Nutritional Status Assessment Before, During, and After Long-Duration Head-Down Bed Rest

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Introduction: Bed rest is a valuable ground-based model for many of the physiological changes that are associated with spaceflight. Nutritional changes during and after 60 or 90 d of head-down bed rest were evaluated. **Methods:** A total of 13 subjects (8 men, 5 women; ages 26–54 yr) participated in either 60 or 90 d of bed rest. Blood and urine were collected twice before bed rest and about once per month during bed rest. Samples were stored frozen and batch analyzed. Data were analyzed using repeated-measures analysis of variance. **Results:** During bed rest, markers of bone resorption (such as N-telopeptide excretion, $P < 0.001$) increased and serum concentration of parathyroid hormone decreased ($P < 0.001$). Also, oxidative damage markers such as superoxide dismutase increased ($P < 0.05$), and after 90 d of bed rest, total antioxidant capacity decreased ($P < 0.05$). During bed rest, iron status indices showed patterns of increased iron stores with a decreased concentration of transferrin receptors ($P < 0.01$). **Discussion:** These changes are similar to some of those observed during spaceflight, and further document the utility of bed rest as a model of spaceflight.

Keywords: spaceflight, nutrition, weightlessness, bone resorption, parathyroid hormone, oxidative stress, antioxidants, iron, transferrin.

SOME OF THE CLINICAL concerns for long-duration spaceflight (longer than 30 d) are bone and muscle loss, inadequate dietary intake, decreased nutrient stores, increased oxidative damage due to factors such as radiation exposure and increased iron stores, and altered nutrient metabolism (41). Designing and testing countermeasures to mitigate these negative physiological effects of spaceflight require a solid understanding of changes in nutrient status during spaceflight, because macro- and micronutrients are essential for every cell and function in the body. Data from 11 International Space Station (ISS) astronauts suggest that their nutritional status is compromised after long-duration spaceflight (40). Some of the most striking changes are decrements in vitamin D, folate, vitamin K, and vitamin E status, and increases in markers of bone resorption and oxidative damage. Changes in urinary excretion of phosphorus and magnesium were also evident after 4 to 6 mo of spaceflight (40).

That vitamin D status is decreased after long-duration spaceflight is clearly indicated by results of studies from Skylab, Mir, and ISS missions (17,33,39,40). Even for crewmembers who used supplements, 25-hydroxyvitamin D status decreased (17,40). The low levels of vitamin D in the space food supply and the absence of ultraviolet light during spaceflight likely contribute to this phenomenon. Altered vitamin D status is accompanied by evidence of increased bone resorption during and after spaceflight (31,33,40). During spaceflight, the excretion rates of urinary markers of bone resorption are typically 100–150% of their preflight values (3,5,31,33,39). Loss of bone mineral is increased by skeletal unloading during weightlessness (10,21,33,36,39,41).

Evidence exists that during and after spaceflight, along with changes in nutrient status, the metabolism of certain nutrients is altered (41), and iron is one of these nutrients. The data suggest that as a result of microgravity, storage pools of iron are shifted so that less iron is in red blood cells and more is stored in ferritin, and less iron is transported (less transferrin) (35,40). Serum ferritin concentration is significantly increased, the amount of ferritin iron is slightly greater than before launch, and the amount of transferrin is decreased. Red blood cell mass also decreases (1,6,14,43). After landing, a delay in replacing red blood cells often leads to decreased hemoglobin, hematocrit, and mean corpuscular volume (34,40).

Like other physiological effects of weightlessness, changes in nutrient status and metabolism can be studied in the bed rest analog of weightlessness. The qualitative effects of bed rest on bone and calcium homeostasis are similar to the effects of spaceflight, but the quantitative effects are generally less than (about half) those of spaceflight (41). As reviewed by Meck and colleagues (23) and others (25), bed rest is a good model for spaceflight, but lack of standard procedures has limited the ability to draw conclusions across studies. This report is one of a series of reports on the Flight Analogs Project, which is designed to lay the groundwork for a standard bed rest protocol. Standard procedures were developed

and implemented in the bed rest study presented here. As part of that larger study, we sought to determine the effect of bed rest on nutrient status and metabolism, and to compare these observations with what is observed after long-duration spaceflight.

METHODS

Study methods were those described by Meck et al. (23). 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, and the UTMB General Clinical Research Center Science Advisory Committee. Subjects received verbal and written explanations of the bed rest and test protocols before they provided written informed consent. Blood and urine samples were collected before, during, and after bed rest (details below) for analysis of markers of nutritional status.

Subjects

A total of 13 healthy subjects (8 men, 5 women) participated in the study. The mean ($\pm$ SD) age of the 13 subjects was 35.5 yr $\pm$ 9.6. The subjects had an average height of 168 cm $\pm$ 9, and weighed 72.6 kg $\pm$ 16.2. The study was designed to maintain subjects' weight throughout their participation. Details of the diet protocol are provided in another paper (13).

Biochemical Analyses

Most biochemical analyses were performed by standard commercial techniques, as described previously (7,28,38,40). Fat-soluble vitamins were analyzed by high-performance liquid chromatography with electrochemical detection (28). The collection profile for some analytes was altered slightly; specifically, some samples were not collected on BR+7 (7 d after bed rest ended). Radioimmunoassay was used to determine 1,25 dihydroxyvitamin D in picograms per milliliter ($pg \cdot ml^{-1}$), which was converted to picomoles per liter ($pmol \cdot L^{-1}$) using a conversion factor of 2.4.

Statistical Analysis

Data were analyzed using repeated-measures analysis of variance (ANOVA) techniques. Because the bed rest periods varied in length, two separate repeated-measures ANOVAs were performed. Initially, a two-way repeated-measures ANOVA was performed with data from subjects 1-13, using a pre-bed rest average and data from bed rest days 28 and 60. Gender and time effects were tested with this model. In a second analysis, a one-way repeated-measures ANOVA was performed with data from the 90-d subjects only, using all time points: pre-bed rest average, BR28, BR60, BR90, and BR+7, $N = 6$ total (2 men, 4 women). The effect of the longer bed rest was specifically tested in this model. Post hoc Bonferroni tests were performed with each model to assess pairwise differences between the pre-bed rest time point and each time point during and after bed rest. Vitamin

D intake was plotted against 25-hydroxyvitamin D status, and dietary magnesium was plotted against urinary magnesium, and correlation coefficients were determined. The data in the tables include all data (pre-bed rest, $N = 13$; BR28, $N = 13$; BR60, $N = 13$; BR90, $N = 6$; BR+7, $N = 9$), and significant effects and interactions found using the two statistical models are indicated.

RESULTS

Calcium and Bone Metabolism

Bone metabolism markers determined in serum and urine are presented in **Table I**. The serum concentration of parathyroid hormone (PTH) was lower at BR28 and BR60 than before bed rest ($P < 0.001$). During bed rest and recovery, neither 25-hydroxyvitamin D nor 1,25 dihydroxyvitamin D changed significantly. Dietary vitamin D (13) was positively correlated with 25-hydroxyvitamin D status ($r = 0.4$, $P < 0.01$).

Urinary markers of bone resorption, including N-telopeptide, pyridinium crosslinks, and deoxypyridinoline, when expressed per 24 h ($nmol \cdot d^{-1}$), were increased during bed rest (Table I, $P < 0.001$). Similar trends were observed for N-telopeptide and crosslinks when the data were normalized to creatinine. The activity of serum bone-specific alkaline phosphatase, a marker of bone formation, did not change significantly during or after bed rest; however, total alkaline phosphatase was significantly elevated on BR60. The blood concentration of osteocalcin, which is produced by osteoblasts and is often used as a marker of bone formation, did not change significantly. There was no effect of gender or bed rest on serum calcium or ionized calcium.

Vitamins

Red blood cell folate concentration (**Table II**) increased during bed rest (BR60, $P < 0.05$), but on BR60 it was lower in men than in women ($P < 0.05$). Plasma γ-tocopherol was lower on BR28 and BR60 ($P < 0.05$) than it was before bed rest when all subjects were combined, and on BR60 when 90-d subjects' data were combined. When all subjects' data were analyzed, no difference in α-tocopherol was detected during or after bed rest. Beta-carotene was higher on BR28, BR60, and BR90, and during recovery ($P < 0.001$, Bonferroni tests on all subjects for BR28 and BR60, and on 90-d subjects for BR90 and BR+7), but no change in retinol or retinol-binding protein occurred over time. Retinol was in general higher in men than in women ($P < 0.05$). The concentration of retinyl palmitate was too low to be detected in all but three samples from one subject (data not shown).

Oxidative Damage Markers and Antioxidants

Oxidative stress during bed rest was evident from the findings that superoxide dismutase activity increased from before to during bed rest (BR28, $P < 0.05$), and that total antioxidant capacity decreased during recovery ($P < 0.05$) (**Table III**). When data from the 90-d subjects were analyzed, there was a significant effect of time for glutathione peroxidase activity ($P < 0.001$, no significant

TABLE I. MARKERS OF BONE METABOLISM BEFORE, DURING, AND AFTER BED REST.

	Pre	BR28	BR60	BR90	BR+7
PTH[b,e] (pg · ml^{-1})	32.5 ± 15.6	21.1 ± 8.0*	24.1 ± 13.6*	23.0 ± 9.9	32.3 ± 14.1
25-hydroxyvitamin D (nmol · L^{-1})	62.3 ± 32.2	58.8 ± 20.3	57.1 ± 19.7	60.7 ± 25.8	51.3 ± 14.8
1,25-dihydroxyvitamin D (pmol · L^{-1})	97.8 ± 33.2	78.6 ± 34.2	90.6 ± 32.2	90.5 ± 24.8	82.5 ± 27.6
N-telopeptide[b,g] (nmol · d^{-1})	461 ± 229	772 ± 397*	772 ± 403*	604 ± 300*	526 ± 267
N-telopeptide (nmol · mmol^{-1} creatinine)	35.5 ± 17.0	56.0 ± 23.3*	56.4 ± 26.4*	61.2 ± 41.1*	48.9 ± 23.6
Pyridinium crosslinks[b,g] (nmol · d^{-1})	305.9 ± 121.3	430.0 ± 183.2*	438.7 ± 199.7*	427.6 ± 110.3*	382.9 ± 145.8*
Pyridinium crosslinks[b,g] (nmol · mmol^{-1} creatinine)	23.6 ± 6.6	31.4 ± 9.9*	32.5 ± 13.8*	41.3 ± 15.7*	35.0 ± 11.7*
Deoxypyridinoline[b,g] (nmol · d^{-1})	58.7 ± 20.9	88.1 ± 31.0*	94.9 ± 32.7*	81.3 ± 32.7*	73.5 ± 28.4
Deoxypyridinoline[b,g] (nmol · mmol^{-1} creatinine)	4.6 ± 1.5	6.6 ± 2.3*	7.2 ± 2.6*	8.0 ± 4.1*	6.7 ± 2.5*
BSAP (U · L^{-1})	22.7 ± 3.5	25.1 ± 8.0	24.2 ± 5.2	26.3 ± 3.2	23.9 ± 5.5
Alkaline phosphatase[a] (U · L^{-1})	60.4 ± 7.6	64.3 ± 9.6	67.3 ± 9.6*	67.2 ± 3.3	59.3 ± 7.4
Osteocalcin (ng · ml^{-1})	12.8 ± 4.8	12.4 ± 2.6	13.5 ± 4.1	14.1 ± 5.0	13.8 ± 4.1
Serum calcium (mg · dl^{-1})	8.9 ± 0.4	9.0 ± 0.4	9.0 ± 0.4	8.9 ± 0.3	
PCBA ionized calcium (mmol · L^{-1})	1.19 ± 0.04	1.19 ± 0.03	1.19 ± 0.04	1.20 ± 0.03	1.18 ± 0.04
Calcium[a] (mmol · d^{-1})	5.6 ± 2.2	6.3 ± 2.9	6.5 ± 2.6	7.3 ± 3.8	5.0 ± 2.5

PTH = parathyroid hormone; BSAP = bone-specific alkaline phosphatase; PCBA = portable clinical blood analyzer (whole blood was used for this analysis).
Data are means ± SD. Pre, N = 13; BR28, N = 13; BR60, N = 13; BR90, N = 6; BR+7, N = 9.
[a,b] Significant effect of time when data from all 13 subjects were combined for pre-bed rest, bed rest day 28 (BR28), and BR60, [a] $P < 0.05$, [b] $P < 0.001$. [c] Significant effect of gender when all 13 subjects were combined, $P < 0.05$. [d] Significant interaction between time and gender, $P < 0.05$. [e,f,g] Significant effect of time when the six subjects who completed 90 d of bed rest were combined, [e] $P < 0.05$, [f] $P < 0.01$, [g] $P < 0.001$. * Significantly ($P < 0.05$) different from pre-bed rest as determined by a post hoc Bonferroni t-test. Differences at BR28 and BR60 were found using data from all subjects; differences at BR90 and BR+7 were found using data from only the 90-d subjects.

differences with a Bonferroni posttest), and serum lipid peroxides were lower at all time points during bed rest and recovery than they were before bed rest ($P < 0.001$). Also, urinary excretion of 8-hydroxy 2'-deoxyguanosine was greater during bed rest ($P < 0.05$), but the difference was not significant when data for all subjects from the first 60 d were analyzed together.

Urine Chemistry

Urinary excretion of phosphorus (**Table IV**) showed a significant ($P < 0.01$) effect of gender, being greater in men. When data from the 90-d subjects were combined, urinary magnesium was significantly ($P < 0.01$) lower at BR90 and BR+7. Urinary and dietary magnesium were not significantly correlated. Urinary sulfate was significantly ($P < 0.01$) lower at BR+7 than it was before bed rest and showed a significant ($P < 0.01$) interaction between time and gender when data from all subjects were combined. Urinary total volume showed a significant

($P < 0.01$) effect of gender but no effect of time, and no significant effect of time or gender was detected on citrate or oxalate. Urinary 3-methyl histidine, a marker of protein catabolism, and urinary 4-pyridoxic acid, a marker of vitamin B6 status and muscle mass (4), were both significantly ($P < 0.05$) affected by gender (men > women).

Blood Chemistry

Blood sodium, whether measured in serum or with the portable clinical blood analyzer (PCBA) (30), did not change significantly during bed rest and was not affected by gender (**Table V**), but men had higher potassium concentrations than women. No time or gender effect on serum chloride was found. Men had higher magnesium concentrations than women, and the serum phosphorus of the 90-d subjects and all subjects combined was significantly ($P < 0.01$ for 90-d subjects and $P < 0.001$ for all subjects combined) higher on BR28 and BR60 than before bed rest started.

TABLE II. VITAMINS BEFORE, DURING, AND AFTER BED REST.

	Pre	BR28	BR60	BR90	BR+7
RBC folate[a,d] (ng · ml^{-1})	573.3 ± 161.7	612.2 ± 166.5	640.8 ± 177.8*	792.3 ± 224.3	807.0 ± 213.2
γ-tocopherol[a,e] (μg · ml^{-1})	1.6 ± 0.6	1.0 ± 0.4*	1.0 ± 0.5*	0.9 ± 0.2	1.1 ± 0.3
α-tocopherol (μg · ml^{-1})	10.7 ± 3.2	10.4 ± 3.8	10.9 ± 6.1	7.7 ± 1.9	10.5 ± 4.3
β-carotene[b,f] (μg · ml^{-1})	0.22 ± 0.10	0.42 ± 0.20*	0.45 ± 0.26*	0.42 ± 0.18*	0.38 ± 0.17*
Retinol[c] (ng · ml^{-1})	415.1 ± 99.7	451.7 ± 113.6	434.3 ± 148.4	353.3 ± 65.0	390.3 ± 80.6
Retinol-binding protein (mg · L^{-1})	44.7 ± 8.9	46.9 ± 9.6	46.6 ± 13.8	46.4 ± 13.9	45.0 ± 8.5

RBC = red blood cell.
Data are means ± SD. Pre, N = 13; BR28, N = 13; BR60, N = 13; BR90, N = 6; BR+7 (when data were available), N = 9.
[a,b] Significant effect of time when data from all 13 subjects were combined for pre-bed rest, bed rest day 28 (BR28), and BR60, [a] $P < 0.05$, [b] $P < 0.01$. [c] Significant effect of gender when all 13 subjects were combined, $P < 0.05$ (men > women). [d] Significant interaction between time and gender, [d] $P < 0.05$. [e,f] Significant effect of time when the six subjects who completed 90 d of bed rest were combined, [e] $P < 0.05$, [f] $P < 0.001$. * Significantly ($P < 0.05$) different from pre-bed rest as determined by a post hoc Bonferroni t-test. Differences at BR28 and BR60 were found using data from all subjects; differences at BR90 and BR+7 were found using data from only the 90-d subjects.

TABLE III. MARKERS OF OXIDATIVE DAMAGE AND ANTIOXIDANTS BEFORE, DURING, AND AFTER BED REST.

	Pre	BR28	BR60	BR90	BR+7
SOD[a] (U · g^{-1} hgb)	1126 ± 231	1536 ± 409*	1358 ± 195	1078 ± 281	1245 ± 222
TAC[b] (mmol · L^{-1})	1.81 ± 0.23	1.77 ± 0.22	1.77 ± 0.22	1.64 ± 0.15	1.60 ± 0.27*
GPX[c] (U · g^{-1} hgb)	42.4 ± 9.7	41.1 ± 12.8	38.7 ± 10.4	49.4 ± 14.6	47.1 ± 20.0
Lipid peroxides[c] (μmol · L^{-1})	0.44 ± 0.08	0.44 ± 0.28*	0.30 ± 0.12*	0.31 ± 0.07*	0.30 ± 0.12*
8-hydroxy 2'-deoxyguanosine[b] (μg · g^{-1} creatinine)	2.6 ± 0.9	3.1 ± 1.2	2.8 ± 1.0	2.9 ± 1.4	2.4 ± 0.6

SOD, superoxide dismutase; hgb, hemoglobin; TAC, total antioxidant capacity; GPX, glutathione peroxidase. Data are means ± SD. Pre, $N = 13$; BR28, $N = 13$; BR60, $N = 13$; BR90, $N = 6$; BR+7, $N = 9$.
[a] Significant effect of time when data from all 13 subjects were combined for pre-bed rest, bed rest day 28 (BR28), and BR60, $P < 0.05$. [b,c] Significant effect of time when the six subjects who completed 90 d of bed rest were combined, [b] $P < 0.05$, [c] $P < 0.001$. * Significantly ($P < 0.05$) different from pre-bed rest as determined by a post hoc Bonferroni t-test. In some cases, despite the occurrence of a significant main effect, the Bonferroni post hoc test failed to identify specific differences between time points. This is likely related to the conservative nature of this test.

Serum pH showed no effect of bed rest, but pH of the 90-d subjects, measured in whole blood with the PCBA, was significantly ($P < 0.05$) higher on BR60 than before bed rest. Men had higher whole-blood pH ($P < 0.05$), serum carbon dioxide (bicarbonate) ($P < 0.05$), serum uric acid ($P < 0.001$), and serum creatinine ($P < 0.001$) than women. In the 90-d subjects, blood urea nitrogen was significantly ($P < 0.05$) lower on BR90 than it was before bed rest. Blood glucose was not significantly affected by bed rest or gender, but glucose measured with the PCBA showed a significant interaction between time and gender. Serum triglycerides were highly variable on BR60, related to one outlier. Serum triglycerides and cholesterol were unchanged during bed rest, and were similar for men and women.

Aspartate transaminase activity was lower on BR28 and BR60 in men but not women ($P < 0.01$). Activity of the enzymes alanine transaminase and glutamyltransferase were not changed during bed rest, but were significantly greater in men than women during the study. Creatine kinase activity was significantly affected by time, but post hoc testing did not localize this effect to a particular day. Neither time nor gender significantly affected lactate dehydrogenase.

Men had significantly ($P < 0.01$) greater serum albumin concentrations than women, and total serum protein showed a significant ($P < 0.05$) effect of gender and

a significant ($P < 0.05$) interaction between time and gender. The globulin fractions, C-reactive protein, thyroxine, and thyroid stimulating hormone were not significantly affected by time or gender. Serum concentrations of minerals, including copper, selenium, and zinc, were not significantly changed during bed rest, but in the 90-d subjects copper was significantly lower on BR90 and BR+7 than it was before bed rest ($P < 0.05$).

Hematology

The results of hematologic analysis are shown in **Table VI**. Significant effects of gender (higher in men than in women) were found for red blood cell count, red cell distribution width, bilirubin (the end product of hemoglobin metabolism), and lymphocytes, and a significant interaction between time and gender was found for mean corpuscular volume. A significant effect of time was detected for platelet count, bilirubin, and neutrophils when data from all 13 subjects were combined for pre-bed rest and bed rest days 28 and 60, and for mean corpuscular hemoglobin, bilirubin, and monocytes when all data from the 90-d subjects were combined.

Iron Metabolism Indicators

Mean hematocrit and hemoglobin concentration tended to be lower at the end of bed rest and during

TABLE IV. URINE CHEMISTRY BEFORE, DURING, AND AFTER BED REST.

	Pre	BR28	BR60	BR90	BR+7
Phosphorus[c] (mmol · d^{-1})	23.1 ± 5.4	25.4 ± 6.4	23.2 ± 5.4	19.1 ± 3.6	25.0 ± 11.6
Magnesium[e] (mmol · d^{-1})	4.5 ± 0.8	4.1 ± 0.9	4.2 ± 0.7	3.9 ± 0.4*	3.3 ± 0.7*
Sulfate[c,g] (mmol · d^{-1})	19.4 ± 2.9	19.7 ± 5.4	20.1 ± 6.0	17.8 ± 5.5	16.2 ± 4.3*
Total volume[c] (L)	2.9 ± 0.6	3.0 ± 0.6	2.9 ± 0.6	2.3 ± 0.3	2.7 ± 0.8
pH[c]	6.3 ± 0.3	6.4 ± 0.1	6.3 ± 0.5	6.2 ± 0.2	6.2 ± 0.2
Citrate (mg · d^{-1})	689.4 ± 202.4	657.4 ± 214.8	651.4 ± 200.2	704.4 ± 222.0	702.1 ± 222.7
Oxalate (mg · d^{-1})	34.7 ± 8.4	39.7 ± 13.3	31.8 ± 7.7	36.8 ± 11.4	30.6 ± 6.9
3-methyl histidine[b] (μmol · d^{-1})	221.7 ± 67.9	232.4 ± 79.3	206.3 ± 58.3	197.1 ± 92.6	206.4 ± 70.7
4-pyridoxic acid[b] (μmol · d^{-1})	10.9 ± 6.3	9.4 ± 4.0	10.9 ± 5.5	8.0 ± 2.4	12.1 ± 5.6
Creatinine[c] (mmol · d)	13.2 ± 3.2	13.8 ± 4.5	14.0 ± 4.7	11.2 ± 4.3	11.3 ± 3.4

Data are means ± SD. Pre, $N = 13$; BR28, $N = 13$; BR60, $N = 13$; BR90, $N = 6$; BR+7, $N = 9$.
[a] Significant effect of time when data from all 13 subjects were combined, $P < 0.05$. [b,c] Significant effect of gender when all 13 subjects were combined, [b] $P < 0.05$, [c] $P < 0.01$ (men > women). [d,e,f] Significant effect of time when the six subjects who completed 90 d of bed rest were combined, [d] $P < 0.05$, [e] $P < 0.01$, [f] $P < 0.001$. [g] Significant interaction between time and gender when all 13 subjects were combined, $P < 0.01$. * Significantly ($P < 0.05$) different from pre-bed rest as determined by a post hoc Bonferroni t-test. Differences at BR28 and BR60 were found using data from all subjects; differences at BR90 and BR+7 were found using data from only the 90-d subjects.

TABLE V. BLOOD CHEMISTRY BEFORE, DURING, AND AFTER BED REST.

	Pre	BR28	BR60	BR90	BR + 7
Sodium (mmol · L^{-1})**	137.6 ± 3.6	138.8 ± 1.8	139.2 ± 1.9	140.0 ± 1.8	134.8 ± 12.1
PCBA sodium (mmol · L^{-1})	139.3 ± 1.1	138.9 ± 1.4	139.2 ± 1.1	140.5 ± 1.5	140.0 ± 1.4
Potassium[f] (mmol · L^{-1})**	4.1 ± 0.2	4.2 ± 0.2	4.1 ± 0.3	4.1 ± 0.3	3.8 ± 0.3
PCBA potassium[e] (mmol · L^{-1})	3.9 ± 0.2	3.9 ± 0.2	3.9 ± 0.3	3.8 ± 0.2	3.7 ± 0.1
Chloride (mmol · L^{-1})**	103.3 ± 3.3	104.3 ± 2.9	103.5 ± 2.7	106.7 ± 3.3	102.1 ± 9.0
Magnesium[d] (mg · dl^{-1})**	2.0 ± 0.2	2.0 ± 0.2	2.1 ± 0.2	1.9 ± 0.1	
Phosphorus[c,j] (mg · dl^{-1})**	3.5 ± 0.4	4.1 ± 0.3*	3.8 ± 0.5*	3.9 ± 0.3	
pH**	7.36 ± 0.03	7.37 ± 0.04	7.35 ± 0.04	7.38 ± 0.05	
PCBA pH[d,i]	7.37 ± 0.04	7.36 ± 0.04	7.38 ± 0.04*	7.38 ± 0.05	7.40 ± 0.04
Carbon dioxide[d] (mmol · L^{-1})**	25.5 ± 4.6	25.7 ± 2.7	26.3 ± 2.3	24.6 ± 1.0	
Uric acid[f] (mg · dl^{-1})**	5.12 ± 1.91	4.81 ± 1.39	4.92 ± 1.55	4.60 ± 1.70	
Creatinine[b,f] (mg · dl^{-1})	0.9 ± 0.2	0.9 ± 0.2*	0.9 ± 0.2*	0.8 ± 0.2	
Blood urea nitrogen[i] (mg · dl^{-1})**	13.3 ± 2.4	12.9 ± 1.6	12.0 ± 2.3	11.2 ± 1.9*	
Glucose (mg · dl^{-1})**	83.6 ± 19.6	82.9 ± 13.4	76.6 ± 14.1	74.0 ± 10.6	
PCBA glucose[g] (mg · dl^{-1})	89.5 ± 12.9	86.5 ± 10.4	87.8 ± 11.4	84.0 ± 4.2	88.2 ± 12.0
Triglycerides (mg · dl^{-1})**	116.5 ± 57.0	118.2 ± 73.9	163.6 ± 195.0	100.5 ± 59.3	113.6 ± 68.3
Cholesterol (mg · dl^{-1})**	171.7 ± 37.7	164.7 ± 38.5	175.2 ± 51.0	150.5 ± 38.7	164.9 ± 52.0
AST[h] (U · L^{-1})**	21.5 ± 7.3	17.0 ± 3.0	17.8 ± 3.6	16.5 ± 3.1	19.8 ± 6.9
ALT[d] (U · L^{-1})**	20.1 ± 14.1	17.1 ± 5.6	19.8 ± 8.1	14.5 ± 4.8	16.0 ± 11.2
Glutamyltransferase[e] (U · L^{-1})**	15.5 ± 7.9	14.6 ± 6.6	16.5 ± 7.9	13.3 ± 8.1	
Creatine kinase[a] (U · L^{-1})**	155.1 ± 127.3	75.7 ± 42.4	74.8 ± 45.1	69.3 ± 22.9	
Lactate dehydrogenase (U · L^{-1})**	123.5 ± 18.2	113.7 ± 24.5	125.4 ± 23.4	130.8 ± 28.8	
Albumin[e] (g · dl^{-1})**	4.2 ± 0.4	4.1 ± 0.4	4.2 ± 0.5	3.9 ± 0.5	3.8 ± 0.6
Transthyretin[e] (mg · dl^{-1})**	24.2 ± 4.8	25.1 ± 5	25.6 ± 5.9	21.4 ± 2.7	22.3 ± 4.4
Total protein[d,g] (g · dl^{-1})**	6.7 ± 0.5	6.6 ± 0.5	6.7 ± 0.5	6.5 ± 0.4	6.4 ± 0.7
Alpha-1 globulin (g · dl^{-1})**	0.21 ± 0.02	0.19 ± 0.03	0.22 ± 0.04	0.20 ± 0.00	0.21 ± 0.03
Alpha-2 globulin (g · dl^{-1})**	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
Beta globulin (g · dl^{-1})**	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.2
Gamma globulin (g · dl^{-1})**	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	0.9 ± 0.2
Ceruloplasmin (mg · dl^{-1})**	26.6 ± 5.2	25.8 ± 6.0	25.6 ± 6.8	22.7 ± 3.1	28.1 ± 6.0
C-reactive protein (mg · dl^{-1})**	1.72 ± 2.23	2.04 ± 2.54	2.99 ± 4.43	1.90 ± 1.78	
Thyroxine (free T4, ng · cl^{-1})**	1.26 ± 0.18	1.25 ± 0.13	1.23 ± 0.20	1.28 ± 0.20	
Thyroid stimulating hormone (μIU · ml^{-1})**	1.95 ± 1.20	2.21 ± 1.27	2.53 ± 1.53	2.08 ± 0.59	
Copper[i] (μmol · L^{-1})	14.6 + 2.6	14.0 + 2.1	14.2 + 2.0	14.8 + 1.7*	14.5 + 1.4*
Selenium (μmol · L^{-1})	2.3 ± 0.3	2.4 + 0.2	2.5 + 0.3	2.4 ± 0.3	2.4 ± 0.3
Zinc (μmol · L^{-1})	11.5 + 1.9	12.5 + 1.7	12.7 + 2.4	12.2 + 1.3	11.4 + 1.9

PCBA = portable clinical blood analyzer (whole blood was used for these analyses); AST = aspartate transaminase; ALT = alanine transaminase. Except for measurements made with the PCBA, all analytes were measured in serum. Data are means ± SD. Pre, N = 13; BR28, N = 13; BR60, N = 13; BR90, N = 6; BR+7 (when data were available), N = 9.

[a,b,c] Significant effect of time when data from all 13 subjects were combined for pre-bed rest, bed rest day 28 (BR28), and BR60; [a] P < 0.05, [b] P < 0.01, [c] P < 0.001. [d,e,f] Significant effect of gender when all 13 subjects were combined, [d] P < 0.05, [e] P < 0.01, [f] P < 0.001 (men > women). [g,h] Significant interaction between time and gender, [g] P < 0.05, [h] P < 0.01. [i,j,k] Significant effect of time when the six subjects who completed 90 d of bed rest were combined, [i] P < 0.05, [j] P < 0.01, [k] P < 0.001. * Significantly (P < 0.05) different from pre-bed rest as determined by a post hoc Bonferroni t-test. Differences at BR28 and BR60 were found using data from all subjects; differences at BR90 and BR+7 were found using data from only the 90-d subjects. ** Analyzed by the Johnson Space Center Clinical Laboratory.

recovery than they were before bed rest (P = 0.06 and P = 0.08, respectively) (Table VI). The serum concentration of transferrin receptors decreased significantly, about 25%, during bed rest (P < 0.01), but ferritin and serum iron concentrations (data not shown) did not change significantly. Concentrations of ferritin at BR90 seem to be much lower than concentrations at the other time points (which were highly variable), but this reflects the smaller number of subjects for that data point, and the small N explains why the decrease was not statistically significant. The subject-to-subject variability for each time point was accounted for in the repeated-measures statistical model.

DISCUSSION

Findings that many physiological systems are affected by spaceflight have implications for nutrition and nutri-

ents as a casualty of these effects, or potentially as a countermeasure against these effects (9,11,16,18,19,24,29,32,41). Understanding the role of nutrition in the physiological adaptation to spaceflight will require much additional effort, including the use of ground-based analogs such as bed rest. Our results from 60 and 90 d of bed rest, conducted using standard procedures, showed that this analog produced many of the effects of spaceflight on nutritional status.

Bed rest provides a valuable model for bone loss associated with spaceflight (22,25,41). In the study presented here, three biochemical markers of bone resorption indicated that a sustained increase in bone resorption occurred during bed rest. These data are consistent with results of previous studies, which showed that markers of bone resorption are elevated within the first few days to weeks of bed rest (2,12,15,37,44). On the

TABLE VI. HEMATOLOGY AND IRON STATUS BEFORE, DURING, AND AFTER BED REST.

	Pre	BR28	BR60	BR90	BR + 7
Red blood cell count[e] (10^6/mm³)**	4.98 ± 0.60	5.00 ± 0.70	4.96 ± 0.77	4.55 ± 0.68	
Mean corpuscular volume[f,g] (fl)**	89.63 ± 2.96	89.06 ± 2.94	89.25 ± 3.27	91.30 ± 4.25*	
Mean corpuscular hemoglobin[g] (pg)**	29.87 ± 1.29	30.10 ± 1.26	30.42 ± 1.18	30.82 ± 1.72*	
Mean corpuscular hemoglobin concentration (g · dl⁻¹)**	33.27 ± 0.74	33.75 ± 0.92	33.87 ± 0.52	33.62 ± 0.47	
Red cell distribution width[d] (%)**	12.67 ± 1.09	12.53 ± 0.81	12.68 ± 0.86	12.75 ± 0.98	
Platelet count[a] (10^3/mm³)**	234.92 ± 59.71	255.62 ± 69.30	260.62 ± 73.45	237.67 ± 94.84	
Bilirubin[a,d,i] (mg · dl⁻¹)**	0.64 ± 0.15	0.49 ± 0.22*	0.57 ± 0.27*	0.38 ± 0.10*	
Monocytes[g] (%)**	8.77 ± 1.24	8.23 ± 1.83	7.96 ± 1.59	7.85 ± 1.49	
Lymphocytes[d] (%)**	34.54 ± 8.57	34.32 ± 7.58	31.45 ± 7.11	31.58 ± 3.52	
Neutrophils[a] (%)**	53.38 ± 7.93	53.78 ± 7.20	57.53 ± 7.13*	57.70 ± 4.14	
Eosinophils (%)**	3.00 ± 2.17	3.18 ± 1.70	2.66 ± 1.68	2.33 ± 0.99	
Hematocrit[e] (%)**	44.3 ± 5.1	45.2 ± 4.5	43.5 ± 5.6	41.4 ± 4.8	39.3 ± 4.5
Hemoglobin[e] (g · dl⁻¹)**	14.7 ± 1.7	14.9 ± 1.8	14.9 ± 1.9	13.9 ± 1.7	13.1 ± 1.5
Transferrin receptors[b] (μg · ml⁻¹)	5.5 ± 1.6	4.7 ± 0.8*	4.2 ± 0.9*	4.7 ± 1.0	4.6 ± 1.1
Ferritin (ng · ml⁻¹)**	55.4 ± 55.4	58.5 ± 55.5	59.8 ± 56.2	20.0 ± 11.6	47.7 ± 45.4
Transferrin[c,h] (mg · dl⁻¹)**	265.8 ± 35.2	232.2 ± 29.6*	235.8 ± 36.2*	225.3 ± 24.0	222.4 ± 26.8*
Total iron binding capacity[h] (μg · dl⁻¹)**	309.77 ± 85.22	293.23 ± 26.88	299.38 ± 39.86	290.83 ± 24.38*	
Transferrin saturation (%)**	33.69 ± 18.90	28.23 ± 7.12	27.15 ± 4.62	23.17 ± 8.13	26.67 ± 2.31

Data are means ± SD. Pre, $N = 13$; BR28, $N = 13$; BR60, $N = 13$; BR90, $N = 6$.
[a,b,c] Significant effect of time when data from all 13 subjects were combined for pre-bed rest, bed rest day 28 (BR28), and BR60, [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$. [d,e] Significant effect of gender when all 13 subjects were combined, [d] $P < 0.05$, [e] $P < 0.001$ (men > women). [f] Significant interaction between time and gender, $P < 0.01$. [g,h,i] Significant effect of time when the six subjects who completed 90 d of bed rest were combined, [g] $P < 0.05$, [h] $P < 0.01$, [i] $P < 0.001$. * Significantly ($P < 0.05$) different from pre-bed rest as determined by a post hoc Bonferroni t-test. Differences at BR28 and BR60 were found using data from all subjects; differences at BR90 and BR+7 were found using data from only the 90-d subjects. ** Analyzed by the Johnson Space Center Clinical Laboratory.

other hand, blood concentrations of parathyroid hormone, which generally promotes bone resorption, have tended to be lower during spaceflight than before spaceflight (39), and PTH was significantly lower during than before bed rest. After spaceflight, PTH is unchanged or elevated (39,40); in the present bed rest study, it was unchanged.

Similar to what is observed during spaceflight (39,40), the increase in bone resorption during bed rest is accompanied by little to no change in markers of bone formation (37). Vitamin D status, as assessed by serum 25-hydroxyvitamin D concentration, typically decreases during and immediately after spaceflight (17,39,40), but was not changed in the bed rest study presented here, although vitamin D status was positively correlated with vitamin D intake during bed rest.

Our results provide evidence that increased oxidative stress, which occurs during spaceflight, also occurs during bed rest. Several theories exist to explain why oxidative damage would increase during bed rest. Red blood cells are normally exposed to reactive oxygen species from the superoxide radical anion formed during oxidation of hemoglobin to methemoglobin, and from the catalytic actions of ferrous and ferric ions in the cytoplasm, which lead to the production of hydroxyl radicals (27). Preventing excess formation of reactive oxygen species (which would cause an increase in oxidative damage) in erythrocytes requires the two antioxidant enzymes superoxide dismutase and glutathione peroxidase. In the present study, both superoxide dismutase and glutathione peroxidase were elevated at some point during or after bed rest, indicating that a derangement had occurred in the balance between formation and re-

moval of reactive oxygen species. Consistent with these data, total antioxidant capacity tended to be lower during bed rest and was significantly lower 7 d after bed rest ended. A marker of oxidative damage, 8-hydroxy 2'-deoxyguanosine, was higher during bed rest in the 90-d subjects. These data are also corroborated by results of several other bed rest studies that show evidence for elevated oxidative stress and increased reactive oxygen species (26,27,45). The serum concentration of the antioxidant γ-tocopherol was also lower during bed rest (BR28, BR60) and recovery, similar to what is observed after spaceflight. Oxidative damage could occur because of increased iron stores, which are apparent in both spaceflight and this bed rest model. However, further studies would need to be done to determine the precise source of oxidative damage during bed rest.

Another similarity between results from spaceflight and the bed rest study described here involves magnesium excretion. After 4 to 6 mo of spaceflight, urinary magnesium is decreased about 45% (17,20,40). In this report, urinary magnesium was decreased about 30% after 60 or 90 d of bed rest, and during bed rest urinary magnesium was not correlated with dietary magnesium. Decreased urinary magnesium could be a point of concern for long-duration spaceflights because magnesium plays a role in inhibiting calcium oxalate renal stone formation (8,42). Magnesium is also critical for cardiovascular health. Whether the decrease observed here and after spaceflight is related to altered dietary intake or intestinal absorption of magnesium, or to altered bone metabolism, warrants further investigation.

For variables such as blood concentrations of copper, selenium, and zinc, slight changes from pre-bed rest

values occurred during bed rest. These changes suggest that the 11-d acclimatization period before bed rest may not have been long enough to normalize these variables. These details will need to be addressed if standardization of nutritional status during bed rest is an ultimate goal.

The findings reported here for hematologic variables and iron indices are qualitatively consistent with spaceflight data (1,34,40,43). The concentration of transferrin receptors decreased about 10% after 4 to 6 mo of spaceflight (40), and the bed rest findings reported here show that transferrin receptors decreased about 28%. The concentration of transferrin also decreased during bed rest, as it does during spaceflight. Unlike spaceflight results, however, no changes were observed in serum ferritin. Also, after long-duration spaceflight, hematocrit and hemoglobin are decreased 2–4% (40), but we did not see these changes in bed rest. Obviously, while bed rest is a valuable analog for spaceflight, it cannot mimic the complexities and uniqueness of spaceflight for all systems.

The fact that we observed gender differences for several variables cannot be ignored. Some of these differences were expected due to a difference in normal ranges for healthy men and women (hematocrit, hemoglobin, potassium, glutamyltransferase, uric acid). Some of the interactions are more difficult to explain, such as the decrease in aspartate transaminase during bed rest in men but not women. Other changes, such as the lower red blood cell folate concentration in men than in women on BR60, might be explained by diet, or intake related to body size.

The nutritional data from this bed rest study provide evidence that chronic responses to bed rest and weightlessness are qualitatively similar, and they provide a model for researching the mechanisms behind deconditioning associated with disuse. Additional work is needed to elucidate the effects of deconditioning on macronutrient metabolism, and the mechanism of oxidative damage during bed rest should be further investigated. The evaluation of nutritional status during bed rest studies will also be critical as countermeasures are developed and evaluated.

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