

# Calcium metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes

SCOTT M. SMITH,<sup>1</sup> MERYL E. WASTNEY,<sup>2</sup> BORIS V. MORUKOV,<sup>3</sup> IRINA M. LARINA,<sup>3</sup> LAURENCE E. NYQUIST,<sup>4</sup> STEVEN A. ABRAMS,<sup>5</sup> ELENA N. TARAN,<sup>6</sup> CHIH-YU SHIH,<sup>7</sup> JEANNIE L. NILLEN,<sup>8</sup> JANIS E. DAVIS-STREET,<sup>8</sup> BARBARA L. RICE,<sup>8</sup> AND HELEN W. LANE<sup>9</sup>  
<sup>1</sup>Nutritional Biochemistry Laboratory, Life Sciences Research Laboratories, <sup>4</sup>Planetary Sciences Division, <sup>9</sup>Space and Life Sciences Directorate, National Aeronautics and Space Administration Lyndon B. Johnson Space Center, Houston 77058; <sup>5</sup>Department of Pediatrics, United States Department of Agriculture/Agricultural Research Service Children's Nutrition Research Center, Baylor College of Medicine, Houston 77030; <sup>7</sup>Lockheed Martin Corporation, Houston 77058; <sup>8</sup>Enterprise Advisory Services, Incorporated, Houston, Texas 77058; <sup>2</sup>Department of Pediatrics, Georgetown University Medical Center, Washington, District of Columbia 20007; <sup>3</sup>Institute for Biomedical Problems, Moscow 123007; and <sup>6</sup>Gagarin Cosmonaut Training Center, Star City 141160, Russia

**Smith, Scott M., Meryl E. Wastney, Boris V. Morukov, Irina M. Larina, Laurence E. Nyquist, Steven A. Abrams, Elena N. Taran, Chih-Yu Shih, Jeannie L. Nillen, Janis E. Davis-Street, Barbara L. Rice, and Helen W. Lane.** Calcium metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes. *Am. J. Physiol.* 277 (Regulatory Integrative Comp. Physiol. 46): R1–R10, 1999.—The loss of bone during spaceflight is considered a physiological obstacle for the exploration of other planets. This report of calcium metabolism before, during, and after long-duration spaceflight extends results from Skylab missions in the 1970s. Biochemical and endocrine indexes of calcium and bone metabolism were measured together with calcium absorption, excretion, and bone turnover using stable isotopes. Studies were conducted before, during, and after flight in three male subjects. Subjects varied in physical activity, yet all lost weight during flight. During flight, calcium intake and absorption decreased up to 50%, urinary calcium excretion increased up to 50%, and bone resorption (determined by kinetics or bone markers) increased by over 50%. Osteocalcin and bone-specific alkaline phosphatase, markers of bone formation, increased after flight. Subjects lost ~250 mg bone calcium per day during flight and regained bone calcium at a slower rate of ~100 mg/day for up to 3 mo after landing. Further studies are required to determine the time course of changes in calcium homeostasis during flight to develop and assess countermeasures against flight-induced bone loss.

weightlessness; calcium absorption; mathematical modeling; stable isotope; microgravity

---

THE EFFECT OF near weightlessness on the human skeletal system is one of the most critical concerns in safely extending space missions. Bone tissue is lost during flight as a result of skeletal unloading (14, 21, 27), thereby increasing calcium excretion in the urine (27, 34). The subsequent bone loss and increased risk of renal stone formation during and after flight (35) are significant.

---

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Few in-flight studies of bone loss have been possible, and the most comprehensive were conducted almost 25 years ago during the Skylab missions (34). The Skylab flights lasted up to 84 days and documented negative calcium balance based on intake and excretion data of up to 300 mg/day (10). Bone resorption markers were significantly elevated during these long-duration flights compared with preflight levels (29). In-flight and ground-based studies (using bed rest as an analog of weightlessness) have shown that the loss of calcium from bones is variable between sites within a subject and that the degree of loss varies between subjects (12, 14). Countermeasures, including exercise, increased calcium intake, vitamin D supplementation, exposure to ultraviolet light, and administration of bisphosphonates have not, to date, proven effective in counteracting the loss of bone calcium during spaceflight or bed rest (13, 16, 17).

Insight into the mechanisms of flight-induced bone loss comes from the study of factors known to regulate bone and calcium homeostasis; key among these factors are parathyroid hormone, osteocalcin, calcitonin, bone-specific alkaline phosphatase, and vitamin D. Together, these factors regulate calcium homeostasis through modulation of the processes of intestinal calcium absorption, bone calcium deposition and resorption, and renal calcium excretion. Each of these processes may be measured through the use of kinetic studies using calcium isotope tracers (20).

Recently, flights to the Russian Space Station Mir have provided opportunities to examine bone loss during and after extended-duration flights. The aim of the studies reported here was to measure changes in calcium and bone metabolism during spaceflight and to assess the rates of recovery by comparing endocrine and kinetic changes before, during, and after a 115-day stay on board the Mir Space Station. The studies in three subjects provide preliminary data for exploring the underlying mechanisms of bone loss and examining individual differences in response to spaceflight.

## SUBJECTS AND METHODS

These studies were conducted on crew members of the Mir 18 mission, which was part of the joint United States and

Russian Shuttle/Mir Science Program. This mission included their launch on board a Russian vehicle, transfer to the Mir Space Station, and return to Earth on board the Space Shuttle Atlantis. The entire flight duration was 115 days.

### Subjects

Subjects were three males: two cosmonauts and one astronaut ( $47 \pm 12$  yr of age, mean  $\pm$  SD,  $77.9 \pm 7.2$  kg body wt). Two subjects had previous spaceflight experience, and all were required to pass an Air Force Class III physical for flight clearance. These studies were reviewed by the Johnson Space Center Institutional Review Board and the Russian Academy of Science Bioethics Review Committee. Subjects provided informed consent before participation.

### Study Design

Each subject was studied eight times: preflight (3 times), in flight (2 times), and postflight (3 times). Preflight studies were conducted at  $\sim$ 5, 2, and 1 mo before launch (designated L-151, L-61, and L-36 days). The L-151 session was completed in Houston; the other two were completed in Star City, Russia. In-flight studies were conducted after 2 wk and 3 mo of weightlessness (designated *flight day 14*, or FD14, and FD110). Postflight studies were performed within 3–4 h of landing (return + 0 days, designated R+0) at the Kennedy Space Center in Florida, and at 9 days (R+9) and 3 mo after landing in Houston, although the last study was completed in Russia for the two cosmonauts. The last postflight study is designated R>75, as this was performed at R+75 for the two cosmonauts, and R+115 for the astronaut.

During each study, blood and urine were sampled for endocrine and biochemical measurements, dietary intake was recorded, and kinetic studies were performed over 5 days. Kinetic studies were conducted only once in flight (FD110), during the final phase of the mission on board the Space Shuttle Atlantis. Body mass was determined in flight using the Mir body mass measuring device.

### Sample Collection

Blood samples were collected by standard phlebotomy techniques into evacuated serum separator collection tubes. With the exception of samples collected on the day of landing, all blood samples were collected in the morning after an 8-h fast.

Ground-based urine voids were collected into single-void urine containers (Cole-Palmer, Niles, IL). Samples were stored with ice packs or refrigerated until processing within 24 h of collection. Aliquots were prepared for individual analysis and stored frozen at  $-80^{\circ}\text{C}$  until analysis.

In-flight urine voids were collected into urine collection devices containing 1 ml of a LiCl solution as a volume marker. Samples were mixed, syringe aliquots were obtained, and aliquots were frozen until return to Earth. Postflight, these samples were analyzed for lithium concentration to determine void volume. Twenty-four-hour pools were created from the individual voids for applicable analyses.

Saliva samples were collected onto dried dental cotton rolls using Salivettes (Sarstedt, Newton, NC). Fecal samples (pre- and postflight only) were collected into preweighed individual fecal collection containers (Sage Products, Crystal Lake, IL) and frozen until analysis.

### Biochemical Analyses

Total calcium was measured in serum using spectrophotometric (Beckman CX5, Beckman Instruments, Anaheim, CA)

techniques. Ionized calcium was determined using ion-sensitive electrode (i-STAT, Princeton, NJ) techniques. This instrument was previously verified for use on the ground and during spaceflight (28).

Urinary and salivary total calcium were measured with a Perkin-Elmer 4000 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, MA). Fecal total calcium content was determined by flame atomic absorption spectrophotometry with a Smith-Hieftje 4000 (Thermo Jarrell Ash, Franklin, MA). Fecal polyethylene glycol content was determined spectrophotometrically.

Serum osteocalcin (Biomedical Technologies, Stoughton, MA), calcitonin (Nichols Institute, San Juan Capistrano, CA), and intact parathyroid hormone (Nichols Institute) were measured by radioimmunoassay. Vitamin D metabolites were also determined using commercially available kits (Diasorin, Stillwater, MN). Bone-specific alkaline phosphatase was measured by ELISA (Metra Biosystems, Palo Alto, CA).

Urinary pyridinium crosslinks were analyzed with the Pylinks kit (Metra Biosystems). This ELISA assay detects pyridinium crosslinks (i.e., both pyridinoline and deoxypyridinoline) in urine. Deoxypyridinoline was analyzed with the Pylinks-D kit (Metra Biosystems). *n*-Telopeptide concentrations in urine were determined with the Osteomark ELISA kit (Ostex International, Seattle, WA), which specifically detects the *n*-telopeptide region of bone collagen in human urine. Coefficients of variation for the low-level control for pyridinium crosslinks, deoxypyridinoline, and *n*-telopeptide were 11.4, 8.6, and 10.2%, respectively; the high-level control yielded coefficients of variation of 11.2, 9.9, and 9.9%, respectively.

### Dietary Intake

During pre- and postflight studies, dietary intake was determined by providing weighed meals to the crew members for 5 days beginning with the day of tracer administration. The Research Dietitian (BLR) met with the crews to train them on recording intake of any additional items. Crew members were also asked to record any medications or dietary supplements taken. The dietitian met with the crew after data collection sessions to review records and verify information. In-flight intake of food and fluids were recorded by means of a bar code reader, which logs information regarding subject identification, time and date of entry, and quantity of each item consumed. Five days of dietary monitoring were completed in flight, beginning with the day of tracer administration.

Preflight and postflight dietary intake data were analyzed using the Minnesota Nutrition Data System software, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN (Food Database version 2.8; Nutrient Database version 2.8) (26). In-flight intakes were analyzed using the database created from the chemical analysis of space foods performed by the National Aeronautics and Space Administration Johnson Space Center Water and Food Analytical Laboratory.

### Tracer Kinetics Studies

Oral doses of calcium ( $160 \mu\text{g Ca}$ ,  $\sim$ 84%  $^{43}\text{Ca}$  enriched; Oakridge National Laboratories, Oakridge, TN) were delivered in 2 ml 0.006 N HCl. Intravenous doses ( $25 \mu\text{g Ca}$ ,  $\sim$ 30%  $^{46}\text{Ca}$  enriched; Oakridge National Laboratories) were delivered in 1 ml of sterile injectable 0.16 M sodium lactate. Preparation of these doses was described previously (30). Tracers were packaged for use in weightlessness, and identical syringes were used for both ground and flight studies.

A carrier dose of calcium and the polyethylene glycol were ingested immediately after the stable isotope tracer. Calcium carbonate, administered in two capsules, was the carrier (625 mg,  $\pm 2\%$  CaCO<sub>3</sub>, 250 mg calcium; ACS grade, #C3049; Sigma, St. Louis, MO). Polyethylene glycol, used as a fecal marker (pre- and postflight only), was contained in two gelatin capsules (750 mg,  $\pm 2\%$ , molecular weight 3350; Sigma).

Subjects initially received the oral <sup>43</sup>Ca dose. One hour later, blood and saliva samples were taken and the <sup>46</sup>Ca was injected intravenously. Subjects were not allowed food for 1 h after the intravenous dose. Another blood sample was collected ~24 h after the oral dose. All urine and fecal samples and daily saliva samples were collected over the following 5 days. The in-flight tracer study did not include fecal collections.

### Stable Isotope Analyses

Urine, serum, and saliva samples (100–200  $\mu$ l) were digested in concentrated HNO<sub>3</sub> and HClO<sub>4</sub>. Fecal samples were ashed in a muffle furnace and reconstituted in 0.3 N HCl before digestion. Stable isotopes were analyzed using thermal ionization mass spectrometry techniques, as described previously (1, 30).

### Kinetic Modeling

**Data.** Enrichment of stable isotope tracer (<sup>43</sup>Ca and <sup>46</sup>Ca) in all samples (serum, saliva, urine, and feces) was converted to milligrams of stable isotope and expressed as percentage of dose. Cumulative appearance data were calculated for urine and feces. Other data consisted of dietary calcium intake (mg) during each study and urinary and fecal excretion of calcium.

**Model.** Data were fitted by a compartmental model for calcium in humans (33) on the basis of the model of Neer et al. (20) using the Windows version of the SAAM/Consaam software, WinSAAM (6). The compartments represent calcium pools in the body that turn over at different rates (Fig. 1). Pathways between the compartments, designated L(I,J), represent the fraction of compartment J that is transported to compartment I per unit time. The model consists of a compartment that contains serum (compartment 1), two extravascular compartments (compartments 2 and 3), and three compartments in the intestine (compartments 8, 9, and 10). Absorption occurs from the first gastrointestinal compartment into plasma via L(1,8), and endogenous excretion occurs from plasma into the third intestinal compartment via L(9,1). Compartments 5 and 6 represent feces and urine, respectively.

**Data fitting.** Oral and intravenous tracer in serum, saliva, urine, and feces (as % of dose) and calcium (mg) excreted in urine and feces from the three subjects were fitted by the model. Data for each of the seven studies (3 preflight, 1 in flight, 3 postflight) per subject were fitted separately initially to identify which parameters changed between studies. Data from all seven studies were subsequently fitted simultaneously for each subject. This approach accounted for carryover of tracer between studies and, by using the minimum change postulate, the minimum number of parameters that were required to differ between studies to explain the changes in kinetics were identified (3). During the data fitting, parameter values were weighted to the population values from previous studies (20, 33). If there were insufficient data to define a parameter, it therefore remained at the population value.

**Assumptions.** An assumption in fitting the data was that the subjects were in steady state during the 5-day study (although the steady-state level could differ between studies). The initial volume of distribution was fixed at 10 l, or four

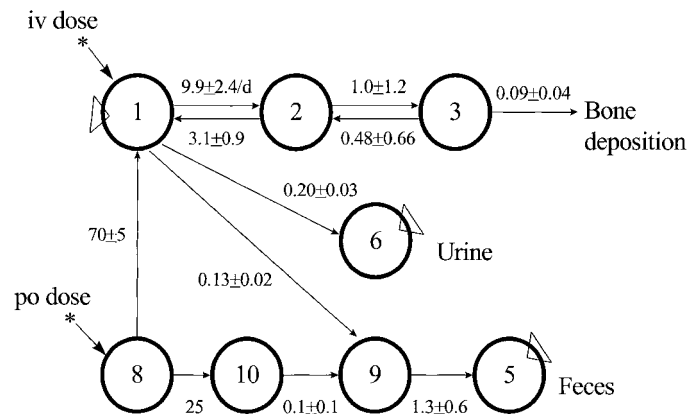


Fig. 1. Model for calcium metabolism. Arrows with asterisk indicate site of tracer entry (iv, intravenous dose; po, oral dose). Triangles represent sampled compartments, and numbers in circles are compartment numbers: compartment 1, serum; compartment 2, calcium in extravascular fluid; compartment 3, exchangeable calcium on bone; compartment 5, feces; 6, urine; and 8, 9 and 10, pools in gastrointestinal tract. Compartment 8, site of absorption; compartment 10, delay of orally administered isotope before appearance in feces; compartment 9, site where endogenously excreted calcium enters gut. Values/day above or below arrows are fractional rate constants (fraction/day, mean  $\pm$  SD,  $n = 3$ ). Transfer from compartment 8 to compartment 10 was fixed at 25/day. Transfer between compartments 1, 2, 3, 5, 9, and 10 were constant within subject between studies. Transfer from compartment 8 to compartment 1 and from compartment 1 into 6 varied for each subject between studies, and values shown for these pathways are average preflight values. Volume of distribution of compartment 1 was fixed at 10 l. Saliva data were fitted as a fraction of compartment 1 and averaged  $0.41 \pm 0.11$ .

times vascular space, on the basis of previous studies in adults (20, 33). Saliva data were fitted as a fraction of serum data, as described previously (30).

**Calculations.** The model was fitted to the data using the least squares iterative procedure and the values of the rate constants [L(I,J), fraction/day], compartment mass [M(J), mg], and transport rates [R(I,J), mg/day, as the product of L(I,J) and M(J)] were calculated. Rates of calcium transport determined included dietary intake ( $V_i$ ), absorption ( $V_a$ ), urine excretion ( $V_u$ ), endogenous excretion ( $V_e$ ), fecal excretion ( $V_f$ ), bone deposition ( $V_{o+}$ ), bone resorption ( $V_{o-}$ ), and bone balance. Bone balance was calculated as  $V_{o+}$  minus  $V_{o-}$  and is equivalent to dietary intake minus urinary and fecal excretion ( $V_i - V_u - V_f$ ). During flight, when fecal samples were not collected,  $V_f$  was calculated as the difference between  $V_i$  and  $V_a$  with the addition of  $V_e$  (on the basis of the assumption that endogenous excretion did not change from preflight levels). Fractional absorption was calculated as the fraction of material entering plasma (compartment 1) from the intestine (compartment 8) (Fig. 1) as  $L(1,8)/[L(1,8) + L(10,8)]$ .

### Statistical Analyses

Repeated-measures ANOVA was performed to assess whether the preflight data within subject were similar. Mean preflight data were compared with in-flight and postflight data. Repeated-measures ANOVA were performed on biochemical data to determine if there were differences between any of the sessions. Post hoc Tukey tests were performed to assess specific differences between sessions. Significance was assigned to  $P < 0.05$ . Statistical analyses were performed using SigmaStat (SPSS, Chicago, IL).

Table 1. *Body weight and markers of calcium and bone metabolism before, during, and after 115-day spaceflight*

	Preflight	Inflight		Postflight		
		FD14	FD110	R+0	R+9	R>75
Body wt, kg	79.6 ± 8.4*	76.1 ± 7.7*†	73.1 ± 6.8†	74.8 ± 7.1††	79.1 ± 9.1*†	84.9 ± 9.5§
Total calcium, mmol/l	2.47 ± 0.09*††	2.35 ± 0.05*	2.35 ± 0.06*†	2.53 ± 0.09†	2.43 ± 0.06*†	2.36 ± 0.06*
Ionized calcium, mmol/l	1.14 ± 0.02*	1.17 ± 0.03*†	1.21 ± 0.01†	1.18 ± 0.01*†	1.23 ± 0.02†	1.21 ± 0.05†
25(OH)-vitamin D, nmol/l	37.3 ± 9.7*†	34.4 ± 8.9*†	22.2 ± 5.1*	23.8 ± 5.3*	28.5 ± 5.8*	45.6 ± 13.1†
1,25(OH) <sub>2</sub> -vitamin D, pmol/l	81.5 ± 5.3*†	56.4 ± 4.3*	66.7 ± 3.3*	61.0 ± 4.0*	72.2 ± 7.8*†	103.5 ± 25.6†
Parathyroid hormone, pg/ml	20.0 ± 7.6	16.1 ± 3.6	18.1 ± 3.0	31.2 ± 13.1	33.5 ± 20.1	28.2 ± 3.5
Bone-specific alkaline phosphatase, µg/l	8.7 ± 3.7*††	5.3 ± 2.1*	5.6 ± 5.6*†	9.7 ± 2.9††	7.5 ± 4.2*†	11.9 ± 2.1†
Osteocalcin, ng/ml	7.5 ± 4.1*†	8.3 ± 1.5*	7.0 ± 6.7*†	14.3 ± 3.8*†	19.8 ± 8.9*†	24.5 ± 8.2†
Calcitonin, pg/ml	8.1 ± 4.5	8.2 ± 4.0	8.1 ± 3.9	9.0 ± 4.9	8.5 ± 3.9	8.6 ± 5.8
<i>n</i> -Telopeptide, nmol/mmol creatinine	25.7 ± 6.3*	51.7 ± 15.1†	49.1 ± 8.0†	32.2 ± 4.0*	32.1 ± 7.5*	24.6 ± 6.2*
Pyridinium crosslinks, nmol/mmol creatinine	17.1 ± 3.3*	25.6 ± 3.8†	28.9 ± 5.4†	24.5 ± 0.7†	26.4 ± 3.7†	27.8 ± 4.3†
Deoxypyridinoline, nmol/mmol creatinine	3.1 ± 0.7*	4.5 ± 0.6†	4.6 ± 0.7†	4.5 ± 0.9†	4.9 ± 0.6†	4.1 ± 1.0†

Data are means ± SD for 3 subjects. For each parameter, values with different superscripts denote significant ( $P < 0.05$ ) differences between studies. FD, flight day; R +  $x$ , return +  $x$  days, where R + 0 is day of landing. Blood data are from analysis of serum samples, except ionized calcium, which was determined on whole blood. Collagen crosslink data are normalized to creatinine excretion.

## RESULTS

All three subjects lost weight during the flight, and two of them lost almost 10% of preflight body mass (Table 1, Fig. 2). Thus it is clear that the crew members were in negative energy balance. Although detailed information regarding in-flight exercise was not recorded, reports from the crew members indicated that there was significant variability between crew members with regard to exercise. This ranged from one subject who reported exercising twice per day almost every day to another who did little to no exercise throughout the flight.

In two cases, within-subject differences occurred in preflight data: ionized calcium and 25-hydroxyvitamin D. For ionized calcium, the final preflight session (L-36) was significantly lower than the other two sessions. As the primary effect of interest in the study was that of spaceflight, only the data from last session (L-36) was used, as it was assumed that this most

closely approximated the preflight data. For the 25-hydroxyvitamin D, the difference was considered to be related to the seasonal effect of training in Russia, with resultant low levels of ultraviolet light exposure. Therefore, only data from the two preflight sessions (L-61 and L-36) conducted in Russia were used.

Results of biochemical and endocrine determinations are presented as mean data with statistical results (Table 1). Individual data for the biochemical and endocrine parameters, normalized to percent change from preflight, are depicted in Fig. 3 to show individual responses and the magnitude of their change. Few parameters changed significantly during flight (Table 1). Whole blood ionized calcium increased significantly late in flight, whereas osteocalcin and calcitonin were unchanged. 25-Hydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone, and bone-specific alkaline phosphatase showed a decreasing trend in flight (Fig. 3). Several parameters changed significantly post-flight compared with in flight, including total calcium, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, bone-specific alkaline phosphatase, and osteocalcin (Table 1). Parathyroid hormone showed a tendency to increase postflight (Fig. 3).

Collagen crosslink excretion was significantly elevated during flight (Table 1) by >40% above preflight levels for all three crosslinks measured. *n*-Telopeptide excretion returned to baseline within days after flight, but pyridinium and deoxypyridinoline remained elevated at R>75 by up to 60% above preflight levels.

Observed tracer data and the model-calculated fits are shown in Figs. 4 and 5. Preflight parameter values (mean ± SD for the 3 subjects) are shown in Fig. 1. Data for each subject were fitted by allowing only three parameters to vary between studies: absorption [L(1,8)], urinary excretion [L(6,1)], and the fraction of serum tracer observed in saliva. Changes in these three parameters were sufficient to explain the differences in kinetics between the studies. In addition, in *subject 1*, L(2,3) increased 300% during the in-flight session and endogenous excretion [L(9,1)] increased by 80% during the R+9 session. Changes in absorption and urine

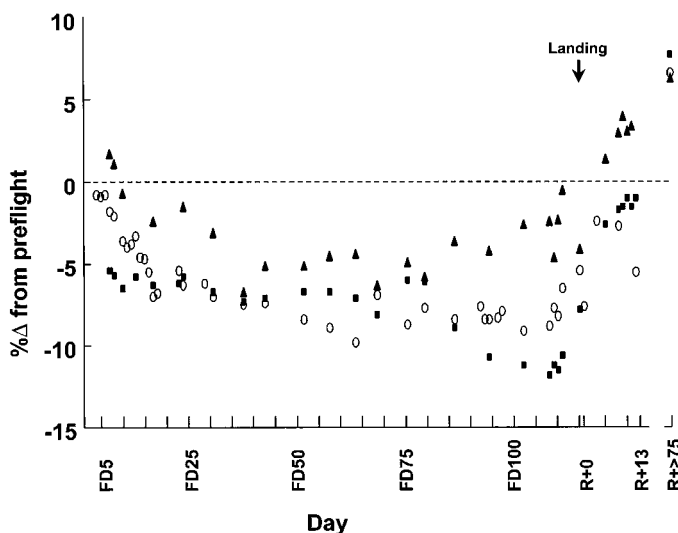


Fig. 2. Body mass during and after a 115-day spaceflight. Data are presented as percent change (%Δ) from preflight values for each subject on days during flight (FD) or postflight (R). ■, *subject 1*; ▲, *subject 2*; ○, *subject 3*.

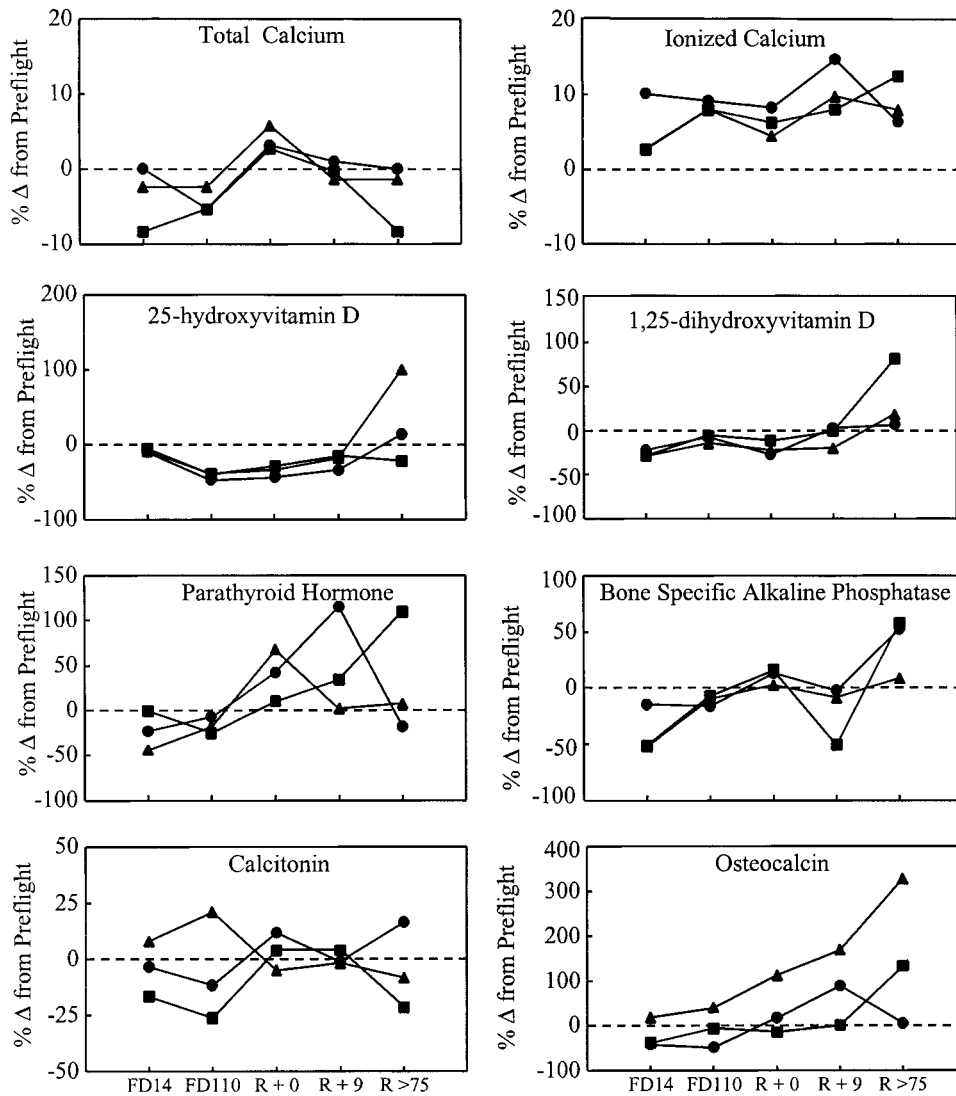


Fig. 3. Biochemical and endocrine markers of bone and calcium homeostasis. Data are presented as % $\Delta$  from preflight values for each subject on days during flight or postflight. ■, subject 1; ▲, subject 2; ●, subject 3.

excretion between studies are shown in Table 2. The fraction of serum tracer observed in saliva changed from  $0.41 \pm 0.11$  preflight to  $0.31 \pm 0.09$  in flight,  $0.31 \pm 0.07$  at R+0,  $0.48 \pm 0.07$  at R+9, and  $0.43 \pm 0.12$  at R>75. The in-flight value was significantly lower than the preflight value, whereas the postflight values did not differ from preflight. No fecal data were collected in the last postflight session (R>75) for this subject, so it was assumed that endogenous excretion remained at the level of the R+9 session. In *subject 2*, endogenous excretion also increased immediately after landing. In *subject 3*, data indicated that the oral and intravenous doses had been reversed for the R+0 session (based on the large amount of intravenous tracer appearing in feces), and data were fitted using this assumption.

Data from kinetic studies for individual subjects are shown in Table 2. Calcium intake and fractional absorption decreased  $\sim 50\%$  during flight and returned to preflight levels by R>75. Urine calcium increased by up to 100% during flight. Bone deposition decreased during flight in one subject, but did not change in the other

two. All three subjects had elevated levels of bone resorption ( $\sim 50\%$ ) and negative bone calcium balance during flight (Fig. 6). Bone resorption returned to baseline by the R+9 session.

## DISCUSSION

The results reported in this paper are consistent with earlier findings and extend them by providing the first in-flight assessment of calcium absorption and kinetics during extended-duration spaceflight. Previous flight and ground-based studies of calcium and bone homeostasis during weightlessness have been summarized in Table 3. The data presented in this paper show that bone calcium is lost at rates in excess of 140 mg/day during spaceflight. Biochemical and kinetic measurements indicate that the loss is due to a combination of lower calcium intake, reduced fractional absorption, increased calcium excretion, and increased bone resorption. Recovery, as indicated by positive calcium balance and increased bone formation markers, continued for

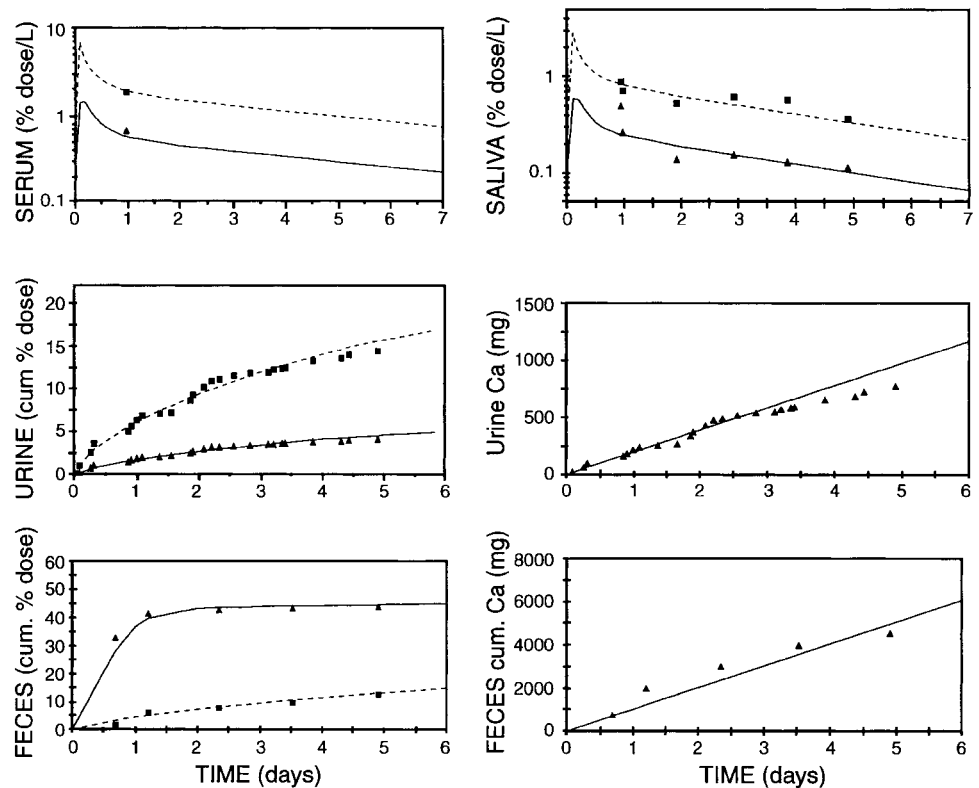


Fig. 4. Observed data following oral (▲) or intravenous (■) tracer and model (Fig. 1)-calculated fits (lines). Serum, saliva, urine, urine calcium, feces, and fecal calcium data for 1 of 7 studies on *subject 2*. cum, Cumulative.

several months after flight at a rate slower than the in-flight loss.

Negative calcium balance was observed during the Skylab missions (10, 27, 34), evidenced by increased urinary and fecal calcium excretion (10, 27, 34, 35). The intestinal absorption and urinary excretion data in this paper corroborate the Skylab metabolic balance studies. While the fecal excretion data presented here were calculated to be lower in flight than preflight, this is likely related to decreased dietary intake of calcium and not a physiological phenomenon. Decreased calcium intake is usually associated with increased fractional absorption. Thus the decreased fractional absorption in this study, associated with decreased intake, implies altered physiology. We hypothesize that this is a result of calcium release from the bone downregulating intestinal absorption, likely mediated by parathyroid hormone and vitamin D.

Intestinal calcium absorption was high (>65%) in most pre- and postflight studies. As described under SUBJECTS AND METHODS, tracer was administered with a calcium load, but the dose was in liquid form (whereas the load was in capsule form) and was absorbed readily. Neer et al. (20) reported an absorption value of 41% in men, and this value was used to calculate the steady-state values for calcium transport and pool size in studies where absorption of tracer exceeded 41%. As absorption was <41% in most in-flight studies, this did not influence the calculation of calcium balance in flight.

If it is assumed that the rate of bone mineral loss observed in flight is linear throughout the flight (i.e., ~250 mg of calcium are lost from bone every day of

flight) and that the rate of recovery is also linear (i.e., ~100 mg of bone calcium are regained every day postflight), it will take ~2.5 times the mission length to recover the lost bone. Although more data clearly are required to validate this hypothesis, bone mineral density studies from other missions estimated that in-flight calcium loss was  $-227 \pm 63$  mg/day (7); collagen crosslink excretion data (29) support the assumption of relatively linear in-flight loss, and ground-based data (12) support the assumption of linear recovery.

As with previous studies, there was considerable variability between subjects in their response to spaceflight. *Subject 1* (Figs. 3 and 6) appeared to be the most susceptible to change. He had the largest increase in urine calcium and the largest negative balance in flight. However, he appeared to have the most pronounced recovery as indicated by positive bone balance at R+9 and high levels of parathyroid hormone and bone-specific alkaline phosphatase at R>75 (Fig. 3). *Subject 3* (Figs. 3 and 6) maintained a higher calcium intake in flight than the other subjects, absorbed more calcium, and as a result had the smallest change in bone calcium balance during flight. This subject appeared to be more refractory to changes in spaceflight. An early indicator of his ability to resist changes in calcium metabolism in flight may have been the small decrease in bone-specific alkaline phosphatase early in flight (-14% compared with the more than -50% change in the other two subjects). *Subject 2* (Figs. 3 and 6) was intermediate between the other two in terms of changes in calcium and bone metabolism in flight, and

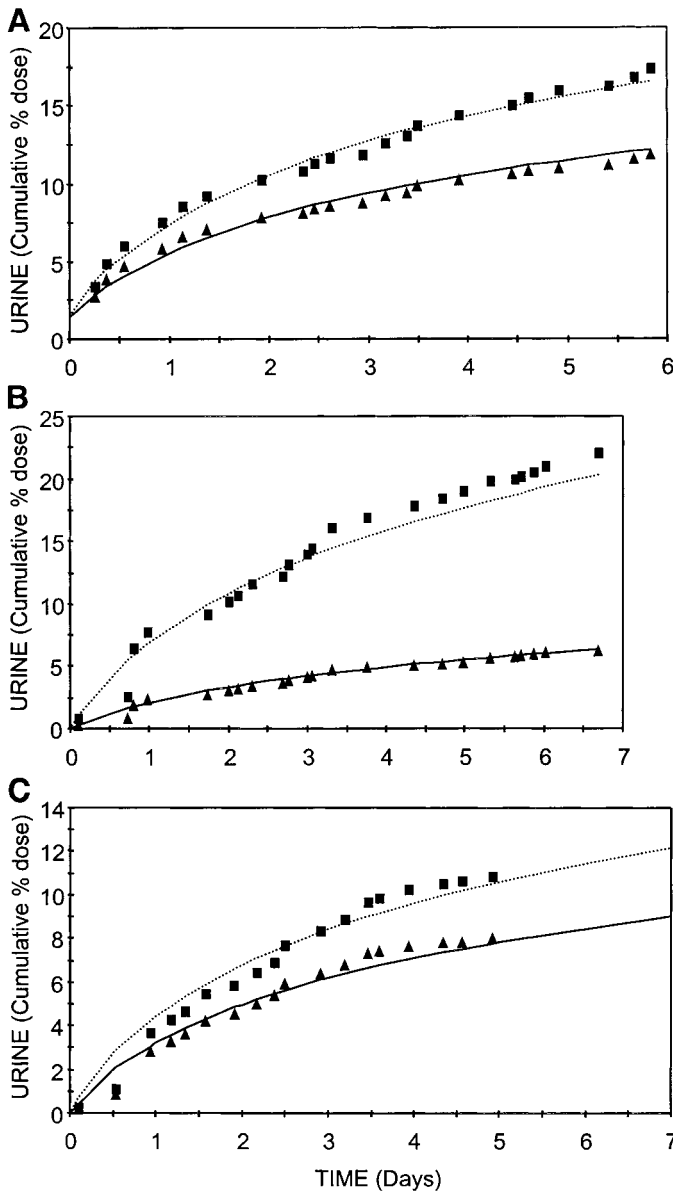


Fig. 5. Observed data after oral ( $\blacktriangle$ ) or intravenous ( $\blacksquare$ ) tracer and model (Fig. 1)-calculated fits (lines). Urine data for preflight (A), R+0 (B), and R>75 days (C) for *subject 1*. Tracers were given on R+0 and R>75, with sample collections occurring over subsequent days. The closer the oral and intravenous curves are, the higher the fractional absorption. On landing, more intravenous tracer was excreted in urine than in other 2 studies (note change in scale), and difference between oral and intravenous curves indicate that fractional absorption was lower.

it appeared that this subject would take longer to recover losses after the flight.

25-Hydroxyvitamin D is considered to reflect the body stores of vitamin D. The absence of ultraviolet light during spaceflight may diminish vitamin D pools in the body and is a concern for lengthy missions. However, despite diet supplements of 500 IU vitamin D on all Skylab missions, plasma 25-hydroxyvitamin D concentrations on landing day after the 84-day Skylab-4 mission (but not after the two shorter missions) were slightly less than preflight values (10). We found also that levels of the active hormone 1,25-dihydroxyvi-

Table 2. Calcium kinetics

	Preflight	FD110	R+0	R+9	R>75
Calcium intake	1,204 ± 287	503	1,618	1,231	1,356
	968 ± 176	400	1,594	1,435	971
	1,025 ± 166	841	788	949	949
Fractional absorption	66 ± 20	36	29	55	74
	77 ± 2	77	24	27	81
	69 ± 5	36	60	80	56
Urinary calcium	259 ± 53	486	256	211	200
	220 ± 17	219	190	204	105
	184 ± 21	300	211	141	151
Endogenous excretion	114	114*	114	194	194
	174	174	318	174	174
	148	148	148	148	148
Fecal excretion	825 ± 170	438	1,266	919	993
	745 ± 104	410	1,529	1,229	747
	753 ± 98	684	613	708	708
Bone calcium deposition	488	166	488	488	488
	521	521	521	521	521
	452	452	452	452	452
Bone calcium resorption	369 ± 109	589	393	388	326
	520 ± 80	751	647	519	403
	365 ± 68	596	489	353	363
Bone calcium balance	119 ± 109	-423	95	100	162
	1 ± 80	-230	-126	2	118
	87 ± 68	-144	-37	99	89

Preflight data are means ± SD for 3 sessions in mg/day, except for fraction absorption (%). Values in each entry are for *subjects 1, 2, and 3*, respectively. \*Because fecal samples were not collected in flight, it was assumed that endogenous excretion remained at preflight levels. See SUBJECTS AND METHODS for details.

tamin D tended to be lower during flight. We hypothesize that this is related to decreased activation as a consequence of lower parathyroid hormone levels, rather than increased disposal of vitamin D. Furthermore, the decrease in 1,25-dihydroxyvitamin D is likely causally related to the decrease in calcium absorption.

Bone formation, as indexed by bone-specific alkaline phosphatase (25) and osteocalcin, tended to decrease during flight but increased by 75 days after flight. Osteocalcin decreased in one subject studied on a recent Mir flight (4). Ground-based studies in humans have also shown no changes in bone formation during bed rest, but increased formation rates after reambulation (15, 36). Osteocalcin concentrations in plasma are

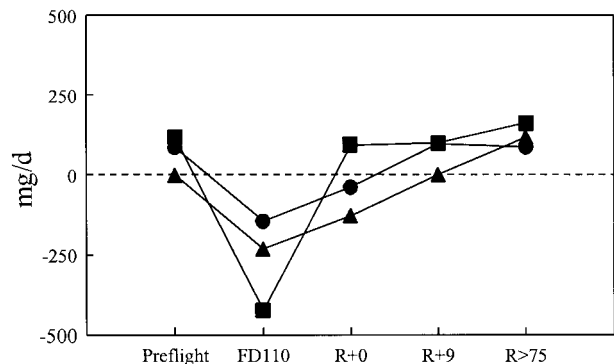


Fig. 6. Bone calcium balance ( $V_{0+} - V_{0-}$ ) during and after spaceflight. Data are presented for each subject. Values were determined during kinetic study beginning at time shown (FD110, R+0, R+9, or R>75 days).  $\blacksquare$ , *subject 1*;  $\blacktriangle$ , *subject 2*;  $\bullet$ , *subject 3*.

Table 3. *Human adaptation to spaceflight and bed rest*

	Spaceflight				Bed Rest				
	Length, day	<i>n</i>	Change	Ref.	Length, day	<i>n</i>	Change	Recovery	Ref.
<b>Bone loss, %Δ/mo</b>									
Os calcis	28	3	+0.5%, -0.9%, 2.7%	27	59	15	-3.5%		27
	59	3	+1.2%, -3.7%, +0.7%	27	84	15	-4%		27
	84	3	-2.8%, -1.6%, +0.25%	27	210-252	3	-5% to -7%	5% to 7%	5
Radius	28	3	-0.5%, +1.4%, +0.2%	27	119	6	NC	NC	12
	59	3	-0.7%, -0.1%, -0.8%	27					
	84	3	-0.4%, +0.4%, -0.2%	27					
Ulna	28	3	-0.9%, +1.9%, +3.1%	27	119	6	NC	NC	12
	59	3	+0.2%, -0.8%, -0.2%	27					
	84	3	-0.6%, +0%, -0.5%	27					
Calcaneous	75	2	-0.4%, -1.31%	31	119	6	-2.4%	1.2%	12
	140	2	-0.6%, -4.2%	31					
	175	2	-1.7%, -0.5%	31					
	184	2	-0.7%, -1.6%	31					
Various sites	120-432	18	-0.04% to -1.58%	7, 14					
<b>Calcium metabolism, %Δ from preflight</b>									
Urinary calcium	28	9	80%	10	7	8	80%		2
	59	6	81%	10	84	11	32%		36
	84	3	47%	10	119	8	138%		15
					144	9	50%		27
					210-252	3	70%		5
Calcium balance	59, 84	6	-200 to -300 mg/d	24	119	8	-160 mg/d		15
					210-252	3	140 mg/d		5
Calcium absorption					84	11	NC		36
					119	8	-22.5%		15
Serum calcium	6 or 7	4	6%	19	7	8	NC	NC	2
	28, 59, 84	9	4%	10	10	14	3%	NC	32
	10-178	1	NC	4	84	11	2%	NC	36
					119	8	NC	-3.8%	15
					210-252	3	NC	NC	5
					119	8	1.5%	-1.5%	15
<b>Ionized Ca</b>	<2 weeks	21	-2%	28					
<b>Calcium regulation, %Δ from preflight</b>									
25-Hydroxyvitamin D	6 or 7	4	17%	19	10	14	NC	NC	32
	28, 59, 84	9	-8.5% at landing	10	84	11	NC	NC	36
1,25-Dihydroxyvitamin D	6 or 7	4	-22%	19	7	8	-15%	NC	2
					10	14	-19%	-25% (1 wk)	32
					84	11	-34%	-24%	36
					119	8	-16.5%		15
Parathyroid hormone	6 or 7	4	-34%	19	7	8	-15%	NC	2
	28, 59, 84	9	NC	10	10	14	NC	NC	32
	10-178	1	-48%	4	84	11	NC	NC	36
					119	8	NC	111%	15
Osteocalcin	10-178	1	-27%	4	7	8	17%	NC	18
					10	14	NC	NC	32
					84	11	NC	NC	36
					119	8	NC	33%	15
Calcitonin	28, 59, 84	9	NC	10	7	8	NC	NC	2
					119	8	NC	NC	15
<b>Bone formation/resorption, %Δ from preflight</b>									
Alkaline phosphatase					10	14	NC	NC	32
					119	8	NC	50%	15
Bone-specific alkaline phosphatase	10-178	1	-28%	4	7	8	NC	NC	18
					84	11	NC	NC	36
Collagen crosslinks	28, 59, 84	9	100-150%	30	7	8	41%	30%	18
	10-178	1	35-78%	4	84	11	49%	NC	36
					119	8	50%	NC	29
Hydroxyproline	28, 59	6	33%	34	7	8	33%	47%	18
					10	14	55%	35%	32
					84	11	45%	16%	36
					210-252	8	8%	NC	5

Units of measure as stated, except for calcium balance, which is mg/day change (Δ) from preflight. NC, No change.



higher in subjects with metabolic bone disease (23) and have been reported to be lower in rats after spaceflight (22). Although the contrast with the animal data is unclear, the data from the R>75 session indicate an increased remodeling, with net formation, of bone. The tracer studies did not detect a change in bone formation ( $V_{0+}$ ) during (or after) flight in this experiment. This may be related to the small number of subjects or to the relatively short duration of the tracer studies. It has been reported that studies of >10 (30) or 20 (8) days are required to define bone calcium kinetics. Studies are currently under development for flights early next century on the International Space Station that will allow for a longer period of measurement (i.e., weeks) of tracer movement through the body and administration of the tracers multiple times during the flight. Data from these proposed studies will allow for a better understanding of the time course of bone adaptation during spaceflight.

Bone resorption, as indexed by urinary hydroxyproline, is increased during spaceflight and bed rest. Urinary hydroxyproline was increased 33% after 84 days of flight (11, 34). Urinary collagen crosslinks, also markers of bone resorption, are elevated by 150% during spaceflight (29). Mathematical modeling of tracer data also indicated that bone resorption ( $V_{0-}$ ) was increased by ~50%. The results from this study indicate that the changes in bone metabolism clearly involve increased bone resorption and possibly decreased bone formation.

Studies of bed rest, an analog for weightlessness, have shown similar qualitative effects on bone and calcium homeostasis, specifically loss of bone mass, decreases in calcium absorption, increases in calcium excretion (2, 15), and decreases in serum concentrations of parathyroid hormone (2) and 1,25-dihydroxyvitamin D (2, 15). Bed rest is associated with elevated hydroxyproline excretion (15), as is paraplegia (9). Quantitatively, collagen crosslink excretion during bed rest (15, 29) is elevated ~50% above control levels compared with the typically >100% increase during flight (29). These data suggest that bed rest, although an analog of spaceflight, does not result in the same magnitude of bone changes.

The changes in endocrine regulation of bone metabolism reflect adaptation to the weightless environment. The decreases in parathyroid hormone, 1,25-dihydroxyvitamin D, and calcium absorption are expected physiological responses to increased resorption of bone that may be occurring as the body attempts to adapt to a lower requirement for weight bearing. Changes in bone mineral content indicate the end point of these changes. By identifying the specific mechanisms that result in mineral loss, countermeasures can be developed to ameliorate the spaceflight-induced bone loss. Whether resistive exercise paradigms or antiresorptive therapies will provide this effect is yet to be determined. Clearly, adequate nutritional support will be required to provide the building blocks when these countermeasures are in place. Ensuring adequate intake of calcium, vitamin D, and other bone-related

nutrients will be required, but does not appear to be the solution to the problem. Other factors that may contribute to the degree of calcium loss are age, fitness, genetics, and dietary history.

### Perspectives

It is well known that spaceflight and other conditions such as aging and immobilization result in loss of bone. This study evaluated changes in calcium metabolism during actual spaceflight. Flight studies, despite the inherent difficulties, are necessary for assessing how well the ground-based analogs and animal studies model spaceflight. From a methodological point of view, tracer kinetic studies provide information with regard to physiological function in vivo, which, together with static measurements of endocrine and biochemical markers, can be used to determine which physiological processes are altered by spaceflight and the degree to which these processes normalize after flight. Defining the mechanism for the loss of bone and calcium during spaceflight and counteracting that loss will not only allow for advances in understanding and treatment of bone diseases on Earth, but will also enable human planetary exploration.

The authors thank Peggy A. Whitson and the Project Science office for support of this project. The authors also thank Vickie L. Kloeris and Daniel L. Feedback for assistance in completion of the ground-based data collection sessions both in the United States and in Star City, Russia. The authors also recognize the time and commitment of the Mir 18 crew members/subjects and the STS-71 crew for assistance in completing the in-flight tracer kinetic studies.

This work was funded by National Aeronautics and Space Administration as part of the Shuttle/Mir Science Program.

Address for reprint requests and other correspondence: S. M. Smith, Life Sciences Research Laboratories/SD3, NASA Johnson Space Center, Houston, TX 77058 (E-mail: smsmith@ems.jsc.nasa.gov).

Received 19 November 1998; accepted in final form 9 March 1999.

### REFERENCES

1. **Abrams, S. A., N. V. Esteban, N. E. Vieira, and A. L. Yergey.** Dual stable isotopic assessment of calcium absorption and endogenous fecal excretion in low birth weight infants. *Pediatr. Res.* 29: 615–618, 1991.
2. **Arnaud, S. B., D. J. Sherrard, N. Maloney, R. T. Whalen, and P. Fung.** Effects of 1-week head-down tilt bed rest on bone formation and the calcium endocrine system. *Aviat. Space Environ. Med.* 63: 14–20, 1992.
3. **Berman, M.** A postulate to aid in model building. *J. Theor. Biol.* 4: 229–236, 1963.
4. **Caillot-Augusseau, A., M.-H. Lafage-Proust, C. Soler, J. Pernod, F. Dubois, and C. Alexandre.** Bone formation and resorption biological markers in cosmonauts during and after 180-day space flight (Euromir 95). *Clin. Chem.* 44: 578–585, 1998.
5. **Donaldson, C. L., S. B. Hulley, J. M. Vogel, R. S. Hattner, J. H. Bayers, and D. E. McMillan.** Effect of prolonged bed rest on bone mineral. *Metabolism* 19: 1071–1084, 1970.
6. **Greif, P., M. E. Wastney, O. Linares, and R. Boston.** Balancing needs, efficiency, and functionality in the provision of modeling software: a perspective of the NIH WinSAAM project. *Adv. Exp. Med. Biol.* 445: 3–20, 1998.
7. **Grigoriev, A. I., V. S. Oganov, A. V. Bakulin, V. V. Poliakov, L. I. Voronin, V. V. Morgun, V. S. Schneider, L. V. Murashko, V. E. Novikov, A. LeBlanc, and L. Shackelford.** Clinical and physiological evaluation of bone changes among astronauts after long-term space flights. *Aviakosm. Ekolog. Med.* 32: 21–25, 1998.

8. **Jung, A., P. Bartholdi, and B. Mermillod.** Critical analysis of methods for analysing human calcium kinetics. *J. Theor. Biol.* 73: 131–157, 1978.
9. **Klein, L., S. van der Noort, and J. J. DeJak.** Sequential studies of urinary hydroxyproline and serum alkaline phosphatase in acute paraplegia. *Med. Serv. J. Can.* 22: 524–533, 1966.
10. **Leach, C. S., and P. C. Rambaut.** Biochemical responses of the Skylab crewmen: an overview. In: *Biomedical Results of Skylab NASA-SP-377*, edited by R. S. Johnson and L. F. Dietlein. Washington, DC: NASA, 1977, p. 204–216.
11. **Leach, C. S., and P. C. Rambaut.** Amino aciduria in weightlessness. *Acta Astronaut.* 6: 1323–1333, 1979.
12. **LeBlanc, A., V. Schneider, H. Evans, and D. A. Engelbretson.** Bone mineral loss and recovery after 17 weeks of bed rest. *J. Bone Miner. Res.* 5: 843–850, 1990.
13. **LeBlanc, A. D., and V. S. Schneider.** Countermeasures against space flight related bone loss. *Acta Astronaut.* 27: 89–92, 1992.
14. **LeBlanc, A., V. Schneider, L. Shackelford, S. West, V. Oganov, A. Bakulin, and L. Veronin.** Bone mineral and lean tissue loss after long duration space flight (Abstract). *J. Bone Miner. Res.* 1, Suppl. 1: S323, 1996.
15. **LeBlanc, A., V. Schneider, E. Spector, H. Evans, R. Rowe, H. Lane, L. Demers, and A. Lipton.** Calcium absorption, endogenous excretion, and endocrine changes during and after long-term bed rest. *Bone* 16: 301S–304S, 1995.
16. **LeBlanc, A., L. Shackelford, and V. Schneider.** Future human bone research in space. *Bone* 22, Suppl. 5: 113S–116S, 1998.
17. **Lockwood, D. R., J. M. Vogel, V. S. Schneider, and S. B. Hulley.** Effect of the diphosphonate EHDP on bone mineral metabolism during prolonged bed rest. *J. Clin. Endocrinol. Metab.* 41: 533–541, 1975.
18. **Lueken, S. A., S. B. Arnaud, A. K. Taylor, and D. J. Baylink.** Changes in markers of bone formation and resorption in a bed rest model of weightlessness. *J. Bone Miner. Res.* 8: 1433–1438, 1993.
19. **Morey-Holton, E. R., H. K. Schoes, H. F. DeLuca, M. E. Phelps, R. F. Klein, R. H. Nissenson, and C. D. Arnaud.** Vitamin D metabolites and bioactive parathyroid hormone levels during Spacelab 2. *Aviat. Space Environ. Med.* 59: 1038–1041, 1988.
20. **Neer, R., M. Berman, L. Fisher, and L. E. Rosenberg.** Multicompartmental analysis of calcium kinetics in normal adult males. *J. Clin. Invest.* 46: 1364–1379, 1967.
21. **Oganov, V. S., A. S. Rakhmanov, V. E. Novikov, S. T. Zatsepin, S. S. Rodionova, and C. Cann.** The state of human bone tissue during space flight. *Acta Astronaut.* 23: 129–133, 1991.
22. **Patterson-Buckendahl, P. E., R. E. Grindeland, R. B. Martin, C. E. Cann, and S. B. Arnaud.** Osteocalcin as an indicator of bone metabolism during space flight. *Physiologist* 28: S227–S228, 1985.
23. **Price, P. A., J. G. Parthemore, and L. J. Deftos.** A new biochemical marker for bone metabolism. *J. Clin. Invest.* 66: 878–883, 1980.
24. **Rambaut, P. C., and P. C. Johnson.** Prolonged weightlessness and calcium loss in man. *Acta Astronaut.* 6: 1113–1122, 1979.
25. **Rosalki, S. B., A. Y. Foo, A. Burlina, W. Prellwitz, P. Stieber, D. Neumeier, G. Klein, W. A. Poppe, and S. Bodenmüller.** Multicenter evaluation of iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. *Clin. Chem.* 39: 648–652, 1993.
26. **Schakel, S. F., Y. A. Sievert, and I. M. Buzzard.** Sources of data for developing and maintaining a nutrient database. *J. Am. Diet. Assoc.* 88: 1268–1271, 1988.
27. **Smith, M. C., P. C. Rambaut, J. M. Vogel, and M. W. Whittle.** Bone mineral measurement (Experiment M078). In: *Biomedical Results of Skylab NASA-SP-377*, edited by R. S. Johnson and L. F. Dietlein. Washington, DC: NASA, 1977, p. 183–190.
28. **Smith, S. M., J. E. Davis-Street, T. B. Fontenot, and H. W. Lane.** Assessment of a portable clinical blood analyzer during space flight. *Clin. Chem.* 43: 1056–1065, 1997.
29. **Smith, S. M., J. L. Nillen, A. LeBlanc, A. Lipton, L. Demers, H. W. Lane, and C. S. Leach.** Collagen crosslink excretion during space flight and bed rest. *J. Clin. Endocrinol. Metab.* 83: 3584–3591, 1998.
30. **Smith, S. M., M. E. Wastney, L. E. Nyquist, C.-Y. Shih, H. Wiesmann, J. L. Nillen, and H. W. Lane.** Calcium kinetics with microgram stable isotope doses and saliva sampling. *J. Mass Spectrom.* 31: 1265–1270, 1996.
31. **Stupakov, G. P., V. S. Kaseykin, A. P. Kolovskiy, and V. V. Korolev.** Evaluation of changes in human axial skeletal bone structure during long-term space flights. *Moscow Kosmicheskaya Biol. I Aviak. Med.* 18: 33–37, 1984.
32. **Van der Wiel, H. E., P. Lips, J. Nauta, J. C. Netelenbos, and G. J. Hazenburg.** Biochemical parameters of bone turnover during ten days of bed rest and subsequent mobilization. *Bone Miner.* 13: 123–129, 1991.
33. **Wastney, M. E., J. Ng, D. Smith, B. R. Martin, M. Peacock, and C. M. Weaver.** Differences in calcium kinetics between adolescent girls and young women. *Am. J. Physiol.* 271 (Regulatory Integrative Comp. Physiol. 40): R208–R216, 1996.
34. **Whedon, G. D., L. Lutwak, P. C. Rambaut, M. W. Whittle, M. C. Smith, J. Reid, C. S. Leach, C. R. Stadler, and D. D. Sanford.** Mineral and nitrogen metabolic studies, experiment M071. In: *Biomedical Results from Skylab NASA SP-377*, edited by R. S. Johnson and L. E. Dietlein. Washington, DC: NASA, 1977, p. 164–174.
35. **Whitson, P. A., R. A. Pietrzyk, and C. Y. C. Pak.** Renal stone risk assessment during space shuttle flights. *J. Urol.* 158: 2305–2310, 1997.
36. **Zerwekh, J. E., L. A. Ruml, F. Gottschalk, and C. Y. C. Pak.** The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects. *J. Bone Miner. Res.* 13: 1594–1601, 1998.