Changes in the sympathetic nervous system induced by 42 days of head-down bed rest

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1Laboratoire de Physiologie de l'Environnement (Groupement d’Intérêt Public Exercice), Faculté de Médecine Lyon Grange-Blanche, F-69373 Lyon Cedex 08, France; and 2Department of Kinesiology, Faculty of Applied Health Sciences, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Sigaudo, Dominique, J jacques-Olivier Forrat, Anne-Marie Allevard, Alain Maillet, Jean-Marie Cottet-Emard, Annick Vouillarmet, Richard L. Hughson, Guillemette Gauquilin-Koch, and Claude Gharib. Changes in the sympathetic nervous system induced by 42 days of head-down bed rest. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1875–H1884, 1998.—Changes in autonomic nervous system activity could be linked to the orthostatic intolerance (OI) that individuals suffer after a spaceflight or head-down bed rest (HDBR). We examined this possibility by assessing the sympathetic nervous system activity during 42 days of HDBR in seven healthy men. Heart rate variability was studied with the use of power spectral analysis, which provided indicators of the sympathetic (SNSi) and parasympathetic (PNSi) nervous system influences on the heart. Urinary catecholamines and the spontaneous baroreflex sensitivity were measured. Urinary catecholamines decreased by 21.3%, showing a decrease in SNSi. Heart rate variability was greatly reduced during 42 days of HDBR with a drop in PNSi but with no significant changes in SNSi. The baroreflex sensitivity was greatly reduced (30.7%) on day 42 of HDBR. These results suggest a dissociation between the catecholamine response and the SNSi of the heart rate. This dissociation could be the consequence of an increase in β-adrenergic receptor density and/or activity induced by a decrease in catecholamines during HDBR. The subjects who suffered from OI also had a greater sympathetic response and much lower baroreflex sensitivity when supine than those who finished the stand test. However, the mean response of all subjects indicated that the sympathetic activity (catecholamine excretion) was probably slightly inhibited during HDBR and could contribute to OI.

orthostatic intolerance; catecholamines; heart rate variability; spontaneous baroreflex

Many of the physiological consequences of weightlessness and the cardiovascular abnormalities that occur on returning to gravity could be due to changes in the endocrine and autonomic nervous systems. Astronauts who have been exposed to microgravity have some degree of orthostatic hypotension during a postflight stand test, with a reduced mean arterial pressure and excessive cardioacceleration (5), which indicates a disturbed autonomic nervous system. Simulated microgravity has been used to obtain a better understanding of the early and long-term cardiovascular alterations induced by spaceflight (21). The sympathetic nervous system (SNS) could be involved in cardiovascular deconditioning, because it plays a major role in blood pressure control and in renal sodium and fluid handling. A change in this system during simulated microgravity could induce several physiological changes and thus influence blood pressure. The plasma concentration of norepinephrine (NE) is frequently used as a marker of SNS activity. There is a change in plasma NE during head-down bed rest (HDBR), and this change could contribute to the appearance of orthostatic intolerance (OI) at the end of HDBR. However, the data obtained during HDBR are often conflicting. The plasma NE and total circulating NE were found in some studies to be decreased after HDBR (11, 19, 20, 33), but this was not confirmed in other studies (8). Urinary catecholamines have also been used as indicators of plasma NE, but few results have been published to date on this parameter during long-term HDBR (20).

Power spectral analysis of heart rate has recently been used to study the influence of autonomic tone on the heart (1, 2). This noninvasive method estimates cardiac sympathetic and parasympathetic nervous inputs to the sinoatrial node. Several studies have used this method during long-term HDBR, and Hughson et al. (24) have observed a significant increase in SNS activity indicator (SNSi) during and following prolonged bed rest, whereas others did not observe any modifications (13, 35). Several different methods of spectral analysis and different methods of time-series analysis have been used to estimate SNSi during different time durations of HDBR. This could be responsible for the contrasting results in SNSi. However, all authors have reported a significant reduction in parasympathetic nervous system (PNS) activity indicator (PNSi) after HDBR (13, 24, 35). There has also been a report of a decrease in vagal-cardiac outflow and vagally mediated responses to changes in arterial baroreceptor input after spaceflight (16). The baroreflex, which includes the PNS/SNS balance, could be involved in cardiovascular deconditioning, and there is good evidence that the baroreflex sensitivity is reduced after long-term HDBR (8, 9, 14, 23).

The present study was therefore carried out to determine the relationships between SNS activity, as indicated by catecholamines, and the end-organ effect of the SNS on the heart, as assessed by power spectral analysis, during a 42-day period of HDBR. The 42-day period was necessary 1) to permit the other physiological adaptations, such as changes in muscle and bone, to occur during the HDBR, and 2) because 42 days of HDBR offered us the opportunity to determine clearly whether the changes in SNSi are effective. We explored the possible relationships between both catecholamines and spectral analysis indicators and studied any reduction...
in orthostatic tolerance during a 10-min stand test to look for cardiovascular deconditioning. We also measured the circulating concentrations of two hormones that are closely associated with the SNS, active renin (AR) and atrial natriuretic peptide (ANP), during HDBR.

METHODS

Subjects and Protocol

Eight healthy men (age 28 ± 0.9 yr, height 176 ± 1.3 cm, weight 74 ± 3.5 kg) volunteered for this study, which was organized by the European Space Agency and the Centre National d'Etudes Spatiales. The subjects were selected after physical and psychological examinations and were in excellent health, with no history of chronic or recent acute illness. All subjects successfully performed the stand test during selection. Medication, smoking, and caffeine-containing drinks were not allowed during the study. Body mass was measured daily in the morning, before breakfast. One subject withdrew from the experiment after 28 days of HDBR because of back pain. Seven subjects completed the 42 days of HDBR at a 6°-tilt. All subjects were thoroughly briefed about the experimental procedures and signed a consent form approved by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale Midi-Pyrénées I (France).

Experimental Procedure

The subjects remained in the bed-rest facility at Hôpital Purpan during a 15-day ambulatory control period for baseline data collection (BDC) before undergoing HDBR. Subjects remained in -6°-tilt HDBR for 42 days, followed by a 12-day ambulatory recovery period. Subjects were given a diet of 2,500 ± 300 kcal per day, and water intake was limited to 2.5 l/day, except during tests with sodium loading: one 12 days before HDBR, during the control period (BDC –2), and three other on days 28 of HDBR. Water intake was limited because of the importance of hydration in OI. Indeed, we have observed in some protocols that the subjects drank large amounts of water (~3.500 ml). The data from the Na⁺-loading experiments were not included in the statistical analysis. Na⁺ excretion and urine volume returned to normal the day after this experiment.

Biological Data

Plasma. Eight blood samples were taken during the experiment: one 5 days before HDBR (BDC –5); one on each of HDBR 2, 14, 21, 35, and 42; and one on each of days 2 and 7 of recovery (R 2 and R 7). A catheter was inserted into an antecubital vein just after the subject awoke, before breakfast. Subjects sat for 30 min (rest period) before the samples were taken. Blood samples for all hormone assays were collected in heparinized tubes. All blood tubes (except the one for AR) were stored in ice, centrifuged (4°C, 20 min, 3,000 rpm), and then stored at –45°C. Hematocrit (Hct) was determined, and the intra-assay variability was 1.04%. Electrolytes (Na⁺, K⁺) were measured on a Radiometer KNa 1 Analyzer (Copenhagen, Denmark). Plasma ANP and AR were measured by radioimmunoassay. The sensitivity for plasma ANP (18) was 1.5 pg/ml, and the intra- and interassay variabilities were 10 and 12%, respectively. AR was measured with a kit (Renin IRMA Pasteur Kit, ref. 79895). The sensitivity was 1.5 pg/ml, and the intra- and interassay variabilities were 5 and 6%, respectively. Catecholamines, NE (intra- and interassay variabilities were 6 and 8%, respectively), and epinephrine (Epi; intra- and interassay variabilities were 7 and 11%, respectively) were measured by HPLC (34) with electrochemical detection.

Urine. A 24-h urine sample was collected to measure hormones. All hormones and cGMP were measured by radioimmunoassay. The cGMP assay (cyclic GMP [125I] RIA Kit, NEN) had a sensitivity of 1 pmol/ml and intra- and interassay variabilities of 8.2 and 3.2%, respectively. Urinary Na⁺ and K⁺ were measured with the Radiometer K Na 1 Analyzer. Creatinine was determined using the Jaffe method (25). Urinary normetanephrine (NME) and metanephrine (ME) were measured using HPLC (31) with electrochemical detection. All plasma and urine osmolalities were measured on a Fisk One-Ten osmometer (Fiske Associates, Uxbridge, MA).

Cardiovascular Data

Autonomic regulation of the cardiovascular system was assessed by measuring the heart rate variability and the spontaneous baroreflex Data collection and analysis have been described (23, 24). Briefly, R peaks were obtained from a standard bipolar lead electrocardiogram (ECG), and blood pressure was measured with a Finapres 2300 (Ohmeda, Englewood, CO). The R-R interval and systolic (SBP) and diastolic blood pressure (DBP) at each beat were stored on a personal computer via an analog-to-digital converter (DAS-16, Metrabyte) for later analysis. Recordings were long enough to obtain at least 570 beats (~10 min). Measurements were made on control days BDC –3 and –4; during tilt on HDBR 1, 6, 15, 22, 33, 38, and 42; and during recovery on R 2, 6, and 10. Arterial blood pressure and ECG were recorded for 25 min during the ambulatory control and recovery periods. The subjects were supine for 60 min before and during these measurements. The measurements during the HDBR period were completed in the –6°-tilt position. The finger blood pressure cuff was kept at the heart level to avoid hydrostatic pressure effects.
Spectral analysis. The data were filtered to eliminate the small number of abnormal R-R intervals caused by failure to pick up the R wave or by the occasional selection of a T wave. Heart rate variability was analyzed by coarse-graining spectral analysis (CGSA; Ref. 37). CGSA allowed extraction of nonharmonic components (noise) from the spectrum of the original data to give the best spectral estimates for harmonic components. Harmonic spectral power components were divided into low-frequency \( (0.0 < P_{\text{Lo}} < 0.15 \text{ Hz}) \) and high-frequency \( (0.15 < P_{\text{Hi}} < 0.50 \text{ Hz}) \) domains. Pharmacological experiments indicate that high-frequency modulations of heart rate are mediated solely by the PNS, whereas the low-frequency modulations of heart rate are mediated by both the PNS and SNS (32). Normalized indicators were calculated because of this distribution of PNS and SNS. PNSi was computed as the \( P_{\text{Hi}} \)-to-\( P_{\text{Tot}} \) ratio (\( P_{\text{Tot}} \) represents total spectral power), and SNSi as the \( P_{\text{Lo}} \)-to-\( P_{\text{Hi}} \) ratio (37).

Spontaneous baroreflex slope. The R-R interval and SBP for each recording were analyzed for spontaneous baroreflex events (3). Baroreflex events were defined by at least three consecutive beats in which the SBP and the R-R interval of the following beat both either increased or decreased. A linear regression was then calculated for each baroreflex event. The slope of each individual event was computed, and the mean slope was determined as the average of all slopes within a given period. This represented the spontaneous baroreflex sensitivity. In this experiment we calculated the baroreflex slope during a 10-min period. At least 50 sequences were included in each average.

Stand Test
The stand test began with a period of 30 min supine (0° angle). Subjects next sat up for 6 min and then stood for \( \pm 10 \) min. The heart rate, SBP, and DBP were monitored during the stand test. The stand test was stopped when SBP decreased by \( -30 \text{ mmHg} \) below the initial value and heart rate increased by \( 15 \text{ beats/min} \), or if the subjects showed signs of presyncope (pallor, nausea, tachycardia, etc.).

Statistical Analysis
Data were analyzed using two-tailed Wilcoxon or Mann-Whitney tests, corrected for ties when appropriate. Values are expressed as means \( \pm SE \), and \( P < 0.05 \) was considered statistically significant. Urine volume, \( Na^+ \) excretion, and urine cGMP data obtained during HDBR were compared with the averages for the last 7 days of the control period.

RESULTS
The subjects were confined during the control period to adapt to the hospital environment and the experiments. The subjects were not psychologically depressed during the HDBR. This adaptation led to a decrease in body mass during the control period. The average body mass decreased by \( -2.59 \pm 0.86\% \) (\( P < 0.05 \)) after 42 days of HDBR (Fig. 1).

Plasma
AR (Fig. 2A) had increased (\( P < 0.05 \)) by 122% after 42 days of HDBR compared with the control value. Plasma ANP (Fig. 2B) had decreased (\( P < 0.05 \)) by 41% after 42 days of HDBR compared with the control value. Resting NE and Epi were not changed during HDBR (Table 1). Similarly, plasma \( Na^+ \) and \( K^+ \) and osmolality during the control and the HDBR periods were unchanged (Table 1).

We have determined the plasma volume (PV) variation according to the equation
\[
\%\text{PV} = 100 \left[ (Hb_b \left( 1 - Hct_A \times 10^{-2} \right) / (Hb_a \left( 1 - Hct_B \times 10^{-2} \right) ) - 1 \right] / \text{Hb}_b - \text{Hct} \text{ values, respectively, measured before (B) BDC and after (A) HDBR 42. This equation indicated a reduction in PV of 10.6% (Table 1). The hematocrit increased during HDBR (Table 1) compared with the control period, which indicates a loss in PV.}

Urine Excretion
The 24-h urine volume was not altered on HDBR 1 (Fig. 3A), whereas \( Na^+ \) excretion was higher than the
control value (the mean of the last 7 days of the control period). Urine volume was decreased (22%, \( P < 0.05 \)) on HDBR 3 below the control value, and Na\(^+\) excretion (Fig. 3B) was also lower (\( P < 0.05 \)) during HDBR than during the control period. Urinary osmolality, solute excretion, K\(^+\), creatinine, and free water clearance were not altered during HDBR (Table 1). Urinary cGMP (Fig. 2C) decreased (36%, \( P < 0.05 \)) during HDBR but returned to baseline during the recovery period. ME and NME (Fig. 4, A and B) were both 20% lower than their respective control values. The excretion rate of NME was higher on R 1 and R 12 than during HDBR. In contrast, the excretion of MN returned to the baseline value during the recovery period.

**Spectral Analysis**

\( P_{\text{Tot}} \) (Fig. 5A) was lower (41%, \( P < 0.05 \)) during the 42 days of HDBR than during the control period, showing a decrease in heart rate variability. These values did not return to baseline after 2 days of recovery. PNSi (\( P_{\text{Hi}}/P_{\text{Tot}} \)) (Fig. 5B) during HDBR was higher (47%) than during control, whereas SNSi (\( P_{\text{Lo}}/P_{\text{Hi}} \)) was unchanged. \( P_{\text{Lo}}/P_{\text{Tot}} \) was also unchanged during HDBR (Fig. 5C).

**Table 1. Plasma electrolyte and catecholamine values**

<table>
<thead>
<tr>
<th></th>
<th>BDC</th>
<th>HDBR 2</th>
<th>HDBR 14</th>
<th>HDBR 21</th>
<th>HDBR 35</th>
<th>HDBR 42</th>
<th>R 2</th>
<th>R 7</th>
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<tr>
<td><strong>Plasma</strong></td>
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</tr>
<tr>
<td>Osmolality, mosmol/kg( \text{H}_2\text{O} )</td>
<td>303 ± 1.6</td>
<td>304 ± 2.2</td>
<td>307 ± 2.9</td>
<td>306 ± 1.8</td>
<td>300 ± 2.4</td>
<td>300 ± 1.4</td>
<td>296 ± 2.3*</td>
<td>296 ± 2.1*</td>
</tr>
<tr>
<td>Na(^+), meq/l</td>
<td>137.9 ± 1.1</td>
<td>137.3 ± 1.1</td>
<td>139 ± 1.1</td>
<td>139.3 ± 2.4</td>
<td>139.7 ± 1.4</td>
<td>138 ± 1.1</td>
<td>137.4 ± 0.8</td>
<td>138 ± 1</td>
</tr>
<tr>
<td>K(^+), meq/l</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>44.0 ± 0.9</td>
<td>45.3 ± 1.3</td>
<td>47.6 ± 0.9*</td>
<td>46.1 ± 1.2</td>
<td>46.0 ± 0.7*</td>
<td>46.3 ± 0.6*</td>
<td>44.1 ± 0.8</td>
<td>39.7 ± 0.6*</td>
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<tr>
<td>NE, pg/ml</td>
<td>146 ± 24</td>
<td>135 ± 18</td>
<td>135 ± 27</td>
<td>126 ± 23</td>
<td>156 ± 29</td>
<td>194 ± 43</td>
<td>199 ± 46</td>
<td>177 ± 26</td>
</tr>
<tr>
<td>Epi, pg/ml</td>
<td>18 ± 1.7</td>
<td>18 ± 1.6</td>
<td>17 ± 1.5</td>
<td>16.7 ± 1.3</td>
<td>17.6 ± 1.4</td>
<td>17.7 ± 1.3</td>
<td>18.4 ± 1.3</td>
<td>18 ± 2.4</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Osmolality, mosmol/kg( \text{H}_2\text{O} )</td>
<td>504 ± 47</td>
<td>469 ± 45</td>
<td>464 ± 32</td>
<td>469 ± 39</td>
<td>463 ± 31</td>
<td>517 ± 69</td>
<td>455 ± 39</td>
<td>347 ± 23*</td>
</tr>
<tr>
<td>Free water clearance, ml/min</td>
<td>0.71 ± 0.14</td>
<td>0.62 ± 0.16</td>
<td>0.64 ± 0.10</td>
<td>0.72 ± 0.15</td>
<td>0.68 ± 0.12</td>
<td>0.72 ± 0.17</td>
<td>0.48 ± 0.08</td>
<td>0.23 ± 0.13*</td>
</tr>
<tr>
<td>Solute excretion, mosmol·kg( \text{H}_2\text{O} )^{-1}·24 h^{-1}</td>
<td>876 ± 280</td>
<td>928 ± 237</td>
<td>929 ± 258</td>
<td>1,091 ± 344</td>
<td>912 ± 218</td>
<td>882 ± 278</td>
<td>632 ± 134*</td>
<td>795 ± 188</td>
</tr>
</tbody>
</table>

Values are means ± SE. Plasma electrolytes, plasma catecholamines, and urinary hydroelectrolytic parameters were measured during the control period [baseline date collection (BDC)] and during and after (R) 42 days of head-down bed rest (HDBR). NE, norepinephrine; Epi, epinephrine. *\( P < 0.01 \), †\( P < 0.05 \) vs. BDC.
Heart Rate, Blood Pressure, and Spontaneous Baroreflex Responses

The resting heart rate increased ($P < 0.05$) during HDBR (Fig. 6C). SBP (Fig. 6B) was increased on HDBR 6, 10, and 15 ($P < 0.05$) and then returned to baseline. The spontaneous baroreflex slopes (Fig. 6A) were lower (31%, $P < 0.01$) during HDBR than during the control period. The drop occurred early in HDBR (HDBR 6) and remained at that level.

Stand Test

All seven subjects successfully performed the stand test during the control period (Table 2). Of the tests done after 42 days of HDBR, four tests were stopped because those subjects (B, E, F, and G) had symptoms of OI with increased heart rate and decreased SBP (Table 2). We have separated the subjects who completed the stand test (no-OI) from those who failed (OI) to get a better perspective on SNS changes after HDBR.

OI and no-OI groups during control period. The OI subjects had a higher plasma NE than the no-OI group (Table 3), and the baroreflex sensitivity of OI subjects was lower than that of the no-OI subjects.

OI and no-OI groups on HDBR 42. The changes in response to HDBR 42 are shown in Table 3. The main differences between the OI and no-OI groups were that the OI group had a greater increase in plasma NE and a lower baroreflex sensitivity between BDC and HDBR 42 compared with the no-OI group. Both OI and no-OI subjects had an increase in AR between BDC and HDBR 42.

OI and no-OI groups during $R_1$ and $R_2$. Four of the seven subjects did not complete the test on $R_1$ because of OI. SBP was increased in both OI and no-OI subjects compared with BDC (Table 3). The main difference on $R_1$ was a great increase in SNSi for OI compared with BDC and the no-OI group. The baroreflex sensitivity was still altered for the OI group compared with BDC and the no-OI group. Urinary NME had increased markedly in the OI group, whereas it had decreased in the no-OI group. Plasma NE, AR, and urinary NME on $R_2$ were more greatly increased for the OI group than for the no-OI group and BDC. PNSi on $R_2$ was lower in the OI group than in the no-OI group and BDC.

DISCUSSION

Orthostatic tolerance can be defined as the capacity of the cardiovascular system to maintain arterial pressure so that an individual can tolerate standing upright. Cardiovascular deconditioning occurs after a
It has been speculated that the increased susceptibility to fainting is due to several factors, including hypovolemia, hormonal changes, altered venous compliance, muscle atrophy, and changes in the cardiovascular system with altered autonomic nervous system regulation. All these factors probably contribute to OI, but we hypothesized, as have other authors (33), that the SNS and catecholamines might play key roles if we consider the increase in heart rate and the hypovolemia. In our study, four of the seven subjects suffered from OI during the stand test at the end of HDBR.

Fig. 5. Total power spectrum ($P_{Tot}$), parasympathetic nervous system activity indicator ($PNSI$) represented by ratio of high-frequency spectral power domain ($P_{Hi}$) to $P_{Tot}$ ($P_{Hi}/P_{Tot}$), and ratio of low-frequency spectral power domain ($P_{Lo}$) to $P_{Tot}$ ($P_{Lo}/P_{Tot}$) before (BDC), during (HDBR), and after (R) 42-day HDBR. Gray bars, HDBR; black bars, control and recovery periods. Values are means ± SE. * $P < 0.05$ vs. BDC.

Fig. 6. Spontaneous baroreflex slope (SBS), heart rate (HR), and systolic blood pressure (SBP) for supine subjects before (control), during (HDBR), and after (R) 42-day HDBR. Gray bars, HDBR; black bars, control and recovery periods. Values are means ± SE. * $P < 0.05$, ** $P < 0.01$ vs. BDC.
Urinary catecholamines were significantly decreased during HDBR, suggesting an overall reduction in the activation of the SNS. Heart rate was increased during HDBR, with an increase in blood pressure followed by a decrease to basal level. As in previous studies, the baroreflex sensitivity decreased, as did heart rate variability, with a reduction in the influence of the PNS on the heart. There were also changes in blood volume-regulating hormones. Thus the experiment caused cardiovascular deconditioning and increased the OI.

**PV and Catecholamine**

Spaceflight and long-term HDBR are known to reduce the PV. Johansen et al. (26) measured the PV in this HDBR by using the Evans blue method and observed a significant decrease (11.9 ± 1.7%) in PV associated with an increase in Hct after 42 days of HDBR. We have determined the PV variation according to the equation described in RESULTS. This equation indicated a reduction in PV of 10.6%, a finding consistent with the results from the Evans blue method. The body weight also decreased up to HDBR 42 (−2.59 ± 0.86%). There was a greater reduction in body weight (>3%) in a previous HDBR experiment (28 days), with the same drop in PV (−11.2%) (29). Some of the changes in blood volume-regulating hormones found in this HDBR could be linked to the decrease in PV. It was reported that the decrease in ANP and in its second messenger, cGMP, were linked to the decrease in PV (18, 21). There was a higher Na⁺ excretion without a change in urine volume on HDBR 1. The fluid balance also changed significantly in response to HDBR (on HDBR 1), although the urine volume was not increased. The fluid intake decreased, and the balance was negative. Urinary Na⁺ excretion gradually decreased, which corresponded with a renal Na⁺ reabsorption, during the 42-day HDBR. This reabsorption could be the consequence of the increases in plasma AR and aldosterone that occur in response to hypovolemia during HDBR (19). The increase in AR (122%) is similar to that described in other studies (19, 35). During the first few hours of HDBR the cephalad fluid shift induces a decrease in renin concentration. These changes in renin are for the most part controlled by the SNS and β-receptors (22). The drop in PV that is associated with an increase in active renin is likely to be due to increased sympathetic activity, but this does not agree with the changes in urinary catecholamine during HDBR. The urinary excretion of NME and ME was significantly lower than during baseline up to HDBR 27. However, NME and ME are not specific markers of the renal sympathetic system but are more generally markers of the whole sympathetic activity. Other factors could explain this discrepancy, such as the glomerular-tubular balance (as there is a loss of Na⁺ during the experiment). A dissociation between plasma NE and AR

### Table 2. Stand test data

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Control</th>
<th>End</th>
<th>Change, %</th>
<th>Control</th>
<th>End</th>
<th>Change, %</th>
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<tbody>
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<td>A</td>
<td>111</td>
<td>117</td>
<td>5.4</td>
<td>77</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>114</td>
<td>135</td>
<td>5.8</td>
<td>84</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>105</td>
<td>112</td>
<td>5.1</td>
<td>88</td>
<td>10</td>
<td></td>
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<tr>
<td>E</td>
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<td>120</td>
<td>3.9</td>
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<tr>
<td>F</td>
<td>106</td>
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<td>6.7</td>
<td>88</td>
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<td>G</td>
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<td>H</td>
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<td>108</td>
<td>6.1</td>
<td>98</td>
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<td></td>
</tr>
</tbody>
</table>

Duration values are duration of stand tests performed during initial control (baseline data collection day – 3 (BDC – 3) and recovery (R 1) periods). Heart rate (HR) and systolic blood pressure (SBP) were measured before (control) and at end of stand test.

### Table 3. OI and no-OI subjects before, during, and after HDBR

<table>
<thead>
<tr>
<th>OI Group (n = 4)</th>
<th>No-OI Group (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR</td>
<td>R 1</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>62 ± 5</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>114 ± 3</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>SNSi</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>PNSi</td>
<td>0.07 ± 0.015</td>
</tr>
<tr>
<td>Baroreflex sensi-</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>tivity, ms/mmHg</td>
<td>AR, pg/ml</td>
</tr>
<tr>
<td>Plasma NE, pg/ml</td>
<td>173 ± 17</td>
</tr>
<tr>
<td>Urine NME, µg/D</td>
<td>178 ± 16</td>
</tr>
</tbody>
</table>

Values are means ± SE for subjects with and without orthostatic intolerance (OI) during control period (BDC), on HDBR 42, and on R 1 and R 2. DBP, diastolic blood pressure; SNSi and PNSi, sympathetic and parasympathetic nervous system activity indicators; AR, active renin; NME, normetanephrine; D, diuresis. *P < 0.05, †P < 0.01 vs. BDC; ‡P < 0.05, §P < 0.01 vs. OI group.
has been reported in men and animals (8, 15). Plasma NE was measured only once a week in this study, and this was not frequent enough for us to use it as a marker of the sympathetic nervous activity. The present urinary catecholamine results suggest that there is sympathetic inhibition during HDBR.

Conflicting results have been obtained concerning the possible changes in sympathoadrenal activity in previous experiments simulating the hemodynamic effects of microgravity. Although no change in plasma NE after long-term HDBR has been observed (8), others have reported a decrease in plasma and urinary NE after 10–14 days of HDBR (11, 20, 28, 33). The conflicting results could be linked to the control period position. There are differences in plasma NE in supine and sitting subjects (8). However, some authors (33) believe that the reduced plasma catecholamine is an adaptation to space and HDBR, which could lead to an increase in the number and affinity of β-adrenoceptors. This hypothesis has also been supported by the results of Convertino et al. (11).

Urinary catecholamines were significantly higher during the recovery period than during the control period. This suggests that the sympathoadrenal system is activated in response to blood pooling and physical activity. A greater sympathetic activity may be necessary to support adequate blood pressure and cerebral perfusion during the orthostatic stress that occurs during readaptation to gravity (36). In our experiment, reexposure to gravity resulted in an enhanced sympathetic response. However, four subjects suffered from OI at the end of HDBR. This could suggest that the increase in catecholamines reported during the recovery period was not effective in the maintenance of blood pressure during the stand tests immediately after HDBR.

Autonomic Regulation of Cardiovascular System

Power spectral analysis was used to study heart rate variability during HDBR. Several factors could affect heart rate variability. The HDBR position modulates the plasma concentration of vasoactive hormones, and these hormones could affect heart rate variability (2, 6, 7). Overall R-R variability was markedly reduced in this study, as indicated by the decrease in $P_{\text{Tot}}$, $P_{\text{Lo}}$, and $P_{\text{PNSi}}$ were significantly decreased, whereas $SNSi$ was unchanged. To eliminate the influence of variation in $P_{\text{Tot}}$ at low frequency, we calculated $P_{\text{Lo}} / P_{\text{Tot}}$, which was unchanged during HDBR. The $P_{\text{NSi}}$ and $SNSi$ results were consistent with those found by Crandall et al. (13), who suggested that increases in heart rate after 15 days of HDBR may be attributed to reduced cardiac vagal activity, whereas changes in cardiac sympathetic activity are less evident. Hughson et al. (24) observed a significant reduction in the $P_{\text{NSi}}$ and a significant increase in the $SNSi$ after a 28-day HDBR. The $SNSi$ of the heart was not statistically altered during HDBR, whereas the urinary NME was significantly lower than the control value. There was no correlation between NME, a marker of overall SNS activity, and the heart rate-specific marker of SNSi. NME is a marker of SNS, whereas SNSi is a heart response. We observed a decrease in SNS activity, but the heart effect of the SNS was unchanged during the HDBR. Kingwell et al. (27) reported a dissociation between heart rate spectral analysis measurements and cardiac NE spillover at rest in healthy subjects. Heart rate variability is an end-organ response determined by nerve firing and electrochemical coupling, but cardiac adrenergic receptor sensitivity, postsynaptic signal transduction, and multiple neural reflexes also play an important role (27). As mentioned previously, one hypothesis could be that an increase in the density and sensitivity of adrenoreceptors during HDBR could compensate for a real decrease in SNS activity and could explain our results (33).

The baroreflex sensitivity was decreased by HDBR 6 and continued so through 42 days of HDBR and into recovery. This alteration occurred before the change in PNS/SNS balance (HDBR 15). Previous studies observed this alteration for different durations of HDBR using the same method or by measuring carotid baroreceptor cardiac responses with a neck chamber (8, 23, 35). There was also an increase in heart rate and a change in the spontaneous baroreflex sensitivity, whereas resting blood pressure was not altered. The reduction in baroreflex sensitivity could reflect changes in the R-R interval. This relationship was also observed in a previous HDBR (8), but other studies have documented no change in reflex gain, whereas the R-R interval was altered (12). Moreover, given the reproducibility of the method (23), the mean slope is not influenced by the number of sequences used to obtain the mean for that subject/test condition.

The neck chamber device for measurement of carotid baroreflex function can provide additional information about the range and operating point of the baroreflex (8, 14, 16). The spontaneous baroreflex focuses on the response of the R-R interval to fluctuations in arterial blood pressure around the operating point (23). In this sense, it does not provide information about the potential to buffer a wider range of arterial blood pressure. We did observe a range in the R-R interval from 800 to 1,300 ms and a range in SBP from 100 to 150 mmHg during the resting measurements, and these ranges were not affected by HDBR. It has been established that the slope of the spontaneous baroreflex is very similar to, and highly correlated with, the tangent to the slope of the full baroreflex-response curve obtained by pharmacological manipulation of arterial blood pressure (30). In the present study, the spontaneous baroreflex method was capable of clearly showing a change in the response of the R-R interval to spontaneous fluctuations in blood pressure following 42 days of HDBR.

Comparison of OI and No-OI Groups

We also compared the subjects who showed an OI (OI group) and those who did not (no-OI group). This comparison was limited by the small number of subjects in each group (4 OI subjects and 3 no-OI subjects). The main difference between the four OI subjects and the others was that the OI subjects had lower spontane-
ous baroreflex sensitivity after 42 days of HDBR and higher plasma AR and NE concentrations in the supine position.

Baroreflex sensitivities were different between OI and no-OI groups during BDC, being lower for OI than for the no-OI group. The OI baroreflex sensitivity on HDBR 42 was still decreased to <10 ms/mmHg, whereas baroreflex sensitivity for no OI was ~20 ms/mmHg. These differences in baroreflex sensitivity between basal and HDBR 42 could be a contributor to the different responses of the cardiovascular system of OI and no-OI subjects to standing. The OI group had a greater sympathetic response, as shown by the large increases in their plasma NE and AR in the ~6°-tilt position before standing, than did the no-OI group. The OI subjects also had greater increases in SNSi plasma NE and AR and a greater decrease in baroreflex sensitivity than the no-OI group during the recovery period in the supine position. These alterations were not surprising because of the differences before HDBR. These differences, observed in the supine position, could be responsible for the appearance of OI during standing. The increase in the SNS activity was not effective with standing for the OI subjects, and the presyncopal state could be favored by a lower spontaneous baroreflex sensitivity before standing. Presyncopal astronauts have a lower peripheral vascular resistance on the day of landing than nonpresyncopal subjects (4). This could reflect an inappropriate vasodilatation rather than inadequate vasoconstriction (33). An alteration of sympathetic efferents or end-organ responsiveness could lead to an altered total peripheral resistance response. Fritsch-Yelle et al. (17) reported differences in venous compliance and/or vascular responsiveness before flight and showed a subnormal increase in NE on standing in those astronauts who experienced presyncope on landing day. Some subjects were susceptible to OI immediately after spaceflight or HDBR and recovered spontaneously within a few days.

In conclusion, the results for subjects who underwent HDBR showed a decrease in the SNS activity as indicated by the significant decrease in urinary catecholamines. The urinary catecholamines were elevated during the recovery period, showing that the SNS was able to respond after HDBR and was not impaired by long-term HDBR. However, the response did not prevent OI for all subjects. This study also suggests that subjects who have a great sympathetic response and a low baroreflex sensitivity during the control period and on the last day of HDBR are susceptible to OI during the stand test, but more subjects are needed to confirm this.

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