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RESEARCH ARTICLE | *Sex Differences in Cardiovascular and Cerebrovascular Physiology, Disease, and Signaling Mechanisms*

Effect of healthy aging and sex on middle cerebral artery blood velocity dynamics during moderate-intensity exercise

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Ward JL, Craig JC, Liu Y, Vidoni ED, Maletsky R, Poole DC, Billinger SA. Effect of healthy aging and sex on middle cerebral artery blood velocity dynamics during moderate intensity exercise. *Am J Physiol Heart Circ Physiol* 315: H492–H501, 2018. First published May 18, 2018; doi:10.1152/ajpheart.00129.2018.—Blood velocity measured in the middle cerebral artery (MCA_V) increases with finite kinetics during moderate-intensity exercise, and the amplitude and dynamics of the response provide invaluable insights into the controlling mechanisms. The MCA_V response after exercise onset is well fit to an exponential model in young individuals but remains to be characterized in their older counterparts. The responsiveness of vasomotor control degrades with advancing age, especially in skeletal muscle. We tested the hypothesis that older subjects would evince a slower and reduced MCA_V response to exercise. Twenty-nine healthy young (25 ± 1 yr old) and older (69 ± 1 yr old) adults each performed a rapid transition from rest to moderate-intensity exercise on a recumbent stepper. Resting MCA_V was lower in older than young subjects (47 ± 2 vs. 64 ± 3 cm/s, $P < 0.001$), and amplitude from rest to steady-state exercise was lower in older than young subjects (12 ± 2 vs. 18 ± 3 cm/s, $P = 0.04$), even after subjects were matched for work rate. As hypothesized, the time constant was significantly longer (slower) in the older than young subjects (51 ± 10 vs. 31 ± 4 s, $P = 0.03$), driven primarily by older women. Neither age-related differences in fitness, end-tidal CO_2, nor blood pressure could account for this effect. Thus, MCA_V kinetic analyses revealed a marked impairment in the cerebrovascular response to exercise in older individuals. Kinetic analysis offers a novel approach to evaluate the efficacy of therapeutic interventions for improving cerebrovascular function in elderly and patient populations.

NEW & NOTEWORTHY Understanding the dynamic cerebrovascular response to exercise has provided insights into sex-related cerebrovascular control mechanisms throughout the aging process. We report novel differences in the kinetics response of cerebrovascular blood velocity after the onset of moderate-intensity exercise. The exponential increase in brain blood flow from rest to exercise revealed that *1*) the kinetics profile of the older group was blunted compared with their young counterparts and *2*) the older women demonstrated a slowed response.

aging; cerebral blood flow; dynamic blood flow response; sex differences

INTRODUCTION

After the onset of physical exercise, neural activation elevates the already considerable resting metabolic requirements of the brain (8, 14, 31, 42, 43, 46, 52, 60, 72). Because of the lack of substantial O_2 or energy storage mechanisms (37, 66, 78) and the inability to increase fractional O_2 extraction above ~0.5 compared with 0.8–0.9 in skeletal muscle (31, 59), cerebral blood flow must increase during exercise to maintain normal brain function (14, 21, 31, 34, 41–43, 46, 60, 72). This exercise-induced elevation of cerebral blood flow is most pronounced for moderate-intensity exercise and is opposed at heavy- and severe-intensity exercise by the concomitant hyperventilation-induced hypocapnia, which constricts the cerebral arterioles (8, 31, 40, 46, 52, 60, 72, 77). This cerebral blood velocity pattern remains accurate for the anterior, but not necessarily the posterior, cerebral circulation (64, 75).

It is known that middle cerebral artery (MCA) blood velocity (MCA_V) at rest is reduced with aging (2, 5, 79) but retains the ability to increase with exercise (27). Recently, we found that, for pulmonary and muscle metabolic [i.e., O_2 consumption ($\dot{V}O_2$)] and hemodynamic responses after the onset of constant-load moderate-intensity exercise (32, 59), the cerebrovascular dynamics are closely fit by a delayed monoexponential increase to the new exercising steady state (8). Our proof-of-concept study demonstrated that the underlying dynamics of the cerebrovascular response to exercise were bilateral (i.e., left and right MCA_V) and highly reproducible within a given subject. Interestingly, preliminary data in three healthy, older (65- to 67-yr-old) subjects suggested that their MCA_V dynamics were considerably slower [longer time constant (τ)] and presented a reduced amplitude compared with their young (23- to 25-yr-old) counterparts (8). Resting MCA_V may be higher in premenopausal women than age-matched men, and this difference seems to disappear after menopause (73); however, whether sex and age conflate to modulate the dynamic MCA_V response remains unknown. The profound and prevalent decrease in cognitive function associated with advancing

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age provides a compelling impetus to resolve the effects of healthy aging on cerebrovascular dynamics.

Healthy aging predicates slowed pulmonary and cardiovascular dynamics across the transition from rest to exercise. Although it is not unequivocal, there is strong evidence that a reduced and slowed cardiovascular response moves the site of $\dot{V}O_2$ control from the muscle mitochondria (i.e., O_2 utilization) upstream to the O_2 transport system (i.e., O_2 supply) (4, 17, 18, 24, 59). Whereas information regarding the effects of aging on cerebrovascular control and, especially, its kinetics with aging is very limited, the skeletal muscle has been extensively studied in this regard. Specifically, the rate of endothelium-dependent vasodilation in aged rat skeletal muscle arterioles, the site of primary resistance to blood flow, is slowed nearly threefold or more in response to acetylcholine or increased flow (6), and capillary hemodynamics are severely impaired during muscle contractions (19). As the amplitude of the submaximal $\dot{V}O_2$ response to contractions is typically not reduced by the aging process but the dynamics of blood flow delivery are slowed, microvascular O_2 pressures fall to lower levels in aged than young muscles (7, 58). These responses, first observed in experimental animals, have been confirmed in human muscle (20, 55), with decreased nitric oxide (NO) bioavailability considered to play a commanding role in the aging effects (16). Similarly, within the aged rat cerebral vasculature, endothelial NO synthase (eNOS), acetylcholine, and ADP-dependent vasodilation are impaired (49). Since NO is thought to play an important role in the cardioprotective effects in younger women (50) through increased expression of eNOS (45, 80) and neuronal NO synthase (25), it is predicted that decrements in the dynamic MCA_V response to exercise will be greater in postmenopausal women.

Based on the evidence from aged muscle and cerebral vasculature stated above, we tested the hypotheses that, after the onset of moderate-intensity exercise, *1*) MCA_V would evidence markedly slowed dynamics, reduced amplitude, and, thus, a grossly diminished rate constant (RC), with greater attenuation in older women; *2*) these age-related effects would not occur secondary to differences in mean arterial pressure (MAP) or heart rate (HR) responses; and *3*) these differences would be retained when young and older subjects were matched for work rate.

MATERIALS AND METHODS

Participants

Thirty healthy individuals [15 young (25 ± 3 yr old) and 15 older (69 ± 4 yr) adults] were recruited for the study by word of mouth or flyer handouts. Ten of the fifteen older adults were considered moderate or high cardiac risk (57), primarily because of their age. Eleven young and ten older subjects were considered recreationally active; however, none was considered a competitive athlete (Table 1). Participants were screened in person on the day of their visit. Inclusion criteria consisted of the following: *1*) 20–85 yr of age, *2*) ability to perform a moderate-intensity exercise bout, and *3*) transportation to the University of Kansas Medical Center for testing. Participants were excluded with the following criteria: *1*) inability of study staff to acquire signal of the MCA using transcranial Doppler ultrasound, *2*) inability of the participant to perform the alternating leg movements on the seated recumbent stepper (model T5XR, NuStep, Ann Arbor, MI), *3*) poor model fit of the MCA data during kinetics analysis, *4*) diagnosis of Parkinson's disease, mild cognitive impairment, Alzheimer's disease, or multiple sclerosis, and *5*) pulmonary disease or dependence on supplemental O_2. Participants were asked to abstain from food for 2 h, caffeine for ≥6 h, and vigorous exercise for 12 h before experimental procedures (28). The laboratory room for the experimental session was dimly lit and quiet, and temperature was maintained between 22 and 24°C (9, 10). External stimuli were kept to a minimum. Hormone levels were not directly assessed; however, premenopausal women performed the exercise session between *days 1* and *7* of their menstrual cycle, when concentrations of plasma estrogen and progesterone are generally low (27, 28, 71). All older female participants were >65 yr of age and considered postmenopausal. The University of Kansas Medical Center Human Subjects Committee approved all experimental procedures, which complied with the Declaration of Helsinki. Institutionally approved written informed consent was obtained from each individual before participation in the study.

Table 1. *Participant demographics*

	Group		
	Young (n = 15)	Older (n = 14)	P Value
Age, yr	25.2 ± 0.8	68.9 ± 1.0	<0.001
Sex (women/men), n (%)	8/7 (53/47)	7/7 (50/50)	0.864
Estimated $\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1}	45.6 ± 1.8	28.5 ± 1.9	<0.001
Cardiovascular disease risk, L:M:H	14:1:0	4:4:6	0.001
Work rate, W	113.7 ± 5.4	94.3 ± 9.3	0.08

Values are means ± SE unless otherwise noted; n, number of participants. $\dot{V}O_{2max}$, maximal O_2 consumption. Cardiovascular disease risk is presented as the number of participants with low (L), moderate (M), or high (H) risk.

Experimental Protocol

After written informed consent was obtained and participants remained seated for 20 min, HR and blood pressure were measured by a hand-held device (Tuffsat Ohmeda, GE Healthcare, Chicago, IL). Participants were familiarized with the reciprocal motion of the recumbent stepper at the prescribed step rate of 120 steps/min (8). Target work rate was identified for moderate-intensity exercise by a 10-W increase in resistance every 30 s until HR was in the moderate-intensity HR zone (8). Moderate-intensity exercise was defined as 45–55% of HR reserve, calculated using the Karvonen formula and age-predicted maximum HR of 220 − age (57). After the target work rate was found, HR was allowed to return to within five beats of resting while the participant completed American College of Sports Medicine cardiovascular risk stratification (57), nonexercise maximum $\dot{V}O_2$ estimate (44), and demographic questionnaire and provided information pertaining to past medical history and physical activity participation.

Beat-to-beat blood pressure (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands), end-tidal P_{CO_2} ($P_{ET_{CO_2}}$, in mmHg, BCI Capnocheck Sleep 9004, Smiths Medical, Dublin, OH), transcranial Doppler ultrasound (Multigon Industries, Yonkers, NY), and ECG (Cardiocard, Nasiff Associates, Central Square, NY) were closely monitored by study staff members. Data were acquired through an analog-to-digital data acquisition unit (model NI-USB-6212, National Instruments, Austin, TX) and custom-written software operating in MATLAB (v2014a, MathWorks, Natick, MA).

Setup

Left and right MCA_V were measured using transcranial Doppler ultrasound at rest and during exercise. An adjustable headband and ultrasonic gel were used to place 2-MHz probes over the cranial temporal bone window (3). The MCA was accurately identified using practice standards for probe positioning and orientation, depth selection, and flow direction (3). After depth and gain settings were

adjusted to ensure optimal signal strength, the probes were fixed in place.

Each participant was set up with a five-lead ECG to monitor HR continuously using the V5 lead and then seated in the semirecumbent stepper. A nasal cannula was positioned in the nares to obtain an optimal $P_{ET_{CO_2}}$ reading. The participant was instructed to breathe through the nose during rest and exercise. During exercise, breathing was monitored by an investigator, and feedback was given as necessary. No subject reported difficulty or discomfort, and nasal breathing compliance was maintained in all subjects.

The left arm was set up with a finger photoplethysmograph to collect continuous MAP with each heartbeat from the middle finger. The left arm was placed on a padded table at heart level (28) and continuously monitored to ensure minimal movement during the experiment. Participants were instructed to keep the left hand as stable as possible throughout the experiment. Right arm brachial artery blood pressure was measured using an automated sphygmomanometer with a microphone (model Tango M2, Suntech, Morrisville, NC) placed on a stable platform at heart level. This allowed for comparison between devices to ensure accurate blood pressure measurements before data collection (28).

Exercise Procedures

During the initial setup, the participant sat quietly for 20 min and was reminded to keep arms and hands relaxed, breathe through the nose, and face forward. The recording period started with 90 s of rest (8). After baseline measurements were obtained, the participant was instructed to begin stepping. All subjects began exercising at 60% of their target work rate for the moderate-intensity exercise bout (8). The workload was increased at 10-s intervals using one-third of the difference between the starting and the target workload, which resulted in achievement of the target work rate by 30 s into exercise (8). Subjects maintained this work rate for 6 min and then cooled down for 2 min (8).

Data Acquisition

All variables were sampled at 500 Hz. The data were divided by RR interval. For each cardiac cycle, mean finger arterial pressure, calculated as area under the pressure curve (MAP; in mmHg), mean left and right MCA_V (in cm/s), $P_{ET_{CO_2}}$ (in mmHg), and HR (in beats/min) were calculated. Data with RR intervals of >5 Hz or changes in peak blood flow velocity of >10 cm/s in a single cardiac cycle were considered artifactual and censored. Acquisitions with >15% of data points censored were discarded. Our previous research showed that the left and right MCAs evinced a high level of agreement before and during exercise (8). Therefore, left MCA_V was used for the analysis of the present study to represent the kinetic response in both arteries. If the left MCA signal was not acquired, then the right MCA was used in analysis. MCA_V, MAP, and $P_{ET_{CO_2}}$ were then interpolated to 2.0 Hz using shape-preserving, piece-wise cubic interpolation to standardize samples across participants. MCA vascular resistance index (VR) was expressed as follows: (MAP/MCA_V).

MCA_V Model Fitting

Kinetic analyses were conducted for the MCA using 3-s time-binned mean values over the entire rest and exercise bout with the following monoexponential model (8): $MCA_V(t) = BL + Amp\{1 - e^{-[(1 - TD)/\tau]}\}$, where $MCA_V(t)$ is MCA_V at any point in time (t), BL is baseline (before the onset of exercise and any appreciable change in MCA_V), Amp is the peak amplitude of the response, TD is the time delay preceding the increase in MCA_V, and τ is the time constant (i.e., time to reach 63% of peak amplitude). RC was calculated as follows: Amp/τ. The RC represents the acceleration of the primary response. The goodness of model fit was determined using the following criteria: *1*) coefficient of determination, *2*) sum of the squared residuals, and *3*) visual inspection and analysis of the model fits to the data and the residuals. Time to 63% of peak amplitude was assessed as a model-independent measure of the response by manual identification of the time to achieve 63% of peak Amp. Specifically, this measurement provides a nonbiased check of the model fitting without assumptions regarding the temporal profile of change.

Hemodynamic Variables: Cross-Correlation

MCA_V, MAP, and HR were compared using cross-correlation to a maximum of 60 s of time shift using R (v3.4.1, R Core Team, Vienna, Austria). Time delay (time shift necessary to achieve the maximum of the cross-correlation function) was calculated from each pair of time-binned signals. In the time series analysis, the position signal of each trial was repeatedly correlated with the target signal while time delay was successively increased between the two signals. The time shift corresponding to the greatest correlation was taken as a measure of temporal correspondence.

Statistical Analysis

All curve fitting and statistical analyses were performed using a commercially available software package (SigmaPlot 12.5, Systat Software, San Jose, CA). Sample size was determined based on our previously described methods (8). We calculated that $n = 10$ in each group would provide a statistical power of 0.9 at a probability level of 0.05. As this was based on data from only three older subjects and there was the possibility, especially in older subjects, that the exercise would not be completed, coupled with potential loss of MCA_V signal and/or poor data fits to the exponential model analysis, we elected to recruit 15 subjects/group. Differences in resting values, exercise responses, and kinetics parameters were analyzed using two-way ANOVA with sex and age group as factors. Significance was verified using Tukey's test when necessary. Differences between workload-matched older and young subjects were analyzed using Student's unpaired *t*-tests. Normality was verified via the Shapiro-Wilk normality test. Differences between older and young temporal signal displacement were tested using the Mann-Whitney *U*-test. Differences were considered significant when $P < 0.05$. Values are means ± SE unless otherwise noted.

RESULTS

Participant Demographics

Twenty-nine participants were included in the final analysis (one older female subject was excluded because of poor model fit, i.e., nonexponential response). Participant demographics and the moderate-intensity work rates for young and older groups are shown in Table 1. Sex-specific work rates are shown in Table 2. Work rates were lower in young and older

Table 2. *MCA_V kinetic parameters for the onset of moderate-intensity exercise*

	Women (n = 15)		Men (n = 14)	
	Young (n = 8)	Older (n = 7)	Young (n = 7)	Older (n = 7)
Baseline, cm/s	65.5 ± 3.0	50.9 ± 2.8*†	60.2 ± 2.5	43.2 ± 2.7*
Amplitude, cm/s	17.1 ± 3.0	9.8 ± 1.8*	21.2 ± 3.0	12.8 ± 2.8*
Total, cm/s	82.6 ± 5.1	60.7 ± 3.4*	81.5 ± 5.1	55.9 ± 4.7*
τ, s	31.9 ± 5.6	62.5 ± 13.3*†	28.6 ± 2.7	32.3 ± 4.3
Time delay, s	45.1 ± 4.4	32.8 ± 11.8	50.4 ± 5.0	49.5 ± 10.8
RC, $cm \cdot s^{-1} \cdot s^{-1}$	0.58 ± 0.06†	0.19 ± 0.04*†	0.78 ± 0.13	0.41 ± 0.07*
Work rate, W	98 ± 3†	72 ± 12*†	132 ± 13	116 ± 8

Values are means ± SE; n, number of participants. RC, rate constant; τ, time constant. *Significantly different from young within sex; †significantly different from men within age (both $P < 0.05$).

women than in their age-matched male counterparts ($P < 0.05$ for both).

Baseline and Exercise-Induced Changes in HR, $P_{ET_{CO_2}}$, and MAP

The baseline and the exercise-induced changes (Δ) in HR, $P_{ET_{CO_2}}$, and MAP are shown in Table 3. Resting HR was lower in both older groups ($P < 0.05$). Resting $P_{ET_{CO_2}}$ was higher in young men than older men ($P = 0.01$), but no other differences were found for resting or $\Delta P_{ET_{CO_2}}$ ($P > 0.05$). Consistent with an unchanged arterial P_{CO_2} of ~40 mmHg from rest to exercise (22, 68, 76), $P_{ET_{CO_2}}$ increased 7.9 ± 1 and 6.1 ± 0.6 mmHg in young and older groups, respectively, with no difference between groups ($P = 0.5$). No differences were found for resting MAP ($P > 0.05$ for all), which increased in all groups with the onset of exercise. ΔMAP was greater for older women than for both of their young counterparts ($P < 0.001$) and age-matched older men ($P = 0.02$). ΔMAP in older men was not significantly different from that in their young counterparts ($P = 0.08$).

MCA_V Kinetic Responses

MCA_V increased from rest to an exercising steady state as determined by real-time visual inspection in all participants. Figure 1 shows the MCA_V response after the onset of moderate-intensity exercise in young and older groups. The MCA_V response for young and older subjects was well fit by a time delay plus exponential model.

Data from the MCA_V kinetics analysis are shown in Table 2. At rest, MCA_V was lower for older than young subjects of both sexes ($P < 0.001$; Fig. 1). There were no between-sex differences for resting MCA_V in young subjects; however, resting MCA_V was significantly higher in older women than older men ($P = 0.03$). After the onset of exercise, MCA_V amplitude ($P < 0.01$) and total response ($P < 0.001$) were reduced in older compared with young subjects; no sex differences were evident. There were no differences in the time delay before the increase in MCA_V after the onset of exercise ($P = 0.51$). Although τ was greater (i.e., longer) in older than young subjects ($P = 0.04$), further analysis revealed that this slowed response was driven by the older women ($P < 0.01$ vs. young women and $P = 0.01$ vs. older men), as τ in older men was not different from that in young men ($P = 0.48$). The blunted response amplitude and slower τ resulted in a substantially lower RC in older than young subjects ($P < 0.001$), with a lower RC in women than their in male counterparts for both age groups ($P = 0.045$ for young subjects and $P = 0.04$ for older subjects).

Table 3. *Baseline and exercise-induced changes in HR, $P_{ET_{CO_2}}$, and MAP*

	Women (n = 15)		Men (n = 14)	
	Young (n = 8)	Older (n = 7)	Young (n = 7)	Older (n = 7)
HR, beats/min				
Baseline	85 ± 3	61 ± 3*	79 ± 4	65 ± 4*
Δ	46 ± 3	42 ± 3	48 ± 3	35 ± 4*
$P_{ET_{CO_2}}$, mmHg				
Baseline	34 ± 1†	35 ± 1	38 ± 1	33 ± 1*
Δ	7 ± 1	5 ± 1	10 ± 2	7 ± 1
MAP, mmHg				
Baseline	72 ± 4	70 ± 5	75 ± 3	77 ± 3
Δ	15 ± 3	36 ± 3*†	19 ± 2	26 ± 3

Values are means ± SE; n, number of participants. HR, heart rate; $P_{ET_{CO_2}}$, end-tidal P_{CO_2}; MAP, mean arterial pressure. *Significantly different from young within sex; †significantly different from men within age (both $P < 0.05$).

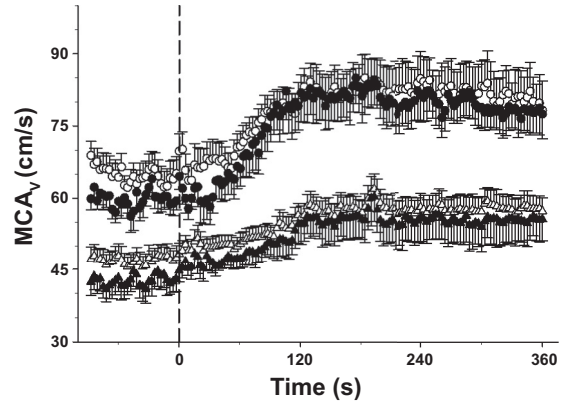

Fig. 1. Group mean blood velocity measured in the middle cerebral artery (MCA_V) before and after the onset of moderate-intensity exercise. Onset of exercise is represented by dashed vertical line (*time 0*). MCA_V increased in all groups after the onset of exercise. MCA_V baseline, amplitude, and total response were increased in young women (○, n = 8) and young men (●, n = 7) compared with their older female (△, n = 8) and male (▲, n = 7) counterparts. Older women also expressed a slowed MCA_V response compared with young women and older men. Values are means ± SE.

Figure 2 shows the MCA VR response after the onset of moderate-intensity exercise in the young and older groups. At rest, VR was higher in the older than young group ($P < 0.001$); there was no sex difference in the young group [1.1 ± 0.1 and 1.3 ± 0.1 arbitrary units (AU) in women and men, respectively, $P = 0.35$], but VR was lower in older women than older men (1.4 ± 0.1 and 1.9 ± 0.2 AU, respectively, $P < 0.01$). After the onset of exercise, VR remained mostly stable and ΔVR was not different between young groups (−0.1 ± 0.1 and −0.1 ± 0.1 AU in women and men, respectively, $P = 0.9$) and

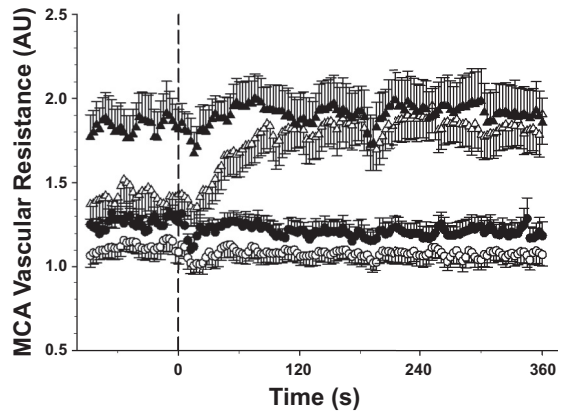

Fig. 2. Group mean middle cerebral artery (MCA) vascular resistance before and after the onset of moderate-intensity exercise. Onset of exercise is represented by dashed vertical line (*time 0*). MCA vascular resistance was lower in young women (○, n = 8) and men (●, n = 7) than in their sex-matched older counterparts. Resting vascular resistance was lower in older women (△, n = 7) than older men (▲, n = 7) but quickly increased to values that were not different following the onset of exercise. Values are means ± SE. AU, arbitrary units.

Table 4. *Baseline and exercise-induced changes in HR, $P_{ET_{CO_2}}$, and MAP in work rate-matched subjects*

	Young Group (n = 11)	Older Group (n = 11)
HR, beats/min		
Baseline	84 ± 3	62 ± 3*
ΔHR	47 ± 2	38 ± 3*
$P_{ET_{CO_2}}$, mmHg		
Baseline	34 ± 1	34 ± 1
Δ	8 ± 1	7 ± 1
MAP, mmHg		
Baseline	72 ± 3	73 ± 3
Δ	17 ± 2	31 ± 3*

Values are means ± SE; n, number of participants. HR, heart rate; $P_{ET_{CO_2}}$, end-tidal P_{CO_2}; MAP, mean arterial pressure. *$P < 0.05$ vs. the young group.

between older and young men (older men: 0.1 ± 0.1 AU, P = 0.26). VR increased rapidly after the onset of exercise in older women (Δ = 0.4 ± 0.1 AU) and was not different from VR in older men at the end of exercise (1.8 ± 0.1 and 1.9 ± 0.2 AU in men and women, respectively, P = 0.54).

Because of the close-to-significant difference in work rate between the young and older groups (P = 0.08), subsets of young and older subjects were matched by work rate to provide insights into whether the differences between the young and older groups demonstrated above might simply have arisen due to lower absolute work rates in the older group. Four young and four older subjects were excluded due to our inability to match them by work rate. Baseline and the exercise-induced changes for HR, $P_{ET_{CO_2}}$, and MAP after subjects were matched by work rate are shown in Table 4. Baseline and ΔHR were lower ($P < 0.001$ and P = 0.03, respectively) and ΔMAP was higher (P = 0.001) in the older than young group. After subjects were matched, work rates were not different (P = 1.0; Table 5). Baseline ($P < 0.001$), response amplitude (P = 0.04), τ (P = 0.04), and RC ($P < 0.01$) were still significantly different between the young and older groups (Fig. 3). Time delay was not different (P = 0.25).

In our cross-correlation analysis, we found no differences in the temporal relationship of MAP, HR, or MCA_V between young and older groups (Fig. 4). The measures were generally tightly coherent in both groups.

DISCUSSION

The principal original findings of the present investigation were as follows: the older group, which had a lower MCA_V at rest and a smaller response amplitude after the onset of moderate-intensity exercise than the young group, demonstrated sex differences for MCA_V kinetics. Older women showed a much slower MCA_V response (τ) than age-matched men and young women. RC for the MCA_V response was substantially lower for the older than the young group, irrespective of sex. Baseline MCA_V, response amplitude, τ, and RC were still significantly different between the older and young groups, even after adjustment for work rate, indicating that the differential MCA_V responses between age groups were work rate independent. These data reveal blunted and slowed MCA_V kinetics after exercise onset in the older group, supporting our primary hypothesis, and could be indicative of a slowed vasomotor control within the aged cerebral vasculature, particularly for aged women. In addition, there was support for our second hypothesis that changes in MCA_V during moderate-intensity exercise occurred independently of changes in MAP, HR, and $P_{ET_{CO_2}}$.

Table 5. *MCA_V kinetic parameters for onset of moderate-intensity exercise in work rate-matched subjects*

	Young Group (n = 11)	Older Group (n = 11)
Baseline, cm/s	63.6 ± 2.6	46.8 ± 2.3*
Amplitude, cm/s	18.1 ± 2.6	11.5 ± 2.1*
Total, cm/s	81.7 ± 4.4	58.3 ± 3.3*
τ, s	30.9 ± 4.0	50.5 ± 10*
Time delay, s	44.6 ± 3.5	35.8 ± 9.0
RC, $cm \cdot s^{-1} \cdot s^{-1}$	0.62 ± 0.06	0.30 ± 0.06*
Work rate, W	107 ± 6	108 ± 7

Values are means ± SE; n, number of participants. MCA_V, middle cerebral artery velocity; τ, time constant; RC, rate constant. *Significantly different from young ($P < 0.05$). Comparisons for sex differences were not made due to the group makeup necessary to match work rate (young group: 8 women and 3 men; old group: 4 women and 7 men).

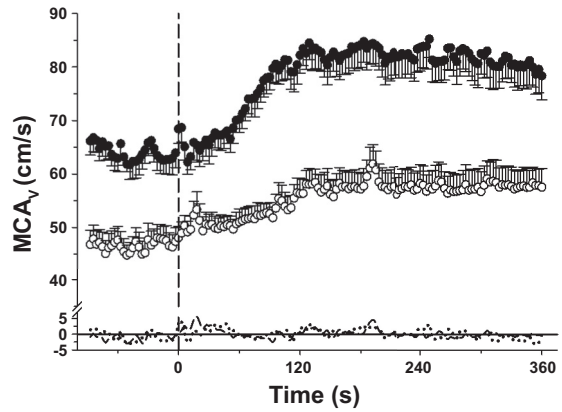

Fig. 3. Group mean blood velocity measured in the middle cerebral artery (MCA_V) before and after the onset of moderate-intensity exercise in subjects matched for work rate. Onset of exercise is represented by dashed vertical line (*time 0*). Filled symbols represent young group, and open symbols represent older group. Residuals are presented just above the horizontal axis for young (dotted line) and older (dashed line) subjects. Analysis included 11 young and 11 older subjects. Values are means ± SE.

Control of Cerebral Perfusion

During large muscle mass exercise, recruited skeletal muscle is the predominant recipient of the augmented cardiac output ($\dot{Q}_{O_2}$), and the impact of advancing age on vascular function, especially across different metabolic rates, has been pharmacologically dissected to the greatest degree. Providing it does not compromise blood flow to other muscles, faster hemodynamic kinetics and higher blood flow at any given metabolic rate ($\dot{V}_{O_2}$) raises muscle microvascular O_2 driving pressures, facilitating blood-muscle O_2 flux and aerobic metabolism. Aging slows arteriolar vasodilation (6) and results in redistribution of muscle blood flow away from metabolically more active fibers toward their less oxidative counterparts (54), increasing $\dot{Q}_{O_2}$-$\dot{V}_{O_2}$ mismatching (7).

In marked contrast to skeletal muscle, the brain is housed within a restrictive skull encasement and may be damaged by excessive increases in cerebral blood flow that elevate intracranial pressures (69). Thus, while the brain's metabolic requirements do increase after exercise onset and cerebral blood flow is elevated, the constraints under which cerebral vascular

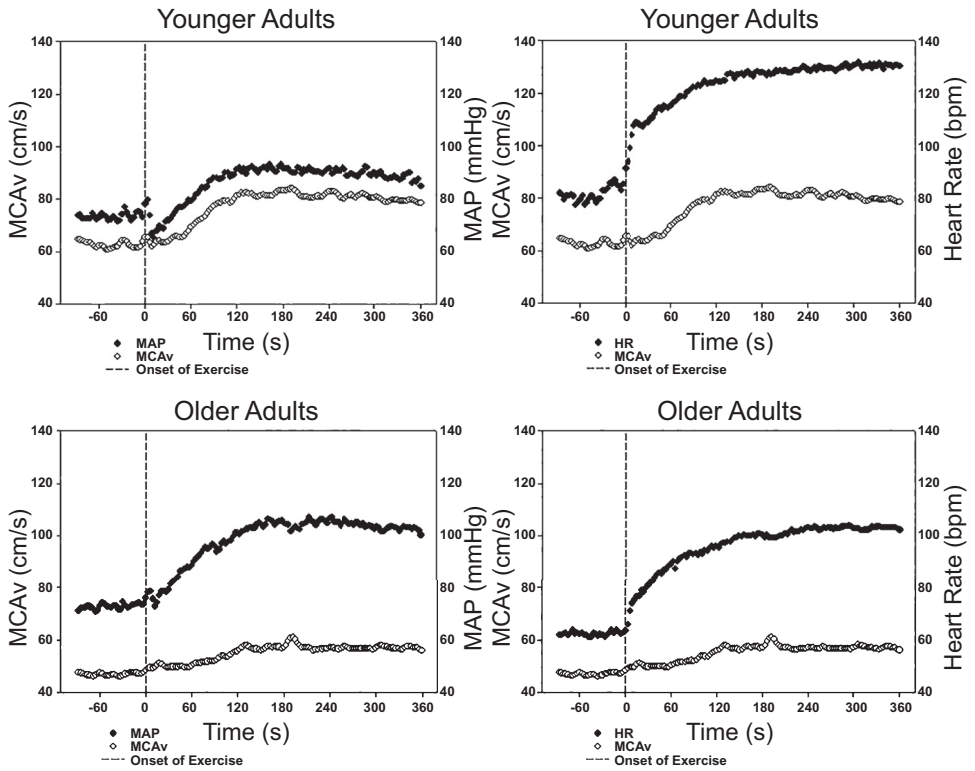

Fig. 4. Relationship between blood velocity measured in the middle cerebral artery (MCA_V), mean arterial pressure (MAP), and heart rate (HR) at rest and during the transition to moderate-intensity exercise in older and young subjects. Onset of exercise is represented by dashed vertical line (*time 0*).

conductance operates are different from those under which skeletal muscle operates; thus cerebral vascular conductance may be controlled very differently in older versus young subjects (47, 69). Compared with our knowledge of skeletal muscle vascular control, especially during exercise in older humans, our knowledge of cerebrovascular control is in its infancy (13). However, in rats there is evidence that cerebrovascular reactivity to acetylcholine and ADP and also eNOS function are impaired with aging (49). Whether this compromises cerebral $\dot{Q}_{O_2}$-$\dot{V}O_2$ matching in aged individuals is not known. It is apparent from the present investigation that if the metabolic demands of the aged brain at a given exercise work rate are the same as those of their younger counterparts [as for skeletal muscle (59)], the more sluggish MCA_V kinetics (in older women) and reduced response amplitude (in both older men and women) observed here predicate a reduced microvascular P_{O_2} and an associated impairment of blood-tissue O_2 flux and, potentially, cerebral metabolic control. Resolution of the kinetics profile of brain deoxygenation across the transition from rest to exercise by near-infrared spectroscopy across the human lifespan would provide valuable insights into this issue.

Arterial blood gases. Given the extreme responsiveness of the cerebral arterioles to arterial P_{CO_2}, a lower exercising arterial P_{CO_2} in aged individuals and/or a lower CO_2 sensitivity in aged individuals could potentially contribute to the MCA_V responses found here. Direct measurements of arterial P_{CO_2} at rest and during moderate-intensity exercise in healthy humans do not support the view that young and older individuals regulate their arterial P_{CO_2} differently (~38–40 mmHg), demonstrating that $P_{ET_{CO_2}}$ underestimates arterial P_{CO_2} at rest and overestimates $P_{ET_{CO_2}}$ during moderate-intensity exercise (22, 68, 76). This latter effect might be expected to be more extreme, given that ventilatory efficiency (ratio of expiratory ventilation to CO_2 production) is decreased in the elderly. Moreover, cerebrovascular responsivity to hypercapnia does not appear to differ between young and older individuals from rest to exercise (13, 53). Similarly, there is no readily available evidence that women and men regulate arterial P_{CO_2} differently during exercise. Arterial hypoxemia (i.e., low arterial P_{O_2}) vasodilates the cerebral arterioles. However, given that, similar to arterial P_{CO_2}, arterial P_{O_2} in healthy individuals does not change systematically as a function of healthy aging (68), it is difficult to conceive how arterial P_{O_2} (or arterial P_{CO_2}) differences could feasibly explain the reduced MCA_V amplitude and slower kinetics demonstrated here.

Neural control. Sympathetic nerve fibers densely innervate the cerebral circulation in humans; however, the degree to which the cerebral blood flow is regulated by sympathetic nerve activity is highly controversial (1, 12, 70). It is well established that sympathetic nerve activity increases with age and may serve to protect the cerebral circulation during hypertensive episodes via arteriolar vasoconstriction (26, 27). Whether the reduced MCA_V response during exercise might result from an impaired functional sympatholysis as identified in skeletal muscle (23) is unknown (13). Interestingly, two lines of evidence provide putative support for involvement of the parasympathetic system in the blunted MCA_V responses documented in the present investigations: *1*) blockade of any parasympathetic cholinergic vasodilation using glycopyrrolate prevents any MCA_V increase during incremental cycle ergometry in young healthy subjects (62) and *2*) cholinergic signaling is reduced with age in the heart and peripheral circulation (13). These are fertile topics for future investigation.

Two additional neural pathways that are likely involved in the MCA_V response to exercise are *1*) the feedforward signals that emanate from the motor cortex and elevate MCA_V in

parallel with neuromuscular innervation (61) and, possibly, *2*) the exercise pressor reflex conveyed via group III and IV afferents in the exercising muscles. How or whether these pathways might modulate the age-induced reductions in MCA_V noted here is unknown.

Blood pressure and HR. It is widely accepted that MAP generally increases with age due to elevated total peripheral resistance (33), and there may be important sex differences in the exercise-induced ΔMAP, with older women expressing a much exaggerated pressure response (48, 74). As for other organs, the cerebral circulation is considered to autoregulate, which is critical during dynamic conditions, such as rhythmic muscular exercise, to counter the dependence of blood flow on MAP (13, 56). The data shown in Fig. 2 demonstrate that the reduced MCA_V baseline, amplitude, and slower response kinetics (for older women) in the older than young cohort were associated with a greater MCA VR. Interestingly, all groups, except older women, maintained a steady MCA VR across the exercise transient. This observation warrants further investigation, as this cerebrovascular response may differ from the peripheral vascular response. Indeed, Trinity et al. (74) reported an augmented increase in systemic VR in older women compared with young women and older men, but older women were able to maintain peripheral perfusion to the working limb. In the present investigation, the near difference in MCA_V baseline ($P = 0.08$) between older women and older men was attenuated by the end of exercise ($P = 0.63$). All groups, including older women, showed no correlation for the increase in MCA_V and ΔMAP during exercise. With respect to HR, the older group demonstrated a smaller increase from rest than their young counterparts, but, as for MAP, there was no correlation between changes in HR and MCA_V within or between groups. These data support the idea that the magnitude of the exercise-induced MCA_V is dissociated from the elevated exercising MAP in the young and older groups. This conclusion agrees with the finding of Fisher and colleagues (27) of an age-invariant transfer function gain between MAP and MCA_V. However, other investigators showed that age may influence the cerebral pressure-flow relationship during low-frequency oscillations in blood pressure (11, 63), fueling speculation that the age-related changes are mediated by alterations in autonomic tone or mechanical properties of vessels. Furthermore, transient squatting exercise-induced hypertension revealed greater relative MCA_V increases per unit change in relative blood pressure with advancing age (11). Thus, increasing age may result in a less efficient dampening of blood pressure during transient hypertension.

Clinical Implications

Addressing the hypotheses posed here has helped respond to important mechanistic questions regarding the control of cerebrovascular hemodynamics in healthy older individuals. Given that therapeutic interventions, such as exercise training (35, 36, 51, 59), dietary supplementation of nitrate to increase NO bioavailability (81, 82), and phosphodiesterase inhibition (67), have improved the muscle vascular response to exercise and exercise tolerance, we reason that resolution of cerebrovascular dynamics in older individuals represents the crucial next step in developing a sensitive MCA_V dynamics-based protocol for assessing putative therapeutic strategies in aged and patient populations.

Experimental Considerations

We recognize several limitations to the present study. First, although transcranial Doppler ultrasound can directly measure cerebral blood velocity, it cannot measure the diameter of a vessel. Therefore, the present study assumes a constant MCA diameter during the exercise transition. There is conflicting evidence as to whether exercise induces changes in MCA diameter (15, 38). However, if MCA diameter does change, that change is likely to be very slight in larger vessels such as the MCA; thus, it would not, in and of itself, affect blood flow velocity to a great extent (29, 30, 39). However, the findings of the present study using the MCA may not reflect the posterior cerebral blood flow response to exercise (65). Second, the sample size was modest, and further work is necessary to support the sex differences. However, the differences agree with age-related sex differences in the periphery (48, 74), increasing confidence in these findings. Third, The exercise protocol implemented a step-wise (over 30 s) increase to the target work rate, as suggested by Billinger et al. (8), to reduce movement artifact and eliminate difficulty in overcoming the increased resistance. As previously mentioned, the smaller step-wise (over 30 s) increase in resistance to the work rate selected likely influenced the MCA_V response kinetics versus those that might be seen with a single immediate step. However, this protocol is necessary to maintain fidelity of the ultrasound signal requisite for measuring MCA_V. Further investigation is needed to fully understand the MCA_V kinetic response among different exercise protocols as well as various exercise intensities. Finally, the present investigation did not measure arterial P_{CO_2} directly but, rather, monitored $P_{ET_{CO_2}}$ principally to ensure that subjects did not hyperventilate, which would decrease MCA_V consequent to cerebral arterial vasoconstriction. As noted above, there were no between-group differences in $P_{ET_{CO_2}}$ that could explain the blunted MCA_V response in the older versus young group. The increased $P_{ET_{CO_2}}$ in both groups during exercise is consistent with the exercise-induced alteration of the breathing pattern and increased rate of CO_2 production (8, 22, 68, 76).

Conclusions

The cerebrovascular kinetic response to exercise, as approximated via MCA_V, is far slower and its amplitude is diminished in older healthy adults compared with their young counterparts. These contrasting responses could not be explained by differences in work rate between age groups or differential responses in MAP, HR, and $P_{ET_{CO_2}}$ (as an indicator of arterial P_{CO_2}), suggesting the presence of altered vasomotor control in older individuals during exercise. These results support the notion that characterization of the dynamic cerebral blood flow response to exercise is a valuable tool to understand mechanistic interrelationships between age and brain health. It is anticipated that such information could potentially lead to improved therapeutic and clinical interventions designed to benefit brain health in aged and patient populations.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.L.W. and Y.L. performed experiments; J.L.W., J.C.C., Y.L., E.D.V., R.M., D.C.P., and S.A.B. analyzed data; J.L.W., J.C.C., Y.L., E.D.V., D.C.P., and S.A.B. interpreted results of experiments; J.L.W., J.C.C., and D.C.P. prepared figures; J.L.W., J.C.C., Y.L., E.D.V., D.C.P., and S.A.B. drafted manuscript; J.L.W., J.C.C., Y.L., E.D.V., D.C.P., and S.A.B. edited and revised manuscript; E.D.V., R.M., and S.A.B. conceived and designed research; S.A.B. approved final version of manuscript.

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