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Cerebral vasomotor reactivity during hypo- and hypercapnia across the adult lifespan

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Abstract

Age is the strongest risk factor for cerebrovascular disease; however, age-related changes in cerebrovascular function are still not well understood. The objective of this study was to measure cerebral vasomotor reactivity (CVMR) during hypo- and hypercapnia across the adult lifespan. One hundred fifty-three healthy participants (21–80 years) underwent measurements of cerebral blood flow velocity (CBFV) via transcranial Doppler, mean arterial pressure (MAP) via plethysmograph, and end-tidal CO_2 ($EtCO_2$) via capnography during hyperventilation (hypocapnia) and a modified rebreathing protocol (hypercapnia). Cerebrovascular conductance (CVCi) and resistance (CVRi) indices were calculated from the ratios of CBFV and MAP. CVMRs were assessed by the slopes of CBFV and CVCi in response to changes in $EtCO_2$. The baseline CBFV and CVCi decreased and CVRi increased with age. Advanced age was associated with progressive declines in CVMR during hypocapnia indicating reduced cerebral vasoconstriction, but increases in CVMR during hypercapnia indicating increased vasodilation. A negative correlation between hypo- and hypercapnic CVMRs was observed across all subjects (CBFV%/ $EtCO_2$: $r = -0.419$, CVCi%/ $EtCO_2$: $r = -0.442$, $P < 0.0001$). Collectively, these findings suggest that aging is associated with decreases in CBFV, increases in cerebrovascular resistance, reduced vasoconstriction during hypocapnia, but increased vasodilatory responsiveness during hypercapnia.

Keywords

Aging, cerebral vasomotor reactivity, hypercapnia, hypocapnia, transcranial Doppler

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Introduction

Cerebral blood flow (CBF) is highly sensitive to partial pressure of arterial carbon dioxide ($PaCO_2$). Elevated $PaCO_2$ (hypercapnia) increases, whereas reduced $PaCO_2$ (hypocapnia) decreases CBF.[1] Cerebral vasomotor reactivity (CVMR) reflects the ability of cerebral blood vessels to dilate or constrict to regulate CBF in response to changes in $PaCO_2$.[1]

CVMR during hypercapnia can be assessed either by using stepwise increases in inspiratory air concentration of CO_2 or a rebreathing method in which a progressive increase in $PaCO_2$ is induced by having subjects rebreathe his/her own expired air.[2–4] Similar results of CVMR between the two methods have been reported previously.[5] Clinically, CVMR measured by the rebreathing method mimics the conditions during sleep apnea where $PaCO_2$ progressively builds up with breathing cessations.[6] On the other hand, CVMR during hypocapnia is commonly assessed by asking study participants to perform a short period of hyperventilation of room air to induce progressive decreases in $PaCO_2$.[5,7,8] Measurement of CVMR has been used

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widely in clinical and research assessment of cerebrovascular function.[9]

Age is the strongest risk factor for cerebrovascular disease[10]; however, age-related changes in CVMR are still not well understood.[8,11–13] In our previous study, we have observed that older adults have reduced cerebral blood flow velocity (CBFV) at rest, increased hypercapnic CVMR, and decreased hypocapnic CVMR when compared with young individuals.[13] Conversely, other studies have reported reduced or no change in hypercapnic CVMR with age.[8,11,12] These discrepancies may reflect the limitations of relatively small sample size, differences in methods used to assess CVMR, or both in these studies.

Measurement of CVMR is also influenced by the marked changes in systemic arterial blood pressure (BP) during hypo- or hypercapnia which are likely to be mediated by the central and peripheral chemoreceptor responses to changes in $PaCO_2$.[14] Recent evidence suggests that aging[15] and clinical conditions, such as cardiovascular disease[16,17] and sleep apnea,[18] may alter peripheral chemoreceptor sensitivity and BP responses to hypercapnia, thus underscoring the necessity to understand the systemic effects of BP responses on CVMR.

The purpose of this study was to extend our previous studies to determine the age-related differences in CVMR during hypo- and hypercapnia across the adult lifespan in a large sample of 153 healthy adults aged 21–80 years. On the basis of our previous study, we hypothesized that advanced age is associated with (1) reduced CBFV and increased cerebrovascular resistance at rest, and (2) reduced hypocapnic CVMR, but increased hypercapnic CVMR in healthy human subjects.

Materials and methods

Subjects

One hundred fifty-three healthy participants aged between 21 and 80 years were recruited through flyers and newspaper advertisements from the Dallas-Fort Worth metropolitan areas. Exclusion criteria were the presence of ischemic or structural heart disease screened by 12-lead ECG and echocardiography, office BP>140/90 mmHg confirmed by ambulatory BP monitoring, carotid artery atherosclerotic plaque or stenosis with > 50% occlusion imaged by ultrasound, diabetes mellitus screened by the presence of symptoms, use of antidiabetic drugs, or fasting blood glucose >126 mg/dL, body mass index (BMI) > 40 kg/m^2, current smoking or a history of smoking within the last two years, active alcohol or drug abuse, history of brain trauma, and the presence or history of cerebrovascular (e.g. stroke), neurological, psychiatric, or inflammatory disease, pregnancy or breast-feeding women. To minimize the confounding effects of aerobic exercise training on CVMR,[19,20] individuals who participate in structured aerobic exercise training program (i.e. moderate intensity, aerobic exercise training over the past two years) were also excluded.

This study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center and Texas Health Presbyterian Hospital Dallas, and was performed in accordance with the guidelines of the Declaration of Helsinki and Belmont Report. All subjects provided informed written consent prior to participation.

Instrumentation and data acquisition

CBFV was measured from the middle cerebral artery (MCA) using a 2-MHz transcranial Doppler (TCD) probe (Multi-Dop X2, Compumedics/DWL, Singen, Germany). The probe was securely attached by an individually created mold to fit the facial bone structure and keep the position and angle of the probe unchanged during the study.[21] End-tidal CO_2 ($EtCO_2$), an estimate of $PaCO_2$,[22] and breathing frequency were monitored using a capnography (Carpnograd, Novamatrix, Wallingford, CT, USA). Arterial blood oxygen saturation (SaO_2) was measured by a pulse oximeter (Biox 3700, Ohmeda Monitoring Systems, Boulder, CO, USA). Brachial BP was intermittently measured from the right upper arm using an electrosphygmomanometer (Suntech, Morrsville, NC, USA). Beat-to-beat mean arterial pressure (MAP) was continuously monitored from the middle finger of the left hand using a Finapres (Finapres Medical Systems, Amsterdam, The Netherlands). The finger pressure transducer was fixed at the heart level during the study. Heart rate (HR) was recorded via a three-lead ECG system (Hewlett-Packard, Palo Alto, CA, USA). All data were collected with a sampling frequency of 1000 Hz and stored in a computer for off-line analysis using a data acquisition and analysis software (Acknowledge, BIOPAC systems, Goleta, CA, USA).

Experimental procedures

All experiments were performed in an environmentally controlled laboratory with an ambient temperature of 22°C. Subjects refrained from high intensity exercise, caffeinated beverages, or alcohol >24 h before experiment. After subjects have rested in the supine position for >10 min, a nose clip was placed and subjects breathed through a mouthpiece with a Y valve, with one end connected to the mouthpiece, one end open to room air, and one end connected to a 5L rebreathing bag.[2,5,13]

First, baseline CBFV, HR, MAP, and $EtCO_2$ were recorded simultaneously for 3 min. During these measurements, subjects were instructed to breathe normally and avoid body movement or Valsalva maneuvers. After baseline data collection, subjects were coached by an investigator to perform voluntary hyperventilation for 20 s (1 breath/second) which induced a brief period of hypocapnia. Following hyperventilation, a > 5-min recovery period was provided until all hemodynamic variables returned to the baseline level.[5] Then, a modified rebreathing protocol was used to induce hypercapnia.[2] Briefly, at the end of a deep inspiration, the Y valve of the mouthpiece was switched to the rebreathing bag to induce a progressive increase in $EtCO_2$ for 3 min.[2] During rebreathing, a small amount of oxygen was added to the rebreathing bag based on each subject's basal metabolic rate (estimated using the Harris-Benedict formula) to maintain constant arterial blood oxygen saturation.[2] Intermittent brachial cuff BP was measured at baseline and during the rebreathing protocol to corroborate finger arterial pressure measurement. The rebreathing protocol was tolerated by all subjects.

Data analysis

Baseline data were obtained by averaging a 1-min steady-state data segment under resting condition before hyperventilation and rebreathing protocol. Cerebrovascular conductance index (CVCi) and resistance (CVRi) indices were calculated from the ratio of mean CBFV and MAP. CVCi was calculated to account for the effects of changes in MAP on CBFV during hypo- and hypercapnia.[2] The magnitude of absolute changes in CBFV, CVCi, MAP, HR, and $EtCO_2$ during hypo- and hypercapnia is presented as ΔCBFV, ΔCVCi, ΔMAP, ΔHR, and $\Delta EtCO_2$, respectively. The percentage changes in hypo- and hypercapnic ΔCBFV% and ΔCVCi% were calculated relative to their corresponding baseline values. The pulsatility index was calculated as systolic minus diastolic CBFV divided by mean CBFV.

Hypocapnic CVMR. During hyperventilation, maximal hemodynamic changes were calculated from the average of three breath cycles after the reduction of $EtCO_2$ reached nadir. Then, CVMR was calculated as the ratio of ΔCFBV% to $\Delta EtCO_2$ and ΔCVCi% to $\Delta EtCO_2$.[2] The breath-by-breath analysis was not performed during hypocapnia because rapid changes in $EtCO_2$ during hyperventilation may not accurately reflect the concurrent changes in $PaCO_2$. Thus, maximal reductions in CBFV% and CVCi% in response to the maximal reduction in $EtCO_2$ were used to assess CVMR when these variables reached the steady-state. Cardiovascular reactivity to changes in $EtCO_2$ was calculated as the ratio of ΔMAP to the corresponding changes in $EtCO_2$.

Hypercapnic CVMR. Baseline data for hypercapnia were obtained by averaging a 1-min steady-state data segment before rebreathing protocol. Breath-by-breath data were extracted for analysis during rebreathing.[2,23] Due to a deep inspiration performed when switching a Y valve, a brief reduction in both $EtCO_2$ and CBFV was observed at the beginning of rebreathing. Hence, to address the effect of hypercapnia, only those data of ΔCBFV% (>100%) and $\Delta EtCO_2$ (> baseline level) were included in analysis. Linear regression analysis of ΔCBFV% vs. $\Delta EtCO_2$ and ΔCVCi% vs. $\Delta EtCO_2$ was performed within each subject and then group averaged for statistical analysis. The slopes of these regression lines were used as the estimates of CVMR during hypercapnia.[2] Cardiovascular reactivity to $\Delta EtCO_2$ was assessed by the slope of linear regression between ΔMAP and $\Delta EtCO_2$. Of note, the rebreathing protocol used in the current study was shorter than the protocol used in our previous studies (5 minutes) and induced moderate increase in $EtCO_2$ and CBFV, thus providing the rationale for using the linear regression analysis.[2] The results of linear fitting were examined by the coefficient of determination (R^2). For data visualization, group-averaged bin plots of CBFV%, CVCi%, and MAP were created based on every 4 mmHg increase in $EtCO_2$ from the baseline (Figure 1).

Statistical analysis

The χ-square test was used to examine the group differences in categorical variables. Two-way analysis of variance (ANOVA) was used to test the main effects of age and sex as well as the interaction effect of age×sex. After observing no notable effects of sex or age×sex on hypo- and hypercapnic CVMR measures, we used one-way ANOVA to focus upon the age-related group differences. Furthermore, linear mixed model (LMM) analysis was conducted to examine the contributions of hypercapnic changes in $\Delta EtCO_2$ and ΔMAP to ΔCBFV% and also account for potential correlations between repeated measures using an unstructured covariance structure.[24] From the LMM analysis, the unstandardized regression coefficients for $EtCO_2$ and MAP were compared among the age groups using one-way ANOVA. The Bonferroni method was used to correct for multiple pairwise comparisons. Pearson's product-moment correlation analysis was used to examine the relation between hypo- and hypercapnic CVMRs. Data are presented as mean ± standard deviation. An α-level of 0.05 was set as the criterion for statistical significance. All statistical analyses were performed using SPSS 19.0 (SPSS, Chicago, IL, USA).

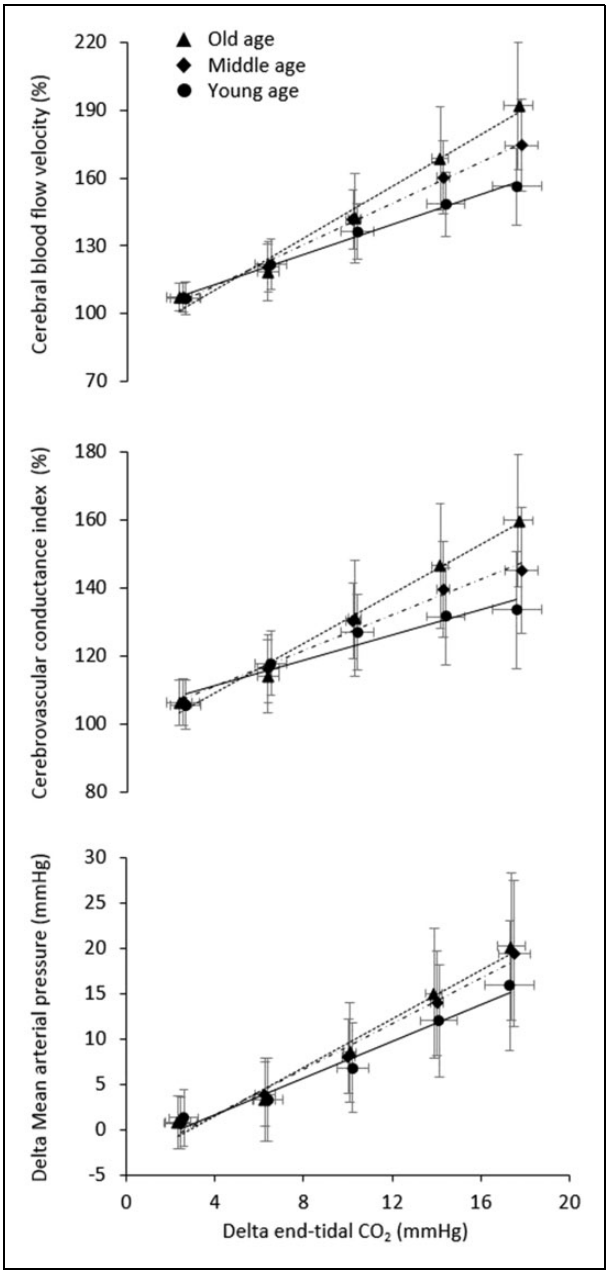

Figure 1. Group-averaged bin plots showing the cerebral blood flow velocity (CBFV, %), cerebrovascular conductance index (CVCi, %), mean arterial pressure (MAP, mmHg), and end-tidal CO_2 ($EtCO_2$, mmHg)) during rebreathing in young, middle-aged, and older adults. The error bars represent standard deviations. Each bin represents 4 mmHg change in $EtCO_2$.

Results

Baseline hemodynamics

Data from 36 subjects were excluded from analysis because of poor TCD, finger pressure, and/or $EtCO_2$ signals during hyperventilation and/or rebreathing. This resulted in the final sample of 117 subjects and their characteristics and baseline hemodynamic data are shown in Table 1. The men and women distributions were similar across the age groups. The brachial systolic BP increased with age, whereas diastolic BP peaked in middle age, and then decreased in the old group. With increasing age, mean, systolic, and diastolic CBFV as well as CVCi were decreased progressively, while the PI was increased. Older adults showed lower $EtCO_2$ than the younger subjects, while SaO_2 was similar across the age groups. Women showed higher mean, systolic, and diastolic CBFVs and CVCi than men.

Hypocapnic CVMR

Hemodynamic data during hyperventilation are presented in Table 2. During hyperventilation, $EtCO_2$, MAP, CBFV, and CVCi decreased, while HR increased from the baseline. Despite similar reductions in $EtCO_2$ across all age groups, the magnitude of CBFV% and CVCi% reductions was attenuated in the old group. The HR response was attenuated in older groups, while the MAP response was similar across all groups. Figure 2 presents the age-related differences in hypocapnic CVMR and cardiovascular reactivity. The $\Delta CBFV\%/\Delta EtCO_2$ and $\Delta CVCi\%/\Delta EtCO_2$ were significantly attenuated in the old group compared with the young and middle-aged groups, while $\Delta MAP/\Delta EtCO_2$ remained at a similar level.

Hypercapnic CVMR

Hemodynamic data during rebreathing are summarized in Table 3. Despite similar increases in $EtCO_2$ across all age groups, elevations of $\Delta CBFV\%$ and $\Delta CVCi\%$ were greater in the older groups. Consistently, hypercapnic CVMRs measured from the slopes of $\Delta CBFV\%$ vs. $EtCO_2$ and $\Delta CVCi\%$ vs. $EtCO_2$ were steeper in the older compared with younger groups (Figures 1 and 2). The goodness of line fit for $\Delta EtCO_2$ with $\Delta CBFV\%$ and $\Delta CVCi\%$ yielded excellent coefficients of determination. The slope of ΔMAP vs. $\Delta EtCO_2$ was greater in the older group than in the young group, while the HR response was similar across the groups.

The LMM analysis demonstrated the greater contribution of $\Delta EtCO_2$ to $\Delta CBFV\%$ in the middle-aged and older groups than in the young group. The contribution of ΔMAP was also greater in the old group compared with young and middle-aged groups (Figure 3).

Relationship between hypo- and hypercapnic CVMRs

Negative correlations were observed between hypo- and hypercapnic CVMRs, as measured by both CBFV% and CVCi% across all subjects (Figure 4).

Table 1. Subject characteristics and baseline cerebral and systemic hemodynamics.

	Young		Middle age		Old		*P*-value		
Variables	Men	Women	Men	Women	Men	Women	Age	Sex	Age×sex
n	16	20	24	20	15	22		0.423	
Age (years)	34 ± 7	33 ± 6	54 ± 6	59 ± 5*	71 ± 3	70 ± 4	<**0.001**	0.354	**0.031**
Height (cm)	177 ± 8	163 ± 6	177 ± 7	165 ± 9	176 ± 5	160 ± 7	0.136	<**0.001**	0.475
Body mass (kg)	81 ± 10	62 ± 12	90 ± 13	71 ± 10	87 ± 10	70 ± 9	**0.001**	<**0.001**	0.930
BMI (kg/m^2)	26 ± 4	23 ± 3	28 ± 4	26 ± 4	28 ± 3	27 ± 4	**0.001**	**0.009**	0.467
Systolic BP (mmHg)	114 ± 11	108 ± 7	117 ± 12	116 ± 7	123 ± 13	122 ± 13	**0.001**	0.170	0.552
Diastolic BP (mmHg)	71 ± 10	69 ± 8	76 ± 7	74 ± 6	74 ± 8	70 ± 9	**0.034**	0.051	0.925
Baseline hemodynamics									
$EtCO_2$ (mmHg)	40 ± 3	38 ± 3	38 ± 3	38 ± 3	37 ± 3	37 ± 2	**0.006**	0.101	0.487
SaO_2 (%)	98 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1	0.417	0.489	0.117
Heart rate (bpm)	68 ± 11	69 ± 9	69 ± 12	71 ± 8	65 ± 9	69 ± 7	0.375	0.183	0.683
MAP (mmHg)	89 ± 14	91 ± 14	93 ± 11	96 ± 11	98 ± 14	98 ± 11	**0.014**	0.446	0.812
Mean CBFV (cm/s)	56 ± 6	65 ± 8	49 ± 9	53 ± 10	43 ± 6	49 ± 9	<**0.001**	<**0.001**	0.449
Systolic CBFV (cm/s)	85 ± 12	95 ± 12	74 ± 13	79 ± 15	69 ± 9	77 ± 13	<**0.001**	**0.001**	0.695
Diatolic CBFV (cm/s)	39 ± 5	42 ± 6	32 ± 6	33 ± 7	26 ± 5	28 ± 6	<**0.001**	**0.036**	0.510
Pulsatility index (a.u.)	0.81 ± 0.17	0.82 ± 0.08	0.85 ± 0.10	0.88 ± 0.07	1.01 ± 0.17	0.99 ± 0.14	<**0.001**	0.858	0.653
CVCi (cm/s×mmHg)	0.64 ± 0.12	0.73 ± 0.14	0.54 ± 0.12	0.56 ± 0.13	0.45 ± 0.08	0.51 ± 0.12	<**0.001**	**0.020**	0.507
CVRi (mmHg/cm/s)	1.61 ± 0.31	4.42 ± 0.28	1.96 ± 0.49	1.89 ± 0.45	2.32 ± 0.46	2.08 ± 0.46	<**0.001**	**0.039**	0.660

Note: Data are mean ± standard deviation. *$P < 0.05$: compared with middle-aged men. Bold values represent $P < 0.05$. BPM: beats per minute; BMI: body mass index; BP: blood pressure; CBFV: cerebral blood flow velocity; CVCi: cerebrovascular conductance index; CVRi: cerebrovascular resistance index; $EtCO_2$: end-tidal CO_2; MAP: mean arterial pressure; SaO_2: arterial blood oxygen saturation.

Table 2. Cerebral and systemic hemodynamics during hypocapnia.

	Young		Middle age		Old		P-value		
Variables	Men	Women	Men	Women	Men	Women	Age	Sex	Age× sex
$\Delta EtCO_2$ (mmHg)	−19 ± 4	−18 ± 3	−19 ± 3	−18 ± 4	−19 ± 4	−18 ± 3	0.997	0.745	0.983
ΔHeart rate (bmp)	19 ± 12	19 ± 8	14 ± 7	11 ± 6	6 ± 5	8 ± 8	<**0.001**	0.955	0.434
ΔMAP (mmHg)	−11 ± 7	−9 ± 7	−11 ± 7	−11 ± 6	−11 ± 7	−10 ± 6	0.773	0.628	0.808
ΔCBFV (cm/s)	−24 ± 7	−27 ± 6	−20 ± 5	−21 ± 7	−15 ± 5	−17 ± 5	<**0.001**	0.109	0.912
ΔCVCi (cm/s×mmHg)	−0.23 ± 0.09	−0.26 ± 0.10	−0.18 ± 0.07	−0.18 ± 0.07	−0.12 ± 0.06	−0.13 ± 0.07	<**0.001**	0.358	0.747
ΔCBFV (%)	−36 ± 13	−35 ± 10	−32 ± 7	−31 ± 7	−26 ± 10	−25 ± 9	<**0.001**	0.380	0.949
ΔCVCi (%)	−44 ± 12	−42 ± 8	−40 ± 6	−39 ± 7	−34 ± 9	−34 ± 7	<**0.001**	0.580	0.993
$\Delta CBFV/\Delta EtCO_2$ (cm/s/mmHg)	1.31 ± 0.27	1.46 ± 0.32	1.06 ± 0.21	1.15 ± 0.30	0.80 ± 0.21	0.90 ± 0.25	**0.001**	**0.028**	0.866
(%/mmHg)	2.34 ± 0.36	2.26 ± 0.35	2.16 ± 0.33	2.14 ± 0.28	1.85 ± 0.32	1.82 ± 0.30	<**0.001**	0.481	0.902
$\Delta CVCi/\Delta EtCO_2$ (cm/s×mmHg/mmHg)	0.012 ± 0.004	0.014 ± 0.005	0.009 ± 0.003	0.010 ± 0.003	0.006 ± 0.002	0.007 ± 0.003	<**0.001**	0.227	0.646
(%/mmHg)	1.89 ± 0.52	1.88 ± 0.47	1.74 ± 0.39	1.71 ± 0.35	1.40 ± 0.41	1.37 ± 0.41	<**0.001**	0.770	0.995
$\Delta MAP/\Delta EtCO_2$ (mmHg/mmHg)	0.57 ± 0.37	0.50 ± 0.41	0.57 ± 0.34	0.60 ± 0.30	0.60 ± 0.34	0.56 ± 0.34	0.796	0.665	0.783

Note: Data are mean ± standard deviation. Bold values represent $P < 0.05$. BPM: beats per minute; CBFV: mean cerebral blood flow velocity; CVCi: cerebrovascular conductance index; $EtCO_2$: end-tidal CO_2; MAP: mean arterial pressure.

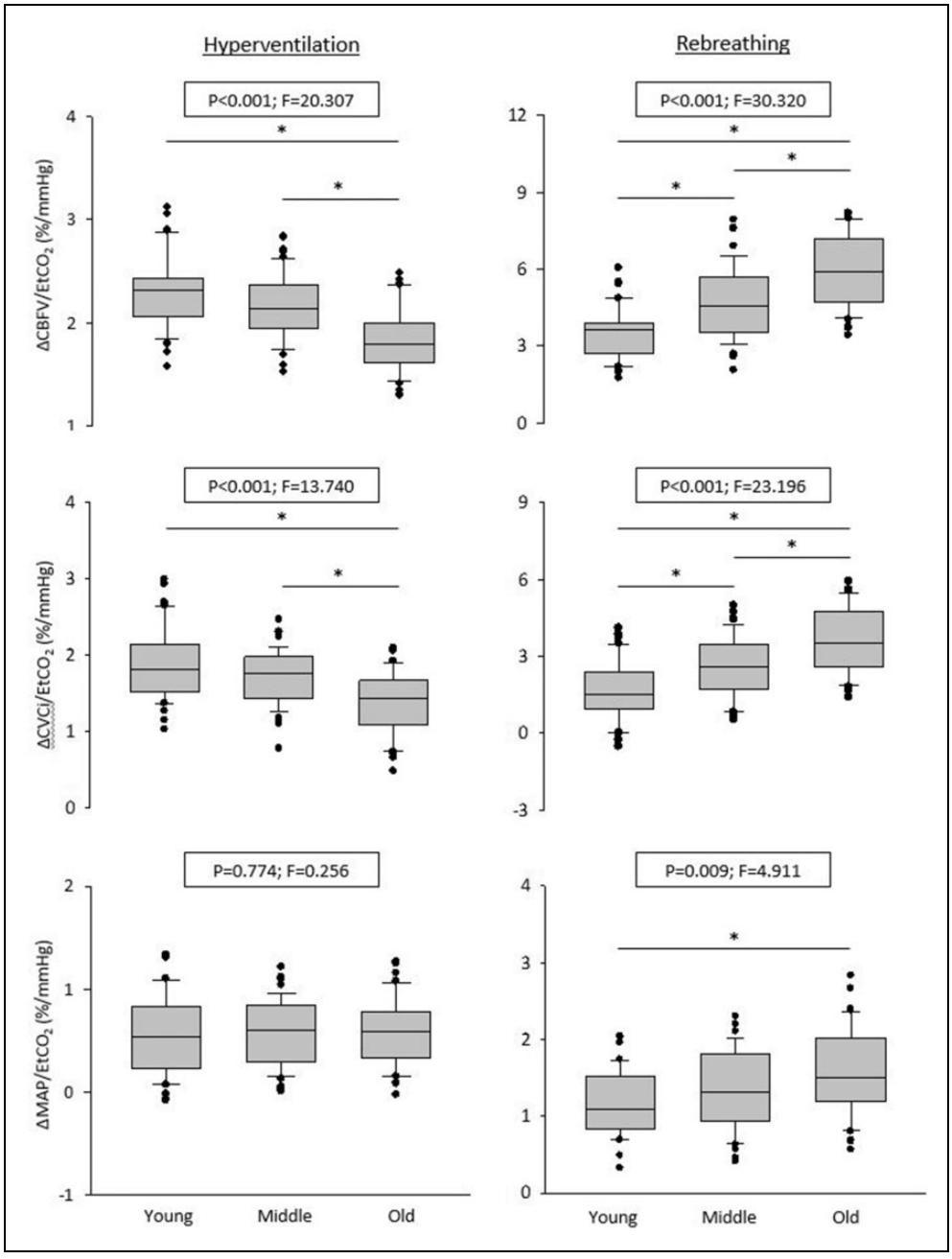

Figure 2. Box-and-whisker plots showing the slopes of mean cerebral blood flow velocity (CBFV), cerebrovascular conductance index (CVCi), and mean arterial pressure (MAP) in response to changes in end-tidal CO_2 ($EtCO_2$) during hypo- and hypercapnia in young, middle-aged, and older adults. *$P < 0.05$.

These correlations indicate that the greater reductions in CBFV or CVCi during hypocapnia are associated with the smaller increases in CBFV or CVCi during hypercapnia.

Discussion

This study examined the effects of normal aging on cerebro- and cardiovascular reactivity to hypo- and hypercapnia across the adult lifespan. The main findings from the present study are as follows. First, aging is associated with reduced CBFV and CVCi and increases in CVRi under resting conditions. Second, aging is associated with a progressive decline in hypocapnic CVMR. Third, hypercapnic CVMR is augmented with increasing age. Fourth, hypo- and hypercapnic CVMRs are negatively correlated with each other across all age groups. Collectively, these

Table 3. Cerebral and systemic hemodynamics during hypercapnia.

	Young		Middle age		Old		*P*-value		
Variables	Men	Women	Men	Women	Men	Women	Age	Sex	Age× sex
$\Delta EtCO_2$ (mmHg)	19 ± 3	18 ± 2	19 ± 2	19 ± 3	19 ± 3	18 ± 3	0.681	0.403	0.500
ΔSaO_2 (%)	−0.6 ± 0.6	−0.5 ± 0.7	−0.6 ± 0.6	−0.6 ± 0.6	−0.7 ± 0.6	−0.5 ± 0.5	0.906	0.448	0.700
ΔHeart rate (bmp)	8 ± 7	9 ± 6	7 ± 6	8 ± 7	4 ± 3	8 ± 5	0.153	0.084	0.497
ΔMAP (mmHg)	17 ± 7	17 ± 7	20 ± 7	21 ± 9	22 ± 8	25 ± 9	**0.002**	0.351	0.779
ΔCBFV (cm/s)	34 ± 9	38 ± 13	37 ± 8	40 ± 11	42 ± 11	46 ± 13	**0.010**	0.093	0.941
ΔCVCi (cm/s×mmHg)	0.22 ± 0.11	0.23 ± 0.08	0.23 ± 0.07	0.24 ± 0.06	0.27 ± 0.08	0.26 ± 0.09	0.056	0.753	0.882
ΔCBFV (%)	60 ± 17	59 ± 19	77 ± 17	78 ± 25	103 ± 34	95 ± 25	<**0.001**	0.516	0.679
ΔCVCi (%)	35 ± 18	34 ± 17	46 ± 17	47 ± 20	64 ± 20	56 ± 19	<**0.001**	0.359	0.566
$\Delta CBFV/\Delta EtCO_2$									
Slope (cm/s/mmHg)	1.98 ± 0.48	2.17 ± 0.85	2.24 ± 0.80	2.49 ± 0.67	2.65 ± 0.65	2.92 ± 0.95	**0.001**	0.105	0.975
Slope (%/mmHg)	3.53 ± 0.99	3.50 ± 1.08	4.71 ± 1.51	4.67 ± 1.40	6.07 ± 1.49	5.85 ± 1.46	<**0.001**	0.699	0.945
R^2	0.88 ± 0.08	0.82 ± 0.23	0.92 ± 0.06	0.91 ± 0.05	0.95 ± 0.02	0.92 ± 0.05	**0.004**	0.104	0.456
$\Delta CVCi/\Delta EtCO_2$									
Slope (cm/s×mmHg/mmHg)	0.011 ± 0.007	0.011 ± 0.007	0.012 ± 0.006	0.015 ± 0.005	0.017 ± 0.005	0.016 ± 0.007	**0.003**	0.485	0.678
Slope (%/mmHg)	1.80 ± 1.14	1.58 ± 1.24	2.46 ± 1.28	2.76 ± 1.15	3.94 ± 1.12	3.43 ± 1.37	<**0.001**	0.532	0.337
R^2	0.60 ± 0.31	0.59 ± 0.31	0.73 ± 0.27	0.76 ± 0.21	0.89 ± 0.07	0.84 ± 0.13	<**0.001**	0.869	0.729
# of data points	37 ± 10	36 ± 9	32 ± 11	34 ± 10	35 ± 9	37 ± 9	0.236	0.556	0.792
$\Delta MAP/\Delta EtCO_2$									
Slope (mmHg/mmHg)	1.18 ± 0.49	1.20 ± 0.40	1.42 ± 0.51	1.28 ± 0.54	1.47 ± 0.55	1.63 ± 0.60	**0.015**	0.876	0.443
R^2	0.80 ± 0.24	0.83 ± 0.08	0.86 ± 0.10	0.84 ± 0.12	0.84 ± 0.11	0.86 ± 0.08	0.421	0.556	0.615

Note: Data are mean ± standard deviation. Bold values represent $P < 0.05$. BPM: beats per minute; CBFV: mean cerebral blood flow velocity; CVCi: cerebrovascular conductance index; $EtCO_2$: end-tidal CO_2; MAP: mean arterial pressure; R^2: coefficient of determination; SaO_2: arterial blood oxygen saturation.

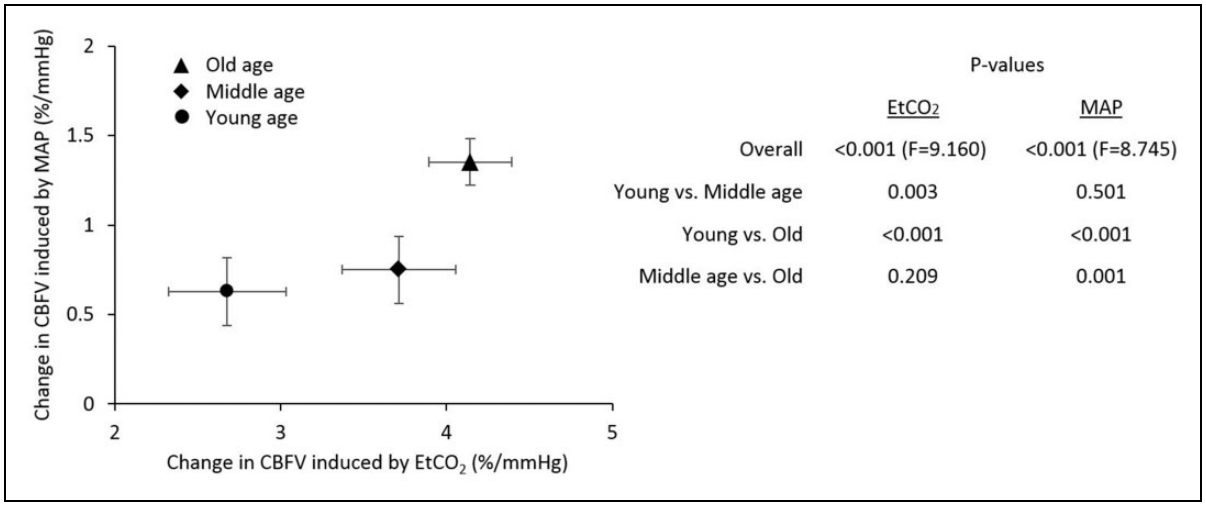

Figure 3. Linear mixed model analysis exhibiting the contributions of hypercapnic changes in end-tidal CO_2 ($EtCO_2$) and mean arterial pressure (MAP) to cerebral blood flow velocity (CBFV) in young, middle-aged, and older groups. The table shows *P*-values for the overall effect of group x $EtCO_2$ and group x MAP as well as their posthoc tests. The error bars represent standard errors.

findings suggest that normal aging is associated with increases in cerebrovascular resistance, leading to the reduced cerebral vasoconstrictive capacity while augmenting the dilatory responsiveness.

Age and baseline hemodynamics

CBF declines with age.[25–27] The magnitude of age-related decline in CBF is approximately 0.3–0.6% per

year across the adult lifespan as measured by positron emission tomography or phase-contrast magnetic resonance imaging.[28] Using TCD, the percentage of CBFV declines we observed in this study was consistent with these previous studies and decreased by ~23% in men and ~25% in women between the age range of 21 to 80 years. These estimates equate to the rate of reductions by ~0.6% in men and ~0.7% in women per year.

The age-related reductions in CBF may be related to the decreases in brain metabolic rate or increases in cerebrovascular resistance and impedance. Age increases systolic BP and MAP while diastolic BP peaks around middle age and decreases in the older age.[29] These systemic changes in BP may cause cerebrovascular remodeling and influence cerebral perfusion pressure.[30] A recent study showed a negative correlation between age and intracranial pressure in patients with traumatic brain injury.[30] If this finding can be extrapolated to normal adults, age may indeed increase cerebral perfusion pressure through the effects of elevated MAP and reduced intracranial pressure. Consequently, cerebrovascular bed in older adults may undergo compensatory remodeling which increases vascular resistance and impedance and protects the downstream capillary beds and vulnerable brain tissues from over perfusion.

The lower resting $EtCO_2$ we observed from older adults is consistent with previous findings.[31,32] In a systematic review of 26 cross-sectional studies, blood $PaCO_2$ decreased by ~3 mmHg in healthy subjects aged between 20 and 80 years.[32] Later, Dhokalia et al. also demonstrated that $EtCO_2$ decreases by 3 mmHg in old adults compared with younger individuals. Further, cross-sectional studies have reported that plasma concentration of bicarbonate is negatively correlated with age, whereas blood concentration of hydrogen is positively correlated with age.[32] These observations then suggest that decreases in $EtCO_2$ with normal aging are due to a compensatory response to progressive metabolic acidosis in the blood.[31]

Cardiovascular and CVMR with aging

In the present study, hypocapnic CVMR (vasoconstriction) was reduced in older adults compared with young individuals. This observation is consistent with previous findings under similar experimental conditions (hyperventilation).[7,8] For example, Gotoh et al.[7] continuously measured the jugular blood flow as an index of CBF during voluntary hyperventilation, and Yamaguchi et al.[8] measured regional CBF by ^{133}Xe inhalation methods during hyperventilation. Both studies demonstrated that the cerebral vasoconstrictor capacity is reduced in older adults compared with young individuals.

In addition, we observed that increases in HR during hypocapnia were attenuated in older adults, while reductions in MAP were similar across the age groups. It is possible that decrease in $EtCO_2$ by hypocapnia stimulates parasympathetic neural system via chemoreceptor and causes vasodilation which can lead to decreased MAP.[33] In response to reductions of BP, HR is increased by the activation of baroreceptor reflex (negative feedback system). Thus, blunted baroreflex sensitivity with aging may have attenuated HR response to hypocapnia in older adults.[15]

In contrast, we observed that hypercapnic CVMR (vasodilation) was enhanced in older adults compared with young individuals. These observations are consistent with our previous report from a small group of young and older participants.[13] However, these findings are not consistent with some of the previous studies using the steady-state (i.e. stepwise increases in inspiratory air concentration of CO_2) and breath-holding techniques.[19,34,35] We suspect a few possible explanations for this discrepancy.

First, rebreathing method may evoke different autonomic and cardiovascular responses compared with the breath-holding and steady-state methods. In terms of the CO_2 stimulus, the magnitude of $PaCO_2$ elevation during rebreathing (~16 mmHg) is greater than the breath-holding method (~5 mmHg when holding breath for 15 seconds) and the steady-state method (~8 mmHg when using the typical 5% CO_2 gas).[36,37] In addition, it has been shown that rebreathing method can elicit the greater responses of peripheral chemoreceptor reflex and sympatho-excitation than the steady-state method.[38,39] On the other hand, breath-holding may evoke greater BP elevation due to the Valsalva effects.[40] Despite these methodological differences, our previous studies have demonstrated that CVMR assessed by the rebreathing and steady-state methods are similar in healthy young individuals,[5] although there is currently no investigation that compared rebreathing and breath-holding methods for CVMR assessment.

Second, our observations of an inverse relationship between hypo- and hypercapnic CVMRs may provide an explanation. In older adults, cerebrovascular disease and/or dysfunction, as manifested, for example, by endothelial dysfunction and blood vessel wall smooth muscle degeneration, increases the basal cerebrovascular tone and risks of cerebral ischemia.[41] Increases in basal cerebrovascular tone may shift the operating (baseline) point of the $PaCO_2$-CBF relationship downward closer to the ischemic threshold and decreases the hypocapnic cerebral vasoconstrictor reserve. On the other hand, the downward shift of the operating point may result in a greater reserve for cerebral vasodilation (Figure 4).

Third, we have observed that advancing age is associated with increases in BP responses to hypercapnia, thus increasing the driving force of CBF. Consistent

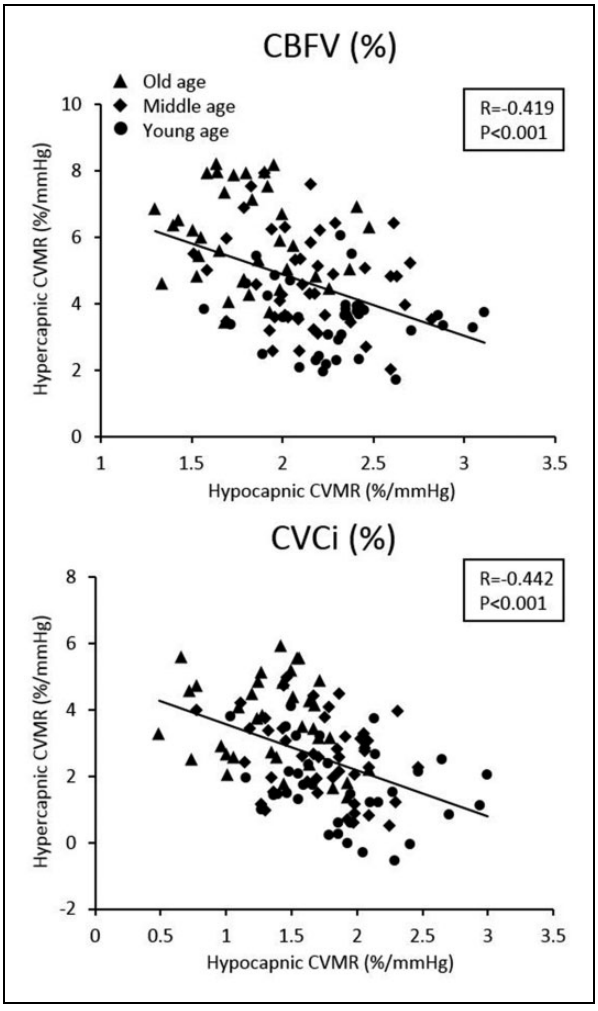

Figure 4. Simple correlation between hypo- and hypercapnic cerebral vasomotor reactivity (CVMR) across all subjects. CVMRs were calculated from the slope of cerebral blood flow velocity (CBFV, %) vs. end-tidal CO_2 (mmHg) and cerebrovascular conductance index (CVCi, %) vs. end-tidal CO_2 (mmHg).

with our observations, peripheral chemoreceptor sensitivity and sympathetic neural responses during hypercapnia were higher in patients with chronic heart failure than in healthy adults.[16,17] Further investigations are needed to understand the physiological mechanisms of age-related differences in CVMR during hypo- and hypercapnia.

Methodological considerations

Prior studies demonstrated that hypo- and hypercapnic CVMRs are asymmetric and that hyperventilation performed immediately before rebreathing may attenuate the magnitude of hypercapnic cerebral vasodilation.[5,42–44] Because of these reasons, hyperventilation and rebreathing protocols were conducted separately in this study. In addition, we used a relatively short period of rebreathing protocol (i.e. 3 min) to induce moderate but sufficiently large increases in $EtCO_2$ to reduce the possibility of discomfort associated with prolonged CO_2 rebreathing. Both CBFV and CVCi responses to moderate changes in $EtCO_2$ can be approximated by a linear relationship to simplify the data analysis (Figure 1).[2,5] Further, linear mixed model was used to examine the independent contributions of hypercapnic changes in $\Delta EtCO_2$ and ΔMAP to $\Delta CBFV\%$ and also account for potential correlations between repeated measures.[13]

It is possible that age alters pulmonary gas exchange efficiency and arterial-pulmonary gradient of CO_2 level, especially during rebreathing where $EtCO_2$ increases rapidly.[45] Although all age groups had similar magnitudes of $EtCO_2$ changes during both hyperventilation and rebreathing, $PaCO_2$ may be different if age influences pulmonary gas exchange.[45] However, no differences in $EtCO_2$ and $PaCO_2$ at rest have been reported from young and older adults,[46] and potential effects of age on the differences between $EtCO_2$ and $PaCO_2$ during rebreathing are likely to be small given a continued rebreathing period of 3 min for pulmonary gas exchange to reach an equilibrium.

Study limitations

There are a few important study limitations which need to be discussed. First, changes in CBFV reflect changes in CBF only if the insolated MCA diameter stays relatively constant. The direct measurement of the MCA diameter during moderate changes in arterial pressure and CO_2 during craniotomy did not show significant changes. In contrast, recent studies using high resolution magnetic resonance angiographic studies showed MCA dilation during moderate hypercapnia ($\sim$15 mmHg) which suggests the potential underestimation of CVMR assessed by TCD.[47] In this study, the magnitude of changes in $EtCO_2$ during both hyperventilation and rebreathing was similar across all the age groups; thus, we assume that hypo- and hypercapnic effects on the MCA diameter are similar across all age groups. In this regard, a recent magnetic resonance angiographic study reported a slight increase in the MCA dimeter with healthy aging.[48] If this is the case, the age-related differences in the MCA diameter may have led to an underestimate of CVMR during rebreathing hypercapnia in older adults. Second, we much acknowledge that similar to previous cross-sectional aging studies, the age-related differences in CVMR observed in this study only suggest, but cannot imply a causal relation between age and CVMR. Third, although our study sample was

vigorously screened for cardiovascular disease and risk factors, other factors such as lifestyle may influence our findings. In this regard, we have excluded individuals who participate in structured aerobic exercise program; however, dietary factor was not considered.[49]

Conclusions

This study demonstrated the presence of substantial age-related differences in cerebral and cardiovascular hemodynamics under resting and hypo- and hypercapnic conditions. We found that CBFV and CVCi are decreased and CVRi is increased at rest in older adults compared with younger adults. Furthermore, advanced age progressively decreased hypocapnic CVMR while increasing hypercapnic CVMR. Notably, we also observed negative correlations between hypo- and hypercapnic CVMRs. Collectively, these findings suggest that aging increases cerebrovascular resistance at rest, thus contributing to the reduction of basal CBF. The increased cerebrovascular resistance at rest diminishes the vasoconstrictor reserve during hypocapnia but augments the dilatory capacity in older adults. From the clinical perspective, these findings suggest that older adults with decreased cerebral vasoconstrictor reserve may have an elevated risk of hypoperfusion or ischemia, as their basal cerebrovascular tone is likely closer to an ischemic threshold. In the future research, investigating the association between hypocapnic CVMR and ischemic brain markers (e.g. white matter hyperintensity) may advance our understanding of the age-related increase in cerebrovascular disease.

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Authors' contributions

T Tomoto, RZ, and T Tarumi conceived and designed the paper; RZ and T Tarumi contributed materials and ideas; T Tomoto, JR, MT, RZ, and T Tarumi analyzed and refined both text and intellectual content. All of the authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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