

## Effects of 21 days of bed rest, with or without artificial gravity, on nutritional status of humans

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**Zwart SR, Crawford GE, Gillman PL, Kala G, Rodgers AS, Rogers A, Inniss AM, Rice BL, Ericson K, Coburn S, Bourbeau Y, Hudson E, Mathew G, DeKerlegand DE, Sams CF, Heer MA, Paloski WH, Smith SM.** Effects of 21 days of bed rest, with or without artificial gravity, on nutritional status of humans. *J Appl Physiol* 107: 54–62, 2009. First published December 12, 2008; doi:10.1152/jappphysiol.91136.2008.—Spaceflight and bed rest models of microgravity have profound effects on physiological systems, including the cardiovascular, musculoskeletal, and immune systems. These effects can be exacerbated by suboptimal nutrient status, and therefore it is critical to monitor nutritional status when evaluating countermeasures to mitigate negative effects of spaceflight. As part of a larger study to investigate the usefulness of artificial gravity as a countermeasure for musculoskeletal and cardiovascular deficits during bed rest, we tested the hypothesis that artificial gravity would have an effect on some aspects of nutritional status. Dietary intake was recorded daily before, during, and after 21 days of bed rest with artificial gravity ( $n = 8$ ) or bed rest alone ( $n = 7$ ). We examined body composition, hematology, general blood chemistry, markers of oxidative damage, and blood levels of selected vitamins and minerals before, during, and after the bed rest period. Several indicators of vitamin status changed in response to diet changes: serum  $\alpha$ - and  $\gamma$ -tocopherol and urinary 4-pyridoxic acid decreased ( $P < 0.001$ ) and plasma  $\beta$ -carotene increased ( $P < 0.001$ ) in both groups during bed rest compared with before bed rest. A decrease in hematocrit ( $P < 0.001$ ) after bed rest was accompanied by a decrease in transferrin ( $P < 0.001$ ), but transferrin receptors were not changed. These data provide evidence that artificial gravity itself does not negatively affect nutritional status during bed rest. Likewise, artificial gravity has no protective effect on nutritional status during bed rest.

microgravity; countermeasure; vitamin E;  $\beta$ -carotene; vitamin B<sub>6</sub>

AS THE U.S. SPACE PROGRAM prepares for exploration-class missions to the moon and Mars, countermeasures are needed to mitigate the adverse cardiovascular, musculoskeletal, and other physiological impacts of spaceflight on the human body. These countermeasures are designed to maintain physiological function and physical capabilities, which are required to perform tasks. It also will be necessary for space explorers to maintain optimal health once they reach the partial gravity environments of the moon and Mars (one-sixth or one-third gravity, respectively). The primary aim of a countermeasure is to mitigate a

known risk, but countermeasures can sometimes be harmful to other body systems that are not being specifically targeted. For example, we previously found that a countermeasure designed to protect muscle during simulated weightlessness unintentionally had a negative effect on bone (53). This emphasizes the fact that multiple systems should be monitored when a countermeasure is evaluated to ensure that all effects (positive or negative) are well characterized.

Spaceflight causes physiological changes that alter nutritional status (39). Ground-based models of spaceflight, including bed rest, are good analogs for some of the nutritional changes observed during spaceflight. For example, hematologic variables and changes in iron metabolism during spaceflight and bed rest are qualitatively similar (3, 38, 40, 46). Another commonality between spaceflight and bed rest is the decrease in urinary magnesium after exposure (40, 54). A change in nutritional status, whether it is due to physiological changes directly caused by spaceflight or bed rest or to altered behavior that is caused by spaceflight or bed rest and leads to changes in diet, could profoundly affect the physiological systems being targeted by a countermeasure.

The Artificial Gravity Pilot Study was a collaborative project designed to test the effectiveness of an artificial gravity countermeasure on negative effects of spaceflight (bone loss, muscle loss, changes in cardiovascular function). As part of this larger study, we sought to identify changes in nutritional status that would occur with an artificial gravity countermeasure during 6° head-down tilt (HDT) bed rest. The nutritional variables chosen for the study were blood or urinary concentrations of a wide variety of macro- and micronutrients, chosen to obtain a sampling of effects on several different physiological systems. The variables included those routinely tested in astronauts before and after long-duration spaceflight and included indicators of the status of vitamins and minerals (40). We hypothesized that artificial gravity would have an effect on some aspects of nutrition but would not affect other aspects. For example, if the apparent increases in iron storage during bed rest and spaceflight are in fact due to a decrease in mechanical load, then we would expect artificial gravity to mitigate this effect. We would expect that artificial gravity would not have an effect on changes in nutritional status that are caused by changes in dietary patterns. A secondary goal of this study was to determine whether changes in nutritional status were related to unintentional changes in nutrient intake

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during the study and also to confirm that no negative nutritional effects were introduced by artificial gravity.

## METHODS

**Bed rest standard conditions.** The environment in the General Clinical Research Center (GCRC) at the University of Texas Medical Branch was carefully controlled. Ambient temperature was maintained at  $72 \pm 2^\circ\text{F}$  and relative humidity at  $70 \pm 5\%$ . Subjects were awakened each morning at 6:00 AM, and room lights were extinguished at 10:00 PM. Napping was prohibited during the waking hours. Sleep medication (zolpidem tartrate) was prescribed as needed during the night hours. During the bed rest phase of the study, subjects were confined to strict  $6^\circ$  HDT bed rest. Subject monitors outside each room ensured round-the-clock compliance. Because many subjects experience headache, backache, and/or constipation during the first few days of HDT bed rest, acetaminophen was provided as needed for pain and docusate sodium was prescribed as needed each evening for stool softening. A  $6^\circ$  HDT gurney was used for transport to the centrifuge facility and the shower. Caloric intake was monitored throughout the study and adjusted as necessary to maintain constant body weight. Initial caloric and fluid intakes were set to 35.7 kcal/kg and 28.5 ml/kg (2,500 kcal/day and 2,000 ml/day for a 70-kg subject), with carbohydrate, fat, and protein provided in a caloric ratio of 55:30:15. Daily mineral consumption was controlled for phosphorus (1,400 mg), sodium (2 mmol/kg), potassium (1.3 mmol/kg), and calcium (1,000 mg). No caffeine, cocoa, chocolate, tea, or herbal beverage was permitted. All urinary and fecal excretions were collected.

**Study schedule.** The study had three distinct phases. For the first 11 days after arriving at the bed rest facility, subjects remained ambulatory (BR-11 through BR-1). During this phase, subjects acclimated physiologically and psychologically to the facility, the study diet regimen, and the circadian cycle regulation, and investigators obtained pre-bed rest baseline measures. Subjects then began the 21-day bed rest phase (BR1 through BR21), throughout which they were confined to strict  $6^\circ$  HDT bed rest. Each was transported daily to the centrifuge, where the subjects in the artificial gravity (AG) treatment group received 1-h AG exposures. The control group (Con) subjects were instrumented and placed on the centrifuge but did not spin. After the 21-day bed rest phase, subjects began an 8-day recovery phase (BR+1 through BR+8), during which they remained in the bed rest facility but returned to ambulation. Investigators obtained post-bed rest baseline measures during this phase. Blood and urine samples were collected before, during, and after bed rest for analysis of markers of nutritional status.

**Centrifuge protocol.** All subjects were transferred daily from their hospital beds to the centrifuge facility by a short (5–10 min) gurney ride (while  $6^\circ$  head down). Subjects were transferred to one arm of the short-radius centrifuge and secured to the subject station in the supine position ( $6^\circ$  head down) using a five-point harness system. Control subjects remained in this orientation for the next hour (sham spin), whereas AG subjects were exposed to inertial loading as follows. The subject was oriented radially (feet out) with the feet placed against a support surface. The padded subject station extended from above the top of the subject's head to just below the subject's hips. It was designed to glide freely in the radial (body  $z$ -axis) direction over a range of 10 cm on a set of low-friction bearings. A counterweight system minimized ( $<4$  kg) the  $z$ -axis loading added to the subject by the moving components of the subject station. Loading was standardized for subjects of different body heights by adjusting the radial distance of the foot support surface from the center of rotation (218–229 cm) and the angular velocity of the SRC arm (30.7–32.1 rpm) to achieve  $z$ -axis loading of 2.5  $g$  at the feet and 1.0  $g$  at the estimated level of the heart for each subject.

**Subjects.** Twenty healthy male subjects were admitted into the study, and 15 completed the protocol. The mean ( $\pm$ SD) age of the 15

subjects was  $31 \pm 3$  yr for the AG group and  $28 \pm 2$  yr for the Con group. The AG and Con groups of subjects had an average height of  $175 \pm 6$  and  $176 \pm 7$  cm, respectively. AG subjects weighed  $81 \pm 9$  kg, and Con subjects weighed  $82 \pm 8$  kg. The protocol for this study was approved by the Johnson Space Center Committee for the Protection of Human Subjects and the University of Texas Medical Branch Institutional Review Board. All subjects provided written, informed consent before they were enrolled in the study.

**Food system.** All meals for the ambulatory and bed rest phases of this study were prepared in a metabolic kitchen where all foods were weighed to  $\pm 0.1$  g using Mettler Toledo balances. Menus were rotated on a 7-day cycle. Subjects were admitted to the GCRC on Sundays, thus ensuring that they consumed identical foods on the same study day (with 1 exception in which 1 subject was offset by 1 day).

At baseline, dietary composition (mean for all subjects combined  $\pm$  SD) was 55% carbohydrates ( $410 \pm 31$  g/day), 30% fat ( $99 \pm 8$  g/day), and 15% protein ( $112 \pm 9$  g/day). Vitamin and mineral supplements were not administered. Caloric requirements were individualized for each subject, and the Harris-Benedict equation (15) for calculation of resting energy expenditure was used to estimate caloric intake. Activity factors of 1.6 and 1.3 were used for ambulatory and bed rest phases, respectively.

Each subject's caloric intake was adjusted as necessary to maintain body weight within 3% of the weight measured on *day 3* of bed rest (BR3), when the initial fluid shift and any diuresis resulting from postural change would have been completed. When necessary, caloric intake was manipulated by increasing carbohydrates and fat while keeping protein constant.

The target intake of nutrients (see Table 1) was based on the National Aeronautics and Space Administration (NASA) spaceflight nutritional requirements (24), with some adaptations to the ground-based model to make a set of requirements for nutrient intake in the Flight Analogs/Bed Rest Research Project at NASA (designated "NASA bed rest requirements"). These requirements reflect daily requirements based on Dietary Reference Intake values (11, 12, 18–20, 28). Calcium and phosphorus intakes were targeted to be 1,000–1,200 mg/day. Sodium was targeted to be  $<3,500$  mg/day, and potassium, 3,500 mg/day. Target fluid intake was 28.5 ml/kg body wt. Filtered water was provided for drinking and used in food preparation. For other nutrients, daily intake was considered acceptable if it averaged 100–125% of NASA bed rest requirements, with no single daily intake being  $<80\%$  of the requirement.

All diets were composed, and actual dietary intakes were calculated, using the Nutrition Data System for Research (NDS-R) software, version 2005, developed by the Nutrition Coordinating Center (University of Minnesota, Minneapolis, MN) (31).

**Body weight, body mass, and body composition.** A bed scale was used to weigh subjects at the same time each morning before the first meal. Body mass and body composition were determined before and after bed rest using dual-energy X-ray absorptiometry (DXA) with a fan-beam densitometer (Hologic QDR 4500W; Hologic, Waltham, MA). Scans of each volunteer's whole body were acquired in triplicate 7 days before and 1 day after bed rest. The average of triplicate measures at each time point was used for statistical analysis. DXA scan images were analyzed by Johnson Space Center Bone and Mineral Laboratory personnel.

**Biological sample collection and processing.** Fasting blood samples were collected 9 days before and immediately before bed rest began. All blood samples were collected between 6:00 and 7:00 AM. Blood was also collected on BR8, BR15, and BR21 (last day of bed rest before subjects stood up) and again on BR+8. Urine was collected throughout the protocol, but most analyses were performed only on samples collected before bed rest on BR-9, BR-8, BR-4, BR-3, BR-2, and BR-1; during bed rest on BR8, BR9, BR15, BR16, BR20, and BR21; and after bed rest on BR+0 (last day of bed rest after subjects stood up), BR+1, BR+6, and BR+7. Subjects were

fasting for 8 h before each blood draw. Blood samples were collected and processed to yield whole blood, plasma, or serum, depending on the specific analytes to be measured. All samples were stored at  $-80^{\circ}\text{C}$  until batch analysis.

All single-void urine samples were collected in individual bottles and stored refrigerated until they were processed (within 3 days). Twenty-four-hour urine pools were created, pH was measured, and aliquots were prepared and frozen for analysis.

**Biochemical analyses.** Most analyses were performed by standard commercial techniques, as described previously (36, 38, 40). Hemoglobin, hematocrit, and mean corpuscular volume were determined using a Coulter MAXM instrument (Beckman Coulter, Brea, CA). Serum ferritin and transferrin were analyzed using the Immulite (Diagnostic Products, Los Angeles, CA) and Olympus AU400E instruments (Olympus America, Irving, TX), respectively. Transferrin receptors were measured using a commercially available ELISA (Ramco Laboratories, Houston, TX). Red blood cell (RBC) folate was measured using a commercially available radioreceptor assay (Diagnostic Products).

The pH, glucose, and concentration of ionized calcium in whole blood were determined by ion-specific electrometry performed with a portable analyzer (i-STAT; Abbott Laboratories, East Windsor, NJ). This instrument had been used in several earlier studies (33, 37, 38).

RBC superoxide dismutase, glutathione peroxidase, and total antioxidant capacity were measured spectrophotometrically using commercially available kits (Randox Laboratories, Crumlin, UK). HPLC techniques were used to determine concentrations of vitamins A, E, and K (10, 34) in plasma, as well as that of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in urine (4). Plasma lipid peroxides were measured using a commercially available kit (MDA-586 assay system; OxisResearch, Portland, OR). The HPLC analysis method for urinary 4-pyridoxic acid has been published (9).

Serum total cholesterol, triglycerides, albumin, creatinine, and total alkaline phosphatase were assayed using an Olympus AU400E automated clinical chemistry system. Transthyretin and ceruloplasmin were analyzed with the Dade Behring BN 100 system (Marburg, Germany). Urinary creatinine was analyzed spectrophotometrically (Jaffe method) on the NExCT clinical chemistry system (Alfa Wassermann, West Caldwell, NJ). Serum sodium, potassium, chloride, aspartate aminotransferase, and alanine aminotransferase were analyzed using the Olympus AU400E automated clinical chemistry system. C-reactive protein (CRP) was analyzed with the Immulite 2000 (Diagnostic Products). Serum protein electrophoresis was performed with the Beckman Appraise (Beckman Coulter). Leptin, cortisol, and testosterone were measured using RIA (Linco Research, St. Charles, MO; DiaSorin, Stillwater, MN; and Diagnostic Products). Endothelin-1 was measured using a chemiluminescent ELISA (R&D Systems, Minneapolis, MN). Serum and urinary minerals were measured using inductively coupled plasma mass spectrometry techniques (17).

Samples for vitamin C analysis were prepared from fresh plasma harvested from blood collected in Vacutainer tubes containing lithium heparin. An aliquot of this plasma was stabilized with 10% metaphosphoric acid containing 2 mM EDTA, and the samples were stored at  $-80^{\circ}\text{C}$ . The ascorbic acid concentration was determined in these samples by reversed-phase HPLC with an Agilent 1200 system coupled to an electrochemical detector. A short ( $4.6 \times 150$  mm) Zorbax SB-C8, 5- $\mu\text{m}$  column was used for the analysis, and the applied potentials were  $-200$  and  $+400$  mV. The mobile phases used were *phase A*, 50 mM sodium phosphate (pH 2.5)-methanol (90:10), and *phase B*, 50 mM sodium phosphate (pH 2.5)-methanol (10:90), with a gradient of 0–50% *phase B* during the first 5 min followed by stabilization of the column with 100% *phase A* for 4 more minutes. This method (26) was determined to be linear for ascorbic acid over its physiological range.

Retinol-binding protein was measured using a radial immunodiffusion kit (The Binding Site, San Diego, CA). Assay of urinary

3-methylhistidine was performed by ion-exchange chromatography on a Hitachi L8800 amino acid analyzer (Hitachi High Technologies, San Jose, CA). Urinary magnesium and phosphorus were measured spectrophotometrically; magnesium was measured using the Olympus AU400E and phosphorus using the NExCT clinical chemistry system (Alfa Wassermann). Urinary citrate, sulfate, and oxalate were determined using ion exchange chromatography (49, 50). RBC aspartate transaminase, glutathione reductase, and transketolase were measured spectrophotometrically (NExCT; Alfa Wasserman) (36, 38).

**Statistical analysis.** Statistical analyses were performed using the raw data. For each variable, the pre-bed rest (BR) mean was used as the pre-BR data point. The data were analyzed using repeated-measures ANOVA, with time and group as repeated factors. The dependent variables were the analytes measured. Post hoc Bonferroni *t*-tests were performed to assess specific differences between times or groups.

When correlations were evaluated, the data were analyzed by simple linear regression, and a Pearson correlation coefficient (*r*) was calculated. Statistical analyses were performed using SigmaStat software version 3.01a (SPSS, Chicago, IL), and  $P < 0.05$  was defined a priori as the level of significance. Data are means  $\pm$  SD.

## RESULTS

**Dietary intake.** AG and Con groups were not significantly different with respect to dietary intake of macronutrients. As planned in the experiment design, energy intake for both groups was reduced during bed rest compared with before bed rest to maintain body weight (Table 1,  $P < 0.001$ ). Because energy intake was less during bed rest, intake of all other nutrients by both groups was less during bed rest than in the ambulatory phases of the study. During all phases of the study, intakes for all nutrients (except vitamin E) were greater than the minimum intake level designed for the study.

**Body composition.** In both groups, body weight was significantly lower on BR16, BR17, BR21, and BR+0 than on BR3 ( $P < 0.001$ ), but otherwise body weight was maintained throughout bed rest (Fig. 1). After bed rest, body mass (as determined by DXA) was not significantly different from body mass before bed rest (Table 2). Total body fat mass was unchanged after bed rest, but lean body mass (excluding bone) was 0.9 and 1.1% less after bed rest in the Con and AG groups, respectively ( $P < 0.05$ ). The Con and AG groups were not different from each other, however. The loss of lean tissue was reflected in total mass, but its impact did not reach statistical significance.

**Oxidative stress.** Glutathione peroxidase, superoxide dismutase, total antioxidant capacity, and urinary 8-OHdG did not change during or after bed rest, and there were no differences between the Con and AG groups (Tables 3 and 4). There was a significant time  $\times$  group interaction for lipid peroxides (Table 3), with lower concentrations on BR21 than before bed rest in the AG subjects.

**Vitamin and electrolyte measurements.** The status of vitamin E and  $\beta$ -carotene changed during bed rest (Table 3), reflecting the changes in intake of those nutrients. Vitamin E intake was significantly lower during bed rest (Table 1), and plasma concentrations of  $\alpha$ - and  $\gamma$ -tocopherol declined during bed rest correspondingly. Intake of  $\beta$ -carotene was significantly lower during bed rest than the mean pre-bed rest intake. Plasma  $\beta$ -carotene was significantly greater than the pre-bed rest mean at all other time points, but  $\beta$ -carotene concentrations at BR-6 and BR-1 were different (BR-1 was greater than BR-6,  $P <$

Table 1. Dietary intake before, during, and after bed rest

	Con			AG			NASA Bed Rest Requirement
	Pre	BR	Post	Pre	BR	Post	
Energy, kcal/day	2,934±220	2,389±175 <sup>a</sup>	2,930±225	2,877±234	2,355±173 <sup>a</sup>	2,906±219	Maintain BW
Total carbohydrate, g/day	414±30	337±25 <sup>a</sup>	414±31	406±33	332±25 <sup>a</sup>	408±31	NA
Total protein, g/day	113±8	92±7 <sup>a</sup>	113±8	111±9	91±7 <sup>a</sup>	112±8	NA
Total fat, g/day	100±8	82±6 <sup>a</sup>	99±8	98±8	80±6 <sup>a</sup>	100±8	NA
Animal protein, g/day	79±6	63±5 <sup>a</sup>	78±6	77±6	62±4 <sup>a</sup>	77±6	NA
Vegetable protein, g/day	34±2	29±2 <sup>a</sup>	35±3	33±4	28±3 <sup>a</sup>	35±3	NA
Cholesterol, mg/day	574±45	460±42 <sup>a</sup>	564±51	552±52	450±40 <sup>a</sup>	558±49	NA
Total dietary fiber, g/day	31±2	25±2 <sup>a</sup>	29±2 <sup>a</sup>	30±3	24±2 <sup>a</sup>	29±2 <sup>a</sup>	10–25
Retinol, µg/day	757±26	652±50 <sup>a</sup>	812±53	731±76	641±53 <sup>a</sup>	807±73	1,000
β-Carotene (provitamin A carotenoid), µg/day	13,087±1,263	10,597±902 <sup>a</sup>	13,100±1,093	12,730±1,524	10,132±1,040 <sup>a</sup>	12,883±1,684	NA
Vitamin D (calciferol), µg/day	10±0	10±1 <sup>a</sup>	12±1 <sup>a</sup>	10±1	9±1 <sup>a</sup>	11±1 <sup>a</sup>	10
Vitamin E (total α-tocopherol equivalents), mg/day	15±1	13±1 <sup>a</sup>	16±1 <sup>a</sup>	14±1	13±1 <sup>a</sup>	15±1 <sup>a</sup>	20
Vitamin C (ascorbic acid), mg/day	309±18	254±16 <sup>a</sup>	308±22	289±27	242±20 <sup>a</sup>	291±27	100
Thiamin (vitamin B <sub>1</sub> ), mg/day	2.18±0.15	1.85±0.14 <sup>a</sup>	2.30±0.18	2.12±0.18	1.82±0.14 <sup>a</sup>	2.27±0.18	1.5
Riboflavin (vitamin B <sub>2</sub> ), mg/day	2.90±0.19	2.40±0.17 <sup>a</sup>	2.96±0.22 <sup>a</sup>	2.80±0.20	2.37±0.17 <sup>a</sup>	2.90±0.22 <sup>a</sup>	2.0
Niacin (vitamin B <sub>3</sub> ), mg/day	32±3	26±2 <sup>a</sup>	31±2 <sup>a</sup>	31±3	26±2 <sup>a</sup>	30±2 <sup>a</sup>	20
Pantothenic acid, mg/day	7±1	6±0 <sup>a</sup>	7±1	7±1	6±0 <sup>a</sup>	7±1	5.0
Vitamin B <sub>6</sub> (pyridoxine HCl, pyridoxal, and pyridoxamine), mg/day	3.16±0.22	2.64±0.15 <sup>a</sup>	3.09±0.15 <sup>a</sup>	3.06±0.22	2.60±0.20 <sup>a</sup>	2.99±0.20 <sup>a</sup>	2.0
Total folate, µg/day	634±34	548±32 <sup>a</sup>	676±44 <sup>a</sup>	605±54	532±44 <sup>a</sup>	658±54 <sup>a</sup>	400
Vitamin B <sub>12</sub> (cobalamin), µg/day	7±1	6±0 <sup>a</sup>	8±1 <sup>a</sup>	7±1	6±0 <sup>a</sup>	8±1 <sup>a</sup>	2.0
Calcium, mg/day	1,427±76	1,222±72 <sup>a</sup>	1,462±105 <sup>a</sup>	1,388±126	1,214±79 <sup>a</sup>	1,444±106 <sup>a</sup>	1,000–1,200
Phosphorus, mg/day	1,887±128	1,565±116 <sup>a</sup>	1,956±156 <sup>a</sup>	1,839±163	1,551±117 <sup>a</sup>	1,939±154 <sup>a</sup>	1,000–1,200
Magnesium, mg/day	391±26	325±23 <sup>a</sup>	393±28	389±37	325±26 <sup>a</sup>	392±32	350
Iron, mg/day	20±1	16±1 <sup>a</sup>	20±1 <sup>a</sup>	19±2	16±1 <sup>a</sup>	19±2 <sup>a</sup>	10
Zinc, mg/day	14±1	12±1 <sup>a</sup>	14±1 <sup>a</sup>	14±1	12±1 <sup>a</sup>	14±1 <sup>a</sup>	15
Selenium, µg/day	169±12	140±10 <sup>a</sup>	177±14 <sup>a</sup>	166±14	138±11 <sup>a</sup>	176±14 <sup>a</sup>	70
Sodium, mg/day	3,335±247	2,698±207 <sup>a</sup>	3,272±235 <sup>a</sup>	3,256±260	2,634±196 <sup>a</sup>	3,194±242 <sup>a</sup>	<3,500
Potassium, mg/day	3,989±310	3,274±244 <sup>a</sup>	3,904±291 <sup>a</sup>	3,921±333	3,237±246 <sup>a</sup>	3,855±310 <sup>a</sup>	3,500
Water, g/day	4,835±549	4,189±328 <sup>a</sup>	4,783±467	4,608±451	4,134±364 <sup>a</sup>	4,601±438	28.5 ml/kg

Values are means ± SD; *n* = 7 for the control (Con) group and *n* = 8 for the artificial gravity (AG) group. Pre, pre-bed rest ambulatory period; BR, bed rest; Post, post-bed rest ambulatory period; BW, body weight; NA, not applicable. Pre means are an average of 10 days immediately before bed rest, BR means are an average of 21 days of bed rest, and Post means are an average of the first 8 days after bed rest. <sup>a</sup>*P* < 0.001 vs. Pre.

0.001; data not shown), indicating that β-carotene intake was likely greater during the study than in the subjects' nominal diets.

Red blood cell folate concentration (Table 3) tended to be lower during bed rest than before bed rest (*P* = 0.077). Serum retinol was not significantly changed during bed rest. Retinyl palmitate was measured, but for most time points it was lower than detectable limits (data not shown). Urinary 4-pyridoxic

acid (Table 4) decreased during and after bed rest in both groups (*P* < 0.001), and for AG subjects it was lower at all time points (*P* < 0.05). The decrease in 4-pyridoxic acid excretion was consistent with the decrease in dietary vitamin B<sub>6</sub> during bed rest compared with before bed rest.

Serum chloride and sodium were not significantly changed during bed rest, but potassium was significantly greater (*P* < 0.001; Table 3). Whole blood electrolyte determinations matched findings in serum, except that a main effect of time was seen for sodium.

*General chemistry, mineral, hormone, blood protein, and lipid measurements.* In both groups, urinary pH was significantly lower on the first day after bed rest than before bed rest (*P* < 0.001; Table 4). Blood pH was significantly increased on the last day of bed rest (BR21) in the AG group only (*P* < 0.05; Table 5).

After a week of bed rest and during recovery, urinary magnesium of both groups was significantly less than baseline values (Table 4). Urinary phosphorus of both groups increased significantly during bed rest.

In both groups of this study, urinary sulfate (*P* < 0.01), citrate (*P* < 0.001), and oxalate (*P* < 0.05) were decreased from baseline at the end of bed rest or immediately after reambulation (or at both times). For oxalate, a significant interaction was observed between treatment group and time: in

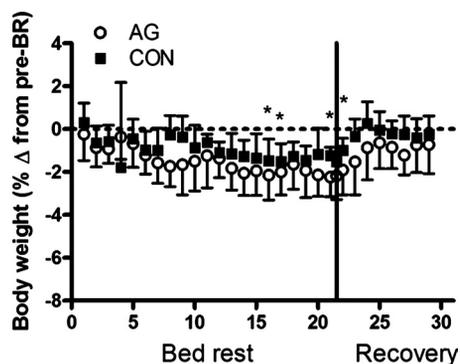


Fig. 1. Body weight [percent change ( $\Delta$ ) from pre-bed rest (BR); mean  $\pm$  SD] before, during, and after 21 days of BR. On BR16, BR17, BR21, and BR+0, mean body weight was significantly different from the mean on BR3 (*P* < 0.001). No other differences in body weight were found during the study.

Table 2. Body composition before and after 21 days of bed rest

	Con		AG	
	Pre	Post	Pre	Post
Whole body total fat, kg	17.4±4.1	17.5±4.0	15.1±5.4	15.3±5.5
Whole body total lean mass (excluding bone mineral content), kg <sup>a</sup>	61.8±6.1	61.2±5.9 <sup>b</sup>	61.9±4.3	61.2±4.3 <sup>b</sup>
Whole body total mass from DXA, kg	81.9±8.1	81.4±8.1	79.9±9.4	79.4±9.5

Values are means ± SD; *n* = 7 for the Con group and *n* = 8 for the AG group. Pre means are an average of 3 dual-energy X-ray absorptiometry (DXA) scans immediately before bed rest, and Post means are an average of 3 DXA scans immediately after bed rest. <sup>a</sup>*P* < 0.05, main effect of time. <sup>b</sup>*P* < 0.05 vs. Pre (determined by a post hoc Bonferroni *t*-test).

the Con group, urinary oxalate excretion was less than the pre-bed rest value only at BR+0/+1, but in the AG group, it was less at all time points during bed rest than it was before bed rest.

CRP was greater on the last day of bed rest in both groups (*P* < 0.01), and serum cortisol was unchanged in both groups (Table 5). Leptin concentrations were lower (*P* < 0.01) on BR+8 than before bed rest.

Serum total protein and most protein fractions (α<sub>1</sub>-globulin, α<sub>2</sub>-globulin, and γ-globulin) were unchanged during bed rest in both groups (data not shown). β-Globulin was significantly (*P* < 0.05) greater at all time points during the study for AG subjects than for Con subjects.

Serum cholesterol was significantly lower on the last day of bed rest than it was before bed rest (*P* < 0.001) for both Con and AG subjects (Table 5). Serum triglycerides were lower

(*P* < 0.001) during the recovery phase than before bed rest. The changes in serum cholesterol and triglycerides were consistent with the lower cholesterol and fat intakes during bed rest than before bed rest (Table 1), which likely also were lower than subjects' typical intakes before they entered the study. In the AG group, serum endothelin-1 tended to be higher at the end of bed rest than before bed rest (*P* = 0.084).

**Hematologic variables.** Hemoglobin increased during bed rest 7.8 ± 4.2 and 2.3 ± 3.7% for Con and AG groups, respectively, but 8 days after bed rest it was ~5% lower than pre-bed rest values in both groups (*P* < 0.001; Table 6). Hematocrit and ferritin also decreased significantly during the recovery period. Transferrin receptors and mean corpuscular volume were not changed. The Con and AG groups were not significantly different for any hematologic variables.

Table 3. Vitamins, minerals, antioxidants, and oxidative damage markers in blood before, during, and after 21 days of bed rest

	Con					AG				
	Pre	BR8	BR15	BR21	BR+8	Pre	BR8	BR15	BR21	BR+8
α-Tocopherol, <sup>c</sup> μg/ml	11.9±2.9	11.1±2.3 <sup>d</sup>	10.6±2.3 <sup>d</sup>	9.3±2.2 <sup>d</sup>	11.4±2.9	11.4±2.4	10.3±2.3 <sup>d</sup>	10.6±2.6 <sup>d</sup>	10.1±2.7 <sup>d</sup>	11.3±2.4
γ-Tocopherol, <sup>c</sup> μg/ml	1.3±0.3	1.2±0.3	1.1±0.3 <sup>d</sup>	0.9±0.2 <sup>d</sup>	1.5±0.6	1.4±0.4	1.2±0.4	1.1±0.4 <sup>d</sup>	1.0±0.4 <sup>d</sup>	1.4±0.5
β-Carotene, <sup>c</sup> μg/ml	0.23±0.06	0.32±0.08 <sup>d</sup>	0.32±0.07 <sup>d</sup>	0.33±0.07 <sup>d</sup>	0.42±0.09 <sup>d</sup>	0.31±0.11	0.42±0.15 <sup>d</sup>	0.49±0.25 <sup>d</sup>	0.45±0.22 <sup>d</sup>	0.56±0.25 <sup>d</sup>
RBC folate, nmol/l	912±238	857±220	884±236	817±202	943±240	942±110	889±129	848±111	876±179	1,018±171
RBC aspartate transaminase, %activation	74±18	74±10	77±13	74±11	69±7	82±9	81±9	83±13	83±14	82±11
RBC glutathione reductase, %activation	11±7	10±9	8±6	11±8	13±9	13±10	9±5	13±11	8±6	8±8
RBC transketolase, %activation	5±2	6±5	5±8	6±7	7±7	6±4	3±3	7±6	6±7	8±6
Vitamin C, <sup>b</sup> μg/ml	11±2	12±3	12±2	11±3	12±1 <sup>d</sup>	10±1	11±1	11±1	11±1	12±2 <sup>d</sup>
Retinol, μg/ml	0.55±0.11	0.55±0.09	0.54±0.09	0.51±0.11	0.52±0.09	0.59±0.13	0.59±0.14	0.61±0.15	0.59±0.18	0.58±0.14
Retinol binding protein, mg/l	51±10	51±9	53±13	49±10	47±7	50±8	49±8	56±7	49±7	50±6
Transthyretin, <sup>a</sup> mg/dl	27±6	28±4	29±6	28±4	24±7	29±3	29±4	30±4	29±4	29±4
Lipid peroxides, <sup>e</sup> μmol/l	0.32±0.15	0.30±0.18	0.33±0.21	0.32±0.15	0.41±0.19	0.42±0.17	0.39±0.16	0.34±0.15	0.30±0.16 <sup>d</sup>	0.31±0.13
Glutathione peroxidase, U/g hemoglobin	43±6	45±8	40±10	40±10	44±15	45±8	45±6	44±7	46±9	47±7
Superoxide dismutase, U/g hemoglobin	1,188±126	1,210±247	1,136±87	1,312±179	1,323±283	1,234±57	1,304±205	1,199±202	1,195±163	1,294±202
Total antioxidant capacity, mmol/l	1.9±0	1.9±0.1	1.9±0.2	1.9±0.2	1.7±0.1	1.9±0.1	1.8±0.1	1.9±0.1	1.9±0.3	1.8±0.1
Serum potassium, <sup>c</sup> mmol/l	3.9±0.1	3.9±0.1	4.0±0.2 <sup>d</sup>	3.9±0.2	3.8±0.1	4.0±0.2	4.1±0.2	4.1±0.1 <sup>d</sup>	4.0±0.1	3.8±0.1
Serum chloride, mmol/l	108±3	108±3	108±3	107±2	108±2	109±2	108±2	109±3	110±2	108±2
Serum sodium, mmol/l	139±2	138±1	140±1	139±1	140±1	138±2	137±2	138±3	138±3	138±2
WB sodium, <sup>c</sup> mmol/l (by PCBA)	138.5±0.6	138.1±1.2	138.4±1.1	138.3±1.1	139.7±0.8 <sup>d</sup>	138.9±0.7	138.4±1.1	139.0±0.8	139.3±1.0	139.3±1.4 <sup>d</sup>
WB potassium, <sup>c</sup> mmol/l (by PCBA)	3.8±0.1	3.8±0.1	3.8±0.2	3.7±0.1	3.6±0.2	3.8±0.1	3.9±0.2	4.0±0.1	3.9±0.2	3.7±0.2
Copper, mmol/l	15.7±2.5	15.3±1.5	15.5±1.6	15.6±2.2	15.1±2.4	15.0±1.4	15.3±2.2	15.3±2.1	15.1±1.8	15.2±2.0
Iron, mmol/l	22.1±4.3	22.8±6.8	23.7±5.9	23.2±5.9	18.5±2.5	29.6±17.3	31.5±14.0	23.0±5.2	29.4±14.7	18.9±4.6
Selenium, mmol/l	2.8±0.2	2.6±0.3	2.6±0.3	2.7±0.4	2.7±0.4	2.5±0.3	2.5±0.3	2.6±0.3	2.5±0.3	2.6±0.3
Zinc, <sup>c</sup> mmol/l	13.4±1.8	13.3±2.9	15.0±2.6 <sup>d</sup>	15.0±1.9 <sup>d</sup>	14.3±2.0 <sup>d</sup>	13.5±2.1	14.1±2.2	14.6±2.1 <sup>d</sup>	15.1±2.3 <sup>d</sup>	14.8±2.4 <sup>d</sup>

Values are means ± SD; *n* = 7 for the Con group and *n* = 8 for the AG group. PCBA, portable clinical blood analyzer; WB, whole blood. BR+8, 8 days post-bed rest. <sup>a</sup>*P* < 0.05; <sup>b</sup>*P* < 0.01; <sup>c</sup>*P* < 0.001, main effect of time. <sup>d</sup>*P* < 0.05 vs. Pre (determined by a post hoc Bonferroni *t*-test). <sup>e</sup>*P* < 0.05, interaction between treatment group and time (within AG, Pre was significantly greater than BR21). Red blood cell (RBC) aspartate transaminase, glutathione reductase, and transketolase are functional tests that assess the in vitro activity of the enzymes, and results are expressed as %activation after the addition of vitamin B<sub>6</sub>, riboflavin, or thiamine to the assay. Statistical analyses were not performed on these functional tests.

Table 4. Urinary markers of general chemistry and nutritional status before, during, and after 21 days of bed rest

	Con					AG						
	Pre	BR8/9	BR15/16	BR20/21	BR +0/+1	BR +6/+7	Pre	BR8/9	BR15/16	BR20/21	BR +0/+1	BR +6/+7
8-OHdG, $\mu\text{g/g}$ creatinine	3.8 $\pm$ 1.3	3.9 $\pm$ 1.5	3.7 $\pm$ 0.9	4.1 $\pm$ 2.2	4.1 $\pm$ 2.4	3.9 $\pm$ 2.5	3.2 $\pm$ 0.7	3.6 $\pm$ 0.9	3.5 $\pm$ 0.8	3.7 $\pm$ 0.9	3.3 $\pm$ 0.8	3.2 $\pm$ 1.1
pH <sup>c,d</sup>	6.4 $\pm$ 0.2	6.5 $\pm$ 0.1	6.4 $\pm$ 0.1	6.5 $\pm$ 0.1	6.1 $\pm$ 0.3 <sup>e</sup>	6.3 $\pm$ 0.2	6.3 $\pm$ 0.2	6.3 $\pm$ 0.2	6.4 $\pm$ 0.4	6.3 $\pm$ 0.2	5.6 $\pm$ 0.3 <sup>e</sup>	6.2 $\pm$ 0.2
Creatinine, mg/day	1967 $\pm$ 209	2019 $\pm$ 241	1996 $\pm$ 269	2005 $\pm$ 258	1941 $\pm$ 186	1950 $\pm$ 212	1959 $\pm$ 106	1969 $\pm$ 253	1951 $\pm$ 210	1970 $\pm$ 159	1900 $\pm$ 133	1944 $\pm$ 118
Magnesium, <sup>e</sup> mg/day	140 $\pm$ 16	125 $\pm$ 18 <sup>e</sup>	131 $\pm$ 15	131 $\pm$ 8	142 $\pm$ 22	121 $\pm$ 24 <sup>e</sup>	128 $\pm$ 35	111 $\pm$ 29 <sup>e</sup>	112 $\pm$ 29	111 $\pm$ 21	130 $\pm$ 44	108 $\pm$ 32 <sup>e</sup>
Volume, <sup>e</sup> ml/day	3,905 $\pm$ 510	3,541 $\pm$ 523 <sup>e</sup>	3,286 $\pm$ 441 <sup>e</sup>	3,600 $\pm$ 439	3,039 $\pm$ 494 <sup>e</sup>	3,631 $\pm$ 622	3,733 $\pm$ 488	3,291 $\pm$ 419 <sup>e</sup>	3,091 $\pm$ 537 <sup>e</sup>	3,518 $\pm$ 784	2,653 $\pm$ 577 <sup>e</sup>	3,478 $\pm$ 653
4-Pyridoxic acid, <sup>ca</sup> $\mu\text{mol/day}$	11.2 $\pm$ 2.8	8.3 $\pm$ 3.0 <sup>e</sup>	8.6 $\pm$ 3.0 <sup>e</sup>	8.4 $\pm$ 1.3 <sup>e</sup>	9.4 $\pm$ 2.9 <sup>e</sup>	8.7 $\pm$ 0.8 <sup>e</sup>	8.8 $\pm$ 1.7	6.5 $\pm$ 0.8 <sup>e</sup>	6.3 $\pm$ 1.7 <sup>e</sup>	8.1 $\pm$ 1.3 <sup>e</sup>	7.4 $\pm$ 1.4 <sup>e</sup>	8.0 $\pm$ 0.9 <sup>e</sup>
3-Methylhistidine, <sup>c</sup> $\mu\text{mol/day}$	329 $\pm$ 38	310 $\pm$ 39 <sup>e</sup>	301 $\pm$ 28 <sup>e</sup>	318 $\pm$ 37	303 $\pm$ 35 <sup>e</sup>	306 $\pm$ 29 <sup>e</sup>	322 $\pm$ 26	285 $\pm$ 29 <sup>e</sup>	274 $\pm$ 21 <sup>e</sup>	296 $\pm$ 19	279 $\pm$ 36 <sup>e</sup>	288 $\pm$ 32 <sup>e</sup>
Phosphorus, <sup>c</sup> mg/day	909 $\pm$ 150	1,068 $\pm$ 135 <sup>e</sup>	1,071 $\pm$ 146 <sup>e</sup>	1,057 $\pm$ 150 <sup>e</sup>	1,039 $\pm$ 167 <sup>e</sup>	977 $\pm$ 154 <sup>e</sup>	830 $\pm$ 181	991 $\pm$ 196 <sup>e</sup>	936 $\pm$ 200 <sup>e</sup>	981 $\pm$ 203 <sup>e</sup>	988 $\pm$ 236 <sup>e</sup>	937 $\pm$ 136 <sup>e</sup>
Sodium, <sup>c</sup> mmol/day	183 $\pm$ 52	175 $\pm$ 56	135 $\pm$ 11 <sup>e</sup>	170 $\pm$ 41	96 $\pm$ 31 <sup>e</sup>	140 $\pm$ 15 <sup>e</sup>	165 $\pm$ 17	134 $\pm$ 20	122 $\pm$ 22 <sup>e</sup>	145 $\pm$ 23	79 $\pm$ 14 <sup>e</sup>	144 $\pm$ 26 <sup>e</sup>
Sulfate, <sup>b</sup> mmol/day	26 $\pm$ 3	24 $\pm$ 3 <sup>e</sup>	25 $\pm$ 4	24 $\pm$ 3 <sup>e</sup>	27 $\pm$ 3	24 $\pm$ 2	27 $\pm$ 3	25 $\pm$ 4 <sup>e</sup>	24 $\pm$ 4	24 $\pm$ 5 <sup>e</sup>	27 $\pm$ 4	26 $\pm$ 3
Citrate, <sup>c</sup> mg/day	537 $\pm$ 177	492 $\pm$ 182	515 $\pm$ 225	494 $\pm$ 182	459 $\pm$ 135 <sup>e</sup>	551 $\pm$ 215	572 $\pm$ 155	572 $\pm$ 160	532 $\pm$ 169	603 $\pm$ 156	457 $\pm$ 128 <sup>e</sup>	612 $\pm$ 180
Oxalate, <sup>ad</sup> mg/day	49 $\pm$ 7	54 $\pm$ 14	51 $\pm$ 12	51 $\pm$ 10	41 $\pm$ 7	47 $\pm$ 6	56 $\pm$ 8	42 $\pm$ 11 <sup>e</sup>	45 $\pm$ 7 <sup>e</sup>	43 $\pm$ 9 <sup>e</sup>	42 $\pm$ 8 <sup>e</sup>	45 $\pm$ 11 <sup>e</sup>
Copper, <sup>f</sup> $\mu\text{mol/day}$	0.6 $\pm$ 0.1	0.6 $\pm$ 0.2	0.7 $\pm$ 0.2	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2	0.7 $\pm$ 0.2	0.7 $\pm$ 0.2	0.8 $\pm$ 0.3 <sup>e</sup>
Selenium, <sup>b</sup> $\mu\text{mol/day}$	1.5 $\pm$ 0.1	1.3 $\pm$ 0.3 <sup>e</sup>	1.3 $\pm$ 0.2 <sup>e</sup>	1.4 $\pm$ 0.2	1.3 $\pm$ 0.2	1.3 $\pm$ 0.2	1.6 $\pm$ 0.3	1.3 $\pm$ 0.3 <sup>e</sup>	1.4 $\pm$ 0.2	1.6 $\pm$ 0.3	1.5 $\pm$ 0.2	1.5 $\pm$ 0.2
Zinc, <sup>c</sup> $\mu\text{mol/day}$	9.6 $\pm$ 3.9	9.7 $\pm$ 4.4	11.9 $\pm$ 5.2	13.0 $\pm$ 4.8 <sup>e</sup>	12.9 $\pm$ 4.7 <sup>e</sup>	9.0 $\pm$ 3.9	10.0 $\pm$ 2.3	11.5 $\pm$ 2.0	11.2 $\pm$ 2.5	13.5 $\pm$ 3.8 <sup>e</sup>	14.6 $\pm$ 3.2 <sup>e</sup>	9.7 $\pm$ 3.2
Cortisol, <sup>g</sup> $\mu\text{g/day}$	85 $\pm$ 19	117 $\pm$ 54 <sup>e</sup>	105 $\pm$ 46	104 $\pm$ 40	98 $\pm$ 36	85 $\pm$ 34	93 $\pm$ 23	91 $\pm$ 21	70 $\pm$ 8	89 $\pm$ 17	79 $\pm$ 18	94 $\pm$ 25

Values are means  $\pm$  SD;  $n = 7$  for the Con group and  $n = 8$  for the AG group. BR +0/+1 and BR +6/+7, 0 to 1 and 6 to 7 days post-bed rest; 8-OHdG, 8-hydroxy-2'-deoxyguanosine. <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ , main effect of time. <sup>d</sup> $P < 0.05$ , effect of treatment group; Con vs. AG. <sup>e</sup> $P < 0.05$  vs. Pre (determined by a post hoc Bonferroni  $t$ -test). <sup>f</sup> $P < 0.05$ ; <sup>g</sup> $P < 0.01$ , interaction between treatment group and time.

## DISCUSSION

The Artificial Gravity Pilot Study was designed to be the first step in testing a multisystem countermeasure to the negative effects of spaceflight on human health and physiology. From a food and nutrition perspective, a primary goal of the study was to provide controlled dietary intake to all subjects, with energy intake at a level to maintain body weight and intakes of micronutrients to meet recommendations. Embedded in this goal is the primary hypothesis for this aspect of the larger study: that bed rest significantly affects nutritional status and nutritional requirements and that artificial gravity can mitigate the effects of bed rest on nutritional status if those effects are truly caused by a lack of mechanical load. Certain aspects of nutritional status, including iron metabolism and oxidative stress, can be altered during 60–90 days of bed rest (54). Calcium, protein, and other nutrients that pertain to muscle and bone health have been studied (5, 8, 22, 32, 42, 52), but considering the importance of nutrient-nutrient interactions and the recognition that assessment of the entire diet is critical for optimal utilization of nutrients, a comprehensive evaluation of diet during bed rest is required. If changes in nutritional status during bed rest are caused by physiological conditions that artificial gravity counteracts, then it is possible that artificial gravity could affect nutritional status.

Energy requirements during spaceflight are unchanged from Earth-based requirements (14, 23, 43), or if a person engages in heavy exercise during flight, energy requirements are increased (43). Despite this, earlier bed rest studies have shown that a 10–15% reduction in energy provision will maintain body weight in healthy human subjects. Use of the Harris-Benedict energy calculations (15) in this study effectively maintained body weight in both groups, suggesting that energy expenditure during bed rest was not altered by artificial gravity. Wade et al. (47) showed in rats that centrifugation at 2.3 or 4.0  $\times$  gravity increased resting energy expenditure  $\sim$ 40%. In those studies, artificial gravity was applied 24 h/day for 14 days, providing a much greater dose than in the present study. These findings were supported by those of another animal study in which rats were exposed to loads up to 2.0  $\times$  gravity for 14 days, 24 h/day. In that study, body mass in the artificial gravity group was significantly less than body mass of controls despite differences in food intake (27). Dysfunction in leptin metabolism has been reported in animals exposed to hypergravity (27, 48), but no change in leptin was observed during bed rest in the present study (a decrease during recovery was the only change), and no differences between groups occurred in food intake, body mass, or body composition during the study. The gravity dose in our study was similar to the gravity doses in the animal studies of leptin, so we do not expect a difference in dose to explain the difference in response. Leptin may have remained unchanged because the response of humans is different from that of rats.

Short-term artificial gravity (1 h/day) for 21 days did not affect the status of most vitamins, including retinol. In contrast, serum levels of retinol and retinol-binding protein (RBP) are decreased after long-duration spaceflight (40); evidence from rodent models has led to this being attributed to a stress response (45). Stress hormones and acute-phase proteins such as serum CRP, cortisol, and ceruloplasmin were not affected by artificial gravity in the current study; if a stress response does

Table 5. General chemistry, lipids, and hormones in blood before, during, and after 21 days of bed rest

	Con					AG				
	Pre	BR8	BR15	BR21	BR +8	Pre	BR8	BR15	BR21	BR +8
Alanine aminotransferase, U/l	20±11	19±6	21±9	19±6	21±8	16±6	16±6	17±6	17±8	20±7
Aspartate aminotransferase, <sup>a</sup> U/l	19±3	17±4 <sup>c</sup>	17±2 <sup>c</sup>	18±2	19±3	18±4	16±2 <sup>c</sup>	16±2 <sup>c</sup>	19±2	21±7
Ceruloplasmin, mg/dl	26±4	26±3	26±6	25±5	26±5	27±4	27±3	26±4	26±3	25±4
Cortisol, µg/dl	29±11	31±12	30±12	28±8	25±7	26±8	26±9	24±8	22±7	26±7
Creatinine, mg/dl	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.1±0.1	1.1±0.2	1.1±0.1	1.0±0.1
C-reactive protein, <sup>a</sup> mg/l	1.1±0.7	0.9±0.5	1.1±0.8	1.6±1.2 <sup>c</sup>	0.9±0.5	0.7±0.4	0.8±0.5	1.1±0.9	1.4±1.0 <sup>c</sup>	0.8±0.4
Glucose, mg/dl	88±5	85±3	86±3	85±4	86±6	88±2	86±4	88±4	86±4	86±3
Triglycerides, <sup>b</sup> mg/dl	162±62	146±49	156±63	144±50	122±40 <sup>c</sup>	128±34	123±47	117±38	124±45	111±28 <sup>c</sup>
Cholesterol, <sup>b</sup> mg/dl	182±40	176±32	180±44	166±39 <sup>c</sup>	175±42	195±20	194±22	190±25	178±23 <sup>c</sup>	197±19
Leptin, <sup>a</sup> ng/ml	5.0±3.5	4.6±2.6	4.8±2.9	4.2±2.3	3.8±2.4 <sup>c</sup>	4.8±4.8	4.5±4.4	4.5±4.4	4.3±4.4	3.8±3.9 <sup>c</sup>
Testosterone, <sup>a</sup> ng/l	595±54	532±45 <sup>c</sup>	569±79	543±61 <sup>c</sup>	535±39 <sup>c</sup>	581±79	536±80 <sup>c</sup>	588±95	531±89 <sup>c</sup>	517±83 <sup>c</sup>
Free testosterone, <sup>bc</sup> pg/ml	17±3	15±3 <sup>c</sup>	16±3	16±3 <sup>c</sup>	15±2 <sup>c</sup>	16±2	13±3 <sup>c</sup>	14±2	12±2 <sup>c</sup>	12±2 <sup>c</sup>
Endothelin-1, pg/ml	2.6±0.2	2.5±0.4	2.9±0.8	2.8±0.4	2.9±0.4	2.7±0.3	2.9±0.7	2.6±0.4	3.1±0.9	3.2±0.7
pH <sup>de</sup>	7.36±0.02	7.36±0.02	7.34±0.01	7.36±0.01	7.36±0.02	7.37±0.02	7.37±0.02	7.38±0.04	7.39±0.03 <sup>c</sup>	7.37±0.02
Total protein, g/dl	6.71±0.34	6.60±0.54	6.89±0.47	6.70±0.50	6.56±0.35	6.89±0.33	6.89±0.47	6.99±0.46	6.83±0.46	6.93±0.47
Albumin, g/dl	3.78±0.20	3.73±0.46	3.86±0.36	3.70±0.16	3.76±0.17	3.91±0.21	3.86±0.15	3.91±0.16	3.85±0.29	3.89±0.29
β-Globulin, <sup>c</sup> g/dl	0.95±0.13	0.93±0.10	0.97±0.15	0.94±0.13	0.90±0.10	1.10±0.10	1.09±0.11	1.16±0.15	1.09±0.14	1.08±0.07

Values are means ± SD;  $n = 7$  for the Con group and  $n = 8$  for the AG group. <sup>a</sup> $P < 0.01$ ; <sup>b</sup> $P < 0.001$ , main effect of time. <sup>c</sup> $P < 0.05$  vs. Pre (determined by a post hoc Bonferroni  $t$ -test). <sup>d</sup> $P < 0.05$ , interaction between treatment group and time (within AG, Pre was significantly lower than BR21). <sup>e</sup> $P < 0.05$ , effect of treatment group.

cause the decrease in serum retinol and RBP after long-duration spaceflight, it is not surprising to see a lack of difference in vitamin A status between the Con and AG groups. CRP level, a measure of overall inflammation, was increased in both groups on the last day of bed rest, whereas urinary cortisol increased in Con subjects in the first week of bed rest. It is unclear whether CRP would have continued to be elevated in longer studies or whether a concomitant change in vitamin A status would have occurred. Furthermore, the lack of stress-mediated responses in retinol and RBP during bed rest may parallel the lack of change in energy metabolism in response to bed rest. It has been proposed that the unchanged or increased energy expenditure during spaceflight is related to stress hormone-induced increases in energy expenditure. The lack of an effect of bed rest on retinol and RBP and the lower energy requirements during bed rest may be related to the lack of a metabolic stress phenomenon in the ground analog.

Oxidative damage is evident during and after long-duration spaceflight, and it is thought to be caused by a combination of radiation, hyperoxic environments (during extravehicular activity, launch, and landing), and increased production of stress hormones (21, 40, 41). In this study, the concentrations of plasma lipid peroxides were less at the end of bed rest in the AG but not in the Con group. The decrease in plasma lipid

peroxides was not related to a change in CRP or cortisol at the end of bed rest. It is not clear why lipid peroxides were lower in AG subjects without an effect also occurring on other markers of oxidative damage, including 8-OHdG, glutathione peroxidase, superoxide dismutase, or total antioxidant capacity.

Although the difference was not significant, during bed rest serum endothelin-1 tended to be higher in the AG group ( $P = 0.084$ ). Endothelin-1 is involved in the regulation of sodium excretion by the kidney, and its expression can affect blood pressure. Endothelin-1 expression is influenced by dietary sodium (16, 30), and it is clear from Table 1 that dietary sodium decreased during bed rest. In this study, the change in endothelin-1 in the AG group from before bed rest to the last week of bed rest was inversely related to the change in sodium intake during bed rest.

Urinary 4-pyridoxic acid significantly decreased in both groups during bed rest ( $P < 0.001$ ). The decrease in status is likely related to the decrease in vitamin B<sub>6</sub> intake during bed rest (Table 1). The decrease in urinary 4-pyridoxic acid reported in our study is similar to the decrease observed in subjects who have low dietary intakes of vitamin B<sub>6</sub> or are undergoing complete fasting (5, 7). In addition to the decreased intake, we expected that a decrease in lean body mass would

Table 6. Hematologic and iron status before, during, and after 21 days of bed rest

	Con					AG				
	Pre	BR8	BR15	BR21	BR +8	Pre	BR8	BR15	BR21	BR +8
Hematocrit, <sup>a</sup> %	46±1	46±5	48±3	46±3	43±1 <sup>b</sup>	47±2	47±3	48±2	46±2	44±2 <sup>b</sup>
Hemoglobin, <sup>a</sup> g/dl	15.3±0.4	15.6±1.7	16.5±0.9 <sup>b</sup>	15.6±1.0	14.5±0.3 <sup>b</sup>	15.6±0.9	15.6±1.0	16.0±0.8 <sup>b</sup>	15.3±0.8	14.6±0.9 <sup>b</sup>
Ferritin, <sup>a</sup> ng/ml	192±90	184±82	193±79	180±79	153±71 <sup>b</sup>	128±84	135±84	142±92	138±87	110±78 <sup>b</sup>
Transferrin, <sup>a</sup> mg/dl	240.0±31.3	226.0±11.7 <sup>b</sup>	229.7±32.2 <sup>b</sup>	222.0±28.4 <sup>b</sup>	215.9±22.4 <sup>b</sup>	229.3±22.9	220.9±21.7 <sup>b</sup>	218.4±23.9 <sup>b</sup>	212.4±25.1 <sup>b</sup>	216.1±22.1 <sup>b</sup>
Transferrin receptors, mg/l	5.8±1.2	6.5±1.8	6.5±0.9	6.3±1.4	6.6±1.2	5.4±1.7	5.5±2.1	5.6±1.7	5.1±1.6	5.4±1.8
Mean corpuscular volume, fl	89±2	88±2	88±2	88±3	89±2	90±2	90±3	90±3	90±2	90±2

Values are means ± SD;  $n = 7$  for the Con group and  $n = 8$  for the AG group. <sup>a</sup> $P < 0.001$ , main effect of time. <sup>b</sup> $P < 0.05$  vs. Pre (determined by a post hoc Bonferroni  $t$ -test).

reduce the amount of the vitamin that is stored in the body, because vitamin B<sub>6</sub> is stored mainly in muscle tissue. The decrease in urinary 4-pyridoxic acid excretion observed after the first week of bed rest suggests there is a rapid-turnover pool of vitamin B<sub>6</sub> that seems to reach a new metabolic steady state. It has been reported that it can take up to 2 wk for a new steady state to be firmly established in ambulatory subjects (6). This is different from what was observed during a 17-wk bed rest study (5), in which urinary 4-pyridoxic acid increased during bed rest. Subjects in the 17-wk study received a daily vitamin supplement that contained 3 mg (15 μmol) of vitamin B<sub>6</sub>, but the subjects in the AG study did not. Baseline urinary 4-pyridoxic acid was about three times as great in the 17-wk bed rest study as it was in the Con and AG groups described in the present study.

Urinary excretion of 3-methylhistidine was less during bed rest and was positively correlated with urinary creatinine excretion in both subject groups. Decreased excretion of 3-methylhistidine indicates that muscle catabolism has decreased (25). Although we expected that the AG treatment would have a protective effect on muscle, these data do not provide evidence of this (given similar changes in controls). Furthermore, there was no group effect on lean body mass (as determined by DXA).

The decrease in urinary magnesium during bed rest and recovery may have been caused by the decrease in magnesium intake during bed rest, but an explanation for the increase in urinary phosphorus during bed rest is less clear. After long-duration spaceflight, urinary magnesium and calcium are both decreased ~45% (40). Decreased urinary magnesium is a point of concern for long-duration flights, because magnesium plays a role in inhibiting calcium oxalate renal stones (13, 44).

Iron metabolism is altered during bed rest (54), and we hypothesized that artificial gravity would have an impact on iron metabolism and RBC metabolism. Decreased RBC mass during spaceflight is believed to be related to the diminished pooling of RBCs in the lower extremities and to the destruction of newly formed RBCs (1–3). When a person becomes weightless, the RBCs in the lower extremities become part of the circulating population of RBCs. This phenomenon, coupled with easier transport of RBCs to peripheral tissues in the absence of gravity, likely leads to the body sensing an excess of available oxygen-carrying capacity (1, 35) and subsequently reducing the circulating RBC volume. Because of the nature of artificial gravity, we predicted that it might cause a transient restoration of the pooling effect, which in turn might stimulate erythropoietin and RBC synthesis. An increase in hemoglobin during bed rest (likely related to loss of plasma volume) was observed in both groups. Iron storage (ferritin) of the two groups was not significantly different, but the decrease in ferritin during the recovery period suggested that iron stores were mobilized after bed rest. A higher dose of AG or a more aggressive blood sampling schedule (to see transient effects soon after daily AG treatment) might have provided more insight into whether these effects were occurring below a physiological threshold for long-term changes in hematology. If we had measured transient responses after an AG spin, we might have been able to definitively say whether the intensity of the spin was adequate to prevent changes in hematology.

Formation of renal stones, along with increased urinary supersaturation of sulfate, oxalate, and citrate, are a docu-

mented risk during spaceflight (49–51). In this study, the decreases from baseline in urinary sulfate, citrate, and oxalate at the end of bed rest or immediately after reambulation (or at both times), in both groups, may or may not have been related to the decrease in dietary protein. In the Con group, urinary oxalate excretion was less than the pre-bed rest value only at BR+0/+1, but in the AG group, oxalate excretion was less at all time points during bed rest than it was before bed rest. Dietary vitamin C and oxalic acid were not significantly different between groups. These results suggest that AG might have had a protective effect on one risk factor (oxalate) for renal stone formation.

In this study, changes in nutritional and physiological variables may be sorted into categories: those altered as a result of bed rest (iron metabolism and hematological variables, markers of lipid oxidation, indicators of stress levels), those not affected by bed rest (oxidative damage markers other than lipid peroxides, serum electrolytes, and retinol), those altered by changes in intake (vitamin E, β-carotene, cholesterol, triglycerides, serum potassium), and those confounded by intake changes and bed rest (4-pyridoxic acid, endothelin-1). In this study, we learned that AG did not have a major effect, positive or negative, on the changes in nutrition status that resulted from bed rest. It is clear that when countermeasures are designed, many factors must be considered, along with a clear understanding of physiological changes caused by the environment, to ensure that the benefits brought about by the countermeasure are not causing any negative consequences. This small step toward understanding the effects and effectiveness of artificial gravity as a multisystem countermeasure was critical, but more work is clearly required.

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