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Impaired Peripheral and Cerebral Microvascular Functon / Reactivity in Healthy Young African Americans

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Impaired Peripheral and Cerebral Microvascular Functon / Reactivity in Healthy Young African Americans

by

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Abstract

Impaired Peripheral and Cerebral Microvascular Function / Reactivity in Healthy Young African Americans

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African Americans (AA) are at an increased risk for cardio and cerebral vascular disease relative to Caucasians (CA) and the underlying impairments manifest as early as the second generation prior to overt signs of risk. The mechanisms of this increased risk are multifactorial; however, evidence suggests that microvascular dysfunction is a primary contributor. This study tested the hypothesis that microvascular function, indexed by the skin vascular conductance (SkVC) response to local heating, is impaired in young otherwise healthy AAs. Furthermore, we hypothesized that AAs have an attenuated cerebral vasodilator response to hypercapnia. Nineteen healthy young individuals were participated in this study (9 AAs, 10 CAs). SkVC was assessed while the skin was clamped at 34 °C and 40 °C and values were normalized to a maximal value obtained during heating at 43 °C for 30 min. Cerebral vasomotor reactivity (CVMR) was assessed by increases in cerebral vascular conductance (CVC) during a rebreathing protocol. SkVC was lower in the AA group at 34 °C (AA: 10±3 % max vs. CA: 16±7 % max; $P < 0.01$) In addition, SkVC was reduced in AAs at 40 °C (AA: 56±15 % max vs.

CA: 68±12 % max; P=0.03). CVMR was significantly attenuated during hypercapnic rebreathing in AAs relative to CAs (AA: 2.8 ± 1.2 %CVC/Torr vs. CA: 5.7 ±0.9 %CVC/Torr; P < 0.001). Our findings suggest that microvascular function is impaired in young otherwise healthy AAs.

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CHAPTER 1

INTRODUCTION

1.1 Background

African Americans are at increased risk for developing a wide variety of cardiovascular diseases including stroke, coronary artery disease, hypertension, and metabolic syndrome compared with Caucasians (Fields et al. 2004; Harris et al. 2011; Melikian et al. 2007; Mensah et al. 2005). The underlying impairments resulting in these disease states manifest as early as in the second generation of life prior to any overt signs of risk (Fields et al. 2004).

The mechanisms resulting in increased risk in this population are likely multifactorial; however, recent evidence suggests that microvascular dysfunction due to decreased nitric oxide (NO) bioavailability and/or vitamin D deficiency in these individuals is a major contributing factor (Harris et al. 2011; Melikian et al. 2007). NO is a primary vasoactive substance that plays a critical role in vascular health and reduced NO bioavailability contributes to endothelial dysfunction and the atherosclerotic process (Fraga et al. 2011). Additionally, Vitamin D deficiency results in endothelial dysfunction, in part through reducing NO bioavailability (Harris et al. 2011), and thereby also contributes to the atherosclerotic process. Reduced NO bioavailability and vitamin D deficiency induce a variety of impairments in microvascular function that initiate the disease progression (Harris et al. 2011; Melikian et al. 2007; Rostand 2010).

These adaptations include; endothelial dysfunction which leads to decreased arterial compliance and increased arterial stiffness, decreased microvascular function (i.e. capillary rarefaction) which leads to reductions in glucose uptake and thus insulin resistance, reductions in oxygen and substrate delivery to the skeletal muscle during periods of increased metabolic demand (i.e. can reduce exercise capacity), and increased vascular resistance which leads to hypertension (Levy et al. 2008; Melikian et al. 2007). Endothelial dysfunction has also been implicated in impaired cerebral vascular regulation which is a precursor for stroke and it also results in impaired vasodilation (Vicenzini et al. 2007).

As in the peripheral circulation, impaired cerebral microvascular function would attenuate the increase in blood flow and thus oxygen and substrate delivery that occurs during periods of increased metabolic demand (Vicenzini et al. 2007) as occurs during tasks that require increased cognitive and executive function. In this regard it has been demonstrated that cerebral microvascular function is partially dependent on NO bioavailability and Vitamin D status, both of which are reduced in African Americans (Harris et al. 2011; Melikian et al. 2007).

To our knowledge, racial differences in peripheral and cerebral microvascular function and reactivity have not been investigated.

1.2 Statement of purpose

The purpose of the present investigation was to determine if there is a difference in microvascular function between healthy young African Americans and Caucasian Americans. The specific objectives of the study were to;

1. Determine race-specific skin blood flow responses to local heating of skin.
2. Determine race-specific cerebral blood flow responses to hypercapnia.

1.3 Hypothesis

In the current study, we tested the following hypotheses;

1. Skin vascular conductance induced by local heating of skin would be lower in healthy young African Americans than Caucasian Americans.
2. Cerebral vasomotor reactivity during a rebreathing protocol to induce hypercapnia would be attenuated in healthy young African Americans relative to Caucasian Americans.

CHAPTER 2

LITERATURE REVIEW

This literature review summarizes the relevant research that shows microvascular function is impaired in healthy young African Americans relative to their Caucasian counterparts. The topics in this review of literature include microvascular function, microvascular dysfunction as it relates to cardiovascular diseases, the evidence of microvascular dysfunction in healthy young African Americans, and the use of local heating and hypercapnic rebreathing procedures to assess microvascular function.

2.1 Microvascular function

Blood vessels in the human body are divided into two types according to their size: macro- and microvasculature. Macrovasculature includes the aorta, arteries, veins, and vena cava, which are primary responsible for the systemic circulation of the blood and nutrients. Microvasculature refers to relatively small vessels when compared to macrovessels and the diameter of microvessels is less than 150 micrometers. Microvasculature consists of small arterioles, capillaries, and venules, and its main functions include the delivery of oxygen and nutrients to the cell, elimination of carbon dioxide and other metabolic byproducts from the cell, controlling blood pressure, and regulation of body temperature. A large proportion of the endothelium, which is a single

thin layer, lies on the internal surface of microvessels and produces vasoactive molecules to keep vascular tone homeostasis (Levy et al. 2001). Endothelium-mediated secretion of NO plays a significant role in vascular dilation (Green et al. 2004).

Endothelial NO synthase activation generates endothelium-dependent NO from L-arginine when shear stress on the blood vessel wall increases. Once NO is produced and released from the endothelial cells, it diffuses to the vascular smooth muscle cells. At the smooth muscle cells NO binds to and thus activate guanylate cyclase which triggers a cascade of intracellular events ultimately resulting in vasodilation. Signaling molecules, such as adenosine, bradykinin, endothelial growth factor, or serotonin, also participate in cell signaling which contributes to endothelium-dependent vasodilation (Deanfield et al. 2007; Kellogg et al. 2008). In addition, NO-independent vasodilator substances exist. Kellogg et al. (2005) administrated ketorolac, an inhibitor of prostaglandin production, directly into the interstitial space within local areas of the skin by using intradermal microdialysis technique. They found that prostaglandins are a potent vasodilator released from endothelial cells (Kellogg et al. 2005). Several recent investigations also suggest that endothelium-derived hyperpolarization factors probably affect NO production via an independent pathway (Busse et al. 2002; Halcox et al. 2001). The endothelium releases not only vasodilator substances such as NO and prostaglandins, but also secretes vasoconstrictor substances. For example, Kinlay and colleagues (2001) reported that endothelin-1 accounts for 39 % of the coronary tone in normal arteries and acts as a vasoconstrictor in atherosclerotic coronary arteries (Kinlay et al. 2001). Vasoconstrictor prostanoids generated from the endothelium and the conversion of angiotensin I to

angiotensin II at the surface of the endothelium also modulate vascular tone, acting as vasoconstrictors (Deanfield et al. 2007; Peiro et al. 2007). Therefore, vascular tone, ultimately the function of the coordinated balance between endothelium-derived vasoconstrictors and vasodilators, is critical to the prevention of microvascular dysfunction and thus overall vascular health.

2.2 Microvascular dysfunction as it relates to cardiovascular diseases

Decreases in NO bioavailability and vascular compliance cause the balance of vascular tone between vasoconstrictors and vasodilators in microcirculation to shift toward vasoconstrictionve forces. This state is defined as microvascular dysfunction. Microvascular dysfunction is a precursor to a number of disease states and is associated with the progression of these diseases. For example, Vuilleumier and colleagues (2002) examined post-ischemic forearm skin reactive hyperemia. They used laser Doppler flowmetry (LDF) to investigate the relationship between skin microvascular function and cardiovascular risk factors in a healthy female population. They found an inverse correlation between cutaneous microvascular function and Framingham cardiovascular risk score (Vuilleumier et al. 2002). Retinopathy, an index of microvascular disease, is also related to increased risk of heart failure, and is an independent predictor of heart failure regardless of existing coronary heart disease, diabetes mellitus, or hypertension (Wong et al. 2005). Abnormalities of microvascular function in the end-organ of human body are related to the metabolic syndrome (Serne et al. 2007). Cerebral white matter

lesions are a marker of cerebral microvascular dysfunction and are associated with an increase in risk of stroke (Pantoni and Garcia 1997). Thus, these findings suggest that impairment of microvascular function has an association with cardiovascular disease and might initiate cardiovascular disease progression.

2.3 Is microvascular dysfunction present in healthy young African Americans

African Americans have higher rates of mortality and morbidity from cardiovascular and metabolic diseases including stroke, coronary artery disease, hypertension, and metabolic syndrome compared to Caucasian Americans (Fields et al. 2004; Mensah et al. 2005). The exact mechanism which increases a risk of cardiovascular diseases in African American is complicated; however, recent studies show that microvascular dysfunction, because of decreased NO bioavailability in this population, is a major contributor for the increased risk of cardiovascular diseases (Harris et al. 2011; Melikian et al. 2007).

Post occlusive reactive or thermal hyperemia on the blood vessels cause increased shear stress which, in turn, leads to an increase in NO production (Deanfield et al. 2007; Secomb and Pries 2011). For this reason many investigators have used venous occlusion plethysmography to test macro and -microvascular reactivity. Previous studies have demonstrated that the vascular dilatory capacity of resistance vessels is reduced in young African Americans relative to Caucasian Americans. For example, Bassett and colleagues (1992) recruited 21 normotensive young African Americans and 20 Caucasian Americans

(mean age 22 years) to investigate vasodilatory capacity in these groups. They found that vasodilatory capacity of the forearm resistance vessels was attenuated in normotensive young African American men when compared to normotensive young Caucasian American men (Bassett et al. 1992). Similarly, forearm vasodilatory response to isoproterenol infusion, which is a β-adrenergic agonist, was blunted in normotensive young African American men relative to Caucasian American men (Lang et al. 1995). NO mediated forearm blood flow (FBF) responses to methacholine and sodium nitroprusside administration were attenuated in healthy young African American men compared with Caucasian American men (Stein et al. 1997). Campia et al. (2002) noted that African Americans have reduced endothelium-dependent vasodilation and endothelium–independent vasodilation of the brachial artery indexed by flow-mediated dilation and nitroglycerin-mediated dilation relative to Caucasian Americans (Campia et al. 2002). These results suggest early onset of microvascular dysfunction in young African Americans prior to any overt signs of risk compared with young Caucasian Americans.

Although most studies on endothelial function in human vessels have focused on macrovessels by using plethysmography, there is an increased interest in how endothelial function contributes to alterations in the microcirculation. Researchers recently have recognized that impairment of microvascular function is involved in cardiovascular and metabolic diseases. Racial differences of macrovascular function have been investigated; however, the role of racial differences in microvascular function has not been fully

understood. Therefore, this study is important to elucidate the role of microcirculation in African Americans.

2.4 Local heating

In humans, neurogenic reflexes and local control factors determine the regulation of skin blood flow (SkBF). Two types of skin, glabrous and non-glabrous (hairy) skin, exist in humans. Non-glabrous skin is predominantly present on most of the body surface (Vinik et al. 2001). In this type of skin, two sympathetic pathways mediate the control of the cutaneous vascular reflexes: a nor-adrenergic vasoconstriction system and an active vasodilation system. In addition, local skin temperature (T_{loc}) is a major factor that regulates the skin vascular function. Cutaneous vasoconstriction, induced by direct local cooling of the skin, reduces SkBF. This is mediated by reflex activation of the sympathetic nervous system and requires norepinephrine release from vasoconstrictor nerve terminals (Pergola et al. 1993). Conversely, an increase in T_{loc} to 42°C for 20-30 minutes on the skin elicits maximal local vasodilation of skin vessels (Kellogg et al. 1995; Pergola et al. 1993).

Local heating of the skin raises T_{loc} of heated skin sites, while the core temperature and thermoregulatory reflex of the body is unaffected. Taylor et al. (1984) studied the effects of a high T_{loc} on skin vasodilation. The result of their study postulated that maximal SkBF on a forearm was obtained when T_{loc} of skin was reached and kept at 42°C, while FBF on the other arm was not increased (Taylor et al. 1984) thus confirming

the local influence of this perturbation. Local heating of skin induces cutaneous blood flow reactivity in two distinct phases. The local axon reflex mechanism primarily mediates the initial peak in SkBF during the first 10 minutes that is only minimally dependent on NO. This is followed by a plateau in SkBF after about 20 to 30 minutes that is primarily a result of NO-dependent vasodilation (Cracowski et al. 2006; Kellogg et al. 1999; Minson et al. 2001).

It has been suggested that the skin vascular circulation serves as a surrogate vascular bed for the assessment of global vascular health. Therefore, due to its accessibility and the opportunity of non-invasive measurements, it is an ideal site for investigation of systemic microvascular dysfunction. For example, impaired cutaneous microvascular reactivity is observed before any clinical signs of microvascular dysfunction during the early stages of diseases (Holowatz et al. 2008; Khan et al. 2008). This result suggests that skin vascular reactivity refers to a systemic microvascular dysfunction. Thus, the skin is a useful site for the assessment of microvascular responses to thermal hyperemia (Fromy et al. 2000; Minson et al. 2002; Stanhewicz et al. 2012).

2.5 Hypercapnic rebreathing

The cerebral blood vessels respond to the partial pressure of arterial carbon dioxide ($PaCO_2$). Hypercapnia (increased $PaCO_2$ tension) and hypocapnia (decrease $PaCO_2$ tension) evoke cerebral vasodilation and vasoconstriction respectively, thus, cerebral blood flow is tightly regulated by $PaCO_2$ (Kety and Schmidt 1948; Wasserman

and Patterson 1961). An increase in $PaCO_2$ a rebreathing protocol, leads to vasodilation of cerebrovascular smooth muscle cells and thus increased cerebral perfusion; this normally happens in the arterioles and the precapillary sphincter (Atkinson et al. 1990; Busija and Heistad 1984). The slope between the increases in cerebral perfusion relative to the Torr increases in $PaCO_2$ is termed cerebral vasomotor reactivity (CVMR) and has an important role in maintaining homeostasis in the brain. (Wei et al. 1980; Wijnhoud et al. 2006).

CVMR has been used as an index of overall cerebrovascular health and provides an index of cerebral vascular endothelial function. The transcranial Doppler (TCD) technique has commonly been used to measure CVMR for the purposes of clinical evaluation and experimental research since Aaslid and his colleagues introduced this non-invasive technique in 1982 for monitoring blood flow velocity in the cerebral arteries (Aaslid et al. 1982). Georgiadis et al. (2000) recruited 50 patients with various degrees of chronic heart failure and examined CVMR in response to hypercapnic rebreathing. The results of this study indicate that patients with cardiac failure have attenuated CVMR to rebreathing when compared with their normal controls (Georgiadis et al. 2000). Impaired CVMR in response to hypercapnia was also observed in patients who have hypertension (Serrador et al. 2005) and stroke (Wijnhoud et al. 2006). Therefore, these findings suggest that Cerebrovasomotor reactivity in response to rebreathing induced hypercapnia is a useful index of cerebrovascular function and a decrease in CVMR is associated with cardiovascular diseases.

CHAPTER 3

METHODOLOGY

3.1 Subjects

Nine African American (3 male and 6 female) and ten Caucasian American (5 male and 5 female) individuals were participated in this study. All individuals were normally active and aged of between 18 and 30. Exclusionary criteria included: obesity (body mass index $\geq$ 30 $kg \cdot m^{-2}$), hypertension (systolic > 140 mmHg and / or diastolic > 90 mmHg), and presence of any other cardiovascular, metabolic, or neurological disease. In addition, those currently taking any medication known to alter cardiovascular function were excluded. Given that smoking can affect the peripheral vascular response, individuals who are currently smokers or quit smoking within the prior two years were excluded (Avery et al. 2009).

Table 1 describes the physical characteristics of the individuals. All experimental procedures were explained to the individuals and they were given an opportunity to ask questions. Prior to participation, all individuals completed a health questionnaire and signed an informed consent approved by the Institutional Research Board at the University of Texas at Austin. Individuals were asked to refrain from any beverage containing alcohol or caffeine and exercise 24 hours prior to the testing

3.2 Experimental Procedures

Instrumentation and measurements

On the experimental day, subjects reported to the laboratory in the morning having refrained from alcohol beverages and strenuous exercise for 24 hours and from caffeine and food for at least 12 hours. Height and weight of the subjects were measured first and then the subjects were asked to lie down on a patient bed in the supine position.

Five electrodes were placed on the subjects for assessment of cardiac rhythms and heart rate measured continuously via an electrocardiogram on a patient monitor (HP patient Monitor, Agilent, Santa clara, CA). A blood pressure cuff was placed on one arm for intermittent assessment of arterial blood pressure via electrosphygmomanometry (Tango+; SunTech, Raleigh, NC). A finger cuff was placed on the hand of the opposite arm and was used for assessment of beat-by-beat arterial blood pressure using the Penaz method (CNAP, Biopac monitor 500, Austria). Mean arterial blood pressure (MAP) was calculated as one-third pulse pressure plus diastolic blood pressure.

Two local heating elements (3-cm diameter, Peritemp4005; Perimed) were placed on one of the subject's forearms and was used for local manipulation of skin temperature during the local heating protocol (see below). An integrating laser Doppler flow probe (MoorLab Laser Doppler Perfusion Monitor, Moor Instruments, Wilmington, DE) was placed into each of the local heating elements. This approach allowed for continuous assessment of SkBF at the site of T_{loc} manipulation via the local heating protocol (see below).

Cerebral blood flow was indexed from the velocity of blood flow in the middle cerebral artery (MCAVmean). Briefly, a 2-MHz Doppler probe (Multi-flow, DWL Elektronische Systeme, Singen, Germany) was adjusted over the temporal window of the right middle cerebral artery (MCA) until an optimal signal was identified at which point

the probe was held in place with a size-adjustable head band. $PaCO_2$ tension was indexed from partial pressure of end-tidal carbon dioxide ($PETCO_2$) (VitalCap Capnograph Monitor, Oridion, Needham, MA, USA). This approach allowed for continuous breathe-by-breathe assessment of MCAVmean and $PETCO_2$ which was used for subsequent calculation of CVMR. Room temperature was kept between 21 ~ 22 °C. Throughout the entire protocol subjects kept in supine position quietly for the remainder of data collection.

Local heating protocol

Following instrumentation there was a 10 min baseline period where the local heaters were clamped at 34 °C while SkBF was continuously determined in one forearm using laser-Doppler flowmetry. The measurement sites were kept at 34 °C for 10 minutes during this baseline period. At the end of tenth minute, this was immediately followed by initiation of local heating of the two sites on skin where SkBF was being measured. This heating protocol was accomplished by increasing the temperature of heating elements to 40°C for a 30 minutes period. Temperature of the local heating elements was increased at a rate of 0.5 °C every 5 seconds to a temperature of 40 °C. At the end of this period, the temperature of the local heating elements was increased to 43 °C for a period of 30 minutes with the same rate that described above. This procedure induces a maximal SkBF response in the cutaneous circulation that is primarily dependent on NO bioavailability (Brothers et al. 2010; Kellogg et al. 1998; Minson et al. 2001; Minson et al. 2002). Immediately following this period the local heating elements were turned off. During this local heating protocol, the subjects were encouraged to maintain the measured arm stable and the measurement sites were monitored to ensure a consistent measurement.

Rebreathing protocol

Following local heating protocol, the subjects remained in the supine position. During this time, a 2-MHz Doppler probe was fixed over the temporal window of the MCA for the assessment of cerebral blood flow which was indexed from MCAVmean using TCD ultrasonography. The probe was fixed and held in place with a size-adjustable head band until an optimal signal was identified. A finger cuff was inflated for the assessment of non-invasive beat-by-beat arterial blood pressure. The subjects were fitted with a nose clip and mouth piece which was connected with a sampling line for continuous assessment of $PETCO_2$. Then, baseline data were collected for 6 minutes with blood pressure assessed via auscultation of the brachial artery occurring in the final minute of this stage. During baseline data collection, the subjects breathed room air. At the end of this 6 minutes baseline data collection, the subjects were asked to breathe in maximally and hold it for a second to change the switch of 3-way valve (2100 series, Hans Rudolph, Kansas City, MO) that connected with a bag and mouthpiece. This allowed the subjects breathe in and out (rebreathe). This initiated the hypercapnic rebreathing test. The subjects rebreathed their own air for approximately 4 minutes which typically increases $PETCO_2$ by approximately 12 -15 Torr (Claassen et al. 2007). According to the subject's basal metabolic rate (estimated by using the Harris-Benedict formula), oxygen gas was supplied into the bag to keep constant arterial oxygen saturation (Harris and Benedict 1918).

3.3 Data analysis

Data were sampled at 125 Hz via a data-acquisition system (Biopac System, Santa Barbara, CA). The last 45 seconds at each local heating phase (i.e. 34, 40, 43 °C) were used for data analysis. SkBF obtained from two sites of the forearm skin during local

heating was averaged. Skin vascular conductance (SkVC) was calculated from the ratio of red blood cell (RBC) flux to MAP. Skin vascular conductance (SkVC) was presented as a percent of each respective maximal SkBF achieved during 43 °C heating (SkVC of % max). Cerebrovascular conductance (CVC) was calculated from the ratio of MCAVmean to MAP which is monitored by using the Penaz method. MCAVmean, MAP, CVC, and $PETCO_2$ tension were assessed on a breath-by-breath basis. The last 45 seconds of the 6 minute baseline period were used for the baseline data analysis. The percent change in CVC from the baseline value during hypercapnia was determined while absolute change in $PETCO_2$ tension from the baseline value was determined

Blood pressures taken at the brachial artery were used for baseline and SkVC data analysis, while beat-by-beat blood pressure was used for CVMR data analysis. $PETCO_2$ provides an indirect and non-invasive index or $PaCO_2$ concentration. $PETCO_2$ was continuously monitored using a capnograph (Capnocheck+; Smiths Medical, Dublin, OH).

3.4 Statistical analysis

Descriptive analyses were conducted to characterize the study sample. The means and standard deviations (SD) were reported for continuous variables (age, height, weight, and BMI), and the numbers of subjects were reported for categorical variables (sex). The means and standard deviations were also reported for continuous variables (heart rate (HR), MAP, $PETCO_2$, MCAVmean, CVC) at the baseline of hypercapnic rebreathing period. Statistically significant differences between African Americans and Caucasian Americans were tested using unpaired t-test for continuous variables. One-way repeated measures analysis of variance (ANOVA) was performed to compare hemodynamic

variables (HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), MAP) in response to local heating among 34 ºC, 40 ºC, and 43 ºC regardless of race type. Additionally, two-way repeated measures ANOVA were employed to assess the hemodynamic variables (HR, SBP, DBP, MAP) in response to local heating of the same individuals at three different temperatures over time between African Americans and Caucasian Americans. Two-way ANOVA were employed to assess SkVC of % max at two different temperatures between two groups. The linear regression was used for calculating the slope of the percent change in CVC from the baseline during the hypercapnic period with respect to the absolute change in $PETCO_2$ from the baseline. Unpaired t-test was used to analyze differences in CVMR between African Americans and Caucasian Americans. The alpha level for statistical significant was used at 0.05. SPSS version 19 (Systat Software, Inc., Chicago, Illinois) was used for statistical analyses.

CHAPTER 4

RESULTS

4.1 Subjects

Nine African Americans and ten Caucasian Americans were enrolled in this study. Physical characteristics are displayed in Table 1. No sex differences in SkBF response to local heating and in CVMR to hypercapnic rebreathing were observed in either group. Thus, the data from men and women were combined for each group. The average age of the subjects was 23.2 yrs. ± 3.3 in the African American group and 22.4 yrs. ± 3.1 in the Caucasian American group (p=0.60). There were no significant difference in height (African American (AA): 171.9 ± 8.9 cm vs. Caucasian American (CA): 173.5 ± 8.9 cm, p=0.70), weight (AA: 73.6 ± 17.5 kg vs. CA: 68.0 ± 10.1 kg, p=0.42), or body mass index (AA: 24.68 kg/m^2 ± 4.3 vs. CA: 22.55 ± 2.7 kg/m^2, p=0.23) between African American and Caucasian American individuals.

Variables	AA (n=9)	CA (n=10)	*P*-value
Age, y	23.2 ± 3.3	22.4 ± 3.1	0.60
Sex (male/female)	3/6	5/5	n/a
Height, cm	171.88 ± 8.88	173.50 ± 8.89	0.70
Weight, kg	73.62 ± 17.53	68.02 ± 10.07	0.42
BMI, kg/m^2	24.68 ± 4.34	22.55 ± 2.72	0.23

Notes:
Values are means ± SD. BMI, body mass index.

Table 1: Subject characteristics

4.2 Local heating

Table 2 shows hemodynamic responses to rise in temperature. One-way and two-way repeated ANOVA were performed to compare HR, SBP, DBP, and MAP in response to local heating among 34 ºC, 40 ºC, and 43 ºC with or without race type each. There were no statistically significant differences in HR, SBP, DBP, and MAP in response to local heating regardless of race type. There were also no statistically significant differences in HR, SBP, DBP, and MAP in response to local heating of the same individuals at three different temperatures over time between African Americans and Caucasian counterparts.

	AA				CA				
Variables	34 ºC	40 ºC	43 ºC	*P*-value[a]	34 ºC	40 ºC	43 ºC	*P*-value[a]	*P*-value[b]
HR (beats × min^{-1})	57.5±11.3	56.2±10.3	57.0±10.8	0.97	55.9±10.7	55.0±8.6	56.4±9.3	0.94	0.80
SBP (mmHg)	108.9±10.8	108.8±12.5	111.3±11.4	0.87	111.5±15.7	107.6±11.8	106.3±16.0	0.71	0.11
DBP (mmHg)	69.8±11.0	73.4±12.0	72.9±8.6	0.74	63.5±5.4	65.0±7.3	67.3±11.9	0.62	0.84
MAP (mmHg)	82.8±10.5	85.2±11.9	85.7±8.8	0.82	79.5± 6.3	79.2±7.2	80.3±6.3	0.92	0.21

Notes:

Values are means ± SD. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure

[a] Results of one-way repeated measures ANOVA

[b] Results of two-way repeated measures ANOVA

Table 2: Hemodynamic responses to rise in temperature

Table 3 shows SkBF response to local heating. SkBF, indexed by RBC flux, increased with increases in T_{loc} of the skin in both groups. SkVC also increased with increases in T_{loc} of the skin. At 34 ºC, SkVC was lower in African Americans than Caucasian Americans (AA: 0.37 ± 0.1 flux/mmHg vs. CA: 0.23 ± 0.1 flux/mmHg, $p<0.01$). SkVC at 40 ºC (AA: 1.46 ± 0.5 flux/mmHg vs. CA: 1.70 ± 0.4 flux/mmHg, $p=0.15$) and 43 ºC (AA: 2.59 ± 0.6 flux/mmHg vs. CA: 2.57 ± 0.7 flux/mmHg, $p=0.48$) were not significantly different between African Americans and Caucasian Americans. However, normalized SkVC to a maximal value (SkVC % max) obtained during local heating at 43 ºC for 30 minutes were significantly lower in African Americans than Caucasian Americans at 34 ºC (AA: 10 ± 2 % of max vs. CA: 16 ± 7 % of max, $p=0.01$) and 40 ºC (AA: 55.6 ± 14.5 % of max vs. CA: 67.9 ± 11.6 % of max, $p=0.03$).

Variables	Temperature (ºC)	AA	CA	*P*-Value
SkBF (RBC flux)	34	18.6 ± 5.7	29.9 ± 10.1	0.01*
	40	117.9 ± 39.3	133.2 ± 40.6	0.33
	43	213.9 ± 31.8	202.8 ± 47.2	0.56
SkVC (flux/mmHg)	34	0.23 ± 0.1	0.37 ± 0.1	0.01*
	40	1.43 ± 0.6	1.70 ± 0.4	0.24
	43	2.54 ± 0.6	2.57 ± 0.7	0.94
SkVC (% of max)	34	9.5 ± 2.5	16.1 ± 7.2	< 0.01*
	40	55.6 ± 14.5	67.9 ± 11.6	0.03*

Note:
Values are means ± SD. SkBF, skin blood flow; SkVC, skin vascular conductance
* Significantly different ($p < 0.05$)

Table 3: Skin blood flow response to local heating

Two-way ANOVA shows that there was a significant difference in SkVC of % max among different temperatures and race, and there was not a statistically significant temperature and race interaction effect (Figure 1).

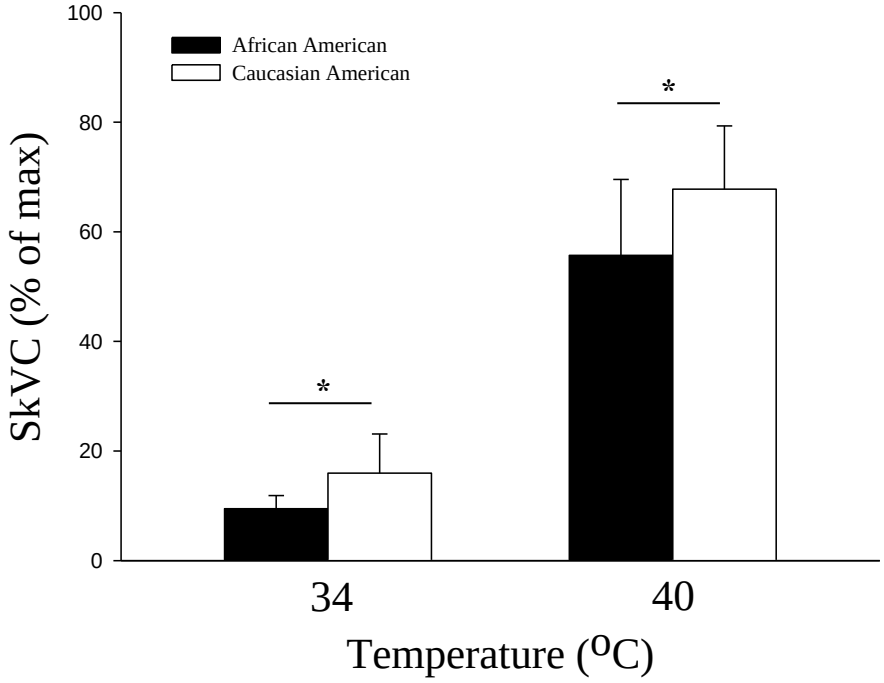

Figure 1: Normalized skin vascular conductance to a maximal value. Abbreviation: SkVC, skin vascular conductance. * Significantly different in African Americans compared to Caucasian Americans ($p < 0.05$)

4.3 Hypercapnic Rebreathing

Hemodynamic information is displayed in Table 4. During eucapnia (baseline), there were no significant differences in HR (AA: 59.5 ± 11.9 bpm vs. CA: 59.0 ± 10.9 bpm, p=0.92), MAP (AA: 84.5 ± 13.8 mmHg vs. CA: 84.8 ± 7.1 mmHg, p=0.95),

MCAVmean (AA: 67.9 ± 12.3 $cm \cdot s^{-1}$ vs. CA: 61.2 ± 13.9 $cm \cdot s^{-1}$, p=0.28), CVC (AA: 0.8 ± 0.2 $cm \cdot s^{-1}$/mmHg vs. CA: 0.7 ± 0.2 $cm \cdot s^{-1}$/mmHg, p=0.34), $PETCO_2$ (AA: 38.4 ± 3.4 mmHg vs. CA: 36.9 ± 4.5 mmHg, p=0.45) between the African American and Caucasian American groups.

Variables	AA	CA	*P*-value
HR (beats × min^{-1})	59.48 ± 11.85	58.95 ± 10.86	0.92
MAP (mmHg)	84.48 ± 13.81	84.78 ± 7.10	0.95
$PETCO_2$ (mmHg)	38.40 ± 3.65	36.93 ± 4.48	0.45
$MCAV_{mean}$ (cm × sec^{-1})	67.91 ± 12.33	61.16 ± 13.86	0.28
CVC (cm × sec^{-1} × $mmHg^{-1}$)	0.82 ± 0.17	0.73 ± 0.20	0.34

Notes:
Values are means ± SD. HR, heart rate; MAP, mean arterial pressure; $PETCO_2$, partial pressure of end-tidal carbon dioxide; MCAVmean, the velocity of blood flow in the middle cerebral artery; CVC, cerebral vascular conductance

Table 4: Hemodynamic responses during eucapnia

The slope of the increase in CVMR per mmHg increase in $PaCO_2$ indexed by $PETCO_2$ were significantly attenuated in African Americans relative to Caucasian Americans (AA: 2.8 ± 1.2 CVC % of baseline/mmHg vs. CA: 5.7 ± 0.9 CVC % of baseline/mmHg, p<0.001). (Figure 2A, B)

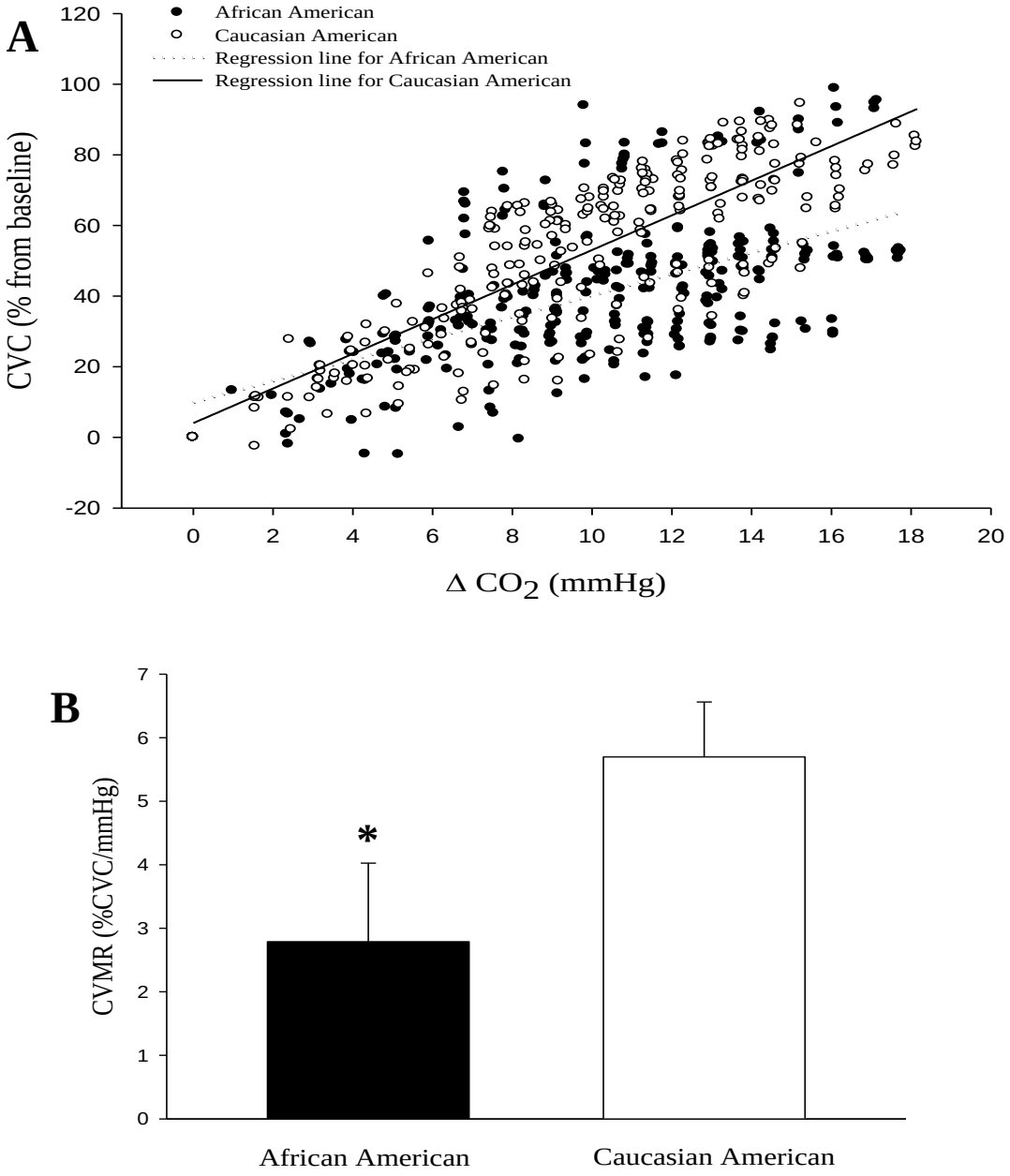

Figure 2: Cerebral vasomotor reactivity in response to rebreathing protocol. Panel A is a tracing of the cerebral vascular conductance response to rebreathing induced hypercapnia in entire subjects. Panel B illustrates the group averaged values for cerebral vasomotor reactivity. The percentage change of CVC from its baseline as a function of PETCO2 was lower in the AA than the CA group. *significantly different (P<0.001).

CHAPTER 5

DISCUSSION

This study investigated peripheral and cerebral microvascular function in African Americans and Caucasian Americans. The results of this study demonstrate that healthy young African American individuals have impaired microvascular function compared with a similar group of Caucasian Americans. This was observed at a relatively young age prior to signs of overt cardiovascular or metabolic disease. This finding suggests that microvascular dysfunction might occur as early as in the second generation of life prior to any overt signs of cardiovascular and/or metabolic disease in African American population. Furthermore, this might partially explain why this population is more likely to develop cardiovascular disease than other ethnic groups as they age.

The main finding of this study is that cutaneous vasodilation in response to local heating of the skin was lower in African Americans than Caucasian Americans. This result, however, differs from previous investigation. Kahn and colleagues (2002) have examined forearm microvessels to test different vasodilatory responses between African Americans and Caucasian Americans. They administrated endothelium-dependent and endothelium-independent agonists into the brachial artery and used venous occlusion plethysmography to measure FBF. They recruited 34 normotensive African Americans and 36 Caucasian Americans who were matched for age, sex, blood pressure, and other risk factors. FBF in response to methacholine infusion, an endothelium-dependent vasodilator, was not significantly difference between African Americans and Caucasian

Americans. In addition, these investigators also observed similar responses to infusion of nitroprusside and verapamil, which are the endothelium-independent vasodilators in African American and Caucasian American subjects (Kahn et al. 2002). The reason for these differing results remains unknown, but is perhaps related to the different techniques for assessing microvascular function used. Kahn et al. (2002) used venous occlusion plethysmography.

Nevertheless, our finding is consistent with some pervious investigations. For example, Lang et al (1995) reported that healthy African Americans have a blunted response to arterial isoproterenol infusion compared with Caucasian Americans (Lang et al. 1995). This research group also found blunted response to methacholine and sodium nitroprusside in healthy African Americans relative to healthy Caucasian Americans (Stein et al. 1997). Similarly, impaired vasodilator responses to acetylcholine, isoproterenol, and sodium nitroprusside were observed in healthy African Americans relative to Caucasian Americans (Cardillo et al. 1999).

We also found that CVMR in response to hypercapnia was attenuated in African Americans when compared with Caucasian Americans. Little is known about the relationship between CVMR and ethnicity. To our knowledge, researchers to date have not investigated racially different CVMR in response to hypercapnia. Thus, it could be a significant finding in this field, although, the underlying mechanisms should be further investigated.

A number of mechanisms might explain this impaired peripheral and cerebral microvascular response and reactivity to local heating and hypercapnia in African

American relative to their Caucasian counterparts. It is possible that microvascular endothelial dysfunction induced by low NO bioavailability might partly lead to reduction of peripheral and cerebral microvascular dilation in African Americans. In support of this possibility, there is evidence that superoxide produced by nicotinamide adenine dinucleotide (phosphate) oxidase leads to excess peroxynitrite formation after endothelial NO synthase stimulation. This suggests that this might lead to the endothelial NO synthase uncoupling, which produces more superoxide (Kalinowski et al. 2004). It has been reported that endothelial dysfunction might be mediated by superoxide (Guzik et al. 2000).

It is also possible that diminished levels of the NO precursor L-arginine might cause attenuated NO-dependent vasodilation in the African American group relative to the Caucasian American group. Houghton et al. (2002) have reported that infusion of L-arginine into the coronary artery elicits significantly greater endothelium-dependent vasodilation in African Americans when compared with matched Caucasian Americans (Houghton et al. 2002). This is supported by the evidence that the intravenous infusion of L-arginine might improve impaired cerebrovascular reactivity in response to inhalation of 5 % CO_2 (Zimmermann and Haberl 2003).

In addition, reduced transduction of the NO signaling in smooth muscle cells in African American could be an alternative explanation of impaired microvascular function. Stein et al. (1997) reported that an increase in SkBF to infusion of sodium nitroprusside iontophoresis is attenuated in African Americans relative to Caucasian Americans (Stein et al. 1997). Sodium nitroprusside is an endothelium-independent

vasodilator acting through NO; thus, reduced SkBF response to infusion of sodium nitroprusside iontophoresis in African Americans suggests that the smooth muscle cells might be losing their NO-dependent dilatory capacity to the same extent as in Caucasian counterparts for a given level of NO.

There is also a possibility that vitamin D deficiency could explain decreased peripheral and cerebral microvascular function in the healthy young African American group relative to the matched Caucasian American group. For example, vitamin D concentration was lower in African Americans compared with Caucasian Americans (Nesby-O'Dell et al. 2002). Interestingly, Harris et al (2011) has reported that vitamin D supplementation for 16 weeks effectively improves NO-dependent vasodilation and endothelial function indexed by flow-mediated vasodilation in African Americans (Harris et al. 2011). This finding suggests that vitamin D supplementation might improve NO bioavailability even though the direct mechanism is not clear.

The exact mechanism resulting in microvascular impairment in healthy young African American is complicated. Therefore, future direction is to extend our findings to design prospective studies to determine whether decreases in NO bioavailability and vitamin deficiency are indeed related to impaired microvascular function in healthy young African American individuals.

CHAPTER 6

LIMITATIONS AND IMPLICATIONS

Female individuals were studied during menstruation (early follicular phase of the menstrual cycle) except for one African American female and one Caucasian American female. This is because the menstrual cycle is known to alter the NO-dependent vasodilation to local heating (Charkoudian et al. 1999).

Although LDF is non-invasive and qualitative SkBF measurement, it does not allow us to compare the absolute SkBF between individuals. This is because substantial variations for point-to-point comparisons among individuals and even in the same individual (Johnson et al. 1984; Smits et al. 1986) which make us questionable to use of absolute LDF measurement. Calculating SkBF measured by LDF to an individual's maximal values might let us control for the disadvantages of LDF. Using laser Doppler flux in a relative term (i.e. % of max) can provide comparison for site-to-site and among different individuals. In addition, we used two laser Doppler flow probes to restrict the influence of site-to-site variations in SkBF within individual in this study. Therefore, the expression of CVC (% of max) for the SkBF measurement is appropriate and necessary for making accurate comparisons.

TCD technique does not allow us to compare the absolute cerebral blood flow (CBF) between individuals. There are three 'windows' including temporal, orbital and, foramen magnum that can be used to insonate different cerebral vessels with TCD. The MCA is useful to be insonated due to its accessibility and the quality of the signal

through the temporal window (Rutgers et al. 2000). Since TCD measures only blood flow velocity, TCD estimation of blood flow velocity can be used only to assess changes in CBF. Nevertheless, a number of studies indicate that changes in CBF and CBF velocity in response to hypercapnia are correlated well. Although correlation between changes in CBF and CBF velocity is acceptable, there is no relationship between absolute CBF and CBF velocity in response to hypercapnia (Clark et al. 1996; Nuttall et al. 1996; ter Minassian et al. 1998). However, absolute values of CBF are not important for the purpose of reliability and repeatability in that CVMR is determined by stimulus-response principles. Thus, MCAVmean is a reliable and valid index when measuring changes in CBF in response to hypercapnic rebreathing.

CHAPTER 7

CONCLUSION

In conclusion, we observed that SkBF, indexed by SkVC (% of max), in response to local heating of the skin is lower in healthy young African American than their Caucasian counterparts. Furthermore, these African American individuals also have attenuated CVMR in response to hypercapnic rebreathing relative to Caucasian American individuals. Taken together, our findings support the hypotheses that peripheral and cerebral microvascular functions are impaired in a relatively young population of healthy African Americans compared with Caucasian Americans. These findings indicate that microvascular dysfunction presents in healthy young African Americans prior to the onset of overt cardiovascular diseases. This is likely related to the greater incidence of cardiovascular and metabolic diseases in this population.

Appendix A: Informed consent form

You are being asked to participate in a research study. This form provides you with information about the study. The Principal Investigator (the person in charge of this research) or his/her representative will provide you with a copy of this form to keep for your reference, and will also describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you don't understand before deciding whether or not to take part. Your participation is entirely voluntary and you can refuse to participate without penalty or loss of benefits to which you are otherwise entitled.

Title of Research Study: Relationship between Mechanisms of Increased Cardiovascular Risk and Reduced Cognitive Performance in African Americans

Principal Investigator(s) (include faculty sponsor), UT affiliation, and Telephone Number(s):

R. Matthew Brothers, Ph.D. Assistant Professor: Department of Kinesiology and Health Education; College of Education; University of Texas at Austin. Phone: (512) 516-1961; Email: r.m.brothers@mail.utexas.edu.

Darla Castelli, Ph.D. Associate Professor: Department of Kinesiology and Health Education; College of Education; University of Texas at Austin. Phone: (512) 232-7636; Email: dacastelli@mail.utexas.edu.

Chansol Hurr, Research assistant: Department of Kinesiology and Health Education; College of Education; University of Texas at Austin.

Kiyoung Kim, Research assistant: Department of Kinesiology and Health Education; College of Education; University of Texas at Austin.

Jungyun Hwang, Research Assistant: Department of Kinesiology and Health Education; College of Education; University of Texas at Austin.

Hildi Nicksic, Research Assistant: Department of Kinesiology and Health Education; College of Education; University of Texas at Austin.

Funding source: University of Texas at Austin

What is the purpose of this study?

African American individuals experience less rise in skin blood flow in response to high skin temperatures, have reduced brain blood flow, and have blood vessels that are stiffer relative to younger individuals. These issues can place these individuals at an

increased risk for heat related illness or death and for developing cardiovascular disease. This project will study peripheral and cerebral microvascular function.
Forty-eight individuals (twenty-four in the African American group and twenty-four in the Caucasian American group) between the ages of 18-30 years will be recruited for this study.

What will be done if you take part in this research study?
Before you can be admitted to the study, you will be given a brief examination during your visit to the laboratory. This examination will include filling out a brief health history questionnaire, which will include questions about your age, sex, and ethnic origin. Additional measurements may include standard measures of bodyweight, height, blood pressure, and heart rate.

If you are deemed eligible for the study you will be enrolled into the study. The expected time for you to complete this study is approximately 2 hours. Please refer to the following section for descriptions of the visits to the laboratory.

Laboratory visits – step-by-step protocol

1st visit - *Experimental trials to identify the effect of flavanol supplementation on skin and brain blood flow.*

1. Arrival at laboratory, health history questionnaire
2. Measures of body height and weight
3. Venous blood sample (obtained from a vein in your upper arm)
4. Measures of blood pressure and heart rate
5. Protocol and measurements

- ***Total time: 120 minutes***

A detailed list and short description of procedures for the study day is described below. Additionally, the potential risks and duration of each procedure are provided. If at any time you wish to discuss the information above or any other risks you may experience, you may ask questions now or call the Principal Investigator listed on the front page of this form.

Control of skin temperature:

- **Description of Procedure:** Control of whole body skin temperature is accomplished by dressing you in a suit made of plastic tubes through which water of different temperatures is passed. Using the suit, skin temperature will be maintained at a normal constant skin temperature of 90 to 93 °F.
- **Potential Risks:** There are no risks associated with these temperature changes.
- **Duration of Procedure:** The total duration of this period will be about 2.5 hours.

Manipulation of skin temperature (local heating):

- **Description of Procedure:** Control of local skin temperature is accomplished by locally heating the site where skin blood flow is measured. The highest temperature we will use is 108° F for 30 minutes.
- **Potential Risks:** There are no risks associated with these temperature changes, although there is a small risk of minor discomfort at the heated sites.
- **Duration of Procedure:** The total duration each session of local heating will be about 30 minutes.

Skin temperature:

- **Description of Procedure:** Skin temperature will be measured by taping temperature probes to your skin.
- **Potential Risks:** There is no risk associated with this procedure.
- **Duration of Procedure:** Skin temperature will be measured during the entire experiment (approximately 1 hour).

Skin blood flow:

- **Description of Procedure:** Skin blood flow will be measured using laser-Doppler devices. These devices use very low power laser light to measure how fast blood is flowing in blood vessels in your skin. A small probe will be placed on one of your forearms.
- **Potential Risks:** There are no risks associated with using these devices to measure skin blood flow; they are painless and harmless in all respects.
- **Duration of Procedure:** Skin blood flow will be measured during the entire experiment (approximately 1 hour).

Electrocardiogram:

- **Description of Procedure:** Sticky patches will be applied to your skin to measure the heart's electrical signals.
- **Potential Risks:** There is no risk or discomfort associated with this procedure.
- **Duration of Procedure:** The electrocardiogram will be measured during the entire experiment (approximately 2 hours).

Finger blood pressure:

- **Description of Procedure:** In order to continuously monitor your blood pressure during the experiment, a small blood pressure cuff will be placed on one of your fingers. Occasionally, some people experience some mild discomfort in the finger after a prolonged period of inflation. If this is the case with you, we can easily deflate the cuff to give the finger a rest.
- **Potential Risks:** Other than some potential discomfort associated with cuff inflation there are no known risks to this procedure.
- **Duration of Procedure:** We will measure your blood pressure during the entire experiment (approximately 2 hours).

Blood pressure:

- **Description of Procedure:** Your blood pressure will also be monitored using a cuff placed on your upper arm that is inflated and deflated periodically.
- **Potential Risks:** Other than some potential discomfort associated with cuff inflation there is no risk to this procedure.
- **Duration of Procedure:** The cuff will be on your upper arm during the entire experiment. We will take blood pressure measurements at different time points during the experiment. Each measurement will last approximately 30 seconds.

Brain blood flow:

- **Description of Procedure:** A gel covered probe will be placed against the temple of your head. Sound waves will be used to record blood flow inside your skull, similar to standard ultrasound tests done to examine the health of fetuses prior to birth.
- **Potential Risks:** There is no risk associated with this procedure.
- **Duration of Procedure:** We will measure your brain blood flow during the entire experiment (approximately 2 hours).

Arm venipuncture:

- **Description of Procedure:** A blood sample will be obtained from a vein in your non-dominant arm. Approximately 3-4 tablespoons of blood to be obtained twice on each day. This method is routinely used to obtain blood for physical examinations.
- **Potential Risks: There is a very small** risk associated with this procedure. This includes minimal discomfort associated with insertion of the needle, a small amount of local bleeding and increased risk of a small bruising (~10% of cases) at the needle insertion site. Also, you may become faint at the site of seeing blood drawn. The likelihood of these complications is very remote when the procedure is carried out by trained personnel and proper equipment is used, as it will be in your case.
- **Duration of Procedure:** This procedure will occur once on the study day.

Brain vasomotor reactivity:

- **Description of Procedure:** We will test how your brain blood flow responds to changes in carbon dioxide concentration. You will be asked to breathe room air through a mouthpiece which is connected to a bag. You will breathe in and out of the bag for another 3 minutes.
- **Potential Risks:** Changing the rate of breathing (i.e. slightly more or slightly less breaths per minute) is safe and well tolerated by subjects. The small amount of carbon dioxide breathed is harmless and is completely washed out in less than 10 minutes. Medical grade oxygen (100%) will be

available throughout the test. You may feel dizzy or short of breathe and may experience a tingling or numbing sensations. Should any of these symptoms occur the test will be stopped and you will be given normal room air and/or 100% medical grade oxygen immediately and the sensations will cease within seconds.

- **Duration of Procedure:** This procedure will last for approximately 8 minutes on the day.

Carbon Dioxide Concentration ($PETCO_2$):

- **Description of Procedure:** Your body is constantly producing carbon dioxide which then leaves the body when you breathe out. This procedure will provide a measurement of how much carbon dioxide is expired each time you breathe out. To do this you will breathe normally with a nasal cannula fitted just outside of your nostrils. This nasal cannula will be unnoticeable to you during the protocol.
- **Potential Risks:** There is no risk associated with this procedure. You will most likely not be able to notice the cannula at all.
- **Duration of Procedure:** This nasal cannula will be in for the entire experiment (approximately 2 hours).

All tests that are to be performed have safely been used in both healthy and diseased individuals. Throughout the tests you will be closely monitored by highly skilled and trained personnel.

Because of your participation in this study, you are at risk for the above mentioned side effects. You should discuss these with the researchers and your regular health care provider. All the tests in this study are designed for research only, not for medical purposes. Even though the researchers are not looking at your tests to find or treat a medical problem, you will be told if they notice something unusual. You and your regular doctor can decide together whether to follow up with more tests or treatment. Because all research tests done in this study are not for medical purposes, the research results will not be sent to you or to your regular doctor.

The Project Duration is: Total time for completion of this study is approximately 3 hours.

What are the possible discomforts and risks?
A description of the risks associated with each of the procedures is described above.

Additional risks include:
Loss of Confidentiality: Any time information is collected; there is a potential risk for loss of confidentiality. Every effort will be made to keep your information confidential; however, this cannot be guaranteed.

There may be other side effects that are unknown at this time including those to women of child bearing potential. If you are concerned about other, unknown side effects, please discuss this with the study doctors and researchers.

What are the possible benefits to you or to others?
If you agree to take part in this study, you will benefit by receiving medical evaluation prior to participating in the study. Occasionally an unknown condition that might require medical attention (such as hypertension, hypotension etc.) is discovered during the routine evaluation. This information will be released to you and we will encourage you to seek further medical care if this is the case.

The new information learned from this study may benefit others. Results from this research may identify significant factors involved in the regulation of blood pressure and blood vessel function.

If you choose to take part in this study, will it cost you anything?
No

Will you receive compensation for your participation in this study?
Yes, you will be compensated $25.00 upon completion of the entire study.
Disclosure of your social security number (SSN) is requested from you in order for The University of Texas at Austin to compensate you as described above for your participation. At all times, any papers that contain you social security number will be stored securely in locked cabinets and in separate files from other study records.

What if you are injured because of the study?
It is important that you report any illness or injury to the research team listed at the top of this form immediately.

Compensation for an injury resulting from your participation in this research is not available from The University of Texas at Austin and therefore there are no plans to compensate you in the event of an injury related to the study.

You retain your legal rights during your participation in this research.

If you do not want to take part in this study, what other options are available to you?
Your participation in this study is entirely voluntary. You are free to refuse to be in the study, and your refusal will not influence current or future relationships with The University of Texas at Austin.

How can you withdraw from this research study and who should you call if you have questions?
If you wish to stop your participation in this research study for any reason, you should contact the principal investigator: R. Matthew Brothers at (512) 232 - 6016. You should also call the principal investigator for any questions, concerns, or complaints about the research. You are free to withdraw your consent and stop participation in this research study at any time without penalty or loss of benefits for which you may be entitled. Throughout the study, the researchers will notify you of new information that may become available and that might affect your decision to remain in the study.

Whom to contact with questions concerning your rights as a research participant?
For questions about your rights or any dissatisfaction with any part of this study, you can contact, anonymously if you wish, the Institutional Review Board by phone at (512) 471-8871 or email at orsc@uts.cc.utexas.edu.

How will your privacy and the confidentiality of your research records be protected?
Each subject will be assigned a unique Subject ID code. This informed consent form and the Health History Questionnaire are the only places where any personal identifying information including your social security number will be recorded. These forms will be stored in a locked file cabinet. In all other cases, your data will only be identifiable by your unique code. Only the directors of the project will have access to a master list that will link your identity to your code.

If in the unlikely event it becomes necessary for the Institutional Review Board to review your research records, then The University of Texas at Austin will protect the confidentiality of those records to the extent permitted by law. Your research records will not be released without your consent unless required by law or a court order. The data resulting from your participation may be made available to other researchers in the future for research purposes not detailed within this consent form. In these cases, the data will contain no identifying information that could associate you with it, or with your participation in any study.

If the research project is sponsored (e.g., receives funding from outside UT-Austin) then the funding institution also have the legal right to review your research records.

If the results of this research are published or presented at scientific meetings, your identity will not be disclosed.

Will the researchers benefit from your participation in this study*?*
The researchers will gain no benefit from your participation in this study beyond the publication and/or presentation of the results obtained from the study.

Signatures:

As a representative of this study, I have explained the purpose, the procedures, the benefits, and the risks that are involved in this research study:

Signature and printed name of person obtaining consent Date

You have been informed about this study's purpose, procedures, possible benefits and risks, and you have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time. You voluntarily agree to participate in this study. By signing this form, you are not waiving any of your legal rights.

Printed Name of Subject Date

Signature of Subject Date

Signature of Principal Investigator Date

Appendix B: Participation medical screening form

Personal Information
Name: _____

Today's Date: _____

Age _____ Sex □ Male
□ Female: Date of Last Menstrual Period: _____

Contact Information:
_____

Physician Name and Phone
Number:_____

Emergency Contact Info:
_____

Symptoms or Signs Suggestive of Disease
Check appropriate box:

Yes	No		
□	□	1.	Have you experienced unusual pain or discomfort in your check, neck, jaw, arms or other areas that may be due to heart problems?
□	□	2.	Have you experienced unusual fatigue or shortness of breath at rest, during usual activities, or during mild-to-moderate exercise (e.g., climbing stairs, carrying groceries, brisk walking, cycling)?
□	□	3.	When you stand up, or sometimes during the night while you are sleeping, do you have difficulty breathing?
□	□	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
□	□	5.	Do you suffer from swelling of the ankles (ankle edema)?
□	□	6.	Have you experienced an unusual and rapid throbbing or fluttering of the heart?
□	□	7.	Have you experienced severe pain in your leg muscles during walking?
□	□	8.	Has a doctor told you that you have a heart murmur?

Chronic Disease Risk Factors
Check appropriate box:

Yes	No		
□	□	9a.	Are you a male over age 45 years or a female over age 55 years?
□	□		b. Are you a female who has experienced premature menopause?

□	□		c. If you answered "yes" to 9b, are you on estrogen replacement therapy?
□	□	10.	Has your father or brother had a heart attack or died suddenly of heart disease before the age of 55; has your mother or sister experienced these heart problems before the age of 65?
□	□	11.	Are you a current cigarette smoker? If quit smoking, when? Date:
□	□	12.	Has a doctor told you that you have high blood pressure (more than 140/90 mm Hg) or a heart condition?
□	□	13.	Is your total serum cholesterol greater than 200 mg/dl, or has a doctor told you that your cholesterol is at a high risk-level?
□	□	14.	Do you have diabetes mellitus?
□	□	15.	Are you physically inactive and sedentary (little physical activity on the job or during leisure time)?
□	□	16.	Do you have a bone or joint problem that could be made worse by a change in your physical activity?
□	□	17.	During the past year, would you say that you have experienced enough stress, strain, and pressure to have a significant effect on your health?
□	□	18.	Do you eat foods nearly every day that are high in fat and cholesterol such as fatty meats, cheese, fried foods, butter, whole milk, or eggs?
□	□	19.	Do you weigh 30 or more pounds than you should?
□	□	20.	Do you know of any other reason you should not do physical activity?

Medical History

21. *Please check which of the following conditions you have had or now have. Also check medical conditions in your family (father, mother, brother(s), or sister(s)). Check as many as apply.*

Self	Family	Medical Condition	Self	Family	Medical Condition
□	□	Coronary heart disease, heart attack; by-pass surgery	□	□	Major injury/fracture to foot, leg, knee
□	□	Arrhythmias	□	□	Major injury to back or neck
□	□	Autonomic	□	□	Diabetic Retinopathy
□	□	Neuropathy			
		Diabetic Nephropathy			
□	□	Angina	□	□	Major injury/fracture to hip or shoulder
□	□	Marfan's syndrome			
□	□	High blood pressure	□	□	Recent leg trauma/injury
□	□	Peripheral vascular disease	□	□	Rheumatoid arthritis

□	□	Phlebitis or emboli	□	□	Osteoarthritis
□	□	Other heart problems	□	□	Osteoporosis
□	□	Stroke	□	□	Fibromyalgia
□	□	Asthma	□	□	Chronic fatigue syndrome
□	□	Bronchitis	□	□	Systemic lupus erythematosus
□	□	C.O.P.D. (emphysema)	□	□	Anemia (low iron)
□	□	Pulmonary embolism (blood clots in lungs)	□	□	Thyroid problems
			□	□	Gout
□	□	Deep vein thrombosis (blood clots in legs)	□	□	Kidney disease
			□	□	Nephrotic (kidney) syndrome
□	□	Antithrombin III deficiency	□	□	Gallstones/gallbladder disease
□	□	Inherited hypercoaguability	□	□	Liver disease (cirrhosis)
□	□	Acquired hypercoaguability	□	□	Hepatitis
□	□	Factor V leiden mutations	□	□	Diabetes mellitus
□	□	Protein C deficiency	□	□	Raynaud's disease
□	□	Protein S deficiency	□	□	Crohn's disease
□	□	Stomach/duodenal ulcer	□	□	Hysterectomy
□	□	Rectal growth or bleeding	□	□	Problems with menstruation
□	□	Irritable bowel syndrome	□	□	Post-menopausal
□	□	Lung cancer			Date:
□	□	Breast cancer	□	□	Allergies
□	□	Prostate cancer	□	□	Depression
□	□	Skin cancer	□	□	Anxiety, phobias
□	□	Colorectal cancer	□	□	Eating disorders
□	□	Other cancer Specify:	□	□	Substance abuse problems (alcohol, other drugs, etc.)
□	□	Hearing loss	□	□	Sleeping problems
□	□	Cataracts	□	□	Other
□	□	Glaucoma			Specify:

Please specify and include information on any recent illnesses, hospitalizations, surgical procedures, or other health problems.

_____

_____

_____

22a. Are you currently pregnant, think you may be pregnant, or are currently trying to get pregnant?

□ Yes □ No □ Not sure □ Not applicable (male or post-menopausal)

b. If you answered "yes" or "not sure" to 22a, do you need a pregnancy test?

□ Yes □ No

23**.** In the past two weeks, have you had a barium test, a nuclear medicine scan, or x-rays with a dye injection?

□ **Yes** □ **No**

24. *Please check any of the following medications you take regularly and give the name and dose of the medication.*

Medication	**Name of Medication**
□ Heart medicine	_____
□ Blood pressure medicine	_____
□ Blood cholesterol medicine	_____
□ Thromboembolic disease medicine	_____
□ Hypercoaguability medicine	_____
□ Steroids	_____
□ Hormones/HRT	_____
□ Birth control medicine	_____
□ Medicine for breathing/lungs	_____
□ Insulin	_____
□ Other medicine for diabetes	_____
□ Arthritis medicine	_____
□ Medicine for depression	_____
□ Medicine for anxiety	_____
□ Thyroid medicine	_____
□ Medicine for ulcers	_____
□ Painkiller medicine	_____
□ Allergy medicine	_____
□ Dietary supplements (herbs, vitamins, etc)	_____
□ Other (please specify)	_____

Body Weight

26. What is the most you have ever weighed? _____

27. Are you now trying to:
□ Lose weight □ Gain weight □ Stay about the same □ Not trying to do anything

Stress

28. During the past month, how would you rate your overall level of stress?
□ Very high □ High □ Moderate □ Low

29. In the past year, how much effect has stress had on your health?
□ A lot □ Some □ Hardly any or none

30. On average, how many hours of sleep do you get in a 24-hour period?
□ Less than 5 □ 5-6 □ 7-9 □ More than 9

Substance Use

31. How would you describe your cigarette smoking habits?
□ Never smoked
□ Used to smoke. How many years has it been since you smoked? _____ years
□ Still smoke. How many cigarettes a day do you smoke on average? _____ cigarettes/day

32. How many alcoholic drinks do you consume? (A "drink" is a glass of wine, a wine cooler, a 16oz bottle/12oz can of beer, a shot glass of liquor, or a mixed drink).
□ Never use alcohol □ Less than 1 per week □ 1-6 per week
□ 1 per day □ 2-3 per day □ More than 3 per day

33. In one sitting, how many drinks do you typically consume? _____
34. How many cups (8 ounces) of coffee do you drink per day? _____
35. How many ounces of sodas containing caffeine do you drink per day? _____

Physical Fitness, Physical Activity/Exercise

36. Considering a **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your **free time** (write on each line the appropriate number).

a) **STRENUOUS EXERCISE (HEART BEATS RAPIDLY)** **Times Per Week**
(i.e. running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling) _____

b) **MODERATE EXERCISE (NOT EXHAUSTING)**
(i.e. fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing) _____

c) **MILD EXERCISE (MINIMAL EFFORT)** **Times Per Week**
(i.e. yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking) _____

37. Considering a 7-Day period (a week), during your leisure-time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

□ OFTEN □ SOMETIMES □ NEVER/RARELY

38. How long have you exercised or played sports regularly?
□ I do not exercise regularly □ Less than 1 year □ 1-2 years
□ 2-5 years □ 5-10 years □ More than 10 years

Fitzpatrick Skin Type Scale

	Type	General	Pigment	Sunburn
	I	Light	Pale white or freckled	Always
	II	Fair	White	Usually
	III	Medium	White to Light Brown	Sometimes
	IV	Olive	Moderate Brown	Rarely
	V	Brown	Dark Brown	Very Rarely
	VI	Black	Very Dark Brown to Black (twice the melanin as type I)	Never

Self-Reported Ethnicity Questionnaire (Optional): (Adapted from Divers et al., 2011).

In the following table please fill in the information in the box, (to the best of your knowledge) that best fits the description for the person requested. For any box if you do not know the answer or choose to not answer the question than please leave it blank.

	White or Caucasian (Country of Descent)	Black or African American (Country of Descent)	Hispanic or Latino (Country of Descent)	Other (Please describe)
Yourself				
Birth Mother				
Birth Father				
Birth Grandmother (Mother Side)				
Birth Grandfather (Mother Side)				
Birth Grandmother (Father Side)				
Birth Grandfather (Father Side)				

Appendix C: Research subject information

Personal Information

Name: _____

Subject ID/SSN: _____

Date of Birth: _____ Age: _____ Sex ☐ Male ☐ Female

Ethnic Background: Hispanic or Latino Not Hispanic or Latino

Race:
White American Indian/Alaskan Native Pacific Islander
Black or African American Asian Other:
_____

Address:
_____

Contact Information:
_____

Emergency Contact Info:
_____

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