flavonoids in

the cerebrovasculature in AA.although the underlying mechanisms in the present study are not known.

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