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Cerebral vasodilatory capacity and reactivity in young African Americans: a comparison of rebreathing and steady-state hypercapnia to assess cerebrovascular health

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Cerebral vasodilatory capacity and reactivity in young African Americans: a comparison of rebreathing and steady-state hypercapnia to assess cerebrovascular health

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Abstract

Cerebral vasodilatory capacity and reactivity in young African Americans: a comparison of rebreathing and steady-state hypercapnia to assess cerebrovascular health

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Evaluation of changes in cerebral blood flow (CBF) in response to manipulation of arterial carbon dioxide tension (P_{CO_2}) is a non-invasive technique to assess cerebrovascular function. Hypercapnia is achieved using respiratory maneuvers that produce either transient or step changes in P_{CO_2}. Although the CBF response is similar between methods, transient hypercapnia through rebreathing provides additional data points and allows for a more dynamic assessment of the relationship between CBF and end-tidal CO_2 tension (P_{ET,CO_2}). The aim of this study was to compare rebreathing and steady-state methodologies to evaluate population differences in the cerebral hemodynamic response to hypercapnia. We hypothesized that during rebreathing, African American (AA) individuals would exhibit a lower maximal increase in cerebrovascular conductance (CVCI) relative to Caucasian Americans (CA) despite comparable cerebrovascular reactivity to CO_2 (CVR), whereas during steady-state hypercapnia, there would be no difference between groups during inhalation of 3% or 6% CO_2, but AA would display reduced CVCI when breathing 9% CO_2. Middle cerebral artery blood flow velocity (CBFV) was measured using transcranial Doppler

ultrasound in young, healthy AA (n = 12) and CA (n = 11) participants during two conditions (randomized): (i) transient, breath-by-breath increases in P_{ET,CO_2} induced by rebreathing; and (ii) steady-state increases in P_{ET,CO_2} induced by inhalation of 3%, 6%, and 9% CO_2. The maximal increase in CVCI (CBFV/mean arterial pressure) with hypercapnia was lower in AA compared to CA in both methods (rebreathing, P = 0.018; steady-state, P = 0.049). Linear regression of CVCI vs. P_{ET,CO_2} during steady-state hypercapnia suggested that CVR was reduced in AA when the slopes from baseline (P = 0.044) or 3% (P = 0.039) through 6% CO_2 were considered, whereas logistic regression of the response to rebreathing indicated no difference in maximal CVR between the two groups (P = 0.59). These results indicate that both rebreathing and steady-state techniques were sufficient to detect differences in the cerebral vasodilatory reserve capacity between populations, whereas linear regression to estimate CVR is influenced by the extent of hypercapnia and may inaccurately describe CVR when data from the asymptotic region of the response is included in analysis.

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Introduction & Purpose

In humans, the cerebral vasculature is tightly regulated by the partial pressure of carbon dioxide (P_{CO_2}) in arterial circulation where cerebral perfusion increases with hypercapnia and decreases with hypocapnia (Kety & Schmidt, 1948; Shapiro *et al.*, 1966). This sensitive response, which is largely mediated by dilation or constriction of arterioles, plays a key role in regulation of central pH (Lambertson *et al.*, 1961; Ainslie & Duffin 2009). In research and clinical settings, the cerebral blood flow (CBF) response to manipulation of P_{CO_2}, referred to as cerebrovascular reactivity (CVR), is used as a safe, inexpensive, and non-invasive technique to assess cerebrovascular function. Alterations in the relationship between changes in P_{CO_2} and cerebral blood flow manifest in clinical conditions including carotid artery disease (Ringelstein *et al.*, 1988), diabetes (Kadoi *et al.*, 2003), and endothelial dysfunction (Lavi *et al.*, 2006), and a diminished response predicts and may contribute to the development of stroke (Gur *et al.*, 1996; Markus & Cullinane, 2001). Thus, CVR is a practical tool that provides powerful insight as an index of cerebral vascular health.

Cerebrovascular reactivity can be assessed using respiratory maneuvers that induce steady-state or transient changes in P_{CO_2}. Steady-state inspiration of a series of fixed concentrations of CO_2 is a commonly used method to elicit hypercapnia (Shapiro *et al.*, 1966; Xie *et al.*, 2005; Peebles *et al.* 2007). Reactivity is then quantified using linear regression of the blood flow response to changes in arterial P_{CO_2}, which is often indexed using end-tidal CO_2 tension (P_{ET,CO_2}). This technique allows sufficient time for changes in medullary concentration of H^+ to stimulate chemoreceptor-mediated hyperventilation; thus it is used when integration between CBF and ventilatory responses to hypercapnia are of interest. Another commonly used approach is rebreathing exhaled gas, which produces breath-by-breath increases in P_{CO_2} (Read & Leigh, 1967; Claassen *et al.*, 2007). Although the cerebrovascular response is similar between steady-state and transient hypercapnia (Brothers *et al.*, 2014), the rebreathing technique, because it produces many additional data points,

provides more information and allows for a more dynamic assessment of the relationship between CBF and P_{CO_2}. Since this relationship is nonlinear (Ringelstein *et al.*, 1988; Claassen *et al.*, 2007), use of linear regression of steady-state data may inaccurately estimate differences in CVR between populations. Additionally, depending on the concentrations employed, inspiration of fixed fractional CO_2 may not produce a sufficient range of change in P_{CO_2} to detect differences in the asymptotic region of the response when comparing groups. Considering these limitations, it is important to assess the sensitivity of each method to detect differences in CVR and cerebral vasodilatory reserve capacity between populations.

The purpose of this study was to compare rebreathing and steady-state methodologies to examine the cerebral hemodynamic response to hypercapnia. To accomplish this, we performed both techniques in a randomized, cross-over design in a population which our lab has previously shown to have a reduced vasodilatory reserve capacity when exposed to rebreathing-induced hypercapnia (Hurr *et al.*, 2014). The responses of African American participants were compared to those of Caucasian American participants matched by age, sex, and BMI. We hypothesized that:

1. The African American group would have a reduced maximal vasodilatory response to changes in P_{CO_2} compared to the Caucasian American group during the rebreathing protocol despite similar cerebrovascular reactivity.
2. The response would be similar between groups during steady-state inhalation of 3% and 6% CO_2.
3. Extending the steady-state stimulus to 9% CO_2 would improve the sensitivity of the technique to detect differences between groups.

Experimental Design & Methods

Participants

Twelve African American (AA) and eleven Caucasian American (CA) adults participated in this study (Table 1). Subjects were free from known cardiovascular, metabolic, or neurological disease. Participants were non-smokers and were not taking medications with the exception of oral contraception. Females were studied during the early follicular phase of the menstrual cycle.

Table 1: Subject Characteristics

Variable	African American (n = 12)	Caucasian American (n = 11)	*P*-Value
Age (years)	24 ± 1	23 ± 1	0.71
Sex (male/female)	7 / 5	7 / 4	0.87
Height (cm)	174 ± 3	173 ± 2	0.92
Weight (kg)	73 ± 4	68 ± 3	0.30
BMI ($kg \cdot m^{-2}$)	24 ± 0.7	23 ± 0.4	0.07

Values are means ± SEM. Abbreviations: BMI, body mass index.

Ethical Approval

The experimental protocol and consent process were approved by The Institutional Review Board at The University of Texas at Austin. The purpose and risks of all study procedures were verbally explained to participants, who provided written, informed consent. The study conformed to provisions established by The Declaration of Helsinki.

Instrumentation & Measurements

All studies took place in the morning following an overnight fast (≥12 hr) in a dimly-lit, temperature-controlled laboratory (24^{O}C, 40% humidity). Participants were required to abstain from exercise, medication, and alcohol for 24 hr prior to the experimental trial and to avoid consumption of food and caffeine for 12 hr prior.

Each subject's right middle cerebral artery (MCA) was imaged through the temporal window to measure cerebral blood flow velocity using a 2 MHz transcranial Doppler (TCD) ultrasound probe (DWL; Compumedics), which was secured in place with a specialized headpiece. Participants were fitted with an oro-nasal face mask (V2 Mask; Hans Rudolph, Inc.) and end-tidal CO_2 was sampled using a capnograph (RespSense; Nonin Medical, Inc.). Beat-to-beat blood pressure was continuously monitored through a finger cuff using the Peňáz method (CNAP Monitor, CNSystems) and blood pressure was measured at regular intervals through auscultation of the brachial artery with electrosphygmomanometry (Tango+; Sun Tech Medical, Inc.). Participants were instrumented with an electrocardiogram to record cardiac rhythms and heart rate and a pulse oximeter to monitor oxygen saturation (GE Dash 4000; General Health Care).

Experimental Protocol

Following collection of anthropometric data, participants rested in a semi-recumbent position for at least 20 min while the TCD signal was optimized. Then, subjects performed two breathing techniques designed to induce hypercapnia in a randomized order separated by 20 min of rest.

Rebreathing

A 5 liter bag was attached to the mask using a three-way valve with a stopcock. Approximately 5 min prior to data collection, participants performed a deep inspiration, then exhaled to fill the empty bag. The valve was returned to room air for 2 min of baseline data collection. Upon switching the valve, subjects rebreathed from the bag for ~3 min. Arterial oxygen saturation was maintained during the rebreathing maneuver by bleeding a small amount of oxygen into the bag (Claassen *et al.*, 2007). Subjects were instructed to continue rebreathing until they began to experience discomfort, at which point the valve was returned to room air.

Steady-State

Baseline data was collected for 2 min while the mask was open to room air, then subjects breathed from pre-mixed gas cylinders containing 3%, 6%, and 9% CO_2 for 2 min per step. Each gas blend contained 21% oxygen and was balanced with nitrogen. The cylinders were attached to the mask with a two-way valve.

Calculations and Data Analysis

Data for cerebral blood flow velocity, blood pressure, $P_{\mathrm{ET,CO_2}}$, and cardiac rhythms were recorded continuously at 125 Hz using a data acquisition program (Biopac System). Baseline values were calculated as 1 min averages, and the final 30 sec of each stage of the steady-state protocol were averaged for each subject. Rebreathing data were obtained using breath-by-breath analysis.

CBFV was expressed as a percentage of baseline. Mean arterial pressure (MAP) was calculated from the automated blood pressure cuff measurements as diastolic blood pressure plus one-third pulse pressure. To account for changes in MAP induced by hypercapnia, an index of cerebrovascular conductance (CVCI) was calculated as CBFV/MAP and expressed as a percentage of baseline.

Values of CVCI during the rebreathing protocol were analyzed using a four parameter logistic regression for sigmoidal curve fitting (Kent *et al.* 1972; Claassen *et al.* 2007):

$$f(x) = y_0 - \left(\frac{a}{1 + e^{b(x - x_0)}}\right)$$

where y_0 represents the maximal value of CVCI; a is the range of change, b indicates the overall sigmoidal property of the curve, and x_0 denotes the value of $P_{\mathrm{ET,CO_2}}$ at which cerebrovascular reactivity was maximal (Figure 1).

Maximal cerebrovascular reactivity (CVR_{max}) was calculated at $x = x_0$ using a first-order derivative of the following function:

$$f(x) = \frac{ab \times e^{b(x-x_0)}}{\{1 + e^{b(x-x_0)}\}^2}$$

Linear regression of the slope of changes in CVCI in response to steady-state changes in $P_{\mathrm{ET,CO_2}}$ was used to assess cerebrovascular reactivity (CVR).

Statistical Analysis

Statistical analyses were performed using commercially available software (SigmaPlot 12.5; Systat Software, Inc.). Student's *t*-tests were used to evaluate subject characteristics and baseline values. The sex ratio between the two groups was analyzed using a χ^2 test. Parameters from the logistic regression, maximal values achieved during rebreathing, and estimates of CVR were compared using Student's *t*-tests. Two-way, repeated measures ANOVA was utilized to evaluate the responses of CVCI, CBFV, and MAP to the steady-state gas stimuli in the AA and CA groups, and post-hoc analyses were performed using Tukey's HSD when appropriate. Planned comparisons of group responses to each gas from the steady-state protocol were performed using the Bonferroni method. A 95% confidence interval was employed to determine significance. All data are presented as means ± SEM.

Results

Subject Characteristics & Baseline Values

There was no difference in age, sex, height, weight, or BMI between groups (Table 1; $P > 0.05$ for all comparisons). During the eucapnic baseline period before each stimulus, there was no difference in HR, MAP, P_{ET,CO_2}, CBFV, or CVCI between groups (Table 2; $P > 0.05$ for all comparisons). Baseline P_{ET,CO_2} was lower preceding the rebreathing trial than the steady-state trial (40 ± 1 vs. 39 ± 1; $P = 0.01$), whereas HR, MAP, CBFV, and CVCI were not different between baselines (Table 2; $P > 0.05$ for all comparisons).

Table 2: Baseline Hemodynamic Values

	Steady-State Baseline			Rebreathing Baseline		
Variable	AA (n = 12)	CA (n = 11)	*P*-Value	AA (n = 12)	CA (n = 11)	*P*-Value
HR (beats·min^{-1})	64 ± 2	61 ± 2	0.41	66 ± 2	62 ± 3	0.33
MAP (mmHg)	92 ± 4	92 ± 3	0.95	93 ± 4	90 ± 3	0.61
CBFV (cm·s^{-1})	75 ± 4	67 ± 4	0.18	75 ± 5	71 ± 3	0.45
CVCI (cm·s^{-1}·mmHg^{-1})	0.8 ± 0.1	0.7 ± 0.0	0.29	0.7 ± 0.0	0.7 ± 0.0	0.56
P_{ET,CO_2} (mmHg)	41 ± 1	40 ± 1	0.50	40 ± 1	39 ± 1	0.63

Values are means ± SEM. Abbreviations: AA, African American; CA, Caucasian American; CBFV, velocity of blood flow in the middle cerebral artery; CVCI, index of cerebral vascular conductance; HR, heart rate; MAP, mean arterial pressure; and P_{ET,CO_2}, partial pressure of end-tidal carbon dioxide.

Cerebrovascular Responses to Hypercapnia

Rebreathing

Maximal CBFV and CVCI reached during rebreathing were lower in AA relative to CA participants (Figure 3; CBFV: AA, 151 ± 5% vs. CA, 165 ± 6%, $P = 0.04$; CVCI: AA, 134 ±

3% vs. CA, 148 ± 5%; $P = 0.018$). Rebreathing increased P_{ET,CO_2} by 17 ± 1 mmHg in AA and 20 ± 1 mmHg in CA ($P = 0.11$).

Data from three subjects were excluded from analysis of logistic function parameters because their responses were not adequately described by the function. For the remaining subjects (AA, $n = 10$; CA, $n = 10$), logistic regression of CVCI vs. P_{ET,CO_2} yielded a correlation coefficient (R^2) of 0.95. Logistic regression curves representing the responses of each group are illustrated in Figure 1. Both the maximal increase (y_0) and the total range of change (a) in CVCI were reduced in AA compared to CA subjects (y_0: AA, 138 ± 4% vs. CA, 151 ± 4%; $P = 0.02$; a: AA, 43 ± 5% vs. CA, 56 ± 5%; $P = 0.03$). Maximal cerebrovascular reactivity (CVR_{max}) and the level of P_{ET,CO_2} at which reactivity was maximal (x_0) were not different between groups (Table 3; CVR_{max}: AA, 3.7 ± 0.5%·mmHg^{-1} vs. CA, 4.1 ± 0.5%·mmHg^{-1}; $P = 0.59$; x_0: AA, 49 ± 2 mmHg vs. CA, 50 ± 1 mmHg; $P = 0.54$).

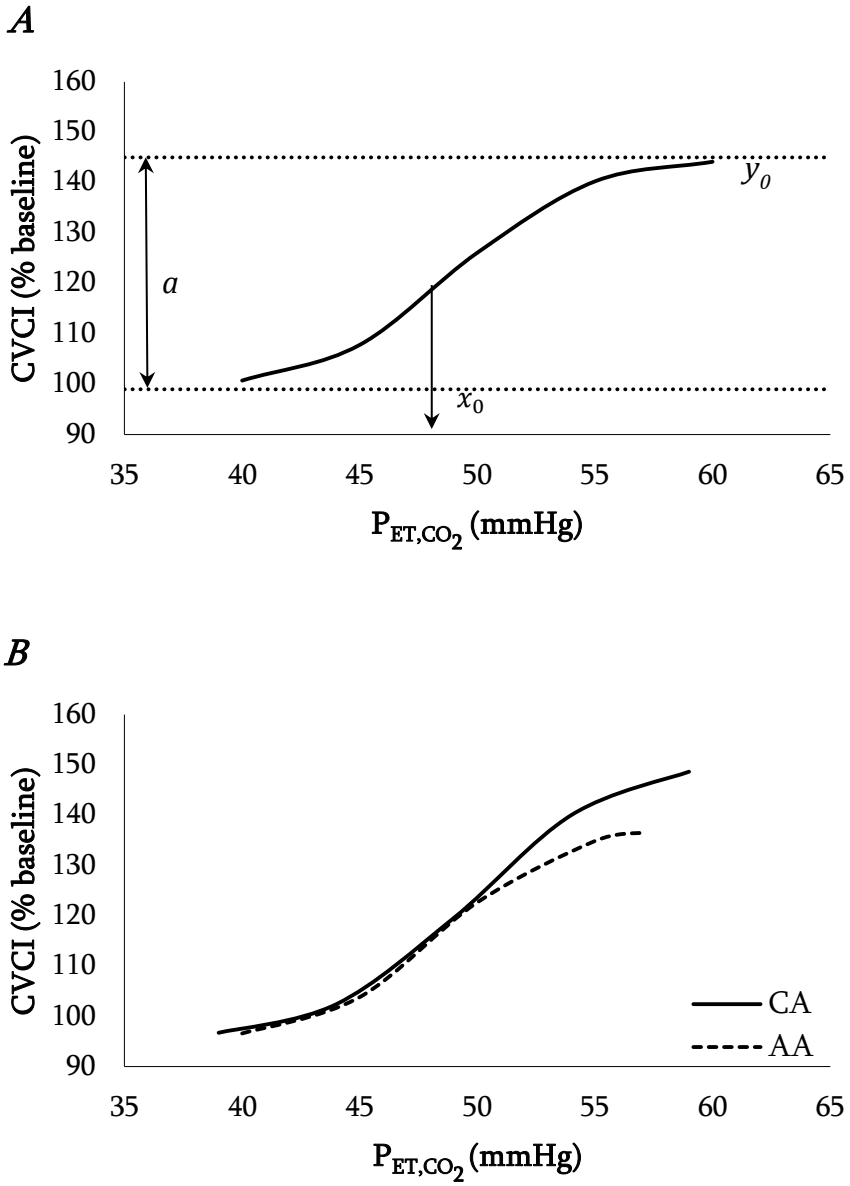

Figure 1: Logistic regressions of CVCI during rebreathing

A schematic diagram illustrating a logistic regression and its parameters is shown in panel A. The parameter y_0 represents the maximal value of CVCI, a is the range of change, x_0 denotes the value of P_{ET,CO_2} at which cerebrovascular reactivity was maximal. Group mean values of the four parameters observed during rebreathing are represented as logistic regression curves in panel B. The parameters y_0 and a were lower in AA relative to CA participants, whereas the maximal cerebrovascular reactivity and x_0 were not different between groups.

Steady-State

Two participants (AA) did not complete the final stage of the steady-state protocol. In these subjects, CBFV appeared to have stabilized, thus data collected after 60 sec of 9% CO_2 inhalation were averaged and included in analysis.

As shown in Figure 2, the increase in CVCI during steady-state hypercapnia was moderated by race ($P = 0.046$). There was no difference in CVCI between groups during 3% CO_2 inhalation (AA, 104 ± 2% vs. CA, 108 ± 2%; $P = 0.42$), whereas CVCI was lower in AA relative to CA participants during 6% and 9% CO_2 inhalation (6%: AA 120 ± 4% vs. CA, 131 ± 3%; $P = 0.026$; 9%: AA, 137 ± 6% vs. CA, 152 ± 6%; $P = 0.006$). The maximal increase in CVCI observed during steady-state hypercapnia was lower in AA than CA participants (Figure 3; AA, 137 ± 6% vs. CA, 152 ± 5%, $P = 0.049$). The magnitude of hypercapnia reached during each stage was similar between groups ($P_{\mathrm{ET,CO_2}}$; $P = 0.43$).

The overall response of CBFV to steady-state hypercapnia was not different between groups ($P = 0.10$). However, planned comparisons for each gas mixture revealed lower CBFV in AA compared to CA participants during inhalation of 9% CO_2 (AA, 158 ± 6% vs. CA, 175 ± 8%; $P = 0.006$). MAP increased with hypercapnia ($P < 0.001$) to a similar degree between groups ($P = 0.83$).

Estimates of CVR obtained from steady-state data are recorded in Table 3. Stepwise linear regression revealed no difference in CVCI reactivity from baseline to 3% CO_2 inhalation or from 6% to 9% CO_2. However, reactivity was reduced in AA when the slopes from 3% to 6% CO_2 inhalation were compared. When multiple data points were included in slope calculations, CVR was lower in AA compared to CA from baseline through 6% CO_2, but there was not a difference in CVR between groups upon regression of all data from baseline through 9% CO_2.

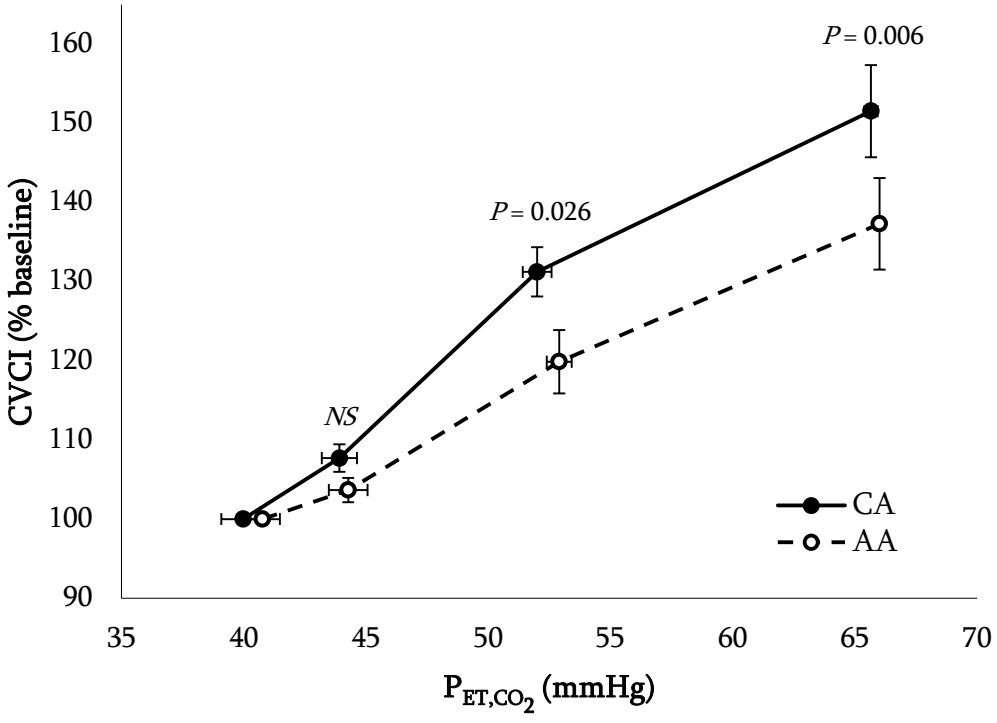

Figure 2: CVCI during steady-state hypercapnia

The response of CVCI to steady-state hypercapnia was moderated by race (group by CO_2 interaction; P = 0.046) such that there was no difference between groups when 3% CO_2 was inhaled, but CVCI increased to a lesser degree in AA compared to CA upon inhalation of 6% and 9% CO_2. Values are shown as means ± SEM.

Table 3: Estimates of Cerebrovascular Reactivity to Carbon Dioxide

Analysis	AA	CA	*P*-Value
Rebreathing			
Logistic Function:			
CVR_{max} at $x = x_0$ (%·mmHg^{-1})	3.7 ± 0.5	4.1 ± 0.5	0.59
Steady-State			
Stepwise Linear Regression:			
Baseline to 3% CO_2 (%·mmHg^{-1})	1.0 ± 0.6	2.1 ± 0.5	0.20
3% CO_2 to 6% CO_2 (%·mmHg^{-1})	1.9 ± 0.4*	3.1 ± 0.4	0.04
6% CO_2 to 9% CO_2 (%·mmHg^{-1})	1.3 ± 0.2	1.5 ± 0.3	0.56
Least Squares Regression:			
Baseline through 6% CO_2 (%·mmHg^{-1})	1.7 ± 0.4*	2.7 ± 0.3	0.04
Baseline through 9% CO_2 (%·mmHg^{-1})	1.5 ± 0.2	2.0 ± 0.2	0.11

Values are means ± SEM. Abbreviations: AA, African American; CA, Caucasian American; CVR_{max} maximal cerebrovascular reactivity. *$P < 0.05$ *vs.* CA.

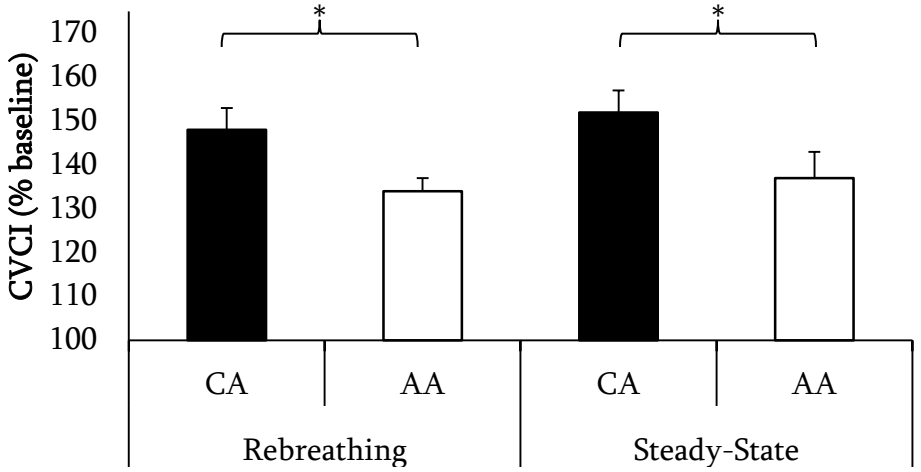

Figure 3: Group differences in the maximal increase in CVCI

The maximal increase in CVCI was lower in AA relative to CA during rebreathing and steady-state hypercapnia. Values are shown as means ± SEM. *$P < 0.05$.

Discussion

The present comparison of rebreathing and steady-state techniques to assess cerebrovascular responses to hypercapnia revealed that each method was sufficient to detect differences between the responses of healthy, college-aged African American and Caucasian American subjects. Both techniques showed similar responses between groups at low levels of hypercapnia with differences emerging as P_{ET,CO_2} progressed. These findings provide insight to group variations in the dynamic relationship between cerebral blood flow and P_{ET,CO_2} with implications for selecting the source and magnitude of hypercapnia and interpreting assessments of cerebrovascular reactivity.

Group Responses to Hypercapnia

As depicted in Figure 1B, the cerebrovascular responses to rebreathing were similar between AA and CA participants until P_{ET,CO_2} reached ~50 mmHg (an increase of ~10 mmHg from baseline). Beyond this point, the responses diverged and revealed a lower maximal increase in CVCI in AA compared to CA subjects. Cerebrovascular reactivity was similar between groups, while the maximal increase and range of change were reduced in AA. These results suggest that, despite equivalent cerebrovascular sensitivity to hypercapnia, AA individuals exhibit a lower cerebral vasodilatory reserve capacity than their CA counterparts.

The cerebrovascular responses to steady-state hypercapnia were similar between groups during inhalation of 3% CO_2, which elicited a P_{ET,CO_2} of 44 mmHg (Figure 2). When the stimulus was raised to 6% and 9% CO_2, which produced P_{ET,CO_2} values of 52 and 66 mmHg, respectively, a distinction emerged between groups in which AA participants exhibited a smaller increase in CVCI than CA participants. Although we hypothesized that the response to 6% CO_2 would be comparable in AA and CA, these results align with our findings from the rebreathing protocol, which suggest that the group responses begin to

separate when P_{ET,CO_2} is ~50 mmHg. Thus, we speculate that 6% CO_2 was a sufficient stimulus to detect differences between these groups, whereas commonly-used intermediate concentrations of 4% or 5% CO_2 (Ringelstein *et al.*, 1988; Xie *et al.*, 2005) would likely fail to catch group differences.

Cerebral Vasodilatory Capacity & Reactivity

The maximal increase in CVCI was reduced in AA relative to CA participants regardless of the technique or analysis employed (Figure 3). This finding is in agreement with previous results from our lab which demonstrated a lower vasodilatory reserve capacity in young African Americans in response to hypercapnic rebreathing (Hurr *et al.*, 2014). However, estimates of cerebrovascular reactivity varied depending on the analysis used (Table 3). Linear regression of the response to steady-state hypercapnia suggested that CVR was reduced in AA when the slopes from baseline or 3% through 6% CO_2 were considered, whereas logistic regression of the response to rebreathing indicated no difference in CVR_{max} between the two groups. These results suggest that estimates of CVCI-CO_2 sensitivity are influenced by the method of analysis and combination of data points examined. Since CVCI exhibits a sigmoidal response to changes in P_{ET,CO_2} (Ringelstein *et al.*, 1988; Claassen *et al.*, 2007), linear regression of steady-state data likely underestimates true CVR when values from the asymptotic region of the response are included in calculations.

Limitations

The diameter of the insonated vessel cannot be obtained through transcranial Doppler measurements; thus, CBFV only accurately reflects CBF if the cross-sectional area of the middle cerebral artery remains constant during hypercapnia. This assumption has been inconclusively debated in recent years. Previous studies have concluded that the diameter is

not altered during moderate changes in plasma CO_2 tension such as those induced by inhalation of 6% CO_2 (Bradac *et al.*, 1976; Poulin & Robbins, 1996; Serrador *et al.*, 2000), while others have challenged these findings (Willie *et al.*, 2012; Coverdale *et al.,* 2014). If the hypercapnic stimuli in the present study did result in dilation of the MCA, it is possible that CBFV measurements underestimated CBF. However, considering the similar blood pressure responses observed in our groups, any discrepancy between CBFV and CBF is likely comparable between AA and CA. Further characterization with more sophisticated imaging modalities is warranted to examine the source and physiological significance of the differences in cerebrovascular reserve capacity.

The degree of hypercapnia was assessed through measurement of P_{ET,CO_2} rather than arterial or venous P_{CO_2}. At high levels, P_{ET,CO_2} has been shown to overestimate arterial P_{CO_2} (Peebles *et al.*, 2007). However, adjusting our P_{ET,CO_2} values to better approximate arterial P_{CO_2} according to the method developed by Peebles *et al.* (2007) did not alter our findings; thus, we feel that P_{ET,CO_2} provided a valid index for interpretation of the cerebrovascular response to hypercapnia. P_{ET,CO_2} was 1 mmHg higher during the normocapnic baseline period preceding the steady-state trial than before rebreathing, which is likely an artifact of the different valves employed. This is supported by the equivalent CBFV and CVCI measurements during the baseline periods.

CONCLUSIONS

In conclusion, we observed that rebreathing and steady-state hypercapnia were each sufficient to detect differences in the maximal increase in cerebrovascular conductance between healthy, young African American and Caucasian American participants. Estimates of cerebrovascular reactivity to hypercapnia differed depending on the method of analysis employed, and it is likely that linear regression inaccurately represents true reactivity when

analysis includes values from the asymptotic region of the response to CO_2. These findings provide insight for selection of an appropriate hypercapnic stimulus to examine group differences in cerebrovascular reserve capacity versus cerebrovascular reactivity. Further investigation is warranted to examine the physiological significance of these two factors.

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