WISE-2005: adrenergic responses of women following 56-days, 6° head-down bed rest with or without exercise countermeasures

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WISE-2005: adrenergic responses of women following 56-days, 6° head-down bed rest (HDBR) with or without exercise countermeasures. Am J Physiol Regul Integr Comp Physiol 293: R2343–R2352, 2007. First published October 10, 2007; doi:10.1152/ajpregu.00187.2007.—We tested the hypotheses that women completing 56 days, 6° head-down bed-rest (HDBR) would have changes in sensitivity of cardiovascular responses to adrenergic receptor stimulation and that frequent aerobic and resistive exercise would prevent these changes. Twenty-four women, eight controls, eight exercisers (lower body negative pressure treadmill and flywheel resistance exercise), and eight receiving nutritional supplement but no exercise were studied in baseline and during administration of the β-agonist isoproterenol (ISO) and the α- and β-agonist norepinephrine (NOR). In the control and nutrition groups, HDBR increased heart rate (HR) and reduced stroke volume (SV), and there was a significantly greater increase in HR with ISO after HDBR. In contrast, the HR and SV of the exercise group were unchanged from pre-HDBR. After HDBR, leg vascular resistance (LVR) was greater than pre-HDBR in the exercise group but reduced in control and nutrition. LVR was reduced with ISO and increased with NOR. Changes in total peripheral resistance were similar to those of LVR but of smaller magnitude, perhaps because changes in cerebrovascular resistance index were directionally opposite to those of LVR. There were no changes in sensitivity of the vascular response to adrenergic stimulation. The HR response might reflect a change in sensitivity or a necessary response to the reduction in SV after HDBR in control and nutrition groups. The reduced peripheral vascular resistance after HDBR might help to explain orthostatic intolerance in women. Exercise was an effective countermeasure to the HDBR effects.

The WISE-2005 60-DAY HEAD-DOWN BED REST (HDBR) study provided an opportunity to study the cardiovascular responses of women to the infused sympathetic agonists isoproterenol, which stimulates primarily β-adrenergic receptors, and norepinephrine, which stimulates both α- and β-adrenergic receptors. In a previous study of men, Convertino and colleagues (8) found that 14 days of HDBR increased the heart rate (HR) and peripheral vasodilatory responses to isoproterenol, while there was no effect of HDBR on the mean arterial pressure (MAP) response to the α1-adrenergic agonist phenylephrine. These observations were interpreted to mean that the chronic reduction in circulating norepinephrine with HDBR had an inverse effect on β-adrenergic receptor responsiveness in both the heart and peripheral vessels with no change in responsiveness of α1-receptors. Data obtained primarily from male astronauts indicated that those who would experience presyncopeal symptoms postflight had lower α1-adrenergic responsiveness to phenylephrine before and after spaceflight (31). These data did not provide any indication of whether the greater impairment in orthostatic tolerance that is observed in women compared with men after spaceflight (46) or HDBR (9) might be due to altered adrenergic receptor responsiveness. In the current study, we tested our first hypothesis that, consistent with studies in men, β-adrenergic receptor responsiveness would be increased in women taking part in complete long-term HDBR (without countermeasures) and that there would be no change in responsiveness of α1-receptors.

To date, research of the effects of spaceflight or HDBR on the adrenergic responsiveness has not attempted to determine whether countermeasures might attenuate the deconditioning response. In WISE-2005, a control group was compared with an exercise group that performed regular endurance and resistive leg exercises [supine position running inside a lower body negative pressure (LBNP) device (4) or flywheel device (1)]. As well in this study, a nutritional protein supplement group, with no exercise, was included primarily to test hypotheses related to muscle atrophy with prolonged HDBR. Regular physical exercise during HDBR has been shown to protect exercise performance on return to an upright posture (21, 45), but there has been less benefit for cardiovascular responses to an orthostatic challenge (22, 26, 37), unless the exercise is combined with artificial gravity (27). Because physical exercise countermeasures would be expected to regularly elevate plasma catecholamines, we tested the study’s second hypothesis that the exercise group would maintain adrenergic receptor responsiveness close to baseline levels, whereas the control and nutrition groups would have altered β-adrenergic receptor responsiveness.

METHODS

Twenty-four healthy women between the ages of 25 and 40 participated in the WISE-2005 bed rest study, which was an international collaboration between the French, European, Canadian, and American space agencies. Each subject completed 60 days of supervised, continuous 6° HDBR, with 20-day periods of additional testing and monitoring before and after bed rest in the Space Medicine...
Research Facility of the Centre National d’Études Spatiales in Toulouse, France. All experimental procedures were approved by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Midi-Pyrénées (France), Committee for the Protection of Human Subjects at Johnson Space Center, and local ethics committees, including the Office of Research Ethics, University of Waterloo. The entire protocol was in accordance with the declaration of Helsinki. Each subject was aware of her right to withdraw from the study for any reason without prejudice.

Subjects were randomly assigned to one of three groups: control, exercise, or nutrition. Throughout the study, hydration was maintained with 60 ml·kg⁻¹·day⁻¹, and caloric intake was set at 120% of the calories dictated by resting metabolic rate. This was modified for the exercise group to account for increased fluid requirements and energy expenditure. No nicotine, alcoholic beverages, or caffeine were permitted. Testing was completed on day 7 before the start of HDBR, and on day 56 of HDBR, for the exercise group day 56, testing took place –24 h after treadmill exercise.

Countermeasures

Subjects in the exercise group walked and ran 3 or 4 times per week in the supine position on a treadmill placed inside an LBNP chamber for 40 min at 40–80% VO₂peak peak in an interval fashion. The applied LBNP (–48 to –55 mmHg) provided 1±1.15 body weight of ground reaction force (4). The treadmill exercise was followed by 10 min of resting LBNP at the same level. Every third day, subjects performed flywheel resistance exercise while maintaining a head-down position (1). The treadmill and flywheel exercises were normally not performed on the same day, and rest days were provided. The nutrition countermeasure group received a supplement of free branched-chain amino acids (3.6 g/day free leucine, 1.8 g/day free isoleucine, and 1.8 g/day free valine) that were added as a supplement (Friliver, Bracco, Italy) given at the main meals to attenuate nitrogen loss during bed rest (43).

Drug Infusion and Blood Sampling

Experiments were conducted in the supine position before HDBR and on day 56 of HDBR. Catheters were placed in the antecubital veins of each arm, one for drug infusion and the other for blood sampling. Following instrumentation, subjects were allowed to rest for 5 min before drawing the baseline blood samples. Drug infusion was always performed in the same order with two doses of isoproterenol (Isuprel, Winthrop, Clichy, France) 0.005 and 0.01 µg·kg⁻¹·min⁻¹ diluted in 5% glucose for 5 min each. Infusion was stopped, and the heart rate and blood pressure were monitored until they returned to preisoproterenol baseline. This was followed by two doses of norepinephrine (noradrenaline tartrate, Laboratoire Agettant, Lyon, France) 10 and 50 ng·kg⁻¹·min⁻¹ diluted in 5% glucose for 5 min each. Blood samples (2 ml) were obtained in the final minute of each infusion, mixed with 50 µl EGTA/glutathione, centrifuged and separated to plasma and packed red cells. Plasma was immediately transferred to sealed, labeled Eppendorf tubes and stored frozen at –30°C. Samples were then packed on dry ice and exported to the University of Waterloo after each series of tests for analysis by HPLC with electrochemical detection (20).

Infusion of isoproterenol was terminated if the increase in heart rate was greater than 35 beats/min. Infusion of norepinephrine was terminated if the systolic blood pressure increased more than 20 mmHg or if the reflex reduction in heart rate exceeded 20 beats/min.

Physiological Measurements

Blood flow velocity was measured by Doppler ultrasound from the aortic root by handheld 2 MHz probe (Mutilgyn, New York, NY) and from the probes fixed above the skin for middle cerebral (2 MHz) and superficial femoral (4 MHz) arteries (Cardiolab, European Space Agency) recorded to digital data acquisition software. Cross-sectional area of the aorta and femoral artery were obtained by ultrasound imaging (Acuson 128XP, Paris, France). Total cardiac output was calculated from the cross-sectional area and the beat-by-beat mean blood flow velocity of the aorta (12), and leg flow was calculated in ml/min and corrected for the muscle mass measured by magnetic resonance imaging (S. Trappe and T. Trappe, personal communication).

MAP was measured using finger-cuff plethysmography (Finometer, Finapres Medical, Amsterdam), and HR was determined from the electrocardiogram with all analog signals sampled in real time at 100 Hz with an online acquisition and analysis system (PowerLab, ADInstruments; Castle Hill, New South Wales, Australia) and stored on a computer for subsequent analysis.

Total peripheral resistance (TPR), leg vascular resistance (LVR), and cerebrovascular resistance index (CVRi) were calculated from MAP divided by cardiac output, leg flow, and cerebral flow velocity, respectively.

Statistical Analyses

Values are presented as the mean and standard deviation for pre- and post-HDBR from the fourth minute of measurement at baseline and the two drug doses. A complete data set is in the supplemental table appended to the electronic version of this paper (reference to location). Statistical comparisons were based on three-way ANOVA of the absolute data with main effects of group (exercise, control, and nutrition), HDBR (pre-HDBR and day 56 of HDBR) and drug (dose of isoproterenol or norepinephrine) using SAS 9.1.3 analysis software (Cary, NC). Important P values are presented in the text and figures for main and interaction effects, and a complete list of P values is in the supplemental table.

RESULTS

For clarity and because the data of the nutrition group did not differ in a physiologically important way from the control group, the presentation in the RESULTS section is confined to comparisons between the control and exercise groups. Absolute values, including full results for the nutrition group, are presented in the supplemental table, which can be found online at the American Journal of Physiology—Regulatory, Integrative and Comparative Physiology website.

Effects of HDBR and Countermeasures on Cardiovascular Function

MAP was not affected by HDBR (P = 0.20), and there were no differences in MAP between groups (complete set of MAP values are in the supplemental table found on the American Journal of Physiology—Regulatory, Integrative and Comparative Physiology website).

The significant increase in HR as a consequence of HDBR is clearly shown in Fig. 1 for the control group (main effect of HDBR, P = 0.0001, Fig. 1A and P = 0.0007, Fig. 1B). The unchanged HR response of the exercise group after HDBR is also evident, and this resulted in a statistically significant group × HDBR interaction effect (P = 0.002, Fig. 1A and P = 0.0008, Fig. 1B).

Stroke volume (SV) was significantly reduced after HDBR (main effect of HDBR, P = 0.006, Fig. 1C and P = 0.007, Fig. 1D). The change in SV was not different between groups, although the exercise group tended to have unchanged SV after HDBR while the control group was reduced (P = 0.125, Fig. 1C).
Cardiac output (Q) was not significantly different after HDBR (Fig. 1E and 1F), and there were no differences between groups after HDBR.

The indicators of vascular resistance in the total body, leg, and cerebral circulations (TPR, LVR, CVRi) were not affected by HDBR (Fig. 2, A and F). The LVR during the norepinephrine phase of the experiment (Fig. 2D) was notable for a significant group times HDBR interaction (P < 0.04), where LVR was increased slightly after HDBR in the exercise group and decreased slightly after HDBR in the control group. The CVRi response during the isoproterenol phase of the experiment revealed a trend to a group times HDBR interaction (P < 0.07) with lower CVRi after HDBR in the exercise group and higher CVRi after HDBR in the control group (Fig. 2E).

**Isoproterenol Responses**

For the isoproterenol infusions, there were significant main effects of drug, as indicated below, but there were no significant drug times group interaction effects with the exception of HR. MAP was not significantly affected by infusion of isoproterenol (0.005 and 0.01 µg·kg⁻¹·min⁻¹) and norepinephrine (10 and 50 ng·kg⁻¹·min⁻¹) in the exercise group (solid) and control (open) groups. Significant main effects of HDBR were observed during the isoproterenol and the norepinephrine conditions for heart rate (HR) (A, P < 0.001 and B, P = 0.0007) and stroke volume (SV) (C, P = 0.006 and D, P = 0.007). There were significant group × HDBR effects on HR (A, P = 0.002 and B, P = 0.0008) with a trend for SV (C, P = 0.125). Values are expressed as means and SD. HR, heart rate; SV, stroke volume; Q, cardiac output.

**Norepinephrine Responses**

For the norepinephrine infusions, there were significant drug effects as highlighted below, but there were no significant drug times HDBR interaction effects on the cardiovascular variables. MAP was significantly increased by norepinephrine infusion (main effect of drug, P = 0.0003, data in supplemental table found online at the American Journal of Physiology—Regulatory, Integrative and Comparative Physiology website). HR decreased during norepinephrine infusion (Fig. 3B, main effect of drug, P = 0.0002). SV was decreased by norepinephrine (Fig. 3D, main effect of drug, P = 0.001). The decrease in both HR and SV during norepinephrine infusion caused Q to be reduced by the drug (main effect of drug, P < 0.0001, Fig. 3F).

Both TPR and LVR were significantly increased by norepinephrine (Fig. 4, B and D, main effect of drug, TPR P < 0.0001 and LVR P = 0.0005). There was a very small trend for CVRi to increase during norepinephrine infusion (Fig. 4F, main effect of drug, P = 0.01).
Plasma Catecholamines

There was a significant HDBR times group interaction for the change in epinephrine concentration during the isoproterenol infusions that reflected primarily the lower epinephrine after HDBR in the exercise group with some elevation in the control group ($P < 0.02$, Fig. 5A). There was a significant elevation of epinephrine in response to norepinephrine infusion (main effect of drug, $P < 0.01$, Fig. 5B), but there were no effects of HDBR.

Plasma norepinephrine increased significantly in response to isoproterenol infusion (main effect of drug, $P < 0.0001$, Fig. 5C) with no changes resulting from HDBR. During the norepinephrine infusion, there was the anticipated increase in plasma norepinephrine concentration (main effect of drug, $P < 0.0001$, Fig. 5D). There was a significant HDBR times drug interaction effect that reflected the relatively lower concentrations of norepinephrine after HDBR ($P = 0.002$, Fig. 5D).

**DISCUSSION**

This was the first study to investigate the cardiovascular responses to infused sympathetic agonists in women after HDBR and the first study to contrast adrenergic responses from control subjects who were totally inactive throughout 56 days of HDBR with responses of subjects who performed exercise or nutritional countermeasures. With regard to our first hypothesis that women would experience similar increases in $\beta$-adrenergic receptor sensitivity as observed in men during HDBR (8) without changes in $\alpha$-adrenergic receptor sensitivity, the increases in HR after HDBR of the control and nutrition groups to isoproterenol were consistent with the hypothesis. However, differences in SV responses need to be considered before accepting the hypothesis of a change in $\beta$-adrenergic receptor sensitivity. Consistent with the hypothesis, there was no evidence for change in sensitivity of the LVR to $\alpha$-adrenergic receptor stimulation after HDBR. Our second hypothesis that the exercise countermeasure group would maintain their responses close to the baseline condition was supported by the similar pre- to post-HDBR HR response, while the LVR was elevated after HDBR. The greater post-HDBR LVR response of the exercise group contrasted with a reduction in the control and nutrition groups, as revealed by the interaction term for group $\times$ HDBR during the norepinephrine infusion. The results for the control and nutrition groups contrasted to the increased LVR observed in men who were inactive throughout 14 days of HDBR (8). Overall, these data reveal signs of cardiovascular deconditioning in women with benefits of an exercise countermeasure on the cardiovascular responses to stimulation of the adrenergic receptors.

**Limitations**

The WISE-2005 study was a large international collaboration with 15 different principal investigators examining a range of questions related to the consequences of prolonged HDBR and the potential benefits of countermeasures. In such a large study, it was necessary to recruit two groups of 12 subjects who were housed two to a room, so that each member of the pair was in the same experimental group and performed the same activities on a given day. Subjects underwent an exten-
sive medical and psychological screening and were stratified by predicted peak oxygen uptake from a cycle ergometer test. The subjects came from eight different European countries, and it was also necessary to group them by common language before randomly assigning them to the control, exercise, or nutrition countermeasure groups. From the perspective of basic data such as MAP, HR, and cardiac output, there were no between-group differences in the baseline measurements (full data in supplemental table found online at the American Journal of Physiology—Regulatory, Integrative and Comparative Physiology). An important limitation for any attempt to relate the responses of women to those of men is that men were not studied with the identical protocol. This restricts the interpretations that can be made with respect to a previous similar study of men who bed rested for only 14 days (8).

In the experimental design, the nutrition countermeasure group was established to test hypotheses related to muscle atrophy during long-term HDBR. It was not anticipated that the nutrition countermeasure in subjects who were otherwise completely inactive throughout the HDBR would have any effect on the cardiovascular responses. Indeed, there is no evidence from the current study that the responses of the nutrition group differed from those of the control group. Thus, for the purpose of this study, the main results are presented only for the control group, although the control and nutrition groups could be considered equivalent.

It was impossible in this study to attempt to account for menstrual cycle phase within the experiments. In the selection criteria, no woman had been on oral contraceptives for at least 2 mo before the start of the experiment, and all had regular cycles. As an indication of relative cycle phase at the times of our testing, three subjects in each group were in the first 10 days of their menstrual cycle during the pre-HDBR tests, while four subjects from the exercise group, two from the control, and zero from the nutrition groups were in the first 10 days of their cycle on day 56 of HDBR. As well, during the post-HDBR tests, three subjects, two in nutrition and one in exercise, had been more than 50 days since their last menstruation. We detected no remarkable difference between subject responses throughout the menstrual cycle, but this could only be resolved by a systematic large-scale study in which individuals were examined with regard to duration of HDBR and menstrual cycle phase. Although the impact of menstrual cycle on adrenergic responses is controversial (25, 32, 33), there is still a possibility that variations in menstrual phase between pre- and post-HDBR testing could have influenced the results, and this will be considered further in later sections.

**Isoproterenol Responses**

In a previous study of men, after 14 days of HDBR, Convertino and colleagues (8) identified increased β-adrener-
adrenergic receptor responsiveness on the basis of higher absolute heart rate and a greater slope for increase in heart rate during isoproterenol infusion after HDBR. The heart rate response in the current study revealed significant main and interaction effects. Overall, heart rate was increased after HDBR, and it was increased in response to isoproterenol. The significant group times HDBR interaction effect reflected the elevated heart rate in the no-exercise groups, as was typically found in Fig. 4. The drug-induced responses of TPR, LVR, and CVRI are shown for both isoproterenol and norepinephrine at baseline with no drug (open column), first drug dose (stippled column, isoproterenol = 0.005 μg·kg⁻¹·min⁻¹, norepinephrine = 10 ng·kg⁻¹·min⁻¹), and second drug dose (cross-hatched column, isoproterenol = 0.01 μg·kg⁻¹·min⁻¹, norepinephrine = 50 ng·kg⁻¹·min⁻¹). There was a significant main effect of drug for all variables for both drugs (see RESULTS for P values). There were no significant interaction terms involving the drug. Pre, before HDBR (open columns); Post, testing on day 56 of HDBR (gray columns). Values are expressed as means and SD.

Fig. 5. The drug-induced changes in plasma concentrations of epinephrine ([Epi]) and norepinephrine ([NE]) are shown during infusion protocols for isoproterenol and norepinephrine at baseline with no drug (open column), first drug dose (stippled column, isoproterenol = 0.005 μg·kg⁻¹·min⁻¹, norepinephrine = 10 ng·kg⁻¹·min⁻¹) and second drug dose (cross-hatched column, isoproterenol = 0.01 μg·kg⁻¹·min⁻¹, norepinephrine = 50 ng·kg⁻¹·min⁻¹). There was a significant main effect of drug on plasma [Epi] during norepinephrine infusion (B) and on plasma [NE] during infusion of both drugs (C and D). See RESULTS for further consideration of main effects and interaction terms. Pre = before HDBR (open columns), Post = testing on day 56 of HDBR (gray columns). Values are expressed as means and SD.
bed rest studies of longer duration (26, 41), and sometimes (27), but not always, with up to 14 days HDBR (24), while the exercise group did not increase heart rate after HDBR as found with a centrifuge plus cycling exercise countermeasure during a 14-day HDBR (27). Further, the significant group times HDBR times drug interaction effect reflected the relatively greater increase in heart rate after HDBR in the no-exercise groups with isoproterenol infusion, thus, confirming our hypothesis that women would respond in a similar manner to the men in the earlier study (8). However, it is not obvious that this is, in fact, an increase in sensitivity of the β-adrenergic receptor to isoproterenol rather than a necessary response to the reduction in SV after HDBR. SV might have been reduced after HDBR (42) due to the reduction in cardiac mass (11), a reduction in total blood volume identified in these subjects (A. R. Hargens and R. L. Hughson, unpublished data from WISE-2005), or reduced venous return due to peripheral blood pooling. Although the interaction term for group × HDBR in the analysis of SV was not significant ($P = 0.125$), the trend in the data suggested differences in response to HDBR between groups. Indeed, Fig. 1C reveals that stroke volume was not reduced after HDBR in the exercise group, but it was in the control group. That is, the HDBR deconditioning effect that results in a reduction in stroke volume was prevented by the exercise countermeasure. As a consequence of the changes in heart rate and stroke volume, there was no significant effect of HDBR on cardiac output in the exercise group, only the significant increase during isoproterenol infusion (Fig. 3C). The responses of the exercise group confirmed our second hypothesis that they would maintain their heart rate response close to that observed before HDBR. A comparison of the heart rate response in our control and exercise groups with the men studied by Convertino et al. (8) is presented in Fig. 6A. There was an elevation in heart rate post-HDBR for the women and men who performed no exercise countermeasures (open circle symbols) but no increase in the exercise group (triangle symbols). In considering the heart rate responses to isoproterenol, it is unlikely that the phase of the menstrual cycle influenced the results. Previously, it has been shown that the dose of isoproterenol necessary to evoke a 15 beat/min increase in heart rate was approximately double in the late luteal phase compared with the follicular phase (25). Thus, for the nutrition group with no members in the follicular phase of their cycle or the control group with two members in the follicular phase of their cycle, it would have been anticipated that the HR response to isoproterenol might be less rather than greater after HDBR. Unfortunately, plasma concentrations of the sex hormones are not available to confirm this proposal.

In the previous study of men during 14 days of HDBR, Convertino and colleagues (8) observed that baseline LVR was elevated and that there was a greater slope for the decrease in LVR with isoproterenol. The current results were not consistent with these previous data and did not support our hypothesis that women completing 56 days of HDBR would respond the same as men after 14 days of HDBR for this cardiovascular variable. Although there was the anticipated reduction in LVR with isoproterenol infusion, there was no overall increase following HDBR in leg vascular resistance as in contrast to the data of Convertino et al. (8) in Fig. 6B. Instead, there was a trend for the control group to decrease LVR after HDBR (from solid small circle to open small circle, Fig. 6B), while the exercise group did have a slight increase in LVR (from solid triangle to open triangle, Fig. 6B). The contrasting responses between the men in the study of Convertino et al. (8) and the women of the current study could simply be a function of the differing durations of HDBR. However, it is possible as well that there are differences in regulation of cardiac output and its distribution. For example, the reduced SV of the women in the current study was compensated by an increase in heart rate to maintain cardiac output, while in men studied after 60 days of HDBR, cardiac output was reduced at rest (42). The responses might also suggest regional differences in how men and women use vasodilation in an attempt to maintain MAP. One study (38) found differences, while another (16) found no difference in muscle sympathetic nerve activity responses of men and women to cardiovascular stress that could be associated with the lower LVR in the current study. Also, it might be anticipated that women would have greater vasodilation than men in response to β-adrenergic stimulation (29). We found differing patterns in the regional vascular resistance responses after HDBR. There were no overall differences in TPR after HDBR during the isoproterenol phase of the study (Fig. 2A),

![Image](http://ajpregu.physiology.org/)

Fig. 6. Data for heart rate and leg vascular resistance from the study of men by Convertino et al. (8) are shown for isoproterenol infusion before and after 14 days of HDBR (● before, □ after HDBR) compared with the control group (○ before, △ after HDBR). Regression lines (dashed for before, solid for after HDBR) are plotted to show trends in data. Units for leg vascular resistance for WISE-2005 are mmHg·ml $^{-1}$·min $^{-1}$·cm $^{-3}$ and for Convertino et al. (8) are peripheral resistance units (PRUs).
there was a nonsignificant decrease in LVR in the control group (Fig. 2C) as well as a trend toward an increase in CVRi after HDBR ($P=0.07$, Fig. 2E). As cerebral flow accounts for ~15% of the total cardiac output at rest, the cerebrovascular response would have an impact on TPR after HDBR. The mechanism responsible for the increased cerebrovascular resistance in the control and nutrition groups might be an increase in myogenic tone as identified in animal models maintained in a head-down position (17, 50), but the mechanism for the reduced resistance in the exercise group is not clear. The increase in CVRi with isoproterenol infusion (Fig. 4E) might be related to an autoregulatory compensation to maintain cerebral blood flow in the face of an elevation in cardiac output. Another important potential contributor to total peripheral resistance is the splanchnic region. It is not known from the current data how splanchnic vascular resistance might have contributed after HDBR. The importance of splanchnic vasoconstriction for maintenance of venous return (2, 14, 44) would be important for both men and women, but differences between men and women for pooling of blood in the splanchnic region (48) might have also affected total peripheral resistance response and point to the importance of measuring this variable in future studies.

Norepinephrine Responses

Evidence from animal studies suggests that $\alpha$-adrenergic responses might be affected by the head-down posture. Chronic tail suspension of rats is associated with reduced nerve terminal density in blood vessels of the lower limb (51), while chronic head-up posture increased terminal density (34). Turnover of norepinephrine is altered by tail suspension (13). The sensitivity of $\alpha_{1B}$-adrenergic receptors of the vena cava of space flown and tail-suspended rats was reduced (36), the sensitivity of $\alpha_1$-adrenergic receptors in the heart was reduced after 90 days of tail suspension (5), and isolated rings from the abdominal aorta of tail-suspended rats had reduced sensitivity and maximal responses to norepinephrine (35). Given these observations in animal models, it might be anticipated that coincident with reduced sympathetic activity in HDBR (18, 19, 39) the sensitivity of the $\alpha$-adrenergic receptors might be altered by 56 days of HDBR. Our data for baseline norepinephrine and epinephrine do not support a reduction in sympathetic activity, but plasma concentrations are susceptible to acute factors such as anticipation of experimental protocols. Measurements of platelet norepinephrine and epinephrine provide an index of chronic changes in sympathetic activity with HDBR (6), but these were not available in this study. The absence of changes in baseline plasma norepinephrine and epinephrine might have influenced the outcome of tests of adrenergic sensitivity in this study.

The current data are consistent with other studies of humans where the $\alpha$-adrenergic receptor responses associated with peripheral vasoconstriction have generally been found to be unchanged after HDBR (8, 28, 30) and spaceflight (31). MAP increased with norepinephrine with no change due to HDBR. HR was increased by HDBR, and it decreased during norepinephrine infusion probably as a consequence of a baroreflex response to the increase in MAP. The elevated arterial blood pressure in response to norepinephrine was a consequence of increased TPR, as the cardiac output was reduced. There was evidence of a group $\times$ HDBR interaction effect for LVR during the norepinephrine infusions ($P=0.04$), suggesting that independent of the norepinephrine effect to increase vascular resistance, LVR was increased in the exercise group but was slightly lower in the control group (Fig. 2D). There was no evidence of impaired vasoconstrictor response to norepinephrine. These results can be compared with those of Convertino et al. (8) to infusion of phenylephrine in Fig. 7. The men studied after 14 days of HDBR without countermeasures had an increase in LVR and no change in slope. The women of the control group in the current study had a reduction in overall LVR, while the exercise group had an increase. Differences in slopes between the men and women in Fig. 7 are arbitrary due to plotting two different adrenergic agonists on the same x-axis. Collectively, these data suggest that any changes in peripheral resistance responses after HDBR were not due to alterations in the adrenergic mechanisms but might be a consequence of deconditioning, perhaps related to changes in vascular structure as observed with HDBR (3) and in animal studies (10).

During the norepinephrine infusions, there was a small significant increase in plasma epinephrine concentration with slightly higher but variable differences after HDBR. The increase in epinephrine during norepinephrine infusion did not have an important role as heart rate was reduced and total peripheral resistance increased, both opposite to the anticipated responses of $\beta$-adrenergic receptor stimulation.

Perspectives

The results of the current study highlighted some mechanisms that might make men or women susceptible to orthostatic intolerance, and they showed the benefits of an exercise countermeasure. While the absence of a group of men studied...
under the same duration of HDBR limits the ability to extrapolate the data, the results do provide a basis for future comparisons between men and women. The elevated HR response in the control and nutrition groups after HDBR might have been a function of increased sensitivity of the β-adrenergic receptors as suggested previously (8); however, the small reduction in stroke volume in our groups, while not statistically significant, was an important contributor to the increase in heart rate. A reduction in stroke volume and the need to increase heart rate in compensation are factors associated with poorer orthostatic tolerance (7, 16, 40, 49). The lower LVR in women of the control and nutrition groups after HDBR is consistent with previous findings (7, 16, 40, 49). This implies that the intrinsic properties of the blood vessels in the lower body might reflect reduced sympathetic activation or alterations in the intrinsic properties of the blood vessels in the lower body. Although absolute leg vascular resistance changed after HDBR, there did not seem to be an impairment or enhancement in the response to sympathetic stimulation of β- or α-adrenergic receptors. The results of the current study showed the benefits of the exercise countermeasure, which consisted of aerobic activity, static LBNP, and resistive exercise for maintaining or even enhancing cardiovascular responses after HDBR. This was evidenced by the preservation of the cardiac stroke volume response that allowed the exercise group to maintain similar HR responses compared with their pre-HDBR investigations. Another consequence of the exercise countermeasure seemed to be the elevation in LVR post-HDBR. Furthermore, in the exercise group, there were no changes in response to stimulation of β- or α-adrenergic receptors. Compared with a previous study of adrenergic responses in men, the HR responses to β-adrenergic receptor stimulation were similar to the men in the women without an exercise countermeasure, but the peripheral vascular responses were quite different. The absolute reduction from pre- to post-HDBR in the LVR during norepinephrine infusion could be due to the longer duration of HDBR in the current study or to differences between men and women. If the latter explanation is supported by future research, the current data might contribute to explaining previous observations that women have lower orthostatic tolerance than men (7, 9, 15, 47) and that female astronauts have a greater incidence of presyncopal symptoms than their male colleagues after spaceflight (23, 31, 46).

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