

Effect of head-down-tilt bed rest and hypovolemia on dynamic regulation of heart rate and blood pressure

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Iwasaki, Ken-Ichi, Rong Zhang, Julie H. Zuckerman, James A. Pawelczyk, and Benjamin D. Levine. Effect of head-down-tilt bed rest and hypovolemia on dynamic regulation of heart rate and blood pressure. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R2189–R2199, 2000.—Adaptation to head-down-tilt bed rest leads to an apparent abnormality of baroreflex regulation of cardiac period. We hypothesized that this “deconditioning response” could primarily be a result of hypovolemia, rather than a unique adaptation of the autonomic nervous system to bed rest. To test this hypothesis, nine healthy subjects underwent 2 wk of -6° head-down bed rest. One year later, five of these same subjects underwent acute hypovolemia with furosemide to produce the same reductions in plasma volume observed after bed rest. We took advantage of power spectral and transfer function analysis to examine the dynamic relationship between blood pressure (BP) and R-R interval. We found that 1) there were no significant differences between these two interventions with respect to changes in numerous cardiovascular indices, including cardiac filling pressures, arterial pressure, cardiac output, or stroke volume; 2) normalized high-frequency (0.15–0.25 Hz) power of R-R interval variability decreased significantly after both conditions, consistent with similar degrees of vagal withdrawal; 3) transfer function gain (BP to R-R interval), used as an index of arterial-cardiac baroreflex sensitivity, decreased significantly to a similar extent after both conditions in the high-frequency range; the gain also decreased similarly when expressed as BP to heart rate \times stroke volume, which provides an index of the ability of the baroreflex to alter BP by modifying systemic flow; and 4) however, the low-frequency (0.05–0.15 Hz) power of systolic BP variability decreased after bed rest (-22%) compared with an increase ($+155\%$) after acute hypovolemia, suggesting a differential response for the regulation of vascular resistance (interaction, $P < 0.05$). The similarity of changes in the reflex control of the circulation under both conditions is consistent with the hypothesis that reductions in plasma volume may be largely responsible for the observed changes in cardiac baroreflex control after bed rest. However, changes in vasomotor function associated with these two conditions may be different and may suggest a cardiovascular remodeling after bed rest.

plasma volume; baroreflex; spectral analysis; men; furosemide; microgravity

SPACEFLIGHT INDUCES A SERIES of cardiovascular changes resulting in reduced postflight orthostatic tolerance and exercise capacity (5, 26). Reflex control of the circulation appears to be altered after cardiovascular adaptation to spaceflight or simulated microgravity (bed rest), leading to orthostatic tachycardia in all, and hypotension in up to two-thirds, of astronauts after short-term (1–2 wk) spaceflight (5). For example, recent data have suggested that the carotid baroreflex regulation of cardiac period may be reduced after spaceflight or bed rest (7, 12, 15, 16) and is associated with the orthostatic intolerance (7). Moreover, studies examining spontaneous changes in R-R interval and blood pressure also have shown that integrated arterial baroreflex control of cardiac period, including both carotid and aortic baroreceptors, may be reduced after bed rest (20, 35). In contrast, isolated aortic baroreflex regulation of heart rate actually may be augmented after short-term bed rest (8).

One of the key adaptations to spaceflight or bed rest “deconditioning” is a reduction in plasma volume (5, 7). Despite extensive study of cardiovascular reflex control after spaceflight or bed rest, it is unclear whether the observed changes in baroreflex function after bed rest are due to hypovolemia alone or represent a unique adaptation of the autonomic nervous system to bed rest deconditioning. Acute hypovolemia seems to change the cardiac baroreflex similarly compared with bed rest or spaceflight. For example, central hypovolemia by head-up tilt reduces carotid-cardiac R-R interval baroreflex gain as assessed with a neck chamber (17); similarly acute reductions in central blood volume by lower body negative pressure (LBNP) or head-up tilt reduce the integrated arterial-cardiac baroreflex by using a sequence method (blood pressure to R-R interval) or transfer function analysis of blood pressure to R-R interval (20, 21) or blood pressure to heart rate (50); acute hypovolemia by furosemide augments aortic-cardiac baroreflex regulation of heart rate (6). Conversely, another study using a neck chamber technique has suggested that carotid baroreflex regulation of

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cardiac period may not change after acute hypovolemia (49). However, there is no available study that directly compares the effect of bed rest on dynamic regulation of the circulation with that of hypovolemia in the same subjects. Thus it is difficult to draw a clear conclusion regarding the role that changes in plasma volume may play in circulatory control after bed rest deconditioning.

In the present study, we directly compared the effects of bed rest (hypovolemia plus deconditioning) and acute hypovolemia alone to determine whether changes in the reflex control of the circulation, including dynamic arterial-cardiac baroreflex control of R-R interval and blood pressure, after bed rest is due to the reduction of plasma volume. We hypothesized that the change in baroreflex sensitivity after bed rest could be reproduced both qualitatively and quantitatively by acute hypovolemia, even in the absence of the deconditioning associated with bed rest. To test this hypothesis, we quantified baroreflex sensitivity by using transfer function analysis between spontaneous changes in arterial pressure and R-R interval before and after both 2 wk of head-down-tilt bed rest and, after recovery, an acute hypovolemia induced by furosemide in the same subjects to reproduce exactly the plasma volume loss of bed rest.

METHODS

Subjects

Nine healthy men, with a mean age of 25 ± 6 yr, height of 178 ± 4 cm, and weight of 78 ± 10 kg, were studied in the head-down bed rest experiment. These subjects are a subset of those previously reported with regard to their left ventricular pressure-volume relationship (28). One year later, five of these same subjects (age 24 ± 6 yr, ht 175 ± 3 cm, wt 76 ± 6 kg at the time of bed rest study; age 25 ± 6 yr, ht 175 ± 3 cm, wt 76 ± 6 kg at the time of acute hypovolemia study) returned to the laboratory and were studied in the acute hypovolemia phase. No subject smoked, used recreational drugs, or had significant chronic medical problems. No subject was an endurance-trained athlete, and subjects were excluded if they exercised for >30 min/day, more than three times per week with either dynamic or static exercise. Subjects were screened with a careful history and physical examination including electrocardiogram (ECG) and echocardiogram. All subjects signed an informed consent form approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center at Dallas and Presbyterian Hospital of Dallas.

Instrumentation

All experiments were performed in the morning, at least 2 h after a light breakfast and >12 h after the last caffeinated beverage or alcohol, in a quiet, environmentally controlled laboratory with an ambient temperature of 25°C . A 6-F balloon-tipped, flow-directed pulmonary arterial catheter (Edwards 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 position, which was confirmed fluoroscopically and by the presence of characteristic pressure waveforms. Right atrial pressure (RAP) and pulmo-

nary capillary wedge pressure (PCWP) were referenced to atmospheric pressure, with the pressure transducer (Transpac IV, Abbott) zero reading set at 5 cm below the sternal angle in the supine position. The pressure waveform was amplified (Hewlett-Packard 78534A and Astromed ASC909) and displayed on a strip-chart recorder (Astromed MT 95000) with 0.5-mmHg resolution. The mean PCWP was determined at end expiration.

An analog ECG was obtained from a CM_5 lead, and continuous arterial blood pressure was obtained at the finger by photoplethysmography (Finapres, Ohmeda) at heart level. Intermittent blood pressure was measured in the arm by electrophygmomanometry (Suntech) with a microphone placed over the brachial artery and the Korotkoff sound detection gated to the ECG.

Protocol

After at least 30 min of supine rest, the initial baseline experiments were started. The subjects were asked to control their respiratory frequency at a fixed rate of 12 breaths/min (0.20 Hz) by following a metronome tone. This rate was chosen to most closely match the subjects' spontaneous respiratory rate, to minimize the influence of respiratory control per se on measured variables. After a 5-min quiet adjustment period of fixed breathing, 5-min segments of arterial blood pressure and ECG were recorded for spectral and transfer function analysis (47). After this data-collection period, plasma volume was measured by using the Evans blue dye technique (14). Orthostatic tolerance was determined on a separate day (bed rest experiments) or the same day (acute hypovolemia experiments) by using LBNP according to the following protocol: -15 mmHg \times 5 min, -30 mmHg \times 5 min, -40 mmHg \times 5 min, and -50 mmHg \times 3 min; then, negative pressure was increased by -10 mmHg for every 3 min to the tolerance limit. The LBNP was discontinued if the subject developed signs and/or symptoms of presyncope. Presyncope was defined as a decrease in systolic blood pressure (SBP) to <80 mmHg; a decrease in SBP to <90 mmHg associated with symptoms of lightheadedness, nausea, or diaphoresis; or progressive symptoms of presyncope accompanied by a request from the subject to discontinue the test. Orthostatic tolerance was assessed by using a cumulative stress index, calculated as the sum of the products of duration of LBNP and the magnitude of the negative pressure at each level (mmHg \times minutes).

The pulmonary arterial catheter was always inserted on the day of the experiment, then removed as soon as the experiment was completed. The average duration of catheter placement was 5–7 h.

Head-Down-Tilt Bed Rest (Simulated Microgravity)

After the initial baseline experiments, the subjects were placed at complete bed rest with -6° head-down tilt. Subjects were allowed to elevate up on one elbow for meals, but otherwise were restricted to the head-down position at all times. Subjects were given a standard diet consisting of $2,827 \pm 609$ cal/day, including 5.2 ± 1.2 mg/day of sodium. Fluids were allowed ad libitum, but all fluid intake and urine output were carefully recorded. The same experiments were repeated after 2 wk of head-down-tilt bed rest.

Acute Hypovolemia

The initial baseline experiments were conducted in the same way as those in the head-down-tilt bed rest and were performed at least 1 yr after recovery from bed rest. For the

acute hypovolemia studies, these experienced subjects were given detailed instructions for diet and fluid balance similar to the diet ingested during the head-down-tilt bed rest studies. One to two weeks after these initial experiments, acute hypovolemia was induced by administration of furosemide 20 mg (iv) for each subject. This dosage was selected in pilot studies to induce a similar degree of reduction of plasma volume as that observed after the head-down-tilt bed rest. After administration of furosemide, the decreases of RAP and PCWP were monitored continuously. When the pressures matched with those observed previously after the head-down-tilt bed rest and were stable for at least 30 min, the second experiments were started. The time duration for the RAP and PCWP to reach this point was ~ 2 h. Of note, although the protocol allowed for additional doses of furosemide in case the target end point was not achieved, or supplemental infusion of saline in case of overshoot, in reality no additional manipulations were necessary for any subject. Then, a blood sample was drawn for measurement of plasma norepinephrine and estimation of changes in plasma volume. The plasma norepinephrine was measured by an independent reference laboratory (Arup Laboratories, Salt Lake City, UT) by using high-precision liquid chromatography. The magnitude of reduction of plasma volume after furosemide was calculated from the changes in hematocrit by using the Dill formula (10). The same data-collection procedures as the initial baseline experiments were then repeated.

Data Analysis

The analog ECG and arterial pressure were sampled simultaneously at 1 kHz and digitized at 12 bits (Metrabyte, DAS-20). The beat-to-beat values of R-R interval, heart rate, and SBP were obtained by using a custom program for peak detection, and were linearly interpolated and resampled at 1 Hz to create an equidistant time series for spectral and transfer function estimation (1). The time series of R-R interval, heart rate, and SBP were first detrended with third-order polynomial fitting and then subdivided into 128-point segments with 50% overlap. This process resulted in four segments of data over the 5-min period of data collection. Fast Fourier transforms were implemented with each Hanning-windowed data segment (21, 22) and then averaged to calculate the autospectra of SBP and R-R interval (Figs. 1 and 2). This data-acquisition and -processing strategy conforms to recommendations of international consensus panels for the assessment of cardiovascular variability (47). The minimal resolution of these spectra is ~ 0.0078 Hz.

High-frequency power of R-R interval variability and SBP in the range of 0.15–0.25 Hz and low-frequency power in the range of 0.05–0.15 Hz were calculated from the integration of the autospectra. The ratio of low-frequency to high-frequency power of R-R interval variability was also calculated because of the suggestion by some investigators that the ratio might reflect cardiac sympathovagal balance (29, 32). These values were also divided by the total spectral power to minimize the effect of the changes in total power on the individual values of low- and high-frequency components (29, 32, 47). This approach derives normalized spectral indexes.

The transfer gain, phase, and coherence (the squared coherence function) between SBP and R-R interval (or heart rate) were estimated by using the cross-spectral method (32, 44, 50). The low- and high-frequency transfer function gain, phase, and coherence were estimated as mean values in the same frequency ranges as above. The transfer function gain between spontaneous changes in the SBP and R-R interval

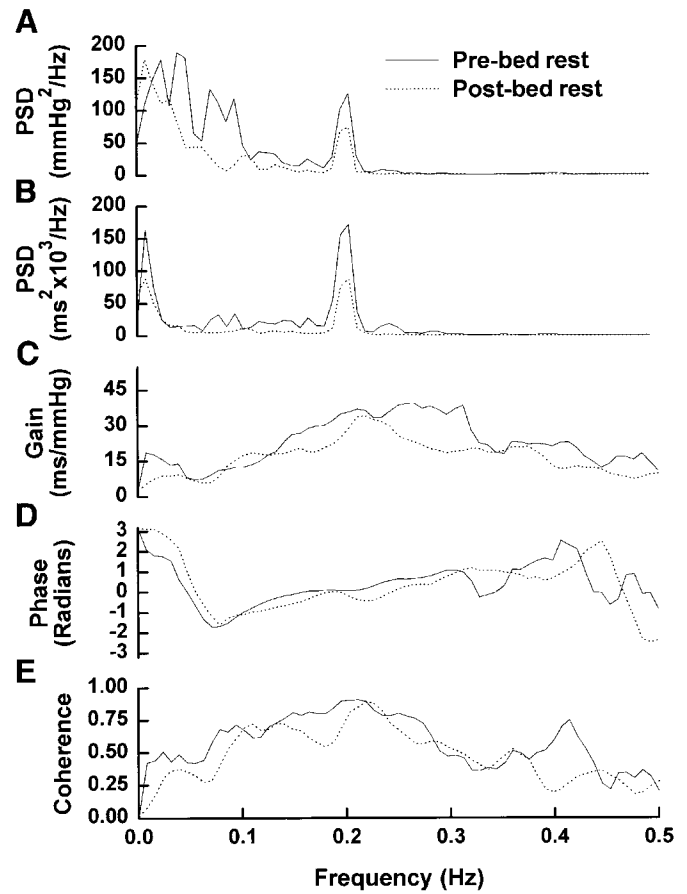


Fig. 1. Representative frequency-domain analysis of changes in R-R interval and systolic blood pressure in a subject before and after head-down-tilt bed rest. *A*: power spectral density (PSD) of systolic blood pressure. *B*: power spectral density of R-R interval. *C*: transfer-function gain between systolic blood pressure and R-R interval. *D*: phase relationship between systolic blood pressure and R-R interval. *E*: coherence function.

was used to reflect changes in R-R interval in response to changes in SBP mediated by baroreflex function, whereas the estimated phase was used to reflect the time relationship between these two variables (44). The assumption of the linearity and reliability of transfer function estimation was evaluated by the coherence, which ranges between zero and one.

In addition, the calculated gain for heart rate (in $\text{beats} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$) was multiplied by the resting stroke volume to determine the effective change in systemic flow (i.e., cardiac output) induced by baroreflex-mediated changes in heart rate, or the “effective gain” of the baroreflex regulation of heart rate (25).

Statistical Analysis

Variables were compared before and after head-down-tilt bed rest ($n = 9$) and before and after acute hypovolemia ($n = 5$) with the paired *t*-test. To strengthen the experimental design of repeated measures with the same five subjects acting as their own controls and to compare possible changes in baseline data between the two conditions, a two-factor repeated-measures ANOVA with time (pre- and post-) and interventions (bed rest and hypovolemia) as factors was performed ($n = 5$), and these comparisons are noted specifically

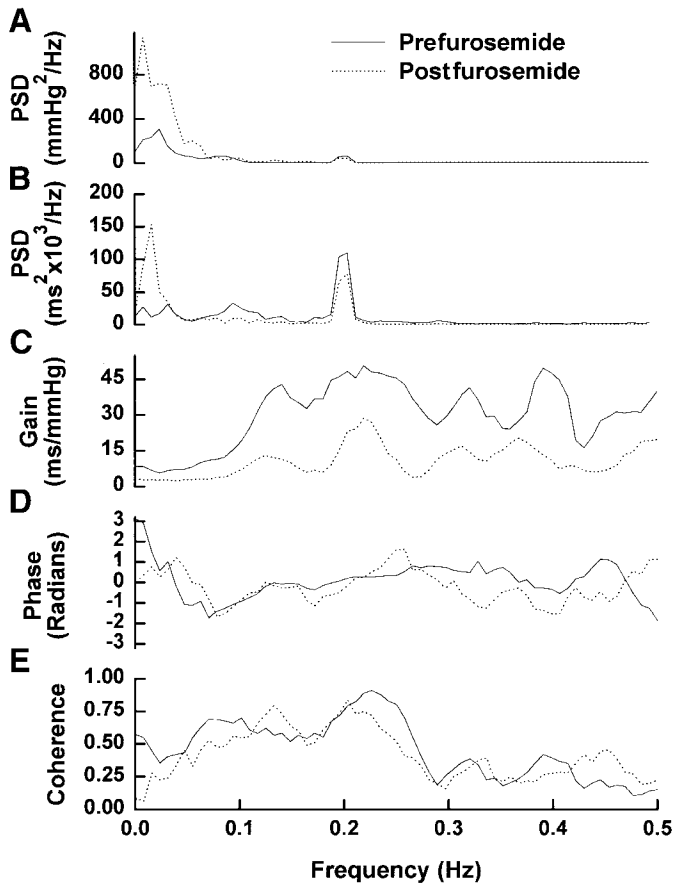


Fig. 2. Representative frequency-domain analysis of changes in R-R interval and systolic blood pressure in a subject before and after acute hypovolemia. A: PSD of systolic blood pressure. B: power spectral density of R-R interval. C: transfer-function gain between systolic blood pressure and R-R interval. D: phase relationship between systolic blood pressure and R-R interval. E: Coherence function.

in RESULTS. A P value < 0.05 was considered statistically significant. The analysis was performed by using PC-based software (ABstat, Anderson Bell). Data are presented as means \pm SE.

RESULTS

Hemodynamics

Two weeks of head-down-tilt bed rest led to a reduction in plasma volume from $3,134 \pm 166$ to $2,676 \pm 124$ ml ($-12 \pm 5\%$) ($P < 0.05$, see Fig. 3A). In comparison, furosemide led to a similar reduction from $3,166 \pm 121$ to $2,833 \pm 132$ ml ($-11 \pm 3\%$) ($P < 0.05$, Fig. 3B). After administration of furosemide, the decreases in RAP and PCWP (RAP: 7.9 ± 0.6 to 4.5 ± 0.5 mmHg; PCWP: 10.3 ± 0.8 to 6.1 ± 0.8 mmHg for hypovolemia) were similar to those observed after the head-down-tilt bed rest in the same subjects (RAP: 8.0 ± 1.1 to 5.4 ± 0.5 mmHg; PCWP: 10.0 ± 1.4 to 7.0 ± 0.6 mmHg for bed rest, $n = 5$) with no difference between bed rest and furosemide (interaction $P = 0.66$; see Table 1). Moreover, cardiac filling pressures remained stable after the achievement of target RAP and PCWP after acute hypovolemia, such that 2 h after beginning data collection (~ 4 h after administration of furosemide), RAP was still 5.3 ± 0.6 mmHg and PCWP was 7.3 ± 0.7 mmHg. Associated with these changes, heart rate increased significantly ($P < 0.05$) after both head-down-tilt bed rest and acute hypovolemia (Table 1). However, no significant changes in SBP and diastolic blood pressure were observed either after head-down-tilt bed rest or after acute hypovolemia (Table 1). There were no significant differences between these two conditions with respect to changes in hemodynamic indexes, including cardiac filling pressures, arterial pressure, stroke volume, or cardiac output.

Spectral Analysis of Cardiovascular Variability

There were no significant differences with regard to baseline data between head-down-tilt bed rest and acute hypovolemia (a 2-factor repeated ANOVA, interventions as a factor, $n = 5$)

Normalized high-frequency power of R-R interval variability decreased significantly in both cases ($P < 0.05$, Table 2 and see Fig. 4), to virtually the same extent in the same subjects (-0.06 ± 0.03 , $20 \pm 9\%$ for

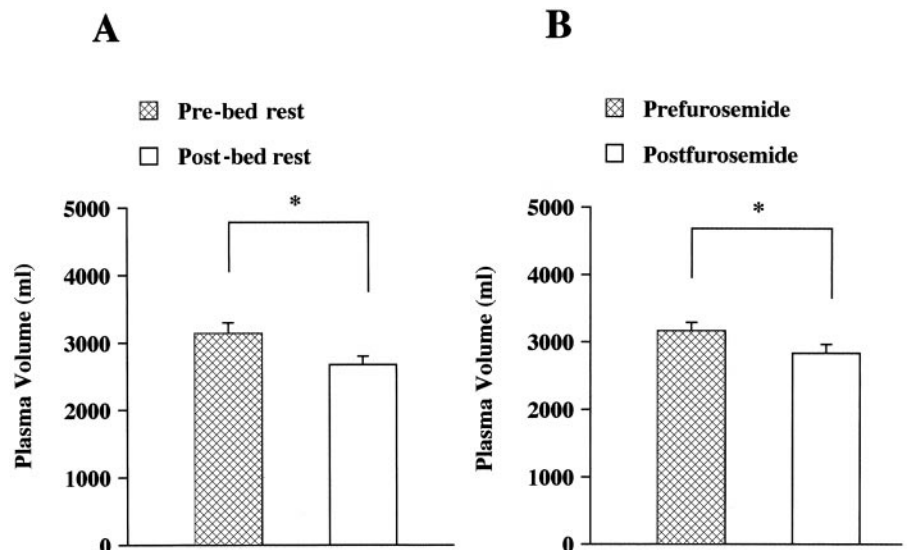


Fig. 3. Mean changes (\pm SE) in plasma volume. A: after 2 wk of head-down-tilt bed rest ($n = 9$), $*P < 0.05$ compared with pre-bed rest. B: 2 h after administration of furosemide ($n = 5$), $*P < 0.05$ compared with preadministration. There was no significant difference between groups with respect to changes in plasma volume.

Table 1. *Hemodynamics after head-down bed rest and acute hypovolemia*

	Head-Down Bed Rest				Hypovolemia	
	Pre (n = 9)	Post (n = 9)	Pre (n = 5)	Post (n = 5)	Pre (n = 5)	Post (n = 5)
Heart rate, bpm	64 ± 3	69 ± 2*	65 ± 4	72 ± 3*	61 ± 3	65 ± 4*
R-R, ms	956 ± 42	871 ± 27*	930 ± 52	838 ± 29*	996 ± 39	938 ± 60*
SBP, mmHg	137 ± 3	139 ± 2	135 ± 4	133 ± 4	122 ± 7	122 ± 4
DBP, mmHg	70 ± 2	72 ± 2	71 ± 3	72 ± 3	68 ± 3	69 ± 4
Stroke volume, ml	91 ± 7	77 ± 3*	95 ± 12	76 ± 4*	96 ± 8	79 ± 8*
Cardiac output, l/min	6.57 ± 0.52	5.90 ± 0.34*	7.25 ± 0.77	5.99 ± 0.37*	6.39 ± 0.30	5.80 ± 0.45
RAP, mmHg	8.4 ± 0.7	5.6 ± 0.5*	8.0 ± 1.1	5.4 ± 0.5*	7.9 ± 0.6	4.5 ± 0.5*
PCWP, mmHg	10.7 ± 1.0	7.6 ± 0.7*	10.0 ± 1.4	7.0 ± 0.6*	10.3 ± 0.8	6.1 ± 0.8*

Values are means ± SE. *n*, No. of men; Pre and Post, before and after experiment, respectively; SBP and DBP, systolic and diastolic blood pressure, respectively; bpm, beats/min; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure. There were no significant differences in control (Pre) values for all indices between bed rest and hypovolemia. **P* < 0.05 (Pre vs. Post).

bed rest; -0.07 ± 0.04 , $20 \pm 12\%$ for hypovolemia; *n* = 5), whereas no significant change was observed in the normalized low-frequency power (see Fig. 4). When the analysis was performed by using heart rate instead of R-R interval, a similar reduction in high-frequency power was observed in both cases (18% for both, Table 2). Associated with these changes, the ratio of low- to high-frequency power increased significantly (*P* < 0.05) after head-down-tilt bed rest, and a trend for an increase was observed after the acute hypovolemia (Table 2). There were no significant differences between these two conditions in the decreases in normalized high-frequency power and the magnitude of changes in other R-R interval spectral indices. However, the changes in the low-frequency

power of SBP variability were significantly different between the two conditions (*P* < 0.05 for the interaction statistic of the 2-way ANOVA; head-down-tilt bed rest: -1.6 ± 1.1 mmHg²; acute hypovolemia: $+3.1 \pm 1.9$ mmHg²; *n* = 5 for both) (Table 2). Figures 1 and 2 show the spectra of changes in R-R interval and SBP in one representative subject, demonstrating a decrease in the low-frequency power spectral density of SBP after bed rest despite an increase after acute hypovolemia.

Transfer Function Analysis of Baroreflex Function

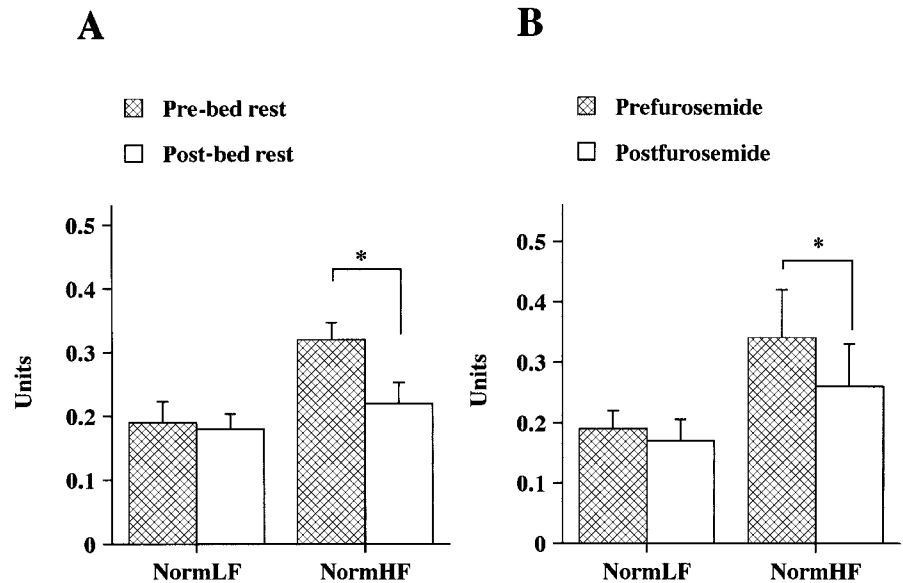
There were no significant differences with regard to baseline data between head-down-tilt bed rest and

Table 2. *Spectral analysis after head-down bed rest and acute hypovolemia*

	Head-Down Bed Rest				Hypovolemia	
	Pre (n = 9)	Post (n = 9)	Pre (n = 5)	Post (n = 5)	Pre (n = 5)	Post (n = 5)
SDR-R, ms	62 ± 9	56 ± 6	58 ± 14	50 ± 6	67.6 ± 7.8	72.4 ± 8.8
LFR-R, ms ²	783 ± 203	552 ± 115	842 ± 317	483 ± 113	961 ± 264	1,015 ± 361
HFR-R, ms ²	1,405 ± 413	886 ± 335	1,327 ± 733	685 ± 251	1,705 ± 632	1,715 ± 804
LF/HFR-R	0.68 ± 0.14	0.92 ± 0.17*	0.87 ± 0.21	0.98 ± 0.27 †	0.84 ± 0.33	1.05 ± 0.46
LFBP, mmHg ²	6.1 ± 1.3	4.9 ± 0.9	5.2 ± 1.2	3.5 ± 0.9	3.70 ± 0.80	6.72 ± 1.94
HFBP, mmHg ²	3.0 ± 0.6	2.5 ± 0.5	2.3 ± 0.6	2.1 ± 0.6	2.26 ± 1.01	1.43 ± 0.64
NormLFRR	0.19 ± 0.03	0.18 ± 0.02	0.24 ± 0.06	0.20 ± 0.04	0.19 ± 0.03	0.17 ± 0.04
NormHFRR	0.32 ± 0.03	0.22 ± 0.03*	0.29 ± 0.02	0.23 ± 0.03*	0.33 ± 0.09	0.26 ± 0.07*
GainLF-RR, ms/mmHg	9 ± 2	8 ± 1	9 ± 2	9 ± 2	15 ± 3.1	10 ± 1.6
GainHF-RR, ms/mmHg	16 ± 3.1	13 ± 2.2*	17 ± 4.9	13 ± 3.4*	28 ± 5.7	17 ± 4.0*
NormLFHR	0.16 ± 0.02	0.18 ± 0.03	0.18 ± 0.03	0.21 ± 0.03	0.19 ± 0.03	0.19 ± 0.03
NormHFHR	0.31 ± 0.04	0.21 ± 0.03*	0.28 ± 0.02	0.23 ± 0.03*	0.33 ± 0.04	0.27 ± 0.02*
GainLF-HR, bpm/mmHg	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	1.0 ± 0.2	0.8 ± 0.1
GainHF-HR, bpm/mmHg	1.0 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.2 ± 0.3	1.7 ± 0.3	1.2 ± 0.3
“Effective gain,” ml · min ⁻¹ · mmHg ⁻¹						
Effective GainLF	53 ± 10	48 ± 8	61 ± 13	57 ± 11	94 ± 16	63 ± 9
Effective GainHF	93 ± 9	81 ± 3*	116 ± 10	88 ± 7*	168 ± 28	101 ± 25*

Values are means ± SE unless noted. *n*, No. of men; SDR-R, SD of R-R intervals; LFR-R, power in low frequency; HFR-R, power in high frequency of R-R variability; LF/HFR-R, the ratio of low- to high-frequency power of R-R interval variability; LFBP, power in low frequency of blood pressure; HFBP, power in high frequency of blood pressure variability; NormLFRR, normalized power in low frequency; NormHFRR, normalized power in high frequency of R-R variability; GainLF-RR, low-frequency transfer function gain (ms/mmHg); GainHF-RR, high-frequency transfer function gain (ms/mmHg); NormLFHR, normalized power in low frequency; NormHFHR, normalized power in high frequency of heart rate variability; GainLF-HR, low-frequency transfer function gain (bpm/mmHg); GainHF-HR, high-frequency transfer function gain (bpm/mmHg); Effective GainLF, low-frequency transfer function gain for heart rate (bpm/mmHg) multiplied by the resting stroke volume; Effective GainHF, high-frequency transfer function gain for heart rate (bpm/mmHg) multiplied by the resting stroke volume. **P* < 0.05 (Pre vs. Post). †*P* < 0.05 (change after bed rest vs. hypovolemia). There were no significant differences in control (Pre) values for all indices between bed rest and hypovolemia.

Fig. 4. Mean changes (\pm SE) in normalized power of R-R interval variability at low frequency (NormLF: 0.05–0.15 Hz) and high frequency (NormHF: 0.15–0.25 Hz). A: after 2 wk of head-down-tilt bed rest, $*P < 0.05$ compared with pre-bed rest ($n = 9$). B: 2 h after administration of furosemide ($n = 5$), $*P < 0.05$ compared with preadministration. There were no significant differences for the baseline data between head-down-tilt bed rest and the acute hypovolemia ($n = 5$, ANOVA).



acute hypovolemia (a 2-factor repeated ANOVA, interventions as a factor, $n = 5$), as shown in Table 2.

High-frequency transfer function gain decreased after head-down-tilt bed rest ($P < 0.05$, see Fig. 5A) and decreased similarly after acute hypovolemia ($P < 0.05$, Fig. 5B). Moreover, there was no significant difference in the magnitude of the decrease in high-frequency transfer function gain between the two conditions (-4 ± 2 ms/mmHg, $-14 \pm 11\%$ for bed rest; -11 ± 5 ms/mmHg, $-30 \pm 17\%$ for hypovolemia; $n = 5$ ANOVA). No significant change in the low-frequency gain was observed after either head-down-tilt bed rest and acute hypovolemia. Coherence was near or above 0.5 under all conditions: 0.61 ± 0.04 in the high-frequency range and 0.51 ± 0.03 in the low-frequency range. A negative phase between the SBP and R-R interval was observed in all cases, and the phase did not change after bed rest or furosemide.

Neither head-down-tilt bed rest nor acute hypovolemia changed transfer function gain when expressed as heart rate instead of R-R interval (Table 2). However, when the gain in terms of heart rate was multiplied by the resting stroke volume to determine the effective change in systemic flow induced by baroreflex-mediated changes in heart rate, there was a reduction in effective gain similar to that observed with R-R interval after both bed rest and furosemide (Table 2).

Orthostatic Tolerance

LBNP tolerance decreased significantly after head-down-tilt bed rest ($P < 0.05$, Fig. 6A). There was a similar trend for a reduction in LBNP tolerance after furosemide that did not achieve statistical significance ($P = 0.08$, Fig. 6B). There were no significant differences with regard to baseline LBNP tolerance between

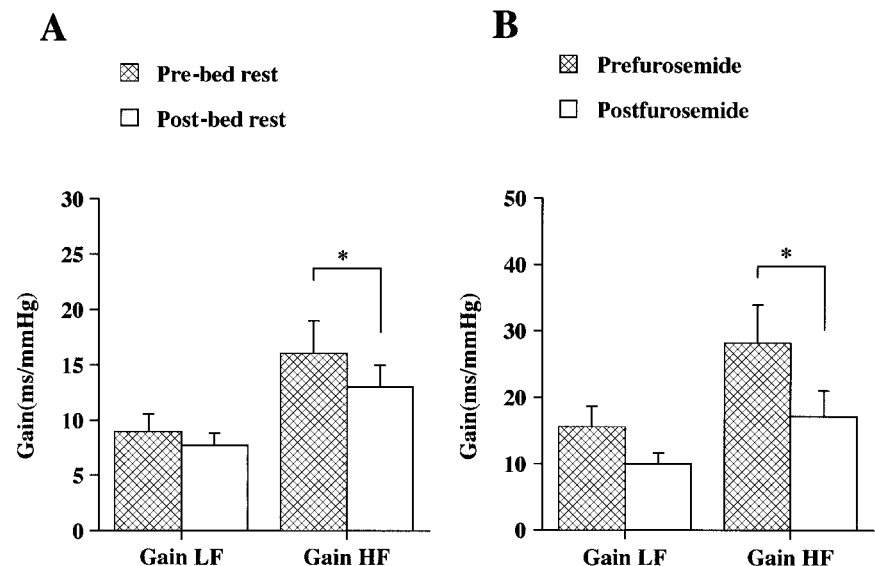


Fig. 5. Mean changes (\pm SE) in gains of transfer function between blood pressure and R-R interval at low frequency (Gain LF) and high frequency (Gain HF). A: after 2 wk of head-down-tilt bed rest, $*P < 0.05$ compared with pre-bed rest ($n = 9$). B: 2 h after administration of furosemide, $*P < 0.05$ compared with preadministration ($n = 5$). Note that the scale for A and B are different; the figures are scaled to depict the relative changes in gain before and after bed rest or furosemide, while preserving the ability to determine the absolute value as well. Both figures therefore begin at 0, with the baseline high-frequency gain scaled to 2/3 of the axis range. There were no significant differences for the baseline data between head-down-tilt bed rest and the acute hypovolemia.

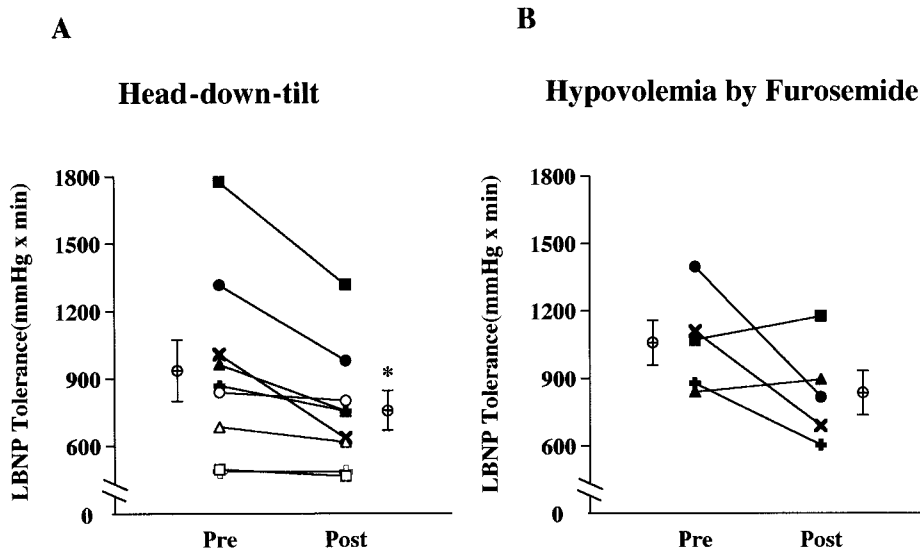


Fig. 6. Individual and group mean changes (\pm SE) in lower body negative pressure (LBNP) tolerance. A: after 2 wk of head-down-tilt bed rest, $*P < 0.05$ compared with pre-bed rest. B: 2 h after administration of furosemide. LBNP tolerance is expressed as a cumulative stress index derived from the sum of the products of negative pressure times duration at each level of LBNP.

head-down-tilt bed rest ($1,186 \pm 165$ mmHg \times min, $n = 5$) and acute hypovolemia ($1,059 \pm 99$ mmHg \times min, $n = 5$). Furthermore, there were no significant differences between the decrease in LBNP tolerance with head-down-tilt bed rest (-297 ± 61 mmHg \times min, $-25 \pm 4\%$) and the decrease that occurred with acute hypovolemia (-223 ± 133 mmHg \times min, $-18 \pm 11\%$) via two-way ANOVA ($n = 5$).

Plasma Norepinephrine

Plasma norepinephrine increased significantly from 1.1 ± 0.2 (187 ± 38 pg/ml) to 1.4 ± 0.3 nM (231 ± 49 pg/ml) after acute hypovolemia ($P < 0.05$), $\sim 10\%$ more than predicted from hemoconcentration alone, consistent with modest sympathetic activation. Although plasma norepinephrine was not measured before and after bed rest, resting supine muscle sympathetic nerve activity was unchanged in these subjects (38). These data will be reported separately.

DISCUSSION

The primary new findings from the present study are twofold. 1) Transfer function gain between blood pressure and R-R interval in the high-frequency range and normalized high-frequency power of R-R interval variability decreased significantly to a similar extent after both head-down-tilt bed rest and acute hypovolemia matched for loss of plasma volume. The remarkable similarity of these responses under both conditions in the same subjects is consistent with the hypothesis that a reduction in plasma volume may be largely responsible for the changes in reflex control of cardiac period observed after head-down-tilt bed rest. 2) However, the changes in low-frequency power of SBP variability were dissimilar between head-down-tilt bed rest and acute hypovolemia. In contrast to changes in control of R-R interval, this observation suggests that the changes in vasomotor function associated with head-down-tilt bed rest and acute hypovolemia may be different.

Methodological Considerations

Transfer function analysis of spontaneous variations between arterial pressure and R-R interval has been employed for the evaluation of dynamic properties of baroreflex function (3, 21, 34, 42, 44, 50). This analysis emphasizes the frequency dependence of baroreflex control of cardiac period (34, 44, 50); that is, the estimates of transfer function in the high-frequency range are predominantly determined by vagal activity, whereas in the low-frequency range, the estimates are influenced by both sympathetic and vagal nerve activity. Moreover, this type of analysis allows the assessment of baroreflex gain from spontaneous fluctuations in blood pressure and R-R interval (3, 21, 34, 42, 44) or heart rate (3, 44, 50) without the injection of vasoactive drugs, or artificial mechanical stimulation of the receptor. Therefore, the baroreflex is not affected by the measuring method itself.

Two possible limitations of this method have been described. 1) Transfer function estimates are limited by a fundamental assumption of linearity between changes in arterial pressure and cardiac period and are reliable only if squared coherence values are near or above 0.5 (42, 44). In the present study, the coherence function was sufficiently high in both the high (0.61)- and low (0.51)-frequency ranges, confirming the validity of using this technique for the assessment of gain and phase. 2) Another possible limitation has been underscored by Saul and Triedman (44, 50). These authors emphasized that the transfer function method reflects a closed-loop relationship between blood pressure and heart rate. However, a mathematical simulation of cardiovascular control showed that the feed-forward effects of heart rate on systolic pressure may be more complicated than simple buffering via the baroreflex (3). For example, Taylor and Eckberg (48) suggested that the phase relationship between systolic pressure and R-R interval may be positive in some conditions such that the pressure oscillations may fol-

low those in R-R interval. As a commonly accepted rule, a positive phase estimate between the input and output variables reflects a phase lead of output to the input of a system; in other words, the output (R-R interval) precedes the input (blood pressure). In the present study, however, the phase was always negative under both conditions (bed rest and hypovolemia) and did not change, thereby minimizing this concern. Finally, transfer function gain correlates significantly with other measures of baroreflex function including the vasoactive drug methods and sequence analysis (21, 34, 39, 42). Therefore, the premise of this study is that the technique of transfer function analysis is a useful tool for the assessment of dynamic baroreflex function, emphasizing the frequency dependence of R-R interval and blood pressure control.

R-R Interval vs. Heart Rate

The hyperbolic relationship between R-R interval, and its hemodynamic correlate, heart rate, confounds a simple or fluent change from one perspective to the other (41, 51). Indeed, in the present study, we observed that when transfer function gain was expressed in conventional units of milliseconds per millimeters mercury we noted reductions in gain similar to those reported previously by other investigators by using both similar (9, 19, 20, 21, 29, 31) and different (7, 12, 15, 17) techniques; however, if gain was expressed as heart rate, the differences before and after bed rest or hypovolemia disappeared.

On a dynamic, beat-by-beat basis, the modulation of phase IV depolarization at the sinus node by baroreflex-mediated changes in efferent cardiac vagal activity is by its nature, time scale independent. That is, the rate of slow, inward Na^+ current (from ~ 120 mV/s in the absence of acetylcholine, to ~ 60 mV/s at acetylcholine concentrations of $0.1 \mu\text{M}$) is independent of larger time scales such as 1 min, 1 h, or 1 day (53). In this regard, animal studies have shown that it is cardiac period that is directly related to efferent vagal activity to the sinus node, as modulated by the arterial baroreflex (24). Whether this relationship is also linear in humans is unknown. However, at least in dogs, because of the reciprocal relationship between cardiac period and heart rate, the relationship between cardiac efferent vagal activity and heart rate is substantially nonlinear. Moreover, heart rate must be calculated after R-R interval is measured on the basis of an arbitrary time scale such as 1 min. Thus heart rate may be a less direct measure of baroreflex output on a beat-by-beat basis. If carotid sinus afferent activity or cardiac vagal and/or sympathetic efferent activity could be measured directly, as the dependent variable during changes in arterial pressure for the assessment of human baroreflex function, this discussion would be moot. However, because an indirect measure such as R-R interval (or heart rate), must be used, it seems reasonable to express the relationship in terms of cardiac period, which is 1) directly measured from the timing between consecutive sinus node depolariza-

tions; 2) time scale independent; and 3) in animal studies, a linear, direct function of cardiac vagal activity.

It is when the question is asked, what is the effect of the changes in baroreflex regulation of cardiac period on steady-state blood pressure control, that the definition of an appropriate time scale for the measurement of heart rate and systemic flow becomes most relevant. Thus the modulation of cardiac period by the baroreflex affects cardiac output (a time scale-dependent variable) on the basis of the prevailing hemodynamic conditions including stroke volume and total peripheral resistance (25). In the present study of dynamic control of heart rate and blood pressure, we have dealt with this issue directly by including an analysis of R-R variability, blood pressure variability, and the closed-loop transfer function between them. Moreover, when the reduction in stroke volume is factored in as the modulator by which changes in heart rate affect changes in blood pressure, there remains a significant reduction in the dynamic regulation of systemic flow (heart rate \times stroke volume) after both bed rest and acute hypovolemia.

Baroreflex Control of Cardiac Period After Central Blood Volume Reduction

In the present study, we sought to determine whether the reduction in cardiac baroreflex sensitivity shown in the recent literature after bed rest by using a neck chamber device or the sequence method could also be identified by transfer function analysis, and asked whether this reduction could be due to a reduction in plasma volume. To address these questions, the effect of head-down-tilt bed rest (chronic hypovolemia plus deconditioning) was compared with acute hypovolemia without bed rest deconditioning, with regard to estimates of the transfer function in the same subjects. We found that transfer function gain in the frequency range of 0.15–0.25 Hz decreased similarly both qualitatively and quantitatively after both head-down-tilt bed rest and acute hypovolemia (Fig. 5). These data suggest that arterial-cardiac baroreflex sensitivity in this frequency range is reduced after head-down-tilt bed rest, and these changes may be due primarily to a reduction in plasma volume associated with head-down-tilt bed rest. We cannot exclude the possibility that our inability to distinguish a significant difference between these two interventions with respect to the magnitude of gain reduction could be due to type II error due to the small subject number. The power of the present study design was 0.72 to detect the differences reported here (52).

However, this finding is somewhat different from a previous report using a neck chamber technique. Convertino et al. (7) reported that during head-down bed rest, the time course of changes in blood volume and the carotid-cardiac period baroreflex were not parallel (7). Therefore, they concluded that the reduction in blood volume may not be the sole cause of baroreflex abnormalities after bed rest. One possible explanation

for this discrepancy could be ascribed to the different methods used for the assessment of baroreflex function. The index of baroreflex sensitivity obtained with the neck chamber technique primarily reflects the specific characteristics of the carotid-cardiac baroreflex loop, whereas estimates of transfer function gain in the present study may reflect an integrated baroreflex sensitivity including both aortic- and carotid-cardiac loops. These two baroreceptor populations may have different characteristics (2) and may adapt differently to microgravity (8). Furthermore, transfer function analysis estimates baroreflex gain during modest spontaneous beat-to-beat oscillations of blood pressure, focused on the "operating point" of the stimulus-response curve, whereas the neck chamber technique gives an estimation of baroreflex gain over greater ranges of pressure, with the maximum gain determined from the steepest slope of the stimulus-response curve (38, 41). The sensitivity obtained with the neck chamber technique is a static index, whereas the transfer function gain reflects dynamic properties of baroreflex function (3, 44). Finally, if the transmission properties of pressure in the neck are changed by bed rest, such as a change in the stiffness of the neck tissue caused by a cephalad shift of fluid, the magnitude of the stimulus to carotid baroreceptors at any given chamber pressure may change during bed rest. Interestingly, decreases in transfer function gain after both head-down-tilt bed rest and acute hypovolemia are consistent with most reports that measured this index during other acute interventions such as head-up tilt (using heart rate) (50) and LBNP (using R-R interval) (21). We speculate that although the mechanisms of changes in plasma volume may be different with these maneuvers, a common feature of central hypovolemia is likely to be a reduction in the transfer function gain and integrated baroreflex control of cardiac period around the operating point.

Spectral Analysis of Cardiovascular Variability

Spectral analysis of blood pressure and R-R interval variability has been shown to be a useful noninvasive tool for quantifying sympathetic and vagal modulations of R-R interval and blood pressure (22, 29, 31, 32, 40). Although there are still arguments over its interpretation (11, 29, 47), when experimental conditions are carefully controlled, changes in R-R interval variability may track changes in autonomic neural control of the heart with reasonable accuracy (1, 13, 23). The major limitation of this technique is the fact that autonomic nervous system activity is estimated by the output variable of a complex system passed through a target organ. Thus this measure is influenced by many factors. In particular, respiration strongly influences the power spectra and therefore must be controlled (4, 18). Conversely, control of respiration itself changes the power spectra (32). Therefore, we chose a frequency of 0.2 Hz that was most closely matched with the subjects' spontaneous respiration rate, recognizing that there may be some harmonic contribution from

low-frequency (0.1 Hz) rhythms. Changes in cardiac mechanics may also be a major factor influencing heart rate variability (27), as may changes in arterial compliance, which influences the amount of baroreceptor distortion during a pressure pulse.

Previous reports from bed rest studies (9, 19, 35) have shown that high-frequency power of R-R interval variability decreases in association with an decrease in R-R interval, suggesting vagal withdrawal. The combination of decreased R-R interval and the relative reduction of high-frequency respiratory sinus arrhythmia observed in the present study is consistent with these previous reports. The reproduction of virtually identical decreases in normalized R-R interval and heart rate variability after bed rest, which includes hypovolemia plus chronic deconditioning and after acute hypovolemia alone (i.e., without superimposed deconditioning) in the same subjects argues against a unique adaptation of the arterial-cardiac baroreflex after bed rest and suggests rather that the observed changes may reflect primarily the loss of plasma volume.

In contrast to R-R interval variability, the low-frequency component of blood pressure variability is presumed to result from sympathetic vasomotor activity (31, 32) and is closely related to low-frequency fluctuations in muscle sympathetic nerve activity (MSNA) (33). In our study, there appeared to be an enhanced vasomotor sympathetic activity after acute hypovolemia on the basis of the results of increases in plasma norepinephrine, and low-frequency SBP variability. This result is further supported by previous reports that low-frequency SBP variability, MSNA, and plasma norepinephrine all increased during central volume reduction (21, 30, 31, 43, 50).

In contrast to acute hypovolemia, there did not appear to be any increase in vasomotor activity after bed rest, as indicated by no change in low-frequency SBP variability. There are two possible mechanisms that can explain this differential response. First, the absence of a change in blood pressure variability after bed rest may be related to a specific adaptation of the coupling of the sympathetic nervous system-arterial vasomotor response. There are limited data available to support or refute such an adaptation. Shoemaker et al. (45) initially suggested, in fact, that MSNA may decrease after bed rest (45). However, more recent data from this same group (46) as well as the present study (37) suggest that MSNA may be unchanged or minimally increased after bed rest in the supine position. A second possible explanation is the presence of cardiovascular remodeling after bed rest, which does not occur after hypovolemia (54). Head-down-tilt bed rest leads to cardiac remodeling, resulting in a smaller, less distensible heart (28), which may influence blood pressure variability. In addition to changes in cardiac mechanics, remodeling of peripheral vessels may develop similarly during bed rest. One preliminary report from these subjects showed reduced leg arterial compliance after bed rest without an effect on the adrenergic

sensitivity of peripheral arterioles (36) and supports this possibility.

Orthostatic Tolerance and Plasma Volume

Previous studies and our present results suggest that a decreased baseline baroreflex R-R interval response could reduce the compensatory reserve of heart rate acceleration during hypotension, which may contribute to orthostatic intolerance. However, no studies have ever demonstrated a lower heart rate during orthostatic stress after spaceflight or bed rest, suggesting that a relatively low heart rate during standing, by itself, cannot be primarily responsible for this type of orthostatic hypotension. Moreover, studies that use the heart rate response during stand tests to estimate the integrity of reflex control of the circulation have provided conflicting results. For example, Buckey et al. (5) reported in 14 astronauts that stand test "finishers" and "nonfinishers" showed similar increases in heart rate. However, the vasoconstrictor response was significantly greater among the finishers. Conversely, Convertino et al. (7) showed that syncopal subjects demonstrated smaller increases in heart rate during a stand test after bed rest compared with nonsyncopal subjects. In the present study, we considered LBNP tolerance to be a continuous rather than a discrete variable, and all subjects ultimately demonstrated frank hypotension both before and after bed rest. Although the reduction in baroreflex control of cardiac period was virtually identical between acute dehydration and chronic bed rest, the loss of LBNP tolerance tended to be greater after bed rest. We speculate that factors related to cardiovascular remodeling during bed rest may therefore compound the hypovolemia, possibly contributing to orthostatic intolerance after bed rest.

Perspectives

Although the present study has limitations such as a small number of subjects and relatively limited data segments, the data demonstrate that both chronic (2 wk) head-down-tilt bed rest and acute induced hypovolemia lead to similar reductions in high-frequency R-R interval variability and baroreflex control of cardiac period. Moreover, the ability of the baroreflex to modify systemic flow (heart rate \times stroke volume) was similarly reduced after both conditions. Such similarities suggest that a reduction in plasma volume rather than a unique autonomic nervous system adaptation to bed rest may be responsible for these changes. However, the results in the low-frequency SBP variability showed specific, directionally opposite differences between head-down-tilt bed rest and acute hypovolemia. These results suggest that the unique cardiovascular remodeling that occurs after bed rest may alter vasomotor function and may contribute to orthostatic intolerance after spaceflight or bed rest.

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