

Cardiovascular Adaptations to Long-Duration Head-Down Bed Rest

STEVEN H. PLATTS, DAVID S. MARTIN,
MICHAEL B. STENGER, SONDRA A. PEREZ,
L. CHRISTINE RIBEIRO, RICHARD SUMMERS,
AND JANICE V. MECK

PLATTS SH, MARTIN DS, STENGER MB, PEREZ SA, RIBEIRO LC, SUMMERS R, MECK JV. *Cardiovascular adaptations to long-duration head-down bed rest*. *Aviat Space Environ Med* 2009; 80(5,Suppl.):A29–36.

Introduction: Orthostatic hypotension is a serious risk for crewmembers returning from spaceflight. Numerous cardiovascular mechanisms have been proposed to account for this problem, including vascular and cardiac dysfunction, which we studied during bed rest. **Methods:** Thirteen subjects were studied before and during bed rest. Statistical analysis was limited to the first 49–60 d of bed rest and compared to pre-bed rest data. Ultrasound data were collected on vascular and cardiac structure and function. Tilt testing was conducted for 30 min or until presyncopal symptoms intervened. **Results:** Plasma volume was significantly reduced (15%) by day 7 of bed rest. Flow-mediated dilation in the leg was significantly increased at bed rest day 49 (6% from pre-bed rest). Arterial responses to nitroglycerin differed in the arm and leg, but did not change as a result of bed rest. Anterior tibial artery intimal-medial thickness markedly decreased at bed rest days 21 (21%), 35 (22%), and 49 (19%). Several cardiac functional parameters, including isovolumic relaxation time (73 ms to 85 ms at day 7) and myocardial performance index, were significantly increased (0.41 to 0.49 by day 7 of bed rest; indicating a decrease in cardiac function) during bed rest. There was a trend for decreased orthostatic tolerance following 60 d of bed rest ($P = 0.1$). **Discussion:** Our data suggest that bed rest altered cardiovascular structure and function in a pattern similar to short-duration spaceflight. Additionally, the vascular alterations were primarily seen in the lower body, while vessels of the upper body were unaffected.

Keywords: spaceflight, orthostatic intolerance, hypotension, fluid-shift, plasma volume.

SPACEFLIGHT PROVOKES numerous untoward effects on the cardiovascular system. These effects have been shown in spaceflight studies (1), bed rest analog studies (21), and animal studies (19). Post-spaceflight orthostatic hypotension has been the subject of intense study during the last 20 yr, both during spaceflight and in head-down bed rest investigations. This problem appears to develop from a series of in-flight cardiovascular changes, including a relative hypovolemia initiated by the cephalad fluid shift and a lack of arterial baroreceptor input (2,3,10,17). Other cardiovascular alterations have also been reported, including decreased cardiac function (22,26), decreased aerobic capacity (13), changes in vascular structure and function (10,30), and possible cardiac rhythm abnormalities (6,9).

Head-down bed rest has become a primary research tool to examine mechanisms and test countermeasures for cardiovascular alterations. There is abundant literature supporting the utility of bed rest as an analog for

multiple organ systems (21). One of the drawbacks of bed rest literature is the lack of standardization. This study is part of a NASA Flight Analogs Project (FAP) initiative to conduct multi-system research on bed rest subjects under strictly standardized conditions.

We tested the hypothesis that arterial flow-mediated dilation responses, dilation in response to nitroglycerin, and arterial structure would all change during bed rest. Additionally, we tested the hypothesis that arteries in the arm would follow different patterns of change than arteries in the leg during head-down bed rest. No bed rest study to date has addressed this possibility. Based on the fluid shifts and cardiovascular deconditioning that occur during bed rest [most recently reviewed by Pavy-Le Traon et al. (21)], we also tested the hypothesis that orthostatic tolerance would decrease following long-duration bed rest.

Spaceflight and bed rest may also induce changes in cardiac function. Recent work has shown a decrease in ventricular mass (22,25) and stroke volume (5,15,22); however, more comprehensive measurements of cardiac function are lacking. In addition to the above standard measurements, we studied the effects of bed rest on numerous other indicators of cardiac function such as isovolumic relaxation time and myocardial performance index. We tested the hypothesis that cardiac function, as measured by echocardiography, would be decreased by exposure to bed rest.

METHODS

Refer to Meck et al. (18) for a description of the protocol, general conditions of the study, and the use of long-duration head-down bed rest as a model for spaceflight. Bed rest and test protocols were reviewed and approved by the Johnson Space Center Committee for the Protection of Human Subjects, the University of Texas Medical Branch (UTMB) Institutional Review Board,

From the NASA Johnson Space Center, Houston, TX; Wyle, Houston, TX; and University of Mississippi, University, MS.

Address reprint requests to: Steven H. Platts, Ph.D., NASA Johnson Space Center, Mail Code SK, 2101 NASA Parkway, Houston, TX 77058; steven.platts-1@nasa.gov.

Reprint & Copyright © by the Aerospace Medical Association, Alexandria, VA.

DOI: 10.3357/ASEM.BR03.2009

and the UTMB General Clinical Research Center Science Advisory Committee. Subjects received verbal and written explanations of the bed rest and test protocols prior to providing written informed consent.

Subjects

Data from 13 subjects are included in this study (8 men and 5 women). Three subjects experienced 60 d of head-down bed rest, six subjects experienced 90 d of head-down bed rest, and four subjects experienced 42–53 d of head-down bed rest (truncated due to Hurricane Rita in September 2005; no post-bed rest data were acquired on these subjects). All pre- and post-bed rest testing was conducted in the supine position with the exception of the tilt test. During the bed rest period, subjects were maintained at 6° head-down tilt for the appropriate tests. Testing on the women was always timed to occur during the first 6 d of the menstrual cycle in order to minimize the effects of estrogen and progesterone on the cardiovascular system. No exercise was performed by these subjects.

Plasma Volume

Plasma volume was measured by the carbon monoxide rebreathing (CORB) technique as previously reported (14,27). In this study, plasma volume was corrected (plasma volume index, PVI) by body surface area (BSA):

$$BSA(m^2) = 0.007184 \times [\text{Weight}(kg)^{0.425}] \times [\text{Height}(cm)^{0.725}].$$

Flow-Mediated Dilation

Subjects were placed in the supine position and instrumented with a three-lead ECG. A Dinamap automatic blood pressure cuff (Johnson & Johnson, Arlington, TX) was placed on the non-imaged arm to obtain a baseline blood pressure. An occlusive pressure cuff (D. E. Hokanson, Inc., Bellevue, WA) was applied to the limb used for imaging. Imaging of the brachial artery was performed by a registered sonographer using a Philips HDI 5000 or Philips iE33 (Bothel, WA) with a 12-MHz transducer above the antecubital fossa; the site was marked with a permanent marker and the same position was used throughout the study. In addition, ultrasound images from the initial procedure were printed and reviewed prior to subsequent imaging sessions. When possible, arterial branches were included as landmarks. Furthermore, a photograph was taken during the procedure to show the position of the transducer on the skin. This photograph was used to ensure proper placement of the ultrasound probe for subsequent tests.

Resting diameter images of the brachial artery in the arm were optimized to allow clear visualization of the intimal-medial borders. The images were then enlarged to improve the accuracy of subsequent measurements. Three images at end-diastole and three at peak systole were stored digitally for off-line analyses. Blood flow parameters, including peak systolic velocity, pulsatility index, and velocity time interval, were also evaluated and stored. After acquisition of baseline images, an

occlusive cuff (Hokanson) placed on the upper arm was inflated to 50 mmHg above the resting systolic pressure and cessation of flow was verified by Doppler ultrasound. If flow was still present, the cuff pressure was increased to a point at which the flow was stopped. The cuff remained inflated for 5 min, at which point the cuff was rapidly deflated. Pulsed Doppler of the flow response was stored at 10 and 20 s post-deflation. Images of the brachial artery were stored every 15 s, starting at 30 s post-deflation and continued until the 3rd min. Additional images were acquired at minutes 4 and 5.

Flow-mediated dilation of the anterior tibial artery in the leg was accomplished following a similar protocol. The anterior tibial artery was chosen because it is of similar size to the brachial artery and is superficial enough to obtain high quality ultrasound images. The occlusive cuff was placed just proximal to the knee and was inflated to 70 mmHg over resting systolic blood pressure. This pressure was maintained for 7 min on the leg prior to deflation. The remainder of the procedure was identical to that used for the brachial artery, described above. Preliminary results in our lab have shown that this protocol achieved a similar hyperemic response to that seen in the arm at 50 mmHg supra-systolic pressure for 5 min (data not shown).

Measurement and analysis of data were performed off-line on a desktop computer using ProSolv Cardiovascular Analyzer 3.0.50 (Indianapolis, IN). Measurements were made at end diastole for consistency. Each image was measured by two experienced sonographers in a double-blind fashion. If a significant (greater than 10%) discrepancy between the two analyses was found, a third sonographer repeated the measurement. Doppler measurements and calculations were performed by an automated analysis feature of the ultrasound system.

For intimal-medial thickness measurements, three diastolic brachial artery images were stored at the R wave and three systolic images were stored at the end of the T wave from a superimposed ECG. The still frame images were stored in DICOM format and analyzed off line on a ProSolv Cardiovascular Analyzer 3.0.50 (Indianapolis, IN). The posterior segment of the artery was enhanced with a region of interest (ROI) magnification tool. Intimal-medial thickness measurements were made by two independent and blinded sonographers from three sequential images taken at the R wave. The measurements were made, with a caliper tool, from the central region of each image. The anterior interface of the intima was selected as the first linear interface echo; the second caliper was set at the medial adventitia line, defined as the next linear interface followed by a dark separating line.

Direct Arterial Dilation

Imaging of the brachial and anterior tibial arteries after administration of nitroglycerin (0.4 mg sublingual) was performed on the same day as, but no sooner than 1 h after the completion of, the flow-mediated dilation procedure. The same imaging techniques and imaging locations were used as previously described. Three

baseline images were acquired and digitally stored at end-diastole prior to administration of sublingual nitroglycerin. The artery was imaged continuously throughout the session to assure the same position was maintained. Starting at 3 min after the nitroglycerin administration, three images per minute were stored digitally for off-line analysis. One image per minute was measured live to monitor the time course of dilation. Imaging was continued until 2 min following the peak dilator response. The images were analyzed off line by two experienced sonographers who were blinded to the subject information. Any discrepancies greater than 10% were re-evaluated by a third sonographer.

Cardiac Function

Subjects were placed in the left lateral decubitus position. During head-down bed rest, the subjects maintained the 6° head-down tilt. American Society of Echocardiography standards were used in acquiring images (HDI 5000, Philips Medical, Bothel, WA). M-mode echocardiography was used to measure interventricular septal thickness (IVST), posterior wall thickness (PWT), and left ventricular diameter in systole (LVESD) and diastole (LVEDD). From these measurements, calculations of systolic function were made, including ejection fraction, stroke volume, velocity of circumferential shortening, and left ventricular mass. Left ventricular mass was calculated as follows: $LVM (g) = 0.8\{1.05 [(LVEDD + IVST + PWT)^3 - (LVEDD)^3] + 0.6\}$. Myocardial performance index (MPI), a global measurement of both systolic and diastolic function, was calculated as the ratio of left ventricular isovolumic contraction time plus isovolumic relaxation time divided by ejection time. Assessment of all the valves was accomplished using pulsed, continuous, and color Doppler. Images were stored digitally and on videotape for subsequent off-line analysis. The echocardiography protocol used views consistent with standards established by the American Society of Echocardiography.

Tilt Test

For the 90-d bed rest, tilt tests were nominally performed on BR-5, BR60, BR+0, and BR+3. For the 60-d bed rest, tilt tests were nominally performed on BR-5, BR60, and BR+3. Subjects were placed on a tilt table and a Dinamap blood pressure cuff was placed on the upper arm. A Finapres (Ohmeda Medical, Amsterdam, Netherlands) finger blood pressure cuff was placed on a finger of the opposite hand for continuous blood pressure measurement. Continuous measures of heart rate, ECG, arterial pressure, and aortic blood flow were obtained by Doppler ultrasound using a non-imaging 2-MHz probe (Biosound, MyLab 30, Indianapolis, IN) of the proximal ascending aorta. These were recorded for 5 min while supine (pre-bed rest) or at 6° head-down tilt (BR42–BR+0), and during tilt. This technique has been validated by previous studies (20,24). The systolic velocity integral was determined from off-line analysis of the Doppler waveform using Prosolv Cardiovascular soft-

ware, level 3.0.50 (Indianapolis, IN). Beat to beat stroke volume was calculated as the product of ascending aortic systolic velocity integral \times cross-sectional area (determined by two-dimensional ultrasound at the point of cusp insertion using a 2–4 MHz phased array probe). Cardiac output (stroke volume \times heart rate) and total peripheral resistance (mean arterial pressure/cardiac output) were all calculated off-line. The subjects were then tilted upright to 80° for 30 min or until symptoms of presyncope intervened.

Data and Statistical Analysis

All vascular measurements and plasma volumes were taken as close as possible to BR-5 (baseline) and at days BR7, BR21, BR35, BR49, BR60, BR90, and at BR+3 post-bed rest. Statistical analysis was only performed for the first 60 d of the study due to the low number of subjects who completed the entire 90 d protocol. Data are presented as mean \pm SE unless otherwise noted. Statistics were performed on a desktop computer using SigmaStat® commercial software v. 3.1 (Richmond, CA).

All data were tested for normality (Kolmogorov-Smirnov test) and equal variance (Levene Median test). Plasma volume, cardiac parameters, and vital statistics were analyzed using 1-way repeated measures ANOVA with a Bonferroni corrected pairwise comparison. Intimal-medial thickness was compared with a 2-way ANOVA with a Bonferroni correction for pairwise comparisons. Flow-mediated dilation and nitroglycerin data failed the normality test and were compared using Friedman's repeated measures ANOVA on ranks. Pairwise comparisons were made using Tukey's test. Tilt data were analyzed with a Kaplan-Meier survival analysis comparing pre-bed rest data with data from BR42–BR60. Significance for all tests was accepted at $P \leq 0.05$.

RESULTS

Vital statistics and baseline hemodynamic measurements are shown in **Table I**. No differences were found during bed rest for systolic or diastolic blood pressure, heart rate, weight, or body mass index.

Plasma Volume

PVI is shown in **Fig. 1** for baseline through 90 d of bed rest. Baseline PVI was $1.49 \pm 0.126 \text{ L} \cdot \text{m}^{-2}$. By day BR7, PVI fell significantly by 15%, to $1.26 \pm 0.166 \text{ L} \cdot \text{m}^{-2}$ ($P < 0.001$). By day BR49 of bed rest, PVI was $1.22 \pm 0.095 \text{ L} \cdot \text{m}^{-2}$ ($P < 0.001$), a total decrease of 18%. There were no differences between days BR7, BR21, BR35, or BR49 of bed rest ($P = \text{NS}$), although they were all significantly reduced from baseline ($P < 0.001$).

Arterial Function

Flow-mediated dilation: Flow-mediated arterial dilations (**Fig. 2**) are expressed, on the y-axis, as the difference between the percent dilation on the bed rest day minus the percent dilation during the pre-bed rest baseline (delta). The responses of the brachial artery did not

TABLE I. VITAL STATISTICS AND BASELINE HEMODYNAMICS.

	Pre-Bed Rest	BR7	BR21	BR35	BR49	BR60	BR75	BR90	BR+3
Height (m)	1.7 ± 0.1								
Weight (kg)	72.6 ± 16.6	72.0 ± 17.0	72.9 ± 14.3	71.4 ± 16.0	69.8 ± 16.1	65.8 ± 14.2	71.4 ± 24.2	62.9 ± 16.8	65.8 ± 15.2
Body Mass Index (kg · m ⁻²)	25.4 ± 4.2	24.6 ± 4.4	25.4 ± 3.7	24.7 ± 4.3	24.5 ± 4.1	23.6 ± 4.2	26.5 ± 5.9	23.4 ± 4.6	23.7 ± 4.5
Systolic Pressure (mmHg)	118.8 ± 13.2	116.1 ± 8.1	118.7 ± 16.2	115.9 ± 10.9	118.2 ± 13.4	116.7 ± 13.9	105.2 ± 8.2	112.7 ± 15.3	109.8 ± 12.0
Diastolic Pressure (mmHg)	67.8 ± 8.4	68.3 ± 7.2	69.4 ± 8.8	70.9 ± 6.7	70.5 ± 6.9	69.1 ± 8.3	69.3 ± 7.1	73.5 ± 12.8	68.2 ± 8.8
Heart Rate (bpm)	65.3 ± 8.9	63.0 ± 10.3	65.5 ± 7.7	65.7 ± 11.0	71.5 ± 9.5	69.7 ± 10.2	69.5 ± 13.1	67.8 ± 11.9	69.3 ± 13.6

change ($P = NS$); however, dilation was significantly increased in the anterior tibial artery on BR49 (day effect, $P = 0.001$).

Direct arterial dilation: There were no differences between bed rest days for dilations induced by the nitric oxide (NO) donor nitroglycerin (Fig. 3, $P = NS$ in the brachial or the anterior tibial artery). However, there was a significant difference in the dilator response between the brachial artery and anterior tibial artery (treatment effect, $P < 0.001$).

Intimal-medial thickness: The thickness of the arterial wall, shown in Fig. 4, decreased significantly during bed rest for the anterior tibial artery ($P < 0.001$), but not in the brachial artery, when compared to the baseline value. This effect was seen at days BR21, BR35, and BR49. There was also a significant difference between the brachial and anterior tibial artery (treatment effect, $P = 0.001$).

Cardiac Function

Measures of cardiac function are shown in Table II. Significant decreases during bed rest were seen in left ventricular systolic diameter (BR7, $P = 0.035$; BR49, $P = 0.005$), left ventricular diastolic diameter ($P = 0.015$ at BR21), IVRT ($P < 0.025$ for days BR7, BR21, BR31, and BR49), and ejection time ($P < 0.03$ on days BR21, BR31, and BR49). Myocardial performance index increased, which indicates a decrease in performance, and became statistically significant by day BR7 ($P < 0.033$) and was also significantly higher at days BR31 and BR49 ($P < 0.005$).

Tilt Test

The ability of test subjects to tolerate 80° upright tilt was analyzed with a Kaplan-Meier survival analysis (Fig. 5). There was a trend ($P = 0.1$) for survival to be lower following 60 d of bed rest when compared to pre-bed rest results.

DISCUSSION

Resources on the remaining Space Shuttle flights and the International Space Station are extremely limited. It is clear that ground-based analogs to spaceflight will be critical to expand our understanding of the causes of spaceflight-induced decrements in cardiovascular function. Head-down bed rest has been used as a model for spaceflight, however, the lack of standardization and varying durations of bed rest complicate interpretation. Our data show that cardiovascular alterations during

60- and 90-d head-down bed rest are similar to those seen in spaceflight.

Plasma Volume

The time course and magnitude of plasma volume loss is critical to the understanding of the mechanisms linking bed rest and spaceflight. While plasma volume is not the separating factor in determining presyncope in crewmembers (17,28), it is believed to be the triggering mechanism that leads to subsequent dysfunction. Previous bed rest reports detail plasma volume losses ranging from 4 to 17%, depending on the protocol (27), which is similar to the data presented here. Our data are also similar to those reported from spaceflight (17,27), which show plasma volume losses ranging from 7 to 19.5%. Our new data show that the entire plasma volume loss occurs within the first 3 d (our earliest time point), and is maintained in a steady state throughout 60 d of bed rest (Fig. 1). The fluid balance data shown in the overview section of this issue (18) shows an early, marked diuresis (likely due to the cardiopulmonary receptors sensing the fluid shift and leading to increased ANP), which confirms the plasma volume data. These results are consistent with spaceflight data as well. It has been shown that plasma volume is reduced by 16% by day 2 of spaceflight and 11% at day 7–8 of spaceflight (12). In these bed rest subjects, there was a trend for decreased orthostatic tolerance and 3 of these 10 (30%)

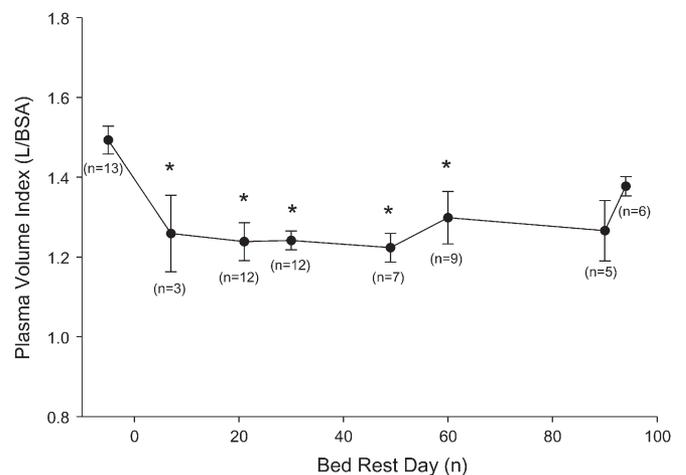


Fig. 1. Plasma volume changes during bed rest. Plasma volume index (PVI) is the plasma volume corrected by body surface area. * $P \leq 0.05$ within group for bed rest day. Statistical testing performed through day 60 due to low subject count beyond BR60.

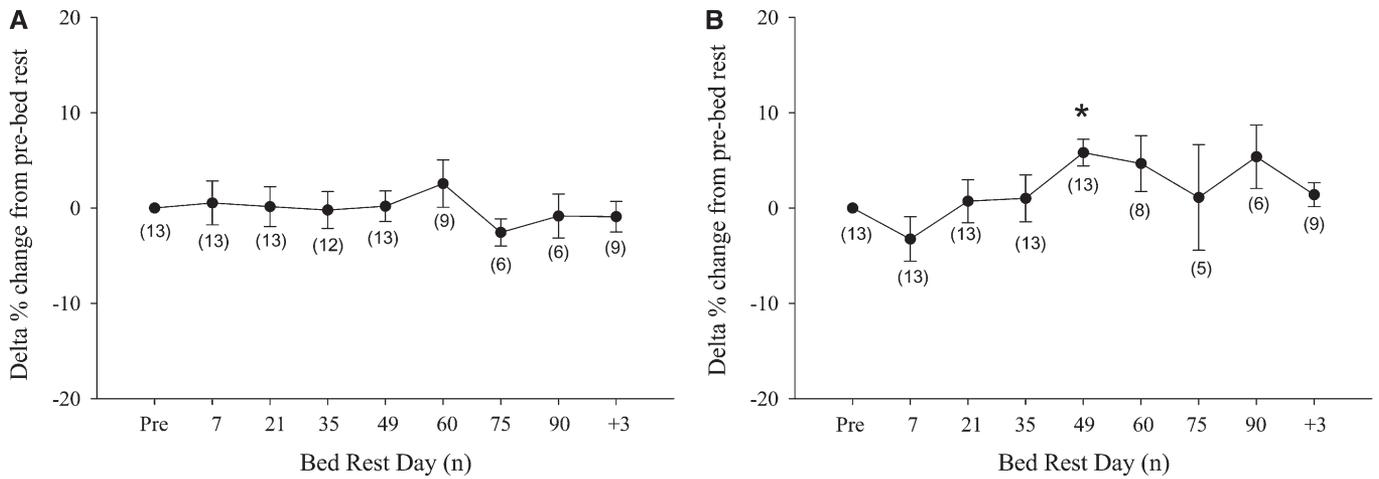


Fig. 2. Reactive hyperemic responses during bed rest in the A) brachial artery and B) anterior tibial artery. These graphs show the difference between pre- and post-occlusion arterial diameter (delta) for each time point, compared to the change during the pre-bed rest measurement. * $P \leq 0.05$ within group for bed rest day compared to pre-bed rest. Statistical testing performed through day 60 due to low subject count beyond BR60.

were unable to complete 10 min of tilt (Fig. 5). This is nearly identical to the percentage of crewmembers unable to complete 10 min of tilt following short-duration spaceflight (28). However, the rate of presyncope following long-duration spaceflight (~ 6 mo) is significantly higher, ~80% (16). This suggests either that a longer relative period of bed rest is required to replicate spaceflight, or that there is a nonlinear relationship between the duration of cardiovascular deconditioning and presyncope. Taken together, these data show that bed rest is a good model for spaceflight-induced plasma volume losses in both magnitude and time course and is similar to short-duration spaceflight for the rate of presyncope during 10 min of tilt testing.

Arterial Function

This study used a new approach to study arteries by measuring both structure and function. In addition, we compared and contrasted responses from an artery in

the arm (brachial) and a similar sized artery in the leg (anterior tibial) during bed rest. This approach has added important new information. In the brachial artery, none of the measured responses changed over the course of bed rest. Conversely, in the anterior tibial artery there were two important changes. First, the intimal-medial thickness was profoundly reduced. This confirms findings in animal studies and suggests that the vascular smooth muscle itself remodels in response to disuse during bed rest. Prior work from this laboratory suggests that the fluid shifts and relative hypovolemia precipitated by both bed rest and spaceflight may cause a loss of interstitial fluid volume (25), which could cause a change in function independent of contractile dysfunction. In cardiac tissue, dehydration causes diastolic dysfunction due to ventricular stiffening (26). In smooth muscle it could also increase stiffness. In addition, dehydration could reduce the diffusion distance from the endothelial layer, so that a greater concentration of NO reaches the smooth muscle (discussed below).

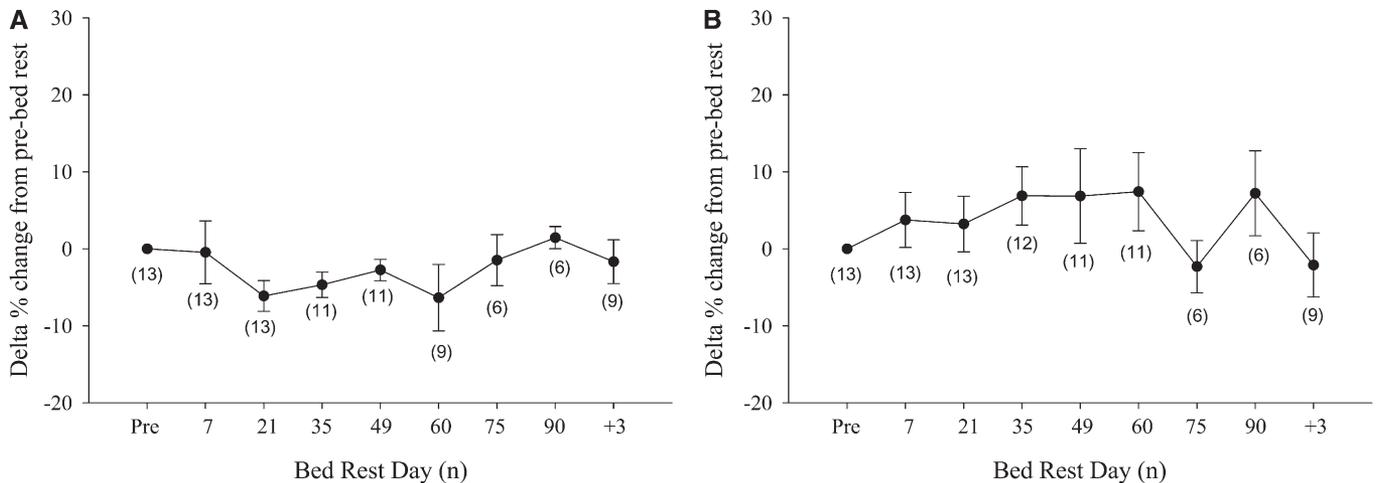


Fig. 3. Direct arterial dilation with nitroglycerin in the A) brachial artery and B) anterior tibial artery. These graphs show the difference between baseline and maximal nitroglycerin-induced dilation (delta) for each time point compared to the change during the pre-bed rest measurement. Statistical testing performed through day 60 due to low subject count beyond BR60.

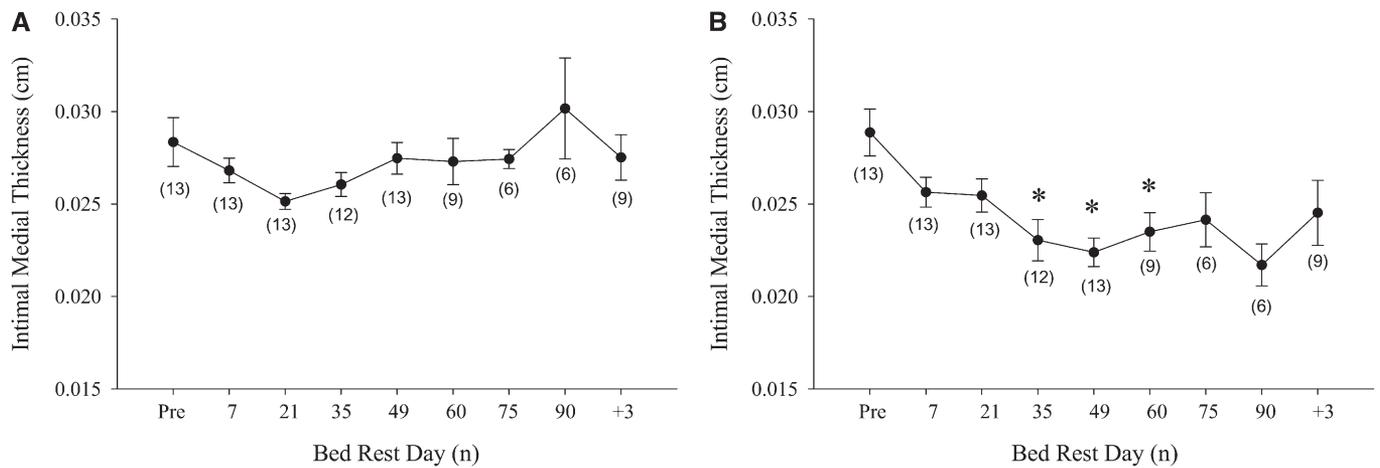


Fig. 4. Intimal medial thickness in the A) brachial artery and B) anterior tibial artery. * $P \leq 0.05$ within group for bed rest day compared to pre-bed rest. Statistical testing performed through day 60 due to low subject count beyond BR60.

Not all vascular beds respond to simulated microgravity in a parallel fashion. There are recent reports from several groups detailing structural remodeling of resistance vasculature in rats after hind limb suspension (8,23,30). In this model, the rat is suspended by the tail to achieve fluid shifts similar to those in microgravity. As a result, the forelimbs are used for locomotion more than during control, and the hind limbs are used less. This causes changes in the two primary mechanical forces that act on the vasculature: transmural pressures and shear stress. During suspension, these forces increase in the forelimbs, but decrease in the hind limbs. Both the smooth muscle and the endothelium respond to these changes. Vasculature in the forelimbs shows hypertrophic remodeling while that in the hind limbs shows atrophic remodeling (29). Following hind limb suspension, vasodilatory responses to acetylcholine injections (an endothelium-dependent response) are reduced in the femoral artery and soleus arterioles (23), both of which have been exposed to reduced blood flow and shear stress, but increased in the carotid artery, which has been exposed to increased blood flow and shear stress (11). The media cross-sectional area in arteries also decreases in the hind limb (8,23), but not the forelimb, suggesting atrophic remodeling in the hind limb (8). Hind limb vessels also have a diminished responsiveness to vasoconstrictors (7),

which shows that function is altered in parallel with structure.

These changes might mirror the arterial changes seen in hypertensive and normotensive patients, or rather their opposites. Hypertensive patients may present with arterial hypertrophy and in our model the leg arteries, where the hydrostatic pressure is greatly decreased, show a significant atrophy. A more intensive study of the mechanisms involved might provide information that benefits the greater clinical community.

The second change in anterior tibial artery function over the course of bed rest was an increase in flow-mediated dilation. This could be related to the change in wall thickness discussed above, as a thinner arterial wall may allow a greater concentration of NO to reach the smooth muscle cells. The third response, dilation in response to sublingual nitroglycerin, was unchanged. At first glance, these findings may seem incongruent; however, they can be reasonably explained. Flow-mediated dilation and sublingual nitroglycerin provide the vascular smooth muscle cells with the dilator nitric oxide by two very different mechanisms. The first provides NO indirectly but locally via the endothelium. The second provides NO directly but systemically. Our results show that an alteration in the NO-sGC signal transduction cascade in the smooth muscle is unlikely, as there is no systemic change in endothelium-independent NO

TABLE II. ECHOCARDIOGRAPHY RESULTS FOR SYSTOLIC FUNCTION, MORPHOLOGY, AND DIASTOLIC FUNCTION DURING PRE-BED REST, AND DAYS BR7, BR21, BR31, AND BR49 OF BED REST.

	Pre-Bed Rest	BR7	P-Value	BR21	P-Value	BR31	P-Value	BR49	P-Value
LV diastolic diameter (cm)	4.94	4.83	NS	4.68	0.015	4.79	NS	4.75	NS
LV systolic diameter (cm)	3.00	2.78	0.035	2.84	NS	2.91	NS	2.72	0.005
Ejection fraction	69	73	NS	70	NS	70	NS	74	0.05
LV mass (g)	118.7	118.7	NS	119.2	NS	114.7	NS	109.9	NS
Stroke volume	80.3	80.0	NS	71.6	NS	75.4	NS	77.6	NS
Isovolumic relaxation time (ms)	72.9	85.0	0.025	83.9	0.041	95.9	< 0.001	89.2	0.002
Isovolumic contraction time (ms)	49.1	53.3	NS	47.5	NS	53.0	NS	48.8	NS
Ejection time(ms)	299	287	NS	281	0.028	282	0.041	275	0.002
Myocardial performance index	0.41	0.49	0.033	0.47	0.082	0.53	< 0.001	0.51	0.004

Statistical analyses are included in separate columns. Significant differences are in bold.

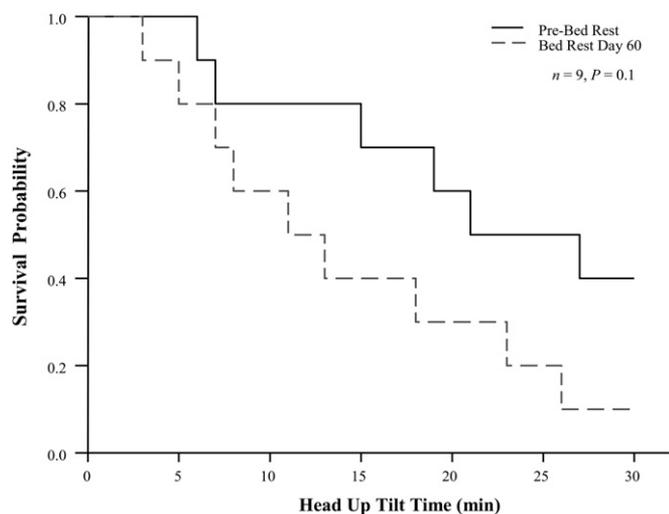


Fig. 5. Survival analysis of tilt test standing times. The solid line represents the probability of standing at each time point of the tilt test before bed rest. The dashed line represents the probability of standing following 42–60 d of bed rest.

dilation. However, as discussed above there may be a relationship between the change in wall thickness and the flow-mediated dilation, which could confound the traditional interpretation differentiating between endothelial dependent and independent vasodilation.

In animal studies, arterial function is usually tested with local intra-arterial injections of vasoactive substances or ex-vivo preparations. Unfortunately, our institutional constraints do not allow intra-arterial injections in bed rest subjects. Thus, we measured arterial function only from the standpoint of changes in the dilatory function of the arteries. Rat data suggests arterial smooth muscle in the lower body would be less able to constrict following head-down tilt (30). We are currently assessing arterial constrictor function in a hypovolemia model that has produced changes in left ventricular (LV) mass similar to spaceflight.

Cardiac Function

This is the most complete echocardiographic assessment of resting cardiac structure and function during long-duration bed rest of which we are aware. We found several measures that indicated a decrement in cardiac structure and in both systolic and diastolic function.

Left ventricular mass did not change during this study. Conversely, LV mass has been reported to be decreased after spaceflight by both MRI (22) and ultrasound techniques (25). We have previously shown, through mathematical modeling, that the post-spaceflight decrease in LV mass is most likely due to interstitial dehydration of the myocardium secondary to spaceflight-induced hypovolemia (25). Unfortunately, LV mass has never been measured during spaceflight, so it is not known whether the same effect would occur in the absence of gravity. In the current study, LV mass was measured during head-down tilt, but not measured after the subjects resumed ambulatory activity. This may explain the absence of change in LV mass. A suggestion for future

bed rest campaigns is to add a measurement early during post-bed rest reconditioning.

Despite the lack of change in LV mass, there were several changes in cardiac function. It is not clear which, if any of these, occurred in response to the decrease in preload caused by the hypovolemia. However, the degree of change suggests true changes in function. In Table II we show that some, but clearly not all measures of cardiac performance are changed following bed rest. There is no consensus as to which measures best represent clinically relevant dysfunction in bed rested subjects; however, some interesting trends can be gleaned from these data. Isovolumic relaxation time increased significantly during bed rest, indicating decreased diastolic function. This is similar to results obtained following spaceflight (25). Myocardial performance index, a global measure consisting of both systolic and diastolic parameters, showed a decrease in performance, suggesting an overall decrease in cardiac function. It is not unusual that echocardiographic measurements of cardiac function seem to contradict each other (4). Each measurement corresponds to parts of the heart that are subject to different degrees of influence by aortic, atrial, and ventricular pressures, and volumes. The heterogeneous nature of these results does not invalidate their usefulness, rather they illustrate that the effects of bed rest on cardiac function are complex and multifactorial. We have previously reported changes in cardiac function following long-duration spaceflight that also show a heterogeneous pattern (15); however, that study lacked some of the more mechanistic measurements that we now present. From the data in this study, it appears as though bed rest and long-duration spaceflight both alter cardiac function. It is not clear whether these changes are the result of decreases in fluid volume and preload, or if they represent a true change in cardiac muscle function. Future spaceflight studies should include more detailed echocardiograms to allow for a more direct comparison.

One primary concern is that most measures of cardiac function are somewhat preload dependent. It is well known that preload changes during bed rest, similar to spaceflight, due to the pronounced fluid redistribution-induced decrease in plasma volume. This could explain the increase in MPI seen at BR7 and BR21 (Table II). However, it does not explain why MPI further increased on BR31 and BR49, because plasma volume had stabilized. It could be that plasma volume accounts for the initial response and another mechanism drives the changes seen following 35 d of bed rest. The second mechanism could include cardiac deconditioning due to the decreased aerobic activity required during bed rest. This could lead to a decrease in contractility or possibly a remodeling of the myocardium (as supported by decreases in LV diameters, but not LV mass). While we were unable to perform statistical analysis on the time points beyond BR49, there are obvious trends that suggest that the cardiac changes persist to 90 d. Further examination of later time points will provide more insight into the ramifications of long-term bed rest, and

potentially longer term spaceflight, on the cardiovascular system.

Limitations

A major limitation of this study is the subject number at the varying time points. This is largely due to the forced evacuation of subjects for Hurricane Rita (September 2005). These subjects only completed 42–53 d of the designed 90-d bed rest protocol, and only a relatively small number of subjects completed 90 d of bed rest. We have chosen to limit our statistical analysis in this report to the first 60 d of bed rest (although we show all data in the figures) in order to use the data points shared by the most subjects.

ACKNOWLEDGMENTS

Sponsored by the NASA Flight Analogs Project; conducted at the NIH-funded (M01 RR 0073) GCRC at the University of Texas Medical Branch, Galveston, TX, and NASA grant NAS9-97005 to JVM.

Authors and affiliations: Steven H. Platts, Ph.D., and Janice V. Meck, Ph.D., NASA Johnson Space Center, Houston, TX; David S. Martin, B.A., Michael B. Stenger, Ph.D., Sondra A. Perez, M.S., and L. Christine Ribeiro, M.S., Wyle Integrated Science and Engineering, Houston, TX; and Richard Summers, Ph.D., University of Mississippi, University, MS.

REFERENCES

- Aubert AE, Beckers F, Verheyden B. Cardiovascular function and basics of physiology in microgravity. *Acta Cardiol* 2005; 60:129–51.
- Buckey JC Jr, Lane LD, Levine BD, Watenpaugh DE, Wright SJ, Moore WE, et al. Orthostatic intolerance after spaceflight. *J Appl Physiol* 1996; 81:7–18.
- Charles JB, Lathers CM. Cardiovascular adaptation to spaceflight. *J Clin Pharmacol* 1991; 31:1010–23.
- Choong CY. Left ventricle V: diastolic function—its principles and evaluation. In: Weyman A, ed. *Principles and practice of echocardiography*. Philadelphia: Lea & Febiger; 1994:575–624.
- Convertino VA. Cardiovascular consequences of bed rest: effect on maximal oxygen uptake. *Med Sci Sports Exerc* 1997; 29:191–6.
- D'Aunno DS, Dougherty AH, deBlock HF, Meck JV. Effect of short- and long-duration spaceflight on QTc intervals in healthy astronauts. *Am J Cardiol* 2003; 91:494–7.
- Delp MD. Myogenic and vasoconstrictor responsiveness of skeletal muscle arterioles is diminished by hindlimb unloading. *J Appl Physiol* 1999; 86:1178–84.
- Delp MD, Colleran PN, Wilkerson MK, McCurdy MR, Muller-Delp J. Structural and functional remodeling of skeletal muscle microvasculature is induced by simulated microgravity. *Am J Physiol Heart Circ Physiol* 2000; 278:H1866–73.
- Fritsch-Yelle JM, Leuenberger UA, D'Aunno DS, Rossum AC, Brown TE, Wood ML, et al. An episode of ventricular tachycardia during long-duration spaceflight. *Am J Cardiol* 1998; 81:1391–2.
- Fritsch-Yelle JM, Whitson PA, Bondar RL, Brown TE. Subnormal norepinephrine release relates to presyncope in astronauts after spaceflight. *J Appl Physiol* 1996; 81:2134–41.
- Jasperse JL, Woodman CR, Price EM, Hasser EM, Laughlin MH. Hindlimb unweighting decreases eNOS gene expression and endothelium-dependent dilation in rat soleus feed arteries. *J Appl Physiol* 1999; 87:1476–82.
- Leach CS, Alfrey CP, Suki WN, Leonard JI, Rambaut PC, Inners LD, et al. Regulation of body fluid compartments during short-term spaceflight. *J Appl Physiol* 1996; 81:105–16.
- Levine BD, Lane LD, Watenpaugh DE, Gaffney FA, Buckley JC, Blomqvist CG. Maximal exercise performance after adaptation to microgravity. *J Appl Physiol* 1996; 81:686–94.
- Maas AH, Hamelink ML, de Leeuw RJ. An evaluation of the spectrophotometric determination of Hb02, HbC0, and HB in blood with the co-oximeter IL 182. *Clin Chim Acta* 1970; 29:303–9.
- Martin DS, South DA, Wood ML, Bungo MW, Meck JV. Comparison of echocardiographic changes after short- and long-duration spaceflight. *Aviat Space Environ Med* 2002; 73:532–6.
- Meck JV, Reyes CJ, Perez SA, Goldberger AL, Ziegler MG. Marked exacerbation of orthostatic intolerance after long- vs. short-duration spaceflight in veteran astronauts. *Psychosom Med* 2001; 63:865–73.
- Meck JV, Waters WW, Ziegler MG, deBlock HF, Mills PJ, Robertson D, et al. Mechanisms of postspaceflight orthostatic hypotension: low alpha1-adrenergic receptor responses before flight and central autonomic dysregulation postflight. *Am J Physiol* 2004; 286:H1486–95.
- Meck JV, Dreyer SA, Warren LE. Long-duration head-down bed rest: project overview, vital signs, and fluid balance. *Aviat Space Environ Med* 2009; 80(5,Suppl.): A1–8.
- Morey-Holton ER, Globus RK. Hindlimb unloading rodent model: technical aspects. *J Appl Physiol* 2002; 92:1367–77.
- Nishimura RA, Callahan MJ, Schaff HV, Ilstrup DM, Miller FA, Tajik AJ. Noninvasive measurement of cardiac output by continuous-wave Doppler echocardiography: initial experience and review of the literature. *Mayo Clin Proc* 1984; 59:484–9.
- Pavy-Le Traon A, Heer M, Narici MV, Rittweger J, Vernikos J. From space to Earth: advances in human physiology from 20 years of bed rest studies (1986–2006). *Eur J Appl Physiol* 1986–2006; 2007 101: 143–94.
- Perhonen MA, Franco F, Lane LD, Buckley JC, Blomqvist CG, Zerwekh JE, et al. Cardiac atrophy after bed rest and spaceflight. *J Appl Physiol* 2001; 91:645–53.
- Schrage WG, Woodman CR, Laughlin MH. Hindlimb unweighting alters endothelium-dependent vasodilation and eNOS expression in soleus arterioles. *J Appl Physiol* 2000; 89:1483–90.
- Shaw JG, Johnson EC, Voyles WF, Greene ER. Noninvasive Doppler determination of cardiac output during submaximal and peak exercise. *J Appl Physiol* 1985; 59:722–31.
- Summers RL, Martin DS, Meck JV, Coleman TG. Mechanism of spaceflight-induced changes in left ventricular mass. *Am J Cardiol* 2005; 95:1128–30.
- Summers RL, Platts SH, Martin DS, Coleman TG. Systems analysis of the mechanisms of cardiac diastolic function changes after microgravity exposure. *Acta Astronautica* 2008; 63:722–6.
- Waters WW, Platts SH, Mitchell BM, Whitson PA, Meck JV. Plasma volume restoration with salt tablets and water after bed rest prevents orthostatic hypotension and changes in supine hemodynamic and endocrine variables. *Am J Physiol* 2005; 288:H839–47.
- Waters WW, Ziegler MG, Meck JV. Post-spaceflight orthostatic hypotension occurs mostly in women and is predicted by low vascular resistance. *J Appl Physiol* 2002; 92:586–94.
- Zhang J, Steiner JP. Nitric oxide synthase, immunophilins and poly (ADP-ribose) synthetase: novel targets for the development of neuroprotective drugs. *Neurol Res* 1995; 17:285–8.
- Zhang LF. Vascular adaptation to microgravity: what have we learned? *J Appl Physiol* 2001; 91:2415–30.