Carotid Artery Stiffness and Hemodynamic Pulsatility During Cognitive Engagement in Healthy Adults: A Pilot Investigation

Kevin S. Heffernan,[1] Nicole L. Spartano,[1] Jacqueline A. Augustine,[1] Wesley K. Lefferts,[1] William E. Hughes,[1] Gary F. Mitchell,[2] Randall S. Jorgensen,[3] and Brooks B. Gump[4]

BACKGROUND
The matching of vascular supply to neuronal metabolic demand during cognitive engagement is known as neurovascular coupling (NVC). Arterial stiffness is a prominent determinant of pulsatility in the systemic circulation and may thus indirectly impact NVC. In this pilot investigation, we explored changes in carotid artery stiffness and cerebrovascular hemodynamic pulsatiltiy during cognitive engagement in healthy adults.

METHODS
Twenty-seven adults (age 39 ± 3 years, BMI 24 ± 1 kg/m^2) underwent Doppler ultrasonography of the common carotid artery (CCA) combined with applanation tonometry to derive (i) CCA elastic modulus (Ep) and β-stiffness index; (ii) CCA flow pulsatility index (PI); (iii) CCA pulse pressure, (iv) CCA augmentation index (AIx). Cerebral PI was assessed using transcranial Doppler at the middle cerebral artery (MCA). All measures were made at rest and during an incongruent Stroop task.

RESULTS
CCA PI was reduced (1.75 ± 0.06 to 1.57 ± 0.06, $P < 0.05$) while MCA PI was unchanged (0.75 ± 0.02 to 0.75 ± 0.02, $P > 0.05$) during Stroop. Brachial pulse pressure increased during Stroop (43 ± 1 to 46 ± 1 mm Hg, $P < 0.05$) while CCA pulse pressure was unchanged (36 ± 1 to 35 ± 1 mm Hg, $P > 0.05$). Similarly, CCA Ep (54.5 ± 5.5 to 53.8 ± 4.9 kPa, $P > 0.05$) and β-stiffness index (4.4 ± 0.4 to 4.2 ± 0.3 aU, $P > 0.05$) were unchanged. CCA AIx increased (1 ± 4 to 13 ± 4%, $P < 0.05$).

CONCLUSION
Carotid pressure pulsatility is unaltered while carotid flow pulsatility is reduced during cognitive engagement. Carotid artery stiffness does not change suggesting that factors other than the dynamic elastic properties of the CCA buffer cerebrovascular hemodynamic pulsatility during cognitive engagement.

Keywords: arterial stiffness; augmentation index; blood pressure; carotid artery; hemodynamics; hypertension; pulsatility.

doi:10.1093/ajh/hpu198

The matching of cerebral oxygen supply to neuronal metabolic demand, also known as neurovascular coupling (NVC), is a significant determinant of cognitive performance.[1] The brain is an obligate high flow, low impedance organ and is therefore particularly susceptible to hemodynamic pulsatility.[2] Excessive flow pulsatility may damage or impair function of small vessels in the brain, predisposing to microvascular hypoperfusion, ischemia, and focal infarcts.[3–8] Central artery stiffness (i.e., aorta or carotid) directly moderates transmission of pulsatile pressure and flow into the cerebral circulation[5,9] and may thus play an important role in affecting NVC.[9,10] Indeed recent cross-sectional studies note strong associations between central artery stiffness, hemodynamic pulsatility, cerebral perfusion, cerebrovascular damage (i.e., white matter hyperintensities, β-amyloid plaque deposition), and cognitive dysfunction.[2–9,11–19] Ultimately, this hemodynamic sequela may have implications for future risk of dementia and Alzheimer's disease.[20]

Arterial stiffness can be altered acutely by any factor that affects vascular function (i.e., vascular smooth muscle tone). Mental stress with concomitant cognitive engagement and sympathoexcitation is known to increase blood pressure.[21] In theory, the pressor response would affect vessel wall load bearing by transferring stress from elastin fibers to stiffer collagen fibers. Sympathoexcitation may also increase vascular smooth muscle tone, functionally stiffening the vessel.[22] As such, an inappropriate hemodynamic and vascular response to cognitive challenge may have a detrimental effect on NVC; acute increases in arterial stiffness may increase cerebrovascular pulsatility and reduce cognitive performance. Cross-sectional studies note that higher hemodynamic reactivity to cognitive challenge is associated with carotid intima-media thickness,[23] cerebrovascular damage, and cognitive decline.[24–26]

Changes in arterial stiffness *during* cognitive engagement have not been fully elucidated, in part due to inherent constraints of research methodology. Many cognitive tasks include

Correspondence: Kevin S. Heffernan (ksheffer@syr.edu).

Initially submitted July 9, 2014; date of first revision July 8, 2014; accepted for publication September 10, 2014; online publication November 10, 2014.

[1]Department of Exercise Science, Syracuse University, Syracuse, New York, USA; [2]Cardiovascular Engineering, Inc., Norwood, Massachusetts, USA; [3]Department of Psychology, Syracuse University, Syracuse, New York, USA; [4]Department of Public Health, Syracuse University, Syracuse, New York, USA.

a verbal component. Acoustic interference and movement artifact associated with talking severely compromises measures of blood pressure, blood flow, and arterial stiffness taken at the carotid artery. Our lab recently developed a technique that enables the simultaneous assessment of carotid blood pressure, carotid and cerebral blood flow, and carotid artery stiffness during cognitive engagement. Participants remain in the supine position and there is no verbal component. Using this highly novel approach, the purpose of this pilot study was to examine carotid artery stiffness and cerebrovascular hemodynamic pulsatility during cognitive engagement in healthy adults. A secondary purpose was to examine the reproducibility of this approach to elicit consistent changes in central hemodynamics during cognitive perturbation.

METHODS

Twenty-seven healthy adults between the ages of 18 and 58 were recruited to participate in this study. Exclusion criteria were history of smoking, stroke, hypertension, diabetes mellitus, hyperlipidemia, obesity (body mass index (BMI) ≥ 35 kg/m^2), pulmonary disease, renal disease, neurological disease, recent head trauma resulting in concussion or loss of consciousness, depressive symptom score > 18 signifying high depressive symptomology, Montreal Cognitive Assessment score < 24 signifying cognitive impairment, severe arrhythmia, peripheral artery disease, use of vasoactive medications of any kind, and color blindness. This study was approved by the Institutional Review Board of Syracuse University. All participants provided written informed consent.

Study design

All participants reported to the Human Performance Laboratory on two separate occasions for screening (visit 1) and vascular hemodynamic assessment (visit 2). A subset of participants (n = 10) returned a third day for additional vascular and hemodynamic assessment that included a time control condition (visit 3) described below. The initial visit 1 was carried out in the early morning (06:00–09:00) following an overnight fast. Following consent, participants completed a health history questionnaire, International Physical Activity Questionnaire (IPAQ), visual acuity test, Ishihara color-blindness test, body composition assessment (air displacement plethysmography, BodPod; COSMED, Concord, CA), urinalysis (Clinitek Status+ Analyzer, Siemans, IL), fasting glucose and lipid assessment via finger stick (Cholestech LDX, Alere, Waltham, MA), depressive symptomology appraisal via the Center for Epidemiologic Studies Depression Scale (CES-D) and a global cognitive appraisal using the Montreal Cognitive Assessment (MOCA), trails A, and trails B. The difference between trials B performance time and trails A performance time (trails B–trails A) was taken as a measure of executive function and vascular brain senescence.[3]

Immediately following the screening visit 1, participants were outfitted with an accelerometer (Actigraph Model 7164 Manufacturing Technology, FL) on the right hip and instructed to wear the device during all waking hours (at least 10 hours) for 5 consecutive days (including at least 1 weekend day), except during swimming or bathing. The monitors were set at 60-second epochs and total movement counts were calculated for each participant based on data from 3 valid days (defined as >10 hours of wear with <1 hours of no accelerometer data (i.e., continuous zeros).

Participants reported back to the Human Performance Lab approximately 1 week after screening for vascular and hemodynamic data acquisition (visit 2). Participants returned the accelerometer during this visit. Vascular hemodynamic testing was conducted at a consistent time of day (13:00–15:00) in a quiet, dimly lit, temperature-controlled laboratory. Participants were instructed to fast for a minimum of 4 hours and avoid vigorous exercise and consumption of caffeine or alcohol for a minimum of 12 hours prior to testing. Baseline measures of blood pressure (brachial and carotid), blood flow (carotid and cerebral), arterial stiffness, and a salivary cortisol were obtained following a 15-minute supine rest. All hemodynamic variables were then simultaneously measured (in triplicate) during two separate 4 minutes blocks of a cognitive perturbation (incongruent Stroop task); 4 minutes of recovery time was afforded between incongruent Stroop trials and no vascular measures occurred during this recovery epoch (quiet rest).

A third visit was conducted in a subset of participants (n = 10) and occurred within 1 week of visit 2. Visit 3 was designed to recapitulate visit 2 with the exception that one incongruent Stroop task was replaced with a neutral time control condition. Participants reported to the lab in a similar fasting state as described above. Following a 15-minute supine rest period and baseline vascular hemodynamic measures (collected in the same fashion as during visit 2), participants underwent a "time control" condition which consisted of viewing a black screen for 4 minutes. This was then followed by a 4-minute recovery period and finally a 4-minute incongruent Stroop task. All hemodynamic variables were simultaneously measured (in triplicate) during the black screen condition and the incongruent Stroop task. No vascular measures occurred during the recovery epoch (quiet rest).

Brachial blood pressure

Systolic blood pressure (SBP), diastolic brachial blood pressure (DBP), and mean arterial pressure (MAP) was measured on the left arm using a validated, automated oscillometric cuff (EW3109, Panasonic Electric Works, Secaucus, NJ).

Carotid blood pressure

Pressure waveforms were obtained in the right carotid artery using applanation tonometry from a 10-second epoch (SphygmoCor, AtCor Medical, Sydney, Australia). Carotid pressure waveforms were ensemble averaged to a single waveform and calibrated to brachial MAP and DBP. Pulse pressure (PP) was calculated as SBP minus DBP.

Carotid blood flow and arterial stiffness

Images of the left common carotid artery (CCA) were obtained using Doppler ultrasound (ProSound α7, Aloka,

Tokyo, Japan) and 7.5–10.0 MHz linear-array probe. Images were acquired 5–10 mm below the carotid bulb. The distance from the near wall to far wall lumen–intima interface was continuously traced using eTracking software and used to determine CCA diameters. Flow velocity waveforms were measured using range gated spectral Doppler signals averaged along the Doppler beam. An insonation angle ≤ 60° was maintained for all measures and sample volume was manually adjusted to encompass the entire vessel. CCA PI was calculated as: $(V_s - V_d)/V_m$, where V_s is the peak systolic velocity, V_d diastolic velocity, and V_m the mean velocity. CCA volume flow rate per minute was calculated as π × (mean radius)^2 × V_m × 60. CCA mean shear rate was calculated as 4 × (V_m/mean diameter). Arterial stiffness measures included beta stiffness index (β) and Peterson's pressure–strain elastic modulus (Ep):

$$\beta = \ln(P_{Max}/P_{Min})/[(D_{Max} - D_{Min})/D_{Min}]$$
$$\mathrm{Ep} = (P_{Max} - P_{Min})/[(D_{Max} - D_{Min})/D_{Min}]$$

where P and D correspond to pressure and diameter, respectively, and Max and Min refer to maximum (systolic) and minimum (diastolic) values during the cardiac cycle. Augmentation index (AIx) was calculated as augmented pressure (i.e., the difference between the early [P_1] and late [P_2] systolic peaks of both the carotid pressure and distension waveforms) relative to the total PP expressed as a percentage (augmented pressure/PP × 100) and taken as a proxy of global wave reflections.

Cerebral blood flow velocity

Middle cerebral artery (MCA) blood flow velocity was assessed using a 2-MHz transcranial Doppler (TCD) ultrasound probe (DWL Doppler Box-X, Compumedics, Germany) applied to the left temporal window. Mean cerebral blood flow velocity (CBFv) and PI were measured at insonation depths of 45–60 mm. MCA PI was calculated via an automated waveform tracking function using the same equation described for CCA PI.

Cognitive challenge protocol

A modified incongruent Stroop color-word interference task was used to elicit changes in central hemodynamics. The Stroop test is one of the most widely utilized neurocognitive challenges in the field of psychophysiology used to elicit reproducible and reliable changes in neural substrates of executive function.[27–30] Participants remained in the supine position. A specialized wall mount was used to horizontally suspend a 42-inch flat screen television over the participant. The television was approximately 40-inches above the participant as per manufacturer suggestions. Font was focally displayed on a black background at a font size of 3-cm which is comparable to the size of font displayed on a standard 17-inch computer monitor (1.5 cm) with participants sitting 2.5 feet from the monitor. The television was interfaced with a laptop (Dell) and remote response clicker that ran a customized incongruent Stroop protocol (E-Prime, Psychology Software Tools, Sharpsburg, PA).

In single trials, participants were prompted to identify the color of a target word displayed centrally by selecting one of four identifier words appearing in one row on the bottom of the screen using the remote response clicker. The identifier words (1–4) corresponded to separate buttons (1–4) on the response clicker located on the exam table at the participant's side. Buttons 1–4 corresponded to individual digits (index finger–pinky finger). For this incongruent Stroop paradigm, the target word appeared in a color incongruent with the target word (i.e., the word "Red" presented in blue font). Moreover, all identifier words also appeared in colors incongruent with the colors that the identifier words identified. Two 4-minute blocks were presented to participants with a 4-minute rest period afforded between trials. This time block was selected based on previous studies that note prominent changes in BP and HR to occur within this allotted time.[30] Longer time blocks may affect participant valence and cause mental fatigue, resulting in progressive tapering of cardiovascular reactivity. This time block was also sufficient in length to afford ample time to collect all hemodynamic variables in triplicate (determined during pilot testing).

In an attempt to elicit comparable hemodynamic reactivity between trials while controlling for potential individual differences in task performance, each participant's accuracy at target word identification was titrated to ~50% by manipulating presentation times in 400 ms steps (range 300 ms–5 s). For example, three consecutive correct responses prompted shorter response time windows, increasing task difficulty; less accurate performance lengthened response time windows, reducing task difficulty. This protocol has previously been used successfully to assess cardiovascular reactivity and neural activation during functional magnetic resonance imaging.[30] Salivary cortisol was analyzed from 25 µl of sample using a highly sensitive salivary enzyme immunoassay (Salimetrics, State College, PA) at baseline and 30-minute following the Stroop task as a marker of generalized stress responsiveness.

STATISTICAL ANALYSES

All data is reported as mean ± standard error of the mean and statistical significance was established *a priori* as $P < 0.05$. Normality of distribution for variables was assessed qualitatively using histograms and Q–Q plots as well as quantitatively using the Shapiro–Wilk test. Degree of consistency across testing trials between individuals was evaluated by the intraclass correlation coefficient (ICC). To further examine reliability of measures obtained during Stroop task, we computed the coefficient of variation (CV, standard deviation/mean) for all hemodynamic variables. Given high ICCs and low CVs, values were collapsed (averaged) across trials and used for subsequent analyses. We examined differences in central hemodynamic parameters using *a priori* contrasts. Homogeneity of variance was assessed with the Levene statistic, and appropriate comparisons were made (equal variance assumed or not assumed) based on results. Absolute change (Δ) scores for each hemodynamic parameter were tabulated as the average value obtained during Stroop—Baseline. A one sample *t*-test was used to compare the change score to a value of zero (i.e., was the change significantly different than zero). We examined associations of

interest between reactivity scores using Pearson's correlation coefficients.

Exploratory analyses examined the effect of sex and age on the hemodynamic response to Stroop. A 2 × 2 analysis of variance (ANOVA) with repeated measures was used to assess sex differences in the response to Stroop: 2 groups (men vs. women) by 2 time points (baseline vs. Stroop). K-means cluster analysis was used to divide participants into 2 groups, stratified by age: younger and older. A 2 × 2 ANOVA with repeated measures was then used to explore age-group differences in the response to Stroop: 2 groups (younger vs. older) by 2 time points (baseline vs. Stroop). Partial correlations were used to examine associations of interest adjusting for age. All statistical analyses were executed using Statistical Package for the Social Sciences (SPSS, version 21, IBM, Chicago, IL).

RESULTS

Participant characteristics are presented in Table 1. Based on aforementioned inclusion and exclusion criteria, two participants were excluded (CES-D score 34 and MOCA score 21, $n = 1$; BMI 38 kg/m^2, $n = 1$). Brachial SBP, DBP, PP, and MAP increased during Stroop (Table 2, $P < 0.05$). While carotid SBP increased during Stroop ($P < 0.05$), carotid PP was unchanged ($P > 0.05$). The increase in carotid SBP was smaller than the increase in brachial SBP (Δ5 mm Hg vs. Δ 9 mm Hg, $P < 0.05$). HR increased during Stroop ($P < 0.05$). AIx, AP, CCA mean diameter, and CCA flow volume increased during Stroop (Table 3, $P < 0.05$). CCA PI and P_1 were reduced during Stroop ($P < 0.05$). Arterial stiffness (β-stiffness and Ep) and CCA shear rate were unchanged ($P > 0.05$). MCA Vmean increased during Stroop (Table 3, $P < 0.05$). MCA PI remain unchanged ($P > 0.05$). Salivary cortisol was unchanged (0.165 ± 0.016 vs. 0.147 ± 0.015 μg/dl, $P > 0.05$).

A correlation matrix is presented in Table 4. Adjusting for age had negligible effects on the associations between Stroop % Correct and: Δ CCA PI ($r = -0.38$, $P = 0.04$), Δ CCA PP ($r = -0.36$, $P = 0.04$) or Δ CCA diameter ($r = 0.35$, $P = 0.06$). Additional correlations of interest are as follows. Trails B–trails A was associated with Δ MCA PI ($r = 0.41$, $P = 0.03$), Δ CCA PI ($r = 0.49$, $P = 0.01$), Δ CCA PP ($r = 0.32$, $P = 0.061$), and associated negatively with Δ CCA diameter ($r = -0.56$, $P = 0.03$). Finally, trails B–trails A was associated positively with mean reaction time for correct choices during Stroop tasks ($r = 0.34$, $P = 0.04$) and associated negatively with the mean % correct for Stroop performances ($r = -0.48$, $P = 0.02$). After adjusting for age with partial correlation, the association between trails B–trails A performance and Δ MCA PI was attenuated ($r = 0.29$, $P = 0.09$) as was Δ CCA PP ($r = 0.27$, $P = 0.10$). Adjusting for age had no significant effect on the association between trails B–trails A performance and Δ CCA PI ($r = 0.48$, $P = 0.01$) or Δ CCA diameter ($r = -0.40$, $P = 0.04$).

Overall ICCs were high (0.90–0.99) and CVs low (0.88–8.53) suggesting good reproducibility for all hemodynamic measures (See Supplementary Table S1). There was a slight but significant

Table 1. Participant descriptive characteristics

Variable	Mean ± SEM
Age, years	39 ± 3
Women, *n*	13
Body mass index, kg/m^2	24 ± 1
Body fat, %	23 ± 2
Total cholesterol, mg/dl	186 ± 7
HDL cholesterol, mg/dl	69 ± 4
Glucose, mg/dl	88 ± 2
Urinary creatinine, mg/dl	110 ± 18
MOCA, score	27 ± 1
Trails A, s	20 ± 1
Trails B, s	42 ± 3
CES-D, score	9 ± 1
Family history CVD, *n*	3
IPAQ, MET min/week	4,424 ± 560
Accelerometer counts ($n = 21$)	$1.37 \times 10^5 \pm 1.38 \times 10^4$

Abbreviations: CES-D, Center for Epidemiologic Studies Depression scale; CVD, cardiovascular disease; HDL, high-density lipoprotein; IPAQ, International Physical Activity Questionnaire; MOCA, Montreal Cognitive Assessment.

Table 2. Blood pressure and heart rate at rest and during Stroop task

Variable	Baseline	Stroop	Δ Score	*t* (*df*)	*P* value
Brachial SBP, mm Hg	117 ± 2	126 ± 2[a]	9 ± 1[b]	6.1 (24)	<0.001
Brachial DBP, mm Hg	74 ± 1	79 ± 1[a]	5 ± 1[b]	6.5 (24)	<0.001
Brachial PP, mm Hg	43 ± 1	46 ± 1[a]	3 ± 1[b]	3.5 (24)	0.002
Brachial MAP, mm Hg	88 ± 1	95 ± 1[a]	7 ± 1[b]	6.7 (24)	<0.001
Carotid SBP, mm Hg	110 ± 2	114 ± 2[a]	4 ± 1[b]	3.9 (24)	0.001
Carotid PP, mm Hg	36 ± 1	35 ± 1	−1 ± 1	1.0 (24)	0.324
Heart Rate, bpm	60 ± 2	72 ± 3[a]	12 ± 2[b]	6.4 (24)	<0.001

Abbreviations: DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure.
[a]Significantly different from baseline ($P < 0.05$).
[b]Significantly different from zero ($P < 0.05$).

Table 3. Carotid and cerebral hemodynamics during Stroop task

Variable	Baseline	Stroop	Δ Score	*t* (*df*)	*P* value
CCA β-Stiffness, aU	4.4 ± 0.4	4.2 ± 0.3	−0.2 ± 0.2	−1.3 (*23*)	0.224
CCA Ep, kPa	54.5 ± 5.5	53.8 ± 4.9	−1.1 ± 2.5	−0.4 (*23*)	0.670
CCA pressure AIx, %	1 ± 4	13 ± 4[a]	12 ± 3[b]	4.6 (*24*)	<0.001
CCA distension AIx, %	4 ± 2	8 ± 2[a]	4 ± 1[b]	4.0 (*23*)	0.001
CCA P_1, mm Hg	34 ± 8	29 ± 7[a]	−5 ± 1[b]	5.5 (*24*)	0.001
CCA AP, ms	1 ± 6	4 ± 6[a]	3 ± 5[b]	3.4 (*24*)	0.102
CCA mean diameter, mm	5.62 ± 0.13	5.74 ± 0.13[a]	0.09 ± 0.03[b]	2.5 (*22*)	0.010
CCA Vmean, cm/s	38.9 ± 1.2	39.6 ± 1.4	0.8 ± 0.9	0.8 (*24*)	0.423
CCA shear rate, /s	28.6 ± 1.3	28.2 ± 1.6	0.4 ± 0.9	0.4 (*22*)	0.680
CCA flow, ml/min	575.5 ± 21.1	607.4 ± 19.6[a]	31.8 ± 17.1	1.9 (*22*)	0.039
CCA PI	1.75 ± 0.06	1.57 ± 0.06[a]	−0.18 ± 0.08[b]	2.3 (*24*)	0.016
MCA Vmean, cm/sec	49.4 ± 2.3	52.5 ± 2.5[a]	3.1 ± 1.5[b]	1.9 (*24*)	0.035
MCA PI	0.75 ± 0.02	0.75 ± 0.01	0.003 ± 0.01	0.2 (*24*)	0.841

Abbreviations: AIx, augmentation index; AP, augmented pressure; CCA, common carotid artery; Ep, elastic modulus;P_1, pressure at the inflection point; PI, pulsatility index MCA, middle cerebral artery; Vmean, mean velocity.
[a]Significantly different from baseline ($P < 0.05$).
[b]Significantly different than zero ($P < 0.05$).

Table 4. Correlation matrix of hemodynamic reactivity scores with Stroop performance ($n = 25$ participants)

	Stroop %Correct	Stroop RT	Δ CCA PI	Δ MCA PI	Δ CCA PP	Δ CCA P_1	Δ CCA AP
Stroop RT	**−0.72***						
Δ CCA PI	**−0.34***	**0.35***					
Δ MCA PI	−0.21	−0.03	**0.70***				
Δ CCA PP	**−0.45***	0.23	**0.34***	0.19			
Δ CCA P_1	−0.24	0.24	**0.39***	0.11	**0.64***		
Δ CCA AP	−0.07	0.08	−0.15	−0.07	0.17	−0.26	
Δ CCA diameter	**0.35***	**−0.48***	**−0.57***	**−0.42***	**−0.41***	**−0.55***	0.04

Abbreviations: AP, augmented pressure; CCA, common carotid artery; MCA, middle cerebral artery; PP, pulse pressure; P_1, pressure at the inflection point;PI, pulsatility index; RT, reaction time.
Significant univariate association (*$P < 0.05$).

increase in brachial SBP, carotid SBP and MAP during the time control condition ($P < 0.05$; See Supplementary Table S2). No other parameter changed during the time control (See Supplementary Table S2, $P > 0.05$).

Demographic data stratified by age and sex are presented in Supplementary Table S3. There were no sex differences in the vascular hemodynamic response to Stroop (See Supplementary Table S4, $P > 0.05$ for all). There were no differences in the vascular hemodynamic response to Stroop when comparing younger adults to older adults within our group of participants (See Supplementary Table S5, $P > 0.05$ for all).

DISCUSSION

Changes in cerebral blood flow are tightly coupled to level of cognitive perturbation[31] such that cerebral flow increases with increasing neuronal metabolic demand.[32,33] In this pilot study, during increased cognitive demand associated with an incongruent color-word interference task, there were increases in both CCA mean flow and MCA flow velocity (i.e., a surrogate for increased inflow volume) consistent with the premise of NVC. The novel findings of the present pilot study were as follows: (i) increases in flow volume occurred concomitant with reductions in CCA flow pulsatility and no change in MCA flow pulsatility; (ii) although there was an increase in peripheral pressure pulsatility, there was no change in CCA pressure pulsatility; (iii) there was no change in CCA stiffness during cognitive engagement. Our preliminary findings indicate that transmission of both pressure and flow pulsatility into the brain is moderated during cognitive engagement despite an increase in mean flow volume. Changes in CCA pulsatility occur without changes in CCA stiffness *per se* suggesting that mechanisms other than

the dynamic elastic properties of the CCA buffer central hemodynamic pulsatility during cognitive challenge.

Overall, our preliminary findings did not support our hypothesis as cognitive perturbation (Stroop task) did not alter carotid artery stiffness. The response to cognitive perturbation in healthy adults may be vascular bed specific. Muscular arteries such as the radial artery may increase in stiffness during mental stress[22] while elastic arteries such as the CCA do not change. This may ensure optimal transfer of flow into the cerebral circulation. CCA PI was reduced with no change in MCA PI during cognitive engagement. Eighty percent of common carotid blood flow feeds the internal carotid artery with subsequent branching giving rise to the MCA which supplies 80% of the blood supply to the brain.[34] We recently reported an association between resting carotid PI and MCA PI in healthy adults.[35] In the current pilot study, we noted that change in carotid PI during Stroop was associated with MCA PI suggesting that for ideal NVC and preservation of the cerebral hemodynamic milieu, a reduction in CCA flow pulsatility may be necessary.

We noted significant increases in CCA diameter during cognitive engagement and this is consistent with previous findings.[36] Increases in common carotid vasodilatory capacity during cognitive challenge contribute to increases in carotid and MCA flow.[36] Changes in CCA diameter during cognitive engagement reflect carotid endothelial function[36] and may be due to β-adrenergically mediated vasodilation, which is dependent on nitric oxide mediated smooth muscle relaxation.[37,38] Endothelial dysfunction affects blood flow into the cerebral vascular bed,[39] preventing efficient oxygen extraction at the capillary level[40] and ultimately reducing neurocognitive capacity. Inhibition of nitric oxide has been shown to reduce cerebral blood flow[41] without affecting carotid artery stiffness.[42] There were negative associations between carotid dilation and cerebrovascular pressure and flow pulsatility in this study. Therefore, carotid vasodilation during cognitive challenge may help to favorably buffer cerebrovascular pulsatility independent of modulation of carotid stiffness, contributing to optimal NVC.

There was a divergent effect of cognitive perturbation on peripheral (i.e., brachial) vs. central (i.e., carotid) *pressure* pulsatility. While pulse pressure increased in the peripheral vascular bed, there was no change in the central vascular bed. This occurred concomitant with a paradoxical increase in carotid AIx. The AIx quantifies the interaction between forward and reflected travelling pressure waves in the systemic and regional circulation. An increase in AIx is often attributed to an increase in pressure from global wave reflections but may also occur with a fall in the forward travelling pressure wave. In this pilot study, there was a significant increase in augmented pressure as well as a reduction in pressure at the inflection point (P_1, a proxy of forward wave pressure) during cognitive engagement. Increases in augmented pressure from wave reflections may be due to increases in peripheral vascular resistance and stiffness that are known to occur during mental stress.[22] The fall in forward wave pressure was likely due to the significant carotid dilation. It is also interesting to note that reductions in pressure at the inflection point were also associated with favorable changes in carotid pressure and flow pulsatility. These preliminary findings allude to the importance of impedance properties of the extra-cranial vascular bed as an important moderator of hemodynamic pulsatility entry into the cerebral circulation during cognitive engagement and suggest that the CCA may not simply be a passive conduit during NVC.[43]

High cerebrovascular hemodynamic pulsatility is associated with reduced cognitive function.[2] Moreover, interventions that reduce cerebrovascular pulsatility have been shown to improve cognitive function.[44] In the present pilot study, there was an association between change in CCA PI and PP and performance during Stroop task (percentage of correct answers and the reaction time for correct answers), a measure of executive function.[27–30] Change in CCA diameter, CCA PI and MCA PI assessed during Stroop task were also each inversely associated with trails B–trails A performance, an additional measure of executive function and vascular brain aging.[3] These preliminary findings support the possibility that CCA dilation and reductions in cerebrovascular pulsatility during cognitive challenge are associated with optimal NVC and improved cognitive performance.[45]

We did not find a significant effect of age on our overall findings. Age is one of the strongest, most consistent determinants of central artery stiffness[46] which in turn plays an important role in age-related differences in cerebral hemodynamics.[12] The lack of effect of age on changes in central hemodynamics during Stroop and subsequent associations with cognitive performance noted herein is likely due to small sample size (i.e., inability to detect an effect) coupled with lack of inclusion of older adults over 60 years of age (when changes in large artery stiffness and impedance matching may more profoundly impact hemodynamic pulsatility transmission into the brain). Our population consisted of fairly healthy adults. Indeed when statistically separating our participants into a younger group and an older group, results revealed no group differences in resting BP, lipids, fasting glucose, or physical activity levels, established arbiters of arterial stiffness, cerebral reactivity and cognitive performance.[47–49] In the absence of CVD risk factors, several studies have shown maintenance of NVC with advancing age.[36,50–54] Our findings also noted no sex differences in NVC. Carotid stiffness and carotid flow may change across the menstrual cycle,[55,56] as does mental stress mediated hemodynamic reactivity[57] and cognitive function[58] arguing for possible hormonal modulation of NVC. Interestingly, acute administration of estrogen has been shown to improve arterial compliance and endothelial function,[59] attenuate hemodynamic reactivity to cognitive perturbation,[60] and improve cerebral vasoreactivity *in vitro*.[61] Additional studies are needed to specifically explore sex- and age-associated changes in large artery stiffness and cerebrovascular hemodynamic pulsatility during cognitive perturbation.

Limitations to this pilot study should be acknowledged. TCD does not measure CBF directly. It is often argued that the diameter of the MCA does not change during various perturbations such as cognitive engagement suggesting that changes in CBF velocity may be used as a suitable proxy of cerebral volume flow changes.[62] It is possible that noted hemodynamic changes are not due to cognitive engagement *per se* but may be a generalized mental stress response. Indeed there was a slight but significant increase in SBP during the

time control condition. It should be noted that the increases in SBP were greater with Stroop than time control suggesting that hemodynamic change was likely due to a combination of mental stress and cognitive engagement. And the lack of change in salivary cortisol in our participants with perturbation would suggest that this protocol was not particularly stressful. This pilot study used healthy participants across a wide age range, but was not specifically designed to examine the impact of sex/gender or aging per se on hemodynamic pulsatility during cognitive engagement. Given that "typical" vascular and hemodynamic responses *during* cognitive engagement (as assessed using this protocol) are largely unknown, we designed this pilot study to first establish the "typical" response to cognitive perturbation in the absence of disease. We also sought to establish reproducibility of our novel method in eliciting consistent vascular and hemodynamic responses.

In conclusion, carotid pressure and flow pulsatility are buffered during cognitive engagement in healthy adults and this may be an important aspect of optimal NVC. Carotid artery stiffness does not change during cognitive engagement suggesting that factors other than the dynamic elastic properties of the CCA moderate changes in cerebrovascular hemodynamic pulsatility during NVC. Future research is needed to explore potential factors affecting central hemodynamic pulsatility during cognitive engagement such as carotid vasoreactivity and pressure from wave reflections and whether favorably modifying these factors improves NVC and cognitive function.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

ACKNOWLEDGMENTS

The Dairy Research Institute (Dairy Management Inc) Grant1154 (Principle Investigator Heffernan), NIH NIA P30 AG0344645 05 (Project Director Kevin Heffernan, Principle Investigator Douglas Wolf), NIH NIEHS R01 ES023252 02 (Principal Investigator Brooks Gump).

DISCLOSURE

We have no conflicts of interest to disclose.

REFERENCES

1. Novak V, Hajjar I. The relationship between blood pressure and cognitive function. *Nat Rev Cardiol* 2010; 7:686–698.
2. Mitchell GF, van Buchem MA, Sigurdsson S, Gotal JD, Jonsdottir MK, Kjartansson Ó, Garcia M, Aspelund T, Harris TB, Gudnason V, Launer LJ. Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility–Reykjavik study. *Brain* 2011; 134:3398–3407.
3. Tsao CW, Seshadri S, Beiser AS, Westwood AJ, Decarli C, Au R, Himali JJ, Hamburg NM, Vita JA, Levy D, Larson MG, Benjamin EJ, Wolf PA, Vasan RS, Mitchell GF. Relations of arterial stiffness and endothelial function to brain aging in the community. *Neurology* 2013; 81:984–991.
4. Jolly TA, Bateman GA, Levi CR, Parsons MW, Michie PT, Karayanidis F. Early detection of microstructural white matter changes associated with arterial pulsatility. *Front Hum Neurosci* 2013; 7:782.
5. Webb AJ, Simoni M, Mazzucco S, Kuker W, Schulz U, Rothwell PM. Increased cerebral arterial pulsatility in patients with leukoaraiosis: arterial stiffness enhances transmission of aortic pulsatility. *Stroke* 2012; 43:2631–2636.
6. Wåhlin A, Ambarki K, Birgander R, Malm J, Eklund A. Intracranial pulsatility is associated with regional brain volume in elderly individuals. *Neurobiol Aging* 2014; 35:365–372.
7. Xiong YY, Mok V, Wong A, Leung T, Chen XY, Chu WC, Soo Y, Fu JH, Ding D, Hong Z, Wong KS. Evaluation of age-related white matter changes using transcranial Doppler ultrasonography. *J Neuroimag* 2013; 23:53–57.
8. Hughes TM, Kuller LH, Barinas-Mitchell EJ, McDade EM, Klunk WE, Cohen AD, Mathis CA, Dekosky ST, Price JC, Lopez OL. Arterial stiffness and β-amyloid progression in nondemented elderly adults. *JAMA Neurol* 2014; 71:562–568.
9. Kwater A, Gasowski J, Gryglewska B, Wizner B, Grodzicki T. Is blood flow in the middle cerebral artery determined by systemic arterial stiffness? *Blood Press* 2009; 18:130–134.
10. Xu TY, Staessen JA, Wei FF, Xu J, Li FH, Fan WX, Gao PJ, Wang JG, Li Y. Blood flow pattern in the middle cerebral artery in relation to indices of arterial stiffness in the systemic circulation. *Am J Hypertens* 2012; 25:319–324.
11. Tarumi T, Shah F, Tanaka H, Haley AP. Association between central elastic artery stiffness and cerebral perfusion in deep subcortical gray and white matter. *Am J Hypertens* 2011; 24:1108–1113.
12. Tarumi T, Ayaz Khan M, Liu J, Tseng BY, Tseng BM, Parker R, Riley J, Tinajero C, Zhang R. Cerebral hemodynamics in normal aging: central artery stiffness, wave reflection, and pressure pulsatility. *J Cereb Blood Flow Metab* 2014; 34:971–978.
13. Katulska K, Wykrętowicz M, Minczykowski A, Krauze T, Milewska A, Piskorski J, Marciniak R, Stajgis M, Wysocki H, Guzik P, Wykrętowicz A. Gray matter volume in relation to cardio-vascular stiffness. *J Neurol Sci* 2014; 343:100–104.
14. Aribisala BS, Morris Z, Eadie E, Thomas A, Gow A, Valdés Hernández MC, Royle NA, Bastin ME, Starr J, Deary IJ, Wardlaw JM. Blood pressure, internal carotid artery flow parameters, and age-related white matter hyperintensities. *Hypertension* 2014; 63:1011–1018.
15. Flück D, Beaudin AE, Steinback CD, Kumarpillai G, Shobha N, McCreary CR, Peca S, Smith EE, Poulin MJ. Effects of aging on the association between cerebrovascular responses to visual stimulation, hypercapnia and arterial stiffness. *Front Physiol* 2014; 5:49.
16. Xu TY, Staessen JA, Wei FF, Xu J, Li FH, Fan WX, Gao PJ, Wang JG, Li Y. Blood flow pattern in the middle cerebral artery in relation to indices of arterial stiffness in the systemic circulation. *Am J Hypertens* 2012; 25:319–324.
17. Brisset M, Boutouyrie P, Pico F, Zhu Y, Zureik M, Schilling S, Dufouil C, Mazoyer B, Laurent S, Tzourio C, Debette S. Large-vessel correlates of cerebral small-vessel disease. *Neurology* 2013; 80:662–669.
18. Poels MM, Zaccai K, Verwoert GC, Vernooij MW, Hofman A, van der Lugt A, Witteman JC, Breteler MM, Mattace-Raso FU, Ikram MA. Arterial stiffness and cerebral small vessel disease: the Rotterdam Scan Study. *Stroke* 2012; 43:2637–2642.
19. Rosano C, Watson N, Chang Y, Newman AB, Aizenstein HJ, Du Y, Venkatraman V, Harris TB, Barinas-Mitchell E, Sutton-Tyrrell K. Aortic pulse wave velocity predicts focal white matter hyperintensities in a biracial cohort of older adults. *Hypertension* 2013; 61:160–165.
20. Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D, Petersen RC, Schneider JA, Tzourio C, Arnett DK, Bennett DA, Chui HC, Higashida RT, Lindquist R, Nilsson PM, Roman GC, Sellke FW, Seshadri S. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke* 2011; 42:2672–2713.

21. Vlachopoulos C, Kosmopoulou F, Alexopoulos N, Ioakeimidis N, Siasos G, Stefanadis C. Acute mental stress has a prolonged unfavorable effect on arterial stiffness and wave reflections. *Psychosom Med* 2006; 68:231–237.
22. Boutouyrie P, Lacolley P, Girerd X, Beck L, Safar M, Laurent S. Sympathetic activation decreases medium-sized arterial compliance in humans. *Am J Physiol* 1994; 267:H1368–H1376.
23. Spartano NL, Augustine JA, Lefferts WK, Gump BB, Heffernan KS. The relationship between carotid blood pressure reactivity to mental stress and carotid intima-media thickness. *Atherosclerosis* 2014; 236:227–229.
24. Brown JP, Sollers JJ 3rd, Thayer JF, Zonderman AB, Waldstein SR. Blood pressure reactivity and cognitive function in the Baltimore Longitudinal Study of Aging. *Health Psychol* 2009; 28:641–646.
25. Waldstein SR, Katzel LI. Stress-induced blood pressure reactivity and cognitive function. *Neurology* 2005; 64:1746–1749.
26. Waldstein SR, Siegel EL, Lefkowitz D, Maier KJ, Brown JR, Obuchowski AM, Katzel LI. Stress-induced blood pressure reactivity and silent cerebrovascular disease. *Stroke* 2004; 35:1294–1298.
27. Van der Elst W, Van Boxtel MP, Van Breukelen GJ, Jolles J. The Stroop color-word test: influence of age, sex, and education; and normative data for a large sample across the adult age range. *Assessment* 2006; 13:62–79.
28. Bucur B, Madden DJ. Effects of adult age and blood pressure on executive function and speed of processing. *Exp Aging Res* 2010; 36:153–168.
29. Mohtasib RS, Lumley G, Goodwin JA, Emsley HC, Sluming V, Parkes LM. Calibrated fMRI during a cognitive Stroop task reveals reduced metabolic response with increasing age. *Neuroimage* 2012; 59:1143–1151.
30. Sheu LK, Jennings JR, Gianaros PJ. Test-retest reliability of an fMRI paradigm for studies of cardiovascular reactivity. *Psychophysiology* 2012; 49:873–884.
31. Vingerhoets G, Stroobant N. Lateralization of cerebral blood flow velocity changes during cognitive tasks. A simultaneous bilateral transcranial Doppler study. *Stroke* 1999; 30:2152–2158.
32. Stroobant N, Vingerhoets G. Transcranial Doppler ultrasonography monitoring of cerebral hemodynamics during performance of cognitive tasks: a review. *Neuropsychol Rev* 2000; 10:213–231.
33. Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW, Haykowsky MJ, Bellapart J, Ogoh S, Smith KJ, Smirl JD, Day TA, Lucas SJ, Eller LK, Ainslie PN. Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *J Neurosci Methods* 2011; 196:221–237.
34. Farkas E, Luiten PG. Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog Neurobiol* 2001; 64:575–611.
35. Heffernan KS, Lefferts WK, Augustine JA. Hemodynamic correlates of late systolic flow velocity augmentation in the carotid artery. *Int J Hypertens* 2013; 2013:920605.
36. Naqvi TZ, Hyuhn HK. Cerebrovascular mental stress reactivity is impaired in hypertension. *Cardiovasc Ultrasound* 2009; 7:32.
37. Dietz NM, Rivera JM, Eggener SE, Fix RT, Warner DO, Joyner MJ. Nitric oxide contributes to the rise in forearm blood flow during mental stress in humans. *J Physiol* 1994; 480:361–368.
38. Halliwill JR, Lawler LA, Eickhoff TJ, Dietz NM, Nauss LA, Joyner MJ. Forearm sympathetic withdrawal and vasodilatation during mental stress in humans. *J Physiol* 1997; 504:211–220.
39. Boveris DL, Boveris A. Oxygen delivery to the tissues and mitochondrial respiration. *Front Biosci* 2007; 12:1014–1023.
40. Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, O'Farrell FM, Buchan AM, Lauritzen M, Attwell D. Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* 2014; 508:55–60.
41. White RP, Deane C, Vallance P, Markus HS. Nitric oxide synthase inhibition in humans reduces cerebral blood flow but not the hyperemic response to hypercapnia. *Stroke* 1998; 29:467–472.
42. Sugawara J, Saito Y, Maeda S, Yoshizawa M, Komine H, Nakamura M, Ajisaka R, Tanaka H. Lack of changes in carotid artery compliance with systemic nitric oxide synthase inhibition. *J Hum Hypertens* 2014; 28:494–499.
43. Willie CK, Tzeng YC, Fisher JA, Ainslie PN. Integrative regulation of human brain blood flow. *J Physiol* 2014; 592:841–859.
44. Heyer EJ, Mergeche JL, Connolly ES Jr. Middle cerebral artery pulsatility index and cognitive improvement after carotid endarterectomy for symptomatic stenosis. *J Neurosurg* 2014; 120:126–131.
45. Fu GX, Miao Y, Yan H, Zhong Y. Common carotid flow velocity is associated with cognition in older adults. *Can J Neurol Sci* 2012; 39:502–507.
46. Cecelja M, Chowienczyk P. Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension: a systematic review. *Hypertension* 2009; 54:1328–1336.
47. Suboc TB, Strath SJ, Dharmashankar K, Coulliard A, Miller N, Wang J, Tanner MJ, Widlansky ME. Relative importance of step count, intensity, and duration on physical activity's impact on vascular structure and function in previously sedentary older adults. *J Am Heart Assoc* 2014; 3:e000702.
48. Bailey DM, Marley CJ, Brugniaux JV, Hodson D, New KJ, Ogoh S, Ainslie PN. Elevated aerobic fitness sustained throughout the adult lifespan is associated with improved cerebral hemodynamics. *Stroke* 2013; 44:3235–3238.
49. Fabiani M, Gordon BA, Maclin EL, Pearson MA, Brumback-Peltz CR, Low KA, McAuley E, Sutton BP, Kramer AF, Gratton G. Neurovascular coupling in normal aging: a combined optical, ERP and fMRI study. *Neuroimage* 2014; 85:592–607.
50. Gröschel K, Terborg C, Schnaudigel S, Ringer T, Riecker A, Witte OW, Kastrup A. Effects of physiological aging and cerebrovascular risk factors on the hemodynamic response to brain activation: a functional transcranial Doppler study. *Eur J Neurol* 2007; 14:125–131.
51. Panczel G, Daffertshofer M, Ries S, Spiegel D, Hennerici M. Age and stimulus dependency of visually evoked cerebral blood flow responses. *Stroke* 1999; 30:619–623.
52. Gur RC, Gur RE, Obrist WD, Skolnick BE, Reivich M. Age and regional cerebral blood flow at rest and during cognitive activity. *Arch Gen Psychiatry* 1987; 44:617–621.
53. Rosengarten B, Aldinger C, Spiller A, Kaps M. Neurovascular coupling remains unaffected during normal aging. *J Neuroimag* 2003; 13:43–47.
54. Sorond FA, Schnyer DM, Serrador JM, Milberg WP, Lipsitz LA. Cerebral blood flow regulation during cognitive tasks: effects of healthy aging. *Cortex* 2008; 44:179–184.
55. Hayashi K, Miyachi M, Seno N, Takahashi K, Yamazaki K, Sugawara J, Yokoi T, Onodera S, Mesaki N. Variations in carotid arterial compliance during the menstrual cycle in young women. *Exp Physiol* 2006; 91:465–472.
56. Krejza J, Mariak Z, Huba M, Wolczynski S, Lewko J. Effect of endogenous estrogen on blood flow through carotid arteries. *Stroke* 2001; 32:30–36.
57. Gordon JL, Girdler SS. Mechanisms underlying hemodynamic and neuroendocrine stress reactivity at different phases of the menstrual cycle. *Psychophysiology* 2014; 51:309–318.
58. Hatta T, Nagaya K. Menstrual cycle phase effects on memory and Stroop task performance. *Arch Sex Behav* 2009; 38:821–827.
59. Clapauch R, Mecenas AS, Maranhão PA, Bouskela E. Early postmenopausal women with cardiovascular risk factors improve microvascular dysfunction after acute estradiol administration. *Menopause* 2012; 19:672–679.
60. Manhem K, Brandin L, Ghanoum B, Rosengren A, Gustafsson H. Acute effects of transdermal estrogen on hemodynamic and vascular reactivity in elderly postmenopausal healthy women. *J Hypertens* 2003; 21:387–394.
61. Lund CO, Nilas L, Dalsgaard T, Pedersen SH, Ottesen B. Acute effects of tibolone on cerebral vascular reactivity *in vitro*. *Climacteric* 2003; 6:228–237.
62. Sorond FA, Hollenberg NK, Panych LP, Fisher ND. Brain blood flow and velocity: correlations between magnetic resonance imaging and transcranial Doppler sonography. *J Ultrasound Med* 2010; 29:1017–1022.