

Effects of 14 days of head-down tilt bed rest on cutaneous vasoconstrictor responses in humans

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Wilson, Thad E., Manabu Shibasaki, Jian Cui, Benjamin D. Levine, and Craig G. Crandall. Effects of 14 days of head-down tilt bed rest on cutaneous vasoconstrictor responses in humans. *J Appl Physiol* 94: 2113–2118, 2003. First published January 10, 2003; 10.1152/jappphysiol.00067.2002.—This study tested the hypothesis that head-down tilt bed rest (HDBR) reduces adrenergic and nonadrenergic cutaneous vasoconstrictor responsiveness. Additionally, an exercise countermeasure group was included to identify whether exercise during bed rest might counteract any vasoconstrictor deficits that arose during HDBR. Twenty-two subjects underwent 14 days of strict 6° HDBR. Eight of these 22 subjects did not exercise during HDBR, while 14 of these subjects exercised on a supine cycle ergometer for 90 min a day at 75% of pre-bed rest heart rate maximum. To assess α -adrenergic vasoconstrictor responsiveness, intradermal microdialysis was used to locally administer norepinephrine (NE), while forearm skin blood flow (SkBF; laser-Doppler flowmetry) was monitored over microdialysis membranes. Nonlinear regression modeling was used to identify the effective drug concentration that caused 50% of the cutaneous vasoconstrictor response (EC_{50}) and minimum values from the SkBF-NE dose-response curves. In addition, the effects of HDBR on nonadrenergic cutaneous vasoconstriction were assessed via the venoarteriolar response of the forearm and leg. HDBR did not alter EC_{50} or the magnitude of cutaneous vasoconstriction to exogenous NE administration regardless of whether the subjects exercised during HDBR. Moreover, HDBR did not alter the forearm venoarteriolar response in either the control or exercise groups during HDBR. However, HDBR significantly reduced the magnitude of cutaneous vasoconstriction due to the venoarteriolar response in the leg, and this response was similarly reduced in the exercise group. These data suggest that HDBR does not alter cutaneous vasoconstrictor responses to exogenous NE administration, whereas cutaneous vasoconstriction of the leg due to the venoarteriolar response is reduced after HDBR. It remains unclear whether attenuated venoarteriolar responses in the lower limbs contribute to reduced orthostatic tolerance after bed rest and spaceflight.

venoarteriolar reflex; norepinephrine; intradermal microdialysis; skin blood flow; simulated microgravity

ORTHOSTATIC TOLERANCE IS REDUCED after bed rest and spaceflight (2, 12, 33). The mechanisms leading to this

response are probably multifactorial, including both central and peripheral mechanisms. Nevertheless, individuals who are prone to syncope after spaceflight do not increase total peripheral vascular resistance to the extent observed in more tolerant astronauts (3). This lack of an appropriate increase in peripheral resistance during orthostasis is not likely due to altered neural mechanisms, because recent observations show that head-down tilt bed rest (HDBR) and spaceflight do not alter reflex control of muscle sympathetic nerve activity (7, 20, 25).

In this regard, a number of microgravity-related studies have focused on postsynaptic responses in animals and humans. Published studies have yielded mixed results, with the bulk of the animal data suggesting attenuated vasoconstrictor responses after hindlimb suspension and spaceflight (10, 11, 22, 26, 28), whereas human studies show unaltered or impaired vascular responses after bed rest (1, 4, 6, 13, 23, 30). However, a potential confounding variable for the majority of the cited human-based research is that the perturbation applied (i.e., graded orthostatic challenges or systemically administered vasoactive drugs) makes it impossible to assess the effects of HDBR on vascular responses independent of other modifiers such as baroreflexes. Hence, the effect of HDBR on vasoconstrictor function in humans independent of other factors remains relatively unclear.

Another issue, which deserves mention with respect to differences in responses due to simulated or actual microgravity exposure between human and animal, is the route of entry of vasoactive agents used to assess vascular reactivity. To date, in human studies vasoactive substances have been infused into the lumen of an artery or vein. This method is in contrast to the majority of the animal data in which vasoactive drugs were primarily applied to the outer vessel wall (i.e., abluminally) in isolated preparations. Thus, if in humans the vasoactive substance used to assess vascular reactivity were administered via the interstitial space, perhaps the responses would be more consistent with the previously cited animal data (10, 11, 22, 26, 28).

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In addition to adrenergic-mediated vasoconstriction, vascular resistance can be increased by other means such as the venoarteriolar response (15–17). Although the exact mechanism causing vasoconstriction due to the venoarteriolar responses is unknown, vasoconstriction occurs via a nonadrenergic local mechanism (9) secondary to venous congestion (15–17). It is possible that this response is mediated through a local neural network or a myogenic response (9). During orthostasis, it has been reported that as much as 45% of the increase in systemic vascular resistance is due to vasoconstriction of cutaneous, subcutaneous, and muscle vascular beds as a result of this response (16, 18). Moreover, the venoarteriolar response likely contributes to the maintenance of orthostatic tolerance in individuals with spinal cord injuries (27). Given the importance of the venoarteriolar response in maintaining vascular resistance during orthostasis (16, 31), coupled with findings that insufficient increases in systemic vascular resistance contribute to orthostatic intolerance after spaceflight (3), reduced vasoconstriction associated with an impairment of the venoarteriolar response may contribute to the increased incidence of orthostatic intolerance after HDBR and spaceflight.

Thus the purpose of this project was to test the hypothesis that peripheral vasoconstrictor responses to both exogenous norepinephrine (NE) administration (i.e., α -adrenergic mechanism) and the venoarteriolar response (i.e., nonadrenergic mechanism) are impaired after 14 days of HDBR and that exercise during HDBR would preserve these impaired responses. Exercise was chosen as the countermeasure to HDBR because it has been demonstrated to attenuate a variety of bed rest deconditioning responses (5). To investigate these questions, cutaneous vascular responses were assessed during microdialysis administration of varying concentrations of NE in forearm skin, as well as during the engagement of the venoarteriolar response in both the arm and leg. It is recognized that under normothermic conditions the cutaneous vasculature probably does not appreciably contribute to changes in systemic vascular resistance during orthostasis. Nevertheless, the proposed questions can be assessed in this readily accessible vascular bed, and findings from this study may provide insight into the effects of HDBR on the control of other vascular regions more important in blood pressure regulation but less easily measured in humans.

METHODS

Subjects. Twenty-two subjects (19 men and 3 women) participated in this study in which they were exposed to 14 days of strict 6° HDBR. Fourteen of these subjects were assigned to an exercise group (2 women), while eight subjects (1 woman) served as control subjects and thus did not exercise during HDBR. This study was a component of an 18-day HDBR study addressing issues related to cardiac function and bed rest. Data reported in the present study were obtained from subjects after 14 days of HDBR. On the final day of HDBR (i.e., day 18), the subjects in the exercise group were

further subdivided to investigate questions unrelated to the present findings. Thus, for the reported data, there are an unequal number of subjects in the control and exercise groups. The subjects' average age was 34 ± 2 yr, and all were of normal height (180 ± 2 cm), weight (82 ± 4 kg), and health. A written, informed consent from each subject was obtained before participation in the institutionally approved study.

Bed rest and exercise. Strict bed rest was maintained in the 6° head-down tilt position for 14 days. Subjects remained in this position at all times, except they were allowed to elevate on one elbow for meals and were horizontal for exercise, transport, and bathing. Subjects were housed in a hospital-based General Clinical Research Center and were given a standard diet, with fluids allowed ad libitum. Exercise was performed at 75% of pre-bed rest heart rate maximum for 90 min/day on a supine cycle ergometer (Collins). Subjects chose to exercise for this duration either in two bouts of 45 min or three bouts of 30 min. Heart rate was monitored (Polar) during these exercise bouts to confirm exercise intensity.

Cutaneous vasoconstrictor responsiveness. Two intradermal microdialysis membranes were placed in dorsal forearm skin. This technique involved placing a small (200- μ m outer diameter, 10-mm length) sterile, semipermeable membrane intradermally by using a 25-gauge needle. Construction and insertion of the microdialysis probe are reported elsewhere (8, 19). The microdialysis membranes were perfused with Ringer solution at a rate of 2 μ l/min via an infusion pump (Harvard). The protocol commenced once skin blood flow (SkBF) returned to normal levels after needle insertion trauma (~60–120 min). Local temperature was controlled at 34°C via a local heating element (Perimed) that housed a laser-Doppler flow probe centered directly above the microdialysis membranes. Once SkBF stabilized, eight doses of NE were delivered (at 10-fold increments) at a flow rate of 2 μ l/min for 5 min per dose. Data are presented from doses of 1×10^{-9} to 1×10^{-2} M, which represent threshold and saturation of the dose-response curve, respectively. SkBF was normalized relative to predrug levels and is reported as a percentage of this baseline flow. Microdialysis membranes were placed at similar regions of the forearm between pre- and post-HDBR trials. However, it is recognized that slight variations in probe placement likely occurred between these periods. For each period (i.e., pre- and post-HDBR), responses between the two sites for each dose were averaged.

Venoarteriolar reflex. With the subject in the supine position and the arms and legs at heart level, the forearm venoarteriolar response was assessed by inflating a cuff placed around the upper arm to 45 mmHg (Hokanson). The same procedure was performed to assess the venoarteriolar response of the lower leg except the cuff was placed around the thigh. Previously, it has been reported that venous stasis needs to be above 25 mmHg to elicit the venoarteriolar response (15). The cuff remained inflated at this pressure for a minimum of 2 min. SkBF, assessed via laser-Doppler flowmetry (Perimed), was measured at two sites on the dorsal forearm. For the lower leg assessment, laser-Doppler measures were obtained from nonglabrous skin over the tibialis anterior region, 39 ± 1 cm from the bottom of the foot. Anatomic measurements were obtained to place the laser-Doppler flow probes in the same region between pre- and post-HDBR. However, as indicated above, given the small sampling area of the laser-Doppler flow probes, it is doubtful that the probes were placed at exactly the same location for each trial.

Data analysis. Data were continuously acquired throughout both protocols at a sampling rate of 20 Hz by using a data

collection system (Biopac). For the vasoconstrictor responsiveness protocol, SkBF measurements were obtained and analyzed during the final minute of each dose of NE. These values were mathematically modeled via nonlinear regression curve fitting by using a four-parameter logistic equation (Prism, GraphPad). The model identified the effective drug concentration that caused 50% of the cutaneous vasoconstrictor response (EC_{50}) as well as minimum SkBF responses. For the venoarteriolar response, the 30-s period before the engagement of the response was compared with the 30-s period surrounding the nadir of the response (typically the last 30 s). The percent reduction in SkBF during the venoarteriolar response was calculated from these two values. Data were statistically analyzed via a two-way repeated-measures ANOVA with main factors of testing period (pre- and post-HDBR) and group (exercise and nonexercise). All values are reported as means \pm SE. The α -level for all statistical analyses was set at 0.05.

RESULTS

Cutaneous vasoconstrictor responses to exogenous NE were obtained on 20 of the 22 subjects, because a subject from the exercise group was excluded because of prolonged hyperemia after needle insertion and a subject from the control group experienced an allergic reaction to the adhesive used to attach the laser housing unit to the skin. The mean goodness of fit (R^2) of the dose-response model was high both pre-HDBR (exercise: 0.94 ± 0.02 and nonexercise: 0.95 ± 0.03) and post-HDBR (exercise: 0.93 ± 0.02 and nonexercise: 0.95 ± 0.03). Neither HDBR alone nor exercise during HDBR had an effect on the EC_{50} of NE (HDBR: $P > 0.30$; exercise during HDBR: $P > 0.15$); see Fig. 1 and Table 1. Similarly, minimal responses from the dose-response model were not altered by HDBR ($P > 0.40$) or exercise during HDBR ($P > 0.15$). Finally, no interactions were observed between main factors of the ANOVA for any of the dose-response variables.

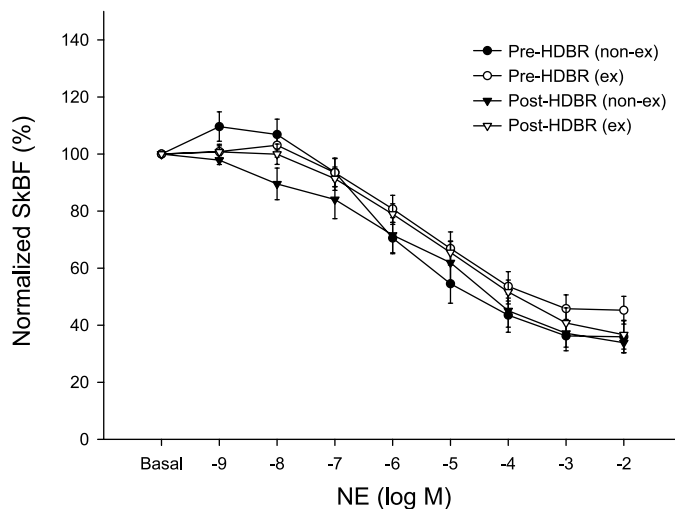


Fig. 1. Dose-response relationships showing the effect of varying doses of norepinephrine (NE) on normalized skin blood flow (SkBF) both pre- and post-head-down tilt bed rest (HDBR) both with (ex) and without (non-ex) an exercise countermeasure. Absolute SkBF flux values before drug delivery were not different across conditions (i.e., pre- and post-HDBR) or groups. SkBF values are normalized relative to predrug baseline and are shown as means \pm SE.

Table 1. Effect of 14 days of 6° HDBR, with and without an exercise countermeasure, on modeled responses during exogenous NE administration

Countermeasure	EC_{50} , log M dose of NE	Minimum SkBF, % of baseline
<i>Pre-HDBR</i>		
Exercise	-5.5 ± 0.2	47 ± 3
Nonexercise	-6.0 ± 0.2	37 ± 5
<i>Post-HDBR</i>		
Exercise	-5.4 ± 0.2	42 ± 5
Non-exercise	-5.6 ± 0.5	38 ± 3

Values are means \pm SE. HDBR, head-down tilt bed rest; EC_{50} , effective drug concentration that caused 50% of the cutaneous vasoconstrictor response; SkBF, skin blood flow (normalized to a percentage of predrug baseline); NE, norepinephrine. No significant differences in the EC_{50} or minimum SkBF were observed between conditions (pre- and post-HDBR) or countermeasures (exercise or no-exercise during HDBR). Skin temperature was held constant (34° C) throughout all NE doses. Both the EC_{50} and minimum values were derived from the mathematical model. Absolute SkBF flux values before drug delivery were not different across conditions (pre- and post-HDBR) or groups.

The magnitude of forearm vasoconstriction associated with the venoarteriolar response was not affected by HDBR regardless of the group (pre-HDBR nonexercise: $53.1 \pm 4.2\%$, post-HDBR nonexercise: $52.2 \pm 4.5\%$; pre-HDBR exercise: $56.6 \pm 2.1\%$, post-HDBR exercise: $52.7 \pm 3.0\%$); see Fig. 2A. Probability values from the ANOVA were $P > 0.30$ for HDBR, $P > 0.70$ for exercise during HDBR, and $P > 0.50$ for the interaction between the pre-/post-HDBR and the exercise/nonexercise main factors.

In contrast, cutaneous vasoconstriction of the lower leg due to the venoarteriolar response was significantly attenuated after HDBR in both groups (pre-HDBR nonexercise: $51.0 \pm 5.0\%$, post-HDBR nonexercise: $43.0 \pm 6.6\%$; pre-HDBR exercise: $48.4 \pm 7.4\%$, post-HDBR exercise: $36.4 \pm 8.5\%$); see Figs. 2B and 3. Probability values from the ANOVA were $P < 0.03$ for HDBR, $P > 0.50$ for exercise during HDBR, and $P > 0.60$ for the interaction between pre-/post-HDBR and the exercise/nonexercise main factors.

DISCUSSION

The major new findings of the present investigation are the following: 1) 14 days of 6° HDBR, both with and without exercise during HDBR, does not alter forearm cutaneous vasoconstrictor responsiveness to exogenous NE; 2) HDBR does not alter cutaneous vasoconstriction associated with the venoarteriolar response in the forearm; and 3) HDBR attenuates the magnitude of cutaneous vasoconstriction due to the venoarteriolar response in the lower leg, and this effect is not prevented by exercise during HDBR. Taken together, these data suggest that forearm cutaneous vasoconstrictor responses are preserved after HDBR, whereas cutaneous vasoconstrictor responses to the venoarteriolar response in the lower limb are impaired by HDBR exposure.

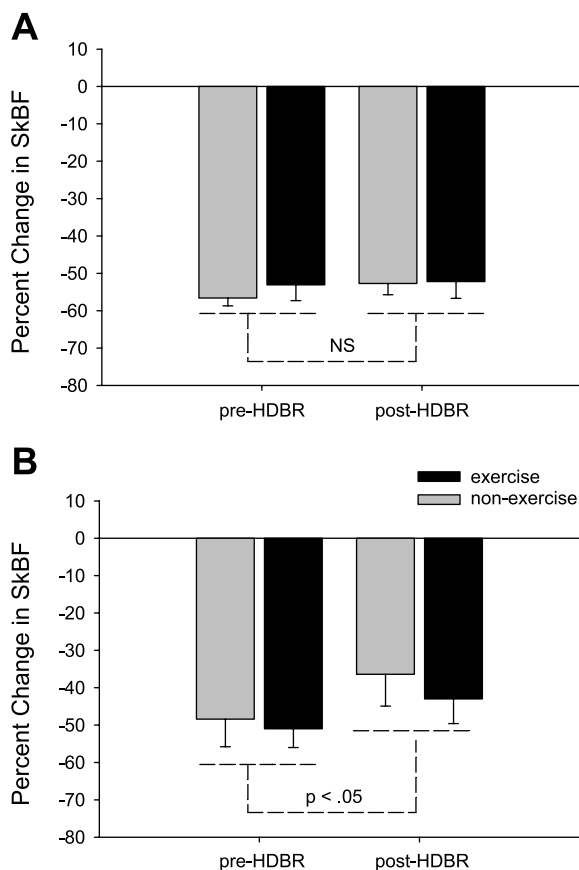


Fig. 2. Cutaneous venoarterial responses between pre- and post-HDBR with and without an exercise countermeasure. *A*: forearm responses. *B*: lower leg responses. In both groups, cutaneous vasoconstrictor responses in the leg due to the venoarterial response were significantly reduced after HDBR. Data are expressed as a percent change from averaged values before cuff inflation. Values are means \pm SE. NS, not significant.

No differences were observed in the EC_{50} or minimum SkBFs for the NE protocol between pre- and post-HDBR, regardless of the group (i.e., exercise or control group). These data suggest HDBR does not affect cutaneous postsynaptic α -adrenergic vasoconstrictor responsiveness even when NE is infused via the interstitial space (i.e., abluminally). A lack of an effect of bed rest on vasoconstrictor responsiveness confirms prior work in humans in which the elevation of blood pressure due to systemic infusions of NE (4) and phenylephrine (6) were similar between pre- and post-HDBR conditions. In contrast, Shoemaker et al. (30) reported that cold pressor-induced forearm vasoconstriction of a previously vasodilated limb was attenuated after HDBR. However, in that study, postsynaptic responsiveness was not specifically investigated independent of other modifiers (e.g., baroreflexes, cold pressor-induced sympathoexcitation, etc). Finally, Maass et al. (21) reported that platelet α_2 -adrenergic-receptor density increased during HDBR. It remains unclear whether changes in α -adrenergic receptor density on platelets would mirror responses in the vasculature. However, a lack of differences in vasoconstrictor responses due to exogenous NE administration in

the present study suggests that if α_2 -adrenergic-receptor density increased in the vasculature during HDBR, this event was not translated to increased vasoconstrictor responsiveness.

To our knowledge only one study specifically investigated the effect of HDBR on postsynaptic responses without the potential confounding influence of the aforementioned modifiers (23). However, in that study, comprehensive dose-response curves were not modeled because of the limited number of doses administered intra-arterially. Moreover, it may be that responses to drugs administered via the vascular space are different relative to delivery of drugs via the interstitial space, as is the case with microdialysis. Despite these potential differences between studies, results from the present study confirm the findings of Pawelczyk and Levine (23) in that HDBR did not alter postsynaptic responsiveness to adrenergic agents.

We recognize that in the present study the effects of HDBR on α -adrenergic vasoconstrictor responsiveness were confined to the skin, which may or may not be representative of the effects of HDBR on vascular beds more important to the regulation of blood pressure during orthostasis in normothermic individuals. Thus direct comparisons between the present study and the findings of others (1, 4, 6, 23, 30) may not be appropriate. Nevertheless, the advantage of the present study is the capability of assessing postsynaptic responses without the possible confounding effects of systemic administration of vasoactive agents. Furthermore,

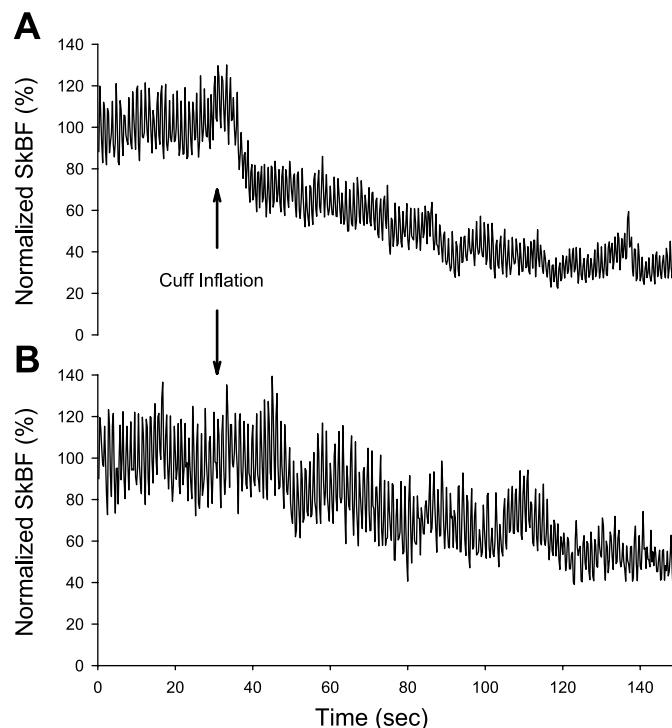


Fig. 3. Example from a representative subject comparing normalized SkBF during venous stasis via cuff inflation (45 mmHg) of the lower leg. *A*: pre-HDBR responses. *B*: post-HDBR responses. Data were originally sampled at 20 Hz; however, for display purposes these data were resampled at 4 Hz.

with the present methodology, comprehensive dose-response curves across a wide range of NE doses can easily be obtained and analyzed (see Fig. 1).

Findings from the present study do not support findings from previous animal studies (primarily in the rat) in which vasoconstrictor responsiveness is diminished after simulated and actual microgravity exposure (10, 11, 22, 26, 28), despite similarities in the method of delivering the vasoactive drug (i.e., abluminally). Differences between the present findings and those from the animal literature could be related to the vascular bed investigated, method of assessing vascular responsiveness, species, and/or magnitude of change in hydrostatic gradients due to the mode of simulating weightlessness (e.g., HDBR vs. hindlimb suspension).

Vasoconstriction associated with the venoarteriolar response of the leg was attenuated after HDBR. In a previous study, Gabrielsen et al. (14) evaluated the venoarteriolar response in the skin of the dorsal foot after 20 days of HDBR (14). They reported that this response was not affected by HDBR. In that study, the venoarteriolar response was engaged by lowering the foot below heart level. In contrast, in the present study, the venoarteriolar response was engaged via inflating a cuff placed proximal to the site of measurement to 45 mmHg. It is not clear whether these differing techniques in assessing the venoarteriolar response, differences in the site of measurement (i.e., dorsal foot vs. anterior tibialis), or a combination of both resulted in the different findings between the present and previously cited work (14).

The observed differences in the venoarteriolar response between the arm and leg are interesting but not entirely unexpected. In a normal gravity (1 G_z) environment, during orthostasis the legs experience greater increases in vascular pressures relative to the arms. However, during HDBR, this increase in leg vascular pressure is eliminated. This concept led others to hypothesize that the legs have the greatest potential for vascular adjustment to microgravity or HDBR (33). Moreover, the leg arterial vascular bed is more sensitive to α -adrenergic vasoconstriction when compared with the arm (24). In contrast, changes in vascular pressure in the arms during HDBR, relative to those occurring in a 1- G_z condition, would be smaller than the leg. Although speculative, it may be that these differences in vasculature pressures in the arms relative to the legs during orthostasis, and subsequent removal of this stimulus during HDBR, contribute to the observed responses after HDBR.

The venoarteriolar response may be integral in the vasoconstrictor response during orthostasis (15, 17, 31). Thus reductions in the contribution of the venoarteriolar response in maintaining vascular resistance during orthostatic stress after HDBR may increase the incidence of orthostatic intolerance. Unfortunately, in the present investigation orthostatic tolerance was not assessed until after a second countermeasure was added on *day 18* of HDBR, thereby precluding us from identifying a relationship between the impairment of the venoarteriolar response and reduced orthostatic

tolerance in these subjects. Nevertheless, an attenuated venoarteriolar response, without appropriate compensation from the baroreflexes, may contribute to the inability of fainters to appropriately increase total peripheral resistance after HDBR or spaceflight (3).

It is interesting that the exercise countermeasure used in this study did not prevent the attenuation of the leg venoarteriolar response. The exercise countermeasure, however, was strenuous enough to prevent HDBR-induced decreases in maximal oxygen uptake and cardiac mass (unpublished data). Hence, reduced venoarteriolar responsiveness of the leg occurred independent of changes in blood flow and arterial pressure associated with supine exercise during HDBR. One possible reason for the lack of an effect of exercise in altering the venoarteriolar response is an absence of an orthostatic challenge associated with exercise. Exercise in combination with an orthostatic component, such as lower body negative pressure, throughout HDBR maintains exercise capacity (32) and preserves cardiovascular responses during subtolerant lower body negative pressure (29). Thus had an orthostatic challenge been included with exercise the venoarteriolar response may have been preserved in this group after HDBR.

Limitations. A primary limitation of this study is the use of the cutaneous vasculature as a representative model of the effects of HDBR on the vasculature in general. Assessment of the skin as a model to identify the effects of a perturbation on the vasculature has a number of advantages, many of which are highlighted in the present study. However, it is presently unknown whether the effects of HDBR, with and without exercise, on the cutaneous circulation are representative of the effects of these perturbations on circulatory beds recognized to be more important in the regulation of blood pressure in normothermic individuals. Nevertheless, assessment of the cutaneous circulation may provide insight into vascular mechanisms that contribute to impaired orthostatic tolerance after HDBR and spaceflight in humans, which may not be as readily assessed in other vascular beds.

The mechanism by which the venoarteriolar response was reduced in the leg after HDBR is unclear. Because of methodological constraints, the effects of HDBR on leg vasoconstrictor responsiveness to exogenous NE were not assessed in the present study. However, given our recent finding that the venoarteriolar response is not mediated by α -adrenergic mechanisms (9), it is doubtful that an assessment of NE-mediated vasoconstriction in the leg would provide additional insight into the mechanisms of reduced cutaneous vasoconstriction associated the venoarteriolar response after HDBR. Thus, although in hindsight it may have been insightful to assess cutaneous vasoconstrictor responses to NE in both the leg and the arm, we do not believe the absence of these data significantly impact the interpretation of the venoarteriolar response data.

Conclusions. Fourteen days of 6° HDBR, with and without exercise during HDBR, did not alter forearm cutaneous vasoconstrictor responsiveness to exogenous

NE administration, indicating that α -adrenergic-mediated vasoconstriction is not altered in forearm cutaneous tissue by HDBR. In contrast, in both groups (i.e., exercise and nonexercise) cutaneous vasoconstriction associated with the venoarteriolar response was attenuated in the lower leg, but not in the forearm, after HDBR. This study provides the first evidence of an attenuated venoarteriolar response after simulated microgravity exposure. A reduced venoarteriolar response in the leg could contribute to reduced orthostatic tolerance after simulated and actual spaceflight.

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