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# Benefits for Bone From Resistance Exercise and Nutrition in Long-Duration Spaceflight: Evidence From Biochemistry and Densitometry

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## ABSTRACT

Exercise has shown little success in mitigating bone loss from long-duration spaceflight. The first crews of the International Space Station (ISS) used the "interim resistive exercise device" (iRED), which allowed loads of up to 297 $lb_f$ (or 1337 N) but provided little protection of bone or no greater protection than aerobic exercise. In 2008, the Advanced Resistive Exercise Device (ARED), which allowed absolute loads of up to 600 $lb_f$ (1675 N), was launched to the ISS. We report dietary intake, bone densitometry, and biochemical markers in 13 crewmembers on ISS missions from 2006 to 2009. Of these 13, 8 had access to the iRED and 5 had access to the ARED. In both groups, bone-specific alkaline phosphatase tended to increase during flight toward the end of the mission ($p = 0.06$) and increased 30 days after landing ($p < 0.001$). Most markers of bone resorption were also increased in both groups during flight and 30 days after landing ($p < 0.05$). Bone densitometry revealed significant interactions (time and exercise device) for pelvis bone mineral density (BMD) and bone mineral content ($p < 0.01$), hip femoral neck BMD ($p < 0.05$), trochanter BMD ($p < 0.05$), and total hip BMD ($p < 0.05$). These variables were unchanged from preflight only for ARED crewmembers, who also returned from flight with higher percent lean mass and lower percent fat mass. Body mass was unchanged after flight in both groups. All crewmembers had nominal vitamin D status ($75 \pm 17$ nmol/L) before and during flight. These data document that resistance exercise, coupled with adequate energy intake (shown by maintenance of body mass determined by dual-energy X-ray absorptiometry [DXA]) and vitamin D, can maintain bone in most regions during 4- to 6-month missions in microgravity. This is the first evidence that improving nutrition and resistance exercise during spaceflight can attenuate the expected BMD deficits previously observed after prolonged missions. © 2012 American Society for Bone and Mineral Research.

KEY WORDS: VITAMIN D; BONE LOSS; BONE TURNOVER MARKERS; SPACE FLIGHT; WEIGHTLESSNESS

## Introduction

Bone loss is a seemingly inevitable outcome of spaceflight.[1–4] Of the many countermeasures evaluated to date, none have been fully effective during flight.[5] Exercise in particular has been applied in many forms over the past 4 decades of spaceflight, with little success in mitigating net bone loss. This was initially assumed to be related to the types of exercise available. That is, before the International Space Station (ISS) was constructed, only aerobic (treadmill and cycle) and muscular endurance (elastic expanders or Exer-Genie) exercise equipment was available on long-duration space missions (Mir and Skylab). It is well recognized by researchers in exercise physiology and bone biomechanics that bone needs to be optimally overloaded to have a stimulatory effect, which cannot always be provided by aerobic exercises.[6] The ability of resistance exercise to mitigate bone loss in bed rest (a ground-based analog of spaceflight) fueled this research-based projection.[7,8]

The first long-duration crew on the ISS launched in late 2000 with a treadmill and cycle ergometer available for exercise and the interim resistive exercise device (iRED) assembled 6 weeks later.[4,9,10] The iRED had a maximum load equivalent of 297 $lb_f$ (1337 N), an elastic force curve at the higher load ranges, and an eccentric force that was 60% to 80% percent of the concentric force. It is not surprising that in-flight exercise on the iRED produced unimpressive results, as ground-based (1g) training

with the iRED failed to affect bone.[9,11] As expected, early reports from the ISS[2,4] indicated that the iRED provided no greater bone protection than devices used in previous space programs (such as Mir) in which only aerobic and muscular endurance exercises were available. Studies of bone loss countermeasures on these early ISS missions were additionally confounded by the fact that the iRED suffered several technical problems and was not usable for long periods. Although some improvements were made to the iRED (during Expedition 9), they did not overcome its inherent load quality and quantity limitations.

Good nutrition is obviously required for overall health, but particularly for exercise to be effective. In reviewing findings from spaceflight, nutritional factors complicate the situation. Dietary intake in general has been inadequate on many space missions, and many space medicine specialists assume that a spaceflight "anorexia" is unavoidable (despite Skylab data to the contrary).[5,12] With regard to the early reports of ISS crews losing bone at rates similar to those of crews on previous missions,[2] overall energy intake was low, with crews consuming 70% to 80% of their energy requirements and losing 5% to 10% of their preflight body mass.[13] Additionally, on early ISS missions, vitamin D status started at levels now considered suboptimal ($<50$ nmol/L), and despite provision of supplements (400 IU/d) during flight, vitamin D status declined in many crewmembers over the course of the mission.[13] This decline was likely caused in part by suboptimal vitamin D intakes, and in part by inconsistent compliance with vitamin D supplementation (at the time, 400-IU/d supplements were provided for all ISS crews).[5,12,13]

In late 2008, the Advanced Resistive Exercise Device (ARED) was launched to the ISS. A more robust device, it has much greater resistance capability.[14] It allows greater absolute loads (600 lb$_f$, 2675 N), provides a constant load throughout the range of motion, is quickly reconfigured to engage flywheels and provide variable force simulating the inertia associated with gravitational loading, and has an improved eccentric:concentric ratio of about 90%. In addition the ARED allows performance of a much greater variety of exercises. Given the site-specificity of bone loss during spaceflight to weight-bearing bones,[15] a highly periodized exercise program with a variety of lower-body exercises was designed to target mechanical loads to those skeletal sites displaying the greatest declines in bone mass.

We report here findings from evaluations of bone biochemical markers and bone densitometry testing in 13 crewmembers on ISS missions flown between 2006 and 2009. Of these 13 crewmembers, 8 had access to the iRED (after the aforementioned modifications and improvements) and 5 had access to the ARED. All had access to aerobic devices (treadmill and cycle ergometer). Blood and urine samples were collected before, during, and after flight. Dual-energy X-ray absorptiometry (DXA) measurements were performed before and after flight. Dietary intake data are presented, along with vitamin D status, both critical factors in bone health.

## Subjects and Methods

The studies described herein were part of the Nutritional Status Assessment experiment. Although other aspects of the study have been published,[16–18] none of the biochemical or nutritional data reported here have been previously reported. Of the DXA data, the only element previously reported is whole-body bone mineral density (BMD) data, examined in relation to dietary intake of fish.[19] The subjects whose data sets are included here are those for whom we have in-flight biochemical data, and those not participating in other bone loss counter-measure studies (such as evaluation of bisphosphonates). The study was reviewed and approved by the NASA Johnson Space Center Committee for the Protection of Human Subjects, and written informed consent was obtained from all subjects.

Subjects ($n = 13$) were astronauts on ISS Expeditions 14–20 (missions of 48–215 days duration, flown between 2006 and 2009). Of the 13 subjects, 8 (6 male and 2 female) had access to the iRED, and 5 (3 male and 2 female) had access to the ARED. Two subjects flew in the transition between ARED and iRED and therefore used both devices during their missions. Fifty percent was used as the threshold for group placement, thus 1 subject (49% of sessions on ARED) was placed in the iRED group and 1 subject (57%) was placed in the ARED group. The iRED hardware provided the ability to perform eight exercises (squats, single-leg squats, heel raises, single-leg heel raises, deadlifts, Romanian deadlifts, upright rows, and bent-over rows). The ARED included these, and added nine more (back squat, sumo squat, sumo deadlift, shrugs, shoulder press, bench press, bicep curl, triceps extension, and single-arm row). All crewmembers had access to treadmill and cycle ergometer exercise devices. Mean ± SD flight duration was 5.33 ± 2.06 months (160 ± 62 days) and 4.47 ± 2.03 months (134 ± 61 days) for the iRED and ARED groups, respectively. The mean ± SD age of crewmembers was 45.0 ± 3.9 years and 46.6 ± 2.0 years for the iRED and ARED groups, respectively. Within the iRED group, the mean ages split by gender were 45.3 ± 4.1 years and 44.0 ± 4.2 years for males and females, respectively. Within the ARED subjects, the mean ages were 47.7 ± 1.5 years and 45.0 ± 1.4 years for males and females, respectively.

Blood and two consecutive 24-hour urine samples were collected about 180 and 45 days before launch, and again on landing day (return, or R + 0 days) and 30 days after landing. During flight, crewmembers provided five blood and 24-hour urine collections during spaceflight at about flight day 15 (designated FD15), FD30, FD60, FD120, and FD180. Except for samples collected on R + 0, all blood samples were collected at least 8 hours after food intake or exercise. Because flight durations varied, not all crews had five in-flight sessions. Blood samples were collected using standard phlebotomy techniques, as described.[16–18] After centrifugation, tubes were frozen at $-96°C$ until they were returned to Earth (within 6 to 12 months) aboard the Space Shuttle. An additional blood sample was collected about 10 days before launch (L–10), and those tubes were frozen after centrifugation. The other preflight blood samples were aliquotted into smaller amounts before they were frozen, to avoid repeated freeze/thaws when the samples were analyzed. The in-flight samples were transported from the landing site to Houston, TX, USA, where they were stored in a $-80°C$ freezer until they were analyzed.

Urine collected before and after flight was collected into single-void urine containers (Cole-Parmer, Niles, IL, USA).

Samples were stored with ice packs or refrigerated until they were processed, within 24 hours of collection. In-flight urine voids were collected into urine collection devices containing 1 mL of a LiCl solution as a volume marker. These samples were processed as described.[3,16–18,20]

## Biochemical analyses

Blood and urine samples were analyzed for indices of bone and calcium metabolism, and vitamin D status. Serum bone-specific alkaline phosphatase (BSAP, 5.5% coefficient of variation [CV]) and osteocalcin (9% CV) served as markers of bone formation, and were evaluated using commercially available kits.[3,8,21] Total alkaline phosphatase (0.9% CV) was determined enzymatically using an Olympus AU480 analyzer made by Olympus America Inc. (Center Valley, PA, USA). Vitamin D metabolites (1,25-dihydroxyvitamin D, 19% CV, and 25-hydroxyvitamin D, 15% CV) were determined by radioimmunoassay (DiaSorin, Inc, Stillwater, MN, USA), as described.[3,8,13,21–24]

Serum intact parathyroid hormone (iPTH) was measured using radioimmunoassay techniques. Changes in manufacturing required changing the kit used for iPTH assays. Thus, for some subjects ($n = 4$; all iRED), the samples were analyzed for iPTH using the Nichols RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), as described.[3,20,21] For other subjects ($n = 7$; 2 iRED and 2 ARED), samples were analyzed for iPTH using an immunoradiometric assay (Scantibodies Inc., Santee, CA, USA; 5.6% CV). Only subjects with all samples analyzed by the same kit were included in this aspect of the study (necessitating exclusion of 2 subjects). Because of differences in the assay results, data are expressed as percentage change within individuals to allow comparisons between groups.

Several biomarkers of collagen degradation were analyzed, including N-telopeptide (NTX, 5% CV), C-telopeptide (CTX, 6.9% CV), pyridinium crosslinks (PYD, 10.3% CV), deoxypyridinoline (DPD, 14.4% CV), and helical peptide (HP, 8.1% CV). All were analyzed using commercially available kits, as described.[8,13,21] Collagen crosslink data are expressed as both crosslink excretion per day and crosslink excretion per millimole creatinine. Serum total and urinary calcium (2.05% CV) were determined by atomic absorption spectrophotometry,[3,8,21] and whole-blood ionized calcium (<1% CV) was determined (before and after flight only) using a portable analyzer.[3,13,25] Urinary creatinine (5.1% CV) was analyzed using a colorimetric assay with a clinical analyzer (Ace Alera; Alfa Wassermann, West Caldwell, NJ, USA).

## Densitometry

As described,[8,13,19,26,27] areal bone mineral density (BMD) was determined once between 31 and 453 (average was $129 \pm 123$) days before and again between 5 and 45 days (average was $12 \pm 11$ days) after each flight by DXA with a fan beam densitometer (Hologic Discovery; Hologic, Inc., Waltham, MA, USA). Three International Society for Clinical Densitometry (ISCD)-certified bone densitometry technologists scanned the crewmembers and analyzed the scans for this study. At each session, a series of six scans was performed including whole-body, lumbar spine, right hip, left hip, left heel, and left forearm. Baseline measurements in 8 astronauts, 3 iRED and 5 ARED, were

the average of duplicate scans. This study reports bone changes from baseline for the whole body (5.47% CV for bone mineral content [BMC], and 0.52% CV for BMD) and pelvis (1.50% CV for BMC, and 1.52% CV for BMD) from the whole-body scans. Regional bone scan changes reported include lumbar spine (1.40% CV for BMC, and 0.88% CV for BMD of $L_1$–$L_4$), and both hips (0.76% CV and 0.85% CV for BMD of the total hip, 1.28% CV and 1.62% CV for BMD of the femoral neck, and 1.00% CV and 0.89% CV for BMD of the greater trochanter, right and left, respectively). Total body mass (0.16% CV), lean body mass (0.61% CV), and fat mass (2.35% CV) were determined from the whole-body DXA scan. Standard manufacturer-recommended methods were used for scan acquisition. Densitometer calibration was verified through daily quality control using automated calibration software. During analysis, the global hip box was manually determined, in accordance with procedures used for analyzing and reporting earlier spaceflight and bed rest data,[8,21] with the lateral margin placed adjacent to the greater trochanter lateral cortex and the distal border placed relative to the distal margin of the lesser trochanter. Precision of the manual procedure is equivalent to that of the automated procedures available on current DXA analysis software. All other reported analyses were standard.

## Dietary intake

During long-duration spaceflight, crewmembers record their dietary intake once per week using a food frequency questionnaire (FFQ).[13,28] A unique FFQ is designed for each crew based on the foods flown on that mission (NutritionQuest; www.NutritionQuest.com). The FFQ is designed to provide estimates of seven nutrients of interest: energy, protein, water, sodium, calcium, iron, and potassium. Given that (1) space food systems are largely closed (because the number of food items is limited and the menu cycle is repetitive), (2) portion sizes are known, and (3) the precise nutrient content is known for each food item in the system, the FFQ designed for spaceflight is more reliable than a standard food questionnaire.[19,28,29] Furthermore, crews on ISS missions are often encouraged to consume the entire quantity of opened food items, to minimize leftovers (and subsequent bacterial growth). No dietary data were collected before or after flight and only in-flight data are presented.

Astronauts were provided with vitamin D supplements (800 IU/d). Supplement lots were analyzed for actual content before flight to verify content. Our previous studies have shown degradation of vitamin D supplements on ISS to be minimal[30] well beyond the time of expected use. Vitamin D supplement intake was specifically reported in the FFQ.

## Exercise prescription

All crewmembers were provided with a specific exercise prescription to follow during the mission. Crew were scheduled 2.5 hours per day for exercise and this included time for setup and personal hygiene. Aerobic exercise was prescribed for cycle ergometer or treadmill 6 days per week for approximately 30 minutes. Detailed data on intensity and adherence are not available. Resistance exercise was prescribed 6 days per week and the lower body exercises included squats, heel raises, and

deadlifts on both iRED and ARED. ARED allows the subject to vary the stance width, and accordingly the ARED prescription includes both normal and wide stance postures for squat and deadlift whereas iRED exercise was limited to a narrow stance. Detailed data on prescribed load and participant adherence are available. The squat exercise was selected for presentation of load data because it is considered one of the most important exercises and it involves the greatest muscle mass. The squat loads were self-reported but were compared to the prescribed load for quality control. The daily loads were averaged over the entire mission and between groups. The adherence rate was calculated as the ratio of the number of resistance exercise sessions completed to the number of resistance sessions scheduled.

## Statistical analyses

A repeated-measures two-way ANOVA was performed on spaceflight data. When overall significant differences were detected, a post hoc Bonferroni $t$ test was performed to establish differences from preflight values or within treatments. Pearson correlation coefficients were determined for some dietary intake and DXA measurements. Statistical analyses were performed using SigmaPlot 12.0 (Systat Software, Inc., San Jose, CA, USA). Log transformation had to be used to normalize the data for some measures (25-hydroxyvitamin D, osteocalcin, iPTH, 1,25-dihydroxyvitamin D, urinary calcium, HP, PYD, and NTX). In these cases the log-transformed data were analyzed; however, raw data are presented in the tables. Serum calcium could not be normalized and in that case the raw data were analyzed. A Student's $t$ test was performed to determine whether differences in lean body mass and fat mass could be due to the length of time after landing that the DXA measurements were collected. Values of $p < 0.05$ were considered statistically significant.

# Results

There were no differences between the ARED and iRED groups in adherence to the resistance exercise program, with both groups achieving approximately 75% adherence to the scheduled exercise sessions. Somewhat surprising was the observation that average squat load and average squat load relative to body weight did not differ between groups ($p > 0.05$). The ARED subjects used a squat load of 163 lb$_f$ (726 N) that averaged 84% of their body weight and the iRED subjects used a squat load of 176 lb$_f$ (785 N) that averaged 91% of their body weight. One subject was close to the threshold value of 50% for group placement and with only 49% of the exercise sessions performed on the ARED this individual was assigned to the iRED group. Bone and exercise data were reanalyzed with this participant assigned to the ARED group; there was no major effect on the results regardless of group assignment of this individual.

Bone densitometry data (Table 1) revealed significant interactions (time × exercise) for pelvis BMC and BMD, right hip neck BMD (and a trend for left hip neck BMD, $p = 0.07$), trochanter (left and right) BMD, and total hip (right and left) BMD. Total lumbar spine BMC and BMD were both affected by spaceflight, and there was a trend for an interaction ($p < 0.06$). Whole-body BMC was also affected by spaceflight, with a trend

for ARED crewmembers to have less of a decrease ($p = 0.08$). In the regions with an interactive effect, the post hoc test revealed that postflight values for ARED crewmembers were unchanged from preflight values, but those for iRED crewmembers were not. There was a significant correlation between protein intake (g/kg body weight; Pearson $r = -0.65$, $p < 0.05$) or energy intake (expressed as a percentage of the WHO requirement, Pearson $r = -0.67$, $p < 0.05$) and the percentage change in pelvis BMC after spaceflight (Fig. 1). Specifically, higher intakes of energy or protein were associated with lower loss of pelvic bone mineral content. No other bone site was found to have a significant relationship with energy or protein intake.

Total body mass determined by DXA was unchanged after flight in both groups (Table 1). Body composition analysis revealed that ARED crewmembers returned from flight with significantly greater lean tissue mass (there was a significant interaction effect, but the post hoc test only shows a trend with $p = 0.055$) and less fat mass (expressed as g, or as % lean body mass) than iRED crewmembers (Table 1). ARED crewmembers tended to have higher energy intakes than iRED crewmembers (Table 2). There were no differences between groups with respect to the number of days between landing day and the day measurements were collected; therefore, differences between groups in the effects of spaceflight cannot be attributed to rehabilitation ($10 \pm 7$ versus $16 \pm 17$ days after landing for the iRED and ARED groups, respectively).

Dietary intake data are presented in Table 2. Data were available for 4 of the ARED subjects and all of the 8 iRED subjects. There was no significant difference between groups for any of the dietary intake variables measured. Protein intake (g/kg body weight) tended to be higher in the ARED group ($p = 0.09$).

Blood biochemistry data are shown in Table 3, and urine data in Table 4. In both groups, 30 days after landing, bone turnover and/or formation biomarkers were increased relative to preflight concentrations (Table 3). Activity of BSAP tended to be greater at the end of spaceflight ($p = 0.06$). Osteocalcin was affected by spaceflight; however, a post hoc analysis revealed no differences between individual time points. In the iRED group, iPTH was significantly lower during flight than before flight, but in the ARED group it was unchanged (Fig. 2). These data are expressed as percentage change because of the difference in assays described in the Subjects and Methods section.

Vitamin D status, estimated with 25-hydroxyvitamin D, was higher during flight than in the early preflight sessions (2 to 12 months before flight), but not higher than at L–10 (Fig. 3, Table 3). Measured values of the active form of vitamin D, 1,25-dihydroxyvitamin D, were lower during spaceflight in both groups (Table 3).

Serum total calcium was not affected by exercise group or spaceflight (Table 3). Serum ionized calcium was lower after flight than before flight. Urinary calcium excretion (Table 4) was increased during flight in both groups. Urine volumes were lower during flight than before or after flight, with no differences between groups. Urinary creatinine was significantly affected by spaceflight (Table 4). Post hoc analysis revealed two time points (FD30 and FD180) that were significantly different from preflight, but all in-flight averages were greater than the preflight average.

**Table 1.** Bone Densitometry Data Before and After Long-Duration Spaceflight

| | iRED pre | iRED post | ARED pre | ARED post | iRED, % change ± SD | ARED, % change ± SD |
|---|---|---|---|---|---|---|
| N | 8 | 8 | 5 | 5 | | |
| Bone densitometry | | | | | | |
| Total BMC, g* | $2774 \pm 469^a$ | $2658 \pm 435^b$ | $2612 \pm 531^a$ | $2595 \pm 527^b$ | $-4 \pm 3$ | $-1 \pm 1$ |
| Total BMD, g/cm$^2$ | $1.213 \pm 0.098$ | $1.175 \pm 0.076$ | $1.147 \pm 0.135$ | $1.148 \pm 0.137$ | $-3 \pm 4$ | $0 \pm 1$ |
| Pelvis BMC, g** | $301 \pm 52^a$ | $265 \pm 47^b$ | $288 \pm 65^a$ | $285 \pm 71^a$ | $-12 \pm 5$ | $-2 \pm 5$ |
| Pelvis BMD, g/cm$^{2**}$ | $1.204 \pm 0.103^a$ | $1.105 \pm 0.085^b$ | $1.124 \pm 0.127^a$ | $1.108 \pm 0.123^a$ | $-8 \pm 3$ | $-1 \pm 1$ |
| L hip neck BMD, g/cm$^{2*}$ | $0.865 \pm 0.112^a$ | $0.796 \pm 0.101^b$ | $0.822 \pm 0.139^a$ | $0.798 \pm 0.143^b$ | $-8 \pm 5$ | $-3 \pm 3$ |
| R hip neck BMD, g/cm$^{2**}$ | $0.848 \pm 0.094^a$ | $0.793 \pm 0.089^b$ | $0.793 \pm 0.109^a$ | $0.784 \pm 0.11^a$ | $-6 \pm 3$ | $-1 \pm 2$ |
| L hip trochanter BMD, g/cm$^{2***}$ | $0.840 \pm 0.141^a$ | $0.771 \pm 0.114^b$ | $0.742 \pm 0.099^a$ | $0.728 \pm 0.095^a$ | $-8 \pm 5$ | $-2 \pm 1$ |
| R hip trochanter BMD, g/cm$^{2***}$ | $0.792 \pm 0.087^a$ | $0.734 \pm 0.08^b$ | $0.740 \pm 0.109^a$ | $0.722 \pm 0.088^a$ | $-7 \pm 3$ | $-2 \pm 3$ |
| Total L hip BMD, g/cm$^{2**}$ | $1.077 \pm 0.128^a$ | $0.991 \pm 0.095^b$ | $0.981 \pm 0.143^a$ | $0.957 \pm 0.141^a$ | $-8 \pm 3$ | $-2 \pm 1$ |
| Total R hip BMD, g/cm$^{2***}$ | $1.037 \pm 0.080^a$ | $0.978 \pm 0.066^b$ | $0.991 \pm 0.122^a$ | $0.970 \pm 0.104^a$ | $-6 \pm 3$ | $-2 \pm 2$ |
| Total lumbar spine BMC, g* | $65 \pm 21^a$ | $61 \pm 19^b$ | $68 \pm 10^a$ | $64 \pm 8^b$ | $-5 \pm 5$ | $-5 \pm 10$ |
| Total lumbar spine BMD, g/cm$^{2*}$ | $1.040 \pm 0.125^a$ | $0.993 \pm 0.099^b$ | $0.997 \pm 0.051^a$ | $0.995 \pm 0.051^b$ | $-4 \pm 4$ | $0 \pm 2$ |
| Body mass and composition | | | | | | |
| Total fat, g*** | $15611 \pm 4271^a$ | $16076 \pm 6083^a$ | $15399 \pm 1584^a$ | $13409 \pm 1851^b$ | $1 \pm 10$ | $-13 \pm 8$ |
| Total fat, % fat** | $20 \pm 4^a$ | $21 \pm 5^a$ | $22 \pm 4^a$ | $19 \pm 3^b$ | $2 \pm 8$ | $-12 \pm 7$ |
| Total lean, % lean** | $76 \pm 4^a$ | $76 \pm 5^a$ | $75 \pm 4^a$ | $77 \pm 3^b$ | $-1 \pm 3$ | $4 \pm 2$ |
| Total lean, g*** | $59099 \pm 8465$ | $58014 \pm 8693$ | $54181 \pm 11948$ | $55484 \pm 11071$ | $-2 \pm 2$ | $3 \pm 4$ |
| Total mass, g | $77483 \pm 11454$ | $76749 \pm 12712$ | $72191 \pm 13000$ | $71488 \pm 12245$ | $-1 \pm 2$ | $-1 \pm 3$ |
| Body weight (scale), kg*,c | $79 \pm 12^a$ | $77 \pm 13^b$ | $74 \pm 13^a$ | $73 \pm 13^b$ | $-4 \pm 3$ | $-1 \pm 1$ |

Values are mean ± SD.

iRED = interim resistive exercise device; pre = before flight; post = 5 days after landing; ARED = advanced resistive exercise device; BMC = bone mineral content; BMD = bone mineral density; L = left; R = right.

[a,b]Different letters mark values that are significant from each other, according to the Bonferroni post hoc *t* test.

[c]Body weight was determined using calibrated scales before and after flight (these data were collected within 2–24 hours of landing).

*Significant effect of spaceflight, $p < 0.05$.

**Significant interactive effect of spaceflight and exercise type, $p < 0.01$.

***Significant interactive effect of spaceflight and exercise type, $p < 0.05$.

Bone resorption was estimated by determining urinary excretion of several collagen degradation markers (Table 4). When expressed as crosslink excretion per day, all of these markers (NTX, CTX, PYD, DPD, and HP) were significantly affected by spaceflight. When HP was normalized to creatinine, the mean for ARED subjects was lower than the mean for iRED subjects during flight. When NTX was normalized to creatinine, ARED subjects had a higher mean NTX 30 days after landing than iRED subjects. These data are obviously confounded by the urinary creatinine results.

Neither flight duration nor crewmember age was significantly different between iRED and ARED groups. Vitamin D compliance was also not significantly different between groups, although it tended to be higher in ARED subjects (compliance was $65\% \pm 25\%$ for iRED and $91\% \pm 17\%$ for ARED).

## Discussion

The data presented here document that astronauts who have access to sufficient resistance exercise, coupled with adequate energy intake and vitamin D status, can return from spaceflight missions of 4 to 6 months with measured bone mass and BMDs seemingly no different from baseline measures—for most skeletal regions. Although further work is needed to refine these factors (by developing optimal exercise prescriptions and optimal nutrition), the results provide the first evidence that nutrition and exercise may be able to mitigate bone loss and reduce risk for spaceflight-induced osteoporosis. Comparison of the data from this study with data from crewmembers on earlier ISS missions and from the Russian space station Mir missions (Fig. 4) shows that when data are normalized to percentage change per month, the benefits of ARED over those of earlier exercise regimens are clear. Additionally, the nutrition findings document that some of the assumptions about human spaceflight are not always true. Specifically, energy intake, body mass, and lean body mass can be maintained during flight, and the same is true for vitamin D status.

The biochemical evidence reported here fits nicely with evidence from earlier flight studies[3] and bed rest studies evaluating resistive exercise.[7,8] Resistive exercise was associated with increased concentrations of bone formation markers (BSAP), and use of the ARED was associated with preventing PTH decline during spaceflight. Data from Mir missions in the 1990s consistently showed no effect of spaceflight on bone formation markers, or if anything, a slight decrease.[3,20,31] Urinary excretion of some but not all bone resorption biomarkers suggests that resistive exercise with ARED lowers bone resorption relative to
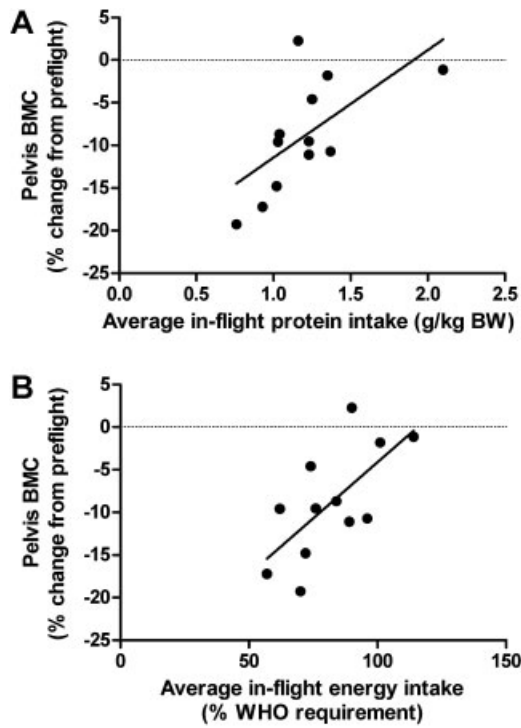
**Fig. 1.** Correlation of percentage change in pelvis bone mineral content (BMC) with (A) protein intake (expressed in g protein/kg body weight, Pearson $r = -0.65$, $p < 0.05$) and (B) dietary intake of energy (expressed as percentage of WHO estimated requirement, Pearson $r = -0.67$, $p < 0.05$).

exercise with iRED, although increased resorptive activity (relative to before flight) was still observed during flight. The maintenance of baseline serum PTH levels, however, suggests that ARED exercise can still sustain bone mineral in a eucalcemic state. Similar effects of resistance exercise on PTH during bed rest have been documented.[7,8] It is important to note that there were no differences between the ARED and iRED groups for average squat load or adherence to the prescribed exercises. The ARED participants in the current study represent the first several individuals to use ARED during a time when the device was new and not well understood, thus prescriptions were very conservative. There are important differences between ARED and iRED with respect to the quality of loading. ARED provides a more constant load and a ~90% eccentric:concentric ratio, and simulates the inertia of free weights. ARED also allows the user to vary the stance width of squat and deadlift exercises. From a biomechanics standpoint, varying the stance width will change

the angle of loading on the femoral neck and other lower body bone structures. Because of the site-specificity of bone loading and response, it seems highly likely that greater variation in loading sites would be associated with better outcomes. Clearly more research is needed to understand the best loading pattern and intensity to protect bone.

Although the importance of both exercise and nutrition for successful bone and muscle outcomes is obvious to most researchers, controlling these factors has been challenging in spaceflight and related ground analog studies. On too many missions, and in too many bed rest studies, subjects have not consumed adequate amounts of calories and/or protein and have lost weight, and this has compromised results. The recent review by Stein and Blanc[32] of amino acid supplementation studies captures this eloquently. Additional evidence to expand the findings reported here will be important, but the recognition that nutrition and existing exercise regimens can mitigate bone loss is a major advance. Although the pelvis is not a typical site for clinical monitoring by densitometry, it is routinely evaluated for spaceflight effects because it has consistently displayed declines with spaceflight and the measures of BMD and BMC for this large skeletal region show good precision. Thus, the observation that pelvis BMC changed less after spaceflight in crewmembers who consumed more calories (closer to their WHO requirement) or more protein per kilogram of body weight supports the argument that adequate energy intake is required to maintain bone health. It is important to note that ground-based studies restricting calories have shown similar findings.[33]

Food and energy intake are critical for general health, especially in high-stress environments like spaceflight. On Shuttle missions, which were relatively short (1–2 weeks), intake was often inadequate because crews did not want to use more of their limited time in space for meals. On longer missions, the timelines are a bit more relaxed, and mealtimes often become times for camaraderie and a once- or twice-daily meeting of the crew to discuss the status of vehicle and scientific operations. Although it has been shown that energy requirements are unchanged during flight,[34,35] anecdotal reports from many crewmembers indicate it is plausible that the sensation of fullness is different in microgravity. Crewmembers need to be cognizant of this, and work to consume additional food/energy throughout the day. Nonetheless, the assumption that a spaceflight "anorexia" is unavoidable is unfounded. Energy intake of the 13 crewmembers participating in this study ranged from 57% to 114% of their WHO requirements (the median was 80%). Whereas energy, protein/muscle, and exercise in space-

**Table 2.** Dietary Intake During Long-Duration Spaceflight

| | Water (mL/d) | Energy (kcal/d) | Energy (%WHO) | Protein (g/d) | Protein (g/kg BW) | Calcium (mg/d) | Sodium (mg/d) | Iron (mg/d) | Potassium (mg/d) |
|---|---|---|---|---|---|---|---|---|---|
| iRED | 1921 ± 306 | 2234 ± 363 | 78 ± 16 | 85 ± 13 | 1.12 ± 0.21 | 912 ± 229 | 4159 ± 656 | 24 ± 8 | 2980 ± 435 |
| ARED | 2159 ± 529 | 2430 ± 893 | 91 ± 17 | 102 ± 57 | 1.39 ± 0.48 | 1025 ± 309 | 5327 ± 2617 | 19 ± 6 | 3465 ± 1435 |

Data are mean ± SD. iRED, $n = 8$. ARED, $n = 4$.
BW = body weight; iRED = interim resistive exercise device; ARED = advanced resistive exercise device.

**Table 3.** Blood Markers of Bone and Calcium Metabolism Before, During, and After Long-Duration Spaceflight

| | Preflight (L–2 to L–12 months)[a] | L–10 days | FD15 | FD30 | FD60 | FD120 | FD180 | R + 0 | R + 30 |
|---|---|---|---|---|---|---|---|---|---|
| *N* | | | | | | | | | |
| iRED | 8 | 4 | 8 | 8 | 7 | 6 | 5 | 8 | 8 |
| ARED | 5 | 5 | 5 | 5 | 5 | 3 | 2 | 5 | 5 |
| BSAP, U/L* | | | | | | | | | |
| iRED | 24 ± 10 | 29 ± 11 | 25 ± 10 | 26 ± 11 | 30 ± 12 | 30 ± 11 | 30 ± 17 | 32 ± 18 | 42 ± 27** |
| ARED | 21 ± 9 | 18 ± 4 | 19 ± 7 | 15 ± 2 | 21 ± 5 | 27 ± 5 | 29 ± 1 | 27 ± 12 | 25 ± 9** |
| Alkaline phosphatase, U/L | | | | | | | | | |
| iRED | 54 ± 16 | 57 ± 19 | 54 ± 21 | 53 ± 19 | 63 ± 21 | 60 ± 23 | 64 ± 33** | 59 ± 23 | 71 ± 23** |
| ARED | 39 ± 7 | 39 ± 12 | 36 ± 11 | 35 ± 9 | 37 ± 9 | 42 ± 6 | 56 ± 5** | 51 ± 16 | 56 ± 17** |
| Osteocalcin, ng/mL* | | | | | | | | | |
| iRED | 10 ± 3 | 9 ± 2 | 10 ± 2 | 11 ± 2 | 10 ± 1 | 13 ± 2 | 14 ± 3 | 11 ± 3 | 14 ± 5 |
| ARED | 11 ± 2 | 9 ± 1 | 8 ± 1 | 9 ± 1 | 10 ± 1 | 10 ± 2 | 11 ± 2 | 9 ± 1 | 11 ± 2 |
| 1,25-Dihydroxyvitamin D, pmol/L* | | | | | | | | | |
| iRED | 132 ± 48 | 116 ± 51 | 103 ± 36 | 86 ± 25** | 97 ± 34** | 90 ± 29** | 94 ± 56 | 178 ± 84 | 153 ± 48 |
| ARED | 140 ± 17 | 142 ± 60 | 119 ± 32 | 81 ± 19** | 103 ± 38** | 109 ± 38** | 103 ± 2 | 154 ± 49 | 130 ± 35 |
| 25-Hydroxyvitamin D, nmol/L*** | | | | | | | | | |
| iRED | 75 ± 18 | 84 ± 22 | 86 ± 20 | 84 ± 15 | 88 ± 15 | 78 ± 9 | 87 ± 33 | 74 ± 19 | 68 ± 16 |
| ARED | 75 ± 16 | 76 ± 14 | 73 ± 12 | 71 ± 11 | 73 ± 15 | 68 ± 19 | 55 ± 18 | 65 ± 13 | 65 ± 16 |
| Calcium, mmol/L | | | | | | | | | |
| iRED | 2.3 ± 0.1 | 2.3 ± 0.1 | 2.3 ± 0.1 | 2.3 ± 0.2 | 2.3 ± 0.1 | 2.3 ± 0.1 | 2.3 ± 0.1 | 2.3 ± 0.2 | 2.3 ± 0.1 |
| ARED | 2.3 ± 0 | 2.3 ± 0.1 | 2.2 ± 0.1 | 2.3 ± 0.1 | 2.3 ± 0.1 | 2.2 ± 0.1 | 2.3 ± 0.1 | 2.2 ± 0.1 | 2.3 ± 0.1 |
| Ionized calcium, mmol/L[†] | | | | | | | | | |
| iRED | 1.24 ± 0.04 | n/a[b] | n/a[b] | n/a[b] | n/a[b] | n/a[b] | n/a[b] | 1.17 ± 0.06** | 1.21 ± 0.04 |
| ARED | 1.22 ± 0.03 | n/a[b] | n/a[b] | n/a[b] | n/a[b] | n/a[b] | n/a[b] | 1.19 ± 0.04** | 1.18 ± 0.04 |

Data are mean ± SD.

L = launch; FD = flight day; R = recovery; iRED = interim resistive exercise device; ARED = advanced resistive exercise device; BSAP = bone-specific alkaline phosphatase; n/a = not available.

[a]"Preflight" represents the mean of two data collection points at about 2–4 months and 6–12 months before flight.

[b]Ionized calcium data were collected only before and after flight.

*Significant effect of spaceflight, $p < 0.001$.

**Significantly different from preflight as determined by a post hoc Bonferroni t test. For some tests, there was a significant main effect of spaceflight, but the post hoc test did not reveal any differences at individual time points.

***Significant effect of spaceflight, $p < 0.01$.

[†]Significant effect of spaceflight, $p < 0.05$.

flight (and bed rest) have been closely linked in attempts to understand muscle loss in microgravity,[36–38] bone has not received the same degree of attention until now, despite ground-based evidence that it has intertwining relationships with muscle, exercise, and nutrition.[33,39]

Vitamin D supplementation was initiated for all ISS crews when it was found that on Mir flights vitamin D status declined as a result of inadequate intake and lack of UV light exposure.[3,20,28] The 400-IU supplements did not prevent decrements in vitamin D status of astronauts on later ISS missions.[13] In the mid-2000s, amid a flurry of vitamin D research and reports in the media of the importance of vitamin D in curing everything from cancer to tuberculosis, awareness of this vitamin increased in the space community as well. In late 2006, the recommended supplement dose for crewmembers on the ISS was doubled, from 400 IU/d to 800 IU/d. The data reported here document that this level is adequate to maintain vitamin D status in an environment with zero UV light exposure and few food sources of vitamin D. Although these data do not constitute a direct

evaluation, they provide useful information in support of the 2011 Dietary Reference Intakes of vitamin D.[40]

The data reported here also provide valuable analytical insight into the cause of a technical problem on the ISS. In the summer of 2009, the Urine Processing Assembly (UPA) was brought online on the ISS to recycle urine into potable water, a critical resource. A few months later, the UPA broke down because of what was ultimately determined to be precipitation of calcium sulfate within the system. The sulfur came from the sulfuric acid flush solution used in the system, and the calcium was from urine (Table 3). The UPA uses distillation and evaporation to reclaim water, and the limits of the system were based on typical (Earth-based) urinary calcium concentrations. Another critical factor, also reported here (Table 4), is that urine volumes are lower during flight, likely due to a combination of reduced fluid intake and low cabin humidity on the ISS. The increased calcium excretion and decreased urine volume both led to a significantly higher concentration of calcium in the urine during the mission, leading to breakdown of the UPA. The UPA waste tanks are

**Table 4.** Urinary Markers of Bone Turnover and Calcium Status Before, During, and After Long-Duration Spaceflight

| | Preflight | FD15 | FD30 | FD60 | FD120 | FD180 | R + 0 | R + 30 |
|---|---|---|---|---|---|---|---|---|
| *N* | | | | | | | | |
| iRED | 8 | 8 | 8 | 7 | 5 | 4 | 8 | 8 |
| ARED | 5 | 5 | 5 | 5 | 3 | 2 | 5 | 5 |
| 24-hour volume, mL* | | | | | | | | |
| iRED | 1730 ± 305 | 1307 ± 539 | 1342 ± 516 | 1434 ± 501 | 1204 ± 377 | 1589 ± 469 | 1665 ± 648 | 1744 ± 504 |
| ARED | 1671 ± 642 | 1037 ± 371 | 1109 ± 253 | 1533 ± 866 | 1270 ± 403 | 1408 ± 167 | 1784 ± 1164 | 1564 ± 811 |
| Creatinine, mg/d** | | | | | | | | |
| iRED | 1741 ± 399 | 1895 ± 490 | 1889 ± 437*** | 1783 ± 325 | 1668 ± 475 | 1778 ± 440*** | 1703 ± 305 | 1617 ± 224 |
| ARED | 1539 ± 435 | 1938 ± 476 | 2039 ± 472*** | 1934 ± 731 | 1801 ± 62 | 2417 ± 30*** | 1695 ± 513 | 1681 ± 324 |
| Calcium, mmol/d† | | | | | | | | |
| iRED | 4.4 ± 2.3 | 6.4 ± 3.8*** | 6.7 ± 2.7*** | 6.0 ± 2.5*** | 4.9 ± 2.6 | 4.9 ± 2.1 | 4.3 ± 1.5 | 3.2 ± 1.6 |
| ARED | 4.4 ± 1.7 | 6.6 ± 2.6*** | 6.6 ± 2*** | 6.5 ± 2.9*** | 5.2 ± 1.5 | 8.0 ± 0.4 | 5.7 ± 2.1 | 4.3 ± 1.4 |
| HP, μg/d† | | | | | | | | |
| iRED | 611 ± 301 | 1221 ± 578*** | 1218 ± 470*** | 1186 ± 415*** | 1281 ± 511*** | 1166 ± 333*** | 1131 ± 465*** | 597 ± 198 |
| ARED | 433 ± 228 | 946 ± 381*** | 912 ± 325*** | 766 ± 221*** | 888 ± 341*** | 1185 ± 120*** | 1008 ± 410*** | 622 ± 196 |
| HP, μg/mmol Cr‡ | | | | | | | | |
| iRED | 39 ± 15 | 72 ± 24*** | 74 ± 24***a | 75 ± 21***a | 86 ± 19*** | 75 ± 18*** | 77 ± 28*** | 42 ± 14 |
| ARED | 32 ± 15 | 54 ± 12 | 50 ± 12b | 47 ± 13b | 56 ± 22*** | 55 ± 6 | 70 ± 26*** | 45 ± 22 |
| DPD, nmol/d† | | | | | | | | |
| iRED | 48 ± 9 | 87 ± 20*** | 98 ± 18*** | 93 ± 12*** | 88 ± 20*** | 100 ± 24*** | 90 ± 19*** | 87 ± 20*** |
| ARED | 43 ± 10 | 80 ± 26*** | 89 ± 28*** | 85 ± 32*** | 83 ± 27*** | 109 ± 3*** | 74 ± 19*** | 84 ± 20*** |
| DPD, nmol/mmol Cr† | | | | | | | | |
| iRED | 3 ± 1 | 5 ± 1*** | 6 ± 1*** | 6 ± 1*** | 6 ± 1*** | 6 ± 1*** | 6 ± 1*** | 6 ± 1*** |
| ARED | 3 ± 2 | 5 ± 1*** | 5 ± 1*** | 5 ± 1*** | 5 ± 2*** | 5 ± 0*** | 5 ± 2*** | 6 ± 3*** |
| NTX, nmol/d‡ | | | | | | | | |
| iRED | 375 ± 127 | 683 ± 307*** | 720 ± 222*** | 688 ± 216*** | 745 ± 289*** | 607 ± 154*** | 571 ± 205*** | 364 ± 87 |
| ARED | 361 ± 143 | 758 ± 398*** | 719 ± 255*** | 614 ± 260*** | 770 ± 405*** | 1170 ± 61*** | 692 ± 290*** | 603 ± 269*** |
| NTX, nmol/mmol Cr§ | | | | | | | | |
| iRED | 25 ± 7 | 40 ± 9*** | 44 ± 12*** | 43 ± 9*** | 49 ± 7*** | 39 ± 3*** | 38 ± 11*** | 26 ± 6a |
| ARED | 26 ± 7 | 42 ± 12*** | 39 ± 8*** | 36 ± 12 | 48 ± 25*** | 55 ± 4*** | 48 ± 19*** | 42 ± 21***b |
| PYD, nmol/d† | | | | | | | | |
| iRED | 222 ± 40 | 426 ± 114 | 472 ± 136*** | 493 ± 54*** | 487 ± 150*** | 504 ± 207*** | 448 ± 147*** | 464 ± 155*** |
| ARED | 197 ± 33 | 354 ± 74 | 366 ± 72*** | 373 ± 94*** | 382 ± 55*** | 526 ± 13*** | 362 ± 74*** | 403 ± 55*** |
| PYD, nmol/mmol Cr† | | | | | | | | |
| iRED | 15 ± 2 | 25 ± 4*** | 28 ± 5*** | 32 ± 5*** | 33 ± 6*** | 31 ± 7*** | 29 ± 5*** | 32 ± 8*** |
| ARED | 15 ± 5 | 21 ± 2*** | 21 ± 4*** | 23 ± 3*** | 24 ± 3*** | 25 ± 1*** | 25 ± 5*** | 29 ± 10*** |
| CTX, μg/d‡ | | | | | | | | |
| iRED | 2058 ± 691 | 4216 ± 1982*** | 4480 ± 1507*** | 4605 ± 1416*** | 4569 ± 1828*** | 3669 ± 689*** | 3756 ± 1258*** | 2187 ± 391 |
| ARED | 1810 ± 787 | 4795 ± 3445*** | 3517 ± 1694*** | 3363 ± 1807*** | 4483 ± 2413*** | 7703 ± 490*** | 4080 ± 1885*** | 2991 ± 1796*** |
| CTX, μg/mmol Cr‡ | | | | | | | | |
| iRED | 135 ± 36 | 248 ± 71*** | 272 ± 79*** | 289 ± 62*** | 302 ± 48*** | 238 ± 35*** | 252 ± 75*** | 154 ± 31 |
| ARED | 133 ± 44 | 257 ± 132*** | 193 ± 82 | 195 ± 107 | 281 ± 150*** | 360 ± 27*** | 267 ± 118*** | 206 ± 125 |

Data are mean ± SD.

FD = flight day; R = recovery; iRED = interim resistive exercise device; ARED = advanced resistive exercise device; HP = helical peptide; Cr = creatinine; DPD = deoxypyridinoline; NTX = N-telopeptide; PYD = pyridinium crosslinks; CTX = C-telopeptide.

[a,b]Different letters in different rows represent differences between iRED and ARED exercise as determined by Bonferroni *t* test.

*Significant effect of spaceflight, $p < 0.05$.

**Significant effect of spaceflight, $p < 0.01$.

***Significantly different from preflight as determined by Bonferroni *t* test.

†Significant effect of spaceflight, $p < 0.001$.

‡Significant interaction between spaceflight and exercise type, $p < 0.05$.

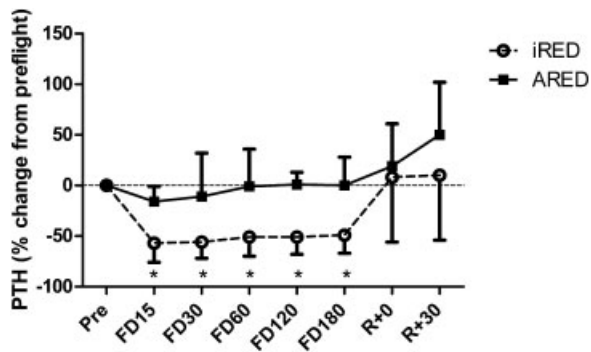§Significant interaction between spaceflight and exercise type, $p < 0.01$.

**Fig. 2.** Percentage change in serum intact parathyroid hormone (PTH) concentrations before, during, and after flight. Dashed line is iRED group, solid line is ARED group. These data are expressed as percentage change because of the difference in assays described in the Subjects and Methods section. $n = 6$ iRED and $n = 5$ ARED crewmembers. Data are mean ± SD. A repeated-measures ANOVA and post hoc Bonferroni $t$ test revealed a significant ($p < 0.05$) interaction of group × time. A significant decrease (*) in PTH occurred during flight in the iRED crewmembers only. Raw data were used for the statistical analyses.

now replaced more frequently, on a schedule based on spaceflight calcium excretion data (reported herein), to avoid repeating this problem. Crewmembers are also advised that the chemistry which led to calcium precipitation in the UPA is quite similar to the chemistry of nephrolithiasis, and that increased fluid intake could help prevent a mission-shortening kidney stone.

The bone resorption markers in general were lower after flight than at the last in-flight session, but all had not returned to preflight levels by the R + 30 session. Given that bone metabolism is believed to be the slowest to fully recover from spaceflight effects on the body, this is not unexpected. The fact that resorption remained elevated after flight may provide further evidence that bone strength must also be determined in astronauts, as remodeling seemed to continue despite maintenance of bone mass in the ARED subjects in particular. From an analytical point of view, these data show the limitations of spot urine collections and/or the use of creatinine to normalize crosslink (or other) data from 24-hour collections, especially in exercise studies. Although we, and others, have shown this
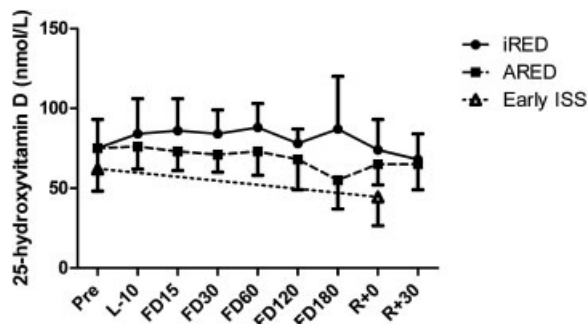
before, the difficulty in obtaining 24-hour urine collections is often an impediment to reliable data collection.

One important limitation of this study is the inability to separate the influence of resistance exercise and adequate nutrition (protein and energy intakes in particular). This interaction between exercise and nutrition will be important to understand for exploration missions of very long duration, on which there will be pressure to minimize the mass and volume of both exercise equipment and food. Future work should include optimizing countermeasure effectiveness by exploring thresholds of exercise and nutrition required for maintenance of bone health.

In summary, we document here evidence that exercise and nutrition can partially maintain bone mass during spaceflight as assessed by the DXA modality. Although DXA densitometry may be insufficient for capturing the complex changes in bone mass, structure, and integrity observed in astronauts,[41] the data from this clinically accepted modality are encouraging. Further studies should expand the subject population and evaluate individual differences (such as gender and dietary factors). More importantly, the present data show that the mitigation of bone loss on the typical long-duration spaceflight missions of ~180 days could be controlled without the use of pharmaceutical agents routinely used to treat the osteoporosis patient population.

## Disclosures

All authors state that they have no conflicts of interest.

## Acknowledgments

**Fig. 3.** Serum 25-hydroxyvitamin D concentrations before, during, and after flight. Dashed line is ARED group, solid line is iRED group. The triangles at Pre and R + 0, connected by a dotted line, represent previously published data from early ISS crewmembers.[13] Data are mean ± SD.
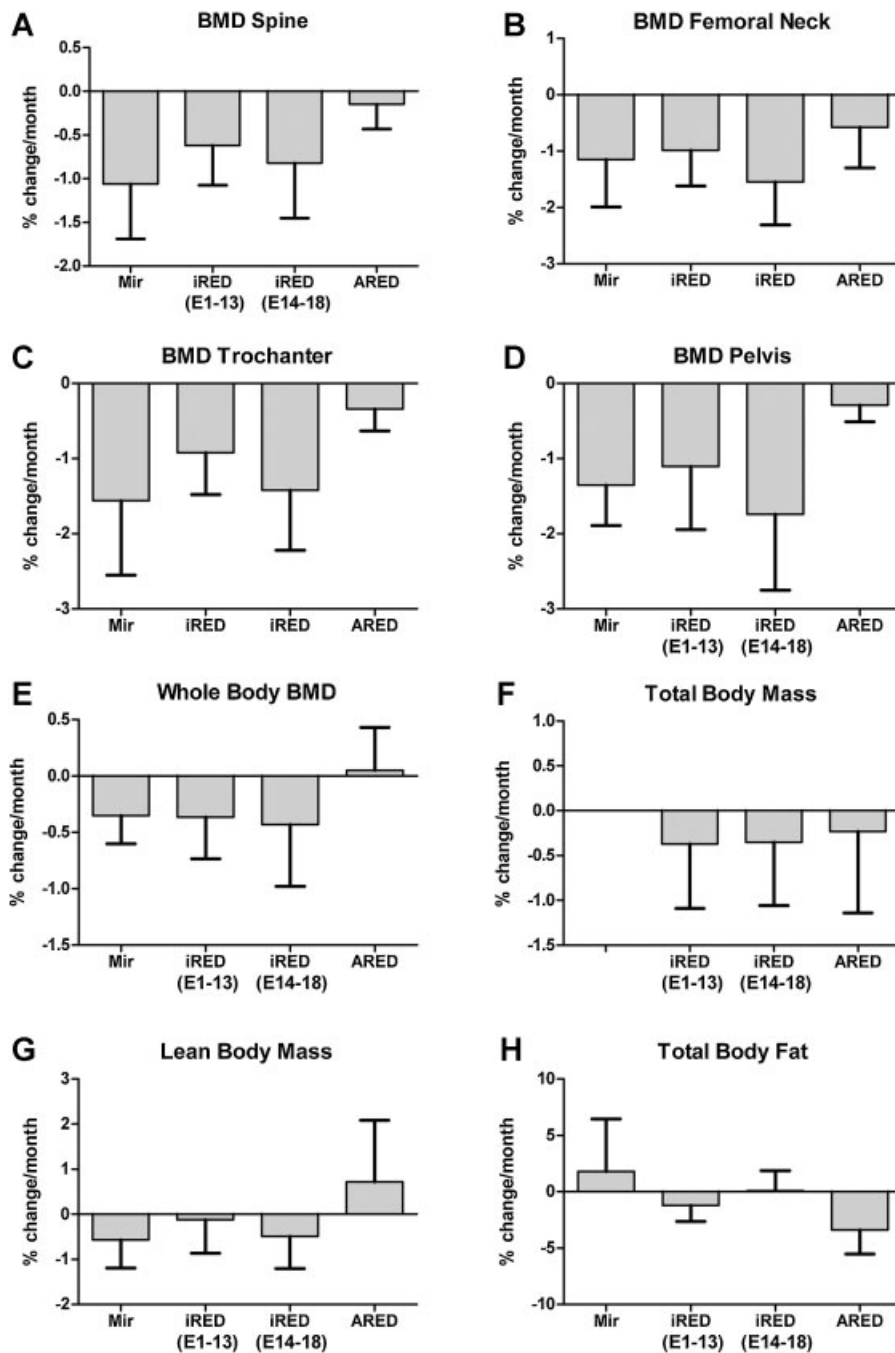
**Fig. 4.** Bone mineral density (BMD) and body composition (expressed as % change per month $\pm$ SD) in crewmembers on the Russian space station Mir, on earlier ISS flights (iRED, Expeditions 1–13), and reported in this work (iRED, Expeditions 14–18, and ARED). Femoral neck, trochanter, and pelvis changes are for the left hip for the iRED and ARED subjects. The Mir data have been published in other reports.[4,15] (A) Spine BMD. (B) Femoral neck BMD. (C) Trochanter BMD. (D) Pelvis BMD. (E) Whole-body BMD. (F) Total body mass. Mir total body mass data were not available. (G) Lean body mass. (H) Total body fat.

resulting data. JDS and LCS managed the densitometry analyses. LPS managed the exercise data analysis. All authors contributed to the interpretation of the data and writing of the manuscript.

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