Insufficient flow reduction during LBNP in both splanchnic and lower limb areas is associated with orthostatic intolerance after bedrest

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Arbeille P, Kerbeci P, Mattar L, Shoemaker JK, Hughson R. Insufficient flow reduction during LBNP in both splanchnic and lower limb areas is associated with orthostatic intolerance after bedrest. Am J Physiol Heart Circ Physiol 295: H1846–H1854, 2008. —We quantified the impact of a 60-day head-down tilt bed rest (HDBR) with countermeasures on the arterial response to supine lower body negative pressure (LBNP). Twenty-four women [8 control (Con), 8 exercise + LBNP (Ex-LBNP), and 8 nutrition (Nut) subjects] were studied during LBNP (0 to −45 mmHg) before (pre) and on HDBR day 55 (HDBR-55). Left ventricle diastolic volume (LVDV) and mass, flow velocities in the middle cerebral artery (MCA flow) and femoral artery (femoral flow), portal vein cross-sectional area (portal flow), and lower limb resistance (femoral resistance index) were measured. Muscle sympathetic nerve activity (MSNA) was measured in the fibular nerve. Subjects were identified as finishers or nonfinishers of the 10-min post-HDBR tilt test. At HDBR-55, LVDV, mass, and portal flow were decreased from pre-HDBR (P < 0.05) in the Con and Nut groups only. During LBNP at HDBR-55, femoral and portal flow decreased less, whereas leg MSNA increased similarly, compared with pre-HDBR in the Con, Nut, and NF groups; 11 of 13 nonfinishers showed smaller LBNP-induced reductions in both femoral and portal flow (less vasoconstriction), whereas 10 of 11 finishers maintained vasoconstriction in either one or both regions. The relative distribution of blood flow in the cerebral versus portal and femoral beds during LBNP [MCA flow/(femoral + portal flow)] increased or reduced <15% from pre-HDBR in 10 of 11 finishers but decreased >15% from pre-HDBR in 11 of 13 nonfinishers. Abnormal vasoconstriction in both the portal and femoral vascular areas was associated with orthostatic intolerance. The vascular deconditioning was partially prevented by Ex-LBNP.

Different countermeasures, such as lower body negative pressure (LBNP) and isotonic or isometric exercise, have been proposed to minimize the effects of microgravity on the cardiovascular system and to reduce its deconditioning impact after spaceflight. Exercise is used mainly for maintaining cardiopulmonary and muscle functional capacity and to prevent major muscle mass reduction and bone demineralization. LBNP, which induces a fluid shift from the cephalad part of the body toward the lower limbs, is used to partially simulate the effects of upright posture during HDBR and spaceflight. When used as a countermeasure, LBNP in association with exercise has been found to restore plasma volume and reduce peripheral vascular deconditioning as well as the subsequent orthostatic intolerance during HDBR (3, 26, 27) and is used extensively at the end of long-term flights in preparation for the return to Earth’s gravity. Nutrition countermeasure (daily amino acid supplementation), designed to protect muscle and bone against the deconditioning effects of HDBR (48), may also act on the cardiovascular system.

After long-term bedrest (30, 42, or 90 days), the reduced capacity for the lower limb or splanchnic bed to vasoconstrict in response to an orthostatic fluid shift was related to orthostatic intolerance (47, 7), but the real-time arterial responses in these two areas were never investigated at the same time in the same subject.

The objective of the present study was to quantify the impact of a 60-day bed rest protocol on the cardiac output redistribution between the cerebral and peripheral (splanchnic and lower limb) vascular areas in response to LBNP. A further purpose was to evaluate the effect of two countermeasures [exercise + LBNP (Ex-LBNP) and nutrition (Nut)] on these arterial responses. It was hypothesized that 1) HDBR should induce a reduction in the sympathetically mediated vascular response to fluid shift (local vasoconstriction) in the lower limbs and splanchnic territories, 2) the loss of vasoconstrictor potential in one or both of the splanchnic and femoral vascular beds would be related to a loss of orthostatic tolerance, and 3) a countermeasure that combines LBNP and exercise would minimize bed rest-induced changes in each of the lower limb and splanchnic responses to orthostatic fluid shifts.

MATERIALS AND METHODS

Women international space simulation for exploration study and subjects. The 60 days at 6° HDBR was organized by the Institute for Space Physiology and Medicine Space Clinic, located at Rangueil...
Hospital in Toulouse, France, in 2005. The experiment was approved by a French committee for health (Comité Consultatif pour la Protection des Personnes dans les Recherches).

Twenty-four healthy women signed a consent form after having been informed of the risks and benefits of this long-term bed rest study. The population was randomly assigned to one of three groups: the Ex-LBNP (n = 8) and Nut (n = 8) countermeasures groups and the control (Con) group (n = 8). Ex-LBNP subjects performed flywheel resistance exercise in the 6° head-down position (2, 48) every third day plus 40-min supine treadmill exercise within LBNP (35) 3–4 days/wk with interspersed rest days. The treadmill within the Ex-LBNP protocol was always followed by 10 min of passive LBNP. Nut subjects consumed a daily supplement of protein (0.6 g·kg⁻¹·day⁻¹) during meals. This supplement included 3.6 g/day free leucine, 1.8 g/day valine, and 1.8 g/day free isoleucine (48).

**LBNP test.** The LBNP test was performed pre-HDBR (day −6) and on HDBR day 55 (HDBR-55), with the HDBR duration being 60 days. After the instrumentation with echographic and Doppler probes (below), the subject was exposed to −45 mmHg of LBNP for 3 min. LBNP was used to induce a passive fluid shift without any muscle contraction or neurosensorial stimulation, which may interfere with the cardiovascular response to the fluid shift.

**Echographic and Doppler measurements.** At rest, cardiac function was investigated by measuring left ventricular systolic volume (LVSV) and left ventricle diastolic volume (LVDV) changes using two-dimensional echocardiography (45). The stroke volume and ejection fraction were calculated from these measured variables. Also, the thickness of the posterior wall and septum were measured to evaluate the cardiac mass index (39).

During LBNP, maximal and mean aortic blood flow velocities (aortic flow, in cm/s) were measured from the aortic Doppler spectrum assuming an angle of 0° between the Doppler beam and the aortic axis (2-MHz pulsed Doppler). The cerebral flow velocity [middle cerebral artery (MCA) flow] was recorded using a 2-MHz transcranial Doppler probe fixed over the temporal window to insonate the right MCA. The angle of insonation of the MCA was also considered to be 0°. Superficial femoral artery flow velocity (femoral flow) was investigated using a flat Doppler probe of 4 MHz fixed by two straps passing around the upper part of the thigh and around the abdomen. The Doppler beam was steered at 45° from the front face of the probe, and the angle between the Doppler beam and the vessel axis remained unchanged during the session. The Doppler spectrum was recorded and analyzed by the Cardiolab ground module (ESA-CNES device). Based on a previous report (4), it was assumed that the diameter of these vessels remained constant during the LBNP test and that mean velocity changed in proportion with flow volume (in ml/min) as calculated from velocity and vessel cross section.

The changes in vascular resistance to LBNP were estimated from the maximal (max) and minimal (min) flow velocities in the MCA [cerebral resistance index (CRI) = [(max − min)/max]] and femoral artery [femoral resistance index (FRI) = min/max, with min being the amplitude of the reverse flow] (1, 4).

Portal vein flow volume changes were assessed from portal vein cross-sectional area changes (measured by echography) as these two parameters have been shown to change in parallel in previous studies (6, 7, 24, 32). Thus, as a convention in this report, the term “portal flow” was used instead of portal vein cross-sectional area.

From these vascular data, three ratios were calculated to estimate the cardiac output redistribution between 1) the leg and brain [cerebral-to-femoral flow ratio = (MCA flow/femoral flow)], 2) the splanchnic area and brain [cerebral-to-portal flow ratio = (MCA flow/portal flow)], and 3) the brain and these two territories [MCA flow/(femoral flow + portal flow)].

Finally, the percent change of the last ratio [MCA flow/(femoral flow + portal flow)] from 0- to −45 mmHg LBNP provided an index that quantified the cardiac output redistribution in favor of the brain; the higher the ratio, the greater the redistribution toward the brain. When the percent [MCA flow/(femoral flow + portal flow)] ratio change during LBNP at HDBR-55 was higher than it was during pre-HDBR LBNP, we concluded that the reduction in both the splanchnic and lower limb flow was a positive adaptation that allowed relatively more of the cardiac output to redistribute toward the brain after HDBR. Conversely, a reduction in the percent [MCA flow/(femoral flow + portal flow)] ratio at HDBR-55 compared with pre-HDBR reflected a poorer adaptation in the splanchnic and femoral territories that reduced the proportion of the cardiac output redistributed toward the brain during the LBNP challenge. The hypothesis predicted that a proportionately greater number of individuals who could not finish the HDBR-55 orthostatic tolerance test (nonfinishers) would demonstrate the latter response.

The percent femoral flow or percent portal flow from 0- to −45 mmHg LBNP provided an index by which the flow reduction in each of the splanchnic and lower limb areas could be quantified. For interpretation, if the decrease in flow from 0- to −45 mmHg LBNP was lower during LBNP on day 55 (less vasoconstriction), the difference [change in percent femoral flow on day 55 − percent femoral flow pre-HDBR] was positive. Similarly, when the splanchnic vasoconstrictor response was reduced at HDBR-55, the difference [change in percent portal flow = (percent portal flow on day 55 − percent portal flow pre-HDBR)] was positive. Thus, the change in percent femoral flow or change in percent portal flow quantified the changes in the vasoconstrictor response at HDBR-55 in the corresponding area revealing the HDBR and countermeasure effects on vascular responses.

**Sympathetic nerve recordings during LBNP.** Multunit recordings of postganglionic muscle sympathetic nerve activity (MSNA) were successfully collected for both physiological test sessions in five subjects for the Con group, four subjects in the Ex-LBNP group, and six subjects in the Nut group. Tungsten microelectrodes were inserted percutaneously into muscle fascicles of the right common peroneal nerve with a reference electrode positioned subcutaneously 1–3 cm from the recording site. Neural activity was amplified 1,000 times by a preamplifier and an additional 75 times through a variable-gain isolated amplifier. The signal was band pass filtered (0.7–2.0 kHz), full wave rectified, and integrated with a resistance-capacitance circuit (0.1-s time constant). Criteria for an acceptable MSNA recording included pulse synchrony with the cardiac cycle as well as increased activity to a voluntary apnea but not to emotional arousal (i.e., a loud noise).

**Measurement schedule.** Echocardiographic, portal, cerebral, and femoral measurements were performed with the participant in the supine rest position both pre-HDBR (day −6) and at HDBR-55. Vascular echographic and Doppler changes (cerebral, aortic, portal, and femoral) from 0- to −45 mmHg LBNP were measured pre-HDBR (day −5) and at HDBR-55.

**Orthostatic tolerance test.** Orthostatic tolerance was evaluated by comparing the tolerance to a 10-min 80° head-up tilt test performed immediately post-HDBR to the same test performed within 16 days pre-HDBR. The tests were terminated at symptoms of presyncpe including a reduction in systolic blood pressure below 70 mmHg, a sudden drop in heart rate (>15 beats/min), or at the subject’s request due to sensations of dizziness or nausea. All subjects completed the 10-min test pre-HDBR, whereas in the post-HDBR test, two of eight Ex-LBNP (25%) subjects, five of eight Con (63%) subjects, and six of eight Nut (75%) subjects failed to complete the full 10-min tilt test. Based on these results, we further subdivided the subjects as finishers and nonfinishers of the 10-min tilt test for additional comparison of the hypothesis that completion of the 10-min tilt test was related to a higher aortic or cerebral flow drop or to a more reduced leg and/or splanchnic arterial vasoconstriction in nonfinishers.

**Statistical analysis.** The absolute values at rest and the percent change from 0- to −45 mmHg LBNP were analyzed with the data grouped according to 1) HDBR effect, 2) the countermeasure used, and 3) whether they finished the post-HDT 10-min tilt test (finishers)
or not (nonfinishers). In figures, values are presented as means ± SD. Statistical comparisons were based on three-way ANOVA of the data with main effects of group (Ex-LBNP, Con, and Nut), HDBR (pre-HDBR and HDBR-55), and tolerance to tilt (pre-HBR and post-HDBR) using SAS 9.1.3 analysis software (Cary, NC). Differences were considered as significant at P < 0.05.

The accuracy of the flow redistribution parameters (percent femoral flow, percent portal flow, and [MCA flow/femoral flow + portal flow]) to predict orthostatic intolerance by the end of the HDBR was determined on the basis of the sensitivity and specificity of responses to the LBNP test and expressed as positive and negative predictive values.

RESULTS

At rest, LVDV was diminished at HDBR-55 in both the Con and Nut groups (−12 ± 4% and −7 ± 7%, P < 0.01; Fig. 1). However, LVDV was maintained in the Ex-LBNP group. Cardiac mass was reduced in the Con (−19 ± 4%, P < 0.01) and Nut (−14 ± 5%, P < 0.05) groups and maintained in the Ex-LBNP group (Fig. 1). The ejection fraction at rest was not affected by HDBR in any group (Fig. 1).

The reductions in LVDV and cardiac mass after 55 days of HDBR were not significantly different in the finishers (LVDV: −4 ± 10% and cardiac mass: −6 ± 7%) compared with the nonfinishers (LVDV: −8 ± 11% and cardiac mass: −11 ± 11%).

Compared with pre-HDBR, portal flow (portal cross-sectional area) at rest was lower at HDBR-55 in the Con and Nut groups (−19 ± 13% and −15 ± 16%, respectively, P < 0.01) but remained unchanged in the Ex-LBNP group (Fig. 1). Similarly, baseline portal flow at rest was reduced in the nonfinishers (−15 ± 23% P < 0.05) at HDBR-55 but not in the finishers.

At HDBR-55, MCA and femoral flow velocities as well as the CRI and FRI at rest were not significantly different in any of the groups compared with pre-HDBR. The femoral artery cross-sectional area at rest was reduced during HDBR by 20% in the Con group and 32% in the Nut group (P < 0.05), whereas it increased very slightly in the Ex-LBNP group by 9%.

At LBNP. From 0- to −45-mmHg LBNP, the aortic flow decreased similarly in all groups pre-HDBR and at HDBR-55 (approximately −28% to 33%, P < 0.05).

MCA flow (Fig. 2A) and CRI (Fig. 3A) decreased significantly but similarly in both pre-HDBR and HDBR-55 tests in all groups (MCA flow: −2% to −8%, P < 0.05, and CRI: −12% to −20%, P < 0.05).

Compared with baseline, portal flow (Fig. 2B) decreased during pre-HDBR in all groups (approximately −28 to −43%, P < 0.05). At HDBR-55, this reduction in portal flow was still significant (approximately −28% to −32%, P < 0.05) but smaller compared with pre-HDBR (P < 0.05) in the Con, Nut, and nonfinisher groups. In contrast, portal flow at HDBR-55 decreased similarly as during pre-HDBR in the finisher group and decreased more in the Ex-LBNP group (P < 0.05).

FRI increased significantly from 0- to −45-mmHg LBNP in all groups by ~30% to 60% (P < 0.05) at pre-HDBR (Fig. 3B). Compared with pre-HDBR, FRI during the HDBR-55 LBNP test increased less (P < 0.05) in the Con, Nut, and nonfinisher groups, whereas it increased similarly in the Ex-LBNP and finisher groups. As a consequence of changes in FRI, the femoral flow decreased during LBNP in all groups pre-HDBR (approximately −26% to −39%, P < 0.05) and decreased less in Con, Nut, and nonfinisher groups at HDBR-55 (P < 0.05), whereas it decreased similarly in the Ex-LBNP and finisher groups (Fig. 2C).

MSNA increased with −45-mmHg LBNP (P < 0.05) in each group, but the increases in both burst frequency and total activity during LBNP were not affected by the bed rest or either countermeasure (Fig. 3C).

From 0- to −45-mmHg LBNP, the decrease in MCA flow was small (−2% to −8%), whereas femoral flow and portal flow decreased markedly (femoral flow: −25% to −40% and portal flow: −25% to −50%, P < 0.05; Fig. 2, A–C). Thus, the ratios used for quantifying the redistribution of blood flow between the brain and splanchnic or leg areas increased markedly [(MCA flow/femoral flow): 25% to 60%, (MCA flow/portal flow): 40% to 90%, P < 0.05, and [MCA flow/femoral flow + portal flow]): 35 to 70%, P < 0.05; Fig. 2, D and E]. The percent [MCA flow/femoral flow + portal flow] ratio increased less at HDBR-55 compared with pre-HDBR in the Con, Nut, and nonfinisher groups as a consequence of smaller reduction in the sum of (femoral flow + portal flow) (P < 0.05). Conversely, the percent [MCA flow/(femoral flow + portal flow)] increased more in the Ex-LBNP and finisher group due to greater reductions in (femoral flow + portal flow) during LBNP.

Figure 4A shows, subject by subject, the variation of the [MCA flow/(femoral flow + portal flow)] ratio during LBNP. Compared with pre-HDBR, the ratio was higher or reduced by <15% in 10 of 11 finishers at HDBR-55. In contrast, 11 of 13 nonfinishers had a reduction in this ratio of >15% at HDBR-55 compared with pre-HDBR (sensitivity: 92%, specificity: 83%, positive predictive value: 85%, and negative predictive value: 91%).

Fig. 1. A: left ventricular diastolic volume (LVDV) in the exercise + lower body negative pressure (LBNP) countermeasure (Ex-Lb), control (Con), nutrition (Nut), finisher (F), and nonfinisher (NF) groups. Measurements were performed before (pre; open bars) head-down bed rest (HDBR) and on HDBR day 55 (shaded bars). B: left ventricular myocardium mass. *P < 0.05; **P < 0.01.
Figure 4 shows the difference in the percent change of femoral flow and portal flow from 0- to 45-mmHg LBNP between pre-HDBR and HDBR-55 (change in percent femoral flow and change in percent portal flow) for each individual. In the finisher group, at least one of the two differences (change in percent femoral flow or change in percent portal flow) was negative in 10 of 11 finishers (91%). In the nonfinisher group, both the change in percent femoral flow and change in percent portal flow were positive (less vasoconstriction) in 11 of nonfinishers (84.6%) (sensitivity: 92%, specificity: 83%, positive predictive value: 85%, and negative predictive value: 91%).

DISCUSSION

The main finding of this study was that the Ex-LBNP countermeasure was effective in minimizing the impact of prolonged physical deconditioning on cardiac mass, volume, and function as well as on changes in leg and portal circulations during the simulated orthostatic stress of LBNP. This likely was due to a differential effect of HDBR on femoral and portal hemodynamic responses in the Con and Nut groups compared with the Ex-LBNP group. The quantification of the cardiac output redistribution between the brain and leg or
between the brain and splanchnic areas during LBNP [(MCA flow/femoral flow) and (MCA flow/portal flow)] emphasized the changes in the leg or splanchnic hemodynamic response. The quantification of the regional redistribution of blood flow using a ratio of flow in the cerebral region (MCA flow) relative to that in the splanchnic (portal flow) and lower limbs (femoral flow) allowed the identification of important differences between nonfinishers compared with finishers. Furthermore, the lower increase of the flow redistribution ratio in the nonfinishers compared with the finishers was more significant for [MCA flow/(femoral flow + portal flow)], a ratio designed for this study that takes into account both peripheral territories.

Fig. 4. A: percent change of the [MCA flow/(FEM flow + portal flow)] ratio, which represents the flow redistribution ratio between the brain area and both the splanchnic and leg areas from 0- to −45-mmHg LBNP. Percent changes at pre-HDBR are shown as open bars and those on HDBR day 55 as shaded bars. The higher the ratio increases (from 0- to −45-mmHg LBNP), the more efficient the flow redistribution toward the brain. For finishers, the percent ratio increase at HDBR day 55 was either higher or at least no lower than 15% compared with pre-HDBR (left part of the x-axis). For nonfinishers, the percent ratio increase at HDBR day 55 was always lower than 15% compared with pre-HDBR. There were only three exceptions: subject K1 showed a much lower increase of the ratio at HDBR day 55 even though she was a finisher (false positive), and subject D1 showed a higher increase of the ratio at HDBR day 55 and subject I2 showed a decrease of the ratio no lower than 15% even though they were nonfinishers (two false negatives). B: percent change of the mean femoral flow velocity at HDBR day 55 minus the percent femoral flow change at pre-HDBR [%FEM flow (FEM flow at day 55 - %FEM flow pre-HDBR)] as well as the percent change of the mean portal vein cross-sectional area at HDBR day 55 minus the percent change of the mean portal vein cross-sectional area at pre-HDBR [%portal flow (portal flow at day 55 - %portal flow pre-HDBR)]. As an example, if %FEM flow pre-HDBR = −30% and %FEM flow at day 55 = −10%, then Δ%FEM flow = +20%. A positive difference in the percent value means that the FEM or portal flow reduced less at HDBR day 55 compared with pre-HDBR, suggesting that the vasoconstrictor response in the corresponding area was lower after 55 days of HDBR. For finishers, at least one of these %FEM flow or %portal flow changes was negative, whereas for nonfinishers, both the Δ%FEM flow and Δ%portal flow were positive. There were only three exceptions: subject C2 did not have at least one negative change in percent value even though she was a finisher (false positive), whereas subjects D1 and H2 did not have two positive values even though they were both nonfinishers (false negative).
Generally, members of the Ex-LBNP group formed the majority of the finishers in the tilt test, and their responses were characterized by a smaller decrement in vasoconstrictor control. This sustained vasomotor control was identified by a greater reduction in flow volume in the leg and/or splanchic area during LBNP after HDBR. Moreover, the study of individual responses in both the femoral and splanchic hemodynamic response showed that the probability of being classified as a nonfinisher was much higher in those subjects with reduced vasoconstriction in both vascular territories at HDBR-55, whereas the probability of being a finisher was much higher in those individuals where the vasoconstrictor response was altered in only one of these regions (lower limb or splanchic area). Finally, the study demonstrated that the insufficient reduction in femoral flow during LBNP was not related to an altered MSNA response. Therefore, it was concluded that 1) HDBR reduced the vascular constrictor response in visceral and limb regions; 2) this altered constrictor response was related to reductions in the end-organ vasomotor response rather than sympathetic outflow; and 3) such neurovascular alterations are minimized by the Ex-LBNP countermeasure.

Cardiovascular changes at rest. Exposure to HDBR induced a reduction in LVDV (approximately −12% and −7%) in the Con and Nut groups but not in the Ex-LBNP group. These findings are in agreement with the decrease in cardiac chambers as measured by MRI (19). The cardiac mass, evaluated from the direct visualization of the left ventricular posterior wall and septum, was reduced in both the Con (−19%) and Nut (−14%) groups only, which is not totally in agreement with cardiac MRI measurements, which showed no cardiac mass reduction in the Nut group. Echography is certainly less accurate than MRI, but this does not mean that what we measure is in contradiction with the MRI data as 1) the changes remain in the range of 15%; 2) the mass reduction (by echography) was much more significant for the Con group than for the Nut group; 3) the subject was not in the same position for both methods at HDBR-55 (MRI: supine and echocardiography: head down); and 4) some error is expected in MRI measures. On the other hand, one may notice that the reduction in cardiac mass, as measured by ultrasound in the Nut group, was found both at HDBR day 25 and HDBR-55 and that both methods found a similar decrease in left ventricular volume in the Con and Nut groups, probably due to the absence of daily physical activity and the reduced mechanical requirements for the heart to pump blood in both groups.

On the basis of previous HDBR studies (11, 26) that demonstrated proportionate changes in LVDV and plasma volume, it was concluded that Con and Nut subjects were probably both hypovolemic and had a myocardial atrophy that was more evident in the Con group than in the Nut group. The aerobic and resistive exercise combined with LBNP prevented the decrease in cardiac volume and mass, whereas in a previous study (11) of men, the resistive exercise alone did not. Nevertheless, reductions in cardiac muscle mass did not predict whether the participants would be finishers or nonfinishers in the post-HDBR tilt test. Even pre-HDBR, these variables did not identify subjects who could finish the tilt test used in this protocol.

At rest, portal flow (an index of splanchic flow) was reduced in Con and Nut subjects and was coincident with a reduction in cardiac volume (i.e., circulating blood volume).

Conversely, in Ex-LBNP subjects (without cardiac volume decrease), portal flow did not change. The reduction of the femoral artery cross-sectional area at rest in the Con and Nut groups and its slight increase in the Ex-LBNP group was probably a consequence of the patterns of physical activity and the demand for muscle blood flow (13). Therefore, while the Nut countermeasure might have provided some defence against cardiac muscle mass changes during HDBR, the Ex-LBNP countermeasure prevented the cardiac volume and splanchic flow volume decrease as well as the myocardial atrophy. None of the variables measured at rest and that were influenced by HDBR (cardiac volume, cardiac mass, and portal vein flow) were related to the orthostatic tolerance.

HDBR and countermeasure effects on aortic, cerebral, splanchic, and femoral flow responses to LBNP. From 0- to −45-mmHg LBNP, aortic flow decreased similarly as pre-HDBR in all groups (Ex-LBNP, Con, Nut, finishers, and nonfinishers). As only the non-Ex-LBNP subjects had a reduced circulating blood volume, hypovolemia did not interfere directly with the cardiac output response to the fluid shift induced by LBNP.

Similar reductions in cerebral flow were observed in both pre-HDBR and HDBR-55 LBNP tests in the Con, Ex-LBNP, Nut, finisher, and nonfinisher groups, whereas previous experiments on men demonstrated a greater reduction in cerebral flow during the LBNP or stand tests performed before HDBR or spaceflight in subjects with reduced orthostatic tolerance after bedrest or flight (47). However, it is noteworthy that the duration of the −45-mmHg LBNP period (3 min) was much shorter than in the study mentioned above (7 min at −45-mmHg LBNP or 10-min stand test). Other studies (14, 28, 33) have reported that cerebral autoregulation was not significantly affected by HDBR or by induced changes in plasma volume, in support of the present observations. Another study (49) reported that reduced orthostatic tolerance after HDBR was associated with greater decreases in cardiac output and cerebral flow velocity as well as a greater increase in total peripheral resistance. These investigators concluded that cerebral autoregulation may be affected by HDBR. However, no such differences were identified in the cerebral, cardiac, or lower limb circulations of the present study; variations in the methods used (greater negative pressures in LBNP) and the parameters measured in these two studies may explain these differences.

The lower limb vascular resistance, as measured by FRI, increased less during LBNP at HDBR-55 than at pre-HDBR in the Con and Nut groups and also in the nonfinisher group, as already observed in previous HDBR and spaceflight studies (29, 47). Thus, only the Ex-LBNP countermeasure prevented the degradation of the lower limb arterial hemodynamic response to LBNP in this group of women. A similar response was observed during a 30-day HDBR with men using aerobic and resistive exercise and LBNP (3, 27), whereas the use of resistive exercise only during a 90-day HDBR had little effect on the lower limb vascular resistance response to a stand test (10). A rodent study (34) reported that hindlimb unloading induced a reduction of the perivascular sympathetic nerve density that could be responsible for the lack of vasoconstriction even if the sympathetic outflow was not affected by the immobility. Other studies (20, 21) have also pointed to a reduction in vasoconstrictor responsiveness in isolated vascular tissue following hindlimb unloading due to remodelling of

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microvascular diameter and wall thickness. The microneurographic recordings in the present study indicated that postganglionic sympathetic activity at baseline and during −45-mmHg LBNP was not affected by the bed rest period or the countermeasures. These data support the hypothesis that adaptations in the functional and/or morphological regulation of peripheral vascular targets develop with physical deconditioning and that this limits the ability of a given sympathetic signal to elicit an effective constriction. These results are consistent with earlier observations in men where acute sympathetic responses to Valsalva’s maneuver were not affected by 14 days of bed rest (25, 41). The present conclusion of altered end-organ responsiveness to sympathetic input is also consistent with previous findings of a diminished ability to constrict a dilated vascular bed following bed rest (40).

The smaller decrease in portal vein flow (i.e., splanchic flow) at −45-mmHg LBNP in the Con, Nut, and nonfinisher groups compared with pre-HDBR and with the Ex-LBNP and finisher groups further confirms that the Ex-LBNP countermeasure minimized the HDBR-induced decline in the splanchic vasoconstriction to the fluid shift and that this vascular territory contributes, with the legs, to maintain an appropriate flow redistribution toward the brain during orthostasis (7, 32). The insufficient decrease on portal flow (i.e., splanchic flow) is likely the consequence of insufficient vasoconstriction in the splanchic territory. Altered vascular contractile behavior could be due to HDBR-induced changes in intracellular calcium mobilization, loss of arteriolar and venous responsiveness to adrenergic stimulation, or anatomic changes in the surrounding parenchymal muscle tissue (increased fast-twitch glycolytic fibers) that affect vascular geometry (9, 17, 36). In this same group of women, we have previously reported that subjects who received no Ex-LBNP countermeasures tended to have lower absolute leg vascular resistance post-HDBR, but there were no differences compared with pre-HDBR in responses to isoproterenol or norepinephrine, suggesting that the adrenergic vascular response was intact (23).

Failure to achieve adequate arterial vasoconstriction, as evaluated by total peripheral or local resistance changes, has been suggested as an important mechanism to explain orthostatic intolerance after spaceflight (29, 38). On the basis of previous HDBR studies during which the lower limb or the portal vein flow was assessed (7, 47), we should have expected a similar alternation of the lower limb and splanchic hemodynamics with the HDBR intervention. Nevertheless, the assessment of both femoral and portal flow during LBNP showed that even in the nonfinishers, the magnitudes of these responses were not similar. This observation raises the question of the respective role of these regions in the cardiac output redistribution of blood flow and the time course of the vascular changes that occur with physical deconditioning.

In this study, we used a new parameter (percent \[MCA flow/(femoral flow + portal flow)\]) to reflect the relative redistribution of peripheral blood flow toward the brain (Fig. 4A). With this ratio, the reduction in flow to both the lower limb and splanchic regions with LBNP was less at HDBR-55 compared with pre-HDBR in the Con and Nut groups but not in the Ex-LBNP group. Also, this ratio was more significant than the single ratios \[\{MCA flow/femoral flow\} or (MCA flow/portal flow)\]. Similarly, the LBNP-induced reduction in limb and splanchic flow volume was less at HDBR-55 in nonfinishers compared with finishers. These results confirm the positive effect of the Ex-LBNP countermeasure on the global response to orthostatic stress, whereas the Nut countermeasure had no benefit.

Another study (44) on normal subjects using the impedance method reported a decrease in blood volume (in ml) in the limbs and an increase in the blood volume within the splanchic area during LBNP. While impedance methods provide valuable time-course data pertaining to total blood volume, they cannot provide data on the instantaneous arterial response [flow reduction (in ml/min) or vasoconstriction] nor do they provide information on the constrictor response that mediates cardiac output redistribution. This may explain why that study (44) reported opposite responses within the splanchic and limb vascular beds during LBNP and in a stand test, whereas we found similar arterial flow responses in both areas in both tests.

The percent change of the \[MCA flow/(femoral flow + portal flow)\] ratio from 0- to −45-mmHg LBNP was studied on an individual basis. In the finisher group, the relative increase (in %) in this ratio during LBNP at HDBR-55 was either higher or at least no lower than 15% of the pre-HDBR value, suggesting that the vasoconstrictor response was not negatively altered in these territories even after 55 days of HDBR. In the nonfinisher group, the relative increase in this ratio at HDBR-55 was always lower by >15% compared with pre-HDBR, suggesting a less effective vasoconstrictor response in those territories. Thus, this parameter quantified the flow redistribution among two major vascular areas involved in blood pressure defence during orthostasis and differentiated the physiologic basis of the finisher and nonfinisher groups in the post-HDBR tilt test with a positive predictive value of 85% and a negative predictive value of 91%.

Nevertheless, the ratio of global flow redistribution could not identify the territory that provided the lowest vasoconstrictor response. Thus, we displayed the percent change of femoral flow (lower limbs) and portal flow (splanchic area) from 0- to −45-mmHg LBNP subject by subject between pre-HDBR and HDBR-55 (Fig. 4B). This analysis showed that these two territories did not respond similarly after 55 days of HDBR. When the femoral flow or portal flow decreased less at HDBR-55 compared with pre-HDBR (less vasoconstriction), the difference [change in percent femoral flow = (percent femoral flow on day 55 − percent femoral flow pre-HDBR)] or [change in portal flow = (percent portal flow on day 55 − percent portal flow pre-HDBR)] was positive (see Fig. 4B). Conversely, if one of these variables decreased more (higher vasoconstriction) at HDBR-55, the difference (percent femoral flow on day 55 − percent femoral flow pre-HDBR) or (percent portal flow on day 55 − percent portal flow pre-HDBR) will become negative.

The graphic display of the change in percent femoral flow and change in percent portal flow in each subject (Fig. 4B) showed that 5 of 11 finishers had a normal response in both femoral and portal vascular territories (both change in percent flow values negative), whereas the 6 other finishers showed a lower reduction of only one area (e.g., reduced lower limb vasoconstriction but maintained splanchic vasoconstriction). Conversely, a lower reduction at HDBR-55 was observed in both regions (two change in percent flow values positive) in 11 of 13 nonfinishers. In addition, the lack of response was more
common in the splanchnic territory than in the lower limb in 9 of 13 nonfinishers (69%). Thus, a reduced vasoconstrictive response in only one of the two territories may not be sufficient to induce orthostatic intolerance at post-HDBR tilt, whereas an altered response in both territories may be associated with orthostatic intolerance at post-HDBR tilt (positive predictive value: 85% and negative predictive value: 91%).

A previous investigation of splanchnic and lower limb responses to tilt in patients with postural tachycardia syndrome (POTS) showed that the splanchnic blood flow did not change during incremental tilt despite marked peripheral lower limb vasoconstriction (43). In combination with the present results, this observation in POTS patients suggests that the splanchnic flow regulation may be influenced by changes in plasma volume. Finally, the HDBR-induced impairment in vasoconstriction should be greater at the splanchnic than at the lower limb level as observed in the majority of nonfinishers (69%). This suggests that inclusion of a LBNP countermeasure (as included in the Ex-LBNP group) may have a higher protective effect against orthostatic intolerance than other countermeasures such as exercise alone, for example, by minimizing the plasma volume loss and stimulating the splanchnic response to fluid shift (12).

On the other hand, the assessment of the calf veins and calf superficial tissue thickness demonstrated that the LBNP at HDBR-55 induced a higher distension of the tibial and gastrocnemius veins and a higher increase of the superficial tissue thickness in the Con, Nut, and nonfinisher groups compared with the Ex-LBNP and finisher groups (8). Thus, the lack of both lower limb and splanchnic arterial vasoconstriction, the higher leg vein distensibility, and the higher amount of liquid stored into the superficial tissue occurred more frequently in the Con and Nut groups and were associated with orthostatic intolerance (nonfinisher group) while the sympathetic nervous response was not significantly different between any group. This emphasizes the major role of the distal arterial, venous, and microcirculatory targets in the adaptation to long-term bed rest and in the development of orthostatic intolerance.

Conclusions. The combined results lead to the following conclusions about the impact of 60 days of HDBR in women that support our hypothesis: 1) none of the resting parameters affected by HDBR (cardiac volume, cardiac mass, and portal flow) were related to the orthostatic tolerance; 2) the Ex-LBNP countermeasure prevented the myocardial atrophy, decrements in cardiac volume, and alterations of orthostatic flow regulation in the splanchnic and leg vascular regions; 3) the ratio between the proportion of cardiac output sent toward the brain and toward both the splanchnic and lower limb area identified the subjects at high risk of orthostatic intolerance; 4) only a reduction in the vasoconstrictor response in both territories was associated with orthostatic intolerance at post-HDBR tilt, whereas an altered response in only one of them was not; and 5) the lack of vasoconstriction at the lower limb level appeared to be related to altered end-organ responses to an unaltered sympathetic neural stimulus.

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