Separate and Combined Effects of Hypoxia and Horizontal Bed Rest on Retinal Blood Vessel Diameters

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Purpose. To assess the separate and combined effects of exposure to prolonged and sustained recumbency (bed rest) and hypoxia on retinal microcirculation.

Methods. Eleven healthy male subjects (mean ± SD age = 27 ± 6 years; body mass index [BMI] = 23.7 ± 3.0 kg m^{-2}) participated in a repeated-measures crossover design study comprising three 21-day interventions: normoxic bed rest (NBR; partial pressure of inspired O_2, P_iO_2 = 133.1 ± 0.3 mm Hg); hypoxic ambulation (HAMB; P_iO_2 = 90.0 ± 0.4 mm Hg), and hypoxic bed rest (HBR; P_iO_2 = 90.0 ± 0.4 mm Hg). Central retinal arteriolar (CRAE) and venular (CRVE) equivalents were measured at baseline and at regular intervals during each 21-day intervention.

Results. Normoxic bed rest caused a progressive reduction in CRAE, with the change in CRAE relative to baseline being highest on day 15 (ΔCRAE = −7.5 µm; 95% confidence interval [CI]: −10.8 to −4.2; $P < 0.0001$). Hypoxic ambulation resulted in a persistent 21-day increase in CRAE, reaching a maximum on day 4 (ΔCRAE = 9.4 µm; 95% CI: 6.0–12.7; $P < 0.0001$). During HBR, the increase in CRAE was highest on day 3 (ΔCRAE = 4.5 µm; 95% CI: 1.2–7.8; $P = 0.007$), but CRAE returned to baseline levels thereafter. Central retinal venular equivalent decreased during NBR and increased during HAMB and HBR. The reduction in CRVE during NBR was highest on day 1 (ΔCRVE = −7.9 µm; 95 CI: −13.3 to −2.5), and the maximum ΔCRVE during HAMB (24.6 µm; 95% CI: 18.9–30.3) and HBR (15.2 µm; 95% CI: 9.8–20.5) was observed on days 10 and 3, respectively.

Conclusions. The diameters of retinal blood vessels exhibited a dynamic response to hypoxia and bed rest, such that retinal vasodilation was smaller during combined bed rest and hypoxia than during hypoxic exposure.

Keywords: bed rest, physical inactivity, hypoxia, microvasculature, retina

Missions on the International Space Station can cause changes in visual acuity of astronauts. The observed hyperopic shifts were secondary to the observations of globe flattening, optic disc edema, choroidal folds, and in some individuals elevated intracranial pressure.[1–3] Based on post-flight questionnaires distributed among 300 astronauts, Mader and colleagues concluded that 29% of astronauts on short-term missions and 60% of astronauts on long-term missions reported impairment of near and distant visual acuity, which in some cases did not return to preflight conditions after several years post flight.[1]

The etiology of this visual syndrome remains unresolved. The changes in the structure and function of the eye observed in space are now being investigated in ground-based studies using the bed rest experimental model. This analogue model is widely used to study physiological adaptations to unloading and microgravity.[4] Intraocular pressure, ocular blood flow, and retinal blood vessel dimensions appear to be affected during bed rest experiments, but current understanding of ocular adaptations is based on only scattered reports.[5] Ocular effects during bed rest are now being studied more systematically, with a recent investigation showing that a 70-day bed rest induced greater peripapillary retinal thickening than a 14-day bed rest.[6]

The present study was part of a larger research program investigating the effect of the anticipated environments in future planetary habitats,[7] which will be hypobaric and hypoxic, on physiological systems. The purpose of establishing a hypobaric hypoxic environment in space habitats is primarily to eliminate the need for long decompression profiles requiring oxygen breathing prior to extravehicular activities. The research program, in general, examines the separate and combined effects of physical inactivity/unloading (simulating the effects of

Investigative Ophthalmology & Visual Science

reduced gravity on the musculoskeletal[8] and cardiovascular[9] systems) and hypoxia on physiological systems. The specific aim of the present study, within this program, was to assess the separate and combined effects of hypoxia and bed rest on the diameters of the retinal arterioles and venules.

Methods

Subjects

Fourteen healthy male subjects participated in this repeated-measures crossover design study. Their participation was subject to physicians' approval following a thorough medical examination, which included a complete ophthalmologic examination. The study was conducted according to European Space Agency recommendations for standardization of bed rest studies.[10,11] These include inclusion/exclusion criteria (e.g., exclusion of people with osteoporosis, blood-clotting disorders, history of deep vein thrombosis, lower back pain, respiratory disorders). Subjects with any ophthalmologic disorders[12] (e.g., best-corrected visual acuity [BCVA] < 0.8, refractive error of spherical equivalent $> \pm 3$ diopters, any eye disease affecting visual function or ocular motility, any retinal or choroidal disease, glaucoma, optic nerve disease, orbital pathology [dysthyroid], ongoing medication [topical or systemic for eye condition], any previous eye surgery), or recent (<2 months) exposure to altitudes above 2000 m were also ineligible to participate.

The subjects were familiarized with the study protocol and gave their written consent for participation in the study. All participants were healthy, near-sea-level residents (<500 m) with the following baseline characteristics (mean ± SD) obtained during the medical screening: age = 27 ± 6 years; weight = 76.7 ± 11.8 kg, height = 179 ± 3 cm, body mass index [BMI] = 23.7 ± 3.0 kg m^{-2}; maximal oxygen uptake = 44.3 ± 6.1 mL kg^{-1} min^{-1} (determined with an incremental load exercise on a cycle ergometer).

Two participants did not return for the last campaign due to personal reasons, and one participant had to be withdrawn from the study during the last campaign as a result of gastrointestinal health problems. Ultimately, of the 14 subjects enrolled in the study, 11 completed all three interventions, and their data are reported.

The study protocol was approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia and conformed to the guidelines of the Declaration of Helsinki (registration number NCT02637921 at www.ClinicalTrials.gov).

Procedures

Subjects ($n = 11$) participated in three experimental interventions in a counterbalanced fashion: (1) normoxic horizontal bed rest (NBR; fraction of ambient O_2 [FO_2] = 0.209; partial pressure of inspired O_2 [P_iO_2] = 133.1 ± 0.3 mm Hg); (2) hypoxic ambulatory confinement (HAMB; FO_2 = 0.141 ± 0.004; P_iO_2 = 90.0 ± 0.4 mm Hg; ~4000-m simulated altitude); and (3) hypoxic horizontal bed rest (HBR; FO_2 = 0.141 ± 0.004; P_iO_2 = 90.0 ± 0.4 mm Hg; ~4000-m simulated altitude). The experimental campaigns were conducted in the hypoxic facility at the Olympic Sport Centre Planica (Rateče, Slovenia), situated at an altitude of 940 m. The participants entered each campaign in a sequential and fixed order, with two participants entering each day. Campaigns lasted 32 days and had three distinct phases. The initial testing phase comprised the first 7 days upon arrival at the facility. This phase allowed the participants to acclimate to the facility, diet, and circadian cycles. Baseline measures were obtained during this period. The second phase was the 21-day confinement phase during which the participants were exposed to their designated condition (NBR, HAMB, or HBR). This was followed by a 4-day recovery phase at the Olympic Sport Centre that enabled the researchers to obtain the postconfinement measurements. This period also allowed for cautious reambulation of the participants. All experiments and measurements were performed on the same days and time periods during the three interventions. A minimum wash-out period of at least 3 months was implemented between the experimental campaigns.

Normoxic and Hypoxic Bed Rest and Confinement Protocols

During the bed rest interventions (NBR and HBR), subjects were confined to the horizontal position for 24 hours a day. All daily activities were conducted in the horizontal position. Subjects were allowed one pillow for head support, and to rest on their elbows during eating and transfer to a gurney. A special gurney was used for showering, and hospital equipment was used for bowel movements and urine collection. Video surveillance cameras provided continuous monitoring of the subjects' activities for safety reasons and to ascertain compliance with the bed rest protocol. Subjects were instructed not to conduct any physical activity during NBR and HBR campaigns.

During HAMB, subjects were confined to the hypoxic area but were ambulatory throughout the day. They were required to be upright at all times (i.e., no resting or napping), and replicated their habitual bone loading during the confinement periods by performing low-level physical activity in two 30-minute bouts per day. These exercise bouts comprised stepping, cycling, or dancing. During the exercises, the targeted heart rate of 123 ± 4 beats min^{-1} was achieved, which was the heart rate observed at 50% of the subjects' normoxic peak power output assessed prior to the start of the intervention.

During the hypoxic campaigns (HBR and HAMB), hypoxia within the facility was maintained by a vacuum pressure swing adsorption system (b-Cat, Tiel, The Netherlands). Samples of air were drawn from all 10 rooms in the facility at 15-minute intervals and analyzed for O_2 and CO_2 concentrations. Any deviation in these concentrations from the preset values was automatically corrected. In addition, each subject was issued a portable O_2 sensor (Rae PGM-1100; RAE Systems, Inc., San Jose, CA, USA), which initiated an audible alarm in the event that the O_2 concentration decreased below the preset value.

Throughout the three interventions, the ambient temperature and relative humidity in the facility were maintained at 24 ± 1°C and 53 ± 5%, respectively. Ambient pressure during the interventions was 91 ± 5 kPa.

Retinal Photography

A Canon 45° 6.3 megapixel digital nonmydriatic camera (Hospithera, Brussels, Belgium) was used to obtain high-resolution images. The fundi of subjects' right and left eyes were photographed twice at distinct time points: a baseline measurement 1 day (–1) before the intervention; on days 1, 2, 3, 4, 6, 8, 10, 15, and 21 of the intervention; and on recovery days 1 (R1) and 2 (R2). The tests were conducted at the same time of day between 1630 and 1830 hours. During the procedure, the subjects' posture depended on the intervention. In the HAMB intervention subjects were seated, whereas during the NBR and HBR interventions they assumed a prone position. During the fundus photography in the NBR and HBR interventions, subjects supported themselves on their elbows and rested their head on the chin holder in front of the camera.

Standard digital photographs centered on the optic disc for each eye were taken at each time point, according to

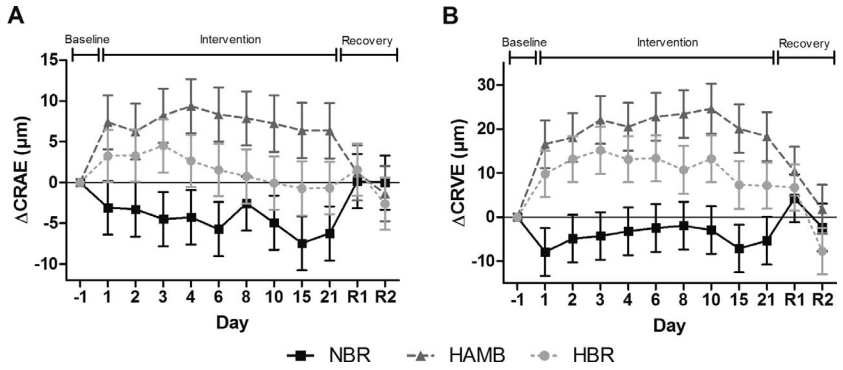

Figure 1. Change in retinal arteriolar response (central retinal arteriolar equivalent; CRAE) and retinal venular response (central retinal venular equivalent; CRVE) relative to baseline during a crossover study with normoxic bed rest (NBR), hypoxic ambulation (HAMB), and hypoxic bed rest (HBR) as experimental conditions. Symbols represent (**A**) change in CRAE (ΔCRAE) and (**B**) change in CRVE (ΔCRVE) relative to baseline (day −1) during the 21-day intervention (days 1–21) and during recovery (R1, R2). *Error bars* are 95% confidence intervals. Estimates are statistically significant when confidence intervals do not include zero.

procedures described elsewhere.[13] An experienced grader, masked to participants' characteristics, identified each vessel as either an arteriole or venule. IVAN retinal image analysis software (University of Wisconsin-Madison, WI, USA) was used to summarize retinal vessel diameters as the central retinal arteriolar equivalent (CRAE) and the central retinal venular equivalent (CRVE) for each image.[14] The equivalents represent a summary of vessel diameters within an area equal to 0.5 to 1 disc diameter from the optic disc margin. Average CRAE and CRVE values were calculated for each time point. Due to high intereye correlation, reported in previous studies, we averaged the values for the left and right eye and used this value in our statistical analysis.[15]

In addition, all images were screened by an ophthalmologist for any signs of high-altitude retinopathy.

Arterial (Systolic, SAP; Diastolic, DAP) and Intraocular (IOP) Pressures

Systolic (SAP) and diastolic (DAP) arterial pressures were measured with a noninvasive oscillometric automated sphygmomanometer (Omron M6; Omron, Kyoto, Japan). Intraocular pressure (IOP) measurements of both eyes were obtained with a Pulsair IntelliPuff noncontact tonometer immediately after the retina scan (Halma India Pvt. Ltd., Mumbai, India). During the IOP measurements, subjects were supine in all three interventions.

Capillary Oxyhemoglobin Saturation

Oxyhemoglobin saturation (SpO_2) was measured daily at 700 and 1700 hours and during the night as part of a sleep polysomographic study, with a 3100 WristOx device (Nonin Medicals, Inc., Plymouth, MN, USA).

Altitude Sickness

Participants were administered the Lake Louise Mountain Sickness questionnaire on a daily basis to assess whether they had any signs or symptoms of acute mountain sickness.

Statistical Analysis

Repeated CRAE and CRVE measurements were analyzed by using linear mixed-effects models (SAS, version 9.2; SAS Institute, Inc., Cary, NC, USA). The models included the sequence in which the interventions were completed, the period when each intervention was conducted, the intervention (NBR, HAMB, HBR), time (day of the examination), and time-by-intervention interaction as categorical fixed effects and subject nested within sequence as random effect. The interaction term examines the effect of the intervention on changes in CRAE and CRVE, and the random effect accounts for the correlation between repeated measures of the same subject.

Results

Clinical Examination

All subjects were asymptomatic during all three interventions.

An ophthalmologist confirmed that all images were free of any sign of high-altitude retinopathy. Participants did not exhibit any signs or symptoms of high altitude sickness.

Capillary Oxyhemoglobin Saturation

Average ± SD daily SpO_2 was 97 ± 1% during NBR, and was significantly ($P < 0.001$) lower during the HBR (88 ± 1%) and HAMB (87 ± 1%) interventions. During the course of the 21-day hypoxic interventions (HAMB and HBR) there was a slight but progressive increase in SpO_2.

Arterial (Systolic, Diastolic) and Intraocular Pressures

There was no significant difference in systolic (NBR: 117 ± 6 mm Hg; HAMB: 116 ± 8 mm Hg; HBR: 120 ± 9 mm Hg) and diastolic (NBR: 69 ± 6 mm Hg; HAMB: 71 ± 6 mm Hg; HBR: 75 ± 7 mm Hg) pressures between the three interventions.

There was also no significant difference in the intraocular pressures observed during the NBR (IOP = 13 ± 1 mm Hg), HBR (IOP = 14 ± 2 mm Hg), and HAMB (IOP = 14 ± 1 mm Hg) interventions. Intraocular pressure remained stable throughout each 21-day intervention.

Central Retinal Arteriolar Equivalent

Figure 1A depicts the change in CRAE (ΔCRAE) relative to the baseline value on the day before the intervention. There was a 3.1-μm decrease (95% confidence interval [CI]: −6.4 to 0.2; $P = 0.0681$) in CRAE on day 1 of the NBR intervention. Thereafter, CRAE continued to decrease progressively, finally attaining a reduction of 7.5 μm (95% CI: −10.8 to −4.2; $P < 0.0001$) on day 15 of the intervention. During the recovery period (R1 and R2), ΔCRAE returned to zero.

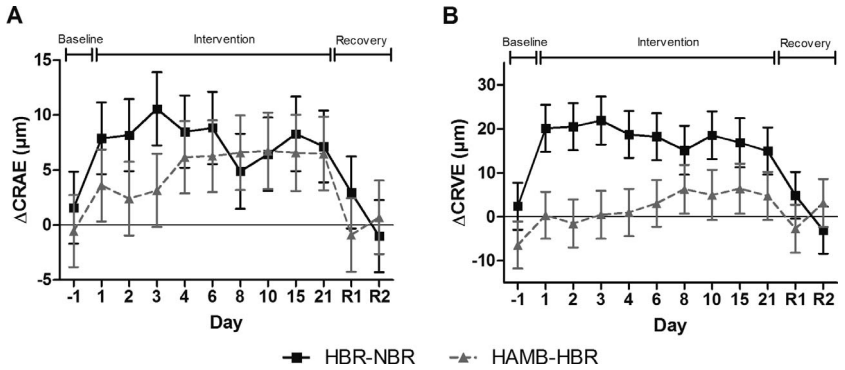

FIGURE 2. Differences in (**A**) retinal arteriolar response (central retinal arteriolar equivalent; CRAE) and (**B**) retinal venular response (central retinal venular equivalent; CRVE) between interventions: normoxic bed rest (NBR), hypoxic ambulation (HAMB), and hypoxic bed rest (HBR). Symbols represent (**A**) difference in CRAE (ΔCRAE) and (**B**) difference in CRVE (ΔCRVE) between interventions at baseline (day −1), during the 21-day intervention (days 1–21), and during recovery (R1, R2). *Error bars* are 95% confidence intervals. Estimates are statistically significant when confidence intervals do not include zero.

In contrast to the NBR intervention, there was a 7.4-μm increase (95% CI: 4.1–10.7; $P < 0.0001$) in CRAE on day 1 of the HAMB intervention. This increase in diameter was maintained throughout the 21-day HAMB intervention, with a maximum ΔCRAE of 9.4 μm (95% CI: 6.0–12.7; $P < 0.0001$) on day 4. Central retinal arteriolar equivalent returned to baseline values upon completion of the intervention on days R1 and R2.

In the HBR condition, CRAE increased by 3.2 μm (95% CI: 0–6.4; $P = 0.0481$) on day 1 of the intervention. The response in HBR was transient in contrast to the response observed in HAMB. Change in CRAE was 4.5 μm (95% CI: 1.2–7.8; $P = 0.0070$) on day 3 of HBR but decreased afterward, reaching zero on day 10. Change in CRAE values remained around zero thereafter.

Central Retinal Venular Equivalent

The decrease in CRVE (ΔCRVE) relative to baseline was 7.9 μm (95 CI: −13.3 to −2.5; $P = 0.0044$) on day 1 of NBR (Fig. 1B). Although the reduction in CRVE was maintained throughout the intervention, it was significant only on day 15 (ΔCRVE = −7.2 μm; 95 CI: −12.6 to −1.7; $P = 0.0098$). During the remainder of the intervention and recovery days, ΔCRVE was not significantly different from zero.

Hypoxic ambulation caused a significant increase in CRVE of 16.6 μm (95 CI: 11.1–22.0; $P < 0.0001$) on day 1. This significant increase persisted during the entire intervention, and ΔCRVE was highest on day 10 (24.6 μm; 95 CI: 18.9–30.3; $P < 0.0001$). Although a trend toward a return of ΔCRVE toward zero was observed on R1, CRVE was still significantly elevated compared to baseline. Change in CRVE returned to zero on R2.

During the HBR intervention, CRVE followed the same trend as during HAMB. A significant increase of 9.8 μm (95% CI: 4.6–15.0; $P < 0.001$) was observed on day 1, with CRVE remaining elevated during the entire intervention period (maximum ΔCRVE = 15.2 μm; 95% CI: 9.8–20.5; $P < 0.0001$), as well as on R1. In contrast to the responses observed in the NBR and HAMB interventions during recovery, HBR caused a significant 7.8-μm undershoot (95% CI: −13.0 to −2.5; $P = 0.004$) of CRVE on R2.

The Effects of Hypoxia and Activity on Retinal Vessel Responses

The effect of hypoxia on retinal vessel responses is reflected in the difference between the responses observed in HBR and NBR (Fig. 2). For both the arteriolar (CRAE) and venular (CRVE) responses, this difference was highest on day 3 (ΔCRAE = 10.6 μm; 95% CI: 7.23–13.89; $P < 0.0001$, and ΔCRVE = 21.88 μm; 95% CI: 16.43–27.32; $P < 0.0001$).

The effect of activity on the retinal vessel responses is reflected in the difference in CRAE (Fig. 2A) and CRVE (Fig. 2B) between HAMB and HBR. For CRAE this difference was significantly greater than zero, from day 4 to day 21, and was highest on day 10 (ΔCRAE = 6.7 μm; 95% CI: 3.25–10.22; $P = 0.0002$). Change in CRVE between HAMB and HBR was significant at baseline (–6.41 μm; 95% CI: −11.75 to −1.07; $P = 0.0188$) and on days 8 and 15 (6.40 μm; 95% CI: 0.73–12.07; $P = 0.0270$).

Discussion

The principal finding of this study is that the hypoxia caused an increase in the diameters of the retinal arterioles and venules, with the effect being greater during hypoxic ambulation (HAMB). The results demonstrate that bed rest attenuates the increase during the combined hypoxia and inactivity/unloading (HBR). The increase in vessel diameter observed during hypoxia was greater for the venules than for the arterioles. Normoxic bed rest, however, decreased the retinal arteriolar and venular diameters.

The retinal blood vessels emanate from the ophthalmic artery, which is the first branch of the internal carotid artery.[16,17] These vessels lack sympathetic innervations, and changes in the diameter of retinal blood vessels are a consequence of local autoregulatory factors that maintain a constant blood flow despite perturbations in perfusion pressure or metabolic activity.[18,19] The former modifies smooth muscle tone as a consequence of intraluminal pressure, the latter by partial pressures of oxygen and carbon dioxide, pH, and lactate.[19–21] The principal factors that contributed to the observed changes in CRAE were the hydrostatic effect and hypoxia.

Retinal perfusion pressure is the difference between the mean pressure in the ophthalmic artery and the pressure in the central retinal vein. The hydrostatic effect is largely determined by the position of the body. During HAMB, participants were in the upright-seated posture. Therefore the hydrostatic effect reduced the arterial pressure at the level of the eye by approximately 22 mm Hg compared to the heart level.[22] In a prone position, which was assumed during NBR and HBR, the hydrostatic column (i.e., distance from the level of the heart to the level of the eyes) was smaller than in HAMB. As a consequence, the absence of the hydrostatic effect during NBR

and HBR increased the retinal perfusion pressure, which may have triggered myogenic autoregulation and caused the observed arteriolar vasoconstriction during NBR.

The autoregulatory system evolved to meet the metabolic demands of a tissue, thus increasing flow when oxygen supply is reduced, as reflected in decreasing tissue PO_2. In the present study, SpO_2 was 88% during the hypoxic conditions and 97% in the normoxic condition. This decrease in SpO_2 most likely initiated the arteriolar and venular vasodilation in HAMB and HBR. This response should have provided adequate oxygenation to the retinal tissues during the hypoxic exposure. These responses are consistent with the universally observed retinal changes associated with altitude exposure.[23]

In HBR, arteriolar vasodilation did not increase by the same magnitude as in HAMB. In this condition, the hydrostatic effect (vasoconstriction) and hypoxia (vasodilation) act oppositely. Assuming that the metabolic demands of the retinal tissue were similar in the hypoxic conditions, then the retinal arteriolar response in HBR may suggest either that oxygenation might have been compromised due to the inadequate increase in arteriolar diameter or that it was maintained due to the regulation of blood flow. As discussed above, the smaller increase in arteriolar diameter in HBR can most likely be attributed to a higher perfusion pressure.

The polarity of the venular response was similar to that of the arterioles, but the venular responses in the HBR and HAMB interventions were almost a magnitude greater than those observed for arterioles. Studies investigating the diameter of arterioles and venules during breathing of a hyperoxic (100% O_2) gas mixture have also noted a difference in the reactivity of the vessels; the venular constriction is much greater than that observed in the arterioles.[24–26] These findings are not equivocal.[27,28] A likely explanation is the compliance of the venular wall, which is much greater than that of the arteriolar wall, which has a strongly developed smooth muscle cell layer. As a consequence, venular responses can be larger.

Narrowing of arteriolar vessels in the NBR trial is in line with cardiovascular observations in other bed rest studies that observed reduction in the diameter of the conduit arteries.[29,30] We are aware of only two other studies that performed individual retinal vessel analysis in response to a 48-hour 10° head-down tilt, and our results are consistent with these.[31,32] Our study has a much longer time frame, and we used retinal vessel equivalents, derived according to the suggestions of Knudtson et al.,[14] that have been shown to be more robust measurements for assessing retinal microvasculature.

Comparison of the difference in the retinal vessel responses (ΔCRAE and ΔCRVE) observed in the HBR and NBR interventions reflects the effect of hypoxia, whereas comparison of the responses observed in the HAMB and HBR interventions reflects the effect of activity. On this basis we conclude that hypoxia and inactivity had a similar effect on ΔCRAE (Fig. 2A). In contrast, only hypoxia appears to have had a significant effect on the venules (ΔCRVE in Fig. 2B).

The strength of the present study is the design that carefully controlled for the daily routines, physical (in)activity schemes, and nutritional intake. Furthermore, repeated measurements were obtained in a homogenous study population, which reduces between-individual variability and increases the statistical power. Limitations of the study are the lack of a more integrated analysis of ocular fluid shifts and other cardiovascular outcomes such as endothelial function or blood flow. For example, it is known that head-down bed rest and stay in microgravity can increase intraocular pressure.[33] We measured intraocular pressures, but the values did not differ between the three interventions. Measurements of ocular blood flow or cerebral blood flow might have shed additional light on the dynamic cardiovascular responses related to changes in retinal vessel diameters. However, this requires additional burden for the study participants and is not always possible from a technical point of view, considering that bed rest patients should be measured in the supine position.

In conclusion, the diameters of arteriolar and venular blood vessels exhibited a dynamic response to hypoxia and bed rest in healthy individuals who were participating in a well-controlled 21-day crossover study. It appears plausible that the vascular autoregulatory response to hypoxia caused the differences in arteriolar and venular response observed between the HBR and NBR conditions. The different responses of the vessel diameters between HBR and HAMB conditions were likely due to vascular responses to differences in perfusion pressure, caused by the different arterial pressures at the eye level.

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