Physiological Measurement

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To cite this article before publication: Max E Weston *et al* 2023 *Physiol. Meas.* in press https://doi.org/10.1088/1361-6579/ace49d

Agreement between left and right middle cerebral artery blood velocity responses to incremental and constant work-rate exercise in healthy males and females

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Abstract

Objective To quantify the agreement between left and right middle cerebral artery blood velocity (MCAv) responses to incremental and constant work-rate exercise in adults.

Approach Seventeen healthy adults (23.8±2.4 years, 9 females) completed a ramp incremental test to exhaustion on a cycle ergometer, three 6-minute transitions at a moderate-intensity, and three at a heavy-intensity, all on separate days. Bilateral MCAv was measured throughout using transcranial Doppler ultrasonography, with left and right MCAv data analysed separately. Data were analysed at baseline, gas exchange threshold, respiratory compensation point and exhaustion during ramp incremental exercise. MCAv responses to constant work-rate exercise were analysed using a mono-exponential model, to determine time- and amplitude-based kinetic response parameters.

Main Results Left and right MCAv responses to incremental and constant work-rate exercise were significantly, strongly and positively correlated ($r \geq 0.61$, $P<0.01$). Coefficient of variation (left *vs* right) ranged from 7.3-20.7%, 6.4-26.2% and 5.9-22.5% for ramp, moderate and heavy-intensity exercise, respectively. The relative change in MCAv from baseline was higher in the right compared to left MCAv during ramp, moderate and heavy-intensity exercise (all $P<0.05$), but the effect sizes were small ($d \leq 0.4$). Small mean left-right differences were present during ramp incremental exercise at all time-points (<6 cm/s; <4%), and for all kinetic parameters during moderate and heavy-intensity exercise (<3 cm/s, <3%, <4 s).

Significance These findings demonstrate similarities between left and right MCAv responses to incremental and constant-work rate exercise in adults on a group-level, but also highlight individual variation in the agreement between left and right MCAv exercise responses.

Key words: exercise, cerebral blood flow, transcranial Doppler ultrasound

Introduction

Transcranial Doppler (TCD) ultrasonography has been widely utilised to estimate the cerebral blood flow (CBF) response to exercise through measuring the velocity of blood in the middle cerebral artery (MCAv) (Ogoh and Ainslie, 2009; Smith and Ainslie, 2017; Ainslie and Hoiland, 2014). Studies have often assumed that the left and right middle cerebral arteries respond similarly to exercise, or indeed other stimuli (Billinger *et al.*, 2017; Ward *et al.*, 2018; Witte *et al.*, 2019; Weston *et al.*, 2021, 2022a; Ellis *et al.*, 2017; Weston *et al.*, 2022b). Consequently, some exercise studies measure MCAv unilaterally (Witte *et al.*, 2019; Weston *et al.*, 2022b), whilst others measure bilaterally. When measured bilaterally, there is not a specific decision making process for handling such data, and therefore some studies average left and right MCAv together (Weston *et al.*, 2021, 2022a), whilst others take one side as a representation of both (Ward *et al.*, 2018), choose the side with the best signal quality and highest resting mean (Klein *et al.*, 2019), or do not report how bilateral data are handled (Ellis *et al.*, 2017). The agreement between left and right MCAv responses to exercise has received little investigation, which likely underpins the varied approaches of previous research. This highlights the need for such data, with a view to challenging this assumption, and to provide recommendations for consistent and appropriate handling and reporting of MCAv data.

Although left-right differences in resting blood flow have been observed in the vertebral artery (feeding the posterior circulation), blood flow in the internal carotid artery (delivering blood to the MCA) is similar in the left and right sides at rest (Khan *et al.*, 2017; Schoning *et al.*, 1994). In addition, data indicate that the Circle of Willis presents anatomical anomalies in ~50% of adult brains (Iqbal, 2013; Kapoor *et al.*, 2008), with unilateral variations the most commonly observed (Enyedi *et al.*, 2021). Furthermore, even in monozygotic twins, structural differences are seen in the structure of the Circle of Willis (Thomas *et al.*, 2020). Therefore, it cannot be assumed that the left and right MCAv responses to exercise are the same. However, to our knowledge, only one study to date has reported the agreement between left and right MCAv responses to exercise. In a seminal study by Billinger *et al.* (2017), the time- and amplitude-based kinetic parameters of MCAv to moderate intensity exercise showed coefficients of variation (CV) between 7.6 and 22.8% for kinetic parameters. However, these analyses were conducted on a sample of size of eight healthy adults, and during moderate intensity stepping exercise only. Given the intensity-dependent changes in MCAv observed during incremental exercise (Smith and Ainslie, 2017) and the greater MCAv response observed during heavy-intensity exercise compared to moderate (Weston *et al.*, 2022a), further investigation on left-right MCAv responses to exercise is warranted, on larger sample sizes across commonly used protocols.

Therefore, the purpose of this study was to investigate the agreement between left and right MCAv responses to: 1) ramp incremental exercise, 2) constant work-rate moderate-intensity exercise, and 3) constant work-rate heavy-intensity exercise in healthy young males and females.

Methods

Participants

Seventeen healthy young adults (eight males, nine females) participated in this study. The mean ± standard deviation (SD) age, stature and mass were: 23.8 ± 2.4 years, 173.0 ± 9.3 cm, and 70.9 ± 13.3 kg, respectively (8 males: 22.8 ± 2.6 years, 181.0 ± 5.3 cm, 80.7 ± 9.8 kg; 9 females: 24.1 ± 2.2 years, 164.8 ± 4.6 cm, 60.5 ± 5.1 kg). Following approval from the Sport and Health Sciences Ethics Committee, University of Exeter (190327/B/01), written informed consent was obtained for all participants. All research was conducted in accordance with the Declaration of Helsinki. Participants were screened for the study exclusion criteria, which included contraindications to maximal exercise, current use of any supplement or medication known to influence blood vessel function and current or previous metabolic, cardiovascular, or cerebrovascular disease. This study formed part of a wider data collection, but the data presented here have not been analysed or published elsewhere and solely focus on the left-right MCAv agreement.

Experimental protocol

Each participant visited the laboratory seven times, on separate days, completed in a mean of 25 ± 8 days (13–39 days). On the first visit, stature and body mass were measured using standard procedures, before participants completed a ramp incremental test to volitional exhaustion on a cycle ergometer (Lode Excalibur, Lode, Groningen, the Netherlands). On visits 2-7, participants completed a single bout of constant work-rate exercise.

Ramp Incremental Exercise

Participants completed 3 mins of seated rest on the cycle ergometer, before completing a ramp incremental test to exhaustion at a rate of 20-30 W.min^{-1}. Participants were asked to maintain a consistent cadence between 70-90 revolutions per minute (rpm) throughout the test. Exhaustion was deemed to have been reached when the cadence fell below 70 rpm for 5 consecutive seconds, despite strong verbal encouragement from the researchers. After 10 mins of rest on the ergometer, participants completed a supramaximal verification test, performed at 105% of their ramp test peak power, until exhaustion (Poole and Jones, 2017). Participants wore a leak-free facemask (Hans-Rudolph, Kansas, USA) connected to a metabolic cart (Medgraphics Cardiorespiratory Diagnostics, UK). Breath-by-breath pulmonary oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and minute ventilation ($\dot{V}_E$) were collected and exported as 10 s stationary averages. $\dot{V}O_{2max}$ was defined as the highest 10 s averaged $\dot{V}O_2$ achieved during the ramp test or the verification bout (Poole and Jones, 2017). The $\dot{V}O_2$ corresponding to the GET was determined as the disproportionate increase in $\dot{V}CO_2$ relative to $\dot{V}O_2$ during the ramp test (Beaver *et al.*, 1986), and verified by an increase in the ventilatory equivalent of oxygen ($\dot{V}_E/\dot{V}O_2$) without an increase in the ventilatory equivalent of carbon dioxide ($\dot{V}_E/\dot{V}CO_2$). The respiratory compensation point (RCP) was identified as the inflection in the $\dot{V}_E/\dot{V}CO_2$ slope and an increase in both $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ (Beaver *et al.*, 1986). Both the GET and RCP were independently verified by two researchers.

Constant work-rate exercise

Participants then visited the laboratory six times at the same time of day (±1 h), with ≥24 h between visits. Participants arrived following a ≥2 h fast and were requested to avoid caffeine (Perod *et al.*, 2000), alcohol (Mathew and Wilson, 1986) and vigorous exercise (Burma *et al.*, 2020) for the 24 h preceding the visit. On each of these six visits, participants completed either a bout of moderate intensity cycling (performed at a $\dot{V}O_2$ corresponding to 90% GET), or a bout of heavy intensity cycling (performed at a $\dot{V}O_2$ corresponding to 40% of the difference between GET and $\dot{V}O_{2max}$, 40%Δ) (Lansley *et al.*, 2011). The work-rates corresponding to these $\dot{V}O_2$ values were obtained from the linear relationship between work rate and $\dot{V}O_2$ during the ramp test, and adjusted for a 30 s mean response time (Whipp *et al.*, 1982). In total, participants completed three bouts of moderate and three bouts of heavy intensity cycling, performed in a counterbalanced order. Each bout consisted of a 3 mins, seated, stationary baseline on the cycle ergometer, before an instantaneous transition to the target work rate. Participants cycled for 6 min and maintained a consistent cadence between 70 and 90 rpm.

Experimental Measures

MCAv was measured bilaterally in all participants on every visit using TCD ultrasonography (DWL, Compumedics, Germany). Insonation of the left and right MCA was performed from an initial depth of 45-50 mm using two 2 MHz probes, secured in place with an adjustable headset (DiaMon, DWL, Germany). The position and depth of the probe was recorded for each participant, and replicated between days. MCAv data were collected at 200 Hz using an analogue-to-digital converter (Powerlab; model – 8/30, ADInstruments, USA) interfaced with a laptop computer, and stored for off-line analysis (LabChart 8, ADInstruments, USA). Breath-by-breath gas exchange was also measured during each bout using the metabolic cart, to verify the intensity domain the participants were working in.

Data Analyses

Left and right MCAv data were collected and analysed separately across all visits. In line with previous work (Weston *et al.*, 2021; Ellis *et al.*, 2017), MCAv data from ramp incremental exercise were exported as 10 s averages and analysed at baseline, GET, RCP and exhaustion. Baseline was taken as the average of the last 60 s of seated, stationary rest on the ergometer prior to commencing the incremental ramp test. Left and right MCAv data were reported as absolute values ($cm.s^{-1}$) and as a relative change from baseline (Δ%).

For moderate and heavy-intensity exercise, mean MCAv data were exported as 1 s averages and time aligned to exercise onset. Left and right MCAv data for each participant were separately ensemble-averaged across each repeat transition for each intensity. This created a single left and right MCAv response for moderate and heavy intensity exercise for each participant. Each left and right MCAv response was baseline-corrected for the 60 s preceding exercise onset, and analysed using a mono-exponential model with a time delay (equation 1) using GraphPad Prism (GraphPad Software, San Diego, CA):

$$MCAv(t) = \Delta MCAv_A \left(1 - e^{-(t-TD/\tau)}\right) \quad \text{(equation 1)}$$

where MCAv(t) is the MCAv at a given time (t), $\Delta MCAv_A$ is the amplitude change of MCAv from baseline to its asymptote, TD is the time delay and τ is the time constant. The mean response time (MRT) was calculated as the sum of the τ and TD. $MCAv_{end}$ is taken from an average of the last 10 s during constant work-rate exercise. The standard error of the τ and amplitude was also extracted.

Data were analysed with this exponential model using the same approach that has been detailed elsewhere, with appropriate test-retest reproducibility (Weston *et al.*, 2022a). The model was fit from the start of the exponential rise until a deviation from steady state was observed, or until the end of exercise if MCAv maintained a steady state following the initial exponential rise. Whilst the start and end of the model differed for each participant and for each intensity, the left and right MCAv responses were modelled from the same start and end point *within* each participant. Any deviation from attainment of a steady state was the same in left and right MCAv within a person (as can be seen in both figure 1 and figure 4).

Statistical Analyses

All data are presented as mean ± SD. Statistical analyses were performed using SPSS version 26 (IBM, USA) and GraphPad Prism (Graphpad Software, San Diego, CA), with statistical significance set *a priori* at an α-level of 0.05.

Mean differences in left and right responses to ramp incremental exercise and kinetic parameters during moderate and heavy-intensity exercise were explored using paired sampled t-tests and effect sizes (*d*). A two-way repeated measures ANOVA (side*intensity) was used to compare left and right MCAv during ramp incremental exercise. Significant differences from the ANOVA were located using pairwise comparisons and interpreted using the P value and effect sizes (*d*). An effect size (*d*) was interpreted as trivial if <0.2, small if 0.2-0.49, moderate if 0.5-0.79 and large if ≥0.8 (Cohen, 1977). 95% confidence intervals (CI) were also calculated for the mean difference.

Agreement between left and right MCAv responses to exercise were explored using linear regression and the Pearson correlation coefficient. Outliers from the linear regression were identified and removed if the residuals were outside of ±3 SDs. The typical errors, both in absolute terms (± 95% CIs) and as a coefficient of variation (CV, ± 95% CIs) (Hopkins, 2000) were also calculated. All data were analysed using the whole sample, and then for males and females separately.

Results

Due to MCAv data loss in one participant, ramp incremental data are presented on n=16 (8 females), whereas moderate and heavy data are presented for the full sample (n=17, 9 females). Ramp test responses are presented in Table 1.

Table 1. Ramp test responses.

	n=16
$\dot{V}O_{2max}$ (L·min^{-1})	2.69 ± 0.69
$\dot{V}O_{2max}$ (mL·kg·min^{-1})	38 ± 8
Peak power (W)	280 ± 69
GET (L·min^{-1})	1.28 ± 0.32
GET (%$\dot{V}O_{2max}$)	48 ± 6
RCP (L·min^{-1})	2.24 ± 0.55
RCP (%$\dot{V}O_{2max}$)	84 ± 5

$\dot{V}O_{2max}$, maximal oxygen uptake. GET, gas exchange threshold. RCP, respiratory compensation point

The group-averaged left and right MCAv response to ramp incremental, constant work-rate moderate (80 ± 22 W) and heavy (159 ± 35 W) intensity exercise are shown in Figure 1.

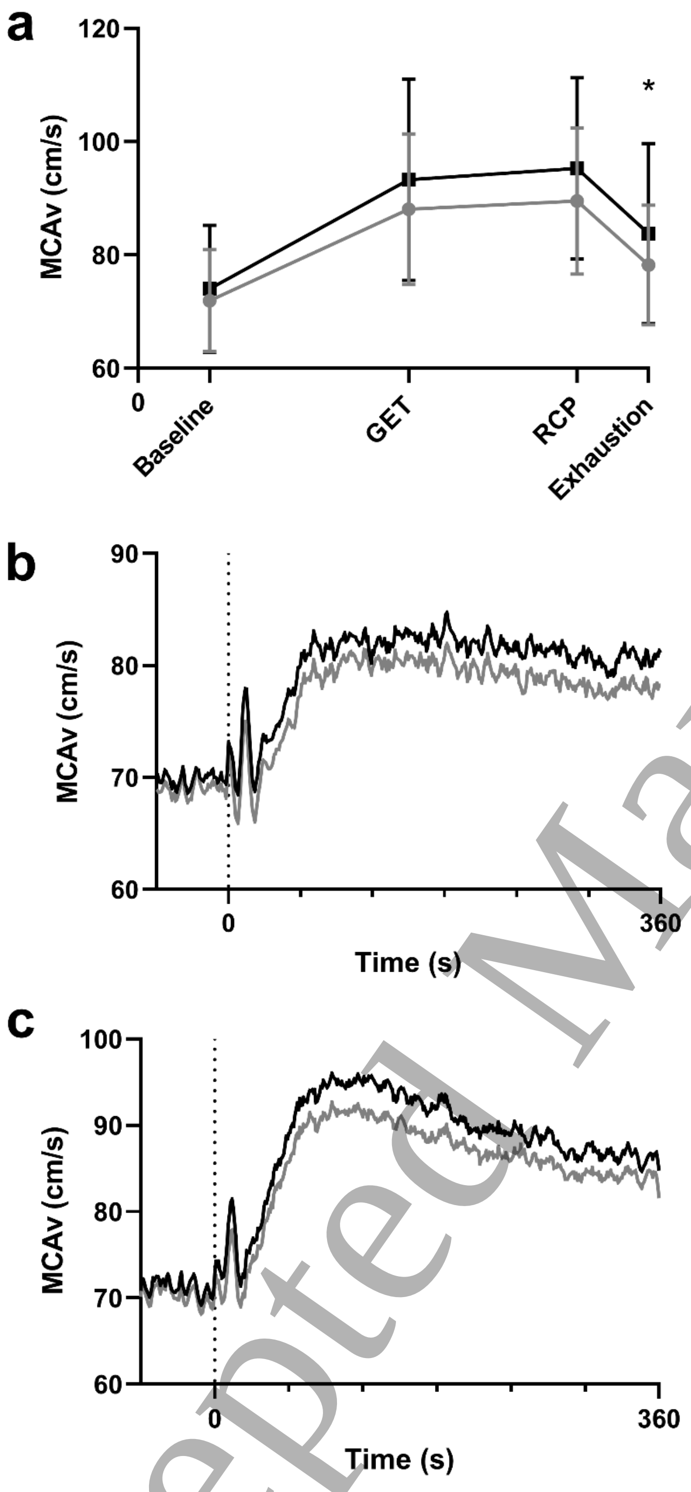

Figure 1. Left (grey) and right (black) middle cerebral artery blood velocity (MCAv) responses to ramp incremental (a), constant work-rate moderate (b) and heavy (c) intensity exercise. GET, gas exchange threshold. RCP, respiratory compensation point. Dashed line indicates start of constant work-rate exercise. * $P<0.05$.

Ramp incremental exercise

Table 2 presents the left and right MCAv responses to ramp incremental exercise in both absolute terms, and as a relative change from baseline. There was a significant side*intensity interaction for MCAv during ramp incremental exercise (P=0.015) (Figure 1), with right MCAv greater at exhaustion compared to the left (P=0.014, *d*=0.4). Δ%MCAv was significantly greater in the right MCAv at the GET (P<0.01, *d*=0.3), and at exhaustion (P=0.04, *d*=0.2). The CV (left *vs* right) ranged from 7.3-20.7% during ramp incremental exercise. Data for males and females separately can be found in Supplementary Tables 1 and 2, respectively. No significant differences in left and right MCAv, in both absolute and relative terms, was observed in males (P>0.16, *d*≤0.2). In females, right MCAv was significantly higher at baseline and all intensities, both in absolute and relative terms, compared to left MCAv (all P<0.05, *d*≥0.4).

Line of identity plots for left and right MCAv responses to ramp incremental exercise in the whole sample, males and females are shown in Figure 2. Left and right MCAv data at all timepoints were significantly, strongly and positively correlated for the whole sample and in males (Table 3). In females, left and right MCAv data were significantly, strongly and positively correlated except for baseline and GET, expressed as cm/s.

Table 2. Mean left and right MCAv kinetic responses during ramp incremental, moderate and heavy intensity exercise for the whole sample.

	Left MCAv	**Right MCAv**	**Mean difference (95% CI)**	**P**	***d***	**Typical error (95% CI)**	**Typical error as CV% (95% CI)**
Ramp Exercise (n=16)							
Baseline MCAv (cm/s)	71.9 ± 9.0	74.0 ± 11.2	-2.2 (-5.8 to 1.5)	0.22	0.2	4.8 (3.6 to 7.5)	7.3 (5.4 to 11.6)
$MCAv_{GET}$ (cm/s)	88.1 ± 13.3	93.3 ± 17.8	-5.2 (-10.4 to 0.0)	0.05	0.3	6.9 (5.1 to 10.7)	8.5 (6.2 to 13.5)
$MCAv_{GET}$ *(Δ%)*	22.6 ± 9.4	25.7 ± 11.0	-3.1 (-4.8 to -1.4)	**0.001***	0.3	2.2 (1.7 to 3.5)	16.8 (12.2 to 27.2)
$MCAv_{RCP}$ (cm/s)	89.5 ± 12.9	95.3 ± 16.0	-5.8 (-11.7 to 0.1)	0.06	0.4	7.8 (5.8 to 12.1)	9.6 (7.0 to 15.2)
$MCAv_{RCP}$ *(Δ%)*	25.3 ± 16.8	29.1 ± 14.9	-3.8 (-7.8 to 0.1)	0.06	0.2	5.2 (3.9 to 8.1)	20.7 (14.9 to 33.8)
$MCAv_{Exhaustion}$ (cm/s)	78.2 ± 10.6	83.8 ± 15.9	-5.6 (-9.9 to -1.3)	**0.01***	0.4	6.1 (4.2 to 11.2)	9.1 (6.2 to 17.3)
$MCAv_{Exhaustion}$ *(Δ%)*	9.8 ± 16.7	13.6 ± 17.2	-3.8 (-7.3 to -0.3)	**0.04***	0.2	4.7 (3.3 to 8.7)	-
Moderate Intensity (n=17)							
Baseline MCAv (cm/s)	69.0 ± 10.5	69.9 ± 8.6	-0.9 (-3.9 to 2.2)	0.56	0.1	4.2 (3.1 to 6.4)	6.4 (4.7 to 9.9)
MCAv τ (s)	25.1 ± 8.3	26.3 ± 12.7	-1.2 (-5.4 to 3.1)	0.58	0.1	5.9 (4.4 to 8.9)	19.0 (13.9 to 30.4)
MCAv amplitude (cm/s)	12.0 ± 4.8	13.4 ± 4.2	-1.4 (-2.5 to -0.2)	**0.02***	0.3	1.5 (1.2 to 2.3)	26.2 (18.9 to 42.5)
MCAv amplitude (%)	17.9 ± 8.2	19.5 ± 7.2	-1.6 (-3.2 to -0.1)	**0.04***	0.2	2.1 (1.6 to 3.3)	23.6 (17.1 to 38.1)
MCAv TD (s)	32.1 ± 14.4	27.1 ± 10.9	5.0 (0.8 to 9.1)	**0.02***	0.4	5.7 (4.2 to 8.6)	18.0 (13.1 to 28.6)
MCAv MRT (s)	57.2 ± 15.4	53.4 ± 12.3	3.8 (-2.6 to 10.3)	0.23	0.3	8.9 (6.6 to 13.5)	13.8 (10.1 to 21.8)
$MCAv_{end}$ (cm/s)	77.9 ± 9.0	80.7 ± 7.8	-2.8 (-6.4 to 0.8)	0.12	0.3	5.0 (3.7 to 7.6)	6.6 (4.9 to 10.3)
$MCAv_{end}$ (%)	13.6 ± 7.7	16.1 ± 8.2	-2.4 (-3.9 to -1.0)	**0.002***	0.3	2.0 (1.5 to 2.3)	26.7 (19.2 to 43.3)
Heavy Intensity (n=17)							
Baseline MCAv (cm/s)	70.3 ± 9.1	71.3 ± 8.7	-1.0 (-3.9 to 2.0)	0.50	0.1	4.1 (3.0 to 6.2)	5.9 (4.3 to 9.0)
MCAv τ (s)	26.2 ± 8.5	26.2 ± 7.8	0.1 (-2.3 to 2.5)	0.95	<0.1	3.3 (2.5 to 5.1)	13.9 (10.2 to 21.9)

MCAv amplitude (cm/s)	22.6 ± 9.4	25.2 ± 10.8	-2.7 (-4.1 to -1.2)	**0.001***	0.3	2.0 (1.5 to 3.0)	11.4 (8.4 to 17.9)
MCAv amplitude (%)	32.7 ± 14.3	35.6 ± 14.7	-2.9 (-4.4 to -1.5)	**0.001***	0.2	2.0 (1.5 to 3.0)	8.7 (6.4 to 13.5)
MCAv TD (s)	30.0 ± 11.1	28.5 ± 8.7	1.5 (-0.3 to 3.3)	0.10	0.2	2.4 (1.8 to 3.7)	7.5 (5.6 to 11.7)
MCAv MRT (s)	56.2 ± 14.3	54.6 ± 10.9	1.5 (-1.6 to 4.7)	0.32	0.1	4.4 (3.3 to 6.7)	6.6 (4.9 to 10.3)
$MCAv_{end}$ (cm/s)	84.1 ± 9.0	86.3 ± 11.3	-2.3 (-5.9 to 1.4)	0.20	0.2	5.0 (3.7 to 7.6)	6.1 (4.5 to 9.5)
$MCAv_{end}$ (%)	20.5 ± 12.5	21.7 ± 13.2	-1.2 (-3.0 to 0.6)	0.17	0.1	2.5 (1.9 to 3.8)	22.5 (16.3 to 36.1)

Data shown as mean ± standard deviation. MCAv, middle cerebral artery blood velocity. τ, time constant. TD, time delay. MRT, mean response time. $MCAv_{end}$, MCAv at end of exercise bout. CV, coefficient of variation. **Bold** * indicates significant difference ($P<0.05$). Mean difference calculated as left – right (i.e. a positive value indicates greater value in the left MCA). "–" CV could not be calculated due to presence of negative values for this variable.

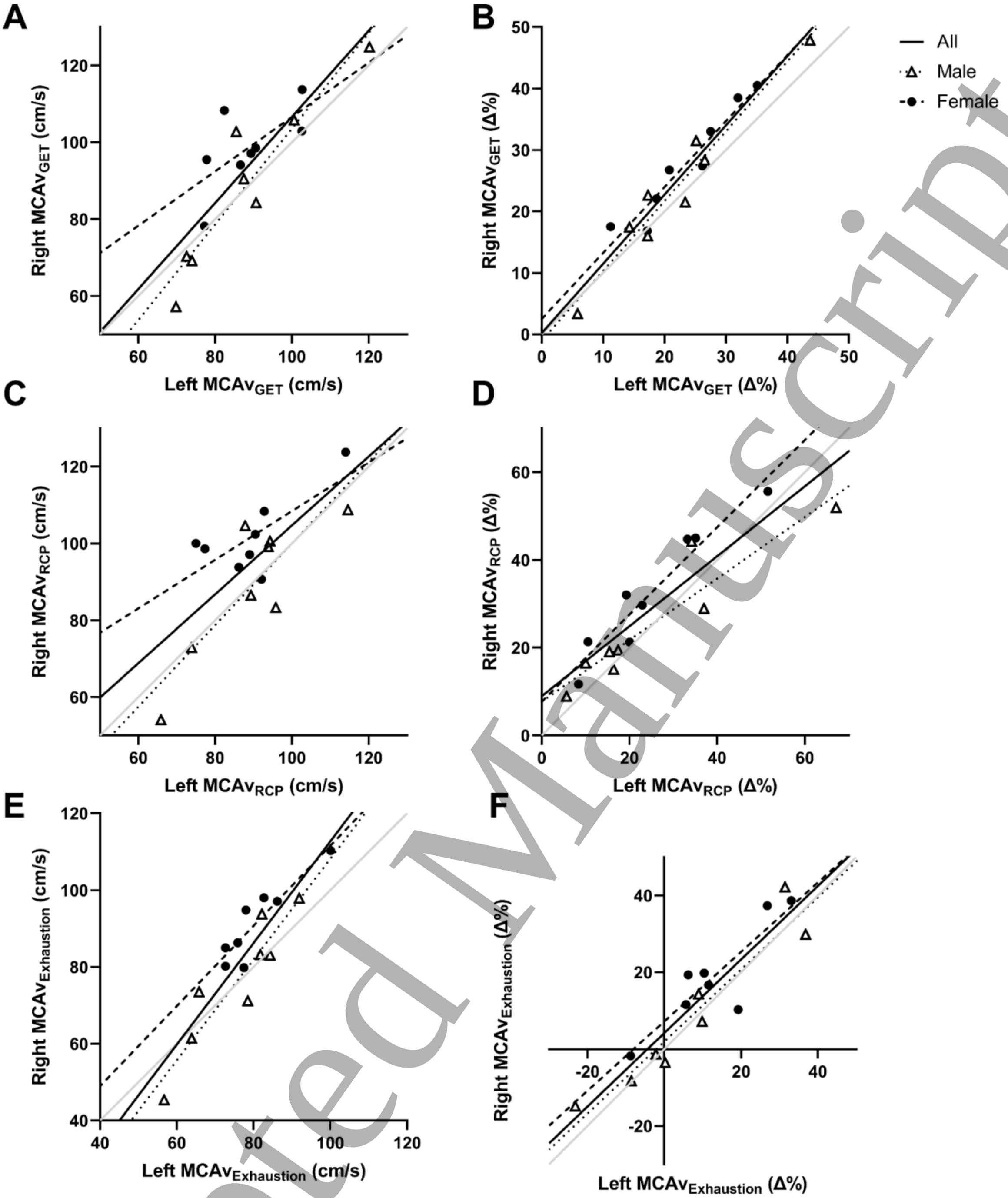

Figure 2. Line of identity plots showing the relationship between the left and right MCAv in absolute and relative terms (Δ%) at the GET (A and B), RCP (C and D) and exhaustion (E and F) during ramp incremental exercise in the whole sample (n=16), males (n=8) and females (n=8). All plots show the linear regressions (black lines) and line of identity (y=x, grey line). Correlation coefficients and values for the regression slope and intercept can be seen in Table 3.

238 **Table 3.** Pearson correlation coefficients and linear regression slope and y-intercept values for the agreement between left and right MCAv to incremental,
239 moderate and heavy-intensity exercise in the whole sample, males and females.

	All			Males			Females		
	r	Slope	Y-intercept	*r*	Slope	Y-intercept	*r*	Slope	Y-intercept
Ramp Exercise		*n=16*			*n=8*			*n=8*	
Baseline MCAv (cm/s)	**0.80***	1.00	2.4	**0.92***	1.27	-20.1	0.60	0.44	45.7
$MCAv_{GET}$ (cm/s)	**0.84***	1.12	-5.5	**0.93***	1.25	-5.5	0.66	0.71	35.8
$MCAv_{GET}$ (Δ%)	**0.96***	1.13	0.2	**0.97***	1.14	-1.1	**0.96***	1.07	2.5
$MCAv_{RCP}$ (cm/s)	**0.73***	0.90	15.0	**0.85***	1.06	-6.3	**0.73***	0.63	45.1
$MCAv_{RCP}$ (Δ%)	**0.90***	0.80	9.0	**0.93***	0.70	7.7	**0.96***	0.99	7.7
$MCAv_{Exhaustion}$ (cm/s)	**0.89***	1.32	-19.6	**0.92***	1.31	-23.0	**0.90***	1.04	7.2
$MCAv_{Exhaustion}$ (Δ%)	**0.93***	0.96	4.2	**0.95***	0.94	2.0	**0.88***	0.90	7.2
Moderate Intensity		*n=17*			*n=8*			*n=9*	
Baseline MCAv (cm/s)	**0.83***	0.67	23.4	**0.85***	0.73	19.1	**0.79***	0.58	30.7
MCAv τ (s)	**0.77***	1.17	-3.2	**0.91***	1.08	-2.8	**0.73***	1.67	-12.1
MCAv amplitude (cm/s)	**0.89***	0.79	3.9	**0.85***	0.70	4.9	**0.91***	0.84	3.3
MCAv amplitude (%)	**0.93***	0.81	5.0	**0.87***	0.86	4.4	**0.95***	0.80	4.9
MCAv TD (s)	**0.92***	0.91	-0.5	0.54	0.50	9.6	**0.99***	0.95	-1.0
$MCAv_{end}$ (cm/s)	**0.66***	0.57	36.3	**0.72***	0.64	30.3	0.51	0.42	48.9
$MCAv_{end}$ (%)	**0.94***	1.01	2.3	**0.95***	1.19	0.1	**0.95***	0.90	3.3
Heavy Intensity (n=17)		*n=17*			*n=8*			*n=9*	
Baseline MCAv (cm/s)	**0.79***	0.77	17.5	**0.90***	1.00	-0.1	**0.67***	0.53	35.1
MCAv τ (s)	**0.84***	0.76	6.2	**0.96***	0.82	4.0	**0.78***	0.74	7.3
MCAv amplitude (cm/s)	**0.97***	1.12	0.0	**0.98***	1.21	-2.7	**0.96***	1.07	1.2

MCAv amplitude (%)	**0.98***	1.01	2.7	**0.96***	1.05	1.7	**0.99***	0.96	3.3
MCAv TD (s)	**0.97***	0.76	5.8	**0.97***	0.89	2.5	**0.98***	0.71	7.3
$MCAv_{end}$ (cm/s)	**0.78***	0.98	4.3	**0.93***	1.12	-8.3	0.38	0.54	40.9
$MCAv_{end}$ (%)	**0.96***	1.02	0.8	**0.96***	0.95	3.0	**0.98***	1.06	-0.7

MCAv, middle cerebral artery blood velocity. τ, time constant. TD, time delay. MRT, mean response time. $MCAv_{end}$, MCAv at end of exercise bout. CV, coefficient of variation. **Bold** * indicates significant correlation (P<0.05).

Moderate Intensity Exercise Mean left and right MCAv kinetic parameters to moderate intensity exercise are presented in Table 2. Data for males and females separately can be found in Supplementary Tables 1 and 2, respectively. All responses were able to be modelled using the mono-exponential model, with a standard error of 2.9±1.1 s and 3.0±1.2 s for the left and right τ, respectively, and 0.3±0.2 cm/s and 0.3±0.1 cm/s for the left and right amplitude, respectively. There were no significant differences between left and right MCAv at baseline, nor the τ of the exponential increase at exercise onset (both P>0.05, *d*=0.1). The TD was greater in the left MCAv (P=0.02; *d*=0.4). The amplitude of the exponential rise following exercise onset was higher in the right MCAv compared to the left, when expressed both in absolute terms and as a relative change from baseline (both P≤0.04; *d*≤0.3). MCAv at the end of exercise was not different between left and right (P>0.05, *d*=0.3), but when expressed as a relative change from baseline, was greater in the right MCAv (P<0.01; *d*=0.3). In both males and females, the only significant left-right differences were observed in $MCAv_{end}$ (Δ%), with right MCAv significantly higher than the left (P<0.05, *d*=0.3-0.4). The CV for left *vs* right for the whole sample ranged from 18.0 to 26.2% for kinetic parameters. Figure 3 shows the line of identity plots for MCAv kinetic parameters during moderate intensity exercise in the whole sample, males and females. All parameters were strongly, positively, and significantly correlated between left and right MCAv across the whole sample (Table 3).

One statistical outlier was identified and removed from the regression analysis of MCAv TD (Figure 3b). Although not identified or removed as an outlier, a different participant was identified as having a large disagreement between left and right MCAv τ (Figure 3a). These individual left-right MCAv responses (both female) have been highlighted, and are shown in Figure 4.

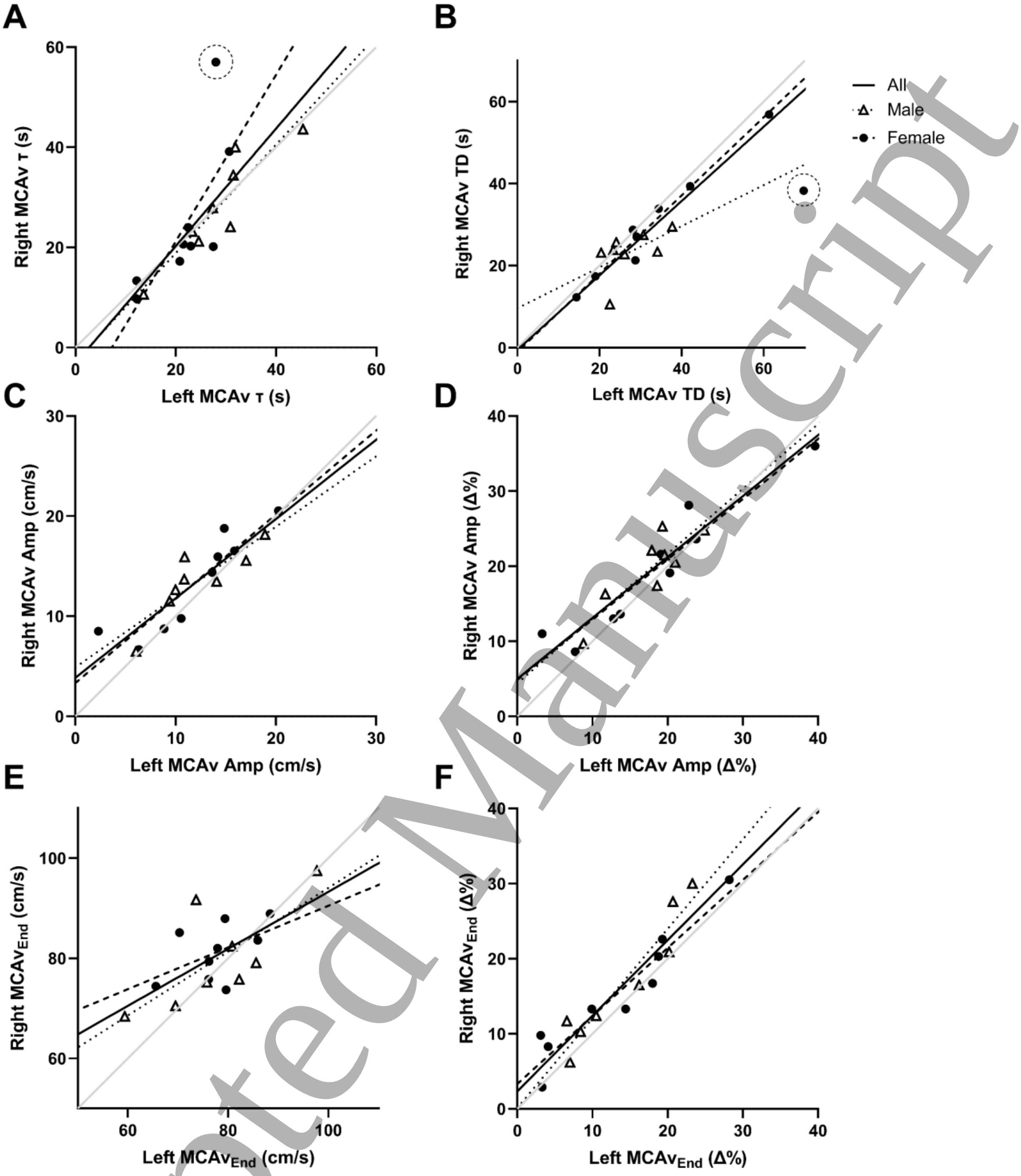

Figure 3. Line of identity plots showing the relationship between left and right MCAv time constant τ (A), time delay (B), amplitude (C – absolute, D – relative) and end-exercise amplitude (E – absolute, F – relative) during moderate intensity cycling in the whole sample (n=17), males (n=8) and females (n=9). The statistical outlier circled in (B) has been removed from the regression analyses for MCAv time delay. One individual, circled in (A), was observed to have a large disagreement between left and right MCAv time constant, but was not identified or removed as an outlier. All plots show the linear regressions (black lines) and line of identity (y=x, grey line). Correlation coefficients and values for the regression slope and intercept can be seen in Table 3.

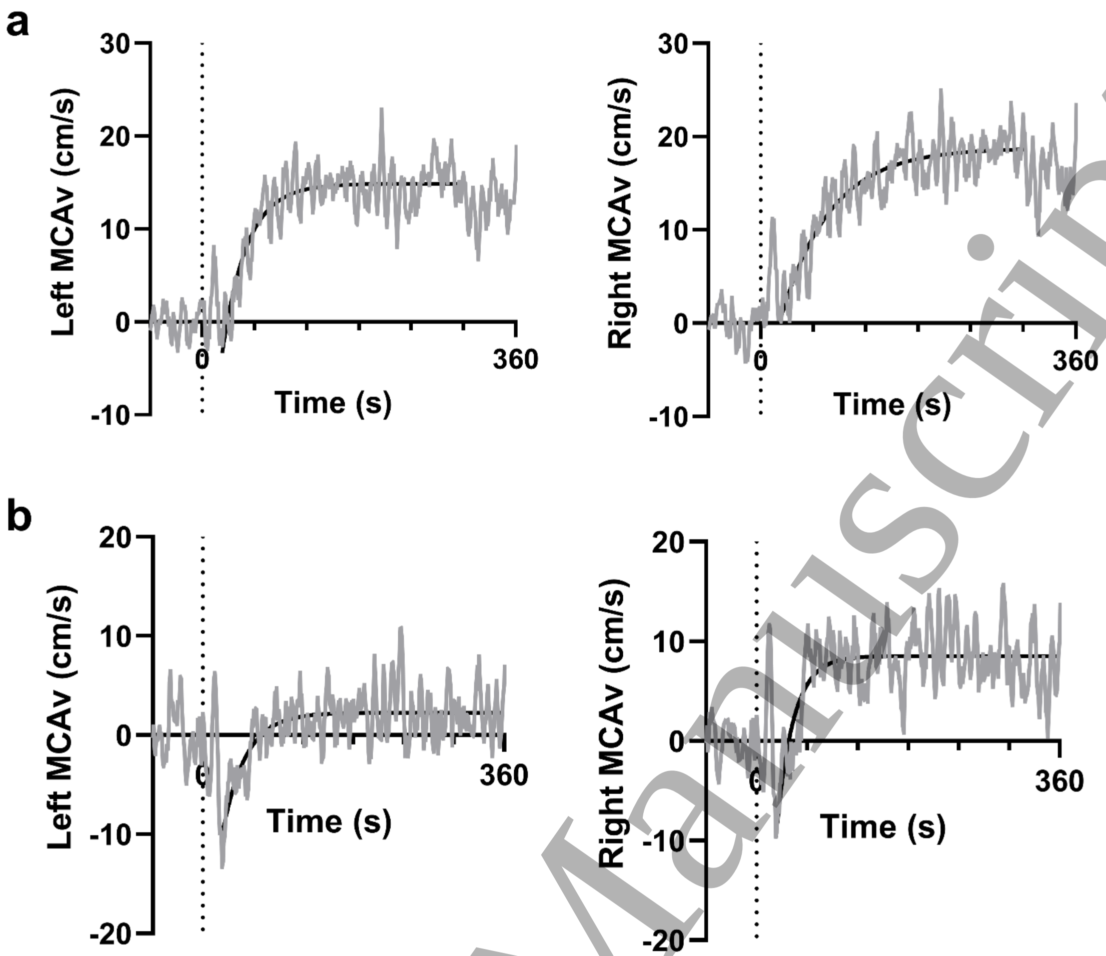

Figure 4. Left and right MCAv responses to moderate intensity cycling in two different participants (a and b) who displayed a disagreement between left and right τ (a: 28.3 *vs* 56.8 s) and TD (b: 69.7 *vs* 38.2 s). These observations were consistent across all three repeat transitions within these individuals.

Heavy Intensity Exercise Table 2 shows the mean left and right MCAv kinetic parameters to heavy intensity exercise. Data for males and females separately can be found in Supplementary Tables 1 and 2, respectively. All responses were able to be modelled using the mono-exponential model, with a standard error of 2.6±1.7 s and 2.4±1.3 s for the left and right τ, respectively, and 0.5±0.3 cm/s and 0.5±0.3 cm/s for the left and right amplitude, respectively. No significant differences were observed between left and right MCAv at baseline or end of exercise, in both absolute terms and relative to baseline (all $P>0.05$, $d\leq0.2$). The τ and TD of the exponential increase in MCAv were also similar between left and right sides (both $P>0.05$, $d\leq0.2$). The amplitude of the exponential rise was greater in the right MCAv in both absolute and relative terms (both $P<0.05$), though the effect sizes were small ($d\leq0.3$). When males and females were analysed separately, only MCAv amplitude (cm/s and Δ%) differed between left and right MCAv, with significantly higher values observed in the right (all $P<0.05$, $d \leq 0.3$). The CV of these parameters for the whole sample ranged from 7.5 to 13.9% during heavy intensity exercise The response parameters of the left and right MCAv were strongly, positively and significantly correlated across the whole sample ($r=0.78$-0.98, $P<0.01$, Table 3).

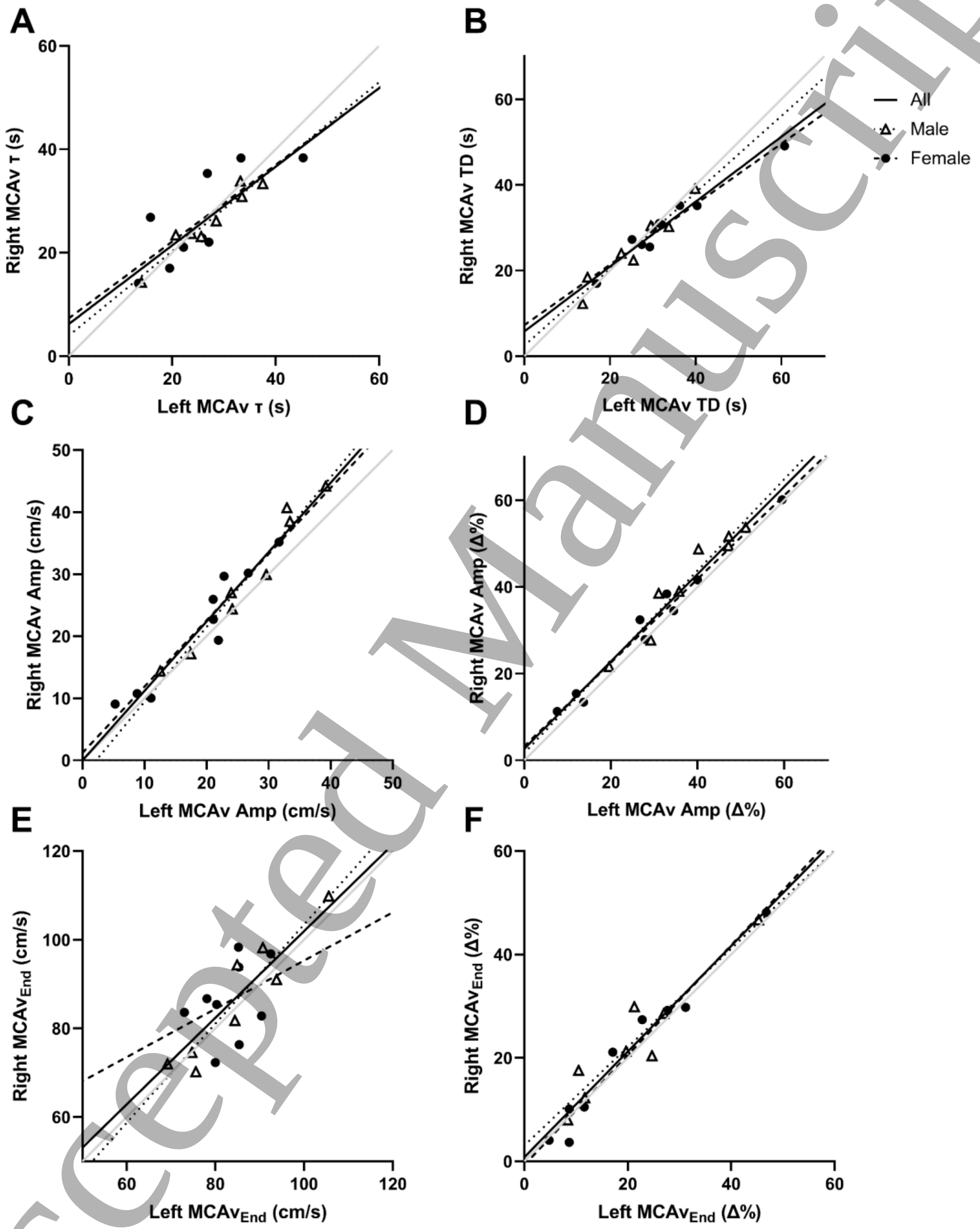

Fig 5. Line of identity plots showing the relationship between left and right MCAv time constant (A), time delay (B), amplitude (C – absolute, D – relative) and end-exercise amplitude (E – absolute, F –

relative) during heavy intensity cycling in the whole sample (n=17), males (n=8) and females (n=9). All plots show the linear regressions (black lines) and line of identity (y=x, grey line). Correlation coefficients and values for the regression slope and intercept can be seen in Table 3.

Discussion

This is the first study to investigate the agreement of left and right MCAv responses to incremental, moderate and heavy intensity exercise in healthy young adults. The main finding from this study was that a close correspondence between left and right MCAv responses to incremental and constant work-rate exercise was observed on a group level, but individual variations were observed across the measured parameters. In particular, two participants presented some distinctly different left and right MCAv response parameters to moderate-intensity exercise.

During incremental, moderate, and heavy intensity exercise, the group-averaged profile of the left and right MCAv response was similar, as can be observed in Figure 1, and all response parameters were strongly, positively and significantly correlated across the whole sample. However, the amplitude of increase in the right MCAv was greater compared to the left in both relative and absolute terms, despite no left-right differences in baseline MCAv. This was a reproducible finding, observed during incremental, moderate and heavy-intensity exercise. The reasons for this remain unclear, and it is not possible to determine if this is biological or technical variability or error, or a characteristic of the sample of the present study. In particular, when data were analysed separately for males and females, significantly greater MCAv values were observed during incremental exercise in the right *vs* left MCAv in females only. However, during constant work-rate exercise, the agreement between left and right MCAv responses were similar between males and females. Whilst this study was not powered to explore differences in the left-right MCAv agreement between the sexes, this interesting observation forms an important area of future research to investigate the potential effects of both sex and exercise protocol (incremental vs constant work-rate) on left-right MCAv agreement in males and females.

There is acknowledged variability in the anatomy of the Circle of Willis (Iqbal, 2013; Kapoor *et al.*, 2008; Thomas *et al.*, 2020), with data suggesting unilateral variations are the most common (Enyedi *et al.*, 2021). An incomplete Circle of Willis could possibly lead to compensatory increases in CBF in other arteries or regions of the brain, and without cerebral imaging data, it is not possible to determine if this is contributing to the observations of the present study. Furthermore, due to the bilateral measurement of MCAv in this study, it was also not possible to measure regional left-right differences in cerebral blood velocity, for example in the posterior cerebral artery. Given data indicating that the right vertebral artery has ~20-30% lower resting blood flow than the left, but resting flow in the internal carotid arteries are similar (Khan *et al.*, 2017; Schoning *et al.*, 1994), left-right cerebral blood velocity differences during exercise may be more pronounced in the posterior circulation.

However, it is important to note that, even when mean differences were present in left and right MCAv responses to exercise, the magnitude of these effect sizes were always small on a group level. Furthermore, the left-right amplitude differences were in the order of ~2-6 cm/s and ~2-4%. When contextualising these results with previous literature investigating differences in MCAv during exercise, intensity-dependent differences in MCAv amplitude of ~15% during moderate compared to heavy intensity exercise in adults has been observed (Weston *et al.*, 2022a), and age-related differences of ~9-14% during incremental exercise in children, adolescents and adults (Weston *et al.*, 2021). Other groups have also reported MCAv amplitude differences of 7-8 cm/s in young compared to older adults during moderate intensity exercise (Ward *et al.*, 2018). Furthermore, baseline MCAv measured in the same vessel (same side) has been shown to differ by ~3-4 cm/s within- and between-day (Koep *et al.*, 2021), which is greater than resting left-right MCAv differences observed in this study. Collectively, these data suggest that the day-to-day variation in MCAv and the intensity- and age-dependent effects on MCAv responses to exercise are greater than the magnitude of left-right mean differences observed in the present study. Therefore, left-right MCAv differences are unlikely to be confounding previous results or interpretations.

The agreement between left and right MCAv responses to exercise has only been reported once before. Billinger *et al.* (2017) were the first to model the exponential rise in MCAv at exercise onset, and observed a close correspondence of left and right MCAv MRT to moderate-intensity stepping exercise in eight healthy young adults (r=0.82, CV=7.6%). The present study builds on previous evidence by using a larger sample size, separated by sex and utilising whole-body exercise above and below the GET. On a group level, there were small mean differences during moderate and heavy intensity exercise (typically ~≤3 sec, ~≤3 cm/s and ~≤3%), with kinetic parameters showing strong, positive and significant correlations between left and right MCAv. These findings were consistent for both males and females during constant work-rate exercise.

These data support previous literature, and suggest that, on a group level, it is appropriate for future research to continue to average left and right MCAv responses to exercise. Furthermore, it also appears appropriate to use data from one MCA in young healthy adults, in instances where only unilateral measurement is possible, or when one bilateral signal is lost. However, given the presence of left-right variation on an individual basis, we would recommend that researchers remain consistent on the side in which they take MCAv measurements in studies employing repeated visits or comparing between groups, so as to limit the potential for any left-right differences to confound interpretation of such data.

An important finding of the present study was two different individuals who presented some striking differences in the left-right MCAv response parameters to moderate-intensity exercise. Both of these participants were female, which may suggest a role of sex hormones in the agreement between left and right MCAv during exercise. Importantly, these were consistent observations within these individuals, as data are taken from an ensemble-average of three repeat transitions, all performed on separate days, so these data are likely not underpinned by changes in hormones across the menstrual cycle. Furthermore, the VO_{2max} of these participants (35 and 44 ml/kg/min) falls in the middle of the range of values in the present study (24-55 ml/kg/min), and it appears unlikely that differences in fitness status are underpinning these observations. Overall, the underpinning reasons for these observations remain unclear and are likely due to underlying individual characteristics of these individuals. These data suggest that it may not be appropriate to average left and right MCAv responses in these two individuals (representing ~12% of the sample), and reinforce that researchers should be consistent in the side in which unilateral measurements are taken across repeated visits. We recommended that future research continue to collect bilateral measurements (and report how these data are handled), to better understand the prevalence of these observations, and explore the underpinning mechanisms, for example through exploring regional differences in CBF during exercise in these individuals, and coupling with cerebral imaging techniques.

Study considerations

This study has a number of methodological strengths, including utilisation of commonly used exercise protocols and analysis of data using previously published approaches. Furthermore, the use of ensemble-averaging across repeat transitions during constant work-rate exercise is an important strength of the present study to improve the signal-to-noise ratio of the responses, thus limiting the potential for technical error to be confounding the main findings. However, there are some pertinent considerations. The separate analysis of left and right MCAv during constant work-rate exercise may decrease the signal-to-noise ratio, compared to previous work that has averaged the two together, and therefore decrease the confidence of the model fits. However, the standard errors of the τ and amplitude in the present study are similar to previously published work averaging left and right MCAv together (Weston *et al.*, 2022a), suggesting appropriate confidence of the model fits in the present study. Furthermore, whilst potential effects of sex were observed during incremental exercise in the present study, this study was not powered to explore differences in left-right MCAv agreement between males and females, so these data must be interpreted with caution. It is also not known if the findings of the present study are consistent across different groups, who are known to have different resting MCAv and cerebrovascular responses to exercise. Future research investigating left-right MCAv agreement in males and females using larger sample sizes, in different populations, such as children and adolescents (Weston *et al.*, 2021) and older adults (Ward *et al.*, 2018), is warranted.

Conclusion

In conclusion, this study is the first to investigate the agreement of left and right MCAv responses to incremental and constant work-rate exercise in healthy young adults. Overall, based on these results, it appears that the assumption of averaging left and right MCAv data, and using unilateral measurements during exercise are generally appropriate on a group-level. However, these findings highlight individual variation, and recommend that researchers are consistent in the side in which they take unilateral measurements across repeated visits. In particular, two individuals (~12% of the sample) presented distinctly different characteristics of the left and right MCAv responses to moderate-intensity exercise, where caution may need to applied when averaging left and right MCAv responses.

Practical recommendations:

- We recommend that researchers collect bilateral MCAv measurements, if possible. After verifying the agreement of the responses on an individual level (using the data from the present study as a reference point), left and right MCAv data can be averaged together. If one signal is lost, it is appropriate to use the remaining (unilateral) signal.
- If only unilateral measurement is available, researchers should be consistent with the side in which they take measurements in studies that 1) utilise repeated visits within an

individual, and 2) compare different groups, so as to minimise any confounding influence of left-right variation on the main observations.

- These findings show some interesting potential sex effects, which requires further investigation on left-right MCAv agreement during exercise in males and females
- Finally, we recommend that future research reports how bilateral MCAv data are collected, handled and reported.

Acknowledgements

The authors thank all participants for their time and dedication to the study.

Funding

This work was supported by the QUEX Institute (University of Queensland and University of Exeter).

Disclosures

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

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