

## Regulation of muscle sympathetic nerve activity after bed rest deconditioning

JAMES A. PAWELCZYK,<sup>1,2</sup> JULIE H. ZUCKERMAN,<sup>2</sup>  
C. GUNNAR BLOMQUIST,<sup>2</sup> AND BENJAMIN D. LEVINE<sup>2</sup>

<sup>1</sup>Noll Physiological Research Center, The Pennsylvania State University,  
University Park, Pennsylvania 16802; and <sup>2</sup>Institute for Exercise and  
Environmental Medicine, University of Texas Southwestern Medical  
Center and Presbyterian Hospital of Dallas, Dallas, Texas 75231

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**Pawelczyk, James A., Julie H. Zuckerman, C. Gunnar Blomqvist, and Benjamin D. Levine.** Regulation of muscle sympathetic nerve activity after bed rest deconditioning. *Am J Physiol Heart Circ Physiol* 280: H2230–H2239, 2001.—Cardiovascular deconditioning reduces orthostatic tolerance. To determine whether changes in autonomic function might produce this effect, we developed stimulus-response curves relating limb vascular resistance, muscle sympathetic nerve activity (MSNA), and pulmonary capillary wedge pressure (PCWP) with seven subjects before and after 18 days of  $-6^\circ$  head-down bed rest. Both lower body negative pressure (LBNP;  $-15$  and  $-30$  mmHg) and rapid saline infusion ( $15$  and  $30$  ml/kg body wt) were used to produce a wide variation in PCWP. Orthostatic tolerance was assessed with graded LBNP to presyncope. Bed rest reduced LBNP tolerance from  $23.9 \pm 2.1$  to  $21.2 \pm 1.5$  min, respectively (means  $\pm$  SE,  $P = 0.02$ ). The MSNA-PCWP relationship was unchanged after bed rest, though at any stage of the LBNP protocol PCWP was lower, and MSNA was greater. Thus bed rest deconditioning produced hypovolemia, causing a shift in operating point on the stimulus-response curve. The relationship between limb vascular resistance and MSNA was not significantly altered after bed rest. We conclude that bed rest deconditioning does not alter reflex control of MSNA, but may produce orthostatic intolerance through a combination of hypovolemia and cardiac atrophy.

vasoconstriction; adrenergic nervous system; sympathetic reflex

ORTHOSTATIC INTOLERANCE OCCURS after prolonged hypodynamic periods with reduced gravitational gradients, such as bed rest deconditioning and spaceflight (5, 27). These stresses lead to changes throughout the cardiovascular system, including hypovolemia (16), reductions in carotid-cardiac baroreflex responsiveness (7, 8, 11, 17, 19), and increased venous pooling (9). An equally attractive hypothesis is that adrenergically mediated vascular control changes in a way that diminishes vasoconstriction. Several reports suggest that sympathetic nervous system responsiveness may be attenuated after spaceflight in astronauts prone to

orthostatic intolerance, evidenced by smaller increases in plasma levels of norepinephrine (18) and systemic vascular resistance (5) on standing after spaceflight compared with those without orthostatic intolerance. These changes are thought to result from diminished release of norepinephrine from sympathetic nerve terminals rather than changes in norepinephrine clearance (21, 33, 39). However, the mechanisms underlying these adaptations are not fully defined.

Recently, we (27) demonstrated that 18 days of bed rest deconditioning with  $-6^\circ$  head-down tilt bed rest (HDBR) resulted in cardiac atrophy associated with reduced orthostatic tolerance, as indicated by 1) an increase in the slope of the Starling curves that relate stroke volume (SV) to left ventricular end-diastolic pressure and 2) a reduction in the zero pressure intercept of the left ventricular diastolic pressure-volume relationship. These findings reveal that nonneural mechanisms can contribute to orthostatic intolerance in otherwise healthy individuals. However, others (37) have shown that resting muscle sympathetic nerve activity (MSNA) increases in individuals who become susceptible to orthostatic intolerance after HDBR, whereas the change in MSNA associated with head-up tilt becomes blunted. Thus changes in neural control of vascular resistance, in addition to nonneural mechanisms, may contribute to orthostatic intolerance after HDBR.

Two possibilities could explain the diminished MSNA response to head-up tilt. First, the increase in resting MSNA observed in orthostatically intolerant individuals, in combination with cardiac atrophy, could predispose subjects to the hyperadrenergic variant of vasodepressor syncope. Alternatively, HDBR could attenuate MSNA and vasoconstriction during orthostatism by decreasing baroreflex responsiveness. Accordingly, we examined the changes in baroreflex control of sympathetic activity and regional vascular tone by studying the responses of MSNA, limb vascular resistance, and hemodynamic parameters during an 18-day

Address for reprint requests and other correspondence: J. A. Pawelczyk, Noll Physiological Research Center, The Pennsylvania State University, 119 Noll Laboratory, University Park, PA 16802 (E-mail: jap18@psu.edu).

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period of HDBR to mimic comparable periods of space flight.

We hypothesized that HDBR would reduce the slope of baroreflex-mediated stimulus-response relationships, which were characterized as changes in MSNA and vascular resistance in response to decreases and increases in left ventricular end-diastolic pressure produced by graded lower body negative pressure (LBNP) and rapid infusion of saline, respectively. This paradigm produces physiological changes in filling pressure, heart rate (HR), and SV that elicit reflex increases and decreases in MSNA from the combined effect of cardiopulmonary and carotid baroreceptors (26, 45). Our results reveal that sympathetic nervous system responsiveness (slope of the MSNA stimulus-response curve) does not change after HDBR. Rather, the operating point on the stimulus-response relationship is shifted to lower cardiac filling pressures.

## METHODS AND PROCEDURES

### *Subjects*

Twelve healthy subjects (11 men, 1 woman) were studied before and after HDBR deconditioning. The physical characteristics included a mean age of  $24 \pm 2$  (range 18–35), height of  $185 \pm 23$  cm, and weight of  $79 \pm 3$  kg. No subject smoked, used recreational drugs, or had significant chronic medical problems. The subjects were excluded if they were endurance athletes (25) or exercised for more than 30 min/day, 3 times/wk with either dynamic or static exercise. The subjects were screened for medical history and with a physical examination, resting electrocardiogram (ECG), and resting echocardiogram, and were excluded if high-quality two-dimensional echocardiographic images could not be obtained. Body composition was obtained by densitometry with the use of underwater weighing (1). All of the subjects provided voluntary informed consent; protocols were approved by the Institutional Review Boards of both the University of Texas Southwestern Medical Center and the Presbyterian Hospital of Dallas.

### *Measurements*

**Hemodynamics.** A 6-Fr, balloon-tipped, flow-directed pulmonary arterial catheter (Swan-Ganz, Baxter) was placed under fluoroscopic guidance through an antecubital vein into the pulmonary artery. With the balloon inflated, the catheter was advanced into the pulmonary capillary wedge (PCW) position, which was confirmed both fluoroscopically and by the presence of characteristic pressure waveforms. Intracardiac pressures were referenced to atmospheric pressure, with the pressure transducer zero set at 5 cm below the sternal angle in the supine position. Pressure waveforms were amplified (model 78534A, Hewlett-Packard; and model ASC909, Astromed), transduced (Transpac IV, Abbott), and displayed on a strip-chart recorder (model MT95000, Astromed) with  $\pm 0.5$  mmHg resolution. The mean PCW pressure (PCWP) was determined visually at end expiration and was used as an index of left ventricular end-diastolic pressure.

HR was monitored from lead II of the ECG. Arterial pressure was monitored by optoplethysmography of the middle digit (Finapres, Ohmeda). The arterial pressure waveform was integrated in real time at a rate of 200 Hz and stored with the instantaneous HR for every beat throughout the study. Mean HR and blood pressure (BP) were calculated

from the time-weighted average of these data. Blood pressure was also measured in the arm intermittently by electro-sphygmomanometry (model 4240, Suntech) with a microphone placed over the brachial artery and the Korotkoff sounds gated to the ECG.

Limb blood flow was determined by venous occlusion plethysmography. During LBNP trials, measurements were made by using the forearm to avoid interference with the legs. During volume infusion, measurements were made by using the calf because saline was being infused through the pulmonary artery catheter and a catheter placed in the antecubital vein of the contralateral arm. The use of different limbs was justified on the basis of previous reports (32, 34) demonstrating little difference in neural control of arm and leg blood flow. Changes in limb volume were transduced with the use of dual-strand mercury-in-Silastic strain gauges (22), amplified (model EC-4; Hokansen), and displayed on a strip-chart recorder for post hoc analysis. Venous occlusion pressures of 50 mmHg were employed, and foot or hand blood flow was excluded from the measurement with an arresting cuff placed around the ankle or wrist inflated to 250 mmHg. Limb vascular resistance was calculated as the quotient of mean arterial pressure and limb blood flow. Arm and leg data were combined by normalizing responses to the resting blood flow of the respective limb.

Cardiac output ( $\dot{Q}_c$ ) was measured by foreign gas rebreathing, using acetylene as the soluble and helium as the insoluble gas (44). Pulmonary blood flow was calculated from the disappearance rate of acetylene in expired air, measured with a mass spectrometer (model MGA1100; Marquette), after adequate mixing was confirmed by a stable helium concentration. This method has been validated in our laboratory against standard invasive techniques, including thermodilution and direct Fick, over a range of cardiac outputs from 2.75 to 27.00 l/min, with an  $r^2$  of 0.91 and standard error of estimate of 1.1 l/min (30). SV was calculated as the quotient of  $\dot{Q}_c$  and the HR measured during rebreathing. Total peripheral resistance (TPR) was calculated as the quotient of the auscultatory blood pressure and  $\dot{Q}_c$ .

**Muscle sympathetic nerve activity.** Multiunit postganglionic recordings of MSNA were made using 200- $\mu$ m-diameter stainless steel microelectrodes of 2–3 M $\Omega$  impedance (Frederick Haer) inserted percutaneously into muscle nerve fascicles of the peroneal nerve. A recording electrode was placed in the peroneal nerve at the fibular head or the popliteal fossa, and a reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The nerve signal was amplified (total amplification 40,000–80,000), band-pass filtered (high pass of 0.3–0.7 kHz and low pass of 2–3 kHz), and then full-wave rectified and smoothed with a resistance-capacitance circuit (time constant, 0.1 s) to produce a recording of “integrated” MSNA.

Adjustments of the recording electrode were made until sites were found in which clear spontaneously occurring sympathetic bursts were recorded. The electrode was then micromanipulated until the signal-to-noise ratio of the band-pass filtered MSNA (peak-to-peak burst amplitude compared with that of baseline noise) exceeded two. This criterion helped ensure that recording electrodes were intrafascicular (not simply near fascicles) to facilitate detection of small bursts of MSNA. Criteria for adequate MSNA recording and discrimination from skin activity included: 1) pulse synchrony, 2) facilitation during the hypotensive phase of the Valsalva maneuver, and suppression during the hypertensive overshoot after release, 3) increases in response to breath holding, and 4) insensitivity to emotional stimuli (loud noises or stressful mental arithmetic). Furthermore,

muscle afferent activity could be identified by stretching the muscle innervated by the fascicle being recorded, but not by stroking the skin.

The ECG, pressure, neural, and plethysmographic recordings were stored on a chart recorder (model MT95000; Astromed) and videotape (model 4000; Vetter) for later analysis. Neural activity was monitored on a storage oscilloscope (model COR5521U; Kikusui) and a loudspeaker during the experiment. Respiratory excursions were identified from large changes in pulmonary artery pressure to detect inadvertent respiratory maneuvers that might have affected MSNA. Records were inspected visually and rejected if skin sympathetic, electromyographic, or unusual ventilatory activity was identified. The amplitude of each mass sympathetic discharge was quantified by digitization of the resistance-capacitance-filtered neurogram (SigmaScan, version 2.1, Jandel Scientific). Sympathetic activity was expressed as the sum of discharges per minute (total activity) and the frequency of discharges (burst frequency).

The amplitude of bursts of sympathetic activity depends critically on electrode position. Whereas determinations of burst frequency are stable between recording sessions (40), measurements of total activity are not because the position of a recording electrode relative to nerve fascicles will vary with repeated procedures. Thus total activity was normalized to the resting supine value to allow before and after bed rest comparisons that might otherwise be confounded by subtle differences in intrafascicular electrode location.

### Procedures

**Protocol.** All of the experiments were performed in the morning, at least 2 h after a light breakfast, and more than 12 h after the last caffeinated or alcoholic beverage, in a quiet, environmentally controlled laboratory, with an ambient temperature of  $25 \pm 1^\circ\text{C}$ . After at least 30 min of quiet rest in the supine position, plasma volume was measured by using Evans blue dye (15). To decrease and increase ventricular filling, we used a sequence of LBNP and rapid saline infusion as previously reported (26). The supine position was maintained throughout the experiment. LBNP was performed by placing the subject in a Plexiglas box, sealed at the level of the iliac crests. Suction was provided by a vacuum pump controlled with a variable autotransformer. Limb blood flow, HR, BP, and MSNA were recorded at baseline and then after 3 min each of LBNP at  $-15$  and  $-30$  mmHg.

Measurements of  $\dot{Q}_c$  and PCWP immediately followed the beat-to-beat recordings. The LBNP was then released. After baseline measurements were repeated to confirm return to hemodynamic steady-state (usually 20–30 min), warm, isotonic saline ( $37^\circ\text{C}$ ) was infused rapidly at a rate of 100 ml/min. Measurements were repeated after 15 ml/kg and 30 ml/kg had been infused.

Seventy-two hours after this session, maximal orthostatic tolerance was measured using a standardized, graded LBNP test. No invasive measurements were made during this protocol. A cumulative stress index (CSI) was calculated for this specific protocol by summing the product of negative pressure and duration at each level of LBNP. Both CSI and the time achieved with the LBNP protocol were used as continuous measures of orthostatic tolerance. LBNP was begun at  $-15$  mmHg for 5 min ( $\text{CSI} = 75 \text{ mmHg}\cdot\text{min}$ ) and then increased to  $-30$  and  $-40$  mmHg for 5 min each ( $\text{CSI} = 225$  and  $445 \text{ mmHg}\cdot\text{min}$ , respectively), followed by an increase in LBNP by  $-10$  mmHg every 3 min until signs or symptoms of presyncope were achieved. Presyncope was defined as one of the following: 1) a decrease in systolic blood pressure (SBP)

below 80 mmHg; 2) a decrease in SBP below 90 mmHg associated with symptoms of light headedness, nausea, or diaphoresis; or 3) progressive symptoms of lipothymia accompanied by subject request to discontinue the test. For the 24 tests, a true hemodynamic endpoint was reached in the majority of circumstances (95%).

**Microgravity simulation with HDBR.** After the initial series of experiments, head-to-foot gravitational gradients were reduced by placing the subjects at complete bed rest, with  $-6^\circ$  head-down tilt. The subjects were allowed to elevate on one elbow for meals, but otherwise were restricted to the head-down position at all times. Subjects were housed in the General Clinical Research Center at University of Texas Southwestern Medical Center and given a standard diet, which consisted of  $2,827 \pm 609$  cal/day, including  $5.2 \pm 1.2$  g/day of sodium. Fluids were allowed ad libitum, but all fluid intake and urine output were carefully recorded. The same series of experiments were repeated after 2 wk of HDBR. The head-down tilt was maintained during the 72 h between the measurement of MSNA and the orthostatic tolerance test, for a total of 18 days.

### Statistical Analyses

Resting variables were compared by using paired Student's *t*-tests. Nonlinear regression was used to model the relationship between limb vascular resistance and MSNA as a first-order exponential (SigmaPlot, version 2.1, Jandel Scientific). Data collected during the infusion/LBNP protocol were compared by using two-factor (filling pressure  $\times$  deconditioning) repeated-measures analysis of variance. Significant differences were probed post hoc by using *t*-tests with the Bonferroni correction for multiple comparisons. In all cases, the probability of rejecting the null hypothesis (no difference with changes in filling pressure or after deconditioning) was set at 5%.

## RESULTS

Figure 1 illustrates the dramatic changes in MSNA that could be obtained from the use of volume infusion and LBNP to alter cardiac filling. In some cases, spontaneous MSNA was virtually abolished during volume infusion, but could still be elicited with breath holding or a Valsalva maneuver. Unfortunately, complete recordings for all segments of the protocol (i.e., baseline, LBNP, and saline infusion) could not be obtained for two subjects before HDBR and three other subjects after HDBR because of shifts in electrode position or failure to meet signal-to-noise criteria. As a result, only seven complete sets of paired data were available for analysis. Thus results are reported only for this subpopulation of subjects; the hemodynamic data for the complete set of subjects are summarized in our related work (27). A practical result of the missing MSNA data was that the critical difference in MSNA that could be identified (assuming a statistical power of 0.8) increased from 7 to 10 bursts/min. This compares favorably with other published reports (36).

### Hemodynamics and Orthostatic Tolerance

Resting hemodynamic data are reported in Table 1. Eighteen days of bed rest resulted in a significant

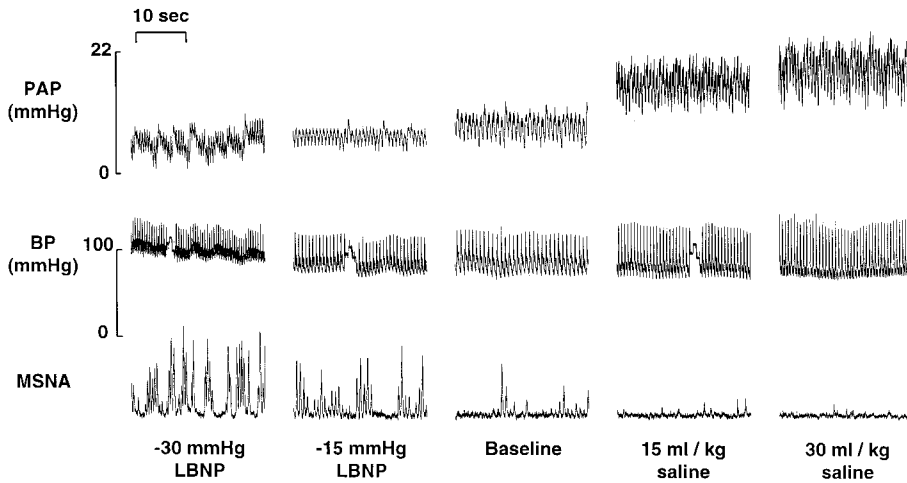


Fig. 1. Changes in muscle sympathetic nerve activity (MSNA) and blood pressure (BP) in response to lower body negative pressure (LBNP) and volume infusion for a representative subject. Volume loading at 30 ml/kg virtually abolished MSNA. PAP, pulmonary arterial pressure.

decrease in LBNP tolerance, expressed as CSI ( $P < 0.001$ ). Although individual LBNP tolerance was variable, ranging from 492 to 1,775 mmHg·min before HDBR and 467 to 1,318 mmHg·min after HDBR, the two levels of LBNP used for hemodynamic comparisons (corresponding to CSI of 75 and 225 mmHg·min, respectively) were well tolerated with no evidence of hemodynamic instability or presyncope. The decrease in LBNP tolerance was associated with a 10% decrease in plasma volume ( $P = 0.06$ ), and a decrease in resting PCW pressure ( $P < 0.05$ ). SV was lower at baseline in the supine position ( $P < 0.001$ ).

Changes in response to perturbations in cardiac filling are shown graphically in Fig. 2. SV decreased significantly at both levels of LBNP ( $P < 0.05$  for each comparison) and fell to a greater extent after HDBR ( $P < 0.01$ ). HR was unchanged at baseline but tended to increase at low-level LBNP and was significantly increased at -30 mmHg LBNP. After HDBR, HR tended to be higher than before HDBR at -15 mmHg,

Table 1. Resting variables before and after 18-day bed rest with -6° head-down tilt

Variable	Before Bed Rest	After Bed Rest
Weight, kg	77.1 ± 2.7	75.5 ± 3.0
Plasma volume, l	2.95 ± 0.15	2.70 ± 0.16*
LBNP tolerance, mmHg·min	1,015 ± 160	789 ± 106*
PCW pressure, mmHg	10.6 ± 1.2	7.7 ± 1.3*
Heart rate, beat/min	67.6 ± 2.7	71.2 ± 1.7
Mean arterial pressure, mmHg	98.9 ± 4.1	99.3 ± 3.3
Arterial pulse pressure, mmHg	50.6 ± 3.0	48.2 ± 3.4
Cardiac output, l/min	7.18 ± 0.36	6.41 ± 0.38
Stroke volume, ml/beat	96.2 ± 7.5	76.1 ± 4.9*
Forearm vascular resistance, PRU	26.9 ± 1.9	27.4 ± 3.3
Calf vascular resistance, PRU	43.8 ± 4.3	59.0 ± 4.9*
Total peripheral resistance, dyn·cm <sup>-5</sup>	891 ± 67	1,099 ± 76*
Muscle sympathetic nerve activity, bursts/min	22.1 ± 3.6	23.9 ± 3.4

Values are means ± SE;  $n = 7$  subjects. LBNP, lower body negative pressure; PCW, pulmonary capillary wedge; PRU, peripheral resistance unit. \* $P < 0.05$ , significantly different than pre-bed rest values.

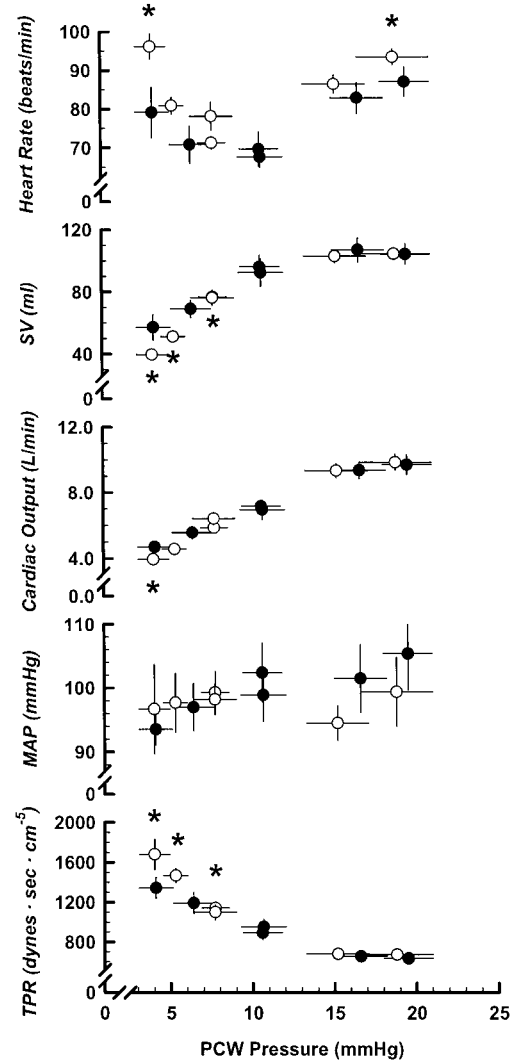


Fig. 2. Hemodynamic responses to graded increases and reductions in cardiac filling pressure before (●) and after (○) 18 days head-down tilt bed rest (HDBR). Heart rate and mean arterial pressure (MAP) represent steady-state responses during a 3-min data collection period. TPR, total peripheral resistance; PCW, pulmonary capillary wedge; SV, stroke volume. \* $P < 0.05$  compared with corresponding pre-bed rest value.

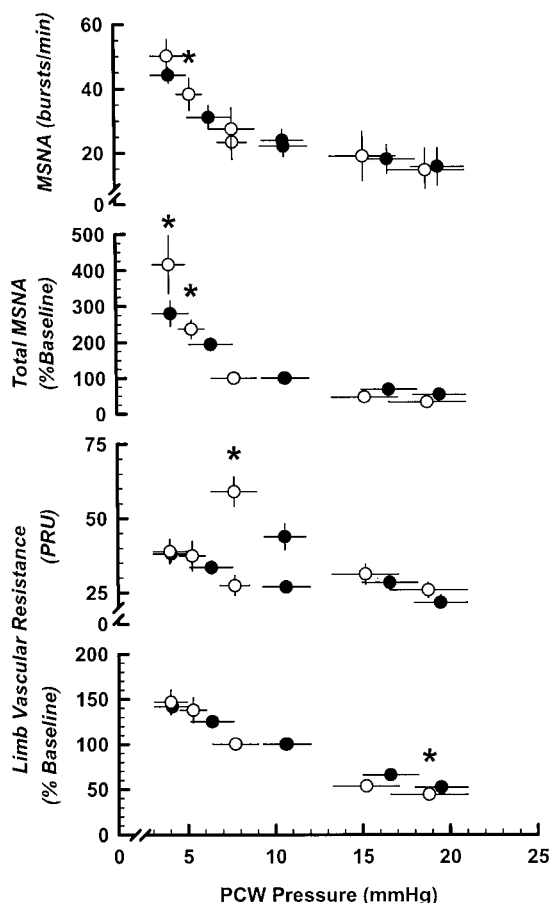


Fig. 3. Changes in MSNA in bursts per minute and total activity normalized to the supine resting value, and limb vascular resistance in response to graded increases and reductions in cardiac filling pressure before (●) and after (○) 18 days of HDBR. MSNA data represent steady-state responses during a 3-min data collection period. PRU, peripheral resistance unit. \* $P < 0.05$  compared with corresponding pre-bed rest value.

( $P = 0.07$ ), and was elevated significantly more at  $-30$  mmHg ( $P = 0.006$ ). Cardiac output was thereby well maintained at rest and mild LBNP, although cardiac output was lower at LBNP  $-30$  mmHg after bed rest ( $P < 0.05$ ). Of note, HR also increased during saline infusion, both before and after bed rest, consistent with an active Bainbridge reflex in humans, as we previously reported (29). After bed rest, this response was significantly greater at the higher level of volume infusion ( $P < 0.05$ ).

LBNP produced significant reductions in SBP ( $P < 0.001$ ) and pulse pressure (PP;  $P < 0.001$ ) without a change in diastolic blood pressure, whereas both SBP and PP increased during volume infusion ( $P < 0.001$ ). However, neither systolic, diastolic, pulse, nor mean blood pressure responses to LBNP and volume infusion were different after HDBR.

#### MSNA and Limb Vascular Resistance

Changes in MSNA in response to changes in left ventricular filling pressure are displayed in Fig. 3. Despite the reduction in resting PCW pressure, base-

line MSNA, expressed in terms of bursts per minute, was unchanged after bed rest. During LBNP, when left ventricular filling was decreased after bed rest, MSNA was appropriately increased compared with before bed rest ( $P < 0.05$  for  $-15$  mmHg). In fact, the relationship between PCW pressure and MSNA followed a curvilinear relationship that was indistinguishable between the two conditions. When MSNA was expressed in terms of total nerve activity (as a percentage of change from baseline), MSNA was increased at  $-15$  and  $-30$  mmHg compared with before bed rest ( $P < 0.05$  in both cases).

The changes in resting limb vascular resistance after bed rest were heterogenous; whereas resting calf resistance increased after HDBR, forearm vascular resistance did not change (Table 1). This finding is consistent with the observation that TPR was significantly elevated over pre-bed rest levels at baseline and each level of LBNP. During LBNP, the increase in forearm vascular resistance was not greater despite a greater rise in MSNA (Figs. 3 and 4). Thus although total MSNA increased during LBNP of  $-30$  mmHg to  $417 \pm 80\%$  of baseline after bed rest compared with  $280 \pm 38\%$  before bed rest ( $P < 0.05$ ), the increase in arm vascular resistance was no different:  $47 \pm 13\%$  or  $11.2 \pm 4.4$  peripheral resistance unit (PRU) after bed rest versus  $42 \pm 6\%$  or  $11.5 \pm 3.0$  PRU before bed rest ( $P = \text{not significant}$ ). Similarly, during volume infusion the decrease in leg vascular resistance was not different after bed rest compared with before bed rest ( $56 \pm 3\%$  or  $33.2 \pm 3.4$  PRU after vs.  $48 \pm 7\%$  or  $22.3 \pm 5.8$  PRU,  $P = 0.08$ ). When arm and limb data were related to MSNA, no differences in the slope of the relationships for either limb could be identified after HDBR (Fig. 4), suggesting that vascular smooth muscle responses to sympathetic stimulation were not affected by bed rest deconditioning.

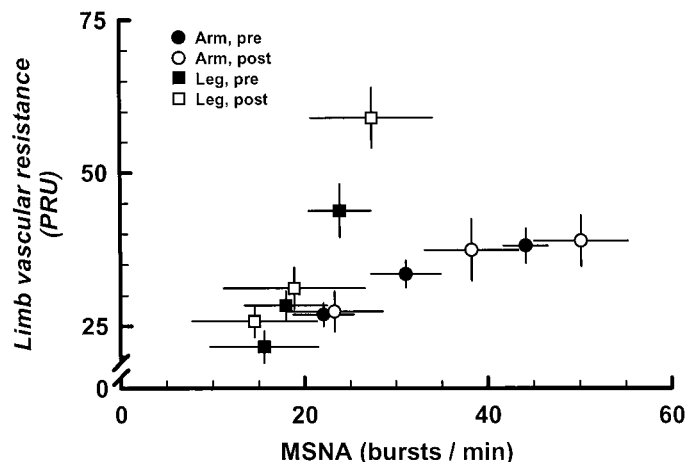


Fig. 4. Arm and leg vascular resistance as a function of MSNA before (filled symbols) and after (open symbols) 18 days of HDBR. Although arm vascular resistance was less than leg vascular resistance for any given level of MSNA, the slope of these relationships was not altered by bed rest.

## DISCUSSION

Three novel observations were made in this investigation. First, the relationship between MSNA and cardiac filling pressure is fundamentally unchanged after 18 days of HDBR deconditioning. Although MSNA was augmented during LBNP after HDBR, this response was consistent with the greater reduction in SV and cardiac filling pressure attendant to deconditioning; i.e., sympathetic neural responses were altered appropriately for the cardiac adaptations produced by HDBR. Therefore, we rejected the hypothesis that impaired baroreflex regulation of MSNA is a primary cause of orthostatic intolerance after bed rest.

Second, MSNA responses after HDBR are difficult to interpret without being referenced to a stimulus variable (e.g., left ventricular end-diastolic pressure). Assuming that measurements were made during periods of hemodynamic stability, one might speculate that previous HDBR studies (28, 36–38) reported discordant information about MSNA because the loading conditions of cardiac mechanoreceptors differed at the time of study. In this investigation we expanded knowledge of reflex regulation of MSNA to the greatest variation of cardiac filling pressure yet reported, which more than doubles the range of previously published data (45) and reveals the curvilinear nature of the filling pressure-MSNA stimulus-response relationship. Though several reports (24, 31, 41) demonstrate increases in MSNA in response to diminished filling pressure, this information has been paired only rarely with increases in filling pressure (45). The results from the present investigation reveal that supine humans can reduce MSNA to extremely low levels with further increases in filling pressure, indicating that before or after HDBR, humans operate within a range of cardiac filling pressures that necessitates continuous regulation of vascular resistance via adrenergic mechanisms.

Third, we have provided the first data relating sympathetic neural and peripheral vascular responses to assess the effects of sympathetic innervation of vascular smooth muscle after HDBR. These relationships were not altered after bed rest, suggesting that neither the release of norepinephrine from sympathetic nerve terminals nor receptor-mediated events in vascular smooth muscle were significantly affected by inactivity and diminished gravitational stress. Thus we did not identify a change in sympathetically mediated regulation of the neuro-effector junction that could account for the reduction in orthostatic tolerance that results from HDBR.

#### *How Would Baroreflex Responsiveness Affect Orthostatic Tolerance?*

During an orthostatic challenge the prevailing level of MSNA is largely established by the combined action of cardiopulmonary and arterial baroreflex disinhibition. Therefore, baroreflex responsiveness can be assessed by relating a stimulus variable (cardiac filling pressure or blood pressure) to the MSNA response. Because orthostatic intolerance increases after HDBR,

we hypothesized, like others, that MSNA would be lower during an orthostatic challenge after HDBR (37). Changes in baroreflex regulation of MSNA could elicit this response by at least one of three potential mechanisms, depicted conceptually in Fig. 5. First, the stimulus-response relationship could move leftward and/or downward, so that a given stimulus would result in a lower response without a change in slope (Fig. 5A).

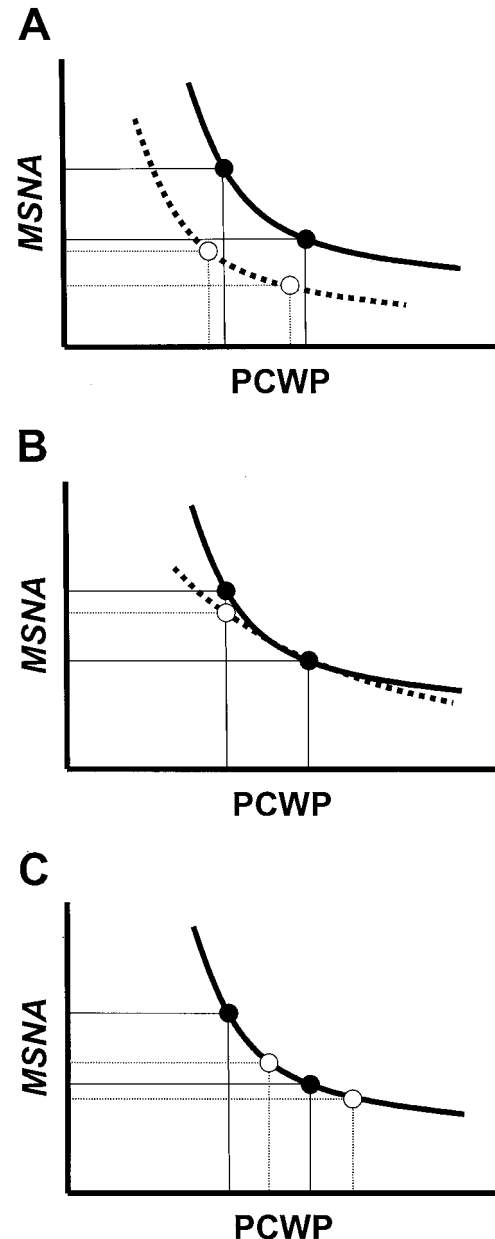


Fig. 5. Schematic representation of changes in baroreflex regulation that might reduce MSNA responses to an orthostatic challenge. ●, Control state when filling pressure is reduced by a fixed amount during an orthostatic challenge. ○, Hypothetical change in the response after HDBR, assuming the same magnitude reduction in filling pressure. Plateau phase of the curve is not shown; vascular collapse when filling pressure falls below zero probably prevents a plateau from being determined. A: baroreflex resetting; B: change in slope (as would be expected with a primary hypoadrenergic state), and (C) a shift in operating point. Combinations of these three changes are possible. PCWP, pulmonary capillary wedge pressure.

This is the classic definition of “resetting” to lower tonic sympathetic activity. Second, the slope of the stimulus-response relationship could decrease (Fig. 5B), which has been defined classically as a reduction in reflex “sensitivity” or “responsiveness.” Third, the stimulus-response relationship could be unaltered, but a new operating point could be established along a less steep portion of the curve (Fig. 5C).

Figure 3 reveals that HDBR did not alter the shape of the MSNA stimulus-response relationship. The only notable change can be categorized as a shift in operating point like Fig. 5C, but with one important difference: at any level of LBNP or volume infusion, PCWP tended to be lower after HDBR, and MSNA responses were appropriately higher. Thus the form of the stimulus-to-response curve was not changed; rather, the operating point was shifted leftward because of the combined effect of hypovolemia (reduction in plasma volume) and cardiac atrophy (27). This finding is consonant with other reports that MSNA responses to head-up tilt are not changed after HDBR (28, 38).

In this report we described baroreflex responsiveness utilizing the PCWP-MSNA stimulus-response relationship. We chose to relate MSNA to PCWP for several reasons. First, PCWP approximates left ventricular end-diastolic pressure, thus representing one of the most accurate methods available to assess left ventricular filling pressure in humans. Second, the left ventricle is richly populated with cardiac mechanoreceptors; deactivation of the mechanoreceptors may disinhibit vasoconstriction during hypovolemia. Moreover, vasodepressor reflexes, such as the von Bezold-Jarisch reflex, originate from the left ventricle (4). Third, measurements of PCWP are independent of geometric assumptions needed to calculate another index of ventricular mechanoreceptor activation (left ventricular end-diastolic volume).

Though it is logical to employ PCWP as a stimulus variable, our findings should be interpreted judiciously in the context of baroreflex regulation for the following reasons. First, both ventricular pressure and volume contribute to regional ventricular wall stress (the likely stimulus to ventricular mechanoreceptors). Second, left ventricular compliance decreases after HDBR because of cardiac atrophy (27). Finally, one might hypothesize that the distribution of mechanoreceptors in the left ventricle would be altered by HDBR (though we have no direct evidence that this hypothesis is correct). Thus the specific HDBR-induced changes in ventricular geometry and morphology that might affect mechanoreceptor activation have yet to be determined accurately.

Orthostatic tolerance, measured by tolerance to progressive LBNP, decreased after HDBR, as has been reported after actual (5, 18) or simulated (2, 3, 6, 8) spaceflight deconditioning. Consistent with previous reports (16), HR was further elevated during mild LBNP after HDBR. This response is consistent with the hypovolemia we noted. Others have suggested that carotid-cardiac baroreflex responsiveness decreases after HDBR (7, 8, 11) or spaceflight (19). Though the

magnitude of tachycardia may be blunted by this mechanism, the consistently greater orthostatic HR reported after HDBR and spaceflight (5, 18, 37) suggest that downregulation of baroreflex control of HR is not a primary mechanism of orthostatic intolerance in this population.

#### *Is Sympathetic Activation Less Likely to Cause Vasoconstriction after HDBR?*

Diminished adrenergic activity has been hypothesized to contribute to the diuresis and orthostatic intolerance that accompanies HDBR (33). Measurements of plasma and urinary catecholamines during HDBR are consistent with this hypothesis (20, 21). However, analysis of sympathetic activity using MSNA are more conflicting. After HDBR, resting MSNA has been reported to increase (28), decrease (36), or remain unchanged (38). In this investigation, we were unable to identify any statistically significant changes in resting MSNA after HDBR, despite the use of extremely sensitive criteria to ensure that all MSNA bursts would be counted (signal-to-noise ratio of band-pass filtered MSNA). One might speculate that hydration state could vary sufficiently between different studies to cause minor shifts in operating point on the stimulus-response relationship, changing resting MSNA. Furthermore, we can only assume that subject postures and hemodynamic stability were maintained in other investigations. Thus without precise knowledge of posture, hemodynamics, and cardiac filling pressure (i.e., stimulus-response characteristics) of the subject groups in the aforementioned studies, further comparison between investigations is equivocal.

Orthostatically intolerant astronauts (i.e., those unable to complete a 10 min “stand test”) have lower levels of systemic vascular resistance (5) and plasma catecholamines (18) compared with those who can complete the test. Yet subjects classified as orthostatically intolerant by comparable criteria have similar levels of MSNA during head-up tilting after HDBR compared with those classified as tolerant (37). This apparent discrepancy can be reconciled if one hypothesizes that central control of sympathetic discharge is not altered by HDBR, but that neuronal release of norepinephrine, or its effect on vascular smooth muscle, is reduced after HDBR.

The closely coupled relationship between MSNA and limb vascular resistance has been well established (35, 45). Accordingly, we related the changes in forearm and calf vascular resistance to MSNA over a wide range of filling pressure. Whether compared as absolute (Fig. 4) or relative values, we were unable to detect any difference in the relationship between MSNA and limb vascular resistance after HDBR. This finding is consistent with other reports demonstrating that leg vascular responses to infused  $\alpha$ -adrenergic agonists change neither after spaceflight (C. G. Blomqvist, personal communication) nor after bed rest deconditioning (10). Thus the effects of norepinephrine on limb vascular smooth muscle contraction did not seem to be affected

by HDBR. However, TPR was greater at rest and during LBNP after HDBR. Therefore we cannot exclude the possibility that sympathetic regulation of regional vascular beds other than those we studied were affected by HDBR, though the particular region(s) remain unidentified at this time.

We can only speculate why our findings differ from the spaceflight findings mentioned above, but several points deserve consideration. First, as mentioned above, TPR such as reported by Buckley et al. (5) includes other circulations not studied in the present investigation. Second, assessments of sympathetic activity by using plasma catecholamines are highly dependent on sampling time, which may be delayed in presyncopal subjects, biasing data to low absolute values. Third, the "intolerant" subjects reported by others (5, 18) may not have been hemodynamically stable at the time of sampling; different than our subjects.

### Limitations

*MSNA analysis.* We quantified MSNA as burst frequency because the recorded amplitude of spontaneous MSNA is influenced by position of the recording electrode relative to the fascicles being recorded (14, 40). Thus important information about MSNA amplitude could not be compared directly, a traditional drawback to sympathetic microneurography. Rather, we related total MSNA (i.e., frequency  $\times$  amplitude) to baseline (initial) values to normalize for changes in MSNA amplitude associated with differences in electrode position that might occur with the test-retest experimental design. With the use of either approach (burst frequency or percentage change in total MSNA) we identified a greater MSNA response to LBNP consistent with a hyperadrenergic, hypovolemic state.

*Interpretation of stimulus-to-response relationships.* What baroreceptor population was responsible for the reflex adjustment of MSNA as filling pressure was changed? It would be improper to conclude that these curves represent either cardiopulmonary or carotid stimulus-response characteristics exclusively. Because filling pressure, SV, and HR changed concomitantly during LBNP and volume infusion, both carotid and cardiopulmonary baroreflexes were engaged. Thus the MSNA responses described here could result from cardiopulmonary baroreflex activity associated with changing central blood volume, and/or arterial baroreceptor activity stimulated by alterations of SV and arterial pulsatility. In fact, the degree of arterial and cardiopulmonary baroreflex involvement may be indeterminate in humans; even mild ( $-5$  mmHg) LBNP dramatically reduces aortic dimensions (42). Thus the curves presented here are based on the recognized baroreflex-mediated association between MSNA, vascular resistance and cardiac filling pressure (45), but the relative contribution of cardiopulmonary and arterial baroreflexes cannot be ascertained.

*Arm and leg blood flow.* The decision to combine arm and leg blood flows to determine limb vascular responses to changes in filling pressure is subject to some

controversy. For example, Essandoh et al. (13) reported differential effects of low-level (less than  $-20$  mmHg) LBNP on calf and forearm blood flow, although a similar degree of vasoconstriction was present when LBNP was increased further. Because of the complexity of the experimental design in the present investigation, the same limb could not be used for both volume infusion and LBNP trials, i.e., LBNP precluded measurement of calf blood flow, and catheter use during volume infusion prevented the measurement of forearm blood flow. Furthermore, because baseline vascular resistance is different between the two limbs (12, 23, 45), responses could be merged and compared only when normalized to their respective baseline value. Two lines of evidence suggest that the approach is valid for the present study. First, the relative distribution of MSNA to arm and leg does not vary when cardiac filling pressure is reduced (32). However, we cannot be certain that this holds true after HDBR. Second, MSNA and vascular resistance respond similarly between the calf and forearm during sustained LBNP (45), and vascular resistance responds similarly in both limbs during steady-state exercise (43). Because of the nonspecific variation in resting forearm and calf vascular resistance, and the differential effect of HDBR on these values, we chose to present data for each limb independently in Fig. 4. For either limb, the slope of the limb vascular resistance-MSNA relationship was unchanged after HDBR, suggesting that HDBR did not alter the effect of norepinephrine release on vascular smooth muscle.

In conclusion, the present investigation demonstrated a reduction in orthostatic tolerance after 18 days of HDBR. Although MSNA responses to LBNP were greater after HDBR, they were appropriate for the hypovolemia and cardiac atrophy associated with deconditioning. Reflex regulation per se was unaltered; the shape of the stimulus-response curve did not change with HDBR, but the operating point on the curve shifted to a lower left ventricular filling pressure.

In summary, neural regulation of limb vascular resistance, assessed by the slope of the MSNA forearm and calf vascular resistance relationship, did not change with HDBR. Thus we conclude that the principal cause of orthostatic intolerance after HDBR is neither a hypoadrenergic state, baroreflex dysfunction, nor a defect in vascular smooth muscle reactivity. Rather, a smaller heart, coupled with a greater increase in sympathetic activity, could produce a hypovolemic, hyperadrenergic form of orthostatic intolerance after HDBR. We speculate that diminished ventricular end-diastolic volume, SV, and sympathetically mediated increases in ventricular inotropic state constitute the substrate for syncope during orthostatism after HDBR. These adaptations may explain, in part, the reduction in orthostatic tolerance that accompanies other deconditioning states such as spaceflight.

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