Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Supplemental Methods

Assessment of VO_{2max} and One-repetition Maximum

During the familiarization visit, maximum oxygen uptake (VO_{2max}) was assessed using an incremental ramp test on an electronically braked supine cycle ergometer (Lode BV, Groningen, Netherlands) in the HDT (–15°) position. Pulmonary ventilation, gas exchange, and HR were continuously measured with a Parvo Medics TrueMax 2400 computerized metabolic measurement system (Parvo Medics, Sandy, UT, USA). After 2 minutes of rest, participants pedaled on the cycle ergometer at a cadence of 60 rpm for 2 minutes at 30 W. After this warm-up, work rate was increased 20 W every minute until each participant reached volitional exhaustion. VO_{2max} was defined as the average of the 2 highest consecutive 30-second averages achieved for VO_2 during the maximal exercise test. Maximal leg press strength was determined for each participant using a one-repetition maximum (1-RM) performed on a modified leg press machine (Quantum Fitness Corporation, Stafford, TX, USA) in the –15° HDT position. All participants performed two 1-RM measurements with the 2nd measurement being obtained approximately 5 minutes after the 1st measurement. The highest weight lifted between the two measurements was considered the participant's 1-RM.

Exercise Equipment Modifications

Significant modifications to a cycle ergometer and leg press machine were required before commencement of the study to create a cephalad fluid shift (eFigures 1 and 2). These modifications were completed and tested between October and December of 2014. Cycle ergometer modifications included adding a wooden step to place the cycle ergometer at a –15° HDT. Leg press modifications included: the addition of steel plates to the leg press machine to create a 15° HDT, shoulder pads and head rest modified to accommodate new tilt angle, and a modified foot plate to accommodate new angle.

Cerebral Hemodynamics

During each postural allocation and experimental exercise condition, blood flow in extracranial arteries (i.e. common carotid artery [CCA], external carotid artery [ECA], internal carotid artery [ICA] and vertebral artery [VA]) and veins (internal jugular vein [IJV] and vertebral vein [VV]) (eFigure 3) were measured by the same two highly-trained operators using color-coded duplex ultrasonography (GE Vivid-q Ultrasound system, GE Medical Systems, Chicago, IL, USA) (eFigure 4). Blood flow in the left ICA and left ECA was measured ~ 1.0 – 1.5 cm distal to the carotid bifurcation, and right CCA blood flow was measured ~ 1.5 cm proximal to the carotid bifurcation. Right VA blood flow was assessed at the midpoint of the V1 segment. For venous outflow, blood flow was measured at the J3 level of the right IJV and at the mid-cervical level of the right IJV (details discussed below). For each subject each vessel was measured in the same location for each condition. For all blood flow measurements, we first used the color flow mode to evaluate flow patterns, and then arterial mean vessel diameter of each artery was acquired using a 2-dimensional longitudinal section. IJV areas were acquired from a transverse, cross-sectional view. Pulse wave mode was then used to obtain Doppler waveforms.(1) Systolic and diastolic diameters were measured with mean diameter (cm) calculated in relation to the blood pressure curve: [mean diameter = [(systolic diameter x 1/3)] + [(diastolic diameter x 2/3)]. Diameter measurements were taken across a region of interest with clearly defined upper and lower arterial walls. Average blood flow velocity measured over ~10-20 cardiac cycles was defined as mean blood flow velocity (V_{mean}) (cm/s). This approach eliminates changes in velocity caused by changes in breath cycles.(2) When making V_{mean} measurements, the ultrasound operator ensured that the probe was stable and the angle of insonation did not change (~60°) and that the sample volume was positioned in the center of the vessel and adjusted to cover the width of the vessel diameter. Blood flow was calculated [(Flow = CSA*V_{mean}*60).

It is impossible to simultaneously evaluate blood flow in ipsilateral cerebral arteries due to the technical limitations of Doppler ultrasound (insufficient space on the neck and interference between Doppler beams from multiple probes).(3) Therefore, we alternated between vessels for each interval or resistance set or continuous aerobic time points. For example, during the first 4-min interval, the left ICA blood flow and right VA blood flow were measured simultaneously. During the second 4-min interval, the left ECA and right CCA were measured simultaneously with the same Doppler ultrasound systems as before. For each exercise session, the upper body was restrained by shoulder straps and a waist belt attached to the exercise equipment (bike or leg press) to limit body and neck movement for cerebral imaging.

Left middle cerebral artery (MCA) blood flow velocity and cerebral outflow pressures (internal jugular venous pressure [IJVP] and external jugular vein pressure [EJVP]) were also assessed at rest and during exercise. Intraocular pressure (IOP) was measured during rest periods on resistance and interval exercise sessions (details discussed below). For the continuous exercise session, participants were asked to briefly stop exercising (~1 min) while IOP was acquired. Global cerebral blood flow was calculated as the sum of the blood flow in the ICA and VA [(ICA blood flow + VA blood flow) x 2 (ml/min)], and total blood flow to the head ($\dot{Q}_{head}$) was calculated by the sum of the blood flow in the CCA and VA [(CCA blood flow +VA blood flow) x 2 (ml/min)] as previously described.(3) The proportion of cardiac output (CO) distributed to the head was calculated as [$\dot{Q}_{head}$/CO x 100%]. Middle cerebral artery (MCA) maximum (V_{max}) and mean blood flow velocity (V_{mean}) was measured by transcranial Doppler (TCD) (GE Vivid-q Ultrasound system, GE Medical Systems, Chicago, IL, USA). A 2 MHz TCD probe was placed over the temporal window of the left MCA and adjusted until an optical image was identified. MCA conductance was calculated as MCA V_{mean} / MAP.

Intraocular pressure (IOP) was non-invasively measured using applanation rebound tonometer (Icare PRO, Finland), and IJVP and EJVP were non-invasively measured using a custom-made prototype compression sonography device (VeinPress 2014, VeinPress GmbH, Switzerland). We did not directly measure ICP, however, IJVP has been shown to be an accurate surrogate measure for ICP.(4, 5) Our group (6), and others (4), have previously reported on the reliability and validity of compression sonography to evaluate internal and external jugular venous pressure (IJVP and EJVP). Estimated translaminar pressure gradient (TLPG) was calculated as the difference of IOP and IJVP [TLPG = IOP – IVJP]. Estimated cerebral transmural pressure (TMP) was calculated as the difference between MAP and IJVP [CTMP = MAP – IJVP], and ocular perfusion pressure (OPP) was calculated as the difference between MAP and IOP [OPP = MAP – IOP]. The coefficient of variation (CV) for between-visit resting supine and HDT cerebral venous pressure measures (primary outcome measures) were as follows: IOP supine = 9.8 ± 6.0 %, IOP HDT = 9.8 ± 4.2 %, IJVP supine = 12.7 ± 4.4%, IJVP HDT = 7.7 ± 4.9%.

Standardized Protocol to Determine Cerebral Outflow from the Internal Jugular Vein.

The right IJV was insonated ≥1 cm from any interference with the omohyoid where transverse and longitudinal transducer locations were marked. Measurements were taken during inspiration and expiration with the body at -20º, 0º, and +90º. Vessel area of the IJV was measured in the transverse plane from the B-mode image. Blood velocity was assessed using a narrow gate placed at the center, medial and lateral walls of the vessel, and with a wide gate encompassing the vessel lumen. Blood flow was calculated as the product of cross-sectional area and time-averaged velocity from 3 cardiac cycles. The jugular CSA was repeated at the same anatomic level in an upright position (90°) and subtracted from the supine value. If the upright CSA was greater than the supine in either jugular, the participant was deemed abnormal for this parameter (Zamboni criterion #5, negative change in the CSA in the jugular vein). Angle-corrected spectral Doppler velocities (cm/s; maximal and minimal velocities in a similar fashion to peak systolic and end diastolic arterial studies) and waveforms in the sagittal plane were generated for both vertebral and jugular veins at 0 and 90°. The PRF was adjusted to optimize the waveform, and the sample gate was set to 1.8 to 3.4 mm (depending on vessel size) and always placed in the center of the vessel according to standard vascular procedures. Spectral waveforms were performed in real time as the patient was instructed to breathe normally. After 5 seconds of normal breathing, the patient was asked to hold their breath (apnea) for 5 seconds following a normal exhalation, being careful not to perform a Valsalva maneuver. After 5 seconds of apnea, the patient was asked to again breathe normally, being careful not to forcefully exhale/inhale, as the spectral gate can be displaced from the vein by excessive movement.

Cardiorespiratory Measures

Heart rate (HR) was determined from R-R intervals using a 3-lead electrocardiogram (ADInstruments, Colorado Springs, CO, USA). Beat-by-beat arterial blood pressure (BP) was continuously measured using finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Arnhem, Netherlands) calibrated to brachial artery BP measured with an automatic electrosphygnomanometer (Tango M2, Suntech Medical Inc, Morrisville, NC). Mean arterial pressure (MAP) was calculated as [(2 x diastolic BP) + systolic BP] / 3. Respiratory variables were determined as described for VO_{2max}, and end-tidal partial pressure of CO_2 (P_{ETCO2}) was measured with this same system (ParvoMedics, TrueMax 2400, Sandy, UT, USA). Resting and exercise echocardiograms were performed by one experienced echosonographer (GE Vivid-q Ultrasound system, GE Medical Systems, Chicago, IL, USA). Left ventricular end-diastolic and end-systolic volumes were assessed using the Simpson's Biplane method. Stroke volume (SV) and CO were calculated using standard formulae.

Swimming Goggles as a Method of Raising IOP

A negative and anteriorly directed TLPG is an established contributor to papilledema.(7) Therefore, given the disproportionate increase in JVP relative to IOP during HDT exercise, we hypothesized that raising IOP would increase the TLPG. We used a simple, yet established method of increasing IOP using swimming goggles(8) in a subset of 10 participants. Participants wore a pair of commercially available swimming goggles (Speedo, Vanquisher, Nottingham, United Kingdom) and adjusted them as they would when entering a swimming pool (eFigure 5).(8) The posterior rubber portion of the goggles pushes into the orbital fat and slightly elevates IOP (~3mmHg) (8). A large hole was cut into the lens for IOP measurements, leaving the posterior rubber portion of the goggles to fit into the orbital soft tissue.

Triggerfish Contact Lens for Continuous Assessment of Corneoscleral Circumference

Previously reported ocular pressure profiles were acquired during a pause in exercise.(9, 10) To partially address this limitation, given that mean 24-hour corneoscleral circumference changes have been correlated with mean 24-hour tonometric curves,(11) we assessed a surrogate of IOP (corneoscleral circumference) using the Sensimed Triggerfish contact lens sensor in 10 subjects. The Triggerfish inbuilt sensor captures circumferential changes at the corneoscleral limbus that occur due to IOP and volume changes and transfers data wirelessly to a portable recorder (eFigure 5). Data was acquired continuously and averaged during supine rest, HDT rest, and during each high-intensity aerobic interval, resistance set, and every 8 minutes during moderate-intensity exercise.

eTable 1. Cardiovascular and Cerebrovascular Responses to Changes in Posture

	EMM Supine	EMM −15° HDT	∆ Posture (95% CIs)	Posture Main Effect (P-value)	Condition Main Effect (P-value)	Interaction (P-value)
Cardiovascular responses						
Heart rate (bpm)	59 ± 8	59 ± 9	0 (-1, 2)	.85	.97	.20
Stroke volume (ml)	90 ± 16	91 ± 15	+ 1 (-1, 3)	.35	.64	.30
Cardiac output (L/min)	5.3 ± 0.9	5.4 ± 1.0	+0.1 (-0.1, 0.2)	.41	.98	.86
SBP (mmHg)	120 ± 8	127 ± 12	+7 (4,10)**	<.001	.85	.39
DBP (mmHg)	74 ± 9	78 ± 7	+4 (2, 6)**	<.001	.30	.21
MAP (mmHg)	90 ± 9	95 ± 8	+5 (3, 7)**	<.001	.47	.20
Cerebrovascular responses						
Intraocular pressure (mmHg)	19.3 ± 3.7	21.6 ± 4.1	+2.3 (1.4, 3.3)**	<.001	.54	.98
Internal jugular venous pressure (mmHg)	21.4 ± 6.0	31.9 ± 6.9	+10.5 (8.9, 12.2)**	<.001	.95	.98
External jugular venous pressure (mmHg)	10.3 ± 4.2	17.6 ± 5.7	+7.3 (5.9, 8.6)**	<.001	.75	.63
Estimated translaminar pressure gradient (mmHg)	-2.1 ± 7.0	-10.3 ± 8.4	-8.2 (-6.3, -10.1)**	<.001	.71	.99
Estimated cerebral transmural pressure (mmHg)	68.6 ± 9.4	62.8 ± 8.1	-5.8 (-3.3, -8.3)**	<.001	.44	.27
OPP (mmHg)	70.9 ± 10.2	73.6 ± 10.3	+ 2.7 (0.1, 5.3)*	.04	.99	.35
Global CBF (ml/min)	1267 ± 275	1270 ± 251	+ 3 (-51, 56)	.92	.76	.24
Total head blood flow (ml/min)	2025 ± 239	1853 ± 297	-172 (-106, -237)**	<.001	.84	.39

% CO to Head	38.5 ± 6.9	35.3 ± 8.1	- 3.2 (-1.4, -5.0)*	.001	.78	.41

CBF: cerebral blood flow; CO: cardiac output; DBP: diastolic blood pressure; EMM: estimated marginal means; HDT: head-down tilt; MAP: mean arterial pressure; OPP: ocular perfusion pressure; SBP: systolic blood pressure. Δ Posture (95% CIs) = EMM –15° HDT minus EMM Supine. ** P<.001 supine versus –15° HDT posture. * P<.05 supine versus –15° HDT posture.

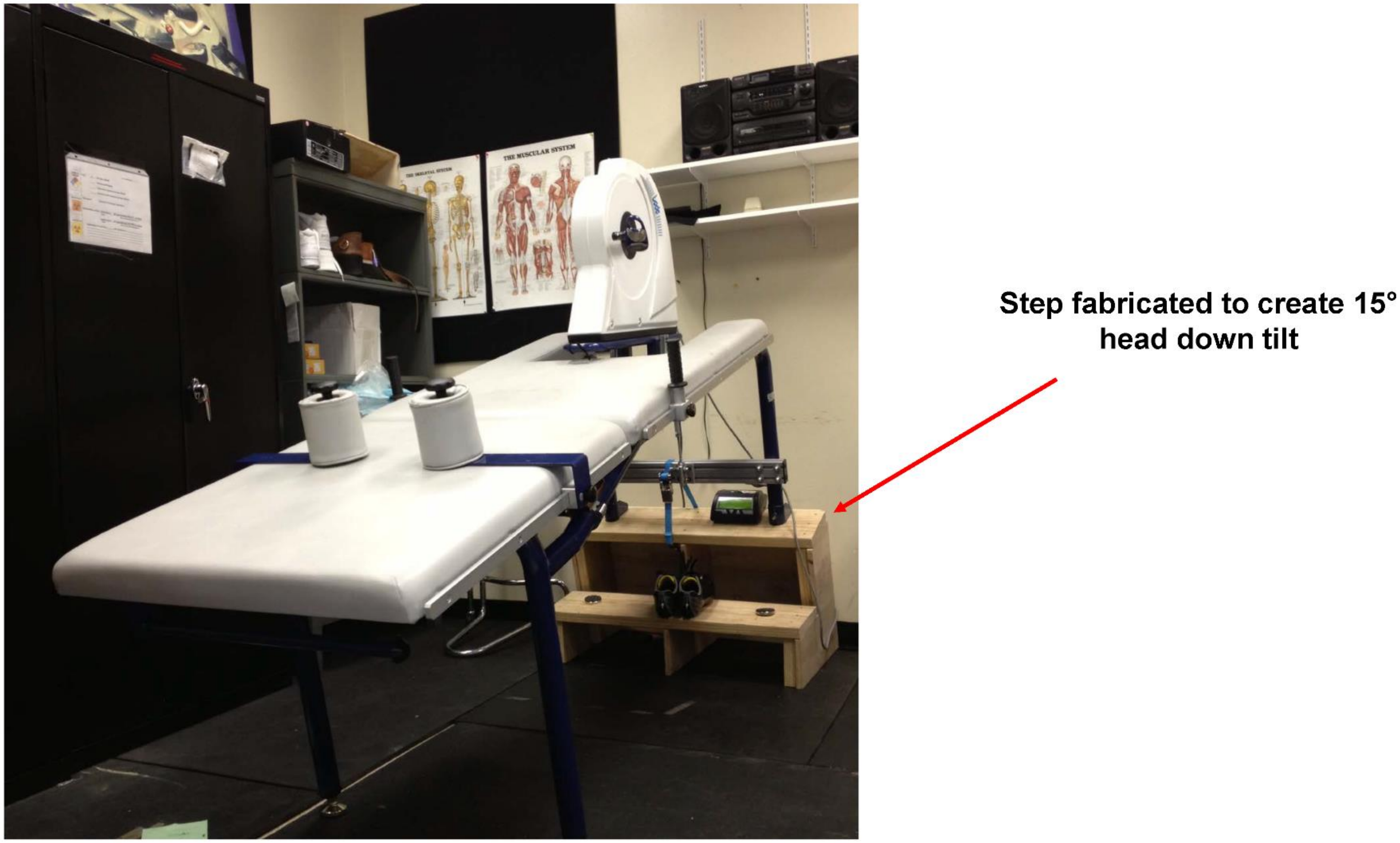
eTable 2. Cerebral Arterial and Venous Blood Flow Responses to Changes in Posture

	EMM Supine	EMM −15° HDT	Δ Posture (95% CIs)	Posture Main Effect (P-value)	Condition Main Effect (P-value)	Interaction (P-value)
Common carotid artery (CCA)						
Blood flow (ml/min)	841 ± 106	765 ± 133	-76 (-49, -103)**	<.001	.55	.58
Diameter (cm)	0.68 ± 0.03	0.69 ± 0.03	+0.01 (0.01, 0.02)**	<.001	.58	.93
Mean blood flow velocity (cm/s)	38.4 ± 5.1	33.9 ± 5.8	-4.5 (-3.3, -5.6)**	<.001	.71	.62
Internal carotid artery (ICA)						
Blood flow (ml/min)	459 ± 102	472 ± 104	+13 (-14, 41)	.32	.92	.45
Diameter (cm)	0.54 ± 0.05	0.55 ± 0.06	+0.1 (-0.01, 0.03)	.06	.85	.93
Mean blood flow velocity (cm/s)	34.0 ± 8.0	33.1 ± 8.7	-0.9 (-2.8, 1.0)	.35	.87	.41
External carotid artery (ECA)						
Blood flow (ml/min)	335 ± 92	297 ± 57	-38 (-18,-59)**	<.001	.53	.61
Diameter (cm)	0.50 ± 0.03	0.50 ± 0.03	+0.01 (-0.01,0.02)	.07	.75	.44
Mean blood flow velocity (cm/s)	29.1 ± 6.1	25.2 ± 4.8	-3.9 (-2.4, -5.2)**	<.001	.10	.67
Vertebral artery (VA)						
Blood flow (ml/min)	169 ± 74	161 ± 59	-8 (-3, 20)	.13	.67	.93
Diameter (cm)	0.40 ± 0.05	0.40 ± 0.04	0.00 (-0.00, 0.00)	.83	.61	.74

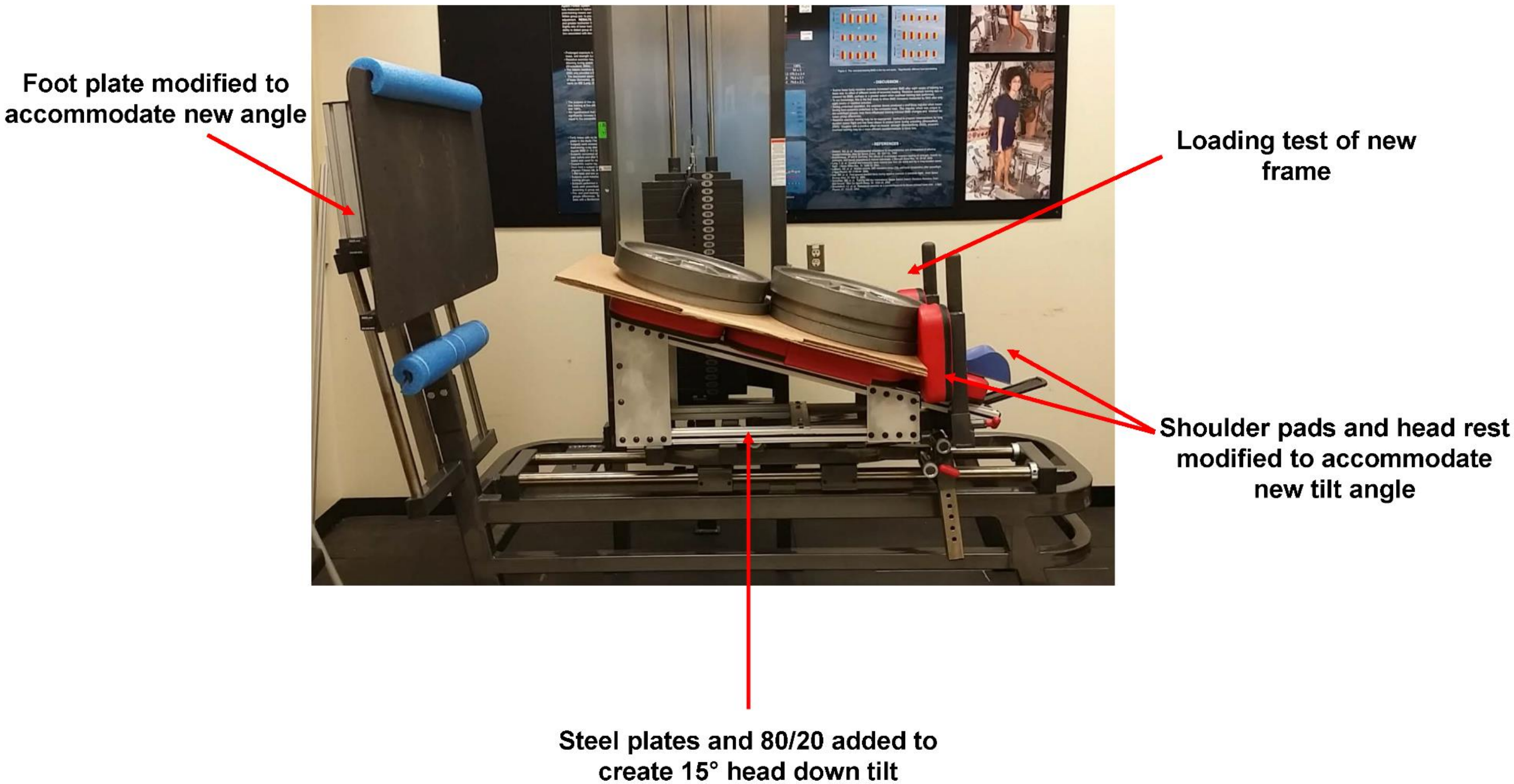
Mean blood flow velocity (cm/s)	21.9 ± 5.0	20.5 ± 4.8	-1.4 (-0.7, 2.6)*	.04	.81	.52
Middle cerebral artery (MCA)						
Mean blood flow velocity (cm/s)	49.1 ± 10.9	51.6 ± 11.8	+2.5 (0.7, 4.3)*	.01	.77	.89
Conductance	0.56 ± 0.14	0.55 ± 0.14	-0.01 (-0.3, 0.2)	.74	.53	.49
Vertebral vein (VV)						
Blood flow (ml/min)	78 ± 63	62 ± 51	-16 (-2, -29)*	.02	.76	.72
Internal jugular vein (IJV)						
Blood flow (ml/min)	947 ± 463	883 ± 372	-64 (-184, 55)	.28	.99	.98

EMM: estimated marginal means; HDT: head-down tilt. Δ Posture (95% CIs) = EMM –15° HDT minus EMM Supine. ** P<.001 supine versus –15° HDT posture. * P<.05 supine versus –15° HDT posture.

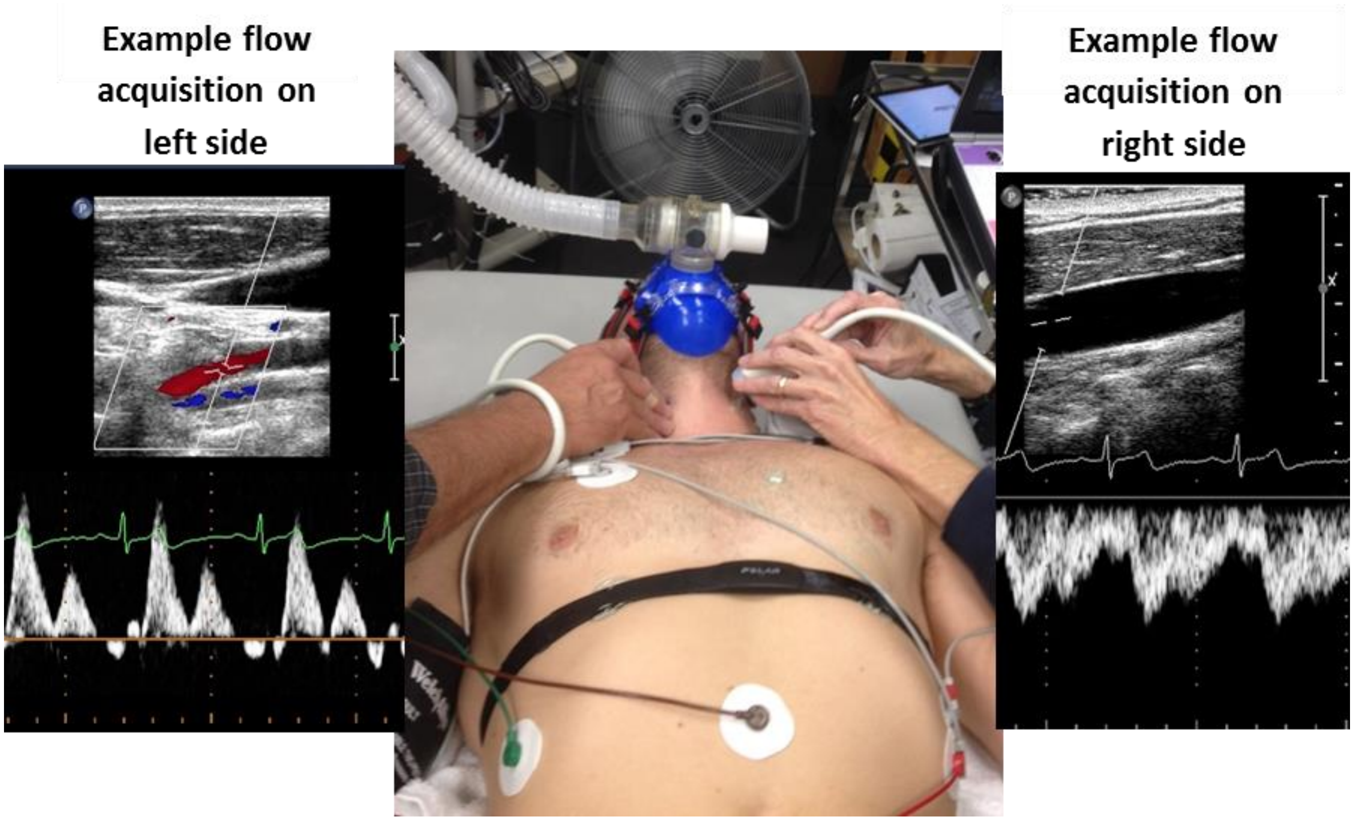
Supplemental Figures



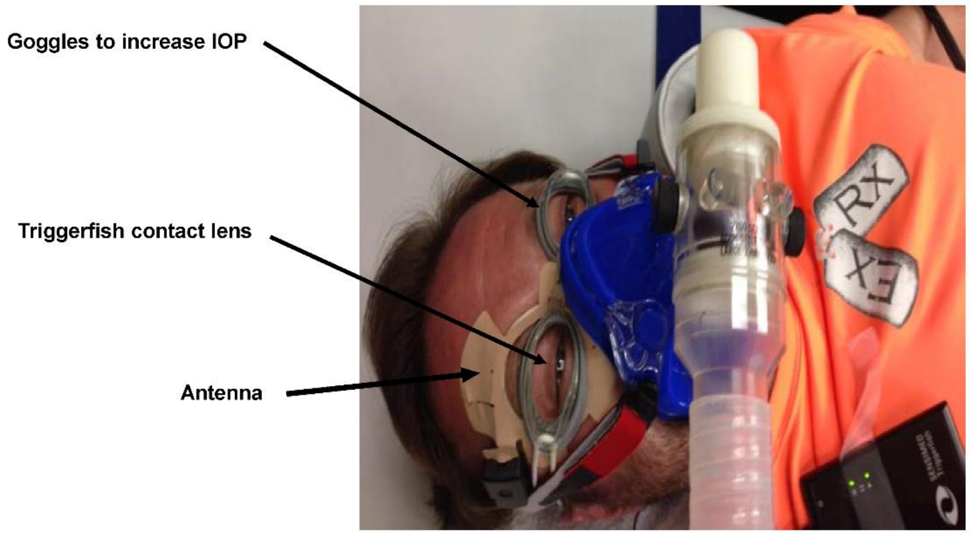
eFigure 1. Cycle ergometer modifications to achieve –15° head-down tilt to induce a cephalad-fluid shift that is analogous to microgravity conditions during spaceflight.



eFigure 2. Leg press modifications to achieve –15° head-down tilt to induce a cephalad-fluid shift that is analogous to microgravity conditions during spaceflight.



eFigure 3. Example of experimental setup for aerobic exercise and cerebral inflow/outflow vascular imaging.



eFigure 4. Example of participant wearing swimming googles with Triggerfish contact lens for continuous measurement of corneoscleral circumference (an estimate of IOP).

eReferences

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