Attenuated sympathetic nerve responses after 24 hours of bed rest

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In this study, we performed 24-h bed rest experiments to examine this issue. We selected 24 h of bed rest because it is sufficient to impair orthostatic tolerance (OT) (14, 29) yet leads to a relatively small reduction in plasma volume and heart muscle mass compared with changes seen with longer bed rest paradigms (17). Significant changes in plasma volume have been shown to contribute to OI. Therefore, we hypothesized that if muscle sympathetic nerve activity (MSNA) responses were impaired after 24 h of bed rest it would strongly point to “peripheral” causes of OI.

During the lower body negative pressure (LBNP) tests we measured HR and blood pressure, made peripheral sympathetic nerve recordings, and calculated stroke volume (SV), peripheral vascular resistance, and cardiac output. The results of these experiments demonstrate that bed rest reduces tolerance to graded LBNP. This effect was associated with a reduced ability to increase MSNA at higher levels of LBNP.

METHODS

A limited echocardiographic examination was performed during the week preceding the study, and only subjects with adequate echocardiographic windows were enrolled. We studied 13 subjects, 9 women and 4 men, with a mean age of 24 ± 1 yr (weight 66 ± 3 kg; height 170 ± 3 cm). We were able to obtain complete nerve data before and after bed rest in 10 subjects. All volunteers were in good health and normotensive as assessed by a prestudy history and physical examination. All subjects were nonsmokers; the male subjects were not taking any medications and seven female subjects were on birth control pills. None of the subjects was an endurance-trained athlete. All volunteers signed an informed consent approved by the Institutional Review Board of the Milton S. Hershey Medical Center.

Best rest. During the 24-h period of bed rest, subjects were confined to bed in a 6° head-down position as described previously in our laboratory (34, 35). A “bed rest monitor”

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was present during this time. Fluids were allowed ad libitum.
Subjects were allowed to rise on one elbow to eat. They were
not allowed to perform any limb exercises. No caffeine was
allowed during this period. The photoperiod was 16 h of light
and 8 h of dark with lights on at 7:00 AM.

**Experimental paradigm.** Subjects presented to the General
Clinical Research Center (GCRC) at least 2 h after a light
meal. Subjects refrained from caffeine ingestion for 12 h
before the experiment. They were placed on a padded table
with their lower body (up to the level of the umbilicus)
positioned inside an LBNP tank. Subjects were instrumented
with electrocardiograph (ECG) leads, a Finapres, and micro-
neurography electrodes. After ~20 min of rest, baseline ECG,
MSNA, and hemodynamic data were recorded over 5 min.

LBNP was applied in a graded fashion starting at -10
mmHg and increasing by -10 mmHg every 3 min until one of
the following occurred: 1) symptoms consistent with LBNP
intolerance developed (nausea, diaphoresis, etc.); 2) systolic
blood pressure fell to a level of 90 mmHg; or 3) the subject
completed 3 min of the sixth and last stage of the paradigm
(i.e., -30 mmHg). Blood pressure, HR, and MSNA were
recorded continuously, and CO was measured during the last
minute of each stage. From these data a “modified cumula-
tive stress index” was calculated by taking the level of LBNP
(mmHg) and multiplying by the time (min) spent in that
stage (7). The values for the various stages were then
summed.

**HR, blood pressure, and echo-Doppler measurements.** HR
was measured by ECG. The blood pressure was measured
with the volume-clamp method (Finapres; Ohmeda, Madison,
WI). CO was calculated with echo-Doppler methods (Accuson
128XP/10; Accuson, Mountain View, CA). Briefly, the flow
across a fixed orifice is equal to the product of flow velocity
and the cross-sectional area (CSA) of that orifice. The left
ventricular outflow tract (LVOT) time velocity interval (TVI)
was measured from the echocardiographic apical long-axis
view just proximal to the aortic annulus. LVOT velocities
were measured during the last minute of each LBNP stage.
From these data the two or three best representative tracings
were selected to obtain TVI. LVOT diameter was measured
in systole with the echocardiographic parasternal long-axis
view (1, 30). This measurement was found to be constant
during the different levels of LBNP and was not influenced
by the 24 h of bed rest. SV was calculated by multiplying TVI
by CSA. Subsequently, CO was determined by multiplying
SV by HR from the same time interval as that of the mea-
sured TVIs. The director of the Pennsylvania State Univer-
sity College of Medicine Echocardiography Laboratory (W. R.
Davidson, Jr.) verified the accuracy of the data. This method
of determining CO is highly concordant with the thermodi-
lation method (23). An index of systemic vascular resistance
(SVR) was derived by dividing MAP by CO. SVR is expressed
in arbitrary units.

The methods for performing the microneurographic tech-
nique were previously described in detail (26, 37). Briefly,
multunit recordings of MSNA are obtained by placing an
electrode in a muscle fascicle within the peroneal or popliteal
nerve and a reference electrode in the adjacent subcutaneous
tissue. The course of the nerve is mapped by
external stimulation with a stimulating pen (40–150 V, 0.2
ms, 1 Hz) to evoke motor activity. The recording electrode is
then passed until the “sound” characteristics and responses
to muscle afferent stimulation (stretching or tapping of ten-
dons) yield a neurogram with the characteristic bursts of
activity. An adequate muscle site of sympathetic nerve activ-
ity is obtained when electric stimulation yields twitch con-
tractions without paresthesias and the integrated neurogram
demonstrates burst activity that is pulse synchronous (39).
The signal is then amplified (50,000–90,000 times), filtered
(700 and 2,000 Hz), rectified, and integrated to obtain a mean
voltage neurogram. Before bed rest, MSNA was recorded
from the right peroneal nerve just posterior to the fibular
head. To avoid possible injury to the nerve from repeated
manipulations after bed rest, MSNA was recorded from the
right peroneal nerve in the popliteal fossa.

The signals for blood pressure, ECG, and MSNA were
sampled at 100 Hz and were stored on a computer with a
commercially available data acquisition system (Power Lab;
ADInstruments, New Castle, Australia).

**Data assessment.** The baseline blood pressure, HR, and
MSNA data were averaged over 5 min. At least three CO
values were obtained and averaged over the baseline period.
Blood pressure, HR, and MSNA were measured over the last
minute of each LBNP level. During the last minute of each
LBNP level, two or three TVI values were obtained and
averaged to calculate CO.

We expressed our MSNA data as bursts per minute and as
bursts per 100 heartbeats. MSNA amplitude (average burst
amplitude × burst count) data are highly dependent on
electrode placement (38). Minute changes in electrode place-
ment are seen frequently at high levels of LBNP. All subjects
completed LBNP of up to -30 mmHg on both days, and
thereafter the number of subjects completing the various
levels declined.

**Statistical analysis.** The design of this study consisted of
two repeated factors for each subject: bed rest (before and
after) and LBNP. To assess the effects of bed rest and LBNP
as well as their possible interaction on the cardiovascular
responses, doubly repeated-measures analysis of variance
models were fit to the data (24). Tests of simple effects
comparing before bed rest to after bed rest at each level of
LBNP were performed. Because the simple effects tested in
the models were determined a priori and because the simple
effects are all orthogonal contrasts, no adjustment for multi-
ple-comparison testing was done.

To examine the rate of change in MSNA as LBNP in-
creased, a piecewise linear random coefficients model was
used that fits a regression line for the correlated data for each
subject and then averages across all regression lines (24, 32).
For each bed rest condition (before and after) in this piece-
wise model, a slope was fit from baseline to -30 mmHg (low
levels of LBNP) and a separate slope was fit from -30 mmHg
to -60 mmHg or the highest level achieved before presyn-
cope (high levels of LBNP). The rates of change in MSNA

![Fig. 1. The modified cumulative stress index before and after 24 h of
bed rest is shown for the 13 subjects. Bold line represents the mean.
*P < 0.023 for difference in index between before and after bed rest
(sign test). LBNP, lower body negative pressure.)](http://ajpheart.physiology.org/)

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from baseline to \(-30\) mmHg, as well as from \(-30\) mmHg to \(-60\) mmHg, were compared between the two bed rest conditions. The fit of all models was assessed with residual diagnostics. A \(P\) value <0.05 was considered statistically significant. The sign test was used to analyze the modified cumulative stress index (16). All data were analyzed with SAS statistical software (SAS Institute, Cary, NC).

RESULTS

The most common symptoms during LBNP at the end of the study before and after bed rest were lightheadedness and dizziness followed by yawning, nausea, feeling of warmth, and abdominal discomfort.

**LBNP tolerance.** Before bed rest five subjects completed the entire paradigm, whereas after bed rest only two did so. Before bed rest the LBNP test was stopped because of hypotension in seven subjects and because of nausea in one subject. After bed rest the test was discontinued because of hypotension in nine subjects, because of nausea in one subject, and because of cold sweats in one subject. The modified cumulative stress index was lower after bed rest \((P < 0.023)\), with 11 of the 13 subjects being less tolerant to the paradigm after bed rest (Fig. 1).

**Cardiovascular responses.** The cardiovascular responses to LBNP before and after bed rest are shown as a change from baseline \((\Delta)\) in Fig. 2. The number of subjects at each level of LBNP for each variable is shown in Table 1. During the LBNP paradigm, \(\Delta\)HR rose to a greater degree after bed rest (bed rest effect

**Table 1. Number of subjects at each level of LBNP**

<table>
<thead>
<tr>
<th></th>
<th>(-10) mmHg</th>
<th>(-20) mmHg</th>
<th>(-30) mmHg</th>
<th>(-40) mmHg</th>
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<td>10</td>
<td>10</td>
<td>9</td>
<td>3</td>
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<tr>
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<td>10</td>
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<td>10</td>
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<td>13</td>
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<tr>
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<td>13</td>
<td>13</td>
<td>13</td>
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<tr>
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<td>13</td>
<td>13</td>
<td>13</td>
<td>9</td>
<td>4</td>
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<tr>
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<td>13</td>
<td>13</td>
<td>10</td>
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<td>13</td>
<td>13</td>
<td>11</td>
<td>9</td>
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<tr>
<td>After bed rest</td>
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<td>13</td>
<td>9</td>
<td>9</td>
<td>5</td>
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<td>13</td>
<td>13</td>
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<tr>
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LBNP, lower body negative pressure; MSNA, muscle sympathetic nerve activity; MAP, mean arterial pressure; SVR, systemic vascular resistance.
After bed rest ΔSV values were generally lower than the values noted at the same level of LBNP before bed rest, but no statistical effect of bed rest was noted. CO, the product of HR and SV, fell with LBNP but was unaffected by bed rest (not significant). ΔSVR and Δ mean arterial pressure (MAP) with LBNP were also unaffected by bed rest. Because we used the blood pressure values at the earliest signs of LBNP intolerance, we did not observe a lower BP at the highest level of tolerable LBNP after bed rest.

To examine the effects of bed rest on MSNA, the rate of change measured as Δ bursts and Δ bursts/100 heartbeats was calculated for “low levels” (baseline to −30 mmHg) and “high levels” (−30 mmHg to end LBNP) of LBNP. This analysis demonstrated that the rate of increase in MSNA at low levels of LBNP was similar before and after bed rest. However, the rate of increase in MSNA for high levels of LBNP was lower after bed rest (Fig. 3). This was noted despite the fact that MAP values tended to be lower. MSNA values before and after LBNP are shown in Table 2.

DISCUSSION

The main findings of this study were that 1) after bed rest subjects were less tolerant of LBNP than before, 2) the ΔCO response to LBNP was not altered by bed rest, and 3) the rate of increase of ΔMSNA during high levels of LBNP was lower after bed rest than before. This latter effect was seen despite the fact that absolute blood pressure at each level of LBNP tended to be lower after bed rest than before. This suggests that the reduced MSNA response to LBNP after bed rest was not due to a smaller hypotensive stimulus. Thus 24 h of bed rest is a sufficient stimulus to alter the MSNA-Δ blood pressure stimulus-response relationship.

The potential causes for this decline in orthostatic tolerance after bed rest (or spaceflight) can be grouped into those that may accentuate the fall in CO and those that lead to an impairment in the vasoconstrictor response seen with orthostatic stress. It is clear that bed rest or spaceflight leads to reductions in plasma volume (33, 36), which can reduce ventricular filling and in the process lead to an accentuated reduction in CO with standing. However, the incidence of OI after head-down bed rest (HDBR) or spaceflight is not well correlated with reductions in plasma volume (6), and restoring plasma volume (and central venous pressure) before bed rest levels does not restore OT (2). In the present report we limited the period of bed rest to 24 h because this time period does not allow the full expression of the fall in plasma volume seen with bed rest (17).

Several investigators have suggested that increased lower limb venous compliance after bed rest or spaceflight may contribute to OI (4, 10, 25). However, orthostatic hypotension has been observed within 4 h of head-down tilt, before any changes in peripheral venous compliance could occur (5, 8).

Work by Levine et al. (22) suggested that the inability of volume replacement to normalize OT may be due

Table 2. MSNA responses to LBNP before and after bed rest

<table>
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<tr>
<th></th>
<th>Baseline</th>
<th>-10 mmHg</th>
<th>-20 mmHg</th>
<th>-30 mmHg</th>
<th>-40 mmHg</th>
<th>-50 mmHg</th>
<th>-60 mmHg</th>
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<td>Bursts</td>
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<tr>
<td>Before bed</td>
<td>16.0 ± 2.2</td>
<td>20.5 ± 2.6</td>
<td>21.5 ± 1.9</td>
<td>23.5 ± 2.6</td>
<td>29.7 ± 4.2</td>
<td>34.1 ± 4.9</td>
<td>54.5 ± 3.2</td>
</tr>
<tr>
<td>After bed</td>
<td>22.0 ± 3.0</td>
<td>25.5 ± 2.5</td>
<td>29.6 ± 3.1</td>
<td>34.2 ± 3.5</td>
<td>37.1 ± 3.6</td>
<td>39.7 ± 4.7</td>
<td>47.3 ± 8.4</td>
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<tr>
<td>Bursts per 100 heartbeats</td>
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<tr>
<td>Before bed</td>
<td>24.6 ± 3.8</td>
<td>31.4 ± 3.9</td>
<td>31.8 ± 3.6</td>
<td>32.8 ± 5.0</td>
<td>37.4 ± 6.8</td>
<td>39.8 ± 8.0</td>
<td>51.0 ± 3.7</td>
</tr>
<tr>
<td>After bed</td>
<td>32.5 ± 4.5</td>
<td>35.8 ± 4.4</td>
<td>39.2 ± 5.4</td>
<td>41.4 ± 5.2</td>
<td>40.2 ± 5.2</td>
<td>38.2 ± 5.2</td>
<td>39.3 ± 8.8</td>
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</table>

* for each level of LBNP is shown in Table 1.
to cardiac atrophy. We doubt that cardiac atrophy contributed to the OI observed in the present study because LBNP intolerance was observed after only 24 h of bed rest. The main stimulus for cardiac protein synthesis is the rate-pressure product (19–21, 24). We are not aware of any data to suggest that the rate-pressure product falls after bed rest. Moreover, even if it did decline, it is doubtful that 24 h of a reduced rate-pressure product would be sufficient stimulus to reduce protein synthesis and cause cardiac atrophy (21, 22), and therefore this response is not likely to explain the present findings.

The autonomic regulatory systems controlling blood pressure responses to postural stress include the cardiopulmonary, aortic, and carotid baroreflexes and vestibular inputs. Hypotension evokes an increase in effent sympathetic vasconstrictor activity as well as parasympathetic withdrawal that leads to an increase in HR. With HDBR, the sensitivities of the arterial (12, 18) and cardiopulmonary (9) baroreflexes appear to be reduced. Furthermore, the greatest reduction in baroreflex gain is found in subjects demonstrating the greatest OI after bed rest (8). This impairment appears to be specific for the vasoconstrictor arm of this reflex because HR responses are not impaired (3, 13). The mechanism for the reduced ability to raise sympathetic nerve activity after immobilization is unclear. Recent work by Moffitt et al. (27) in a rat model suggests that the baroreflex impairment after hindlimb unweighting is due to altered central processing of baroreceptor input.

Recently, it was suggested that myogenic responses are impaired after bed rest (11). Reduced myogenic responses could contribute to impaired vasconstriction with postural stress seen after bed rest. However, this would not explain the reduced MSNA responses after bed rest. Nevertheless, the present report does not allow us to exclude an effect of bed rest on myogenic activity.

As mentioned above, 24 h of bed rest does not lead to large changes in plasma volume and/or cardiac atrophy. Therefore, the results of the present study may not explain OI seen after longer periods of bed rest. In this study, subjects underwent a graded LBNP paradigm until presyncope or –60 mmHg level. Thus presyncope was not observed in 5 of 13 subjects before and 2 of 13 subjects after bed rest. This limits our ability to precisely quantify the magnitude of OI in this study. Nonetheless, 11 of 13 subjects had a reduction in the cumulative LBNP index used. Thus we believe our contention that LBNP tolerance was reduced after HDBR is valid.

In conclusion, after 24 h of bed rest, LBNP tolerance was reduced, HR was greater, CO was unchanged, and the ability to augment MSNA at high levels of LBNP was reduced. These findings suggest that 24 h of bed rest is sufficient to interfere with the ability to raise MSNA normally with LBNP.

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REFERENCES


