



OPEN

## Ventilatory response to hypercapnia is increased after 4 h of head down bed rest

K. R. Murray<sup>1,3</sup>, S. Wasef<sup>1,3</sup> & Heather Edgell<sup>1,2</sup>✉

Head-down bed rest (HDBR) has previously been shown to alter cerebrovascular and autonomic control. Previous work found that sustained HDBR ( $\geq 20$  days) attenuates the hypercapnic ventilatory response (HCVR); however, little is known about shorter-term effects of HDBR nor the influence of HDBR on the hypoxic ventilatory response (HVR). We investigated the effect of 4-h HDBR on HCVR and HVR and hypothesized attenuated ventilatory responses due to greater carotid and brain blood flow. Cardiorespiratory responses of young men ( $n = 11$ ) and women ( $n = 3$ ) to 5%  $\text{CO}_2$  or 10%  $\text{O}_2$  before and after 4-h HDBR were examined. HDBR resulted in lower HR, lower cardiac output index, lower common carotid artery flow, higher  $\text{SpO}_2$ , and higher pulse wave velocity. After HDBR, tidal volume and ventilation responses to 5%  $\text{CO}_2$  were enhanced (all  $P < 0.05$ ), yet no other changes in cardiorespiratory variables were evident. There was no influence of HDBR on the cardiorespiratory responses to hypoxia (all  $P > 0.05$ ). Short-duration HDBR does not alter the HVR, yet enhances the HCVR, which we hypothesize is a consequence of cephalic  $\text{CO}_2$  accumulation from cerebral congestion.

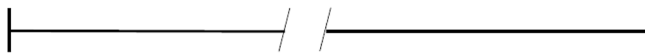
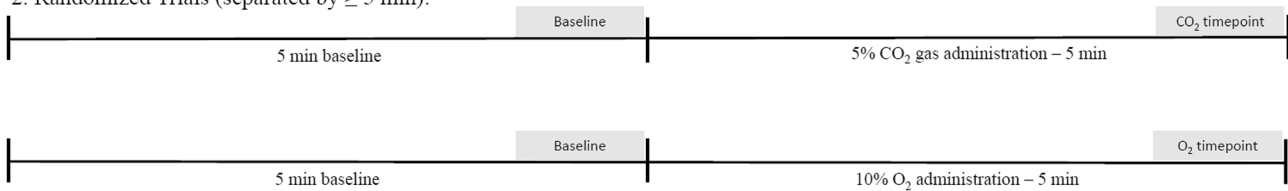
As little as 4 h of head-down bed rest (HDBR) has been used to study the cardiovascular effects of simulated microgravity<sup>1–3</sup>. Short-duration HDBR mimics the cephalic fluid shift that occurs during exposure to microgravity<sup>4,5</sup> and results in reduced indices of venous return<sup>6</sup>, decreased plasma volume<sup>3,7</sup>, and an increased sympathetic influence on heart rate<sup>8</sup>. Autonomic function after HDBR could therefore be influenced by changes in multiple reflexes such as the baroreflex, chemoreflexes, or their interactions. We have recently found that unloading the baroreceptors acutely during 5 min upright tilt can augment the ventilatory response to breathing 5%  $\text{CO}_2$ <sup>9</sup> suggesting that the HDBR posture (i.e. loading the baroreceptors) may attenuate the chemoreflex. Indeed, longer duration HDBR studies have found lower  $\text{CO}_2$  chemosensitivity after 20 or 120 days of HDBR<sup>10,11</sup>. These interactions could also be important to note from a clinical perspective as use of intraabdominal  $\text{CO}_2$  insufflations during surgery in 30–40° head-down posture (i.e., Trendelenburg position) increases arterial  $\text{PCO}_2$  within 1–2 h<sup>12–14</sup>, likely stimulating central chemoreceptors, and influencing autonomic control<sup>15</sup>.

Respiratory control appears to be influenced by complex interactions between the baroreflex, chemoreflex, and postural fluid shifts. Changes in carotid body blood flow may influence the function of the peripheral chemoreceptors. Indeed, carotid artery occlusion and therefore lower carotid body blood flow has been shown to increase the sensitivity of the hypoxic chemoreflex response in animals<sup>16</sup>. Further, reductions of blood pressure have been noted to increase aortic and carotid chemoreceptor firing in animal models<sup>17–19</sup>. These studies in animal models suggest that increased carotid body blood flow may decrease the sensitivity of the hypoxic chemoreflex in humans.

The primary objective of our study was to investigate the impact of 4-h HDBR on the hypercapnic ventilatory response (HCVR) and to determine if changes in brain blood flow were influential. Further, since hypercapnia induced hyperventilation increases the partial pressure of arterial oxygen, and alterations in carotid flow have been shown to alter peripheral chemoreflex sensitivity<sup>9,16,20–22</sup>, our secondary objective was to assess the hypoxic ventilatory response (HVR) in response to 4-h HDBR. Indeed, Prisk et al. found that 16 days of microgravity exposure attenuated the ventilatory response to hypoxia<sup>23</sup>. Therefore, we hypothesized that 4-h HDBR would attenuate both HCVR and HVR.

<sup>1</sup>School of Kinesiology and Health Science, York University, 355 Bethune College, 4700 Keele St, Toronto, ON M3J 1P3, Canada. <sup>2</sup>Muscle Health Research Centre, York University, Toronto, ON, Canada. <sup>3</sup>These authors contributed equally: K. R. Murray and S. Wasef. ✉email: edgell@yorku.ca

## 1. Set-up and familiarization (~30 min):

2. Randomized Trials (separated by  $\geq 5$  min):

**Figure 1.** Depiction of protocol completed before and after completion of 4-h HDBR. Grey areas indicate where the data were used to calculate the  $\Delta$  cardiorespiratory and cerebrovascular response to the respective gas administration.

## Methods

**Ethical approval.** All participants completed written informed consent, procedures were approved by the Office of Research Ethics at York University (ORE #e2018-029), and experiments were in accordance with the Declaration of Helsinki.

**Participant description.** Eleven males and three females ( $23 \pm 5$  years,  $170.4 \pm 10.7$  cm,  $75.3 \pm 17.1$  kg) completed this study. Women were tested on day 8–11 of their menstrual cycle (i.e. late follicular phase to ensure a pre-ovulatory phase, when estrogen is present without progesterone, while avoiding menses), determined by self-report. All participants were free from previously diagnosed cardiovascular or respiratory conditions. Participants were instructed to abstain from fatty foods, alcohol, heavy exercise, smoking/vaping, and caffeine for 12 h prior to testing. They were also asked to consume a breakfast prior to arriving at the testing facility which consisted of 400 cal, 400 mg of sodium and 250 mL of water. Participants came for a single laboratory visit, which spanned approximately 7 h.

**Protocol.** Prior to HDBR participants completed two randomized gas mixture breathing trials to assess baseline chemoreflex function in the supine position (Fig. 1). Each trial included a 5 min baseline period of breathing room air follow by a 5 min period of breathing a gas mixture. Tests were separated by at least 5 min to ensure that cardiorespiratory data returned to baseline. Gas tank mixtures included (1) hypercapnia (5% CO<sub>2</sub>, 21% O<sub>2</sub>, balance nitrogen); and (2) hypoxia (10% O<sub>2</sub>, balance nitrogen) and were administered via a Douglas bag. Participants were blinded to the identity of the gas being administered, and the hypoxia test would have been terminated if arterial O<sub>2</sub> saturation (SpO<sub>2</sub>) dropped below 80%, however, this did not occur. Participants then completed 4hrs of 6° HDBR during which they were supervised to ensure their head remained at a relative position below their feet. After HDBR, participants were briefly upright (~10 s) when transferring between the 6° angled bed and the supine testing bed, after which they again completed randomized gas mixture breathing tests.

**Cardiorespiratory measurements.** A standard 3-lead electrocardiogram (ECG) was used to continuously measure heart rate (HR) (ADInstruments, Colorado Springs, USA). Beat-by-beat continuous blood pressure and stroke volume (SV) were measured using finger plethysmography (Nexfin, Amsterdam, Netherlands). Blood pressure was measured at the beginning of each trial using a BPTru device (Coquitlam, Canada) for subsequent offline calibration of the continuous measurements. Cardiac output (Q) was calculated as the product of HR and SV, and total peripheral resistance (TPR) was calculated as the quotient of mean arterial pressure (MAP) and Q. Changes in cardiac output as measured by the Modelflow algorithm have been shown to be correlated to changes in cardiac output as measured by Doppler echocardiography<sup>24</sup>. Using the Dubois and Dubois formula<sup>25</sup> SV (and therefore Q and TPR) was normalized to body surface area to calculate their respective indices (Qi, SVi, TPRi, respectively). A heated, linear pneumotachometer (Hans Rudolph Inc, Shawnee Mission, USA) was used to collect ventilatory data and was calibrated using a 3L syringe before each test. Tidal volume (V<sub>T</sub>) was calculated as the volume inspired, and respiration rate (RR) was calculated as the rate of inspiration. Ventilation (V<sub>E</sub>) was calculated as the product of RR and V<sub>T</sub>. End-tidal oxygen (ETO<sub>2</sub>) and carbon dioxide (ETCO<sub>2</sub>) were measured using O<sub>2</sub> and CO<sub>2</sub> analyzers (Vacumed, Ventura USA), and SpO<sub>2</sub> was measured using finger pulse oximetry (Nexfin, Amsterdam, Netherlands).

**Carotid and cerebrovascular measurements.** Transcranial Doppler (TCD) ultrasound (Multigon Industries, Elmsford, USA) was used to measure blood velocity through the right middle cerebral artery (MCA) using a 2-MHz probe. Cerebrovascular resistance index (CVRI) was calculated as the quotient of MAP and MCA mean velocity. Resistance index (RI) and pulsatility index (PI) were calculated as (MCA systolic velocity – MCA diastolic velocity) normalized to either MCA systolic velocity or MCA mean velocity, respectively. Longitudinal common carotid artery (CCA) diameter and blood velocity were measured using duplex ultrasonography (GE Medical Systems, Mississauga, Canada). CCA flow was calculated as the product of end-diastolic cross-sectional

	Baseline	After HDBR	P-value
HR (bpm)	64 ± 9	62 ± 9*	0.03
MAP (mmHg)	81 ± 12	85 ± 9	0.19
SVi (mL/m <sup>2</sup> )	62 ± 7	57 ± 9	0.09
Qi (L/min/m <sup>2</sup> )	4.0 ± 0.7	3.5 ± 0.9*	0.03
TPRi (mmHg/L/min/m <sup>2</sup> )	21 ± 4	26 ± 9	0.06
MCA mean velocity (cm/s)	64 ± 10	69 ± 6	0.14
CVRi (mmHg/cm/s)	1.3 ± 0.3	1.3 ± 0.2	0.83
RI	0.59 ± 0.09	0.57 ± 0.06	0.49
PI	0.95 ± 0.23	0.89 ± 0.17	0.36
CCA flow (mL/min)	481 ± 96	412 ± 73*	<0.001
RR (bpm)	15 ± 4	16 ± 3	0.28
V <sub>T</sub> (L)	0.9 ± 0.2	0.9 ± 0.3	0.58
V <sub>E</sub> (L/min)	13 ± 2	14 ± 4	0.75
ETO <sub>2</sub> (mmHg)	106 ± 9	105 ± 7	0.63
ETCO <sub>2</sub> (mmHg)	43 ± 5	43 ± 5	0.68
SpO <sub>2</sub> (%)	98 ± 1	99 ± 1*	0.007

**Table 1.** Cardiorespiratory and cerebrovascular baseline comparisons before and after HDBR. HR heart rate, MAP mean arterial pressure, SVi stroke volume index, Qi cardiac index, TPRi total peripheral resistance, MCA middle cerebral artery, CVRi cerebrovascular resistance index, RI resistance index, PI pulsatility index, CCA common carotid artery, RR respiration rate, V<sub>E</sub> ventilation, V<sub>T</sub> tidal volume, ETO<sub>2</sub> end-tidal oxygen, ETCO<sub>2</sub> end-tidal carbon dioxide, SpO<sub>2</sub> arterial oxygen saturation. \*Significant main effect of HDBR. All values are mean ± SD.

area and time-averaged velocity of 12 heart cycles using EchoPAC software (Version 201, GE Medical Systems, Mississauga, Canada).

**Autonomic indices.** Pulse waveforms were measured simultaneously from the middle finger using the Nexfin waveform, and the ipsilateral great toe using a piezoelectric pulse transducer (ADInstruments, Colorado Springs, USA). The arrival time of both pulse waves was determined. Finger-toe pulse wave velocity (PWV) was calculated as (toe pulse arrival time – finger pulse arrival time)/(distance from suprasternal notch to toe – distance from suprasternal notch to finger). Heart rate variability (HRV; time domain and spectral analysis) was determined from the resting ECG recording before and after HDBR using a Hann (cosine-bell) data window (window overlap of 50%), and a fast Fourier transform size of 1024. A range of 0.04–0.15 Hz and 0.15–0.45 Hz was used for the low frequency (LF) and high frequency (HF) spectrums, respectively (LabChart Pro software; Version 8.1.9, ADInstruments, Colorado Springs, USA). Spontaneous cardiovagal baroreceptor sensitivity (cBRS) was measured at the same timepoints as HRV using the sequence method<sup>26,27</sup>.

**Data and statistical analysis.** For all central hemodynamic, cerebrovascular, and respiratory variables 1 min averages at baseline and at the end of 5 min of gas mixture breathing were analyzed for both the hypercapnia and hypoxia trials. CCA flow was measured during the final minute of baseline and gas mixture breathing, and PWV was calculated during the same timepoints using at least 20 consecutive pulse waves. All continuous data were collected at 1000 Hz using a Powerlab data acquisition system (ADInstruments, Colorado Springs, USA), and accompanying LabChart Pro software.

Statistical analysis was completed using Sigmaplot 13.0 (Systat Software Inc, San Jose, USA). The baseline data for cardiorespiratory, cerebrovascular, and autonomic data were obtained before and after HDBR prior to any gases were administered and were compared using a paired t-test for normally distributed data or a Wilcoxon Signed Rank Test for non-normal distributions (Tables 1 and 2). The changes in the responses to CO<sub>2</sub> or O<sub>2</sub> in all cardiorespiratory and cerebrovascular variables were also compared before and after HDBR using a paired t-test for normally distributed data or a Wilcoxon Signed Rank Test for non-normal distributions (Figs. 2, 3, and 4; Table 3). Gases were not compared to each other. Statistical significance was set a priori at  $P < 0.05$ . Data in tables are presented as mean ± standard deviation. Data in figures are presented as median with interquartile range and 10th and 90th percentile.

## Results

**Baseline changes.** After 4-h HDBR participants had significantly lower HR, Qi, CCA flow, and higher SpO<sub>2</sub> (Table 1). There were no significant main effects of HDBR on MAP, SVi, TPRi, MCA mean velocity, CVRi, RI, PI, RR, V<sub>T</sub>, V<sub>E</sub>, ETO<sub>2</sub>, or ETCO<sub>2</sub> (Table 1). Finger-toe PWV was significantly higher after 4-h HDBR, but there were no significant effects of 4-h HDBR on any HRV variable (SDRR, RMSSD, pRR50, total power, LF, HF, LF/HF, SD1, SD2. cBRS; Table 2).

	Baseline	After HDBR	P-value
SDRR (ms)	75 ± 31	84 ± 47	0.37
RMSSD (ms)	76 ± 58	90 ± 72	0.28
pRR50 (%)	44 ± 25	49 ± 25	0.17
Total power ( $\mu\text{s}^2$ )	6576 ± 6592	9793 ± 14,839	0.40
LF (nu)	31 ± 20	39 ± 23	0.12
HF (nu)	68 ± 19	60 ± 23	0.13
LF/HF	0.63 ± 0.67	0.98 ± 1.05	0.17
SD1 (ms)	54 ± 41	62.1 ± 51.7	0.28
SD2 (ms)	89 ± 26	100.0 ± 48.5	0.44
cBRS slope (ms/mmHg)	22 ± 10	25 ± 13	0.46
Finger-toe PWV (cm/s)	7.5 ± 2.3	8.4 ± 1.9*	0.03

**Table 2.** Heart rate variability and cardiovagal baroreceptor sensitivity before and after HDBR. *SDRR* SD between RR intervals, *RMSSD* square root of the mean difference between adjacent beats, *pRR50* proportion of RR interval differences greater than 50 ms divided by total beats, *LF* low frequency, *HF* high frequency, *SD1* width of the Poincaré plot, *SD2* length of the Poincaré plot, *cBRS* cardiovagal baroreceptor sensitivity, *PWV* pulse wave velocity. \*Significant effect of HDBR. All values are mean ± SD.

**Hypercapnia.** After 4-h HDBR, there were no significant changes in the responses of MAP ( $P=0.23$ ), HR ( $P=0.19$ ), SVi ( $P=0.83$ ), Qi ( $P=0.50$ ), or TPRi ( $P=0.15$ ) to hypercapnia (Fig. 2). Similarly, after 4-h HDBR, there were no significant changes in the responses of MCA mean velocity ( $P=0.94$ ), CVRi ( $P=0.74$ ), RI ( $P=0.69$ ), PI ( $P=0.51$ ), or CCA blood flow ( $P=0.15$ ) to hypercapnia (Fig. 3). However, after 4-h HDBR, while there was no change in the response of RR ( $P=0.73$ ),  $\text{ETO}_2$  ( $P=0.47$ ),  $\text{ETCO}_2$  ( $P=0.20$ ), or  $\text{SpO}_2$  ( $P=0.06$ ) to hypercapnia, there was an augmented  $V_T$  ( $P=0.04$ ) and  $V_E$  ( $P=0.02$ ) response to hypercapnia (Fig. 4).

**Hypoxia.** After 4-h HDBR there were no significant changes in the responses of HR, MAP, SVi, Qi, TPRi, MCA mean velocity, CVRi, RI, PI, CCA flow, RR,  $V_T$ ,  $V_E$ ,  $\text{ETO}_2$ ,  $\text{ETCO}_2$ , or  $\text{SpO}_2$  to hypoxia (Table 3).

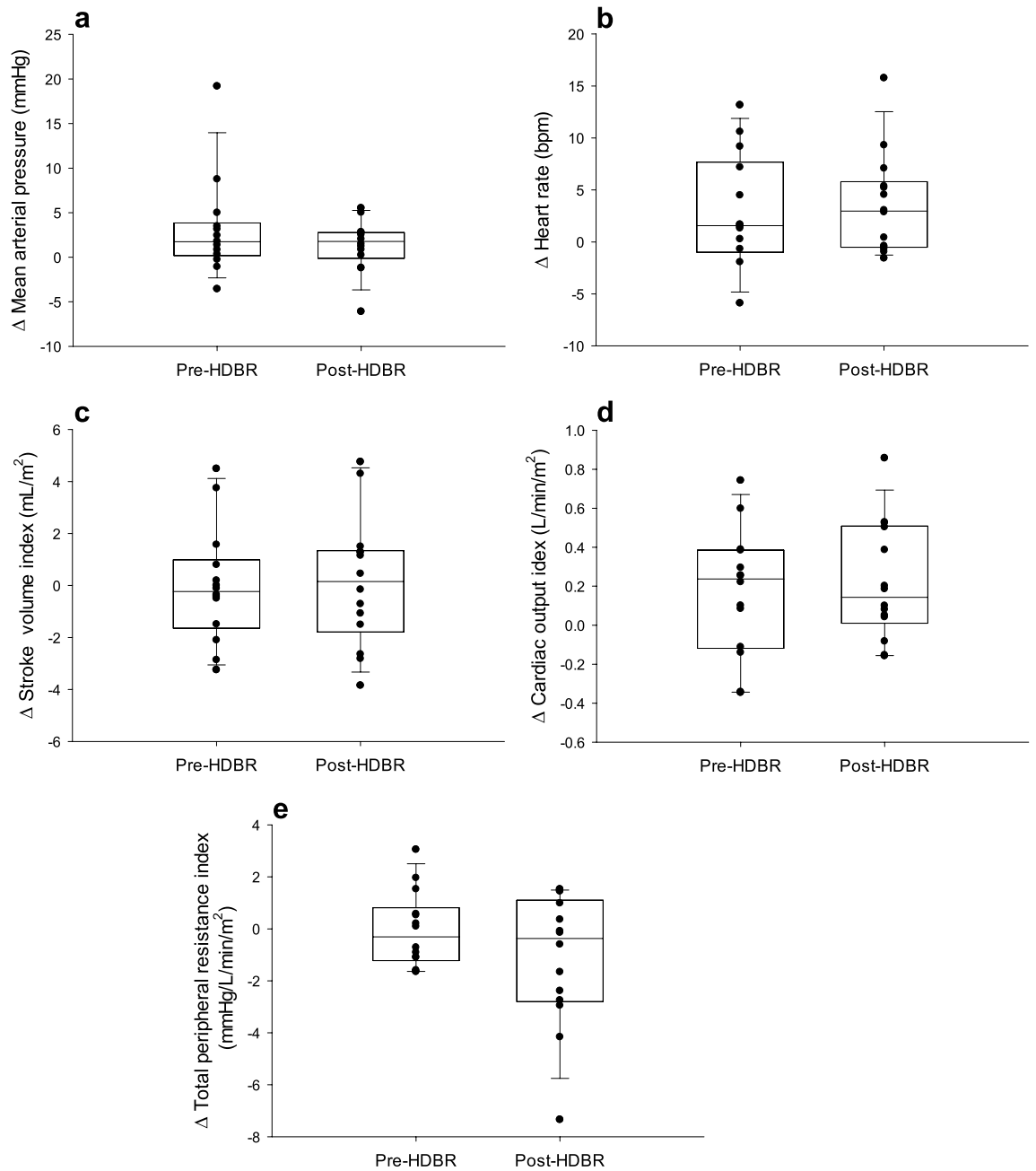
## Discussion

After HDBR, we observed an augmented HCVR, refuting our first hypothesis of a reduced respiratory  $\text{CO}_2$  chemoreflex after 4-h HDBR. Second, there was no effect of HDBR on HVR suggesting no change in the respiratory  $\text{O}_2$  chemoreflex also refuting our second hypothesis. Since indices of cerebrovascular flow were not different after HDBR, we hypothesize that these changes in ventilatory responses (or lack thereof) could be due to exposure to greater cephalic  $\text{CO}_2$  concurrent with greater  $\text{SpO}_2$ .

**Cardiorespiratory dynamics.** After 20 days of HDBR, acute exposure to hypercapnia via rebreathing resulted in a reduced ventilatory response despite no change in  $\text{ETCO}_2$ <sup>10</sup>. Similarly, 120 day HDBR also reduced the ventilatory response to hypercapnia<sup>11</sup>, which conflicts with the results of the present study. While Katayama et al. did not measure arterial  $\text{CO}_2$ , others have documented increased arterial  $\text{CO}_2$  after HDBR<sup>28</sup>, which may be a consequence of hypoventilation from a headward shift of the diaphragm<sup>29,30</sup>. Others have also noted cerebral congestion<sup>31</sup> and reduced cerebrospinal fluid drainage<sup>32</sup> with HDBR which could potentially act in combination to increase cerebral  $\text{CO}_2$ . Thus, the reduced HCVR documented by Katayama et al.<sup>10</sup> and Vorob'yev et al.<sup>11</sup> could have been due to adaptation of the  $\text{CO}_2$  chemoreflex<sup>33</sup>. In the present study, we suggest that the acute cephalic  $\text{CO}_2$  accumulation augmented the  $\text{CO}_2$  chemoreflex as adaptation had not yet occurred; however, these mechanisms remain speculative.

The effect of short-duration HDBR on HVR had not been previously examined. Given that a previous study found a significantly reduced  $\text{O}_2$  chemoreflex after short-duration spaceflight<sup>23</sup>, we expected that increased carotid chemoreceptor perfusion in the head-down posture would also reduce peripheral chemoreflex activity<sup>16,20–22</sup> attenuating the ventilatory response to hypoxia; however, we observed no effect of HDBR on this response. We suggest reduced plasma volume (previously observed after 4-h HDBR)<sup>3</sup> resulted in the observed reduction of Qi, which along with cerebral congestion<sup>31</sup> decreased CCA blood flow. While this reduction in CCA flow might be expected to influence chemoreflex activity, we do not have evidence that flow in the carotid body itself has changed (and indices of baseline cerebrovascular resistance and ventilation are unchanged). Since the carotid body has complex autoregulatory regulation of glomus tissue to maintain flow<sup>34,35</sup>, we suggest this to be the case. Further, after HDBR there was a small but significant increase of  $\text{SpO}_2$  (likely due to better ventilation-perfusion matching) which could have contributed to lower activity of the peripheral chemoreflex.

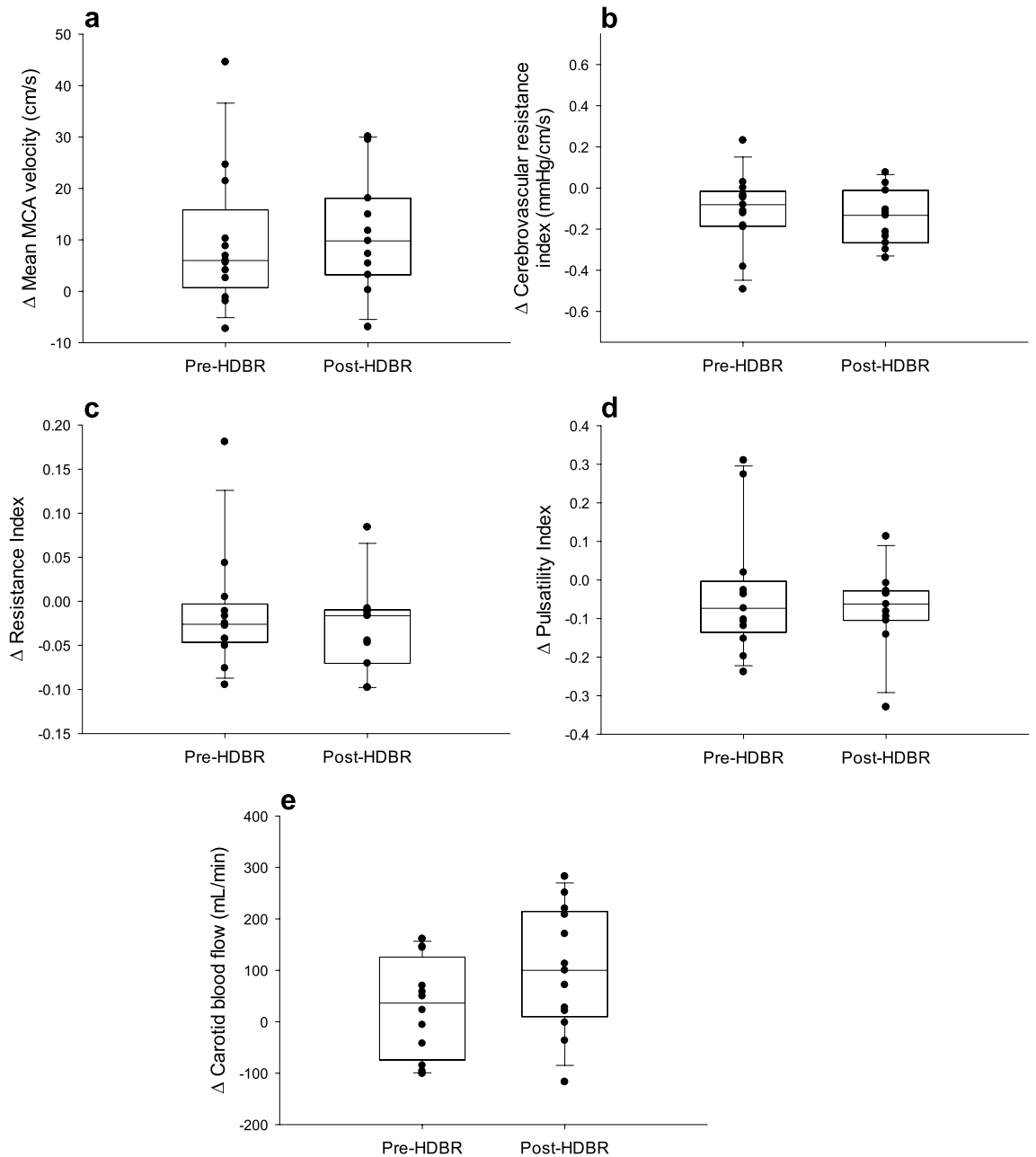
After HDBR, the significantly reduced Qi was also driven by lower HR after HDBR. Edgell et al. (2012) found that resting plasma norepinephrine was lower after 4-h HDBR<sup>3</sup> implying that plasma epinephrine may also be lower after HDBR. These reductions could help to explain the reduction of HR and Qi. MAP remained unchanged after HDBR despite lower Qi which could have been a consequence of greater regional vascular resistance (as observed with increased finger-toe PWV) and may suggest increased sympathetic activity in response to 4-h HDBR, which has been observed previously<sup>8</sup>. However, since there were no significant changes in HRV



**Figure 2.** Change in mean arterial pressure (a), heart rate (b), stroke volume index (c), cardiac index (d), and total peripheral resistance index (e) from baseline to 5 min hypercapnia. The line within each box indicates the median, whereas the lower and upper boundaries indicate the 25th and 75th percentiles. Lower and upper whiskers represent the 10th and 90th percentiles.

indices of sympathetic control of heart rate (LF:HF), and muscle sympathetic nerve activity was not measured, changes in sympathetic activity after short-duration HDBR likely require further investigation. Further, we used the Modelflow algorithm to determine SV (and thus Q and TPR) which may have increased variability in the absolute measurements. However, Modelflow measurements of Q have been shown to be highly correlated to ultrasound measurements when determining the differences between an intervention and baseline measures<sup>24</sup>.

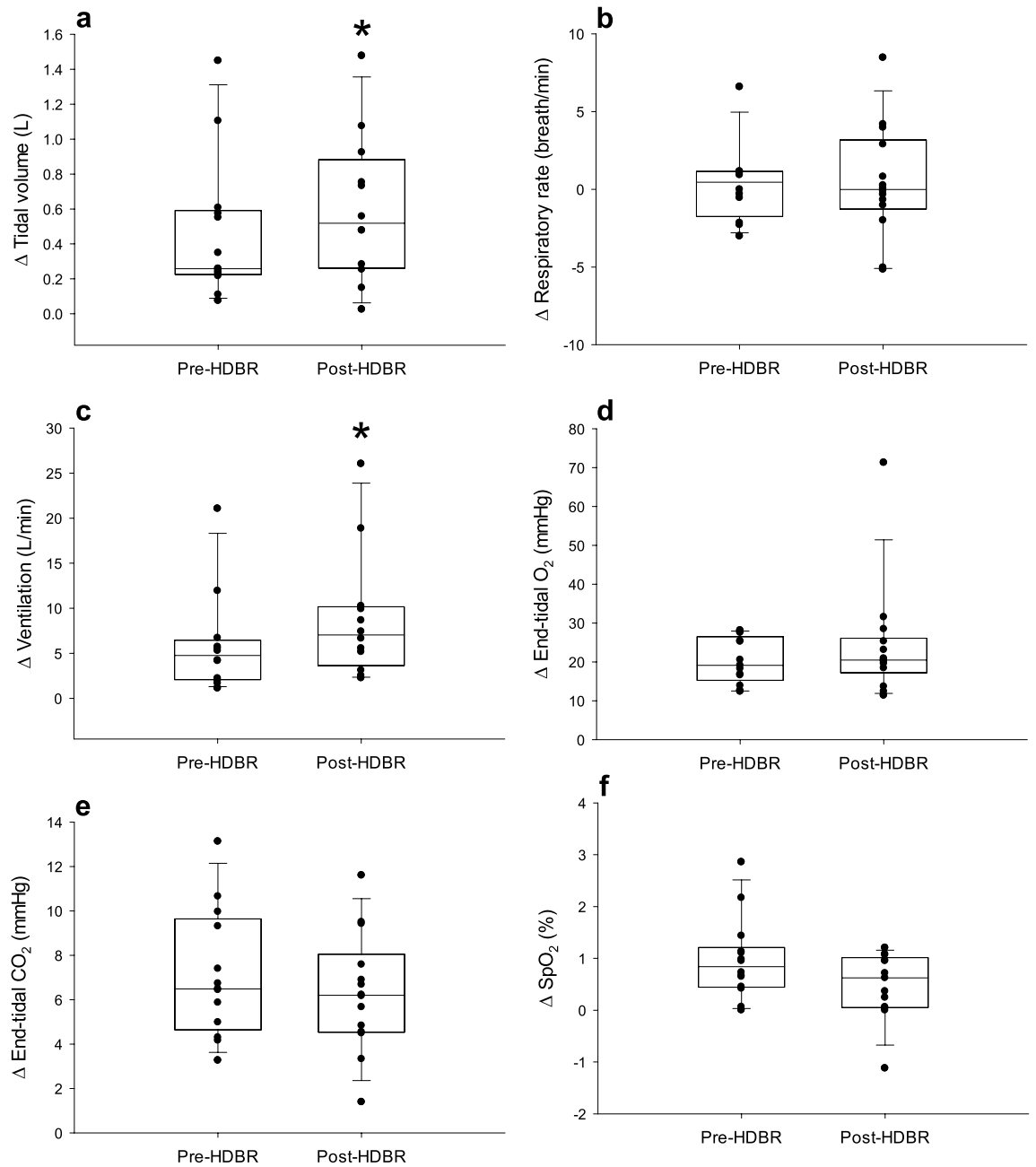
**Cerebrovascular dynamics.** Multiple studies have assessed the effect of HDBR on cerebral hemodynamics, but results are inconsistent and likely depend on the duration. In men, cerebral blood flow, directly measured using MRI, was reported to decrease after ~ 5-h HDBR; however, the authors also reported lower ET<sub>CO</sub><sub>2</sub> which may have confounded the effect of HDBR on brain blood flow<sup>31</sup>. Alternatively, studies in men for longer periods of HDBR (24-48hrs) have reported increased MCA mean velocity<sup>36,37</sup>, whereas no change has been reported after 2 weeks of HDBR<sup>38</sup>, unfortunately these studies did not report ET<sub>CO</sub><sub>2</sub>. In the present study, while CCA



**Figure 3.** Change in middle cerebral artery mean velocity (a), cerebrovascular resistance index (b), resistance index (c), pulsatility index (d), and common carotid artery flow (e) from baseline to 5 min hypercapnia. The line within each box indicates the median, whereas the lower and upper boundaries indicate the 25th and 75th percentiles. Lower and upper whiskers represent the 10th and 90th percentiles.

flow was lower after 4-h HDBR, MCA mean velocity and all of the cerebrovascular indices were not significantly different which was likely due to unchanged  $ETCO_2$  and appropriate autoregulation.

As arterial  $O_2$  decreases, cerebral blood flow increases, via cerebral vasodilation, to maintain cerebral  $O_2$  delivery<sup>39</sup>. However, in the current hypoxia trial, despite decreased  $ETO_2$  and  $SpO_2$ , as well as increased  $Q_i$ , MCA mean velocity did not increase. This was likely the consequence of hyperventilation decreasing  $ETCO_2$  counteracting the expected cerebral vasodilation. Unchanged cerebrovascular indices likely indicate minimal cerebral vasomotion or even slight vasoconstriction during hypoxia in the present study. In response to hypercapnia we observed a characteristic increase in MCA mean velocity, with reductions of CVRi, RI, and PI suggesting cerebral vasodilation, but HDBR did not affect the cerebrovascular responses. To the best of our knowledge no other studies have assessed the cerebrovascular  $CO_2$  reactivity response after 4hrs of 6° HDBR; however, acute and severe head-down tilt (45° and 90° for ~20 min) does not alter MCA mean velocity, CVRi, or cerebrovascular  $CO_2$  reactivity either<sup>40</sup>. Together, these results suggest that the myogenic response of cerebral vascular smooth muscle to  $CO_2$  is preserved during short-duration head-down posture.



**Figure 4.** Change in tidal volume (a), respiration rate (b), ventilation (c), end-tidal oxygen (d), end-tidal carbon dioxide (e), and oxygen saturation (f) from baseline to 5 min hypercapnia. \*Significant main effect of HDBR. The line within each box indicates the median, whereas the lower and upper boundaries indicate the 25th and 75th percentiles. Lower and upper whiskers represent the 10th and 90th percentiles.

**Limitations.** Firstly, only three women participated in and completed our study preventing sex-based comparisons, but previous work has noted similar hemodynamic responses between men and women after 4-h HDBR<sup>3</sup>. Similarly, the cerebral hemodynamic and ventilatory response to supine hypercapnia<sup>9</sup>, and the ventilatory response to hypoxia<sup>41</sup> are not different between men and women. Secondly, since no sedentary upright control group was utilized in this study, previously observed variations in chemoreflex activation due to circadian rhythm<sup>42,43</sup> could potentially confound our results. However, when specifically observing the hours of 8 am–4 pm relevant to this study, HCVR appeared similar<sup>42,43</sup>, and central hemodynamics have been shown to be a function of head-down posture, rather than 4hrs of inactivity, physical confinement, or time of day<sup>3</sup>. Thirdly, participants were upright for approximately 10 s when transitioning between the angled and supine beds for testing. While we assumed that the effects of 4-h HDBR would persist after this very brief transition, it is possible that even brief orthostasis could have affected our results. There are conflicting studies which suggest that 5 min or more of upright posture either does<sup>44</sup>, or does not<sup>45</sup>, influence the CO<sub>2</sub> respiratory chemoreflex. However, we did not expect to see persistent effects of the brief upright posture as the cardiorespiratory responses to

	Baseline	After HDBR	P-value
HR (bpm)	8.7 ± 4.3	8.0 ± 4.7	0.65
MAP (mmHg)	1.5 ± 4.8	1.8 ± 3.5	0.84
SVi (mL/m <sup>2</sup> )	- 0.8 ± 1.8	- 0.9 ± 2.6	0.91
Qi (L/min/m <sup>2</sup> )	0.5 ± 0.2	0.4 ± 0.3	0.56
TPRi (mmHg/L/min/m <sup>2</sup> )	- 1.9 ± 1.5	- 1.6 ± 1.7	0.49
MCA mean velocity (cm/s)	- 1.2 ± 6.4	- 1.8 ± 4.1	0.42
CVRi (mmHg/cm/s)	0.07 ± 0.17	0.06 ± 0.09	1.00
RI	- 0.005 ± 0.046	0.009 ± 0.025	0.47
PI	- 0.007 ± 0.125	0.027 ± 0.072	0.52
CCA flow (mL/min)	14 ± 173	37 ± 76	0.22
RR (bpm)	- 1.0 ± 2.2	- 0.8 ± 2.6	0.96
V <sub>T</sub> (L)	- 0.25 ± 0.22	0.28 ± 0.32	0.65
V <sub>E</sub> (L/min)	2.8 ± 3.4	3.3 ± 5.4	0.62
ETO <sub>2</sub> (mmHg)	- 48 ± 8	- 48 ± 12	0.46
ETCO <sub>2</sub> (mmHg)	- 2.3 ± 2.5	- 3.2 ± 2.5	0.28
SpO <sub>2</sub> (%)	- 6.1 ± 3.7	- 6.0 ± 4.2	0.86

**Table 3.** Change in cardiorespiratory and cerebrovascular responses to hypoxia before and after HDBR. *HR* heart rate, *MAP* mean arterial pressure, *SVi* stroke volume index, *Qi* cardiac index, *TPRi* total peripheral resistance, *MCA* middle cerebral artery, *CVRi* cerebrovascular resistance index, *RI* resistance index, *PI* pulsatility index, *CCA* common carotid artery, *RR* respiration rate, *V<sub>E</sub>* ventilation, *V<sub>T</sub>* tidal volume, *ETO<sub>2</sub>* end-tidal oxygen, *ETCO<sub>2</sub>* end-tidal carbon dioxide, *SpO<sub>2</sub>* arterial oxygen saturation. All values are mean ± SD.

tilt recover within 5 min of the return to supine posture<sup>46</sup> and our participants were supine for longer periods in preparation for testing (i.e. electrode and TCD placement, Nexfin activation, pneumotach placement, etc.). Further, after HDBR we still observed an increase of SpO<sub>2</sub> which persisted past the brief orthostasis. If the orthostatic stress were to have persistently and negatively influenced the ventilation-perfusion matching in the lung, we expect that the SpO<sub>2</sub> would have lowered back to pre-HDBR levels. Lastly, during hypoxic breathing our participants only had a 6% drop of SpO<sub>2</sub> before and after HDBR which was a smaller drop than expected. As our participants were primarily young and healthy kinesiology students, we suggest that a larger than average blood volume could contribute to this and therefore a plateau at the SpO<sub>2</sub> nadir may not have been reached after only 5 min of hypoxia. We suggest that future studies employ a longer period of exposure.

We investigated flow within the CCA rather than the internal carotid artery. The diameter of the internal carotid artery is often difficult to image, and therefore we chose to use the common carotid. While not ideal, this measurement along with the cerebrovascular resistance indices from the TCD measurements, allowed for indices of brain blood flow. Additionally, TCD ultrasound does not allow for estimation of blood vessel diameter, yet MCA velocity is often used as an index of cerebral blood flow. The impact of HDBR on MCA diameter has not been studied, but previous work using similar levels of hypercapnia has shown that CO<sub>2</sub> causes vasodilation of cerebral arteries<sup>47–49</sup>. Therefore, the actual increase in cerebral blood flow in response to hypercapnia was likely larger than the observed increase in MCA velocity. We also observed a discrepancy between arterial oxygen saturation (higher) and ETO<sub>2</sub> (unchanged) after HDBR, but this was potentially due to greater variability in ETO<sub>2</sub> measurements. Future work should also utilize near-infrared spectroscopy (NIRS) or transcutaneous CO<sub>2</sub> monitoring on the head to help characterize potential alterations of cerebral O<sub>2</sub> and CO<sub>2</sub> after 4-h HDBR. Lastly, the results of the present study cannot be generalized to older adult or clinical population as comorbidities and pathologies would likely impact the responses measured.

## Conclusions

Short-duration HDBR resulted in enhanced HCVR, yet no change in HVR. We suggest that these changes could be due to exposure to cephalic CO<sub>2</sub> accumulation and higher peripheral oxygenation while in the head-down posture, respectively. We did not find evidence of changes in cerebrovascular resistance after short-term HDBR; however, Qi was lower due to reductions of HR and potential increases of vascular resistance. This enhancement of HCVR in the HDBR position could potentially influence cardiorespiratory function during cephalic fluid-shifts.

## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Received: 23 November 2020; Accepted: 12 January 2021

Published online: 25 January 2021



## References

- Butler, G. C., Xing, H. C., Northey, D. R. & Hughson, R. L. Reduced orthostatic tolerance following 4 h head-down tilt. *Eur. J. Appl. Physiol. Occup. Physiol.* **62**, 26–30 (1991).
- Fischer, D. M., Arbeille, P., Shoemaker, J. K., O'Leary, D. D. & Hughson, R. L. Altered hormonal regulation and blood flow distribution with cardiovascular deconditioning after short-duration head down bed rest. *J. Appl. Physiol.* **103**, 2018–2025 (2007).
- Edgell, H., Grinberg, A., Gagne, N., Beavers, K. R. & Hughson, R. L. Cardiovascular responses to lower body negative pressure before and after 4 h of head-down bed rest and seated control in men and women. *J. Appl. Physiol.* **1985**(113), 1604–1612. <https://doi.org/10.1152/jappphysiol.00670.2012> (2012).
- Gaffney, F. A. *et al.* Cardiovascular deconditioning produced by 20 hours of bedrest with head-down tilt ( $-5^\circ$ ) in middle-aged healthy men. *Am. J. Cardiol.* **56**, 634–638. [https://doi.org/10.1016/0002-9149\(85\)91025-2](https://doi.org/10.1016/0002-9149(85)91025-2) (1985).
- Hargens, A. R. & Watenpaugh, D. E. Cardiovascular adaptation to spaceflight. *Med. Sci. Sports Exerc.* **28**, 977–982 (1996).
- Edgell, H., Grinberg, A., Gagne, N., Beavers, K. R. & Hughson, R. L. Cardiovascular responses to lower body negative pressure before and after 4 h of head-down bed rest and seated control in men and women. *J. Appl. Physiol.* **113**, 1604–1612. <https://doi.org/10.1152/jappphysiol.00670.2012> (2012).
- Edgell, H., Grinberg, A., Beavers, K. R., Gagné, N. & Hughson, R. L. Efficacy of fluid loading as a countermeasure to the hemodynamic and hormonal changes of 28-h head-down bed rest. *Physiol. Rep.* **6**, e13874 (2018).
- Patwardhan, A. *et al.* Spectral indices of cardiovascular adaptations to short-term simulated microgravity exposure. *Integr. Psychol. Behav. Sci.* **30**, 201–214 (1995).
- Hazlett, C. & Edgell, H. Chemoreflex function and brain blood flow during upright posture in men and women. *Physiol. Rep.* **6**, e13571. <https://doi.org/10.14814/phy2.13571> (2018).
- Katayama, K. *et al.* Ventilatory and cardiovascular responses to hypercapnia after 20 days of head-down bed rest. *Aviat. Space Environ. Med.* **75**, 312–316 (2004).
- Vorob'yev, V., Abdrakhmanov, V., Golikov, A., Stazhadze, L. & Goncharov, I. Effect of 120-day antiorthostatic hypokinesia on gas exchange and pulmonary circulation in humans. *Kosm. Biol. Aviakosm. Med.* **18**, 23–26 (1984).
- Kalmar, A. F. *et al.* Influence of steep Trendelenburg position and CO<sub>2</sub> pneumoperitoneum on cardiovascular, cerebrovascular, and respiratory homeostasis during robotic prostatectomy. *Br. J. Anaesth.* **104**, 433–439 (2010).
- Meininger, D. *et al.* Effects of prolonged pneumoperitoneum on hemodynamics and acid-base balance during totally endoscopic robot-assisted radical prostatectomies. *World J. Surg.* **26**, 1423–1427 (2002).
- Park, E., Koo, B. N., Min, K. & Nam, S. The effect of pneumoperitoneum in the steep Trendelenburg position on cerebral oxygenation. *Acta Anaesthesiol. Scand.* **53**, 895–899 (2009).
- Raimondi, F. *et al.* Effects of laparoscopic radical prostatectomy on intraoperative autonomic nervous system control of hemodynamics. *Miner. Anesthesiol.* **83**, 1265–1273 (2017).
- Ding, Y., Li, Y. L. & Schultz, H. D. Role of blood flow in carotid body chemoreflex function in heart failure. *J. Physiol.* **589**, 245–258. <https://doi.org/10.1113/jphysiol.2010.200584> (2011).
- Lahiri, S., Mulligan, E., Nishino, T., Mokashi, A. & Davies, R. Relative responses of aortic body and carotid body chemoreceptors to carboxyhemoglobinemia. *J. Appl. Physiol.* **50**, 580–586 (1981).
- Heistad, D., Abboud, F. M., Mark, A. L. & Schmid, P. Effect of baroreceptor activity on ventilatory response to chemoreceptor stimulation. *J. Appl. Physiol.* **39**, 411–416 (1975).
- Heistad, D. D., Abboud, F. M., Mark, A. L. & Schmid, P. G. Interaction of baroreceptor and chemoreceptor reflexes. Modulation of the chemoreceptor reflex by changes in baroreceptor activity. *J. Clin. Invest.* **53**, 1226–1236. <https://doi.org/10.1172/JCI107669> (1974).
- Schultz, H. D. Angiotensin and carotid body chemoreception in heart failure. *Curr. Opin. Pharmacol.* **11**, 144–149 (2011).
- Dekker, R. J. *et al.* Endothelial KLF2 links local arterial shear stress levels to the expression of vascular tone-regulating genes. *Am. J. Pathol.* **167**, 609–618 (2005).
- Miyakawa, A. A., de Lourdes Junqueira, M. & Krieger, J. E. Identification of two novel shear stress responsive elements in rat angiotensin I converting enzyme promoter. *Physiol. Genomics* **17**, 107–113 (2004).
- Prisk, G. K., Elliott, A. R. & West, J. B. Sustained microgravity reduces the human ventilatory response to hypoxia but not to hypercapnia. *J. Appl. Physiol.* **88**, 1421–1430 (2000).
- Kim, A. *et al.* Sex differences in carotid baroreflex control of arterial blood pressure in humans: Relative contribution of cardiac output and total vascular conductance. *Am. J. Physiol. Heart Circ. Physiol.* **301**, H2454–2465. <https://doi.org/10.1152/ajpheart.00772.2011> (2011).
- Du Bois, D. & Du Bois, E. F. A formula to estimate the approximate surface area if height and weight be known. *Arch. Intern. Med.* **17**, 863–871. <https://doi.org/10.1001/archinte.1916.00080130010002> (1916).
- Bertinieri, G. *et al.* Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am. J. Physiol.* **254**, H377–H383 (1988).
- Blaber, A. P., Yamamoto, Y. & Hughson, R. L. Methodology of spontaneous baroreflex relationship assessed by surrogate data analysis. *Am. J. Physiol.* **268**, H1682–H1687 (1995).
- Prisk, G. K., Fine, J. M., Elliott, A. R. & West, J. B. Effect of 6 degrees head-down tilt on cardiopulmonary function: Comparison with microgravity. *Aviat. Space Environ. Med.* **73**, 8–16 (2002).
- Laurie, S. S. *et al.* Effects of short-term mild hypercapnia during head-down tilt on intracranial pressure and ocular structures in healthy human subjects. *Physiol. Rep.* **5**, e13302 (2017).
- Prisk, G. K., Fine, J. M., Cooper, T. K. & West, J. B. Vital capacity, respiratory muscle strength, and pulmonary gas exchange during long-duration exposure to microgravity. *J. Appl. Physiol.* **101**, 439–447 (2006).
- Marshall-Goebel, K. *et al.* Effects of short-term exposure to head-down tilt on cerebral hemodynamics: A prospective evaluation of a spaceflight analog using phase-contrast MRI. *J. Appl. Physiol.* **120**, 1466–1473 (2016).
- Lawley, J. S. *et al.* Effect of gravity and microgravity on intracranial pressure. *J. Physiol.* **595**, 2115–2127 (2017).
- Schaefer, K., Hastings, B., Carey, C. & Nichols, G. Jr. Respiratory acclimatization to carbon dioxide. *J. Appl. Physiol.* **18**, 1071–1078 (1963).
- McCloskey, D. I. & Torrance, R. W. Autoregulation of blood flow in the carotid body. *Respir. Physiol.* **13**, 23–35. [https://doi.org/10.1016/0034-5687\(71\)90062-4](https://doi.org/10.1016/0034-5687(71)90062-4) (1971).
- Brogna, F., Felipe, I. S. A., Salgado, H. C. & Paton, J. F. R. Autonomic innervation of the carotid body as a determinant of its sensitivity—Implications for cardiovascular physiology and pathology. *Cardiovasc. Res.* <https://doi.org/10.1093/cvr/cvaa250> (2020).
- Kawai, Y. *et al.* Cerebral blood flow velocity in humans exposed to 24 h of head-down tilt. *J. Appl. Physiol.* **74**, 3046–3051 (1993).
- Frey, M., Mader, T. H., Bagian, J. P., Charles, J. B. & Meehan, R. T. Cerebral blood velocity and other cardiovascular responses to 2 days of head-down tilt. *J. Appl. Physiol.* **74**, 319–325 (1993).
- Zhang, R., Zuckerman, J. H., Pawelczyk, J. A. & Levine, B. D. Effects of head-down-tilt bed rest on cerebral hemodynamics during orthostatic stress. *J. Appl. Physiol.* **83**, 2139–2145 (1997).
- Ogoh, S. & Ainslie, P. N. Cerebral blood flow during exercise: Mechanisms of regulation. *J. Appl. Physiol.* **1985**(107), 1370–1380. <https://doi.org/10.1152/jappphysiol.00573.2009> (2009).

40. Tymko, M. M., Skow, R. J., MacKay, C. M. & Day, T. A. Steady-state tilt has no effect on cerebrovascular CO<sub>2</sub> reactivity in anterior and posterior cerebral circulations. *Exp. Physiol.* **100**, 839–851 (2015).
41. Jones, P. P., Davy, K. P. & Seals, D. R. Influence of gender on the sympathetic neural adjustments to alterations in systemic oxygen levels in humans. *Clin. Physiol.* **19**, 153–160 (1999).
42. Spengler, C. M., Czeisler, C. A. & Shea, S. A. An endogenous circadian rhythm of respiratory control in humans. *J. Physiol.* **526**, 683–694 (2000).
43. Stephenson, R., Mohan, R. M., Duffin, J. & Jarsky, T. M. Circadian rhythms in the chemoreflex control of breathing. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **278**, R282–R286 (2000).
44. Hazlett, C. & Edgell, H. Chemoreflex function and brain blood flow during upright posture in men and women. *Physiol Rep.* <https://doi.org/10.14814/phy2.13571> (2018).
45. Taneja, I., Medow, M. S., Clarke, D. A., Ocon, A. J. & Stewart, J. M. Baroreceptor unloading in postural tachycardia syndrome augments peripheral chemoreceptor sensitivity and decreases central chemoreceptor sensitivity. *Am. J. Physiol. Heart. Circ. Physiol.* **301**, H173–179. <https://doi.org/10.1152/ajpheart.01211.2010> (2011).
46. Serrador, J. M., Hughson, R. L., Kowalchuk, J. M., Bondar, R. L. & Gelb, A. W. Cerebral blood flow during orthostasis: role of arterial CO<sub>2</sub>. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **290**, R1087–R1093 (2006).
47. Coverdale, N. S., Gati, J. S., Opalevych, O., Perrotta, A. & Shoemaker, J. K. Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. *J. Appl. Physiol.* **117**, 1090–1096. <https://doi.org/10.1152/jappphysiol.00285.2014> (2014).
48. Verbree, J. *et al.* Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI. *J. Appl. Physiol.* **117**, 1084–1089. <https://doi.org/10.1152/jappphysiol.00651.2014> (2014).
49. Kellawan, J. M. *et al.* Quantitative cerebrovascular 4D flow MRI at rest and during hypercapnia challenge. *Magn. Reson. Imaging* **34**, 422–428 (2016).

### Author contributions

The experiments were performed in the laboratory of H.E. H.E. and S.W. contributed to the conception and design of the work. H.E., S.W., and K.R.M. contributed to the acquisition, analysis, and interpretation of the data for the work. H.E. and K.R.M. drafted the work and critically revise it for important intellectual content. S.W. and K.R.M. contributed equally to this work. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy of integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

### Funding

The Natural Sciences and Engineering Research Council of Canada (#2016-05289), the Canada Foundation for Innovation, and the Ontario Research Fund provided funding for this study.

### Competing interests

The authors declare no competing interests.

### Additional information

**Correspondence** and requests for materials should be addressed to H.E.

**Reprints and permissions information** is available at [www.nature.com/reprints](http://www.nature.com/reprints).

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



**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2021