Sympathetic discharge and vascular resistance after bed rest

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SYMPTOMS OF AUTONOMIC DYSFUNCTION are evident in astronauts returning from spaceflight and in individuals confined to prolonged bed rest (4, 14, 16, 29), a ground-based model of microgravity (2, 24). In particular, the ability to maintain an upright posture is diminished in many individuals returning from spaceflight (4, 16, 41). The mechanisms of orthostatic intolerance are currently unknown, but recent evidence suggests that the vascular regulation may be altered (4, 16). Fritsch-Yelle et al. (15) related this orthostatic intolerance to reduced plasma concentrations of norepinephrine during upright posture. Furthermore, reductions in plasma norepinephrine after bed rest (9, 18, 20, 29) have been hypothesized that the mechanism for this apparent sympathoinhibition involves a reduction in the synthesis and release of norepinephrine from terminal endings of sympathetic neurons (20). In contrast, some investigators have observed elevated plasma catecholamines in supine subjects after spaceflight (16, 41), suggesting sympathetic excitation in their subjects. Altered sympathetic outflow should result in alterations in peripheral vascular resistance unless compensatory peripheral vascular adaptations occurred simultaneously. Several studies have examined issues related to limb blood flow and vascular resistance after spaceflight, but consistent conclusions cannot be drawn, with both increases (17, 28) and decreases (1) in vascular resistance being reported. Similarly, some reports indicate that forearm blood flow (FBF) (8) and leg (12, 42) blood flow were normal after spaceflight, whereas another study points to reductions in supine leg blood flow (3). Adding to the confusion has been the fact that measurements of sympathetic activity and vascular resistance are rarely performed in the same report.

In the present study, we used microneurography to obtain direct measurements of muscle sympathetic nerve activity (MSNA), together with measurements of mean arterial pressure (MAP), ascending aortic mean blood velocity (MBV), and FBF to assess the relationship between sympathetic discharge and vascular resistance before and after 14 days of head-down-tilt bed rest (HDBR). The data suggest that baseline MSNA burst frequency was reduced, and despite this reduction in sympathetic outflow, forearm vascular resistance (FVR) and our index of total peripheral resistance (TPR) were not altered by HDBR. Therefore, vascular adaptations may have developed to maintain vascular resistance in the face of reduced sympathetic discharge.

METHODS

In total, 25 healthy men volunteered for the study and gave their consent to the experimental procedures that had been approved by the Institutional Review Board at The Milton S. Hershey Medical Center. The mean age of the subjects was 30 yr (range 20–41 yr). Cardiovascular health was evaluated by a detailed questionnaire, a physical examination, and electrocardiogram (ECG).

During HDBR, the average daily caloric intake across subjects was ~2,500 calories (55% carbohydrate, 25% fat, 20% protein). Daily dietary sodium was ~3,000 mg, and a goal of 2,000-mL fluid consumption was encouraged. Each day the photoperiod was 16 h of light and 8 h of dark with lights on at 0700. Blood pressure and heart rate (HR) were assessed four times daily at 4-h intervals during the period when the subjects were awake.

Data acquisition. All data were collected at least 14 days before (pre) and on day 14 of (post) the bed rest period. On each day, at least 30–45 min of supine rest occurred before any baseline measurements were obtained. For the pre-HDBR tests, this stabilization period was used to obtain a 12-lead ECG tracing and perform a physical examination on the subject. Instrumentation for MAP, HR, MSNA, and blood flow measurements was then performed. After instrumentation, the subject rested quietly for at least another 10 min, after which data collection commenced.
HR and blood pressure (n = 25). HR was monitored by standard ECG methods, and systemic blood pressure was estimated with a pneumatic finger cuff (Finapres, Ohmeda, Englewood, CO). Baseline blood pressure values were confirmed by an automated upper arm sphygmomanometer (Dinamap, Criticon, Tampa, FL).

Microneurography (n = 36). Multunit recordings of postganglionic MSNA were obtained from the peroneal nerve with an insulated 200-μm-diameter tungsten electrode that was tapered to an uninsulated 1- to 5-μm-diameter tip. The microelectrode was inserted transcutaneously into the peroneal nerve just posterior to the fibular head. A reference electrode was positioned subcutaneously 1–3 cm from the recording site. Neuronal activity was amplified 1,000 times by a preamplifier and 50–100 times by an amplifier. The signal was band-pass filtered with a bandwidth of 700–2,000 Hz and then was rectified and integrated to obtain a mean voltage neurogram.

Normally, MSNA data are analyzed according to the burst frequency and the burst amplitude (10). Resting burst frequency has been shown to be reproducible (7, 37) when readings are performed on different days. Resting burst amplitude is dependent on electrode placement and signal amplification, both of which may vary from the pre- to post-HDBR experiments. For this reason we will only present burst frequency data in this report.

To further address the issue of MSNA burst frequency repeatability in our study, we have compared baseline MSNA data from 36 men who have had microneurographic tests performed on two separate occasions between the years 1992 and 1996. The mean age of these control subjects was 26.4 yr (range 21–38 yr).

FBF (n = 14). FBF was measured by using venous occlusion (40) and mercury-in-Silastic strain gauge plethysmography (21). While the subject was supine, the forearm was placed ~10 cm above the heart, and the strain gauge was placed ~10 cm below the olecranon process. Care was taken to place the strain gauge in the same place during the pre- and post-HDBR trials. Occlusion cuffs were placed around the upper arm and around the wrist. With the wrist cuff inflated to 250 mmHg to exclude hand blood flow, FBF measurements were made by inflating the upper arm cuff to 50 mmHg for 5–7 s each. FBF at rest was taken as the average of 8–10 repeated measurements over 2–4 min. FVR was calculated as FVR = MAP/FBF.

Ascending aorta MBV (n = 7). To obtain an index of stroke volume, continuous beat-by-beat measurements of aortic mean blood velocity were made by using pulsed Doppler ultrasound (2 MHz; Multigon 500M, Multigon Industries, Yonkers, NY). The depth and gate of the sample volume were adjusted to obtain flow velocity signals from ~2 cm above the aortic ring where left ventricular ejection velocity was still maximal, but wall and valve motion artifacts were minimal. The instantaneous MBV was determined from the Doppler spectra at 100 Hz and collected on a dedicated computer together with continuous blood pressure data also collected at 100 Hz. Beat-by-beat values of MBV and MAP were then determined by averaging the instantaneous values for each variable between adjacent R waves of the ECG tracing. At least 1 min of continuous MBV and MAP data were collected, providing data from at least 60 cardiac cycles. The consecutive beat-by-beat data were averaged to determine a resting value for each variable. MBV was corrected for bed rest-induced changes in cardiac frequency (R–R interval) to provide an index of stroke volume. Because the units of this corrected MBV value were centimeters per beat, the stroke volume index is referred to as stroke distance (SD) (SD = MBV/HR·60). Thus TPRi could be calculated (TPRi = MAP/SD·HR). For these tests, the subject was instructed to relax comfortably, breathe normally, and not move the head or limbs.

Statistics. A Wilcoxon signed-rank test was used to assess the effect of HDBR on MSNA burst frequency, MAP, HR, FVR, SD, and TPRi. A probability level of P < 0.05 was considered statistically significant. All values are expressed as means ± SE.

RESULTS

MAP and HR. Overall, MAP was not different in the pre- and post-HDBR tests (Table 1). The deconditioning effects of bed rest were evident in the HR, which was greater after bed rest compared with the pre-HDBR test (Table 1; P < 0.003).

MSNA and HDBR. MSNA burst frequency was reduced in the post (16.7 ± 2.8 bursts/min)- compared with the pre (25.2 ± 2.6 bursts/min)-HDBR condition (P < 0.01) (Fig. 1A). Because MSNA bursts are pulse synchronous, they are also affected by HR. Thus correction for changes in HR that occurred with HDBR should enhance the link between MSNA and MAP by accounting for the effect of HR-related changes in systemic blood flow. With this approach, the decrease in burst frequency after HDBR was more impressive with 24.0 ± 4.0 bursts/100 heart beats compared with the 40.1 ± 4.7 bursts/100 heart beats in the pre-HDBR tests (P < 0.005; Fig. 1B). In the experimental group, MSNA burst frequency was reduced in 13 of the 16 individuals and was increased in 3 subjects (Fig. 2). Representative neurograms demonstrating the reduction in burst frequency are shown for three individuals in Fig. 3. An example of the increase in MSNA burst frequency with HDBR is shown for a single individual in Fig. 4.

Repeatability of MSNA burst frequency. The mean burst frequency during supine rest for the 36 control subjects on whom repeated MSNA measurements were made but who did not perform the HDBR was 16.3 ± 1.4 beats/min in the initial test and 15.7 ± 1.5 beats/min.

Table 1. Effect of 14-day –6° head-down-tilt bed rest on heart rate, mean arterial pressure, forearm vascular resistance, stroke distance, and total peripheral resistance index

<table>
<thead>
<tr>
<th>n</th>
<th>Pre-HDBR</th>
<th>Post-HDBR</th>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>25</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>Stroke distance, cm/beat</td>
<td>7</td>
<td>20.8 ± 1.5</td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
<td>25</td>
<td>92.7 ± 1.9</td>
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<tr>
<td>Forearm vascular resistance, units</td>
<td>14</td>
<td>40.7 ± 7.7</td>
</tr>
<tr>
<td>Total peripheral resistance index, units</td>
<td>7</td>
<td>0.08 ± 0.01</td>
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Values are means ± SE; n, no. of subjects. HDBR, head-down-tilt bed rest. For the group from which stroke distance data were obtained (n = 7) mean arterial pressure in pre- and post-HDBR tests was 93.2 ± 1.9 and 94.6 ± 1.8 mmHg, respectively. Heart rate values for this subset were 63 ± 1.6 and 71 ± 1.7 beats/min pre- and post-bed rest, respectively. For the group from which forearm blood flow data were obtained (n = 14) pre- and post-HDBR mean arterial pressure values were 88.4 ± 2.4 and 92.3 ± 1.9 mmHg, respectively. Heart rate values for this subset were 64 ± 2.6 and 69 ± 2.5 beats/min pre- and post-bed rest, respectively.*Significantly different from pre-HDBR, P < 0.05.
min for the second test. These values were not statistically different. HR was not different between the initial (65.5 ± 1.6 beats/min) and second (63.8 ± 1.4 beats/min) tests. Therefore, burst frequency, as a function of HR, during the initial test (25.2 ± 2.2 bursts/100 heart beats) was unchanged from the repeated measure (24.7 ± 2.1 bursts/100 heart beats). Compared with the initial test, burst frequency per 100 heart beats during the second study was increased in 16, decreased in 16, and was unaltered in 4 of the control subjects. These data, in part, have been reported previously (39).

Fig. 1. Fourteen days of head-down-tilt bed rest reduced peroneal nerve muscle sympathetic nerve activity burst frequency responses obtained over 3–5 min of supine rest. A: raw burst frequency. B: burst frequency corrected for bed rest-induced changes in heart rate. Values are means ± SE; n = 16 subjects. Pre, before; post, after. *Significantly different from pre-bed rest, P < 0.05.

FVR. FBF, as measured by venous occlusion plethysmography, was not different between the pre (3.60 ± 0.7 ml·100 ml⁻¹·min⁻¹) and post (3.54 ± 0.4 ml·100 ml⁻¹·min⁻¹)-HDBR tests. With no change in either MAP or FBF, FVR was not different between tests (Table 1).

TPRi. TPRi was not different between the pre (22.1 ± 1.4 cm/s) and post (19.5 ± 1.5 cm/s)-HDBR tests. However, SD was significantly reduced after bed rest (Table 1). Bed rest did not alter supine TPRi (Table 1).

Fig. 2. Individual responses for muscle sympathetic nerve activity burst frequency, corrected for differences in heart rate. Baseline muscle sympathetic burst frequency was reduced in 13 of 16 individuals.

Fig. 3. Neurograms of 3 individuals showing reduction in baseline muscle sympathetic nerve activity burst frequency after 14 days of −6° head-down-tilt bed rest.

Fig. 4. Neurogram of a single individual showing an increase in baseline muscle sympathetic nerve activity burst frequency after 14 days of −6° head-down-tilt bed rest.
DISCUSSION

This is the first study to report the effect of prolonged HDBR on MSNA. The new information observed from these experiments was that MSNA was reduced in the majority of subjects after HDBR. However, FVR and TPR, remained unaltered in the face of decreased peripheral vascular adaptations occurred to compensate for attenuated sympathetic discharge. The reduction in SD (an index of stroke volume) was compensated for by an increase in HR that maintained MAP at pre-HDBR levels. The elevated HR with depressed MSNA responses likely reflects adjustments in autonomic function whereby parasympathetic modulation of HR is reduced (19, 22, 23).

Limitations. Although burst frequency may be dependent on electrode placement, previous reports (7, 37) and observations in the present study demonstrate that the mean MSNA frequency response is reproducible on repeated measurements separated by days or weeks. Additionally, 13 of 16 subjects had lower MSNA values after bed rest. In our control subjects, MSNA burst frequency in the second study was less in 16, increased in 16, and the same in 4 subjects. We believe these data provide additional support for the concept that MSNA falls after HDBR.

In this study, MBV was used as an index of stroke volume. For this we assumed that bed rest did not alter the dimensions of the aortic root from which the blood velocity data were obtained. On the basis of prior reports that cardiac output normally is not altered by bed rest [see Fortney et al. (14) for review] and our observations that MBV was not different with HDBR, it can be reasoned that aorta cross-sectional area was also similar in our pre- and post-HDBR tests. The reduction in SD with HDBR observed in the present study is of the same magnitude observed for direct measurements of stroke volume during pre- and post-spaceflight tests (5, 41). In addition, our data support those of other investigators who used direct measurements of cardiac output and observed that spaceflight (4, 16, 41) or hindlimb unweighting in rats (42) did not alter baseline total peripheral resistance.

MSNA. Although plasma catecholamine levels have been correlated with MSNA (32, 38), it is not known whether real or simulated microgravity alters this relationship. Thus it is difficult to predict whether MSNA would increase or decrease after HDBR on the basis of plasma norepinephrine concentrations. Specifically, some researchers have reported that bed rest increases baseline plasma norepinephrine concentrations (11, 16, 41), whereas others have observed reductions (9, 18, 20, 26, 29). Additionally, the ability to predict MSNA on the basis of plasma norepinephrine is complicated by the fact that changes in plasma volume and cardiac output can alter norepinephrine clearance. This can alter the relationship between MSNA and plasma norepinephrine independent of effects of norepinephrine spillover on plasma norepinephrine (20, 25). In addition, products of muscle contraction and metabolism are known to affect norepinephrine release (34). Therefore, the reductions in plasma volume, altered neuromotor recruitment patterns, and muscle atrophy associated with spaceflight and bed rest (14) may account for much of the debate as to the effects of real or simulated microgravity on plasma norepinephrine concentrations.

Recent evidence from Goldstein et al. (20) indicates that a reduction in plasma norepinephrine with HDBR probably is due to diminished synthesis and release from terminal sympathetic nerve endings but that it is obscured if changes in plasma volume or urinary excretion are not taken into consideration. Thus the direct measurement of sympathetic nerve activity in the present study showing that MSNA burst frequency at rest is reduced in many individuals after HDBR is consistent with the observation of lower norepinephrine release after simulated microgravity. Importantly, the present data suggest that the sympathoinhibition is related to an attenuated activation of sympathetic discharge rather than changes at the nerve terminal ending. The mechanism(s) for altered efferent sympathetic nervous system discharge frequency in humans remains to be explored. One potential concern is that tests of autonomic function immediately before the commencement of bed rest may reveal artificially elevated adrenergic responses due to mental stress (26). The 2-wk period between the pre-HDBR test and the onset of bed rest in the present study probably circumvented this concern. Importantly, Fagette et al. (13) examined norepinephrine turnover rates in cardiovascular control regions of the nuclear tractus solitarius and in peripheral organs of rats after prolonged head-down suspension and observed reduced sympathetic activity in both regions. These data suggest that central modulation of efferent sympathetic activity develops during bed rest and could provide a mechanism for the present findings.

From a teleological perspective, the reduction in sympathetic activation at rest may be protective for individuals undergoing prolonged bed rest or spaceflight. Burke et al. (6) first demonstrated greater tolerance for upright posture in those individuals with lower resting MSNA; if baseline MSNA was higher, then the increase in sympathetic activity with standing was diminished. Importantly, Fritsch-Yelle et al. (16) reported that supine plasma norepinephrine levels were somewhat higher, but increased less during standing, in those astronauts who could not complete a 10-min stand test after spaceflight, compared with those who could complete the test. Future experiments will need to examine whether levels of MSNA obtained during supine rest are predictive of the response to an orthostatic challenge.

A second observation of the present study was that TPR, and FVR were maintained despite reductions in MSNA. Under normal circumstances, peripheral vascular resistance is maintained by relatively high levels of sympathetic tone (33), and, during a vasoconstrictor stress, changes in vascular resistance are linked to MSNA (31). Therefore, we would speculate that peripheral adaptations may have occurred to maintain vascular tone in the face of reduced sympathetic discharge. It is unlikely that myogenic influences compensated for...
reduced sympathetic tone because MAP was unchanged by bed rest. We believe our data are most consistent with the concept that there is an upregulation of α-adrenergic receptor populations (26, 29). It is possible that vascular morphological adaptations occurred that compensate for reduced MSNA to maintain vascular tone at rest. Several researchers, using venous occlusion plethysmographic measurements of the hyperemic response to ischemia (reactive hyperemia), have documented that vasodilator capacity increases with muscle conditioning (27, 36) and decreases with muscle deconditioning (35). These results suggest that structural adaptations occur in vascular tissue in response to changes in the level of physical activity. However, we cannot exclude the possibility that HDBR does not alter the postreceptor regulation of vascular tone for a given change in sympathetic stimulus.

Investigations into the effect of spaceflight and bed rest on peripheral vascular tone at rest have produced inconsistent results (14). Differences in blood flow measurement methods, in the site of measurement (i.e., upper vs. lower limbs, or muscle vs. skin vessels), and interindividual variability may all impact on these heterogeneous data. For example, forearm subcutaneous vascular resistance, as measured by $^{133}$Xe washout, tended to be augmented after 10 days of spaceflight (17), but this may not reflect skeletal muscle perfusion, which constitutes the majority of limb blood flow and vascular resistance. Arbelle et al. (1) used Doppler measurements of conduit artery blood flow and reported a reduction in leg vascular resistance at rest after 1–24 days of HDBR and spaceflight. Assuming arterial pressure was not decreased in this latter study, leg blood flow must have increased. In contrast, Blamick et al. (3) used impedance measurements of leg flow and reported reductions in leg arterial pulse volume. Schulz et al. (30) also reported a 20% increase in total peripheral resistance after 10 days of HDBR. The elevated peripheral resistance was due to reductions in cardiac output with little change in MAP. However, many reports show little change in cardiac output with bed rest or spaceflight (14). Despite these varied findings, the results of the present study are consistent with previous reports, also based on venous occlusion plethysmographic measurements, that lower leg blood flow (11) or FBF (8) was unchanged with HDBR.

Summary. The new information of the present study was that resting MSNA burst frequency was reduced after 14 days of HDBR. However, FVR and TPR, were unchanged. Taken together, these data suggest that central modulation of sympathetic discharge and peripheral vascular adaptations may have occurred concurrently during the HDBR period to maintain MAP despite reductions in sympathetic discharge.

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