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ORIGINAL PAPER

The effect of muscle metaboreflex on the distribution of blood flow in cerebral arteries during isometric exercise

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Abstract

The present study examined the effect of muscle metaboreflex on blood flow in different cerebral arteries. Eleven healthy participants performed isometric, one-leg knee extension at 30% maximal voluntary contraction for 2 min. Activated muscle metaboreflex was isolated for 2 min by post-exercise muscle ischemia (PEMI). The contralateral internal carotid (ICA), vertebral (VA), and ipsilateral external carotid arteries (ECA) blood flows were evaluated using Doppler ultrasound. The ICA blood flow increased at the beginning of exercise ($P = 0.004$) but returned to the baseline level at the end of exercise ($P = 0.055$). In contrast, the VA blood flow increased and it was maintained until the end of the exercise ($P = 0.011$), while the ECA blood flow gradually increased throughout the exercise ($P = 0.001$). These findings indicate that isometric exercise causes a heterogeneous cerebral blood flow response in different cerebral arteries. During PEMI, the conductance of the VA as well as that of the ICA was significantly lower compared with the baseline value ($P = 0.020$ and $P = 0.032$, at PEMI90), while the conductance of the ECA was not different from the baseline ($P = 0.587$), suggesting that the posterior and anterior cerebral vasculature were similarly affected during exercise by activation of muscle metaboreceptors, but not in the non-cerebral artery. Since ECA branches from ICA, the balance in the different influence of muscle metaboreflex on ECA (vasodilation via exercise-induced hypertension) and ICA (vasoconstriction) may contribute to the decrease in ICA blood flow at the end of isometric exercise.

Keywords Internal carotid artery · External carotid artery · Vertebral artery · Cerebral blood flow · Post-exercise muscle ischemia · Exercise pressor reflex · Central command

Introduction

Regulation of blood flow in each cerebral artery is considered important for maintaining brain homeostasis. However, the response of cerebral artery blood flow to various physiological conditions (e.g., orthostatic stress, hypoxia, exercise, heat stress) has been reported to be different compared with that observed in other arteries [1–5]. On the other hand, this phenomenon has not yet been fully and physiologically understood. Regarding cerebral vascular response to exercise, it has been well established that low or moderate dynamic exercise increases anterior cerebral blood flow (CBF), internal carotid artery (ICA) blood flow, but heavy dynamic exercise decreases it from moderate intensity despite a large cerebral neural activation [5–9]. In contrast, posterior CBF, vertebral artery (VA) blood flow, gradually increases during increasing exercise workload [5, 10]. Also, previous studies investigated CBF response to isometric exercise and demonstrated that the increase in posterior CBF during isometric exercise is much larger than that of anterior CBF (ICA vs. VA blood flow: 16% vs. 40%, respectively) [10, 11]. Taken together, these previous findings suggest that there is a difference in the impact of exercise on CBF between anterior and posterior cerebral arteries, regardless of exercise mode. In cerebral circulation, the anterior cerebral artery, i.e., the blood flow in the ICA, supplies blood to the cerebral cortex, while the posterior cerebral artery, i.e., the blood flow in the VA, supplies blood to the medulla oblongata. Therefore, these differing blood flow responses

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to exercise conditions observed among cerebral arteries are physiologically reasonable and may be attributed to differing anatomical and functional characteristics between anterior and posterior cerebral circulation. However, the physiological mechanism of a heterogeneous CBF response to exercise has not been elucidated.

Previous studies suggested that muscle metaboreflex, which is responsive to metabolic perturbation (Groups III and VI), plays a vital role in mediating cardiovascular adjustments in response to exercise [12, 13]. Some previous studies suggested that the muscle metaboreflex may play a role as one regulatory mechanism in anterior CBF during exercise [14–21]. Although the influence of the sympathetic nerve activity (SNA) on the regulation of CBF is still controversial during exercise [22–25], we can estimate that the effect of SNA on posterior CBF during exercise is different from that of anterior CBF, since the posterior cerebral circulation has less sympathetic innervation compared with the anterior cerebral portion [26]. Moreover, there is a difference in the CBF regulatory system between the anterior and posterior cerebral arteries, while dynamic cerebral autoregulation or cerebral carbon dioxide (CO_2) reactivity in the anterior cerebral artery is greater than those of the posterior cerebral artery [4, 27]. Activation of muscle metaboreceptors elevates SNA, perfusion pressure and ventilation [28], and therefore it is possible that the effect of muscle metaboreflex on posterior CBF is different from that of anterior cerebral circulation. However, no study has investigated an effect of muscle metaboreflex on posterior cerebral vasculature. Therefore, the role of muscle metaboreflex in the distribution of CBF hemodynamics during exercise, particularly in that of posterior CBF, remains unknown.

In the present study, we expected that isometric exercise also causes a heterogeneous CBF response in different cerebral arteries, with anterior CBF increasing and thereafter decreasing to the baseline level, while posterior CBF gradually increases during isometric exercise. Furthermore, we hypothesized that the muscle metaboreflex also affects posterior cerebral vasculature, but the effect of muscle metaboreflex on posterior cerebral vasculature may be less than that of anterior cerebral circulation because of its weak CBF regulatory mechanisms. Importantly, these findings regarding different effects of muscle metaboreflex on cerebral vasculature may provide the evidence for the mechanism of a heterogonous CBF response to exercise in different cerebral arteries. Previous studies have established several approaches for muscle metaboreflex assessment [29]. A popular approach involves complete circulatory arrest of the exercising skeletal muscle that continues into the recovery period while the muscle is quiescent [i.e., a state of post-exercise muscle ischemia (PEMI)]. Thus, metabolically sensitive skeletal muscle afferents may be separately activated through trapping exercise-induced metabolites within the muscle. Although the external carotid artery (ECA) is a non-cerebral artery, change in the ECA blood flow may affect ICA blood flow regulation since the ECA branches from the ICA. Indeed, previous studies [30–33] demonstrated that the ECA blood flow modified the ICA blood flow regulation especially during hypo- or hypertension conditions as well as during exercise. Therefore, in the present study, we measured the blood flow in the ICA, ECA, and VA during isometric, one-side a leg exercise and PEMI to test the aforementioned hypotheses.

Methods

Participants

Eleven healthy individuals [three males and eight females; age, 21 ± 2 years; height, 165 ± 7 cm; body weight, 58 ± 8 kg; all values presented as mean ± standard deviation (SD)] volunteered to participate in the study. All experimental procedures and protocols conformed to the principles of the Declaration of Helsinki and were approved by the Ethics Committee of the Japan Women's College of Physical Education (No. 2014-13). Each participant provided written informed consent following the detailed explanation of all potential risks and procedures. None of the participants had apparent cardiovascular, pulmonary, metabolic, or neurological disease or receiving medications known to influence cerebral hemodynamic function. In addition, the participants were requested to abstain from strenuous physical activity and the consumption of caffeinated beverages and alcohol for 12 h and at least 24 h, respectively, prior to the initiation of the experimental sessions. Furthermore, all participants were familiarized with the equipment and procedures prior to their participation in the experimental sessions.

Experimental protocols

Before the experimental day, each participant performed two maximal isometric one-legged knee extensions (a 90° angle between the lower and upper leg) using the right leg to determine the maximal voluntary contraction (MVC). The higher (of the two) forces generated during the exercise was recorded. On the experimental day, the participants arrived at the laboratory at least 2 h after consuming a light meal and sat comfortably in a chair during the course of instrument set-up. After a 30-min seated rest, each participant performed an isometric one-knee extension at 30% MVC for 2 min in the seated position with the knee joint angle set at 90°. During the exercise, the participants achieved the required workload using visual feedback. The right thigh cuff was inflated acutely (it took few seconds) to 220 mmHg at 15 s before the end of the exercise to occlude the blood flow

to the exercising muscles and was held inflated for 2 min to isolate the activation of muscle metaboreflex (PEMI). The thigh cuff was wrapped around the groin to avoid mechanical stress against the exercising muscle.

Cardiorespiratory measurements

The heart rate (HR) was continuously monitored using a three-lead electrocardiogram (Bedside Monitor BMS-2400; Nihon Kohden, Japan). The systolic, diastolic, and mean arterial blood pressures (SBP, DBP, and MAP, respectively) were continuously measured through finger photoplethysmography (Finometer; Finapress Medical Systems, Amsterdam, The Netherlands) using the middle or index finger of the left hand. Cardiac output (CO) was calculated offline from the blood pressure waveform using the Modelflow software program incorporated into the BeatScope® v.1.1 software (TNO-TPD; Biomedical Instrumentation, Amsterdam, The Netherlands). Although the Modelflow method overestimates or underestimates absolute CO during exercise, the Modelflow-estimated CO correlated significantly with the simultaneous estimates by the Doppler method in all the subjects [34]. Thus, a previous study [34] recommended to use a relative change in Modelflow-estimated CO during exercise. Accordingly, in the present study, we used a relative (percent) change in Modelflow-estimated CO from resting baseline to evaluate the effect of each condition on change in CO. The respiratory variables were sampled breath-by-breath. The end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) and ventilation (V_E) were measured via capnography (AE-310S; Minato Medical Science, Osaka, Japan).

Measurements of CBF

The blood flow in the right (ipsilateral) ECA, the left (contralateral) ICA and the VA was measured at each time stage (baseline, during, and after the exercise) using two color-coded ultrasound systems (Vivid-i; GE Healthcare, Tokyo, Japan) equipped with 8-MHz linear transducers. Within 30 s at each time stage, all blood flow measurements (ECA, ICA and VA) were performed randomly by two experienced operators as in previous studies [2, 3, 5, 27, 30]. The blood flow in the ECA and ICA was measured approximately 1.0–1.5 cm distal to the carotid bifurcation, whereas the blood flow in the VA was measured at the midpoint of the V1 segment with the participant's chin slightly elevated.

A previous study [17, 19, 35] reported that the contralateral, unlike the ipsilateral, middle cerebral artery blood velocity (MCA *V*) in response to one handgrip exercise was increased. This finding is due to cerebral neural activation in the motor-sensory cortex and supplementary motor area. Conversely, the ipsilateral MCA *V* was unchanged during the handgrip exercise, indicating that the ipsilateral anterior CBF may not be affected by muscle metaboreflex as well as cerebral neural activation [17, 19, 35]. Moreover, previous studies [14, 17, 19, 21] have used the contralateral side artery to examine an effect of exercise on anterior CBF because exercise-induced cerebral neural activation occurs in the contralateral side of the brain. with these backgrounds, in the present study, we focused on the effect of cerebral neural activation in the motor-sensory cortex and supplementary motor area on the blood flow in the contralateral ICA rather than that in the ipsilateral side of the ICA. Regarding posterior cerebral circulation, our previous study did not demonstrate a significant difference between the contralateral and ipsilateral VA blood flow during isometric handgrip exercise [10]. This is because of the integration of both the right and left VA blood flow by the basilar system. In addition, the ECA does not supply blood to the motor-sensory cortex and supplementary motor area because of a non-cerebral artery. Therefore, the response to exercise-induced cerebral neural activation may not be different between the contralateral and ipsilateral CBF in the VA and ECA. Therefore, in contrast to ICA, we do not need to consider the contralateral or ipsilateral side regarding VA and ECA blood flows in the present study.

For the measurement of the blood flow in the ECA, ICA, and VA, we initially used the brightness mode to determine the mean diameter of each vessel in a longitudinal section. Subsequently, the Doppler velocity spectrum was identified using the pulsed-wave mode. Both systolic and diastolic diameters were measured, and the mean diameter (cm) was calculated with regard to the blood pressure curve: mean diameter = (systolic diameter × 1/3) + (diastolic diameter × 2/3). The time-averaged mean blood flow velocity obtained using the pulsed-wave mode was defined as the mean blood flow velocity (m s^{-1}). The blood flow velocity was measured as the mean of approximately 10–20 cardiac cycles to eliminate the effects of respiration at the end of each condition [at baseline; during exercise 30 s (EX30), 60 s (EX 60), 90 s (EX 90), and 120 s (EX 120); PEMI 30 s (PEMI30), 60 s (PEMI60), 90 s (PEMI90), and 120 s (PEMI120) and; recovery 30 s (REC30), 60 s (REC60), 90 s (REC90), and 120 s (REC120)]. The probe was stabilized to prevent variation of the insonation angle (approximately 60° in most cases) during the measurement of the blood flow velocity. Additionally, the sample was positioned in the center of the vessel and the volume was adjusted to cover the width of the vessel diameter. Finally, the blood flow was calculated by multiplying the cross-sectional area of the artery [$\pi \times$ (mean diameter/2)^2] with the mean blood flow velocity: blood flow = mean blood flow velocity × cross-sectional area × 60 (mL min^{-1}). The conductance of the ICA, VA, and ECA was calculated from the ratio of the ICA, VA, and ECA blood flow to MAP, respectively. These Doppler data were

analyzed by investigators randomly and with confidentially, without individual information, to avoid any biases.

Data processing

In addition to Doppler ultrasound, hemodynamic and respiratory measurements were performed at 1 kHz using an analogue-to-digital converter (PowerLab; ADInstruments, Milfored, MA, USA) interfaced with a computer for offline analysis. These variables during exercise were averaged for 30 s at the same data point as for the Doppler measurement.

Statistical analysis

Data were analyzed using the Statistics Package for Social Scientists (IBM SPSS Statistics v.22.0). Differences between values at the baseline, during exercise, PEMI and recovery were evaluated using one-way repeated-measures analysis of variance (ANOVA) followed by Tukey's Honestly Significant (HSD) post hoc tests. The relative (percent) change in blood flow or vascular conductance data for the ICA, ECA, and VA were analyzed using two-way repeated-measures ANOVA (site × time) and Tukey's HSD test whenever an interaction effect was observed. Spearman's rank-order correlation was used to analyze the statistical relationship between relative changes in MAP and the ICA, VA, or ECA blood flow throughout the protocol. Data were expressed as mean ± SD and a $P < 0.05$ denoted statistical significance for all two-tailed tests.

Results

CBF during exercise

The isometric one-legged knee extension exercise rapidly increased the HR, CO, SBP, DBP, and MAP (Table 1). These variables gradually increased during the exercise without a steady-state until the end of the exercise. In addition, the V_E and $P_{ET}CO_2$ gradually increased during the exercise. The contralateral ICA blood flow increased from the baseline and reached its peak value 60 s after initiating the exercise (EX60, $P = 0.004$). At the end of exercise, this value returned to the baseline level ($P = 0.055$ at EX120; Table 1; Fig. 1a). In contrast, the contralateral VA blood flow increased from the baseline to EX60 and this VA value was maintained until the end of the exercise ($P = 0.011$). The ipsilateral ECA blood flow progressively increased throughout the exercise ($P = 0.001$). On the other hand, the conductance of the ICA 90 s after initiation of the exercise decreased from the baseline (EX90, $P = 0.034$), whereas the conductance of the VA and ECA remained unchanged throughout the exercise.

CBF during PEMI

During PEMI, the HR rapidly returned to the resting baseline value, whereas the CO, SBP, DBP, and MAP values decreased from the exercise. However, these values remained higher than the baseline resting values ($P = 0.010$ for CO, $P = 0.017$ for SBP and $P = 0.005$ for DBP and MAP). Similarly, the V_E and $P_{ET}CO_2$ also gradually decreased from the exercise, but remained slightly higher than the baseline values at 30 s (PEMI30, $P = 0.021$ for V_E and $P = 0.033$ for $P_{ET}CO_2$). However, the $P_{ET}CO_2$ during PEMI (60–120 s, PEMI60–120) was not different from the baseline ($P = 0.413$). The contralateral ICA and VA blood flows rapidly returned to that recorded at the baseline immediately after the end of the exercise and remained unchanged during PEMI (Table 1; Fig. 1a). In contrast, exercise-induced increase in the ipsilateral ECA blood flow decreased; however, it remained higher than that recorded at the baseline during PEMI ($P = 0.046$). The percent change in the ECA blood flow from baseline during PEMI was significantly higher than those in the contralateral ICA and VA blood flow ($P = 0.047$ vs. ICA and $P = 0.008$ vs. VA).

The conductance of both the contralateral ICA and VA decreased from the baseline value during PEMI (PEMI90 and PEMI120: $P = 0.020$ and 0.066 for ICA and $P = 0.032$ and 0.003 for VA). In contrast, the conductance of the ipsilateral ECA was not different from the baseline value at PEMI90 and 120 ($P = 0.587$ and $P = 0.567$) although it was elevated at the beginning of PEMI (PEMI30 and 60; $P = 0.002$ and $P = 0.044$, Table 1; Fig. 1b).

Recovery from PEMI

The contralateral ICA blood flow tended to increase during recovery from PEMI but this change was not significant. The contralateral VA blood flow unchanged, while the ipsilateral ECA blood flow decreased to the baseline level from PEMI. On the other hand, there is an overshoot of vascular conductance of contralateral ICA immediately after PEMI (REC30, $P = 0.023$; Fig. 1b). Similarly, the conductance of the contralateral VA also increased acutely to the baseline level at REC30 but it was not significant from the baseline ($P = 0.091$). The conductance of the ipsilateral ECA unchanged from PEMI to recovery.

Interestingly, the Spearman's rank correlation coefficient between relative changes in the blood flow in the ECA ($P = 0.513$, $P = 0.010$ vs. ICA; Table 2) or VA ($P = 0.456$, $P = 0.011$) and MAP was higher compared with that in the ICA ($\rho = 0.128$). In 7 of 11 subjects, there was a significant relationship between relative changes in MAP and the blood flow in the ECA or VA.

Table 1 Cardiorespiratory and cerebrovascular variables during rest, isometric exercise, post-exercise muscle ischemia, and recovery

	BL	EX				PEMI				REC			
		30 s	60 s	90 s	120 s	30 s	60 s	90 s	120 s	30 s	60 s	90 s	120 s
HR (beat min^{-1})	71 ± 13	86 ± 13*	88 ± 12*	92 ± 12*	96 ± 13*	74 ± 15	69 ± 13	69 ± 13	69 ± 13	72 ± 11	71 ± 12	68 ± 12	66 ± 13
SBP (mmHg)	122 ± 13	129 ± 17*	139 ± 14*	147 ± 15*	150 ± 16*	127 ± 12	133 ± 13†	133 ± 13†	133 ± 13†	115 ± 12	119 ± 12	120 ± 12	121 ± 10
DBP (mmHg)	66 ± 10	71 ± 11*	79 ± 9*	85 ± 10*	87 ± 10*	66 ± 9	75 ± 8*	75 ± 8*	75 ± 8*	57 ± 9	64 ± 9	64 ± 9	65 ± 9
MAP (mmHg)	86 ± 11	91 ± 12*	99 ± 10*	107 ± 11*	109 ± 12*	87 ± 10	95 ± 9*	95 ± 9*	95 ± 10*	77 ± 9	83 ± 9	83 ± 10	84 ± 9
CO (%)	0	27.0 ± 10.6*	40.9 ± 20.7*	51.1 ± 30.6*	60.3 ± 35.8*	26.4 ± 26.5*	18.0 ± 20.7†	19.0 ± 18.9†	17.4 ± 18.2†	15.3 ± 16.3†	14.7 ± 16.4†	12.2 ± 15.6	9.2 ± 14.2
V_E (L min^{-1})	9.2 ± 2.7	9.8 ± 3.9†	10.9 ± 4.3†	13.9 ± 6.2*	13.1 ± 28*	12.2 ± 3.4*	11.0 ± 2.8†	9.8 ± 2.4	9.6 ± 3.0	10.0 ± 2.4	10.6 ± 2.2	9.2 ± 2.2	8.8 ± 1.3
$P_{ET}CO_2$ (mmHg)	37.4 ± 4.5	38.7 ± 5.4	39.3 ± 3.7†	40.1 ± 5.1†	41.2 ± 54†	39.4 ± 4.9†	37.3 ± 4.9	36.5 ± 4.7	36.2 ± 4.7	39.6 ± 3.9	39.2 ± 4.0	37.9 ± 3.9	37.1 ± 4.4
ICA blood flow (mL min^{-1})	295 ± 88	335 ± 96*	348 ± 100*	330 ± 102	328 ± 113	279 ± 81	289 ± 83	281 ± 81	297 ± 91	318 ± 112	322 ± 105	319 ± 107	285 ± 86
ECA blood flow (mL min^{-1})	115 ± 49	131 ± 43†	135 ± 51†	152 ± 54*	171 ± 49*	147 ± 60*	152 ± 79†	134 ± 70†	135 ± 66†	114 ± 61	107 ± 50	110 ± 46	106 ± 43
VA blood flow (mL min^{-1})	93 ± 34	111 ± 44*	118 ± 46*	118 ± 46*	115 ± 45†	99 ± 42	94 ± 35	95 ± 39	94 ± 36	92 ± 36	96 ± 35	92 ± 32	92 ± 33
ICA conductance (mL min^{-1} $mmHg^{-1}$)	3.56 ± 1.22	3.84 ± 1.28	3.56 ± 1.01	3.16 ± 0.96†	3.08 ± 1.05	3.26 ± 0.99	3.07 ± 0.88	2.97 ± 0.78†	3.15 ± 0.89	4.24 ± 1.50†	3.99 ± 1.45	3.91 ± 1.39	3.44 ± 1.07
ECA conductance (mL min^{-1} $mmHg^{-1}$)	1.37 ± 0.50	1.48 ± 0.50	1.38 ± 0.49	1.45 ± 0.49	1.60 ± 0.40	1.71 ± 0.70*	1.61 ± 0.79†	1.43 ± 0.72	1.44 ± 0.68	1.52 ± 0.79	1.32 ± 0.60	1.34 ± 0.55	1.27 ± 0.49
VA conductance (mL min^{-1} $mmHg^{-1}$)	1.11 ± 0.41	1.24 ± 0.48	1.21 ± 0.47	1.12 ± 0.42	1.08 ± 0.44	1.14 ± 0.49	1.00 ± 0.38†	1.01 ± 0.42†	1.00 ± 0.37*	1.21 ± 0.46	1.16 ± 0.39	1.10 ± 0.35	1.09 ± 0.37

Data are mean ± SD

BL baseline rest, EX static exercise, PEMI post exercise muscle ischaemia, REC recovery, HR heart rate, SBP systolic blood pressure, DBP diastolic blood pressure, MAP mean arterial pressure, CO relative change in cardiac output from the baseline, V_E minute ventilation, $P_{ET}CO_2$ end-tidal partial pressure of CO_2, ICA internal carotid artery, ECA external carotid artery, VA vertebral artery

*Different from baseline rest ($P < 0.01$)

†Different from baseline rest ($P < 0.05$)

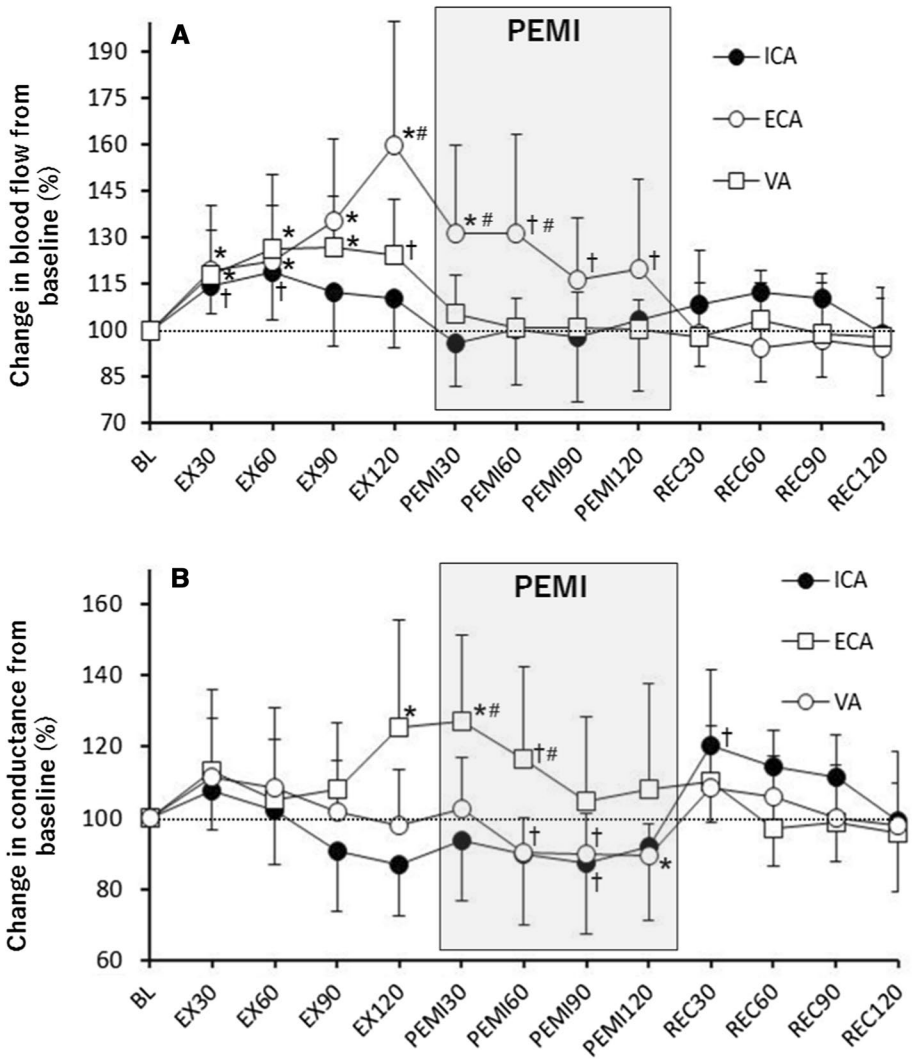

Fig. 1 Percent change in cerebral blood flow (**a**) and conductance (**b**) from baseline (*BL*) during static exercise (*EX*), post-exercise muscle ischemia (*PEMI*), and recovery (*REC*). *ICA* internal carotid artery, *ECA* external carotid artery, *VA* vertebral artery. Data are mean ± SD. *Different from baseline ($P < 0.01$). †Different from baseline ($P < 0.05$). #Different from ICA and VA ($P < 0.05$)

Table 2 Spearman's rank-order correlation between relative changes in MAP and the ICA, VA, or ECA blood flow in individual subject

Subject no.	MAP versus ICA		MAP versus VA		MAP versus ECA	
	P value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient
1	0.878	0.044	**0.006**	0.709	**0.011**	0.670
2	0.179	0.390	**0.019**	0.632	**0.009**	0.681
3	0.806	0.071	0.173	−0.396	0.403	0.247
4	0.269	0.324	**0.000**	0.868	**0.000**	0.802
5	0.392	−0.253	0.892	−0.039	0.132	0.435
6	0.111	0.456	0.055	0.538	**0.006**	0.709
7	0.244	0.341	0.778	0.082	0.221	0.357
8	0.469	0.214	**0.022**	0.621	**0.003**	0.742
9	0.089	0.484	**0.022**	0.621	**0.002**	0.747
10	**0.000**	−0.802	**0.044**	0.560	0.214	−0.363
11	0.629	0.143	**0.000**	0.824	**0.023**	0.615
Average		0.128		0.456*		0.513*
SD		0.359		0.379		0.325

Significant values in bold

*$P < 0.01$ versus ICA

Discussion

The present study provides valuable new information regarding the regulation of CBF during isometric exercise. The contralateral ICA blood flow increased but returned to the baseline level at the end of the exercise. In contrast, the contralateral VA blood flow increased and this value was maintained, while the ipsilateral ECA blood flow gradually increased throughout the exercise (Table 1; Fig. 1a). These findings indicate that the isometric exercise also induced a heterogeneous CBF response in different cerebral arteries such as in dynamic exercise [5]. In addition, the conductance of both the contralateral ICA and VA decreased similarly from the baseline during PEMI (Table 1; Fig. 1b), in contrast, the conductance of the ipsilateral ECA, non-cerebral artery, was not different from the baseline value at PEMI90 and 120. These findings suggest that activation of the metabolically sensitive skeletal muscle afferent fibers modify CBF regulation during isometric exercise in the posterior cerebral artery as well as the anterior cerebral artery but not in the non-cerebral artery (ECA).

Effect of isometric exercise on CBF

During isometric exercise, the VA blood flow increased and was maintained at its peak value until the end (Table 1; Fig. 1). Anatomically, it has been suggested that the posterior CBF supplies blood to the cardiovascular center in the brain for the cardiovascular system [3]. Therefore, physiologically, the elevated VA blood flow induced by exercise may be important to maintain the cardiovascular system function (e.g., high sympathetic nerve activity and hypertension) to perform exercise. During isometric exercise, the conductance of the VA remained unaltered, indicating that the VA blood flow may be affected by the change in cerebral perfusion pressure. Indeed, the change in the perfusion pressure throughout the protocol likely affects the blood flow in the VA more than that in the ICA ($P=0.456$, $P=0.011$ vs. ICA; Table 2). However, the physiological mechanism involved in this process remains unclear.

On the other hand, during isometric one-legged knee extension, the ICA blood flow increased at the beginning of the exercise and returned to the baseline level towards the end of the exercise. This response of the ICA blood flow to isometric exercise was similar to that observed during dynamic exercise. It has been reported that the ICA blood flow increases during mild or moderate dynamic exercise but decreases during heavy dynamic exercise of moderate intensity [5, 7]. During heavy dynamic exercise, the reduction in the ICA blood flow has been suggested to be partly due to hyperventilation-induced hypocapnia [5]. However, in the present study, an isometric one-leg extension did not cause hypocapnia via hyperventilation because of the minimal change in the neural respiratory drive compared with upper body exercise [36–38]. The isometric exercise used in the present study increased the $P_{ET}CO_2$ rather than decreased it. Therefore, it is suggested that the decrease in the ICA blood flow observed towards the end of the isometric exercise was not the result of a change in $P_{ET}CO_2$. Sander et al. [39] reported that, in the anterior brain, isometric exercise increased regional CBF in the motor, somatosensory, and parietal association cortex. In contrast, regional CBF in the midcingulate and anterior cingulate cortices decreased. Therefore, the anterior regional CBF in the midcingulate and anterior cingulate cortices rather than that in the motor, somatosensory, and parietal association cortex may contribute to a decrease in the ICA blood flow to the baseline level at the end of the exercise.

The mechanism of exercise-related change in the ICA blood flow remains controversial. The different responses of the blood flow to isometric exercise between the ICA and VA may be associated with different sympathetic innervation between the anterior and posterior cerebral circulation [26]. The effect of SNA activation on the vasculature may be more pronounced at the ICA compared with the VA. This may be explained by the significant decrease in the conductance of the ICA (occurred vasoconstriction at the ICA; Table 1; Fig. 1b; $P=0.034$) or an overshoot of vascular conductance in ICA immediately after PEMI (removal of sympatho-excitation via muscle metaboreflex), whereas the conductance in the VA remained unchanged throughout the exercise.

In contrast to the ICA and VA blood flow, the ECA blood flow gradually increased until the end of exercise. The ECA blood flow may aid in protecting from hypo- and over-perfusion of the intracranial blood flow [30–32]. Although resistance exercise increases the perfusion pressure, it decreases the blood flow in the ICA or the MCA V [30, 40]. Our previous findings demonstrated that vasoconstriction of the ICA was probably accompanied by vasodilation of the ECA during resistance exercise [30]. Moreover, our recent study [32] demonstrated that an increase in arterial blood pressure via enhanced cardiac contractility and HR through infusion of dobutamine decreased the ICA blood flow, but considerably increased the ECA blood flow. These findings suggest that the ECA downstream vasculature responds differently to acute hypertension versus that of the ICA downstream vasculature. An increase in the vascular conductance of the ECA—reflective of vasodilation in the vascular bed perfused by the ECA—may protect against over-perfusion of the intracranial cerebral arteries during elevated cardiac output and arterial blood pressure [30–32]. In the present study, therefore, a different CBF response between the ICA and ECA may maintain the intracranial CBF adequately during

exercise, despite exercise-induced increase in the perfusion pressure.

Effect of PEMI on CBF

Importantly, activation of muscle metaboreceptors reportedly causes increased regional CBF in the posterior circulation coupled with high sympathetic nerve activity [39, 41]. This region in the posterior brain is supplied by the VA. Thus, we suspected that the activation of muscle metaboreceptors influences the posterior cerebral vasculature and plays an important role in VA blood flow regulation in response to exercise. In the present study, the VA blood flow returned to the baseline level during PEMI. However, the conductance of the VA decreased during PEMI (Fig. 1b), suggesting that muscle metaboreflex affects the posterior cerebral vasculature and thus may contribute to exercise-induced change in the VA blood flow. One possible mechanism of vasoconstriction in the VA is the change in $P_{ET}CO_2$; however, the $P_{ET}CO_2$ during PEMI (60–120 s, PEMI60–120) was not different from the baseline ($P = 0.413$) ,indicating that the effect of change in $P_{ET}CO_2$ on VA may be minimal. Therefore, the physiological mechanism involved in this process remains unclear. Further investigation is needed to identify the mechanism of posterior CBF regulation during exercise.

Similar to the VA, the ICA blood flow remained unchanged during PEMI. This result is consistent with the findings of some previous studies [18, 19, 21] reporting that the exercise-induced (isometric and dynamic) increase in the MCA *V*, as an index of anterior CBF, was not sustained and returned to the baseline level during PEMI. More importantly, the conductance of the ICA decreased significantly during PEMI (Fig. 1b), indicating that the muscle metaboreflex plays a role in the regulation of the ICA blood flow during isometric exercise. Again, the effect of change in $P_{ET}CO_2$ on the ICA could not be ruled out; however, the $P_{ET}CO_2$ during PEMI (60–120 s, PEMI60–120) was not different from the baseline ($P = 0.413$). Thus, it is possible that the effect of change in $P_{ET}CO_2$ on ICA was minimal. Interestingly, there is an overshoot of vascular conductance in the ICA immediately after PEMI. It is likely that the removal of muscle metaboreflex-induced sympatho-excitation causes cerebral vasodilation in the ICA acutely ($P = 0.023$; Fig. 1b).

Several previous studies [14–17, 20] have reported that muscle metaboreflex contributes to the exercise-induced increase in the anterior CBF. For example, it has been reported [15–17] that the blockage of afferent muscle fibers through local anesthesia abolished the exercise-induced increase in MCA *V*, providing the evidence that muscle metaboreflex affects anterior cerebral vasculature. Also, a recent study [14] demonstrated that the effect of muscle metaboreflex on anterior CBF was masked by isometric exercise-induced hypocapnia via hyperventilation using a CO_2 clamp to isolate the hypocapnia condition. However, this exercise condition (handgrip exercise at 40% MVC to exhaustion) substantially increased the neural respiratory drive that increased ventilation (hypocapnia) even during the CO_2 clamp condition. Of note, an isometric arm exercise results in a larger neural respiratory drive compared with an isometric leg exercise [36–38]. Therefore, we cannot rule out the possibility that a high neural respiratory drive may modify the cerebral hemodynamics in this condition (i.e., arm isometric exercise at high workload). In addition, Jorgensen et al. [18] demonstrated that cerebral perfusion during exercise reflects an increase in brain activation that is independent of the muscle metaboreceptors. Thus, we may need further investigations to identify the mechanism of some inconsistent results (no effect of muscle metaboreflex on anterior CBF) in these previous studies.

Physiologically, the cerebral artery blood flow may support an increase in regional CBF to maintain regional brain function. Exercise-induced increase in regional CBF is abolished through the removal of muscle afferent feedback using anesthesia [15–17]. In addition, the exercise-induced elevation in the regional blood flow in the thalamus and right inferior anterior insular region is well maintained during PEMI [42]. These findings suggest that exercise-induced activation of the metaboreceptors increases regional CBF to maintain brain function. However, our previous studies [8, 43, 44] suggested that a reduction in arterial CBF does not attenuate brain function, indicating that a change in arterial CBF may not reflect brain function. The mechanism of the mismatch between arterial and regional CBF responses to the activation of muscle metaboreflex remains unknown.

In contrast to the VA and ICA blood flow, the exercise-induced increase in the ECA blood flow decreased but it is still higher than the baseline value during PEMI. In particular, the relative change in the ECA blood flow was more affected by that in the MAP throughout the protocol compared with ICA ($\rho = 0.513$, $P = 0.010$; Table 2). These findings suggest that the activation of muscle metaboreceptors-induced hypertension may contribute to an increase in the ECA blood flow during exercise and PEMI. The mechanism responsible for the increase in the ECA blood flow during PEMI may involve a lower cerebral autoregulation and lower cerebral CO_2 reactivity in the ECA than those in the ICA and VA [4, 27]. On the other hand, the conductance of the ipsilateral ECA, non-cerebral artery, was not different from the baseline value at PEMI90 and 120 ($P = 0.587$ and $P = 0.567$), although it was elevated at the beginning of PEMI (PEMI30 and PEMI90, $P = 0.002$ and $P = 0.044$; Table 1; Fig. 1b), indicating that the regulation of the ECA blood flow was not affected by exercise-induced activation of muscle metaboreceptors. Considering that blood supplied by the ECA circulates in the external cerebral area,

the downstream flow in the ECA may not be associated with cerebral neural activation via muscle metaboreflex. Therefore, the decrease in the ICA blood flow at end of isometric exercise may be partly explained by the interaction between a small influence of muscle metaboreflex on the ECA (that occurs as vasodilation via exercise-induced hypertension) and sympatho-excitation in the ICA associated with activation of muscle metaboreceptors (vasoconstriction) since the ECA branches from the ICA. Logically, since the VA does not have a bifurcation vessel, the effect of muscle metaboreflex on the VA blood flow was different from that of the ICA blood flow. Taken together, the differing effects of exercise-induced activation of muscle metaboreceptors on cerebral and non-cerebral arteries may partly contribute to a heterogeneous CBF response in different cerebral arteries to isometric execise.

Limitations

The present study has several limitations. Firstly, an external thigh cuff was used for the occlusion of blood flow to the leg to activate muscle metaboreflex. There was no index of muscle metaboreflex activation because the leg blood flow or local metabolic concentration was not measured. However, this maneuver induced a large increase in MAP, indicating that isolated muscle metaboreflex was successfully induced. Secondly, unfortunately, we could not control/synchronize the menstrual cycles in female participants. Previous studies [45, 46] have demonstrated that estrogen affects cerebral vascular responses. However, the effect of estrogen on cerebral vasculature is controversial [47]. To the best of our knowledge, there are no studies which have examined the effect of sex on blood flow in different cerebral arteries. A dedicated study investigating the potential effects of sex on the regulation of CBF in humans is warranted. Thirdly, the simultaneous measurement of the ipsilateral blood flow in the ECA, ICA, and VA was not possible due to interference caused by the ultrasound beam, despite the involvement of two trained investigators using two color-coded ultrasound systems. However, the average of the steady-state data within 15 s was used. In the present study, the time point of VA or ICA blood flow measurement deviated from that of other blood flow; however, VA or ICA blood flow measurement was performed randomly at the same time stage as other measurements. Thus, the impact of this limitation on the present data may be minimal. Finally, the response of the blood flow in the ipsilateral ICA to muscle metaboreflex may be different from that of contralateral ICA. However, previous studies indicate that the blood flow in the ipsilateral ICA unchanged during isometric exercise because of no neural activation in the ipsilateral side of the brain. Thus, in the present study, we focused on the contralateral ICA that was changed during isometric exercise to examine our aims.

Conclusion

Isometric exercise induces a heterogeneous CBF response in different cerebral arteries. In addition, the posterior and anterior CBF regulation were affected similarly by exercise-induced activation of muscle metaboreceptors, but not in the ECA. These findings suggest that the differing effects of exercise-induced activation of muscle metaboreceptors on cerebral and non-cerebral arteries may partly contribute to a heterogeneous CBF response in different cerebral arteries to isometric exercise.

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Author's contributions SO and KS were responsible for the conception and design of the research; SO, AH, and KS performed the experiments; SO, AH, and KS analyzed the data; SO and KS interpreted the results of the experiments; SO and KS prepared the figures; SO drafted the manuscript; all authors edited and revised the manuscript; all authors approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ogoh S, Sato K, Nakahara H, Okazaki K, Subudhi AW, Miyamoto T (2013) Effect of acute hypoxia on blood flow in vertebral and internal carotid arteries. Exp Physiol 98:692–698
2. Ogoh S, Sato K, Okazaki K, Miyamoto T, Hirasawa A, Morimoto K, Shibasaki M (2013) Blood flow distribution during heat stress: cerebral and systemic blood flow. J Cereb Blood Flow Metab 33:1915–1920
3. Ogoh S, Sato K, Okazaki K, Miyamoto T, Hirasawa A, Sadamoto T, Shibasaki M (2015) Blood flow in internal carotid and vertebral arteries during graded lower body negative pressure in humans. Exp Physiol 100:259–266
4. Sato K, Fisher JP, Seifert T, Overgaard M, Secher NH, Ogoh S (2012) Blood flow in internal carotid and vertebral arteries during orthostatic stress. Exp Physiol 97:1272–1280
5. Sato K, Ogoh S, Hirasawa A, Oue A, Sadamoto T (2011) The distribution of blood flow in the carotid and vertebral arteries during dynamic exercise in humans. J Physiol 589:2847–2856
6. Ide K, Pott F, Van Lieshout JJ, Secher NH (1998) Middle cerebral artery blood velocity depends on cardiac output during exercise with a large muscle mass. Acta Physiol Scand 162:13–20
7. Ogoh S, Dalsgaard MK, Yoshiga CC, Dawson EA, Keller DM, Raven PB, Secher NH (2005) Dynamic cerebral autoregulation

during exhaustive exercise in humans. Am J Physiol Heart Circ Physiol 288:H1461–H1467
8. Ogoh S, Tsukamoto H, Hirasawa A, Hasegawa H, Hirose N, Hashimoto T (2014) The effect of changes in cerebral blood flow on cognitive function during exercise. Physiol Rep. https://doi.org/10.14814/phy2.12163
9. Smith KJ, Wong LE, Eves ND, Koelwyn GJ, Smirl JD, Willie CK, Ainslie PN (2012) Regional cerebral blood flow distribution during exercise: influence of oxygen. Respir Physiol Neurobiol 184:97–105
10. Washio T, Sasaki H, Ogoh S (2017) Transcranial Doppler-determined change in posterior cerebral artery blood flow velocity does not reflect vertebral artery blood flow during exercise. Am J Physiol Heart Circ Physiol 312:H827–H831
11. Ogoh S, Sato K, Akimoto T, Oue A, Hirasawa A, Sadamoto T (2010) Dynamic cerebral autoregulation during and after handgrip exercise in humans. J Appl Physiol 108:1701–1705
12. Fisher JP, Young CN, Fadel PJ (2015) Autonomic adjustments to exercise in humans. Compr Physiol 5:475–512
13. Raven PB, Fadel PJ, Ogoh S (2006) Arterial baroreflex resetting during exercise: a current perspective. Exp Physiol 91:37–49
14. Braz ID, Scott C, Simpson LL, Springham EL, Tan BW, Balanos GM, Fisher JP (2014) Influence of muscle metaboreceptor stimulation on middle cerebral artery blood velocity in humans. Exp Physiol 99:1478–1487
15. Friedman DB, Friberg L, Mitchell JH, Secher NH (1991) Effect of axillary blockade on regional cerebral blood flow during static handgrip. J Appl Physiol 71:651–656
16. Friedman DB, Friberg L, Payne G, Mitchell JH, Secher NH (1992) Effects of axillary blockade on regional cerebral blood flow during dynamic hand contractions. J Appl Physiol 73:2120–2125
17. Jorgensen LG, Perko G, Payne G, Secher NH (1993) Effect of limb anesthesia on middle cerebral response to handgrip. Am J Physiol 264:H553–H559
18. Jorgensen LG, Perko M, Hanel B, Schroeder TV, Secher NH (1992) Middle cerebral artery flow velocity and blood flow during exercise and muscle ischemia in humans. J Appl Physiol 72:1123–1132
19. Pott F, Ray CA, Olesen HL, Ide K, Secher NH (1997) Middle cerebral artery blood velocity, arterial diameter and muscle sympathetic nerve activity during post-exercise muscle ischaemia. Acta Physiol Scand 160:43–47
20. Prodel E, Balanos GM, Braz ID, Nobrega AC, Vianna LC, Fisher JP (2016) Muscle metaboreflex and cerebral blood flow regulation in humans: implications for exercise with blood flow restriction. Am J Physiol Heart Circ Physiol 310:H1201–H1209
21. Vianna LC, Araujo CG, Fisher JP (2009) Influence of central command and muscle afferent activation on anterior cerebral artery blood velocity responses to calf exercise in humans. J Appl Physiol 107:1113–1120
22. Brassard P, Tymko MM, Ainslie PN (2017) Sympathetic control of the brain circulation: appreciating the complexities to better understand the controversy. Auton Neurosci 207:37–47
23. Ogoh S (2008) Comments on Point:Counterpoint: sympathetic activity does/does not influence cerebral blood flow. Autonomic nervous system influences dynamic cerebral blood flow. J Appl Physiol 105:1370
24. Ogoh S, Ainslie PN (2009) Cerebral blood flow during exercise: mechanisms of regulation. J Appl Physiol 107:1370–1380
25. Ogoh S, Ainslie PN (2009) Regulatory mechanisms of cerebral blood flow during exercise: new concepts. Exerc Sport Sci Rev 37:123–129
26. Edvinsson L, MacKenzie ET (1976) Amine mechanisms in the cerebral circulation. Pharmacol Rev 28:275–348
27. Sato K, Sadamoto T, Hirasawa A, Oue A, Subudhi AW, Miyazawa T, Ogoh S (2012) Differential blood flow responses to CO_2 in human internal and external carotid and vertebral arteries. J Physiol 590:3277–3290
28. Houssiere A, Najem B, Ciarka A, Velez-Roa S, Naeije R, van de Borne P (2005) Chemoreflex and metaboreflex control during static hypoxic exercise. Am J Physiol Heart Circ Physiol 288:H1724–H1729
29. Rowell LB, Sheriff DD, Wyss CR, Scher AM (1986) The nature of the exercise stimulus. Acta Physiol Scand Suppl 556:7–14
30. Hirasawa AI, Sato K, Yoneya M, Sadamoto T, Bailey DM, Ogoh S (2016) Heterogeneous regulation of brain blood flow during low-intensity resistance exercise. Med Sci Sports Exerc 48:1829–1834
31. Ogoh S, Lericollais R, Hirasawa A, Sakai S, Normand H, Bailey DM (2014) Regional redistribution of blood flow in the external and internal carotid arteries during acute hypotension. Am J Physiol Regul Integr Comp Physiol 306:R747–R751
32. Ogoh S, Moralez G, Washio T, Sarma S, Hieda M, Romero SA, Cramer MN, Shibasaki M, Crandall CG (2017) Effect of increases in cardiac contractility on cerebral blood flow in humans. Am J Physiol Heart Circ Physiol 313:H1155–H1161
33. Ogoh S, Sorensen H, Hirasawa A, Sasaki H, Washio T, Hashimoto T, Bailey DM, Secher NH (2016) Dynamic cerebral autoregulation is unrelated to decrease in external carotid artery blood flow during acute hypotension in healthy young men. Exp Physiol 101:1040–1049
34. Sugawara J, Tanabe T, Miyachi M, Yamamoto K, Takahashi K, Iemitsu M, Otsuki T, Homma S, Maeda S, Ajisaka R, Matsuda M (2003) Non-invasive assessment of cardiac output during exercise in healthy young humans: comparison between Modelflow method and Doppler echocardiography method. Acta Physiol Scand 179:361–366
35. Linkis P, Jorgensen LG, Olesen HL, Madsen PL, Lassen NA, Secher NH (1995) Dynamic exercise enhances regional cerebral artery mean flow velocity. J Appl Physiol 78:12–16
36. Brice AG, Forster HV, Pan LG, Funahashi A, Lowry TF, Murphy CL, Hoffman MD (1988) Ventilatory and PaCO2 responses to voluntary and electrically induced leg exercise. J Appl Physiol 64:218–225
37. Miyamoto T, Manabe K, Ueda S, Nakahara H (2018) Development of an anaesthetized-rat model of exercise hyperpnoea: an integrative model of respiratory control using an equilibrium diagram. Exp Physiol 103:748–760
38. Pendergast DR (1989) Cardiovascular, respiratory, and metabolic responses to upper body exercise. Med Sci Sports Exerc 21:S121–S125
39. Sander M, Macefield VG, Henderson LA (2010) Cortical and brain stem changes in neural activity during static handgrip and postexercise ischemia in humans. J Appl Physiol 108:1691–1700
40. Dickerman RD, McConathy WJ, Smith GH, East JW, Rudder L (2000) Middle cerebral artery blood flow velocity in elite power athletes during maximal weight-lifting. Neurol Res 22:337–340
41. Williamson JW, McColl R, Mathews D (2003) Evidence for central command activation of the human insular cortex during exercise. J Appl Physiol 94:1726–1734
42. Williamson JW, McColl R, Mathews D, Mitchell JH, Raven PB, Morgan WP (2002) Brain activation by central command during actual and imagined handgrip under hypnosis. J Appl Physiol 92:1317–1324
43. Hashimoto T, Tsukamoto H, Takenaka S, Olesen ND, Petersen LG, Sorensen H, Nielsen HB, Secher NH, Ogoh S (2017) Maintained exercise-enhanced brain executive function related to cerebral lactate metabolism in men. FASEB J 32:1417–1427
44. Ogoh S (2017) Relationship between cognitive function and regulation of cerebral blood flow. J Physiol Sci 67:345–351
45. Geary GG, Krause DN, Duckles SP (1998) Estrogen reduces myogenic tone through a nitric oxide-dependent mechanism in rat cerebral arteries. Am J Physiol 275:H292–H300

46. Geary GG, Krause DN, Duckles SP (2000) Estrogen reduces mouse cerebral artery tone through endothelial NOS- and cyclooxygenase-dependent mechanisms. Am J Physiol Heart Circ Physiol 279:H511–H519
47. Gros R, Van Wert R, You X, Thorin E, Husain M (2002) Effects of age, gender, and blood pressure on myogenic responses of mesenteric arteries from C57BL/6 mice. Am J Physiol Heart Circ Physiol 282:H380–H388