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Unchanged cerebrovascular CO_2 reactivity and hypercapnic ventilatory response during strict head-down tilt bed rest in a mild hypercapnic environment

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Key points

- Carbon dioxide levels are mildly elevated on the International Space Station and it is unknown whether this chronic exposure causes physiological changes to astronauts.
- We combined ~4 mmHg ambient P_{CO_2} with the strict head-down tilt bed rest model of spaceflight and this led to the development of optic disc oedema in one-half of the subjects.
- We demonstrate no change in arterialized P_{CO_2}, cerebrovascular reactivity to CO_2 or the hypercapnic ventilatory response.
- Our data suggest that the mild hypercapnic environment does not contribute to the development of spaceflight associated neuro-ocular syndrome.

Abstract Chronically elevated carbon dioxide (CO_2) levels can occur in confined spaces such as the International Space Station. Using the spaceflight analogue 30 days of strict 6° head-down tilt bed rest (HDTBR) in a mild hypercapnic environment (P_{CO_2} = ~4 mmHg), we investigated arterialized P_{CO_2}, cerebrovascular reactivity and the hypercapnic ventilatory response in 11 healthy subjects (five females) before, on days 1, 9, 15 and 30 of bed rest (BR), and 6 and 13 days after HDTBR. During all HDTBR time points, arterialized P_{CO_2} was not significantly different from the pre-HDTBR measured in the 6° HDT posture, with a mean (95% confidence interval) increase of 1.2 mmHg (–0.2 to 2.5 mmHg, $P = 0.122$) on day 30 of HDTBR. Respiratory acidosis was never detected, although a mild metabolic alkalosis developed on day 30 of HDTBR by a mean (95% confidence interval) pH change of 0.032 (0.022–0.043; $P < 0.001$), which remained elevated by 0.021 (0.011–0.031; $P < 0.001$) 6 days after HDTBR. Arterialized pH returned to pre-HDTBR levels 13 days after BR with a change of –0.001 (–0.009 to 0.007; $P = 0.991$).

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Compared to pre-HDTBR, cerebrovascular reactivity during and after HDTBR did not change. Baseline ventilation, ventilatory recruitment threshold and the slope of the ventilatory response were similar between pre-HDTBR and all other time points. Taken together, these data suggest that the mildly increased ambient P_{CO_2} combined with 30 days of strict 6° HDTBR did not change arterialized P_{CO_2} levels. Therefore, the experimental conditions were not sufficient to elicit a detectable physiological response.

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Introduction

We previously reported on the development of optic disc oedema and thickening of the retina surrounding the optic nerve head in five of 11 subjects who participated in this bed rest study (Laurie *et al.* 2019). These findings are similar to those that develop in astronauts during long-duration spaceflight (Mader *et al.* 2011) and characterize the Spaceflight Associated Neuro-ocular Syndrome (SANS) (Stenger *et al.* 2017), yet the underlying cause(s) of these changes remains unclear in both populations. Both weightlessness during spaceflight and the change in angle of the gravitational vector with respect to the body during chronic head-down tilt bed rest (HDTBR) impose a headward fluid shift that is hypothesized to be the primary underlying factor in the development of optic disc oedema. However, the mild hypercapnic environment on the International Space Station (ISS) has been hypothesized as a potential contributing factor to SANS (Stenger *et al.* 2017), as well as being implicated in increasing the risk of headaches during spaceflight (Law *et al.* 2014). This hypothesis suggests the elevated ambient P_{CO_2} increases arterial P_{CO_2} to vasodilate cerebral arterioles and increase intracranial pressure, which would be transmitted down the optic nerve and contribute to the development of optic disc oedema. With future space vehicles being constructed that will take astronauts to the moon and Mars, it is imperative to better understand what effect low levels of ambient P_{CO_2} will have on humans who are also simultaneously exposed to a chronic headward fluid shift.

Exposure to chronically elevated carbon dioxide (CO_2) levels does not typically occur in healthy individuals unless they are confined to spaces with inadequate ventilation, including submarines or spacecraft (Law *et al.* 2014; Rodeheffer *et al.* 2018), become trapped in caves or mines (Monsé *et al.* 2014) or perhaps as a result of 'green buildings'. Increasing ventilation rates in buildings to lower indoor CO_2 levels can decrease the prevalence of 'sick building syndrome' and improve perceptions of air quality (Seppänen *et al.* 1999), and associations between increased ventilation rates and improved student performance (Fisk, 2017) and reductions in absenteeism (Mendell *et al.* 2013) have been reported. However, prospective studies that exposed adults to mild levels of CO_2 have reported decreases (Satish *et al.* 2012), no change (Rodeheffer *et al.* 2018; Zhang *et al.* 2016) and increases (Scully *et al.* 2019) in performance, highlighting the challenges in interpreting a direct link between exposure to mild levels of CO_2 and performance impairments. Because these environments have varied levels and fluctuations of CO_2 (Fisk, 2017), descriptions of the physiological acclimatization to mildly elevated CO_2 levels are scarce and vary considerably. The response(s) to such stimuli probably require an integrated response with both respiratory and renal compensation occurring to protect acid–base balance.

Weightlessness induces a headward fluid shift as a result of the removal of a head-to-foot gravitational gradient, although cerebrovascular reactivity to CO_2 is unchanged during acute tilting between 90° head-up to 90° head-down tilt (Tymko *et al.* 2015). This suggests that the fluid shift changes do not necessarily contribute to changes in cerebrovascular reactivity. Conversely, during acclimatization to high altitude when arterial partial pressure of CO_2 (P_{CO_2}) is chronically low, cerebrovascular reactivity to P_{CO_2} is enhanced (Fan *et al.* 2016), suggesting that changes in arterial P_{CO_2} levels may lead to alterations in cerebrovascular reactivity. In addition, rats exposed to 10% CO_2 for 21 weeks demonstrated an attenuated cerebrovascular response to CO_2 and normalized intracranial pressure (Kondo *et al.* 1999). Exposure of four subjects to 1.7 mmHg ambient CO_2 for 42 days led to enhancement of the hypercapnic ventilatory response (HCVR), suggesting that mild hypercapnia may also have the potential to alter chemoreflexes responsible for maintaining arterial blood gas homeostasis (Elliott *et al.* 1998).

In 2017, the National Aeronautics and Space Administration (NASA) conducted a 30-day HDTBR study at the German Aerospace Center:envihab facility. During the study, 11 subjects remained in a strict 6° head-down tilt position in a mild hypercapnic environment in which the ambient P_{CO_2} was increased to ∼4 mmHg (indoor P_{CO_2} ∼1 mmHg). The 6° HDTBR model has been used as a spaceflight analogue for decades. The novelty of the present study arises from (i) the addition of the mild hypercapnic environment to simulate the atmosphere within the ISS and (ii) the

strict maintenance of subjects in the HDT position by not using a traditional pillow and by instructing subjects not to lift their upper torso onto an elbow during meals. The study was designed to investigate whether physiological adaptations occurred in response to the combination of the chronic headward fluid shift and the mild hypercapnic environment. We hypothesized that arterial P_{CO_2} would be elevated during the early part of HDTBR, whereas chronically elevated arterial P_{CO_2} resulting from exposure to the mildly hypercapnic environment would result in a blunted cerebrovascular reactivity to CO_2.

Methods

Ethical approval

The present study was conducted in response to NASA Research Announcement NNJ14ZSA001N that solicited scientific investigations to investigate 12 subjects before, during and after 30 days of HDTBR in a 0.5% (∼4 mmHg) CO_2 environment. The protocol described here was approved by the Institutional Review Board at the NASA Johnson Space Center (Protocol 2131) and by the Ethics Committee of the Medical Council of North Rhine for the Institute for Aerospace Medicine at the German Aerospace Center (DLR) (Protocol 2016408). All subjects provided their written informed consent to participate and all procedures adhered to the *Declaration of Helsinki*, except for registration in a database. This study was conducted at the DLR:envihab facility at an altitude of ∼50 m as part of the '*VIIP and Psychological:envihab Research (VaPER)*' bed rest campaign. End-tidal P_{CO_2} data from two of the 10 time points (Table 1) have been reported previously (Laurie *et al.* 2019).

Subjects

Twelve subjects (six females) consented to participate in the present study, although one female subject dropped out after the start of data collection on the first day of HDTBR. Data from the remaining 11 subjects are presented here. Subject inclusion and exclusion criteria, along with dietary planning, followed guidelines set forth in *Guidelines for Standardization of Bed Rest Studies in the Spaceflight Context* (Sundblad *et al.* 2014). Nonsmoking healthy male and female subjects needed to be aged 24–55 years, with body mass index of 20 to 30, satisfactorily complete physiological and psychological screening, and undergo a dual-energy X-ray absorptiometry bone density measurement. Laboratory testing included a basic metabolic panel, vitamin D and iron levels, a lipid panel, creatinine kinase levels, thyroid disease testing, drug screening, thrombophilia counselling, pregnancy test, and a complete blood count. Before the study, no medications were allowed, including for the relief of seasonal allergies, heartburn, acid reflux or indigestion, or any other medications that may interfere or interact with blood pressure regulation, the cardiovascular system, sleep patterns or neurological function. Female subjects could not be pregnant, menopausal or postmenopausal, could not use oral contraceptives or contraceptive patches, and were required to have a regular menstrual cycle based on daily body temperature recording for two menstrual cycles before the start of the study.

Environment

Subjects lived at the:envihab facility in Cologne, Germany (altitude 300 feet, ∼750 mmHg) for 14 days before, 30 days during and 14 days after HDTBR. All consumed standardized meals, utilizing a 7 day meal plan for pre- and post-bed rest and a standard 14 day meal plan that has been identified and implemented previously for:envihab long-term bed rest studies. Tailored meals were served three times per day plus an afternoon snack with dietary intake sufficient to maintain body weight within 3% of each subject's measured weight on the third day of HDTBR. Subjects were expected to eat all of their food at each meal and were given an isocaloric diet based on NASA spaceflight nutritional requirements with a balanced intake of macro- and micronutrients and a ratio of 50% to 55% carbohydrate, <35% fat and 12–15% protein. Subjects were given a standardized level for daily water consumption based on their weight (50 mL of fluid intake per 1 kg body weight). Subjects could add lemon juice to water but were not allowed to have cocoa, chocolate, tea, alcoholic beverages, herbal drinks or caffeinated beverages. The results of the vitamin D test from subject screening determined whether a subject received 2000 IU of vitamin D supplementation before the head-down tilt phase, and all subjects received 1000 IU of vitamin D supplementation during the head-down tilt phase.

During the pre- and post-HDT period, subjects were ambulatory, although they are not allowed to leave the facility and slept in a horizontal position with *ad libitum* use of a normal pillow. During the 30 days of HDTBR, a normal pillow was not allowed, although a 5 cm tall support for the head and neck (120 kg m^{-3}) was allowed to be used by subjects during the day and when sleeping on their side only (Fig. 1). No pillows were used when sleeping on their back. During HDTBR, six subjects reported never using the neck support, three reported using it sometimes and two used it for the duration of HDTBR. Of the five subjects who developed optic disc oedema (Laurie *et al.* 2019), two never used the support, two used it some of the time and one subject used it during all nights. Throughout the daytime, subjects maintained the HDT position for all activities, including eating during meals, showering, and the use of a bedpan for urination and defecation.

Table 1. Physiologic parameters at rest and the end of hyperventilation and rebreathing; values are the mean (95% confidence interval) during 10 min of quiet rest, during the last five breaths of hyperventilation and during the last five breaths of rebreathing

	BDC-6		HDT2	HDT9	HDT15	HDT30	R+6		R+13	
Phase and variable	Seated	HDT	HDT	HDT	HDT	HDT	Seated	HDT	Seated	HDT
Baseline										
P_{ETCO_2} (mmHg)	40.3	42.1	40.8	40.8	41.7	41.7	38.4	40.3	38.8	40.7
	(38.7–41.9)	(40.3–43.9)	(39.0–42.7)	(38.4–43.2)	(39.6–43.7)	(39.7–43.6)	(36.3–40.6)	(38.0–42.5)	(37.0–40.5)	(38.9–42.6)
MCA velocity ($cm\ s^{-1}$)	51.1	56.7	54.6	55.3	54	56.5	55.2	59.9	55.2	60.2
	(45.5–56.8)	(50.4–63.0)	(47.9–61.2)	(47.5–63.2)	(46.1–61.8)	(49.2–63.9)	(48.4–62.1)	(52.4–67.4)	(46.5–64.0 0	(51.7–68.6)
Systolic blood pressure (mmHg)	122.8	118.2	120.4	119.0	119.8	122.1	117.7	116	114.9	110
	(116.0–129.7)	(111.8–124.6)	(113.4–127.3)	(113.2–124.9)	(111.6–128.0)	(115.0–129.2)	(112.1–123.4)	(109.3–122.8)	(108.6–121.2)	(105.0–115.0)
Diastolic blood pressure (mmHg)	76.2	68.1	68.6	70.2	71.6	73.4	75.1	66.3	71.7	63.2
	(71.9–80.6)	(64.5–71.7)	(65.0–72.2)	(66.8–73.7)	(67.7–75.5)	(70.8–75.9)	(72.1–78.1)	(63.4–69.2)	(68.2–75.3)	(59.7–66.7)
End of hyperventilation										
P_{ETCO_2} (mmHg)		22.1	21.8	21.9	23.9	22.7		21.4		21.1
		(21.2–23.1)	(21.1–22.5)	(21.3–22.8)	(23.3–24.5)	(22.1–23.3)		(20.3–22.4)		(20.4–21.8)
MCA velocity ($cm\ s^{-1}$)		32.1	31.7	30.8	31.9	33.6		34.8		33.6
		(28.4–35.7)	(26.6–36.8)	(25.2–36.4)	(25.9–37.8)	(28.3–38.9)		(30.2–39.4)		(28.0–39.3)
End of rebreathe										
P_{ETCO_2} (mmHg)		57.1	56.9	57.4	57.5	58.3		55.5		55.8
		(55.2–59.0)	(54.7–59.1)	(54.7–60.2)	(55.6–59.5)	(56.5–60.2)		(53.1–58.0)		(54.2–57.4)
MCA velocity ($cm\ s^{-1}$)		90.5	86.5	82.1	77.5	84.2		83.9		83.8
		(79.9–101.1)	(75.3–97.7)	(67.1–97.1)	(64.1–90.8)	(73.1–95.2)		(72.5–95.2)		(71.5–96.1)

Mean (95% confidence interval) modeled values include inputs at each time point from n = 11 subjects. The 10 min baseline period incorporated up to 10 averages of 1 min for each subject. The end of hyperventilation and end of rebreathe periods included five breaths of data for each subject.

After 14 days of the pre-HDTBR period (BDC-14 to BDC-1), subjects entered the hypercapnic environment (0.5% CO_2 in 21% O_2) and assumed the 6° HDT position at 09.00 h on the first day of HDTBR (HDT1). Therefore, measurements on HDT2 occurred ∼24 h after starting the HDT phase of the study. Subjects maintained the 6° HDT position in the hypercapnic environment through HDT30 and resumed an upright posture in a normocapnic environment on the morning of the first day of recovery (R+1). Subjects remained in the facility during recovery and reconditioning for 2 weeks (R+1 to R+14).

Study timeline, subject instrumentation and rebreathe protocol

One week after a familiarization practice session, subjects were studied in the morning between 09.00 and 11.00 h, 6 days before (BDC-6) and 6 and 13 days following (R+6, R+13) HDTBR. During HDTBR subjects were studied on HDT2, HDT9, HDT15 and HDT30 (Fig. 2). Subjects were instrumented with a nose clip and breathed through a mouthpiece connected to a T-valve, with one end open to room air and the other attached to a 5 L rubber bag containing 100% oxygen. Carbon dioxide was sampled at the base of the mouthpiece and measured using a CO_2 analyser (Silver Series; VacuMed, Ventura, CA, USA). An analogue output signal of breath-by-breath tracing of CO_2 was sent to a data acquisition system at 250 Hz (NOTOCORD-hem v4.4.0.1; Notocord, Inc., Newark, NJ, USA) and used to determine end-tidal P_{CO_2} and inspired P_{CO_2} values. There was a pneumotach and a second gas sampling line between the mouthpiece and the T-valve that was used to measure airflow and inspired and expired O_2 and CO_2 with a metabolic system (Ultima PFX; MedGraphics, Minneapolis, MN, USA). Arterial blood pressure (ABP) was measured noninvasively using finger photoplethysmography (Finometer Model 2; Finapres Medical Systems B.V., Amsterdam, The Netherlands). The middle cerebral artery velocity (MCA_V) was determined from the insonated Doppler ultrasound signal (TOC Neurovision Model ROBOTOC2MD; Multigon Industries, Inc., Yonkers, NY, USA). Baseline resting data were collected for 10 min in the seated (pre- and post-HDTBR) and the 6° HDT (pre-, in and post-HDTBR) positions. Following collection of baseline data in the 6° HDT, subjects were coached to voluntarily hyperventilate for 1 min to lower end-tidal P_{CO_2} (P_{ETCO_2}) to a target of 20–25 mmHg before the T-valve was switched to the bag containing 100% O_2 and subjects rebreathed from this bag until their P_{ETCO_2} reached a target of 55–60 mmHg. The T-valve was then switched back to room air for 5 min of recovery. Raw data collected throughout the rebreathe test were used to determine cerebrovascular reactivity to CO_2 and the hypercapnic ventilatory response (for analysis details, see below).

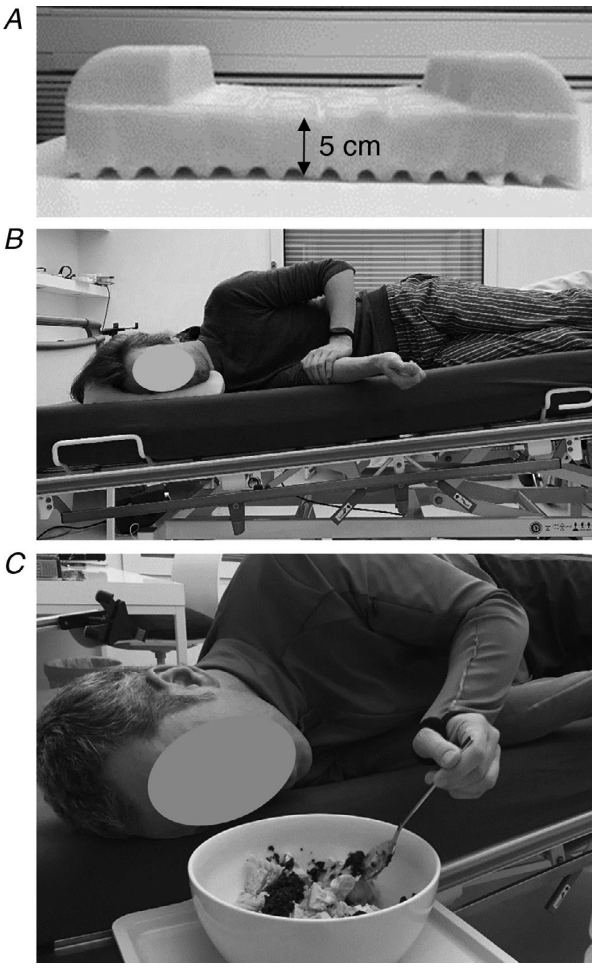

Figure 1. Head and neck support during strict 6° head-down tilt
A, custom designed head and neck support. *B*, subjects used head and neck support when sleeping on side to keep head in line with the rest of their body. *C*, strict head-down tilt was maintained even when eating meals.

Arterialized blood gas collection and analysis

At the beginning of each 10 min baseline period, a heating pad was wrapped around the hand of the subject. Approximately 7 min into the 10 min baseline period, two arterialized capillary blood samples were obtained from the index or middle finger into heparinized glass capillary tubes and immediately analysed for P_{O_2}, P_{CO_2} and pH (ABL 800 FLEX; Radiometer, Brønshøj, Denmark).

Cerebrovascular reactivity

The MCA_V, beat-by-beat blood pressure and CO_2 gas sample were collected using the data acquisition system described above and analysed offline using custom

MATLAB software (MathWorks Inc., Natick, MA, USA) that selected the peak and trough of the P_{CO_2} signal for each breath and calculated the area under the curve for each beat of the MCA_V and Finometer signals. Each breath was identified from the end-tidal P_{CO_2} (P_{ETCO_2}) signal and averages for P_{ETCO_2}, MCA_V and ABP were measured and used to calculate mean values from the baseline period, the last five breaths of the hyperventilation period, the last five breaths of the rebreathe period and to derive the breath-by-breath MCA_V– P_{ETCO_2} relationships. The cerebrovascular conductance index was calculated by dividing the mean MCA_V by the mean ABP for each breath during the rebreathe period to reveal cerebrovascular responses intrinsic to the change in P_{CO_2}. To estimate cerebrovascular reactivity, we adopted previously outlined analysis methods (Claassen *et al.* 2007; Fan *et al.* 2016) that use a four-parameter logistic function and first-order derivative to quantify the midpoint of the sigmoidal relationship of the MCA_V– P_{ETCO_2} relationship, indicative of maximal capacity of the vasculature to dilate or constrict. The maximal slope within the linear portion of the sigmoid curve was determined to quantify cerebrovascular CO_2 reactivity.

Hypercapnic ventilatory response

Minute ventilation (V_E) and P_{ETCO_2} were collected on a breath-by-breath basis during the same protocol outlined above and analysed offline. Each HCVR test was preceded by 1 min of coached, voluntary hyperventilation. The purpose of this was to lower the subject's P_{ETCO_2} within the range of 20–25 mmHg. As previously outlined by Mohan *et al.* (1999), the rationale of prior hyperventilation is to ensure that the subject begins the rebreathing stage at an P_{ETCO_2} value below the ventilatory recruitment threshold (VRT). Furthermore, this technique allows for an accurate measure of baseline ventilation (Duffin *et al.* 2000; Mohan *et al.* 1999). After voluntary hyperventilation, the subject exhaled completely and the T-valve was then directed toward the 5 L rebreathing bag containing 100% O_2. The subject was then asked to take three, deep equilibration breaths. The purpose of this was to rapidly equilibrate P_{CO_2} in the bag, lungs and arterial blood with the subjects mixed venous blood (Slessarev *et al.* 2010). Upon switching to the rebreathe portion of the test, the subjects were encouraged to relax and breathe normally. The test ended when (i) P_{ETCO_2} reached 60 mmHg or (ii) the subject voluntarily ended the test. Breath-by-breath

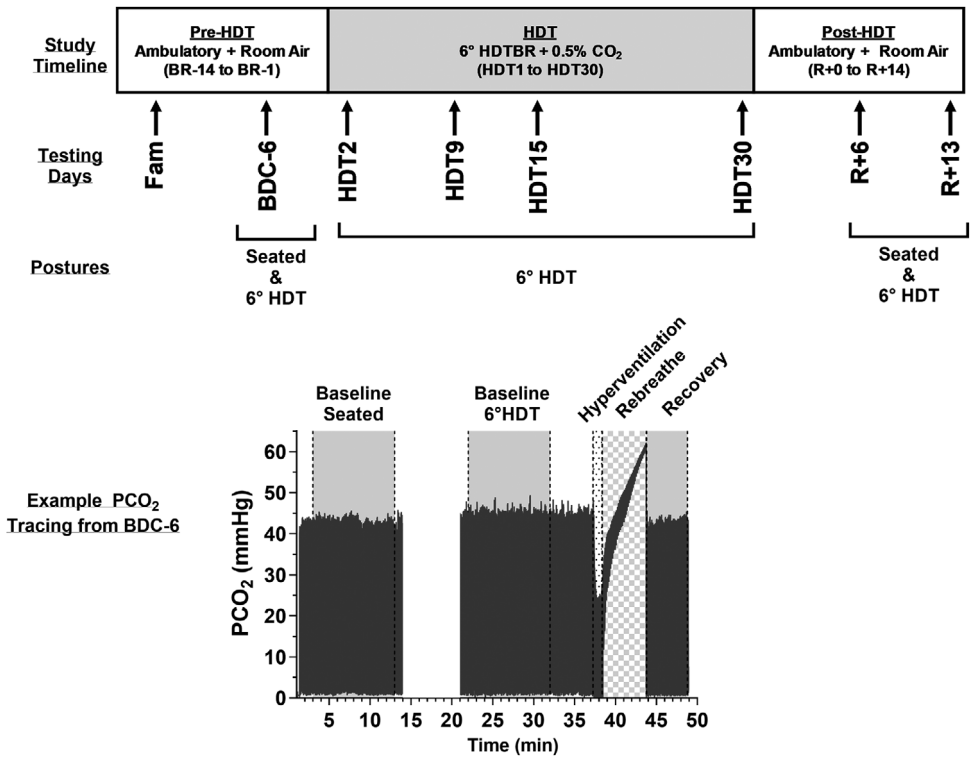

Figure 2. Schematic of study timeline, including data collections time points, subject postures and environmental CO_2 levels
Example of raw tracing of P_{CO_2} levels at BDC-6 when subjects were studied when seated and in the 6° head-down tilt (HDT) posture. Subjects remained in the 6° HDT posture during the rebreathe protocol, including during hyperventilation, rebreathe and recovery. Note trough of P_{CO_2} tracing approaches near zero, reflecting the inspired P_{CO_2}.

data from the metabolic system were then transferred into an Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for further analysis. Using a least squares regression line, P_{ETCO_2} was plotted against time. The regression line was then used to calculate a predicted P_{ETCO_2} against time as a method of data smoothing (Duffin *et al.* 2000). Finally, V_E was plotted against the predicted P_{ETCO_2} for further analyses. For each subject and at each time point, two distinct segments were identified using segmental linear regression (Prism, version 5; GraphPad Software Inc., San Diego, CA, USA). The first segment was a line representing baseline V_E (non-chemoreflex drive) at the beginning of the rebreathe test. The second segment began immediately after the breakpoint (VRT; mmHg) with the slope representing the HCVR (L min^{-1} $mmHg^{-1}$). The segmental linear regression eliminated outlier breaths until an r^2 value of >95% was achieved and was defined as any breath that did not fit within a 95% confidence limit of the two segments. From this procedure, the identification of basal V_E, the VRT and HCVR were easily detectable.

Statistical analysis

Data were analysed using mixed models for each measure. All data were analysed in SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA) using the GLIMMIX procedure and LSMEANS statement for pairwise comparisons. Subject-specific random intercepts were included to address the repeated measures within individuals. Robust standard errors were used to address heteroscedasticity: changes in variance of the measures over time. Study day (BDC-6, HDT2, HDT9, HDT15, HDT30, R+6 and R+13) was included as a fixed effect. Pairwise comparisons with BDC-6 were conducted for all other study days using Dunnett's adjustment to address the multiple testing for each measure. When applicable, the position (upright or HDT) was included as an interaction with bed rest day. When the interaction was significant, pairwise comparisons were completed assessing differences with BDC-6 within each position, as well as between positions at the overlapping days (BDC-6, R+6 and R+13). Again, Dunnett's method was used to adjust for the multiple testing of the pairwise comparisons within a measure. All *P* values reflect adjustments for multiple comparisons.

Results

Subjects had a mean (range) age of 33.4 (25–50) years, height of 173.8 (158–186) cm, weight of 70.8 (55–84) kg and body mass index of 23.4 (20–28). During HDTBR, the environmental control system of the facility successfully increased ambient CO_2 to 0.49% (SD 0.01%) and we measured the inspired P_{CO_2} during the 10 min baseline period as ∼4 mmHg (Fig. 3). Arterialized P_{CO_2}, pH and bicarbonate concentrations ($[HCO_3^-]$, calculated by the blood gas machine) are shown in Fig. 3. The acute posture change from seated to 6° HDT caused arterialized P_{CO_2} to increase by a mean [95% confidence interval (CI)] of 1.8 mmHg (0.6–3.1 mmHg, $P = 0.004$) on BDC-6, 1.5 mmHg (0.6–2.3 mmHg, $P = 0.0007$) on R+6 and 1.3 mmHg (0.7–1.8 mmHg, $P < 0.001$) on R+13 (Fig. 3*B*) and the associated mild respiratory acidosis was reflected in the pH data (Fig. 3*C*) by a mean (95% CI) of –0.007 (–0.017 to 0.004, $P = 0.213$) on BDC-6, –0.005 (–0.010 to 0.0002, $P = 0.0611$) on R+6 and –0.005 (–0.011 to 0.0006, $P = 0.0778$) on R+13. At all time points during HDTBR, arterialized P_{CO_2} was not significantly different from the pre-HDTBR measured in 6° HDT posture, with the largest mean (95% CI) increase of 1.2 mmHg (–0.2 to 2.5 mmHg, $P = 0.122$) on HDT30. Compared to BDC-6 in the 6° HDT posture, arterialized pH during and after HDTBR never demonstrated acidosis. Conversely, on HDT30, the arterialized pH increased by a mean (95% CI) of 0.032 (0.022–0.043, $P < 0.0001$) and remained elevated by 0.021 (0.011–0.031, $P < 0.0001$) on R+6 before returning to pre-HDTBR values on R+13 with a change of –0.001 (–0.009 to 0.007, $P = 0.991$) (Fig. 3*C*). The $[HCO_3^-]$ calculated by the blood gas machine demonstrated the same pattern.

All subjects tolerated and completed the rebreathing protocol on all study days. Figure 4 shows a representative plot of raw tracings from a single subject of the breath-by-breath tidal P_{CO_2}, beat-by-beat MCA velocity and arterial blood pressure collected on HDT30. The 10 min baseline period of quiet rest precedes the 1 min of hyperventilation, the rebreathe test and the recovery period. Note that the trough of the tidal P_{CO_2} tracing during the baseline and recovery periods reveals the elevated inspired P_{CO_2} level. In addition, an example of the plot of cerebrovascular reactivity derived from the P_{ETCO_2} and MCA velocity sigmoidal relationship is provided. Mean values for the group of P_{ETCO_2}, middle cerebral artery velocity, and systolic and diastolic blood pressure during the 10 min baseline period (seated and HDT postures) and the last five breaths of the hyperventilation and rebreathe periods (HDT posture only) are presented in Table 1. Compared to BDC-6 in the HDT position, mean (95% CI) baseline P_{ETCO_2} was significantly less by –1.2 mmHg (–2.4 to 0.0 mmHg, $P = 0.039$) on HDT2, as well as on R+6 by –1.8 mmHg (–3.2 to –0.4 mmHg, $P = 0.006$) and on R+13 by –1.3 mmHg (–2.1 to –0.5 mmHg, $P = 0.0001$). The mean MCA blood flow velocity in the HDT position was not significantly different from BDC-6 at any time during or after HDTBR. Compared to BDC-6, mean (95% CI) systolic blood pressure in the HDT position was significantly less by 8.2 mmHg (–12.2 to –4.2 mmHg, $P < 0.0001$) on R+13 and diastolic blood pressure was significantly greater by 5.3 mmHg (1.2 to 9.4 mmHg,

$P = 0.0016$) on HDT30 and significantly less by 4.9 mmHg (–8.3 to –1.5 mmHg, $P = 0.0021$) on R+13.

Subjects successfully lowered P_{ETCO_2} as a result of hyperventilation to a target of 20–25 mmHg and increased P_{ETCO_2} at the end of rebreathing to target of 55–60 mmHg on all study days before, during and after HDTBR. The P_{ETCO_2} and corresponding MCA velocity at either the end of hyperventilation or the end of the rebreathe period were not significantly different from pre-BR at any time points (Table 1).

Cerebrovascular reactivity as quantified from the steepest slope of the linear portion along the sigmoid relationship between MCA velocity and P_{ETCO_2} is shown in Fig. 5. We report both the absolute and percentage change for both MCA velocity and cerebrovascular conductance that normalizes to the arterial blood pressure. Compared to BDC-6, there was no significant change in any measure of cerebrovascular reactivity at any time point.

Compared to BDC-6, minute ventilation measured during the baseline period did not change during or after HDTBR. The VRT remained similar to BDC-6 during BR, although it shifted to a lower P_{ETCO_2} at R+6 by a mean (95% CI) of –3.9 mmHg (–1.5 to –6.3 mmHg, $P < 0.001$) and at R+13 by –2.6 mmHg (–0.7 to –4.4 mmHg, $P = 0.0025$). The slope of the HCVR after the VRT did not change throughout or after HDTBR (Fig. 6).

Discussion

The main finding of the present study was that subjects exposed to 30 days of a mild hypercapnic environment when in the strict 6° HDT posture did not increase their P_{CO_2} levels evaluated from P_{ETCO_2} or arterialized blood gas samples, nor did acidosis develop. Conversely, a mild alkalosis developed that appeared to be independent of arterialized blood gas levels. Probably as a result of the maintenance of arterialized P_{CO_2} levels, the cerebrovascular response and the hypercapnic ventilatory response to CO_2 were maintained and did not change.

Arterial blood gases

Studies investigating chronic exposure to hypercapnic environments are sparse, with variable experimental conditions and consequently variable findings. Goats exposed to 6% (45.6 mmHg) inspired CO_2 for 30 days

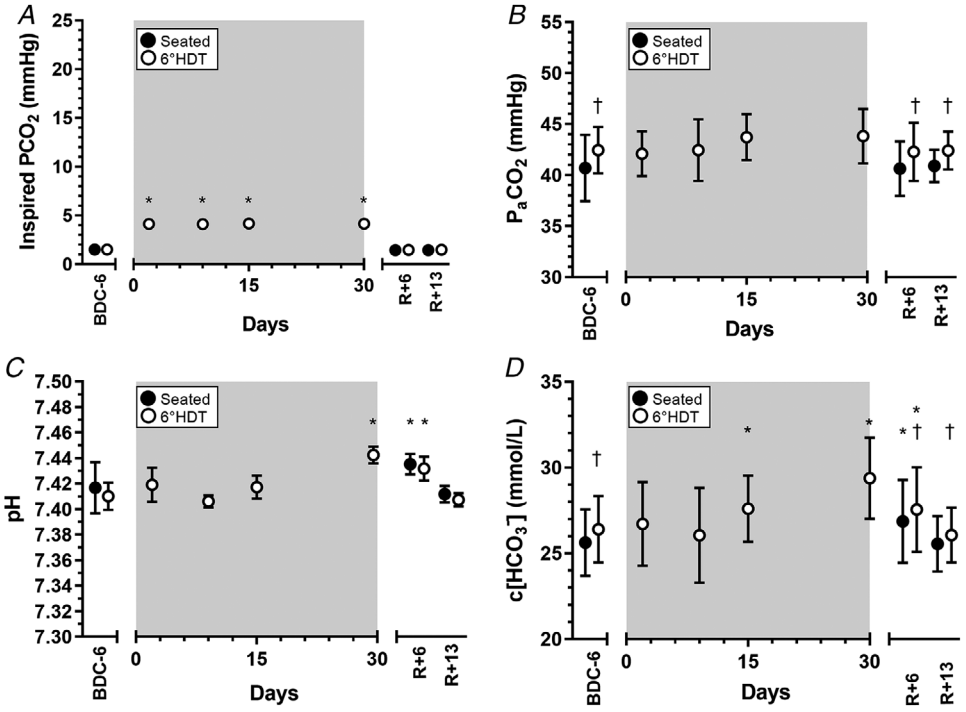

Figure 3. Inspired CO_2, arterialized CO_2 and pH, and calculated HCO_3^- before, during, and after strict 6° head-down tilt bed rest
Inspired P_{CO_2} (*A*), arterialized P_{CO_2} (*B*), pH (*C*) and calculated [HCO_3^-] (*D*) levels measured before, during and after 30 days of head-down tilt bed rest (HDTBR). Inspired P_{CO_2} levels were ∼1.5 mmHg during BDC-6, R+6 and R+13 and were elevated to ∼4 mmHg during HDTBR. Subjects were studied in the seated (black symbols) before and after HDTBR and 6° HDT posture (white symbols) before, during and after HDTBR. Shaded region represents period of HDTBR in the mild hypercapnic environment. Values are the mean ± SD for n = 11 subjects at each time point. *$p < 0.05$ *vs.* BDC-6 within same posture. †$p < 0.05$ *vs.* seated posture within same day. Mixed-model Dunnett-adjusted pairwise *t* tests were conducted to determine significant differences between days and postures.

demonstrated an initial increase of arterial P_{CO_2} by 10 mmHg, and a further increase of 5 mmHg throughout the first week of hypercapnic exposure (Burgraff *et al.* 2018). The P_{CO_2} remained at ∼15 mmHg above baseline throughout the remainder of the study. Data from four subjects exposed to P_{CO_2} of 5.3 mmHg for 26 days demonstrated mild increase during the first 2–3 days before a return towards pre-exposure levels for the duration of the study, probably mediated by the resulting hyperventilation (Sliwka *et al.* 1998). Interestingly, there was an increase in variability between subjects in P_{ETCO_2} throughout the duration of exposure. This suggests that some individuals may experience larger increases in P_{ETCO_2} and thus could have larger physiological effects despite a constant level of inspired CO_2. Across the study as a whole, few results were different from control conditions, yet it was unclear whether those differences were the direct result of the CO_2 level (Wenzel *et al.* 1998). Exposure of 21 subjects to P_{CO_2} of 11.4 mmHg, which is almost three times the level used in the present study, resulted in increased alveolar P_{CO_2} of 2–3 mmHg and increased minute ventilation, although arterial blood gas analysis was not performed. Venous blood pH decreased upon initial exposure to the hypercapnia and gradually returned

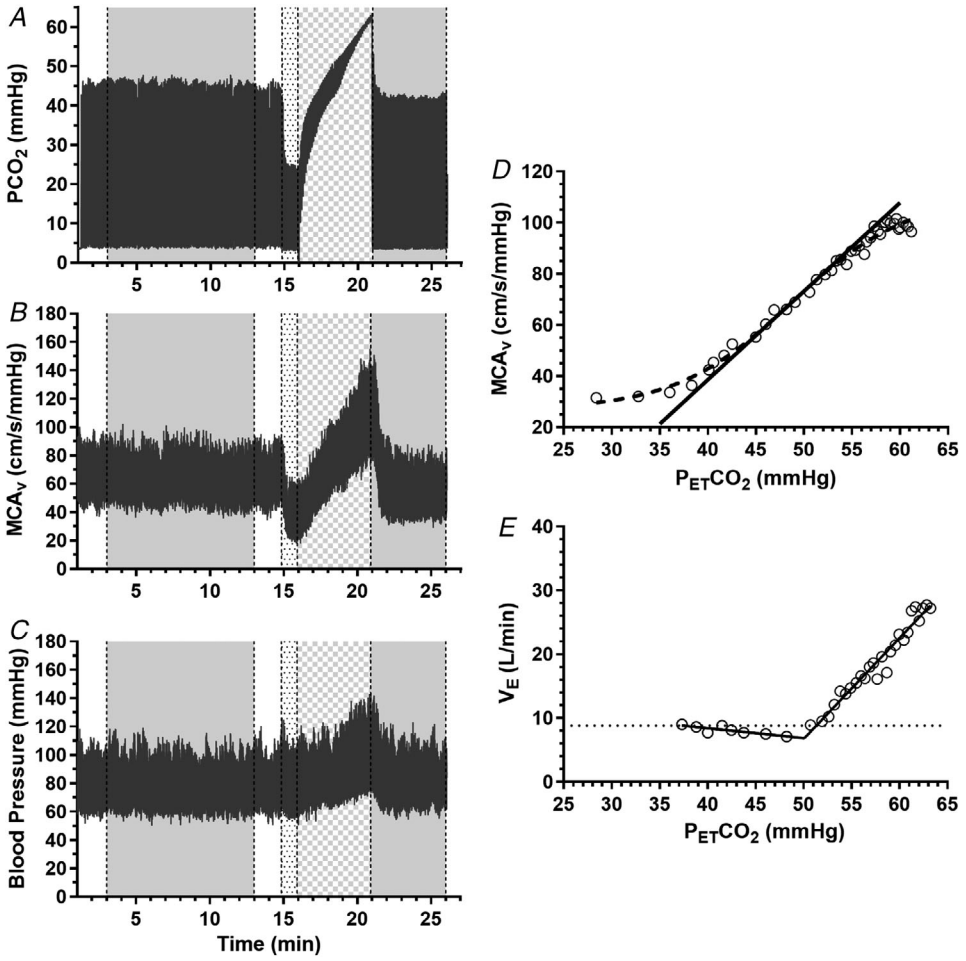

Figure 4. Representative raw tracings and analysis of cerebrovascular reactivity and hypercapnic ventilatory response of a single subject
Original tracing from a representative subject on HDT30 of (*A*) P_{CO_2}, (*B*) middle cerebral artery blood flow velocity, (*C*) arterial blood pressure, (*D*) a representative plot of the cerebrovascular reactivity derived from (*A*) and (*B*), and (*E*) a representative plot of the hypercapnic ventilatory response showing minute ventilation as a function of P_{ETCO_2}. In (*A*) to (*C*), the grey region from ∼3 to 13 min is the 10 min baseline, the white with black dotted region from ∼15 to 16 min is the 1 min of hyperventilation, the grey checkerboard region from ∼16 to 21 min is the rebreathe test, and the grey region from ∼21 to 26 min is 5 min of recovery. Note the trough of the P_{CO_2} tracing is elevated at ∼4 mmHg during baseline. In (*D*), each symbol represents the average MCA_v and P_{ETCO_2} value for each breath of the rebreathe test. The dashed sigmoid curve is the best fit of the data and the solid black line is the steepest slope of the sigmoid. In (*E*), the horizontal dashed line at $V_E = 9$ L min^{-1} represents the mean ventilation during the 10 min baseline period. Two linear best-fit segments were used to identify the minute ventilation, ventilatory recruitment threshold and slope of the hypercapnic ventilatory response.

to baseline over the 42 day exposure (Schaefer *et al.* 1963). Submariners exposed to ∼1% CO_2 demonstrated an initial increase in P_{CO_2} of 1 mmHg, which increased further to a P_{CO_2} ∼3.5 mmHg above baseline after 44 days (Pingree, 1977), which, despite being twice the exposure used in the present study, is in line with our data. By contrast to the present study, however, a mild acidosis developed that did not recover to baseline level until returning to fresh air. Forcing end-tidal P_{CO_2} to remain elevated by 8 mmHg for 5 days led to a rapid acidosis that only partially recovered (Crosby *et al.* 2003).

Taken together, these data suggest that exposure to sufficiently elevated ambient P_{CO_2} leads to a rapid increase in arterial P_{CO_2} and mild acidosis. Renal compensation in response to the acidosis results in the retention of bicarbonate that appears to be completed within the first week of exposure. Conversely, our data do not demonstrate a significant respiratory acidosis ever developing, and the time course of the mild metabolic alkalosis is delayed relative to renal responses that appear to be complete within the first week of exposure in previous studies. When our subjects returned to daily upright posture in a normocapnic environment, again, there was a delayed response of the arterialized pH, which did not recover until almost 2 weeks later. Finally, in all previous studies that demonstrate an acute acidosis and bicarbonate retention to normalize the pH, none demonstrate an overshoot of the pH as we report here. These observations suggest that the stimulus for the mild metabolic alkalosis reported in the present study is not in response to a respiratory acidosis. The cause of this mild metabolic alkalosis remains perplexing to us and we have hypothesized regarding the various mechanisms that may have led to this finding. Plasma volume loss occurs during HDTBR (Platts *et al.* 2009; Westby *et al.* 2016) and may lead to mild renal hypoperfusion and retention of bicarbonate, and also result in mild metabolic alkalosis. Alternatively, given that bone resorption can occur to buffer systemic acidosis (Krieger *et al.* 2004), it is possible that the inactivity of subjects in the present study led to bone resorption that, without acidosis, resulted in the mild alkalosis.

It is important to emphasize that, although real, the magnitude of the metabolic alkalosis in our subjects was relatively small (0.03 increase in pH) and we do not have measurements of pH_{CSF} to determine whether the CSF reflected the mild alkalosis observed in arterialized

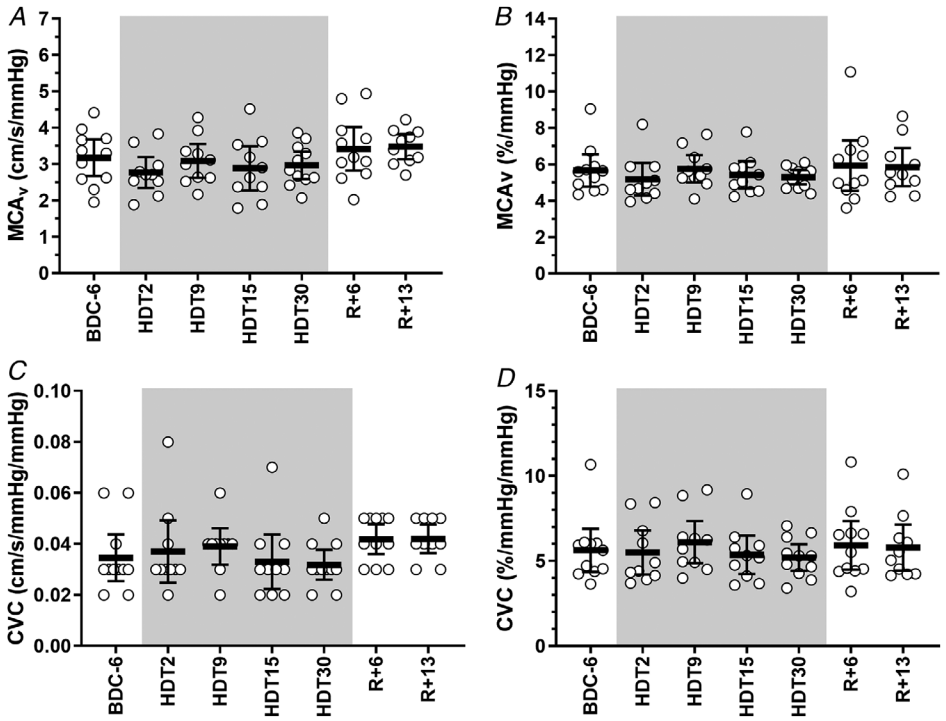

Figure 5. Cerebrovascular reactivity measured in the 6° HDT posture before, during and after HDTBR
Data are presented as an absolute (*A*) and percentage change (*B*) in middle cerebral artery velocity (MCAv), as well as the absolute (*C*) and percentage change (*D*) in middle cerebral artery velocity, normalized to arterial blood pressure (cerebrovascular conductance index, CVCi). Symbols represent data from each subject ($n = 11$) and the thick line represents the mean with 95% CI error bars. We were unable to obtain the Doppler signal in one subject on HDT2, HDT9 and HDT15, resulting in only 10 subjects on those days. The shaded region represents the period of HDTBR in the mild hypercapnic environment. No time points demonstrated statistically significant changes from BDC-6. Mixed-model Dunnett-adjusted pairwise *t* tests were conducted to determine significant differences between days.

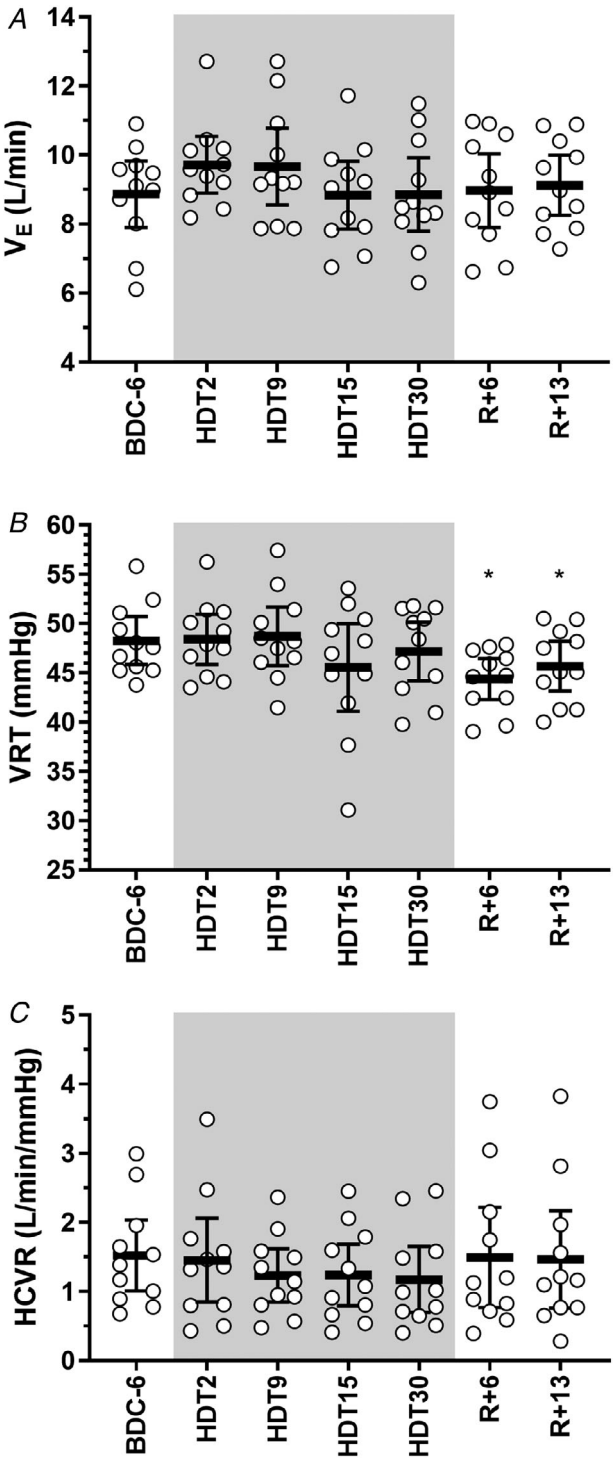

Figure 6. Hypercapnic ventilatory response variables before, during, and after 6-degree strict head-down tilt bed rest
Minute ventilation (V_E) (*A*), ventilatory recruitment threshold (VRT) (*B*) and hypercapnic ventilatory response (HCVR) (*C*) measured in the 6° HDT posture before, during and after HDTBR. Symbols represent data from each subject and the thick line represents the mean with 95% CI error bars. The shaded region represents the period of HDTBR in the mild hypercapnic environment. *$p < 0.05$ *vs*. BDC-6. Mixed-model Dunnett-adjusted pairwise *t* tests were conducted to determine significant differences between days.

blood. Given that hydrogen ions do not directly cross the blood–brain barrier, and P_{CO_2} that readily crosses the blood-brain barrier did not significantly change, we would not expect significant changes to central chemoreceptor input. Conversely, the mild alkalosis could attenuate the stimulus to peripheral chemoreceptors. As reviewed by Dempsey & Forster (1982) across numerous studies, the slope of the $\Delta PaCO_2/\Delta[HCO_3^-]$ averages 0.9 mmHg mEq^{-1} L^{-1} (range 0.8 – 1.2). Using our calculated $\Delta[HCO_3^-]$ from BDC-6 to HDT30, we would predict our arterialized Δ P_{CO_2} to increase by 2.7 mmHg (range 2.4–3.6). We measured an increase in arterialized P_{CO_2} from BDC-6 to HDT30 of 1.4 mmHg. The reported values from other studies were obtained from subjects with a range of arterial $[HCO_3^-]$ from 15 to 35 mEq L^{-1}, and our $\Delta[HCO_3^-]$ estimate falls within that span, although within a narrower range of 26 to 29 mEq L^{-1}. Thus, we view our measurement of an increase in $PaCO_2$ within 1 mmHg of the predicted $PaCO_2$, to reflect a very mild respiratory acidosis in response to the relatively mild metabolic alkalosis comprising part of the integrative physiological response to the experimental conditions.

Arterial blood for analysis of P_{O_2} and P_{CO_2} has not been collected from astronauts when on the ISS. When ambient P_{CO_2} levels on ISS were similar to those used in the present study (~4 mmHg), P_{ETCO_2} (which, in healthy subjects, is in equilibrium with arterial P_{CO_2}) during spaceflight was no different than that measured in the supine position breathing room air either before or after spaceflight (Prisk *et al.* 2006). The headward translation of the diaphragm that occurs as a result of changing positions from seated to supine, or with the removal of gravity during spaceflight, leads to a mild hypoventilation and a resulting increase in P_{ETCO_2}, as demonstrated previously (Elliott *et al.* 1998; Laurie *et al.* 2017; Prisk *et al.* 2006). A recent report suggesting the increase in ambient P_{CO_2} on ISS may have contributed to an elevated P_{ETCO_2} of astronauts on ISS (Hughson *et al.* 2016) should be interpreted with caution because the baseline P_{ETCO_2} on Earth was only measured in the seated upright position and no measuresments were reported in the supine position. Taken together, these data suggest that much larger increases in ambient or inspired P_{CO_2} would be necessary to have a physiologically meaningful increase in arterial P_{CO_2}. Thus, it was not surprising to observe that neither arterialized P_{CO_2}, nor P_{ETCO_2} increased during chronic exposure to this mild hypercapnic environment.

Cerebrovascular reactivity

The responsiveness of the cerebrovasculature to changing CO_2 levels appears to be maintained during acute posture changes (Tymko *et al.* 2015) and stable over time (Spencer *et al.* 2015). However, it has not been investigated during or after exposure to a chronic headward fluid shift, nor

after exposure to a chronic hypercapnic environment. We hypothesized that, if the mild hypercapnic environment had a physiological effect, we would detect increased brain blood flow during the early period of exposure, although the cerebrovascular response to CO_2 would be attenuated by the end of 30 days of HDTBR. This was in part based on the observation that exposure to 10% CO_2 leads to an attenuation of nitric oxide (NO)-dependent vasodilatation (Kondo *et al.* 1999) and that exposure to a high altitude resulting in chronic hypocapnia augments the cerebrovascular response to CO_2 (Fan *et al.* 2016). In addition, dynamic cerebral autoregulation was improved in astronauts after return from short-duration spaceflight (Iwasaki *et al.* 2007). In addition, recent use of head-out water immersion, another spaceflight analogue that also results in an acute headward fluid shift, resulted in a blunted CVR (Sackett *et al.* 2018), although there was no exposure to elevated CO_2 as a stimulus in that study. In the present study, we conducted the rebreathe test in the same 6° HDT posture at all before, during and after HDTBR time points. Despite conducting the studies in the same posture for all time points, we did not detect changes in the resting cerebral blood flow velocity, which is similar to previous bed rest studies (Jeong *et al.* 2014; Kermorgant *et al.* 2019; Zhang *et al.* 1997). Taken together, these data suggest the combination of strict HDTBR along with the mild elevation in ambient P_{CO_2} did not cause a blunting or augmentation of CVR.

Impairment in cerebral autoregulation (CA) was initially investigated as an explanation for orthostatic intolerance following the NeuroLab (STS-90) Space Shuttle mission. Measures of cerebral blood flow velocity with beat-to-beat changes in arterial pressure in four subjects after 16 days in space revealed that static autoregulation was not impaired and dynamic regulation was actually improved (Iwasaki *et al.* 2007). However, these data were collected over a range of lowering arterial pressures induced by lower body negative pressure. Thus, it is still unclear whether CA would have exhibited similar responses when cerebral perfusion pressure is increased as a result of the headward fluid shift. A possible limitation with these data is that none of the astronauts studied developed presyncope upon return to Earth and a subsequent study suggested that only those with orthostatic intolerance demonstrated impaired CA (Blaber *et al.* 2011). Importantly, ambient environmental CO_2 was tightly controlled during flight on the Space Shuttle, remaining <0.1% (Iwasaki *et al.* 2007). Conversely, during long-duration spaceflight on ISS when ambient CO_2 is known to average 0.5 ± 0.2%, with periodic localized spikes, especially during exercise (Law *et al.* 2010), dynamic CA was impaired (Zuj *et al.* 2012).

To our knowledge, no previous data exist assessing cerebrovascular reactivity during BR or spaceflight when the cephalad fluid shift and chronic hypercapnia are still in place. In the isolation study with four subjects exposed to 5.3 and 9.1 mmHg P_{CO_2} for 23 days, subjects did not experience a chronic headward fluid shift (Sliwka *et al.* 1998). Because only four subjects participated in that study, it is unclear whether the increasing variability in cerebral blood flow velocity that occurred throughout the 23-day exposure resulted from differences in cerebral vascular reactivity or reflected the variability in resulting arterial P_{CO_2}, caused by differences in HCVR. Unexpectedly, cerebral blood flow remained elevated for up to 5 days after returning to ambient air, suggesting that a chronic adaptation to higher CO_2 had occurred, specifically from a blunting of the HCVR. Given the challenges associated with repeatedly obtaining MCA velocity in the same location, the small number of subjects may have led to the observed changes in cerebral blood flow velocity. Following long-duration spaceflight, cerebrovascular resistance was not significantly elevated when supine and exposed to a lower body negative pressure stimulus on Earth (Zuj *et al.* 2012). However, these data include four subjects who were tested within hours of landing and three that were tested 1–2 days after landing when re-adaptation to Earth's gravitational environment was already occurring.

Ventilatory response to CO_2

The HCVR can be altered by chronic exposure to hypercapnia, although the impact appears to depend on the length of exposure and level of CO_2. For example, work by Elliott *et al.* (1998) found that mild hypercapnia (0.7% CO_2 for 22 days) can result in a blunted HCVR slope and lower VRT, whereas, in the same study, they found that 1.2% CO_2 for 22 days resulted in a similar HCVR slope and breakpoint. Forcing end-tidal P_{CO_2} to remain elevated by 8 mmHg for 5 days led to no change in ventilatory chemosensitivity to hypercapnia (Crosby *et al.* 2003). In five astronauts who flew a 16 day mission and 6 astronauts who flew a 17 day mission, the slope of the HCVR did not change during or after spaceflight (Prisk *et al.* 2000). The ambient P_{CO_2} during these flights was 2.3 mmHg and 2.8 mmHg, respectively. These values are slightly lower than that used in the present study, although higher than that typically experienced on Earth. Recent data collected from goats exposed to higher levels of CO_2 (6% CO_2) for a similar time period of 30 days results in a transient reduction in CO_2 chemosensitivity followed by a return to normal (Burgraff *et al.* 2018). In the present study, our subjects breathed an ambient CO_2 equivalent to 4 mmHg (0.5%) for 30 days. Under these conditions, there were no detectable changes in P_{ETCO_2} or P_aCO_2. Thus, it may not be surprising that the ventilatory response to CO_2 was not altered at the conclusion of 30 days of strict HDT under the current experimental conditions. Although we detected no statistically significant changes in the HCVR, the VRT

decreased by ∼4 mmHg during the recovery period. Thus, although the HCVR remained unchanged for the duration of the study, the onset of the response occurred at a lower P_{ETCO_2} after HDTBR was completed. The reasons for this are not clear, although they may be related to an altered $[H+] - P_{CO_2}$ relationship as a result of the mild metabolic alkalosis that developed during this time period (Duffin, 2005). Based on the current and existing studies, we would suggest that ambient levels of CO_2 >0.5%, and/or those that elicit sustained increases in arterial P_{CO_2}, are required to substantially impact the ventilatory chemosensitivity to CO_2.

Limitations

The small number of subjects is always a challenge in this type of research. In addition, female subjects were targeted to make up one-half of the study group. Efforts were made to align the start of the female hormonal cycle with the pre-BR data collection, although this then precluded subsequent testing days from occurring during the same phase of their cycle. Whether measures on different days were significantly affected by changes in hormonal levels and possibly contributed to greater variability across the group as a whole remains unknown.

The study design of a strict HDTBR and mild hypercapnic environment was chosen by NASA to most closely match conditions on the ISS to increase the chances of developing a model of SANS using an Earth-based analogue. As a result, the study design did not include a control group exposed to strict HDTBR without elevated ambient P_{CO_2}. Yet, given the lack of change in the majority of our outcome variables between measures obtained before, during or after exposure to the hypercapnic environment, this limitation probably would not affect the interpretation of our data.

As a result of implementation challenges, the methodological approach of the rebreathe test differed from previous studies by only including 100% O_2 and not including elevated CO_2 in the rebreathing gas stimulus. The example tracings in Figs 2 and 4 demonstrate that we substantially reduced (but did not entirely eliminate) the arterial-tissue-venous gradients. Therefore, our measures of P_{ETCO_2}, especially during the early portion of the rebreathe test, may have over-estimated the P_{CO_2} stimulus by a few mmHg, which could have altered our measures of CVR and HCVR, although this would have been consistent across all subjects and time points. Our raw tracings reveal a difference between the inspired and end-tidal P_{CO_2} during the early portion of the rebreathe test of ∼8 mmHg and this narrows to <3 mmHg by the end of the test, which may have caused this stimulus to shift from a pseudo-steady-state to a more traditional rebreathe test. Additionally, the range of absolute CVR reported for all of our subjects falls within the range reported by (Boulet *et al.* 2016), suggesting a minimal effect as a result of methodological differences. Because the rebreathe test was conducted in the same subjects across all time points, we assume that any potential impact would occur at all time points and would not change our interpretation of the data.

As a result of the invasiveness of the procedure, we were unable to conduct arterial blood draws for blood gas analysis. As a result, it is possible that our sampling of arterialized blood from a heated finger contained venous blood, which would have diluted our sample. A meta-analysis of studies comparing arterial and arterialized samples from a fingertip suggest that capillary P_{CO_2} and pH obtained from a heated finger accurately and precisely reflect arterial blood (Zavorsky *et al.* 2007). Because the sampling and analysis technique was consistently applied, and the data show a similar pattern to the P_{ETCO_2} data, we are confident in our approach. To overcome this limitation, future studies should consider arterial blood draws to directly measure arterial blood gases and confirm our findings of a mild alkalosis.

Conclusions

Chronic exposure to strict HDTBR for 30 days in a mild hypercapnic environment resulted in no change in arterialized P_{CO_2} levels, cerebrovascular reactivity or the hypercapnic ventilatory response. These data suggest that mildly elevated ambient CO_2 combined with strict HDTBR does not elicit changes in cerebral blood flow, which could alter intracranial pressure. Thus, the experimental conditions were not sufficient to elicit a detectable physiological response and are probably not a contributing factor to the development of optic disc oedema during bed rest and/or spaceflight.

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Additional information

Competing interests

The authors declare that they have no competing interests.

Author contributions

Data collection occurred at the German Aerospace Center in Cologne, Germany. Data analysis, interpretation, and reporting occurred at NASA Johnson Space Center in Houston, TX. SSL conceived and designed the experiments, oversaw data collection and analysis, created figures, and wrote the manuscript. KC and JK conducted the data analysis. ATL, SMCL, BRM and MBS conceived and designed the experiments, and contributed to intellectual discussions. KC, JK, ATL, SMCL, BRM and MBS contributed to manuscript writing and editing. SM, WS and EM collected data and provided a critical review of the manuscript. MY provided the statistical analysis.

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Keywords

bed rest, carbon dioxide, cerebral blood flow, hypercapnia, spaceflight

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Statistical Summary Document
Supporting Material – Data Analysis