



Acute effects of aerobic exercise duration on blood pressure, pulse wave velocity and cerebral blood flow velocity in middle-aged adults

Sophy J. Perdomo^{1,3} · Jeffrey R. Balzer² · John M. Jakicic¹ · Christopher E. Kline¹ · Bethany Barone Gibbs¹

Received: 23 January 2019 / Accepted: 19 June 2019 / Published online: 27 June 2019
© Springer-Verlag Italia S.r.l., part of Springer Nature 2019

Abstract

Purpose It is important to understand acute dose-response relationships on cardiovascular health and brain health. Thus, we evaluated the acute effects of 10- and 30-min exercise bout on blood pressure (BP), pulse wave velocity (PWV) and cerebral blood flow velocity (CBFv).

Methods Fifteen adults (mean age 45.4 ± 8.9 years, 87% female) participated in this randomized crossover study comprised of three acute experimental sessions: a 10-min exercise bout (EX10), a 30-min exercise bout (EX30) and a sitting control (SIT). Exercise consisted of walking on a treadmill at 70–75% of age-predicted maximum heart rate. BP, PWV and CBFv were measured 30 and 60 min after each experimental session. BP was obtained at the brachial artery while PWV was measured at the carotid-femoral and carotid-radial sites. CBFv was measured at the middle cerebral artery using a 2 MHz transcranial Doppler.

Results Compared to SIT, BP was lower following EX10, and even lower following EX30 ($P < 0.05$). Though EX30 and SIT resulted in similar PWV responses ($P > 0.05$), EX10 resulted in a higher carotid-femoral PWV vs. EX30 and SIT at 30 min (both $P = 0.02$) and a lower carotid-radial PWV vs. SIT at 60 min ($P = 0.004$). CBFv did not differ across conditions (all $P > 0.05$).

Conclusions Our results suggest that 10- and 30-min aerobic exercise bouts have differential effects on BP and PWV. CBFv did not change in the hour following either bout. Further research is needed to elucidate the mechanisms and effects of 10- vs 30-min bouts of exercise.

Keywords Aerobic exercise · Blood flow velocity · Pulsatile flow · Blood pressure · Pulse wave velocity

Abbreviations

| | | | |
|-------|-------------------------------------|---------------------|--------------------------------------|
| bpm | Beats per minute | MCA | Middle cerebral artery |
| BP | Blood pressure | PI | Pulsatility Index |
| CBF | Cerebral blood flow | PP | Pulse pressure |
| CBFv | Cerebral blood flow velocity | PWV | Pulse wave velocity |
| cfPWV | Carotid-femoral pulse wave velocity | SBP | Systolic blood pressure |
| crPWV | Carotid-radial pulse wave velocity | TCD | Transcranial Doppler Ultrasonography |
| DBP | Diastolic blood pressure | VO ₂ max | Maximal oxygen consumption |
| HR | Heart rate | | |

✉ Sophy J. Perdomo
sperdomo@kumc.edu

¹ Department of Health and Physical Activity, University of Pittsburgh, Pittsburgh, PA, USA

² Department of Neurological Surgery, University of Pittsburgh, Pittsburgh, PA, USA

³ Department of Physical Therapy and Rehabilitation Science, University of Kansas Medical Center, 3901 Rainbow Blvd, Mail Stop 2002, Kansas City, KS 66160, USA

Introduction

The 2018 Physical Activity Guidelines Advisory Committee Scientific Report stated that the acute benefits of exercise on brain health and cardiovascular health were important and emphasized the need for understanding dose–response relationships on health outcomes [1]. Furthermore, the American College of Sports Medicine recommends participating in moderate-intensity physical

activity for 30 min per day for a total of 150 min per week in bouts of at least 10 continuous minutes [2].

Hypertension is a significant predictor of cardiovascular and cerebrovascular disease [3, 4], thus blood pressure (BP) reduction is important. Extensive research has shown that aerobic exercise results in an acute reduction in BP [5]. One study compared 10- and 30-min bouts and found that BP reduction was similar irrespective of duration [6]. However, a meta-analysis found that longer duration exercise elicits a greater reduction in BP following exercise [7]. Very few studies include an exercise duration of 10 min, leaving acute effects of the minimally recommended bout length poorly established. Furthermore, the acute effects of aerobic exercise on other vascular outcomes are not well understood. Aortic pulse wave velocity (PWV), for instance, is the gold standard measure of arterial stiffness and is an independent predictor of cardiovascular mortality [8, 9]. Several studies have reported a significant decrease in both aortic and peripheral PWV during this post-exercise period [10–12] while contrasting studies report no change during the hour following a bout of aerobic exercise [13–15]. Further, most studies have used 30–60-min exercise durations, resulting in uncertainty about the effects of a 10-min aerobic exercise bout on PWV.

Cerebral blood flow velocity (CBFv) is another measure of vascular health, and a measure of brain health, that is associated with cerebrovascular disease and cognition [16, 17]. The acute effects of aerobic exercise on CBFv are inconsistent, with some reporting increases following a 30-min bout and others reporting no change following 30- and 40-min bouts [18, 19]. These inconsistencies may be due to variable duration and intensity of the exercise stimulus. To the best of our knowledge, there are currently no studies evaluating the acute effects of a 10-min bout or exercise duration on CBFv. A further limitation of previous research is that the effects of exercise are often studied in a quasi-experimental pre-post design. Since CBFv has known diurnal effects [20, 21], comparing the effects of exercise to a time-matched sitting (no exercise) condition is likely important.

To address the research gaps regarding dose–response relationships on these health outcomes, the purpose of this study was to compare the acute effects of 10- and 30-min bouts of aerobic exercise on BP, PWV, and CBFv. In this study, 10- and 30-min duration of moderate-intensity aerobic exercise were based on physical activity recommendations [2] and were compared to a sitting control condition. We hypothesized that compared to sitting, BP and PWV would be lower while CBFv would be higher following an exercise bout. We further hypothesized that compared to a 10-min exercise bout, BP and PWV would be lower while CBFv would be higher following a 30-min exercise bout.

Methods

Participants

This randomized crossover study was conducted from March to May of 2017. All subjects provided written informed consent prior to any data collection. Approval for this study was granted by the Institutional Review Board at the University of Pittsburgh. Participants were recruited using flyers distributed around the University of Pittsburgh campus. Middle-aged adults were of interest due to their increased risk for arterial stiffness, which predicts sub-clinical cerebrovascular disease, cognitive decline, and increased risk of dementia later in life [22, 23]. Inclusion criteria were: 35–59 years old, willingness to watch pre-selected nature documentaries, ability to attend all four study visits and complete exercise sessions safely, and resting blood pressures < 160/100 mmHg. Participants were excluded for any of the following reasons: inability to complete exercise sessions for any reason, presence or history of cardiovascular or cerebrovascular disorders, use of medication to control heart rate (HR) or BP, or, for women, pregnancy, or attempted or planned pregnancy within the next 2 months. Women were not excluded for use of hormonal contraception. Sixteen individuals consented to participate. One participant failed to complete the protocol due to an inability to complete the 30-min exercise session; thus, fifteen adults completed the study.

Experimental procedure

Participants attended the laboratory on four separate occasions: one baseline visit and three randomly ordered, 2.5-h experimental visits. At the baseline visit, participants were familiarized with the treadmill and underwent a workload estimation. This procedure established the starting speed to reach a moderate-to-vigorous exercise intensity during the experimental sessions (specifically, 70–75% of age-predicted maximum HR calculated as 220 beats per minute (bpm) – age) [12, 24, 25].

An overview of the experimental visit protocols is displayed in Fig. 1. Consecutive visits were at least 48 h and less than 7 days apart. All visits took place between 11:40 AM and 5:00 PM. Start time was standardized within participants across the three experimental conditions to limit potential diurnal variability in outcomes [20, 26, 27]. Upon arrival at each visit, adherence to instructions to abstain from moderate-to-vigorous physical activity for 24 h, caffeine, alcohol, and nicotine for 12 h, and a 4-h fast were verbally confirmed. Then, participants rested in a supine position for 10 min and underwent baseline

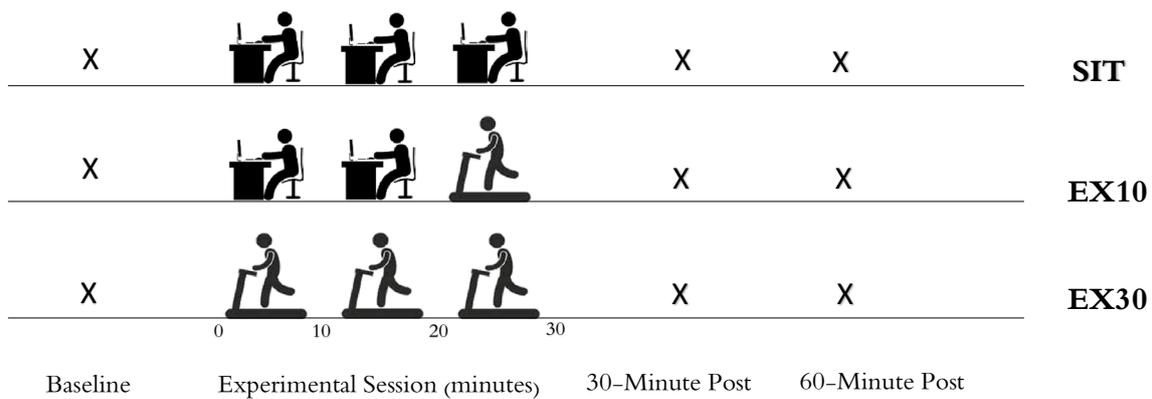


Fig. 1 Overview of experimental visits. All experimental visit followed identical protocols with the exception of the 30-min experimental session, which was either 30 min of sitting (SIT), 20 min of

sitting followed by a 10-min exercise bout (EX10) or a 30-min exercise bout (EX30). BP, TCD and PWV assessments (X) occurred prior to (baseline) and 30- as well as 60-min post-exercise session

assessments which included resting BP, Transcranial Doppler (TCD), and PWV (described in detail in Measurements). Thereafter, experimental visits followed standardized procedures for three distinct experimental sessions, as described below. Experimental sessions were standardized to be 30 min in duration. Immediately following each experimental session, participants laid supine for 1 h and underwent 30- and 60-min post-session assessments.

Seated session (SIT)

Immediately following baseline assessments, participants sat and watched a preselected, neutral documentary series from the National Geographic Channel website for 30 min. This seated session was included as control which allowed us to account for diurnal variations that are not normally accounted for in quasi-experimental studies relying on single-group, pre- to post-intervention assessments. Neutral documentary series were chosen to standardize cognitive activity as this could affect CBFv [28].

Ten-minute exercise bout (EX10)

Immediately following baseline assessments, participants watched the same preselected neutral documentary series from the National Geographic Channel website, but for only 20 min, after which they began their 10-min moderate-to-vigorous exercise bout. This was done to standardize the duration of the experimental session (30 min). The exercise bout was completed on a motorized treadmill and started at the speed and grade established at the baseline visit. Thereafter, HR was monitored each minute, with speed or grade adjusted using a standardized algorithm to maintain the target of 70–75% of age-predicted maximal HR.

Thirty-minute exercise bout (EX30)

Immediately following baseline assessments, participants walked on the same treadmill for 30 min. Also, similar to EX10, the exercise session started at the speed and grade established at the baseline visit, HR was monitored each minute and speed or grade were adjusted using the standardized algorithm to maintain the target of 70–75% of age-predicted maximal HR.

Measurements

BP, CBFv, and PWV were measured in a supine position immediately before and then 30 and 60 min after the experimental sessions during each experimental visit (Fig. 1). Timing of post-exercise assessments was chosen to be comparable to previous studies [13–15, 29–31].

Body height, weight and body mass index

Height and weight were measured at the baseline visit while the participant wore lightweight clothing and no shoes. Bodyweight was measured to the nearest 0.1 kg on a digital scale, and height was measured using a wall-mounted stadiometer.

Waist circumference

Waist circumference was measured using a Gulick measuring tape at the baseline visit while the subject was standing as previously described [32]. Two measurements were made at the end of a normal expiration and were recorded to the nearest millimeter. A third measurement was made if the

first two measurements differed by more than 1.0 cm. The average of the measurements within range were used for data analyses.

Level of physical activity

Physical activity levels were measured at the baseline visit using the Paffenbarger Exercise Habits Questionnaire for descriptive purposes [33]. Total weekly exercise minutes were calculated by adding minutes spent walking briskly and minutes spent in sport, fitness or recreational activities per week.

Cardiorespiratory fitness

Fitness was estimated for descriptive purposes using a validated fitness calculator [34]. This calculator estimates maximal oxygen consumption ($\text{VO}_{2\text{max}}$) using gender, age, height, weight, resting HR, waist circumference, and level of physical activity based on the Paffenbarger Exercise Questionnaire.

Resting blood pressure and heart rate

Supine brachial artery BP and HR were measured in duplicate by an automated blood pressure monitor (HEM-705; Omron Healthcare, Inc., Lake Forest, IL) after a 10-min rest. Pulse pressure (PP) was calculated by subtracting diastolic blood pressure (DBP) from systolic blood pressure (SBP). Pre-condition and post-exercise time course measures of BP and HR were the average of two measurements with a 1-min rest between measures.

Heart rate during exercise sessions

Participants wore a HR monitor (Polar A1; Polar Electro, Inc., Woodbury, NY) throughout each experimental visit. HR was recorded every minute during exercise sessions to evaluate intensity.

Carotid-femoral and carotid-radial pulse wave velocity

Carotid-femoral (cfPWV) and carotid-radial (crPWV) PWV were measured with the subject in a supine position using tonometry and the Complior Analyse (Alam Medical, Vincennes, France). Using a tape measure, aortic distance for cfPWV was estimated by subtracting the distance from the carotid artery site to the sternal notch from the distance of the sternal notch to the femoral artery site [35]. Carotid radial distance was estimated by measuring from the carotid artery site to the radial artery site with the arm in anatomical position [35]. Piezoelectric sensors were placed on the skin surface on the right side of the body over the carotid

and radial artery sites and over thin fabric (e.g., medical gown or similar thin fabric) on the femoral artery where the pulse was most strongly palpated. Sensors were held in place until 10 valid waveforms were captured for each scan with error $\leq 10\%$. Average carotid-radial and carotid-femoral PWV (m/s) were calculated as the distance divided by the average time differential between the foot of the waveform at the carotid and radial or femoral sites [35, 36]. Three scans were measured at each site and were averaged to reduce measurement error. All scans were performed by the principal investigator (SJP); intra-technician ICCs for PWV were 94–98%.

Cerebral blood flow velocity

While in the supine position, CBFv of the middle cerebral artery (MCA) was measured bilaterally for one minute using a 2 MHz noninvasive TCD probe (Terumo; Spencer Technologies; Redmond, WA) by the principal investigator (SJP) (intra-rater correlation coefficient of 85–97% across TCD measures). TCD is a validated measure of cerebral blood flow (CBF) [37]. For MCA insonation, the probe was placed on the skin over the temporal bone window and readings at depths of 40–65 mm were made [38]. The depth, location of the probe, and bony landmarks were recorded so that the TCD recording position remained the same in each subject and the same approach was used for each condition as well as between conditions. Quality of the measurements was rated as poor, adequate, good, or excellent based on how well the envelope captured the waveforms, the strength of the signal, the direction and depth that is consistent with MCA flow. Most scans were rated excellent or good, with no scans rated as poor. Cerebral pulsatile flow was evaluated using the pulsatility index (PI), calculated as the difference between peak systolic and diastolic flow velocities divided by mean flow velocity.

Statistical analyses

Sample size was estimated assuming 80% power, two-sided $\alpha = 0.05$, within-subjects correlation of 0.80 [21], and assuming a small between-condition effect size (SIT, EX10, or EX30) of 0.25 (corresponding to $\eta^2 = 6\%$). Given these assumptions, 12 subjects were required. Based on a 20% estimate of missing data (e.g., inability to insonate vessels), 15 subjects were recruited. Paired t-tests or Wilcoxon signed-rank tests were performed, as appropriate, to evaluate differences in parameters of experimental sessions. Linear mixed models compared baseline values across conditions and estimated effects for HR, BP, PWV, and CBFv over time and across conditions. Analyses investigated whether outcome responses (change from baseline) differed by group, time point, or as a function of group and time (i.e.,

interaction) and adjusted for the order of condition, age, and gender. CBF_v were additionally adjusted for HR. With the exception of HR, condition effects on vascular responses were evaluated separately at the 30- and 60-min post-session assessments due to non-significant interactions and typically non-linear trajectories (adjusted for baseline value, order of condition, age, and gender). To reduce the potential influence of baseline differences, all analyses evaluating pairwise differences across conditions at the 30- and 60-min post-session assessment were adjusted for baseline values. Due to the sample that was mostly females, we repeated analyses restricting to females. We further repeated PWV analyses adjusting for mean arterial BP.

Results

Participant characteristics

Fifteen adults between the ages of 36–59 years completed all research procedures. Participants had a mean (SD) age of 45.4 (8.9) years, BMI of 26.4 (4.2) kg/m² and were mostly non-Hispanic (73.3%), white (66.7%) women (87.0%). Demographic characteristics are reported in Table 1. Combining self-reported minutes spent walking briskly and minutes spent in sport, fitness or recreational activities per week, 46.7% of participants met the current American College of Sports Medicine physical activity guidelines of 150 min per week. Mean (SD) estimated cardiorespiratory fitness was 37.0 (5.3) ml/kg/min.

Comparison of exercise session parameters (EX10 vs EX30)

The mean % of age-predicted maximum HR was within the targeted 70–75% range for both the EX10 (71.1 ± 2.1%) and EX30 (73.5 ± 2.2%) exercise sessions. Despite use of the same algorithm to maintain the targeted HR through speed and grade adjustments, EX10 did result in a minimally though statistically lower session-averaged heart rate as compared to EX30 (124 ± 7 bpm vs. 128 ± 7 bpm, $P=0.001$). Neither mean speed (3.4 ± 0.2 mph vs. 3.4 ± 0.1 mph, $P=0.38$) nor grade (4.8 ± 4.1% vs. 4.7 ± 4.2%, $P=0.79$) was different in EX10 vs. EX30.

Blood pressure and heart rate

Systolic blood pressure

SBP response did not differ by condition at the 30-min post-session assessment (all $P>0.05$, Fig. 2a), but did differ by condition at the 60-min post-session assessment. Compared to SIT which tended to increase from baseline,

Table 1 Participant characteristics

| | Mean (SD) or <i>n</i> , % |
|---|---------------------------|
| Age (years) | 45.4 (8.9) |
| Height (cm) | 165.5 (10.2) |
| Weight (kg) | 73.0 (18.4) |
| Body mass index (kg/m ²) | 26.4 (4.2) |
| Waist circumference (cm) | 91.1 (12.6) |
| Gender | |
| Female | 13, 87.0% |
| Male | 2, 13% |
| Ethnicity | |
| Hispanic | 4, 26.7% |
| Non-Hispanic | 11, 73.3% |
| Race | |
| White or Caucasian | 10, 66.7% |
| Black or African American | 1, 6.7% |
| Asian | 3, 20.0% |
| Other | 1, 6.7% |
| Education | |
| High school | 1, 7.0% |
| College | 8, 53.0% |
| Post graduate | 6, 40.0% |
| Cardiorespiratory fitness and physical activity | |
| Estimated($\dot{V}O_{2max}$) (ml/kg/min)) | 37.0 (5.3) |
| Weekly exercise (minutes) | 201.0 (224.8) |

Values are reported as Mean (SD)

$\dot{V}O_{2max}$ maximal rate of oxygen consumption

SBP was -3.40 ± 1.67 mmHg in EX10 ($P=0.04$) and -7.03 ± 1.68 mmHg in EX30 ($P<0.001$) at the 60-min assessment. Further, the EX30 response was also significantly lower than that of EX10 by -3.64 ± 1.68 mmHg ($P=0.03$). A sensitivity analysis restricting to only the female participants revealed that SBP response did differ by condition at the 30-min post-session assessment. Compared to SIT, SBP was -2.86 ± 1.36 mmHg in EX10 ($P=0.04$) and -3.44 ± 1.38 mmHg in EX30 ($P=0.01$) at the 60-min assessment.

Diastolic blood pressure

DBP response did not differ by condition at the 30-min post-session assessment (all $P>0.05$, Fig. 2b), but did differ by condition at the 60-min post-session assessment. Compared to SIT which also tended to increase from baseline, a similar response was observed in EX10 ($P=0.54$). However, the EX30 response at the 60-min post-session assessment was significantly lower as compared to SIT (-3.43 ± 1.04 mmHg, $P=0.001$) and EX10 (-2.79 ± 1.03 mmHg, $P=0.01$).

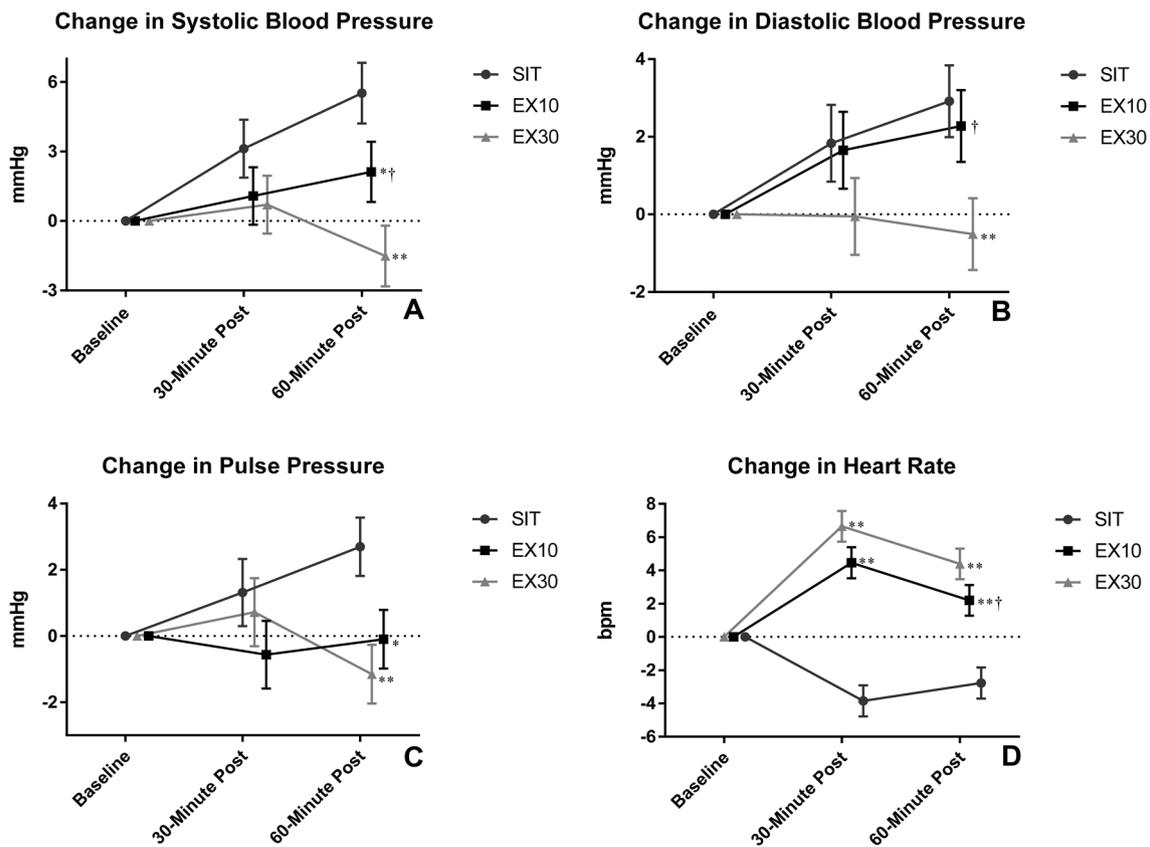


Fig. 2 Response in blood pressure and heart rate. Differences are least square means adjusted for baseline values, condition order, gender, and age. Response in **a** SBP, **b** DBP, **c** PP, and **d** HR at 30- and 60-min post-exercise session assessments. *Significantly different

from SIT ($P < 0.05$). **Significantly different from SIT ($P < 0.01$). †Significantly different from EX30 ($P < 0.05$). ††Significantly different from EX30 ($P < 0.01$)

Pulse pressure

PP response did not differ by condition at the 30-min post-session assessment (all $P > 0.05$, Fig. 2c), but did differ by condition at the 60-min post-session assessment. Compared to SIT which also tended to increase, PP response was lower by -2.79 ± 1.12 mmHg in EX10 ($P = 0.01$) and -3.85 ± 1.13 mmHg in EX30 ($P = 0.001$). PP response was similar in EX30 and EX10 ($P = 0.35$).

Heart rate

There was a small but statistically significant difference in baseline HR between EX10 and SIT ($P = 0.02$) (Table 2). There was a significant condition by time interaction ($P < 0.0001$) for HR. *Post-hoc* testing revealed the HR response in EX10 ($P = 0.001$) and EX30 ($P < 0.0001$) were significantly higher compared to SIT. Additionally, HR response in EX30 was significantly higher compared to EX10 ($P = 0.0002$). Compared to SIT, HR was higher by 8.31 ± 1.23 bpm in EX10 ($P < 0.001$) and by

10.5 ± 1.23 bpm in EX30 ($P < 0.001$) at the 30-min assessment (Fig. 2d). However, EX30 did not significantly differ from EX10 ($P = 0.07$). Compared to SIT, HR was higher by 4.98 ± 1.11 bpm in EX10 ($P < 0.001$) and by 7.16 ± 1.11 bpm in EX30 ($P < 0.001$) at the 60-min assessment. Further, HR response in EX30 was higher by 2.18 ± 1.10 ($P = 0.047$) compared to EX10.

Pulse wave velocity

Carotid-femoral pulse wave velocity

cfPWV response differed by condition at the 30-min post-session assessment (Fig. 3a). While SIT and EX30 were similar ($P = 0.95$), cfPWV response after EX10 was 0.297 ± 0.127 m/s higher vs. SIT ($P = 0.02$) and 0.305 ± 0.127 m/s higher vs. EX30 ($P = 0.02$). cfPWV responses did not differ by condition at the 60-min post-session assessment (all $P > 0.05$). Adjusting cfPWV for mean arterial BP did not alter results.

Table 2 Unadjusted Values across Conditions and Assessment Time Points

| | SIT | EX10 | EX30 |
|--|--------------|----------------|-------------|
| Systolic blood pressure (mmHg) | | | |
| Baseline | 111.3 (11.4) | 110.7 (8.2) | 108.5 (7.9) |
| 30-min post | 114.1 (11.1) | 111.6 (9.2) | 109.6 (6.7) |
| 60-min post | 116.4 (10.2) | 112.6 (9.0) | 107.5 (7.3) |
| Diastolic blood pressure (mmHg) | | | |
| Baseline | 70.0 (7.1) | 69.2 (7.4) | 68.4 (8.1) |
| 30-min post | 71.6 (7.9) | 70.7 (7.7) | 68.6 (5.9) |
| 60-min post | 72.6 (6.9) | 71.5 (6.3) | 68.2 (6.3) |
| Pulse pressure (mmHg) | | | |
| Baseline | 41.3 (6.8) | 41.5 (4.9) | 40.2 (4.9) |
| 30-min post | 42.5 (7.2) | 40.9 (5.3) | 41.0 (6.0) |
| 60-min post | 43.8 (5.9) | 41.1 (6.2) | 39.4 (4.6) |
| Heart rate (bpm) | | | |
| Baseline | 59.6 (10.9) | 62.2 (11.3)* | 61.7 (10.7) |
| 30-min post | 56.2 (9.1) | 66.5 (9.9) | 68.1 (10.1) |
| 60-min post | 57.4 (9.3) | 64.1 (8.6) | 65.8 (10.3) |
| cfPWV (m/s) | | | |
| Baseline | 6.98 (0.73) | 6.80 (0.68) | 6.93 (0.76) |
| 30-min post | 6.92 (0.74) | 7.05 (0.78) | 6.88 (0.79) |
| 60-min post | 7.08 (0.80) | 6.92 (0.73) | 7.05 (0.75) |
| crPWV (m/s) | | | |
| Baseline | 8.98 (1.04) | 8.53 (1.45) | 8.68 (1.40) |
| 30-min post | 9.05 (1.53) | 8.98 (1.14) | 8.64 (1.51) |
| 60-min post | 9.29 (1.20) | 8.22 (1.02) | 8.70 (1.18) |
| Peak MCAv (cm/s) | | | |
| Baseline | 86.7 (14.0) | 90.5 (14.0)**† | 86.4 (15.6) |
| 30-min post | 88.6 (14.2) | 92.0 (13.8) | 89.4 (14.8) |
| 60-min post | 89.4 (14.4) | 92.1 (14.3) | 88.0 (15.7) |
| Mean MCAv (cm/s) | | | |
| Baseline | 62.4 (10.7) | 65.3 (11.1)**† | 62.4 (11.3) |
| 30-min post | 63.5 (11.0) | 66.4 (11.0) | 63.9 (10.6) |
| 60-min post | 64.8 (10.8) | 67.0 (11.0) | 64.0 (11.7) |
| Diastolic MCAv (cm/s) | | | |
| Baseline | 44.5 (8.0) | 46.8 (7.5) | 44.7 (7.9) |
| 30-min post | 45.0 (7.9) | 47.5 (7.9) | 46.0 (7.0) |
| 60-min post | 45.6 (6.6) | 48.1 (7.7) | 46.1 (7.8) |
| Cerebral pulsatile flow | | | |
| Baseline | 0.68 (0.09) | 0.68 (0.08) | 0.67 (0.08) |
| 30-min post | 0.69 (0.09) | 0.68 (0.08) | 0.68 (0.10) |
| 60-min post | 0.68 (0.11) | 0.66 (0.09) | 0.66 (0.08) |

Values are reported as Mean (SD)

cfPWV carotid-femoral pulse wave velocity, crPWV carotid-radial pulse wave velocity, MCAv middle cerebral artery velocity

*Significantly different from SIT ($P < 0.05$)

†Significantly different from EX30 ($P < 0.05$). mmHg indicates millimeters of mercury

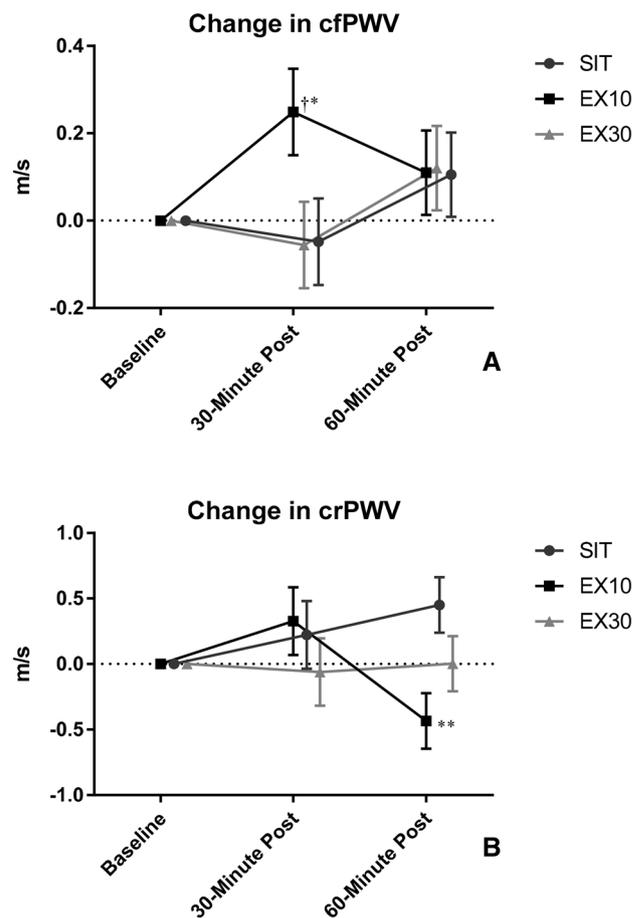


Fig. 3 Response in PWV. Differences are least square means adjusted for baseline values, condition order, gender, and age. Response in **a** cfPWV and **b** crPWV at 30- and 60-min post-exercise session assessments. *Significantly different from SIT ($P < 0.05$). **Significantly different from SIT ($P < 0.01$). †Significantly different from EX30 ($P < 0.05$)

Carotid-radial pulse wave velocity

crPWV response did not differ by condition at the 30-min post-session assessment (all $P > 0.05$, Fig. 3b). However, at the 60-min post-session assessment, crPWV response was lower by -0.883 ± 0.303 m/s after EX10 vs. SIT ($P = 0.004$). crPWV after EX30 was not different than SIT or EX10 ($P = 0.14-0.15$). Adjusting crPWV for mean arterial BP did not alter results.

Cerebral Blood Flow Velocity

There were small but statistically significant baseline differences in peak systolic ($P = 0.02$) and mean MCA CBFv ($P = 0.04$) (Table 2). Peak systolic CBFv (Fig. 4a), mean CBFv (Fig. 4b), diastolic CBFv (Fig. 4c) and PI (Fig. 4d)

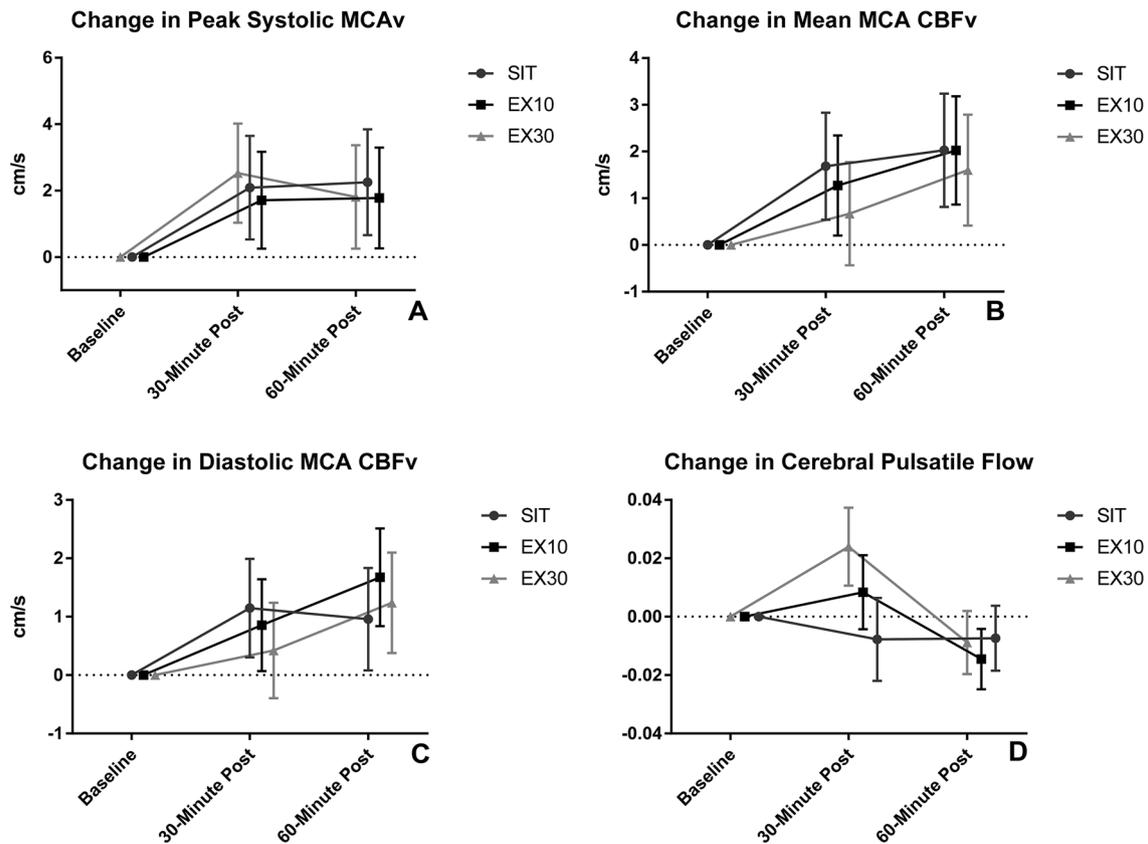


Fig. 4 Response in CBFv. Differences are least square means adjusted for baseline values, condition order, gender, and age. Response in **a** peak systolic CBFv, **b** mean CBFv, **c** diastolic CBFv, and **d** cerebral pulsatile flow at 30- and 60-min post-exercise session assessments

responses were not different at 30- or 60-min post-session assessments (all $P > 0.05$).

Discussion

The purpose of this study was to compare the acute effects of 10- and 30-min exercise bouts on BP, PWV and CBFv. We found that 10- and 30-min bouts had differential effects on BP and PWV. Compared to a sitting control condition, BP was lower 60 min following 10- and 30-min bouts of aerobic exercise, and to a greater extent after the longer, 30-min exercise bout. PWV responded similarly following sitting or a 30-min exercise bout, though responses were variable after a 10-min bout. Specifically, following the 10-min bout, cfPWV response was elevated at 30 min compared to sitting and the 30-min bout and crPWV response were significantly lower at 60 min compared sitting. Furthermore, we found that CBFv did not change significantly within an hour following exercise.

BP and PWV

SBP and DBP responded in the expected dose–response manner at both the 30- and 60-min post-session assessments. Specifically, BP increased from baseline following SIT [39] and decreased from baseline following EX30 [7]. These results, along with the dose–response effects on HR, support that our participants achieved the desired aerobic exercise stimuli. Furthermore, these results are consistent with a meta-analysis which found that a longer duration of exercise elicited a greater reduction in SBP [7]. However, our results are inconsistent with findings from the same meta-analysis that walking does not reduce DBP [7]. We found no significant differences in cfPWV at 30- or 60-min post-exercise following EX30 compared to SIT. These results are consistent with a recent systematic review finding no significant change in arterial stiffness measures 10–60 min following exercise [29].

While there were no significant differences in PWV following EX30 vs. SIT, acute effects on PWV were very different following EX10. We found cfPWV was significantly

higher 30 min following EX10 when compared to SIT and EX30. Additionally, we found crPWV to be significantly lower 60 min following EX10 when compared to SIT. The most similar study to ours, which evaluated waveforms of the carotid and radial arteries following 15 min of moderate-intensity cycling, found greater arterial stiffness (as measured by the augmentation index) 10 min post-exercise [40]. Though their study measured arterial stiffness more proximally to the exercise bout, their findings are consistent with our data of an increased cfPWV response following EX10 vs. SIT at the 30-min post-session assessment.

The mechanism accounting for these EX10-induced results is unclear. Previous studies have demonstrated that intermittent 10-min bouts of exercise are not as effective as a 30-min continuous bout at improving cardiorespiratory fitness, suggesting physiological effects differ during longer durations of continuous aerobic exercise [41, 42]. Thirty minutes of exercise may allow for true steady state and homeostasis, whereas a 10-min bout may allow for only 5–7 min of the steady state. This shorter duration of steady state may not be enough to alter the physiological properties that influence PWV [42]. Thus, a 10-min exercise bout may affect the factors involved in arterial stiffness such as nitric oxide bioavailability, other vasodilators, vasoconstrictors, inflammatory molecules, reactive oxygen species and antioxidants differently than a 30-min bout [43]. For example, the shorter duration of shear stress-induced nitric oxide release for EX10 may not be sufficient to counteract the sympathetic-induced vasoconstriction [44]. Another possible explanation may be differences in timing from the initiation of exercise. At the 30-min post-session assessment, a participant would have initiated exercise 40 min prior in EX10 vs. 60 min prior in EX30. We are not able to evaluate this possibility as we chose to anchor post-session assessments from the time of exercise completion.

Cerebral blood flow velocity

We found that the change in CBFv was not different following a 30- vs 10-min bout. These results add to inconsistent literature reporting acute increases, decreases, or no change in CBF following exercise [45–51]. Inconsistent results across studies may be due to small sample sizes, differences in neuroimaging methodology, measurement of different cerebral vessels or regions, variable duration and intensity of the exercise stimulus, and disparities in the post-exercise observation period.

Most studies finding no change in CBFv following exercise have focused on young, healthy individuals who are likely to have healthy CBF; studies with null results may reflect a ceiling effect. The lack of an acute effect of exercise on CBFv in our study could also be due to a ceiling effect as our participants, though middle-aged, were also healthy

and active on average. Our non-significant changes in CBFv could also be due to a time course where CBFv returns to baseline within 30 min post-exercise; our assessments would have been too late to detect more acute effects. Additionally, autoregulation could explain our results. Autoregulation is the ability to maintain a relatively constant supply of CBF despite changes in blood pressure and a wide range of metabolic demands from neuronal tissue. According to autoregulation, CBF should not change unless blood pressure falls outside of 60 mmHg to 150 mmHg, which was not the case in our participants at 30 or 60 min post-exercise [52]. Future research should evaluate less healthy and older populations, measure CBF more proximally to the exercise bout, and simultaneously measure different cerebral regions and vessels to improve our understanding of acute exercise effects on CBF.

Strengths and limitations

Strengths of this study include the randomized crossover design, standardization of the protocol as well as timing to account for known diurnal variations in cardiovascular and cerebrovascular measures, and recommendation-based durations of aerobic physical activity (30 and 10 min) [2]. Though the 2018 report by Physical Activity Guidelines Committee recently recommended that activity accumulated in any bout length (i.e., < 10 min) should now be counted toward weekly physical activity goals [1], characterizing responses to different exercise durations remains important for exercise prescription. We used PWV to measure arterial stiffness, which is the gold standard [36]. Additionally, we used TCD, which has been validated and has excellent temporal resolution [37], to measure CBFv of the MCA, the cerebral vessel providing flow to the largest cerebral territory. Furthermore, a sole sonographer performed all TCD and PWV measurements, thus eliminating inter-rater variability, to which TCD is particularly sensitive [53]. Another strength is our sitting control condition (SIT) to which we compared the exercise sessions. This control condition allowed us to account for diurnal variations that are not normally accounted for in quasi-experimental studies relying on single-group pre- to post-intervention assessments [20, 26, 27].

Limitations of the study include the small sample size and a lack of measurement of other factors that could affect CBFv (e.g., PaCO₂). Additionally, this was an acute laboratory study, and generalizability to a real-world setting and clinical interpretation is limited. Though a sensitivity analysis restricting to only the female participants yielded similar results (except SBP), it should be noted that most participants were female (87%). This could have affected our results, given the known sex differences in CBFv [54]. Moreover, we did not standardize the phase of menstrual

cycle or use of hormones, which could also have affected our results [55]. Vascular outcomes were assessed at limited time points (30- and 60-min post-exercise sessions), thus potentially missing changes that could be captured with more frequent assessments and using a longer observation period. Another limitation is that we anchored the intensity of the exercise by age-predicted maximum HR rather than individualized intensities based on a cardiopulmonary exercise test. Though age-predicted maximum HR can have large prediction error, it is widely used in exercise prescription and likely resulted in moderate-to-vigorous intensity exercise in our subjects [56]. Regarding TCD measurements, TCD assesses CBFv, which is an indirect measure of CBF and does not account for vessel diameter [57]. Vessel diameter is thought not to change with exercise; however, this has not been well-established [37, 58, 59]. The experimental arrangement (i.e., abstinence from food and caffeine prior to exercise, the time of day, and known effects following acute exercise) resulted in many participants becoming sleepy during the supine post-exercise observation period. This could have affected results as vascular testing is recommended to be performed while participants are awake [8, 36] and CBFv are known to be altered by a subject's level of wakefulness [60].

Conclusions

In summary, we found that BP was lower in the hour following a 10-min and to a greater extent, a 30-min bout of aerobic exercise as compared to sitting. In contrast, these data do not support an acute decrease in PWV following a 30-min exercise bout, though differential effects were observed following a 10-min bout. Elucidating mechanisms and effects of 10- versus 30-min aerobic exercise bouts on BP and PWV warrants further research. Additionally, based on our results, aerobic exercise does not have acute effects on MCA CBFv within the hour following either a 10-min or 30-min exercise bout compared to sitting. These findings should be confirmed in other brain regions or in less healthy populations to improve understanding of whether exercise can acutely benefit CBFv.

Acknowledgements The authors would like to thank the University of Pittsburgh's School of Education Student Research Grant for funding this study as well as the University of Pittsburgh's K. Leroy Irvis Fellowship and the University of Kansas Alzheimer's Disease Center (P30 AG035982) for supporting Dr. Perdomo's time. The authors would also like to thank our research assistants Nanami Mano, Tayler Magda, Celina Cantini and Elliot Fisher for their dedication to this study as well as our participants without which this study would not have been possible.

Compliance with ethical standards

Conflict of interest Dr. Balzer reports no conflicts of interest. Dr. Perdomo discloses research funding from the National Institute on Aging and the University of Kansas Alzheimer's Disease Center. Dr. Barone Gibbs discloses research funding from the National Institutes of Health, Tomayko Foundation, Humanscale, the Virginia Kaufman Fund and the American Heart Association. Dr. Jakicic discloses his position on the scientific advisory board for Weight Watchers International and research funding from the National Institutes of Health, Weight Watchers International, and Humanscale. Dr. Kline discloses research funding from the National Institutes of Health.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Written informed consent was obtained from all participants.

References

1. Physical Activity Guidelines Advisory Committee (2018) Physical Activity Guidelines Advisory Committee Scientific Report. US Department of Health and Human Services, Washington, DC
2. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee I-M, Nieman DC, Swain DP (2011) American College of Sports Medicine position stand Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med Sci Sports Exerc* 43(7):1334–1359
3. Iadecola C, Davisson RL (2008) Hypertension and cerebrovascular dysfunction. *Cell Metab* 7(6):476–484
4. Wang W, Lee ET, Fabsitz RR, Devereux R, Best L, Welty TK, Howard BV (2006) A longitudinal study of hypertension risk factors and their relation to cardiovascular disease: the strong heart study. *Hypertension* 47(3):403–409
5. Halliwill JR (2001) Mechanisms and clinical implications of post-exercise hypotension in humans. *Exerc Sport Sci Rev* 29(2):65–70
6. MacDonald J, MacDougall J, Hogben C (2000) The effects of exercise duration on post-exercise hypotension. *J Hum Hypertens* 14(2):125
7. Carpio-Rivera E, Moncada-Jiménez J, Salazar-Rojas W, Solera-Herrera A (2016) Acute effects of exercise on blood pressure: a meta-analytic investigation. *Arq Bras Cardiol* 106(5):422–433
8. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank J, De Backer T, Filipovsky J, Huybrechts S, Mattace-Raso FU, Protogerou AD (2012) Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens* 30(3):445–448
9. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A (2001) Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertens* 37(5):1236–1241
10. Kingwell BA, Berry KL, Cameron JD, Jennings GL, Dart AM (1997) Arterial compliance increases after moderate-intensity cycling. *Am J Physiol Heart Circ Physiol* 273(5):H2186–H2191
11. Heffernan K, Collier S, Kelly E, Jae S, Fernhall B (2007) Arterial stiffness and baroreflex sensitivity following bouts of aerobic and resistance exercise. *Int J Sports Med* 28(03):197–203

12. Sugawara J, Komine H, Miyazawa T, Imai T, Ogoh S (2015) Influence of single bout of aerobic exercise on aortic pulse pressure. *Eur J Appl Physiol* 115(4):739–746
13. Heffernan KS, Jae SY, Echols GH, Lepine NR, Fernhall B (2007) Arterial stiffness and wave reflection following exercise in resistance-trained men. *Med Sci Sports Exerc* 39(5):842–848
14. Munir S, Jiang B, Guilcher A, Brett S, Redwood S, Marber M, Chowienicz P (2008) Exercise reduces arterial pressure augmentation through vasodilation of muscular arteries in humans. *Am J Physiol Heart Circ Physiol* 294(4):H1645–H1650
15. Gkaliagkousi E, Gavriilaki E, Nikolaidou B, Triantafyllou G, Douma S (2014) Exercise-induced pulse wave velocity changes in untreated patients with essential hypertension: the effect of an angiotensin receptor antagonist. *J Clin Hypertens* 16(7):482–487
16. Bai CH, Chen JR, Chiu HC, Pan WH (2007) Lower blood flow velocity, higher resistance index, and larger diameter of extracranial carotid arteries are associated with ischemic stroke independently of carotid atherosclerosis and cardiovascular risk factors. *J Clin Ultrasound* 35(6):322–330
17. Hartje W, Ringelstein EB, Kisting B, Fabianek D, Willmes K (1994) Transcranial Doppler ultrasonic assessment of middle cerebral artery blood flow velocity changes during verbal and visuospatial cognitive tasks. *Neuropsychologia* 32(12):1443–1452
18. Willie CK, Ainslie PN, Taylor CE, Eves ND, Tzeng Y-C (2013) Maintained cerebrovascular function during post-exercise hypotension. *Eur J Appl Physiol* 113(6):1597–1604
19. Hellstrom G, Fischer-Colbrie W, Wahlgren N, Jogestrand T (1996) Carotid artery blood flow and middle cerebral artery blood flow velocity during physical exercise. *J Appl Physiol* 81(1):413–418
20. Conroy DA, Spielman AJ, Scott RQ (2005) Daily rhythm of cerebral blood flow velocity. *J Circadian Rhythms* 3(1):3
21. Perdomo SJ, Gibbs BB, Kowalsky RJ, Taormina JM, Balzer JR (2019) Effects of alternating standing and sitting compared to prolonged sitting on cerebrovascular hemodynamics. *Sport Scie Health* :1–9
22. Tsao CW, Himali JJ, Beiser AS, Larson MG, DeCarli C, Vasan RS, Mitchell GF, Seshadri S (2016) Association of arterial stiffness with progression of subclinical brain and cognitive disease. *Neurology* 86(7):619–626
23. Debette S, Seshadri S, Beiser A, Au R, Himali J, Palumbo C, Wolf P, DeCarli C (2011) Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. *Neurology* 77(5):461–468
24. Fox S 3rd, Naughton JP, Haskell WL (1971) Physical activity and the prevention of coronary heart disease. *Ann Clin Res* 3(6):404–432
25. Perdomo SJ, Moody AM, McCoy SM, Barinas-Mitchell E, Jakicic JM, Gibbs BB (2016) Effects on carotid–femoral pulse wave velocity 24 h post exercise in young healthy adults. *Hypertens Res* 39(6):435
26. Millar-Craig M, Bishop C, Raftery E (1978) Circadian variation of blood-pressure. *Lancet* 311(8068):795–797
27. Bodlaj G, Berg J, Biesenbach G (2007) Diurnal variation of pulse wave velocity assessed non-invasively by applanation tonometry in young healthy men. *Yonsei Med* 48(4):665–670
28. Stroobant N, Vingerhoets G (2000) Transcranial Doppler ultrasonography monitoring of cerebral hemodynamics during performance of cognitive tasks: a review. *Neuropsychol Rev* 10(4):213–231
29. Mutter AF, Cooke AB, Saleh O, Gomez Y-H, Daskalopoulou SS (2016) A systematic review on the effect of acute aerobic exercise on arterial stiffness reveals a differential response in the upper and lower arterial segments. *Hypertens Res* 40(2):146
30. Chandrakumar D, Boutcher S, Boutcher Y (2015) Acute exercise effects on vascular and autonomic function in overweight men. *J Sports Med Phys Fitness* 55(1–2):91–102
31. Akazawa N, Ra S-G, Sugawara J, Maeda S (2015) Influence of aerobic exercise training on post-exercise responses of aortic pulse pressure and augmentation pressure in postmenopausal women. *Front Physiol* 6:268
32. Centers for Disease Control and Prevention (2007). National health and nutrition examination survey data: anthropometry procedures manual. https://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_an.pdf. Accessed 9 Sep 2016)
33. Paffenbarger R, Wing A, Hyde R (1978) Paffenbarger physical activity questionnaire. *Am J Epidemiol* 108(3):161–175
34. Nes BM, Janszky I, Vatten LJ, Nilsen TIL, Aspenes ST, Wisløff U (2011) Estimating V·O₂ peak from a nonexercise prediction model: the HUNT Study, Norway. *Med Sci Sports Exer* 43(11):2024–2030
35. Weber T, Ammer M, Rammer M, Adji A, O'Rourke MF, Wassertheurer S, Rosenkranz S, Eber B (2009) Noninvasive determination of carotid–femoral pulse wave velocity depends critically on assessment of travel distance: a comparison with invasive measurement. *J Hypertens* 27(8):1624–1630
36. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 27(21):2588–2605
37. Kontos HA (1989) Validity of cerebral arterial blood flow calculations from velocity measurements. *Stroke* 20(1):1–3
38. Alexandrov AV, Sloan MA, Wong LK, Douville C, Razumovsky AY, Koroshetz WJ, Kaps M, Tegeler CH (2007) Practice standards for transcranial Doppler ultrasound: part I—test performance. *J Neuroimaging* 17(1):11–18
39. Vranish JR, Young BE, Kaur J, Patik JC, Padilla J, Fadel PJ (2017) Influence of sex on microvascular and macrovascular responses to prolonged sitting. *Am J Physiol Heart Circ Physiol* 312(4):H800–H805
40. Payne RA, Teh CH, Webb DJ, Maxwell SR (2007) A generalized arterial transfer function derived at rest underestimates augmentation of central pressure after exercise. *J Hypertens* 25(11):2266–2272
41. Jakicic JM, Wing R, Butler B, Robertson R (1995) Prescribing exercise in multiple short bouts versus one continuous bout: effects on adherence, cardiorespiratory fitness, and weight loss in overweight women. *Int J Obes Relat Metab Disord* 19(12):893–901
42. Church TS, Earnest CP, Skinner JS, Blair SN (2007) Effects of different doses of physical activity on cardiorespiratory fitness among sedentary, overweight or obese postmenopausal women with elevated blood pressure: a randomized controlled trial. *JAMA* 297(19):2081–2091
43. Miura H (2012) Arterial function during various acute exercises. *J Sports Med Phys Fitness* 1(4):605–610
44. Aggio A, Grassi D, Onori E, D'Alessandro A, Masedu F, Valenti M, Ferri C (2013) Endothelium/nitric oxide mechanism mediates vasorelaxation and counteracts vasoconstriction induced by low concentration of flavanols. *Eur J Nutr* 52(1):263–272
45. Robertson AD, Crane DE, Rajab AS, Swardfager W, Marzolini S, Shirzadi Z, Middleton LE, MacIntosh BJ (2015) Exercise intensity modulates the change in cerebral blood flow following aerobic exercise in chronic stroke. *Exp Brain Res* 233(8):2467–2475
46. Hellstrom G, Fischer-Colbrie W, Wahlgren N, Jogestrand T (1996) Carotid artery blood flow and middle cerebral artery blood flow velocity during physical exercise. *J Appl Physiol* 81(1):413–418
47. MacIntosh BJ, Crane DE, Sage MD, Rajab AS, Donahue MJ, McIlroy WE, Middleton LE (2014) Impact of a single bout of aerobic exercise on regional brain perfusion and activation responses in healthy young adults. *PLoS ONE* 9(1):e85163

48. Williamson J, Querry R, Mccoll R, Mathews D (2009) Are decreases in insular regional cerebral blood flow sustained during postexercise hypotension? *Med Sci Sports Exerc* 41(3):574
49. Williamson JW, McColl R, Mathews D (2004) Changes in regional cerebral blood flow distribution during postexercise hypotension in humans. *J Appl Physiol* 96(2):719–724
50. Willie CK, Ainslie PN, Taylor CE, Eves ND, Tzeng Y-C (2013) Maintained cerebrovascular function during post-exercise hypotension. *Eur J Appl Physiol* 113(6):1597–1604
51. Ainslie PN, Barach A, Murrell C, Hamlin M, Hellemans J, Ogoh S (2007) Alterations in cerebral autoregulation and cerebral blood flow velocity during acute hypoxia: rest and exercise. *American Journal of Physiology-Heart and Circulatory Physiology* 292(2):H976–H983
52. Paulson O, Strandgaard S, Edvinsson L (1990) Cerebral autoregulation. *Cerebrovasc Brain Metab Rev* 2(2):161–192
53. Babikian VL, Wechsler LR (1999) *Transcranial doppler ultrasonography*. Butterworth-Heinemann Medical, Oxford
54. Vriens E, Kraaier V, Musbach M, Wieneke G, Van Huffelen A (1989) Transcranial pulsed Doppler measurements of blood velocity in the middle cerebral artery: reference values at rest and during hyperventilation in healthy volunteers in relation to age and sex. *Ultrasound Med Biol* 15(1):1–8
55. Brackley K, Ramsay M, Pipkin FB, Rubin P (1999) The effect of the menstrual cycle on human cerebral blood flow: studies using Doppler ultrasound. *Ultrasound Obstet Gynecol* 14(1):52–57
56. Robergs RA, Landwehr R (2002) The surprising history of the “ $HR_{max} = 220 - \text{age}$ ” equation. *Journal of Exercise Physiology Online* 5(2):1–10
57. Naqvi J, Yap KH, Ahmad G, Ghosh J (2013) Transcranial Doppler ultrasound: a review of the physical principles and major applications in critical care. *Int J Vasc Med* 2013
58. Brothers RM, Zhang R (2016) CrossTalk opposing view: the middle cerebral artery diameter does not change during alterations in arterial blood gases and blood pressure. *J Physiol* 594(15):4077–4079
59. Hoiland RL, Ainslie PN (2016) CrossTalk proposal: the middle cerebral artery diameter does change during alterations in arterial blood gases and blood pressure. *J Physiol* 594(15):4073–4075
60. Kuboyama T, Hori A, Sato T, Mikami T, Yamaki T, Ueda S (1997) Changes in cerebral blood flow velocity in healthy young men during overnight sleep and while awake. *Electroencephalogr Clin Neurophysiol* 102(2):125–131

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.