The Thesis Committee for Chansol Hurr
Certifies that this is the approved version of the following thesis:

Impaired Cerebral Vascular Function in College-aged African Americans and Caucasian Americans: Potential Role of Vitamin D and Arterial Stiffness

APPROVED BY
SUPERVISING COMMITTEE:

Supervisor:

Robert Matthew Brothers

Hirofumi Tanaka

Impaired Cerebral Vascular Function in College-aged African Americans and Caucasian Americans: Potential Role of Vitamin D and Arterial Stiffness

by

Chansol Hurr, B.S.

Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Kinesiology

The University of Texas at Austin

August 2013

Abstract

Impaired Cerebral Vascular Function in College-aged African Americans and Caucasian Americans: Potential Role of Vitamin D and Arterial Stiffness

Chansol Hurr, M.S. Kin

The University of Texas at Austin, 2013

Supervisor: Robert Matthew Brothers

African Americans have increased risk for cardiovascular and cerebral vascular disease relative to Caucasian Americans. While it is generally accepted that arteries become stiffer at a younger age in African Americans; less is known regarding cerebral vascular function / reactivity (CVMR) to hypercapnia in African Americans. Furthermore, little is known regarding the relationship between arterial stiffness and CVMR, particularly in young healthy adults. We hypothesized that African Americans have stiffer arteries (i.e. arterial stiffness) and reduced CVMR during hypercapnia relative to Caucasian Americans. We also hypothesized that there would be a negative relationship between arterial stiffness and CVMR. Lastly, we hypothesized that these responses would be related to a decrease in Vitamin D status in this population and there would be correlation between Vitamin D status and CVMR. In 11 African American and

19 Caucasian American subjects central arterial stiffness was indexed from carotid-femoral pulse wave velocity (PWV). CVMR was assessed by the cerebral vascular conductance (CVC) response to rebreathing-induced hypercapnia. Vitamin D status was assessed from plasma 25(OH) Vitamin D. PWV was elevated in the African Americans (African American: 581.16 ± 27.7 cm/sec vs. Caucasian American: 502.98 ± 17.6 cm/sec; $P < 0.01$). CVMR was significantly reduced during hypercapnic rebreathing in the African Americans (African American: 3.05 ± 0.38% of baseline/mmHg vs. Caucasian American: 5.09 ± 0.29% of baseline/mmHg; $P < 0.001$). When data from all subjects was included there was a trend towards a negative relationship (R = 0.32, $P = 0.10$) between PWV and CVMR. Vitamin D status was significantly lower in African Americans (African American: 14.96 ± 0.97 ng/ml vs. Caucasian American: 32.73 ± 0.99 ng/ml; $P < 0.001$); however, there was no significant relationship between Vitamin D status and CVMR (R = 0.23 $P = 0.23$). In conclusion, these data indicate that African Americans have impaired cerebral vascular responses to hypercapnia, stiffer arteries, and lower Vitamin D status when compared with Caucasian Americans. In addition, there may be a negative relationship between CVMR and PWV; however, no significant correlation between Vitamin D status and vascular function including PWV or CVMR was observed in this study.

Table of Contents

List of Tables

List of Figures

CHAPTER 1

INTRODUCTION

1.1 Background

Epidemiological studies (27, 36, 55, 56) have shown that African Americans have a higher incidence of cardiovascular disease including hypertension, stroke, coronary artery disease, and metabolic syndrome relative to Caucasian Americans. The underlying mechanisms remain unresolved and multifactorial. It has been recently suggested, however, that reduced nitric oxide (NO) bioavailability and vitamin D deficiency are major contributing factors in this population (36, 55, 69).

Nitric oxide is an important cellular signaling molecule involved in vascular functioning, e.g., among other important actions it serves as a potent vasodilator. A previous study has demonstrated that NO bioavailability is reduced in relatively young AA men (55) which likely contributes to impaired endothelium-dependent vasodilation in African Americans (69). In addition, it has been shown that impaired endothelium-dependent vasodilation leads to structural loss in microcirculation (48), known as structural rarefaction, thus causing an increase in peripheral vascular resistance, and a subsequent elevation in arterial blood pressure. Therefore, African Americans may have a tendency toward having impaired aortic wall function and, impaired function of the peripheral and cerebral microcirculation, which have been similarly observed in hypertensive patients (46).

A previous study (36) showed that African Americans, compared to Caucasian Americans, have significantly lower serum 25-hydroxyvitamin D concentration, which is

also called calcidiol, and is considered the best indicator of vitamin D status in the body. Furthermore, it has been shown that Vitamin D deficiency causes an increase in oxidative stress in vascular smooth muscle cells (VSMCs) which can induce a variety of physiological changes including elevating renin activity and angiotensin II levels, leading to VSMC remodeling (49, 50, 57, 78, 85). In addition, it has been reported that African Americans have an elevated serum asymmetric dimethylarginine (ADMA) concentration (55), which decreases NO bioavailability by competing with L-arginine as a substrate for endothelial NO synthase (15). This results in uncoupled eNOS and subsequent superoxide production, both of which act to ultimately reduce NO bioavailability.

Therefore, based on these findings, it seems reasonable to speculate that African Americans have stiffer arteries when compared with Caucasian Americans. This comes about, at least in part, from VSMC remodeling caused by Vitamin D deficiency and chronic elevation in blood pressure. The elevated blood pressure associated with endothelial dysfunction leads to arterial wall thickening and the rearrangement of internal elastic properties of the blood vessel wall without growth, which is called eutrophic inward remodeling (102).

Furthermore, Vicenzini et al. (91) indicated that endothelial dysfunction results in impaired vasodilation in the cerebral vasculature, and thus, leads to a dysfunction in cerebral blood flow regulation. In addition, given that cardiovascular disease is closely associated with cognitive impairment (2, 52, 81) and Alzheimer's disease (2, 89) through vascular damage in the brain (87), the impaired cerebral blood flow regulation may be related to those diseases including cognitive decline and Alzheimer's disease. Other studies (36, 55) also suggested that cerebral blood flow regulation is in part dependent on NO bioavailability and Vitamin D status. Therefore, we hypothesized that cerebral vasomotor reactivity is impaired in African Americans relative to Caucasian Americans.

1.2 Statement of purpose

The purpose of the present investigation was to determine whether there is a difference in arterial stiffness, cerebral vasomotor reactivity, and Vitamin D status between healthy young African Americans and Caucasian Americans. The specific objectives of the study were to;

1. Determine the difference in cerebral blood flow responses to hypercapnia between African Americans and Caucasian Americans.
2. Determine the relationship between Vitamin D and cerebral vasomotor reactivity.
3. Determine the difference in arterial stiffness between African Americans and Caucasian Americans.
4. Determine the relationship between arterial stiffness and cerebral vasomotor reactivity.

1.3 Hypothesis

In the current study, we tested the following hypotheses;

1. The cerebral vascular response to hypercapnia (CVMR) would be attenuated in African Americans relative to Caucasian Americans.
2. This impairment would be related to a lower plasma Vitamin D concentration in African Americans.
3. Arterial stiffness, as indexed by PWV, would be higher in African Americans relative to Caucasian Americans.
4. This elevated PWV would be related to impaired CVMR in African Americans.

CHAPTER 2

LITERATURE REVIEW

This literature review includes various aspects of arterial stiffness and cerebral vasomotor reactivity, e.g., development of techniques or several factors that need to be considered. The review also describes the relevant research and evidence that endothelial function is impaired in African Americans and Vitamin D deficiency may be related to the impaired endothelial function in this population.

2.1 Arterial Stiffness

In recent years, many researchers have emphasized on the role of arterial stiffness in the development of cardiovascular disease (CVD) and have increasingly used the measurement of arterial stiffness for the purpose of clinical assessment / detection of risk for CVD. Indeed, arterial stiffness, as indexed by carotid-femoral pulse wave velocity (PWV), is an independent predictor of cardiovascular morbidity and mortality in hypertensive patients, type-2 diabetics, and end-stage renal disease in older populations (47). PWV can be calculated by dividing the distance between two arterial sites by the time difference of pulse wave arrival between the carotid and femoral artery. Several factors contribute to changes in arterial stiffness, such as age (4, 5, 58-60), gender (37, 59), mean arterial blood pressure (MAP) (33), and body mass index (BMI) (103).

Many previous studies (4, 5, 58-60) showed that aortic walls become stiffer throughout the lifespan and this linear relationship between age and arterial stiffness seems more obvious in patients with cardiovascular diseases, including obesity or diabetes (60). Previous studies (32) showed a linear relationship between MAP and

arterial stiffness. Michell (58) stated that MAP increases slightly with advancing age and aortic PWV rises modestly in parallel with an increase in MAP; however, at around 60 years-of-age, peripheral pulse pressure (PPP) increases dramatically and arterial stiffness tends to be accelerated accordingly.

In this regard, Mitchell et al. (61) also investigated changes in central and peripheral arterial stiffening and wave reflection with advancing age to verify the mechanism of elevated PPP in an elderly population to determine whether it occurs due to increased arterial stiffness or increased reflected wave amplitude. Elevated aortic PWV was observed, but the peripheral PWV changed minimally with advancing age and the reflected wave amplitude did not change even though there was an increase in forward wave amplitude. Thus, they concluded that increases in aortic stiffness that increases forward wave amplitude, rather than reflected wave amplitude, would cause an increased peripheral pulse pressure with advancing age. Therefore, beyond 60 years-of-age, reflection from a proximal reflecting site may be reduced due to stiffened artery with advancing age and thus, forward wave amplitude increases, resulting in increased transmission of pulsatile flow into smaller arteries. This hemodynamic change could explain the association between arterial stiffness and cardiovascular diseases (61, 92).

Several studies investigated the relationship between vascular stiffening and mortality in different populations. Sutton-Tyrrell et al. (88) examined whether aortic PWV is associated with total and cardiovascular mortality and other cardiovascular diseases (CVD) in older adults using a longitudinal research methodology. They found that, independent of race, gender, age, and systolic blood pressure, subjects with aortic PWV values > 641 cm/s for men and > 627 cm/s for women had a more than 2-fold increase in the development of CVD, a 2- to 3-fold increase in stroke events in patients with chronic heart failure relative to those with values below this level of aortic PWV. In

addition, in a younger population, markedly increased pulse pressure, which is closely related to arterial stiffening, has been shown to be associated with mortality (6, 7, 20). Therefore, greater aortic PWV (e.g. elevated pulse pressure) is associated with higher CHD, cardiovascular mortality, and stroke. Thus, aortic PWV can be a reliable predictor of long-term cardiovascular disease.

In addition, Din-Dzietham et al. (18) found that African Americans who are free of coronary heart disease or stroke have significantly stiffer arteries relative to Caucasian American, after adjustment for cardiovascular risk factors including hypertension, diabetes, or plasma cholesterol levels. Another study (79) also investigated the association of ethnicity on arterial stiffness from a Brazilian population and supported the idea that pulse wave velocity levels were significantly higher in African descent individuals when compared with other ethnic groups including Amerindian, Caucasian descent, and Mulatto individuals.

There have also been some arguments regarding the accuracy of Doppler ultrasound as a suitable method of assessing arterial stiffness. In recent years, Calabia et al. (10) pointed out that the measurement of PWV using pressure transducer or arterial tonometry has several disadvantages. These include: requiring specific software program and device and difficulty in obtaining clear pulse waves, requiring large amounts of time to obtain data from the optimal signals (53). They examined whether pulse wave velocity measured by mechanical pressure, which is being considered as a gold standard, is similar to that estimated by Doppler. They found that pulse wave velocities measured by the two methods were highly correlated (R=0.91) and concluded that Doppler ultrasound can be used to estimate aortic PWV in a reliable and reproducible way. This showed similar results to the established method (Complior®), which they took as a gold standard.

2.2 Cerebral Vasomotor Reactivity

An increase in arterial PCO_2 ($PaCO_2$) is a potent vasodilator for cerebral vasculature (17), that is, cerebral vessels respond rapidly to changes in the partial pressure of $PaCO_2$. Therefore, it has been thought that cerebral blood flow is tightly regulated by $PaCO_2$ (39, 95). This unique characteristic of cerebral vasculature can be quantified by assessing the cerebral vasodilator response to increases in $PaCO_2$. This response is known as cerebral vasomotor reactivity (CVMR), which is used to provide a non-invasive index of cerebral vascular health (76). The slope of the relationship between cerebral blood flow and the increase in $PaCO_2$ is calculated and is a measure of CVMR.

Phan et al. (70) demonstrated that end-tidal PCO_2 ($PETCO_2$) is correlated well with the arterial partial pressure of carbon dioxide ($PaCO_2$) at rest. The study compared non-invasive technique for estimating the partial pressure of carbon dioxide ($PETCO_2$) with $PaCO_2$ values determined by arterial blood samples in 24 anesthetized subjects. There was a significant correlation between $PETCO_2$ values and the blood gas values. Therefore, the study showed that $PETCO_2$ can be used to estimate $PaCO_2$ with accuracy. Other previous studies supported this idea (93, 97, 100). However, it has been observed that $PETCO_2$ overestimates $PaCO_2$ when CO_2 production, ventilation rate, and tidal volume are elevated during exercise (41). As a result, Jones et al. (42) developed a regression equation to estimate $PaCO_2$ from $PETCO_2$, which is $P_JCO_2 = 5.5 + 0.9 \times PETCO_2 - 2.1 \times V_T$. This equation has been widely used and proven to be a good estimate of $PaCO_2$ in young and older adults (77, 86) and morbidly-obese adults (8).

Blood flow velocity in the large cerebral arteries (i.e., middle cerebral artery (MCA)) determined by TCD is widely used as an index of cerebral blood flow. A critical consideration is that changes in the diameter of the insonated vessels might govern CBF velocity; however, Giller et al. (30) verified that the MCA diameter remains relatively

constant in humans under moderate changes in blood pressure and changes in $PETCO_2$. Therefore, this constancy of diameter of cerebral arteries suggests that the MCA blood velocity reflects blood flow through the insonated artery. In addition, Serrado et al. (83) found that changes in MCA diameter were not observed under the conditions of stimulated orthostatic stress and changes in $PETCO_2$.

Cerebral vasomotor reactivity (CVMR) measurements have been widely used in clinical practice to assess cerebral vascular function. Many research teams have examined CVMR measurements on various types of patients and demonstrated that the CVMR in response to hypercapnic rebreathing was significantly attenuated in patients with carotid artery stenosis (9, 44, 76, 98), hypertension (84), chronic heart failure (29), or internal carotid artery occlusion (98). These results suggest that CVMR assessment is a useful and reliable index of cerebrovascular function and attenuated CVMR may indicate an impairment in systemic vascular function and elevated CVD risk.

Dumville et al. (22) conducted a study to investigate whether cerebrovascular reactivity is affected by blood pressure. They compared conventional methods of assessing CVMR that do not consider the influence of blood pressure with new methods with considering changes in blood pressure. In this study, the authors observed CO_2-induced changes in arterial blood pressure and demonstrated that CVMR results were significantly affected by changes in arterial blood pressure. In addition, other studies also showed that the relationship between changes in CBF and arterial PCO_2 level is affected by CO_2-induced changes in arterial blood pressure (23, 24, 34, 62, 66, 67, 74, 76). Given that CO_2-induced changes in blood pressure may have an effect on cerebral blood flow, an index of cerebral vascular conductance (CVCi), which is calculated from the ratio MCA V_{mean} to MAP, has been widely adopted to solely estimate the CO_2-induced changes in cerebral blood flow (51, 64, 101).

Several studies have focused on the effects of various physiological activities on cerebral vasomotor reactivity. Rasmussen et al. (73) examined cerebrovascular reactivity during strenuous exercise. The relationship between MCA V_{mean} and $PaCO_2$ was linear in a resting condition and this linear relationship became curvilinear during moderate exercise and even more during strenuous exercise. Ogoh et al. (65) also found results that supported the idea that cerebral CO_2 reactivity increases during exercise compared with that in resting condition. Therefore, cerebral CO_2 reactivity elevates as exercise intensity increases and this may indicate that exercise-induced physiological changes govern the cerebrovascular responses to CO_2. On the other hand, Meadows et al. (54) reported that cerebral CO_2 reactivity decreases during sleep compared with wakefulness. Thus, it has been suggested that the level of activation of cerebral vasculature influences cerebral CO_2 reactivity.

2.3 High Cardiovascular Risks in African Americans

Epidemiological studies (27, 36, 55, 56) demonstrated that African Americans are at higher risk of cardiovascular disease and its complications relative to Caucasian Americans. Santos et al. (79) investigated the association of ethnicity on arterial stiffness in a Brazilian population. They concluded that an association between different ethnicities and arterial stiffness exists, and a higher PWV, which is a predictor of cardiovascular disease, is observed in individuals of African descent. Perregaux et al. (69) also demonstrated that post ischemic dilatory responses of the brachial artery were significantly attenuated in healthy young African Americans relative to Caucasian American counterparts and other studies (31, 43) supported the same idea that African Americans have endothelial dysfunction.

Previous studies (11-14, 35, 40, 68) that used an identical methodology showed that patients with hypertension, diabetes mellitus, and hypercholesterolemia had lower post ischemic dilatory responses compared with healthy controls. Given that hypertension, diabetes mellitus, and hypercholesterolemia are risk factors for atherosclerosis or other cardiovascular diseases, the impaired endothelial function could explain a higher incidence of hypertension and atherosclerosis in African Americans.

2.4 Relationship between Vascular Function and Vitamin D Status

Previous study (94) demonstrated that 1,25(OH)2D3 increases the production of prostacyclin, which is thought as an effective vasodilator for vascular smooth muscle cells (VSMCs). This idea is also supported by Wong et al. (99) who showed that 1,25(OH)2D3 reduces calcium influx into endothelial cells, resulting in a reduction in endothelium-dependent VSMC contraction. Thus, the reduced Vitamin D status may cause increase in peripheral resistance and blood pressure. Furthermore, Feihl et al. (25) stated that the prolonged vasoconstriction caused by impaired endothelium-dependent vasodilation of VSMC may lead to eutrophic inward remodeling, eventually causing vascular stiffness.

In addition, Al Mheid et al. (1) reported the negative relationship between Vitamin D status and arterial stiffness. A total of 554 healthy participants with a large multiethnic population over a wide age range (20 to 79 years-old) were recruited for this study. The study demonstrated that increased arterial stiffness measured by PWV was associated with lower 25-OH D levels. Another study (21) verified that Vitamin D supplements over 16 weeks had positive effects on plasma Vitamin D status and caused a decrease in arterial stiffness. From these studies, it would be reasonable to speculate that

Vitamin D deficiency is a major contributing factor for arterial stiffness and endothelial dysfunction in African Americans.

CHAPTER 3

METHODOLOGY

3.1 Subjects and Ethical Approval

The Institutional Review Board at The University of Texas at Austin approved all techniques and protocols used in the present study. Subjects were given a verbal description of all procedures and informed of the purpose and risks involved in the study before providing their informed, written consent.

Nineteen Caucasian Americans (8 males and 11 females in CAs) and eleven African Americans (3 males and 8 females in AAs) participated in this study. Subject characteristics were (mean ± SE) 22.6 ± 3.2 years, 173.3 ± 8.6 cm, 70.8 ± 14 kg, 23.4 ± 3.5 kg/m^2 for age, height, weight, and BMI, respectively (Table 1).

African and Caucasian American individuals (ages 18-30; both genders) were recruited from the University of Texas at Austin and the greater Austin area to participate in the study. All subjects completed a health questionnaire that included questions about the race of their birth parents (19). These questions were used to avoid data collection from African American and/or Caucasian American subjects from a biracial background. Individuals with cardiovascular, neurological, metabolic, orthopedic, or cognitive diseases were excluded from the study. In addition, subjects currently taking medications known to influence the autonomic nervous system were excluded. Pregnant women and children (i.e. younger than 18) were not recruited for the study. Given that smoking has an effect on the peripheral vasculature, current smokers and individuals who regularly smoked within the previous two years were also excluded (3). Subjects were instructed to refrain from strenuous exercise and from consuming alcoholic beverages for 24 hours and

to refrain from consuming caffeine and food for 12 hours prior to the experimental trial. Temperature and relative humidity were maintained at ~24°C and 40% while conducting all experiments and procedures.

3.2 Instrumentation and Measurements

Heart rate and cardiac rhythms were continuously recorded and monitored from an electrocardiogram (ECG)(HP Patient Monitor, Agilent, Santa Clara, CA) interfaced with a cardiotachometer (CWE, Ardmore, PA). Continuous arterial blood pressure was recorded and monitored from a finger using the Penaz method (CNAP, Monitor 500, Austria). In addition, intermittent blood pressure was measured by auscultation of the brachial artery via electrosphygmomanometry (Tango+; SunTech, Raleigh, NC). Mean arterial blood pressure (MAP) was calculated as one-third pulse pressure plus diastolic blood pressure. End-tidal carbon dioxide concentration ($PETCO_2$) was continuously collected and measured through a mouthpiece during all data collection periods using a capnograph (VitalCap Capnograph Monitor, Oridion, Needham, MA) and used as an index of arterial carbon dioxide concentration.

Cerebral blood flow was indexed from the velocity (MCA V_{mean}) of blood flowing through the middle cerebral artery using transcranial Doppler ultrasonography. The middle cerebral artery was imaged through a 2-MHz Doppler probe (Multi-flow, DWL Elektronische Systeme, Singen, Germany) adjusted over the temporal window of the right or left middle cerebral artery until an optimal signal was identified. The probe was fixed securely in place using a head strap to prevent slight movement of the Doppler probe. An index of cerebral vascular conductance (CVCi) was calculated from the ratio of middle cerebral artery blood velocity (MCA V_{mean}) to MAP obtained from beat-to-beat

arterial pressure measurement. Cerebral vasomotor reactivity (CVMR) was assessed by changes in cerebral vascular conductance (CVCi; MCA V_{mean}/ MAP) response to rebreathing-induced hypercapnia. Delta increase in $PETCO_2$ and the % change in CVCi were used to obtain its slope of regression line. During the CVMR test, oxygen (calculated by height and weight) was continuously supplied to avoid hypoxic events (i.e. cerebral hypoxia).

Arterial stiffness was indexed from pulse wave velocity (PWV) using Doppler ultrasound, synchronized with ECG. Pulse wave velocity was calculated from distance divided by transit time (TT). TT was estimated from the time delay between the R wave of QRS and the foot of the waveform, which is defined as the point at which the steep rise begins (10). Five consecutive TTs of the femoral and carotid artery were taken and then averaged. Distance traveled by the pulse wave was measured in duplicate with a non-elastic tape over the surface of the body. The distance (between suprasternal notch and femoral artery – suprasternal notch and carotid artery) was divided by calculated TT (averaged TT of femoral artery – averaged TT of carotid artery).

3.3 Experimental Protocol

Upon arriving at the laboratory, height and weight were measured, which were then used to calculate the rate of oxygen that was continuously supplied during the CVMR test. Subjects rested quietly in a supine position on a patient bed and a 20mL blood sample was obtained from venipuncture of the non-dominant arm (using vacutainers serum separating (SST) and anticoagulant (EDTA) tube), centrifuged, and stored in a -80°C freezer until analysis. Following blood collection, trial participants were instrumented with an ECG to monitor heart rate and with an

electrosphygomanometer to obtain intermittent blood pressure. MAP was calculated as one-third pulse pressure plus diastolic blood pressure. Finger cuffs were placed on 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, which is a necessary step for subjects to be stable before measuring PWV as previously described. After the 6-minute baseline measurement, arterial stiffness was assessed using Doppler ultrasound.

A 2-MHz Doppler probe was then instrumented using head strap to prevent slight movements of the Doppler probe and adjusted over the temporal window until the optimal signal was obtained. This adjustment was followed by the CVMR test. Subjects remained in a supine position and a modified mouthpiece was inserted into the mouth. This mouthpiece allowed for a variety of capabilities including the ability to switch a valve so subjects went from breathing atmospheric air to rebreathing their own expired air from a specialized rubber bag. In addition, a tube connected to a capnograph allowed for continuous monitoring of $PETCO_2$ during the test. Six-minute baseline data were obtained prior to the CVMR test and averaged to represent baseline values for MCA V_{mean}, CVCi, and $PETCO_2$. Following the baseline data collection, subjects underwent a rebreathing procedure (CVMR test). To accomplish this the valve was closed so that the subjects began rebreathe their own air. This causes 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) which was approximately four minutes. The rebreathing procedure was stopped once subjects reached the target increase in $PETCO_2$, which was delta 15 mmHg or if subjects experienced dizziness, shortness of breath, and/or tingling

or numbing sensations, at which point subjects were immediately given normal room air and 100% oxygen.

3.4 Data Analysis

MAP, MCA V_{mean}, subsequent calculation of CVCi and $PETCO_2$ were assessed 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).

3.5 Statistical Analysis

Descriptive analysis was conducted to show the characteristics of the subjects. The means and standard errors (SE) were used for continuous variables (e.g. age, height, weight, and BMI), and the numbers of subjects were reported for categorical variables (sex). The means and standard errors were also reported for continuous variables (HR, MAP, $PETCO_2$, MCA V_{mean}, CVCi) at the 6-minute baseline prior to hypercapnic rebreathing period. Statistical significance between African Americans and Caucasian Americans was tested using unpaired t-test for hemodynamic variables at baseline (BMI, HR, MAP, $PETCO_2$, MCA V_{mean}, CVCi). The linear regression was employed 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. Unpaired t-

test was used to analyze differences in CVMR and PWV between African Americans and Caucasian Americans. 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 4

RESULTS

4.1 Subjects

Nineteen Caucasian Americans and eleven African Americans participated in this study. Physical characteristics are shown in Table 1. The average age of the subjects was 22.2 ± 0.8 yrs in the Caucasian American group and 23.4 ± 1.0 yrs in the African American group (P = 0.36). There were no significant differences in height (Caucasian American: 173.6 ± 2.0 cm vs. African American: 172.8 ± 2.7 cm, P = 0.81), weight (Caucasian American: 68.5 ± 2.7 kg vs. African American: 74.7 ± 5.1 kg, P = 0.25), or body mass index (Caucasian American: 22.6 kg/m^2 ± 0.6 vs. African American: 24.9 ± 1.3 kg/m^2, P = 0.09) between African American and Caucasian American individuals.

Variables	Caucasian Americans (n=19)	African Americans (n=11)	*P*-value
Age (yrs)	22.2 ± 0.8	23.4 ± 1.0	0.36
Sex (m/f)	8/11	3/8	N/A
BMI (kg/m^2)	22.6 ± 0.6	24.9 ± 1.3	0.09
Height (cm)	173.6 ± 2.0	172.8 ± 2.7	0.81
Weight (kg)	68.5 ± 2.7	74.7 ± 5.1	0.25

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

Table 1. Subject Characteristics

4.2 Arterial Stiffness

Figure 1 shows differences in pulse wave velocity between Caucasian Americans and African Americans. The average PWV was 502.98 ± 17.6 cm/sec in Caucasian Americans and 581.16 ± 27.7 cm/sec in African Americans. Unpaired t-test was used to compare PWVs between two ethnicities and there was a significant difference ($P < 0.01$).

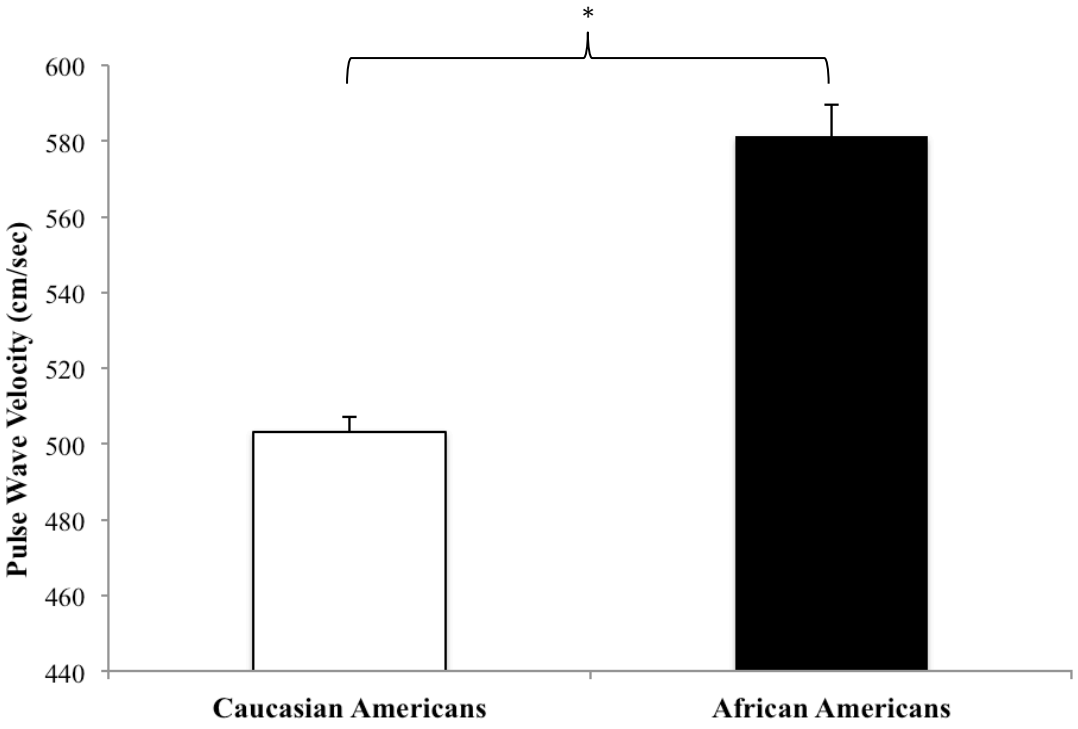

Figure 1. Group Averaged Pulse Wave Velocity. There is a significant difference between Caucasian Americans and African Americans ($P < 0.01$).

4.3 Cerebral Vasomotor Reactivity

Table 2 displays hemodynamic information during eucapnic period (baseline). There were no significant differences in HR (Caucasian American: 55.9 ± 2.5 beats × min^{-1} vs. African American: 57.5 ± 3.4 beats × min^{-1}, P = 0.76), MAP (Caucasian American: 84.7 ± 1.6 mm Hg vs. African American: 84.8 ± 3.6 mmHg, P = 0.99), MCA V_{mean} (Caucasian American: 60.9 ± 3.0 cm × sec^{-1} vs. African American: 67.9 ± 3.3 cm × sec^{-1}, P = 0.15), $PETCO_2$ (Caucasian American: 37.8 ± 1.0 mm Hg vs. African American: 37.9 ± 1.0 mm Hg, P = 0.95), and CVC (Caucasian American: 0.73 ± 0.04 cm × sec^{-1} × $mmHg^{-1}$ vs. African American: 0.81 ± 0.05 cm × sec^{-1} × $mmHg^{-1}$, P = 0.22).

Variables	Caucasian American (n=19)	Caucasian Americans (n=11)	*P*-value
HR (beats×min^{-1})	55.9 ± 2.5	57.5 ± 3.4	0.76
MAP (mmHg)	84.7 ± 1.6	84.8 ± 3.6	0.99
MCA V_{mean} (cm×sec^{-1})	60.9 ± 3.0	67.9 ± 3.3	0.15
$PETCO_2$ (mmHg)	37.8 ± 1.0	37.9 ± 1.0	0.95
CVC (cm × sec^{-1} × $mmHg^{-1}$)	0.73 ± 0.04	0.81 ± 0.05	0.22

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 ± SE.

Table 2. Hemodynamic state during eucapnia

The slopes of the percent increase in cerebral vascular conductance per mmHg increase in $PaCO_2$ indexed by $PETCO_2$ were significantly attenuated in African Americans relative to Caucasian Americans (Caucasian American: 5.09 ± 0.29 CVC % of baseline/mmHg vs. African American: 3.05 ± 0.38 CVC % of baseline/mmHg, $P < 0.001$) (Figure 2).

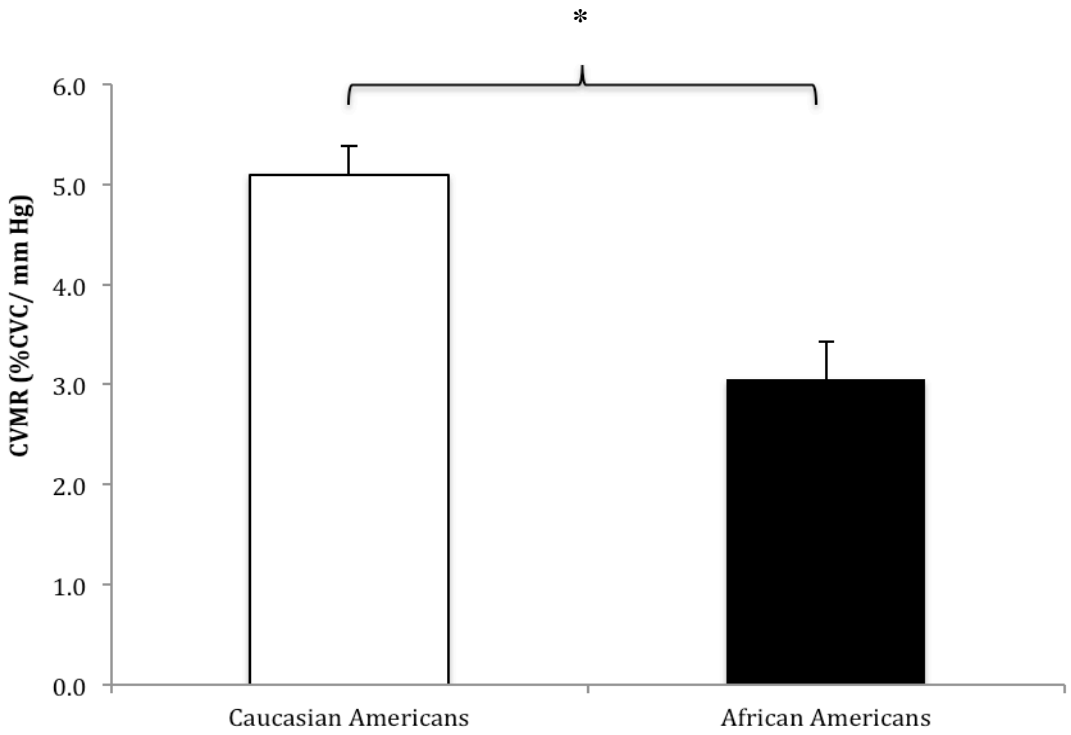

Figure 2. Cerebral Vasomotor Reactivity in Response to Rebreathing. Group averaged values for CVMR are illustrated. The percentage change of CVC from its baseline as a function of $PETCO_2$ was blunted in the African Americans (black bar) relative to Caucasian Americans (white bar). * significant difference ($P < 0.001$).

4.4 Relationship between CVMR and Arterial Stiffness

Figure 3 illustrates the relationship between PWV and CVMR. There is a trend towards a negative relationship (R = 0.32) between two variables but the relationship was not significant ($P = 0.10$).

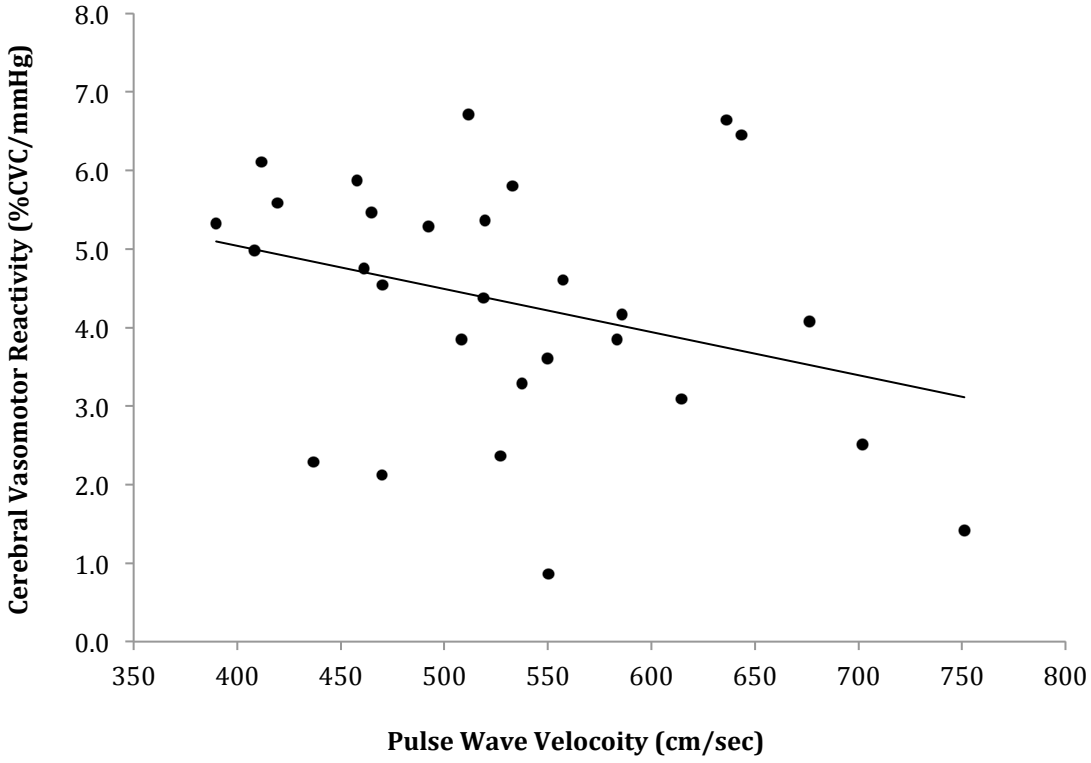

Figure 3. Relationship between Arterial Stiffness and Cerebral Vasomotor Reactivity (R = 0.32, $P = 0.10$).

4.5 Vitamin D Status

Figure 4 shows plasma 25(OH) Vitamin D concentrations between Caucasian Americans and African Americans. The average concentration of 25(OH) Vitamin D was 36.35 ± 0.91 ng/ml in Caucasian Americans and 14.96 ± 0.97 in African Americans. Unpaired t-test was performed to compare 25(OH) Vitamin D concentrations between two ethnicities and there was a significant difference ($P < 0.001$)

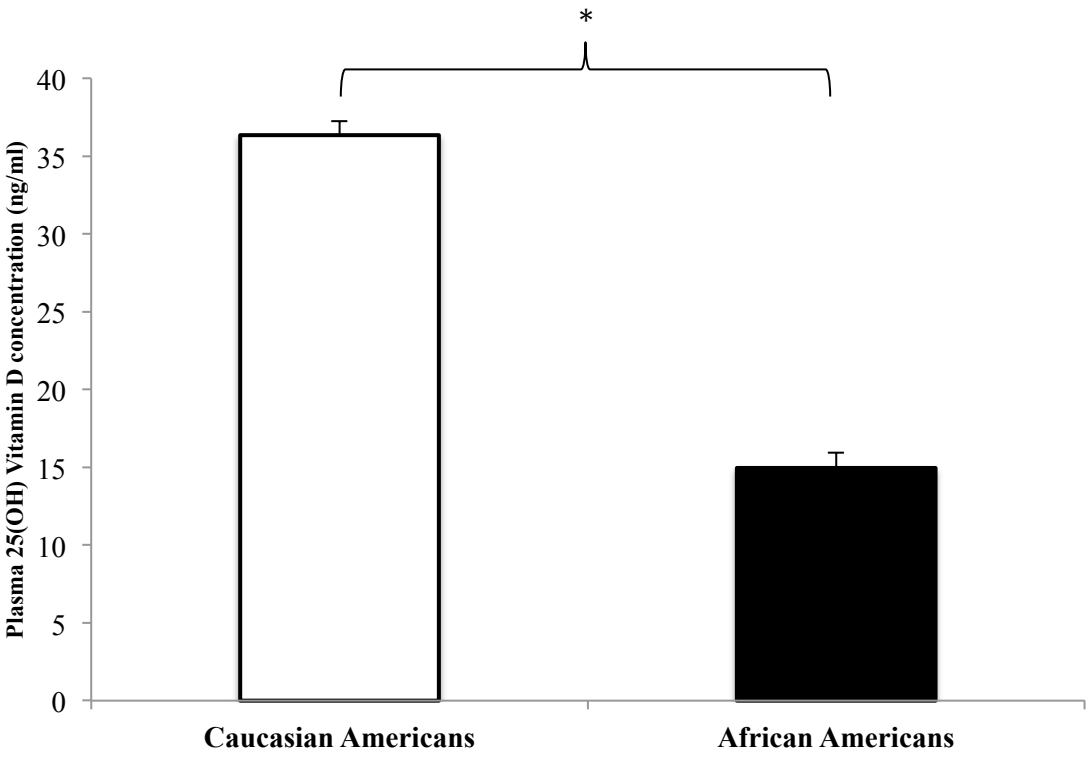

Figure 4: Group Averaged Plasma 25(OH) Vitamin D Concentration. There is a significant difference between two groups ($P < 0.001$).

4.6 Relationship between Vitamin D Status and CVMR

Figure 5 illustrates the relationship between plasma Vitamin D concentration and CVMR and there was no significant correlation (R = 0.23, *P* = 0.23).

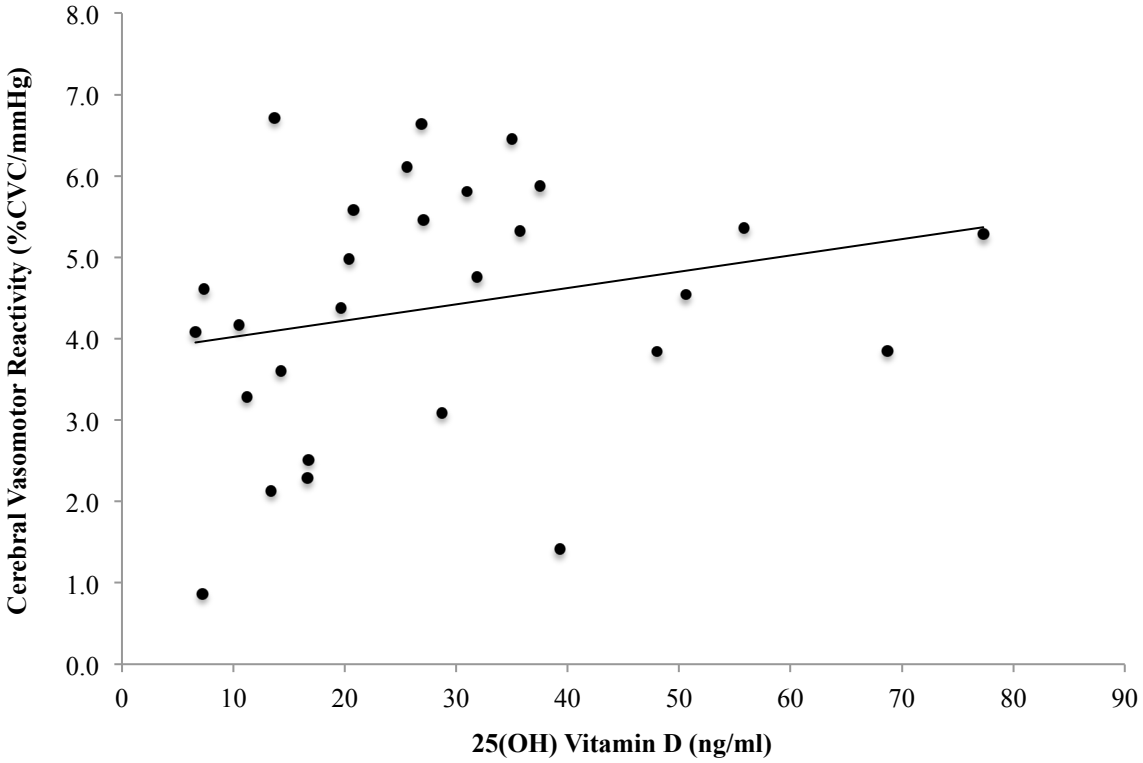

Figure 5: Relationship between 25(OH) Vitamin D and CVMR. There is no significant correlation (R = 0.23, *P* = 0.23).

CHPTER 5

DISCUSSION

The data clearly demonstrates that PWV, which is an index of arterial stiffness, is significantly higher (i.e., stiffer arteries) and CVMR is markedly attenuated in African Americans compared with Caucasian Americans. In addition, this study showed that there was a trend towards a negative relationship between PWV and CVMR. Given that African Americans are at a higher risk of cardiovascular disease such as hypertension, stroke, or coronary artery disease compared with other ethnicities (27, 55, 56), the findings of this study suggest that impaired cardiovascular and cerebral vascular function occurs at a relatively young age prior to the onset of overt disease in African Americans. It is likely that this contributes to the greater incidence in overt cardiovascular and cerebrovascular diseases in this population. Therefore, PWV, CVMR, and the relationship between the two, which were used in this study, could be useful indexes to predict the future development of cardiovascular disease in African Americans.

Furthermore, we found that African Americans have lower Vitamin D status relative to Caucasian Americans; however, we could not observe significant correlation between Vitamin D status and PWV or between Vitamin D status and CVMR. According to previous studies (1, 21, 25, 94, 99), it has been known that Vitamin D status is related to vascular function, thus, Vitamin D deficiency may be a contributing factor for impaired endothelial function or stiffer artery (21). Our finding indicates that Vitamin D status might not be related to vascular function and this confliction might occur, at least in part, due to small sample size of the current study.

The relationship between arterial stiffness and different ethnicities has been studied by Albano et al. (26) who showed that normotensive African Americans have lower mean values of pulse wave velocity than normotensive Caucasian Americans. In comparison, Urbina et al. (90) investigated race differences in carotid artery stiffness in young adults measured by M-mode ultrasonography as Peterson's elastic modulus (Ep) and relative wall thickness-adjusted Young's elastic modulus (YEM). They demonstrated that African Americans had lower arterial elasticity (i.e., higher Ep) when compared with a white population. In addition, Rebecca et al. (18) reported that middle-aged African Americans had a stiffer common carotid artery (CCA) compared with Caucasian Americans.

Given that the current study recruited normotensive young subjects, the results of Albano's study conflict with the current investigation; however, the data regarding the age range of normotensive subjects in their study limit the extrapolation of these results to the current study because the age range of normotensive Caucasian Americans was from 19 to 47, which is significantly different from the age range of normotensive African Americans, which was from 19 to 37.

Since a previous study (4) reported that pulse wave velocity increases with advancing age in a linear fashion, the unbalanced age distribution between the two normotensive groups of Albino's study could be a confounding factor for their results. In addition, it has been shown that pulse wave velocity is higher in men compared with women (37), but they did not report any information regarding gender distribution, which could also be a confounding factor. In the current study, the age ranges between both groups were tightly matched to eliminate age-dependent aspects of pulse wave velocity (e.g., 22.2 ± 0.8 yrs in Caucasian Americans vs. 23.4 ± 1.0 yrs in African Americans). Considering the fact that pulse wave velocity is higher in males relative to females (37),

gender effects on pulse wave velocity were minimal in the current study because the African American group showed a higher pulse wave velocity even though this group had a greater portion of female subjects compared with the Caucasian American group. Therefore, it can be speculated that if the groups were perfectly matched for gender, the differences between the two ethnicities would be even greater.

We also investigated the difference in cerebral vasomotor reactivity between African Americans and Caucasian Americans and found that cerebrovascular responsiveness to hypercapnia was markedly attenuated in African Americans when compared with Caucasian Americans. To the best of our knowledge, the relationship between CVMR and ethnicity remains unknown, thus impaired CVMR in African Americans could be a novel finding in this area although the underlying mechanisms need to be further investigated.

5.1 Potential Mechanisms for Stiffened Artery in African Americans

Ultraviolet light that can be absorbed into skin decreases progressively as the distance from the equator increases and cutaneous vitamin D photosynthesis becomes progressively limited accordingly (17, 96). Furthermore, it has been well documented that skin pigmentation affects the photosynthesis of vitamin D (38). In this regard, the dark skin pigmentation of African Americans contains a high concentration of melanin that impairs the efficiency of vitamin D photosynthesis in the skin. Thus, individuals of African descent with dark skin pigmentation that live far from the equator, such as in Europe or north America are at a higher risk of vitamin D deficiency compared with other ethnicities (28, 63, 80, 82). There are a number of previous studies that support the idea that vitamin D deficiency induces vascular stiffness (i.e., arterial stiffness).

One possible explanation is that vitamin D deficiency causes attenuated vasodilation of vascular smooth muscle cell (VSMC). Wakasugi et al. (94) reported that 1,25(OH)2D3, which is an active form of vitamin D, stimulates the production of prostacyclin, which is known as an effective vasodilator for VSMCs. In addition, Wong et al. (99) found that 1,25(OH)2D3 reduces calcium influx into endothelial cells, causing a reduction in endothelium-dependent VSMC contraction. This impaired endothelium-dependent vasodilation of VSMC may increase peripheral resistance and cause an increase in blood pressure even though it is not in a pathologic state. An increase in pressure brings about myogenic-induced vasoconstriction of small resistance vessels and if this vasoconstriction is prolonged, components of the vessel wall are rearranged without growth, which is called eutrophic inward remodeling (25), eventually resulting in vascular stiffness.

Furthermore, Resnick et al. (75) found an inverse relationship between vitamin D3 concentration and plasma renin activity in patients with hypertension and Li et al. (49) also demonstrated that the expression of renin mRNA and angiotensin 2 were significantly elevated in a mouse model when vitamin D receptors were deactivated. An increase in angiotensin 2 and plasma renin activity caused by vitamin D deficiency may bring about angiotensin 2-mediated inflammation (57) or promote extracellular matrix proteins, resulting in fibrosis of VSMC by increasing TGFB1 and eventually leading to vascular stiffness (85).

In addition, it has been demonstrated that elevated angiotensin 2 may cause oxidative stress in VSMCs by increasing NAD(P)H oxidase. The elevated reactive oxygen species may then promote vascular proliferation and hypertrophy or endothelial fibrosis and dysfunction, causing stiffened vessels (50, 78).

5.2 Potential Mechanisms for Reduced CVMR in African Americans

Previous animal studies (16, 45) found that oxidative stress induces microvascular rarefaction through impaired NO signaling. Therefore, the impaired endothelium-dependent vasodilation caused by NO inactivation that is accelerated by reactive oxygen species (ROS) may contribute to functional rarefaction, in which the number of perfused vessels is reduced without a decrease in the number of vessels (48). Furthermore, it has been reported that prolonged functional rarefaction results in structural rarefaction, in which the number of vessels in the tissue is decreased (72). Given that endothelium-dependent vasodilation is impaired (69) and NO bioavailability is attenuated in African Americans (55), it seems reasonable to speculate that African Americans may have tendency to have structural damage (e.g., rarefaction) in their microcirculation. Since the rarefaction occurs globally, it would be structural rarefaction in their cerebral microcirculation as well as other areas of the body.

In addition, when the aorta stiffens, more blood is transmitted into the capillaries that are located distally since wave reflection occurs more distally and partial reflection becomes smaller when compared with normal aorta (58). The increased pulsatile pressure that is brought by an increase in stiffened artery causes cerebral microbleeds (CMBs) and leads to cerebral microcirculation damage (71).

The structural rarefaction in cerebral microcirculation might explain why CVMR is attenuated in African Americans. In this study, CVMR was indexed from MCA V_{mean} because a previous study (83) demonstrated that changes in MCA V_{mean} reflect changes in cerebral blood flow by providing evidence that the diameter of the middle carotid artery (MCA) does not change significantly during moderate changes in arterial blood pressure and carbon dioxide tension. In this regard, the changes in MCA V_{mean} occur not

due to changes in diameter of the MCA, but due to the vasodilation or vasoconstriction of smaller vessels located deep in the brain (i.e., cerebral microcirculation) that are affected by metabolic demands such as changes in carbon dioxide concentration in plasma.

Therefore, it seems reasonable to speculate that the response of cerebral vasculature to changes in carbon dioxide in plasma is reduced because functional or structural rarefaction in cerebral vasculature prevents the delivery of carbon dioxide, which is known as a strong vasodilator, into deep area of the cerebral vasculature (i.e., reduced perfusion), thus leading to reduced vasodilatory effects of carbon dioxide on cerebral vasculature.

CHAPTER 6

LIMITATIONS

Among a total of 30 subjects, 19 female individuals were studied, which is an unbalance of gender. Previous study (37) showed that males have higher pulse wave velocity compered with females; however, given that African Americans showed significantly higher PWV than Caucasians even though African American group has much higher ratio of females in their group (8 females and 3 males in African Americans group vs. 11 females and 8 males in Caucasian group), the effect of gender on pulse wave velocity seems minimal in this study.

CVMR was indexed from MCA V_{mean}. Blood flow velocity changes if the diameter alters even though blood flow remain unchanged; however, previous study (83) demonstrated that the diameter of the middle carotid artery (MCA) does not change significantly during moderate changes in arterial blood pressure and carbon dioxide tension in plasma, thus changes in MCA V_{mean} reflect changes in cerebral blood flow and can be used as an index of cerebral perfusion.

CHAPTER 7

CONCLUSION

In conclusion, we observed that African Americans had higher PWV and reduced CVMR when compared with Caucasian counterparts. Taken together, our findings support the hypothesis that cerebral and arterial vascular functions are impaired in relatively young, healthy African Americans. In addition, impaired cerebral vascular responses to hypercapnia in this population might be related to stiffer arteries. Given that African Americans are at higher risk of cardiovascular disease, the findings of this study indicate that impaired cardiovascular and cerebrovascular function in early ages of African Americans might become progressively worse and develop into overt cardiovascular diseases and its complications with advancing age. Given that there may be a trend towards a negative relationship between PWV and CVMR, those indexes could be useful to predict future development of cardiovascular disease in African Americans. We observed a significant difference in Vitamin D status between two ethnicities; however, no significant correlation between Vitamin D status and PWV or between Vitamin D status and CVMR was observed in this study.

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	☐	☐	Rheumatoid arthritis

		disease			
☐	☐	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)** (i.e. running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)	**Times Per Week** _____

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:
_____

References

1. **Al Mheid I, Patel R, Murrow J, Morris A, Rahman A, Fike L, Kavtaradze N, Uphoff I, Hooper C, Tangpricha V, Alexander RW, Brigham K, and Quyyumi AA**. Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. *Journal of the American College of Cardiology* 58: 186-192, 2011.
2. **Alzheimer's A**. 2010 Alzheimer's disease facts and figures. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 6: 158-194, 2010.
3. **Avery MR, Voegeli D, Byrne CD, Simpson DM, and Clough GF**. Age and cigarette smoking are independently associated with the cutaneous vascular response to local warming. *Microcirculation* 16: 725-734, 2009.
4. **Avolio AP, Chen SG, Wang RP, Zhang CL, Li MF, and O'Rourke MF**. Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation* 68: 50-58, 1983.
5. **Avolio AP, Deng FQ, Li WQ, Luo YF, Huang ZD, Xing LF, and O'Rourke MF**. Effects of aging on arterial distensibility in populations with high and low prevalence of hypertension: comparison between urban and rural communities in China. *Circulation* 71: 202-210, 1985.
6. **Benetos A, Rudnichi A, Safar M, and Guize L**. Pulse pressure and cardiovascular mortality in normotensive and hypertensive subjects. *Hypertension* 32: 560-564, 1998.
7. **Benetos A, Safar M, Rudnichi A, Smulyan H, Richard JL, Ducimetieere P, and Guize L**. Pulse pressure: a predictor of long-term cardiovascular mortality in a French male population. *Hypertension* 30: 1410-1415, 1997.
8. **Bernhardt V, Lorenzo S, Babb TG, and Zavorsky GS**. Corrected end-tidal P(CO(2)) accurately estimates Pa(CO(2)) at rest and during exercise in morbidly obese adults. *Chest* 143: 471-477, 2013.
9. **Blaser T, Hofmann K, Buerger T, Effenberger O, Wallesch CW, and Goertler M**. Risk of stroke, transient ischemic attack, and vessel occlusion before endarterectomy in patients with symptomatic severe carotid stenosis. *Stroke; a journal of cerebral circulation* 33: 1057-1062, 2002.
10. **Calabia J, Torguet P, Garcia M, Garcia I, Martin N, Guasch B, Faur D, and Valles M**. Doppler ultrasound in the measurement of pulse wave velocity: agreement with the Complior method. *Cardiovascular ultrasound* 9: 13, 2011.
11. **Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, and Deanfield JE**. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 88: 2149-2155, 1993.
12. **Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, and Deanfield JE**. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 340: 1111-1115, 1992.

13. **Chowienczyk PJ, Watts GF, Cockcroft JR, Brett SE, and Ritter JM**. Sex differences in endothelial function in normal and hypercholesterolaemic subjects. *Lancet* 344: 305-306, 1994.
14. **Clarkson P, Celermajer DS, Donald AE, Sampson M, Sorensen KE, Adams M, Yue DK, Betteridge DJ, and Deanfield JE**. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. *Journal of the American College of Cardiology* 28: 573-579, 1996.
15. **Cooke JP**. Asymmetrical dimethylarginine: the Uber marker? *Circulation* 109: 1813-1818, 2004.
16. **DeLano FA, Parks DA, Ruedi JM, Babior BM, and Schmid-Schonbein GW**. Microvascular display of xanthine oxidase and NADPH oxidase in the spontaneously hypertensive rat. *Microcirculation* 13: 551-566, 2006.
17. **Diffey BL**. Solar ultraviolet radiation effects on biological systems. *Physics in medicine and biology* 36: 299-328, 1991.
18. **Din-Dzietham R, Couper D, Evans G, Arnett DK, and Jones DW**. Arterial stiffness is greater in African Americans than in whites: evidence from the Forsyth County, North Carolina, ARIC cohort. *American journal of hypertension* 17: 304-313, 2004.
19. **Divers J, Redden DT, Rice KM, Vaughan LK, Padilla MA, Allison DB, Bluemke DA, Young HJ, and Arnett DK**. Comparing self-reported ethnicity to genetic background measures in the context of the Multi-Ethnic Study of Atherosclerosis (MESA). *BMC genetics* 12: 28, 2011.
20. **Domanski M, Mitchell G, Pfeffer M, Neaton JD, Norman J, Svendsen K, Grimm R, Cohen J, Stamler J, and Group MR**. Pulse pressure and cardiovascular disease-related mortality: follow-up study of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA : the journal of the American Medical Association* 287: 2677-2683, 2002.
21. **Dong Y, Stallmann-Jorgensen IS, Pollock NK, Harris RA, Keeton D, Huang Y, Li K, Bassali R, Guo DH, Thomas J, Pierce GL, White J, Holick MF, and Zhu H**. A 16-week randomized clinical trial of 2000 international units daily vitamin D3 supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial stiffness. *The Journal of clinical endocrinology and metabolism* 95: 4584-4591, 2010.
22. **Dumville J, Panerai RB, Lennard NS, Naylor AR, and Evans DH**. Can cerebrovascular reactivity be assessed without measuring blood pressure in patients with carotid artery disease? *Stroke; a journal of cerebral circulation* 29: 968-974, 1998.
23. **Edwards MR, Topor ZL, and Hughson RL**. A new two-breath technique for extracting the cerebrovascular response to arterial carbon dioxide. *American journal of physiology Regulatory, integrative and comparative physiology* 284: R853-859, 2003.
24. **Ellingsen I, Hauge A, Nicolaysen G, Thoresen M, and Walloe L**. Changes in human cerebral blood flow due to step changes in PAO2 and PACO2. *Acta physiologica Scandinavica* 129: 157-163, 1987.
25. **Feihl F, Liaudet L, Waeber B, and Levy BI**. Hypertension: a disease of the microcirculation? *Hypertension* 48: 1012-1017, 2006.

26. **Ferreira AV, Viana MC, Mill JG, Asmar RG, and Cunha RS**. Racial differences in aortic stiffness in normotensive and hypertensive adults. *Journal of hypertension* 17: 631-637, 1999.
27. **Fields LE, Burt VL, Cutler JA, Hughes J, Roccella EJ, and Sorlie P**. The burden of adult hypertension in the United States 1999 to 2000: a rising tide. *Hypertension* 44: 398-404, 2004.
28. **Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, and Curhan GC**. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* 49: 1063-1069, 2007.
29. **Georgiadis D, Sievert M, Cencetti S, Uhlmann F, Krivokuca M, Zierz S, and Werdan K**. Cerebrovascular reactivity is impaired in patients with cardiac failure. *European heart journal* 21: 407-413, 2000.
30. **Giller CA, Bowman G, Dyer H, Mootz L, and Krippner W**. Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery* 32: 737-741; discussion 741-732, 1993.
31. **Gokce N, Holbrook M, Duffy SJ, Demissie S, Cupples LA, Biegelsen E, Keaney JF, Jr., Loscalzo J, and Vita JA**. Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. *Hypertension* 38: 1349-1354, 2001.
32. **Gunarathne A, Patel JV, Gammon B, Hughes EA, and Lip GY**. Impact of mean arterial blood pressure on higher arterial stiffness indices in South Asians compared to white Europeans. *Journal of hypertension* 26: 1420-1426, 2008.
33. **Gunarathne A, Patel JV, Gammon B, Hughes EA, and Lip GYH**. Impact of mean arterial blood pressure on higher arterial stiffness indices in South Asians compared to white Europeans. *Journal of Hypertension* 26: 1420-1426, 2008.
34. **Harper AM, and Glass HI**. Effect of alterations in the arterial carbon dioxide tension on the blood flow through the cerebral cortex at normal and low arterial blood pressures. *Journal of neurology, neurosurgery, and psychiatry* 28: 449-452, 1965.
35. **Harris LM, Faggioli GL, Shah R, Koerner N, Lillis L, Dandona P, Izzo JL, Snyder B, and Ricotta JJ**. Vascular reactivity in patients with peripheral vascular disease. *The American journal of cardiology* 76: 207-212, 1995.
36. **Harris RA, Pedersen-White J, Guo DH, Stallmann-Jorgensen IS, Keeton D, Huang Y, Shah Y, Zhu H, and Dong Y**. Vitamin D3 supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. *American journal of hypertension* 24: 557-562, 2011.
37. **Hlaing WM, Koutoubi S, and Huffman FG**. Differences in arterial stiffness and its correlates in tri-ethnic young men and women. *Ethnicity & disease* 16: 837-843, 2006.
38. **Holick MF**. Photosynthesis of vitamin D in the skin: effect of environmental and life-style variables. *Federation proceedings* 46: 1876-1882, 1987.
39. **Ichiyanagi K, Matsuki M, Masuko K, Nishisaka T, Watanabe R, and Horikawa H**. Effect of altered arterial carbon dioxide tensions on the electroencephalogram during hypothermia. *Acta anaesthesiologica Scandinavica* 13: 173-183, 1969.

40. **Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, and Creager MA**. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 88: 2510-2516, 1993.
41. **Jones NL, McHardy GJ, Naimark A, and Campbell EJ**. Physiological dead space and alveolar-arterial gas pressure differences during exercise. *Clinical science* 31: 19-29, 1966.
42. **Jones NL, Robertson DG, and Kane JW**. Difference between end-tidal and arterial PCO2 in exercise. *Journal of applied physiology* 47: 954-960, 1979.
43. **Kahn DF, Duffy SJ, Tomasian D, Holbrook M, Rescorl L, Russell J, Gokce N, Loscalzo J, and Vita JA**. Effects of black race on forearm resistance vessel function. *Hypertension* 40: 195-201, 2002.
44. **Kleiser B, and Widder B**. Course of carotid artery occlusions with impaired cerebrovascular reactivity. *Stroke; a journal of cerebral circulation* 23: 171-174, 1992.
45. **Kubis N, Richer C, Domergue V, Giudicelli JF, and Levy BI**. Role of microvascular rarefaction in the increased arterial pressure in mice lacking for the endothelial nitric oxide synthase gene (eNOS3pt-/-). *Journal of hypertension* 20: 1581-1587, 2002.
46. **Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, and Benetos A**. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 37: 1236-1241, 2001.
47. **Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H, and Non-invasive EN**. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *European Heart Journal* 27: 2588-2605, 2006.
48. **Levy BI, Schiffrin EL, Mourad JJ, Agostini D, Vicaut E, Safar ME, and Struijker-Boudier HA**. Impaired tissue perfusion: a pathology common to hypertension, obesity, and diabetes mellitus. *Circulation* 118: 968-976, 2008.
49. **Li YC, Kong J, Wei M, Chen ZF, Liu SQ, and Cao LP**. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *The Journal of clinical investigation* 110: 229-238, 2002.
50. **Liu J, Yang F, Yang XP, Jankowski M, and Pagano PJ**. NAD(P)H oxidase mediates angiotensin II-induced vascular macrophage infiltration and medial hypertrophy. *Arteriosclerosis, thrombosis, and vascular biology* 23: 776-782, 2003.
51. **Low DA, Wingo JE, Keller DM, Davis SL, Zhang R, and Crandall CG**. Cerebrovascular responsiveness to steady-state changes in end-tidal CO2 during passive heat stress. *Journal of applied physiology* 104: 976-981, 2008.
52. **Malmstrom TK, Wolinsky FD, Andresen EM, Miller JP, and Miller DK**. Cognitive ability and physical performance in middle-aged African Americans. *Journal of the American Geriatrics Society* 53: 997-1001, 2005.
53. **Matthys K, and Verdonck P**. Development and modelling of arterial applanation tonometry: a review. *Technology and health care : official journal of the European Society for Engineering and Medicine* 10: 65-76, 2002.

54. **Meadows GE, Dunroy HM, Morrell MJ, and Corfield DR**. Hypercapnic cerebral vascular reactivity is decreased, in humans, during sleep compared with wakefulness. *Journal of applied physiology* 94: 2197-2202, 2003.
55. **Melikian N, Wheatcroft SB, Ogah OS, Murphy C, Chowienczyk PJ, Wierzbicki AS, Sanders TA, Jiang B, Duncan ER, Shah AM, and Kearney MT**. Asymmetric dimethylarginine and reduced nitric oxide bioavailability in young Black African men. *Hypertension* 49: 873-877, 2007.
56. **Mensah GA, Mokdad AH, Ford ES, Greenlund KJ, and Croft JB**. State of disparities in cardiovascular health in the United States. *Circulation* 111: 1233-1241, 2005.
57. **Mervaala E, Muller DN, Schmidt F, Park JK, Gross V, Bader M, Breu V, Ganten D, Haller H, and Luft FC**. Blood pressure-independent effects in rats with human renin and angiotensinogen genes. *Hypertension* 35: 587-594, 2000.
58. **Mitchell GF**. Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. *Journal of applied physiology* 105: 1652-1660, 2008.
59. **Mitchell GF**. Increased aortic stiffness: an unfavorable cardiorenal connection. *Hypertension* 43: 151-153, 2004.
60. **Mitchell GF, Guo CY, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, Vasan RS, and Levy D**. Cross-sectional correlates of increased aortic stiffness in the community: the Framingham Heart Study. *Circulation* 115: 2628-2636, 2007.
61. **Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, Vasan RS, and Levy D**. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. *Hypertension* 43: 1239-1245, 2004.
62. **Mitsis GD, Ainslie PN, Poulin MJ, Robbins PA, and Marmarelis VZ**. Nonlinear modeling of the dynamic effects of arterial pressure and blood gas variations on cerebral blood flow in healthy humans. *Advances in experimental medicine and biology* 551: 259-265, 2004.
63. **Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, Allen C, Doughertly C, Gunter EW, and Bowman BA**. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. *The American journal of clinical nutrition* 76: 187-192, 2002.
64. **Ogoh S, Ainslie PN, and Miyamoto T**. Onset responses of ventilation and cerebral blood flow to hypercapnia in humans: rest and exercise. *Journal of applied physiology* 106: 880-886, 2009.
65. **Ogoh S, Hayashi N, Inagaki M, Ainslie PN, and Miyamoto T**. Interaction between the ventilatory and cerebrovascular responses to hypo- and hypercapnia at rest and during exercise. *The Journal of physiology* 586: 4327-4338, 2008.
66. **Panerai RB, Evans DH, and Naylor AR**. Influence of arterial blood pressure on cerebrovascular reactivity. *Stroke; a journal of cerebral circulation* 30: 1293-1295, 1999.

67. **Panerai RB, Simpson DM, Deverson ST, Mahony P, Hayes P, and Evans DH**. Multivariate dynamic analysis of cerebral blood flow regulation in humans. *IEEE transactions on bio-medical engineering* 47: 419-423, 2000.
68. **Perregaux D, Chaudhuri A, Mohanty P, Bukhari L, Wilson MF, Sung BH, and Dandona P**. Effect of gender differences and estrogen replacement therapy on vascular reactivity. *Metabolism: clinical and experimental* 48: 227-232, 1999.
69. **Perregaux D, Chaudhuri A, Rao S, Airen A, Wilson M, Sung BH, and Dandona P**. Brachial vascular reactivity in blacks. *Hypertension* 36: 866-871, 2000.
70. **Phan CQ, Tremper KK, Lee SE, and Barker SJ**. Noninvasive monitoring of carbon dioxide: a comparison of the partial pressure of transcutaneous and end-tidal carbon dioxide with the partial pressure of arterial carbon dioxide. *Journal of clinical monitoring* 3: 149-154, 1987.
71. **Poels MM, Vernooij MW, Ikram MA, Hofman A, Krestin GP, van der Lugt A, and Breteler MM**. Prevalence and risk factors of cerebral microbleeds: an update of the Rotterdam scan study. *Stroke; a journal of cerebral circulation* 41: S103-106, 2010.
72. **Prewitt RL, Chen, II, and Dowell R**. Development of microvascular rarefaction in the spontaneously hypertensive rat. *The American journal of physiology* 243: H243-251, 1982.
73. **Rasmussen P, Stie H, Nielsen B, and Nybo L**. Enhanced cerebral CO2 reactivity during strenuous exercise in man. *European journal of applied physiology* 96: 299-304, 2006.
74. **Reivich M**. Arterial Pco2 and Cerebral Hemodynamics. *The American journal of physiology* 206: 25-35, 1964.
75. **Resnick LM, Muller FB, and Laragh JH**. Calcium-regulating hormones in essential hypertension. Relation to plasma renin activity and sodium metabolism. *Annals of internal medicine* 105: 649-654, 1986.
76. **Ringelstein EB, Sievers C, Ecker S, Schneider PA, and Otis SM**. Noninvasive assessment of CO2-induced cerebral vasomotor response in normal individuals and patients with internal carotid artery occlusions. *Stroke; a journal of cerebral circulation* 19: 963-969, 1988.
77. **Robbins PA, Conway J, Cunningham DA, Khamnei S, and Paterson DJ**. A comparison of indirect methods for continuous estimation of arterial PCO2 in men. *Journal of applied physiology* 68: 1727-1731, 1990.
78. **Sachse A, and Wolf G**. Angiotensin II-induced reactive oxygen species and the kidney. *Journal of the American Society of Nephrology : JASN* 18: 2439-2446, 2007.
79. **Santos PC, Alvim Rde O, Ferreira NE, de Sa Cunha R, Krieger JE, Mill JG, and Pereira AC**. Ethnicity and arterial stiffness in Brazil. *American journal of hypertension* 24: 278-284, 2011.
80. **Schmitz KJ, Skinner HG, Bautista LE, Fingerlin TE, Langefeld CD, Hicks PJ, Haffner SM, Bryer-Ash M, Wagenknecht LE, Bowden DW, Norris JM, and Engelman CD**. Association of 25-hydroxyvitamin D with blood pressure in predominantly 25-hydroxyvitamin D deficient Hispanic and African Americans. *American journal of hypertension* 22: 867-870, 2009.

81. **Schwartz BS, Glass TA, Bolla KI, Stewart WF, Glass G, Rasmussen M, Bressler J, Shi W, and Bandeen-Roche K**. Disparities in cognitive functioning by race/ethnicity in the Baltimore Memory Study. *Environmental health perspectives* 112: 314-320, 2004.
82. **Scragg R, Sowers M, and Bell C**. Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. *American journal of hypertension* 20: 713-719, 2007.
83. **Serrador JM, Picot PA, Rutt BK, Shoemaker JK, and Bondar RL**. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke; a journal of cerebral circulation* 31: 1672-1678, 2000.
84. **Serrador JM, Sorond FA, Vyas M, Gagnon M, Iloputaife ID, and Lipsitz LA**. Cerebral pressure-flow relations in hypertensive elderly humans: transfer gain in different frequency domains. *Journal of applied physiology* 98: 151-159, 2005.
85. **Sorescu D**. Smad3 mediates angiotensin II- and TGF-beta1-induced vascular fibrosis: Smad3 thickens the plot. *Circulation research* 98: 988-989, 2006.
86. **St Croix CM, Cunningham DA, Kowalchuk JM, McConnell AK, Kirby AS, Scheuermann BW, Petrella RJ, and Paterson DH**. Estimation of arterial PCO2 in the elderly. *Journal of applied physiology* 79: 2086-2093, 1995.
87. **Stampfer MJ**. Cardiovascular disease and Alzheimer's disease: common links. *Journal of internal medicine* 260: 211-223, 2006.
88. **Sutton-Tyrrell K, Najjar SS, Boudreau RM, Venkitachalam L, Kupelian V, Simonsick EM, Havlik R, Lakatta EG, Spurgeon H, Kritchevsky S, Pahor M, Bauer D, Newman A, and Health ABCS**. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation* 111: 3384-3390, 2005.
89. **Tang MX, Cross P, Andrews H, Jacobs DM, Small S, Bell K, Merchant C, Lantigua R, Costa R, Stern Y, and Mayeux R**. Incidence of AD in African-Americans, Caribbean Hispanics, and Caucasians in northern Manhattan. *Neurology* 56: 49-56, 2001.
90. **Urbina EM, Srinivasan SR, Kieltyka RL, Tang R, Bond MG, Chen W, and Berenson GS**. Correlates of carotid artery stiffness in young adults: The Bogalusa Heart Study. *Atherosclerosis* 176: 157-164, 2004.
91. **Vicenzini E, Ricciardi MC, Altieri M, Puccinelli F, Bonaffini N, Di Piero V, and Lenzi GL**. Cerebrovascular reactivity in degenerative and vascular dementia: a transcranial Doppler study. *European neurology* 58: 84-89, 2007.
92. **Vyas M, Izzo JL, Jr., Lacourciere Y, Arnold JM, Dunlap ME, Amato JL, Pfeffer MA, and Mitchell GF**. Augmentation index and central aortic stiffness in middle-aged to elderly individuals. *American journal of hypertension* 20: 642-647, 2007.
93. **Wahba RW, and Tessler MJ**. Misleading end-tidal CO2 tensions. *Canadian journal of anaesthesia = Journal canadien d'anesthesie* 43: 862-866, 1996.
94. **Wakasugi M, Noguchi T, Inoue M, Kazama Y, Tawata M, Kanemaru Y, and Onaya T**. Vitamin D3 stimulates the production of prostacyclin by vascular smooth muscle cells. *Prostaglandins* 42: 127-136, 1991.

95. **Wasserman AJ, and Patterson JL, Jr.** The cerebral vascular response to reduction in arterial carbon dioxide tension. *The Journal of clinical investigation* 40: 1297-1303, 1961.
96. **Webb AR, Kline L, and Holick MF**. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *The Journal of clinical endocrinology and metabolism* 67: 373-378, 1988.
97. **Whitesell R, Asiddao C, Gollman D, and Jablonski J**. Relationship between arterial and peak expired carbon dioxide pressure during anesthesia and factors influencing the difference. *Anesthesia and analgesia* 60: 508-512, 1981.
98. **Widder B, Kleiser B, and Krapf H**. Course of cerebrovascular reactivity in patients with carotid artery occlusions. *Stroke; a journal of cerebral circulation* 25: 1963-1967, 1994.
99. **Wong MS, Delansorne R, Man RY, and Vanhoutte PM**. Vitamin D derivatives acutely reduce endothelium-dependent contractions in the aorta of the spontaneously hypertensive rat. *American journal of physiology Heart and circulatory physiology* 295: H289-296, 2008.
100. **Yosefy C, Hay E, Nasri Y, Magen E, and Reisin L**. End tidal carbon dioxide as a predictor of the arterial PCO2 in the emergency department setting. *Emergency medicine journal : EMJ* 21: 557-559, 2004.
101. **Zhang P, Huang G, and Shi X**. Cerebral vasoreactivity during hypercapnia is reset by augmented sympathetic influence. *Journal of applied physiology* 110: 352-358, 2011.
102. **Zieman SJ, Melenovsky V, and Kass DA**. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arteriosclerosis, thrombosis, and vascular biology* 25: 932-943, 2005.
103. **Zoungas S, and Asmar RP**. Arterial stiffness and cardiovascular outcome. *Clinical and experimental pharmacology & physiology* 34: 647-651, 2007.