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Full Length Article

Resistive exercise in astronauts on prolonged spaceflights provides partial protection against spaceflight-induced bone loss

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ABSTRACT

Bone loss in astronauts during spaceflight may be a risk factor for osteoporosis, fractures and renal stone formation. We previously reported that the bisphosphonate alendronate, combined with exercise that included an Advanced Resistive Exercise Device (ARED), can prevent or attenuate group mean declines in areal bone mineral density (aBMD) measured soon after ~ 6-month spaceflights aboard the International Space Station (ISS). It is unclear however if the beneficial effects on postflight aBMD were due to individual or combined effects of alendronate and ARED. Hence, 10 additional ISS astronauts were recruited who used the ARED (ARED group) without drug administration using similar measurements in the previous study, i.e., densitometry, biochemical assays and analysis of finite element (FE) models. In addition densitometry data (DXA and QCT only) were compared to published data from crewmembers (n = 14–18) flown prior to in-flight access to the ARED (Pre-ARED). Group mean changes from preflight (± SD %) were used to evaluate effects of countermeasures as sequentially modified on the ISS (i.e., Pre-ARED vs. ARED; ARED vs. Bis + ARED). Spaceflight durations were not significantly different between groups. Postflight bone density measurements were significantly reduced from preflight in the Pre-ARED group. As previously reported, combined Bis + ARED prevented declines in all DXA and QCT hip densitometry and in estimates of FE hip strengths; increased the aBMD of lumbar spine; and prevented elevations in urinary markers for bone resorption during spaceflight. ARED without alendronate partially attenuated declines in bone mass but did not suppress biomarkers for bone resorption or prevent trabecular bone loss. Resistive exercise in the ARED group did not prevent declines in hip trabecular vBMD, but prevented reductions in cortical vBMD of the femoral neck, in FE estimate of hip strength for non-linear stance (NLS) and in aBMD of the femoral neck. We conclude that a bisphosphonate, when combined with resistive exercise, enhances the preservation of bone mass because of the added suppression of bone resorption in trabecular bone compartment not evident with ARED alone.

Abbreviations: Bis, bisphosphonate; ISS, International Space Station; ARED, Advanced Resistive Exercise Device; IRED, Interim Resistive Exercise Device; aBMD, areal Bone Mineral Density; vBMD, volumetric Bone Mineral Density; DXA, dual energy X-ray absorptiometry; QCT, quantitative computed tomography; FEM, finite element modeling; NLS, Non-linear Stance; NLF, Non-linear Fall; WHO, World Health Organization

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1. Introduction

Astronauts have served on the International Space Station (ISS) for durations of spaceflight mostly between 4 and 6 months where they have returned from long-duration space missions with lower areal bone mineral densities (aBMDs) compared to preflight, i.e., beyond the Least Significant Change (LSC) for DXA measurements at Johnson Space Center (JSC) [1]. Space, a weightless environment, appears to induce a musculoskeletal adaptation in astronauts that resembles the effects of immobilization or paralysis. The losses in bone mass during spaceflight are not stochastic, but appear to target skeletal sites that are weight-bearing on Earth, suggesting a primary role of biomechanics for skeletal adaptation in space. Overall, it is unclear as to what these spaceflight-induced changes mean to the affected astronaut in terms of fracture risk or impacts to long-term skeletal health.

In another report that focused on resistive exercise and nutrition as countermeasures to mitigate losses in aBMD during spaceflight, resistive exercise on the ARED (Advanced Resistive Exercise Device) was capable of maintaining, after typical 6-month spaceflights, the aBMD (group mean value ± SD) of astronauts at their preflight hip and the lumbar spine [2]; these observations with DXA densitometry suggested possible skeletal benefits with ARED and nutrition alone. The positive aBMD outcome using the ARED has been attributed to the ability to perform resistive exercise that more closely resembles weight-lifting in Earth's 1 g environment. This advanced capability included an increased loading capability (600 pound-force (lbf), or 2669 Newton (N)), and a higher ratio of eccentric to concentric loading than was possible with the previous exercise device (i.e., the interim resistive exercise device, or IRED) [3]. The IRED, in contrast, provided a maximum resistance of only 300 lbf or 1334 N which appears to be insufficient mechanical loading in astronauts to preserve skeletal mass during spaceflight or to facilitate recovery after spaceflight [4,5].

Despite findings with DXA measures that show that the development of osteoporosis (T score < -2.5) did not occur in astronauts after ~6-month spaceflights [6], there are spaceflight-induced changes to bone that have raised concern that the degree of bone loss may be deleterious and that its prevention would be desirable [7]. Collective evidence substantiate i) perturbed bone remodeling during spaceflight with bone resorption increased and uncoupled from bone formation [6,8,9], ii) bone loss still evident in some astronauts who are using ARED (this paper and confidential medical communication) and iii) an associated bone loss that is accelerated relative to bone loss due to aging [1,5,7]. Specifically, the calculated average of hip trabecular vBMD loss over 6 months from reported population data is ~0.4% which is ~20-fold less than the average hip trabecular vBMD loss over 6 months reported for crewmembers using exercise alone (ARED only, no bisphosphonates) in this report. Moreover, it has not been possible to predict which astronauts might lose bone from the data acquired to-date from ~70 long-duration astronauts.

Previously, we have described that 70 mg/week of alendronate, an oral bisphosphonate (Bis), appeared to attenuate BMD loss in astronauts, to prevent an elevation of biomarkers for bone resorption, and to reduce the typically increased urinary excretion of calcium during spaceflight [10]. However, the effects of alendronate were obscured because ISS astronauts who were taking alendronate concurrently performed resistive exercise on the ARED. From the perspective of mission operations and for the planning of missions beyond low-Earth orbit, it is important to delineate whether the postflight benefits observed in the Bis + ARED group can be achieved with ARED alone since each countermeasure is associated with its own in-flight risks and/or requirements for implementation.

To this end, we conducted two flight investigations. One study reported the anti-resorptive effect of a bisphosphonate in space in combination with ARED [10]. The other flight study, described here in, investigated the efficacy of ARED in the absence of a bisphosphonate as a strategy to prevent bone loss during spaceflight. We hypothesized that ARED alone would not completely protect astronauts against bone loss because of an inability to mitigate bone resorption and this inability would be detected by QCT measurement of trabecular bone, a bone sub-region that is sensitive to osteoclast activity.

2. Methods

2.1. Subjects

Following approval by the Institutional Review Boards of NASA and Japan Aerospace Exploration Agency (JAXA), a total of 20 astronauts signed informed consent to participate in the Bisphosphonate flight study. Ten astronauts had originally signed up to take alendronate (Bis + ARED, n = 10) and the study results have been previously reported [10]. Ten additional ISS astronauts signed up for exercise on the ARED only (ARED, n = 10) with no ingestion of alendronate. Densitometry and biochemical data, as similarly acquired in the Bis + ARED subjects, were collected in this ARED group. None of the astronauts in this ARED only group had participated as subjects in a previously reported study on ARED effects [2]. Densitometry included QCT hip scans which had not been used to describe ARED effects in the previous report [2].

In early expeditions of the ISS program there were crewmembers (n = 9 US astronauts and n = 9 Russian cosmonauts) who had provided informed consent to participate in an ISS flight study (Pre-ARED, n = 18) which conducted both hip and lumbar spine QCT scans. Not all of the study data were published [5] but are currently available to augment the comparative data analysis reported in this paper. The years for collecting data specific to our comparative analysis are outlined along with the final subject number per group (Table 1).

In the earlier ISS spaceflights (2001–2008), resistive exercise performed by US crewmembers was conducted on the IRED only – a resistive exercise device that was found to be ineffective for protecting against loss in bone mass [5]. Because the extent of IRED use by cosmonauts could not be confirmed, the data are presented as a group we designate as Pre-ARED.

2.2. Bisphosphonate dosing

The protocol for bisphosphonate dosing in the Bis + ARED subjects was previously reported along with study results [10]. In brief, an oral weekly dose (70 mg) of the alendronate (Fosamax®) was administered to each Bis + ARED subject 3 weeks prior to the scheduled launch date and continued throughout the flight. Before and during spaceflight, crewmembers in the flight study (Bis + ARED and ARED) took a daily vitamin D supplement (cholecalciferol, 400–800 IU). Calcium supplementation (1000 mg/d) coincided with the preflight alendronate dosing; during spaceflight, adequate calcium intake (~1000 mg/d) was provided by diet.

2.3. Exercise

All subjects in both the Bis + ARED and ARED groups followed an individualized exercise prescription assigned by an Astronaut Strength,

Table 1
Timeline for acquisition of flight data.

Description subject group (Final subject number)	Time ranges for bisphosphonate flight study	
	Years of ISS spaceflights	Years of data acquisition
ARED (n = 10)	2012–2016	2012–2017
Bis + ARED (n = 7)	2008–2012	2008–2013
Pre-ARED (n = 18)	2001–2004	2000–2006

Conditioning and Rehabilitation (ASCR) specialist. As previously reported for Bis + ARED subjects [10], this exercise regimen was comprised of ARED resistance training and aerobic exercise (using a cycle ergometer or treadmill). In particular, the ARED device allows 17 different exercise configurations, including 4 types of squats, 3 types of deadlifts, 2 types of heel raises, 3 types of rows, as well as shrugs, shoulder press, bench press, bicep curl, and triceps extension (Personal communication, Resistive Exercise Description Document Reference: JSC 29558).

To provide a historical context, this report includes data from crewmembers who performed resistive exercise on the ISS prior to the availability of the ARED. As mentioned earlier, half of the Pre-ARED group of subjects consisted of US astronauts (n = 9) who performed resistive exercise on the IRED, an earlier exercise hardware which offered a lower maximum load than the ARED (i.e., 300 vs. 600 lbf) and only 8 of the ARED's 17 exercise configurations previously mentioned. The other half of Pre-ARED subjects were cosmonauts (n = 9), Russian crewmembers who had access to IRED but for whom we have no further information on use. Skeletal measurements between the US astronauts and the Russian cosmonauts were not significantly different between the two groups (data not shown) and the data were subsequently pooled into the single Pre-ARED group. For the Bis + ARED and the ARED groups, we compared the total volume of resistive exercise during flight for the lower body (i.e., squats and deadlifts) as a possible confounder. The total resistive exercise volume was calculated by multiplying the number of performed repetitions by the resistive load (lbf), dividing by total body mass and normalizing to total days of spaceflight. In contrast to the ARED, the IRED was not capable of providing resistive load information and therefore the volume of lower body resistive exercise could not be calculated for the entire Pre-ARED group for a comparative analysis.

2.4. Bone imaging

2.4.1. DXA

Serial measurements of areal bone mineral density (aBMD) were conducted with Dual-Energy X-ray Absorptiometry (DXA) scans i) preflight (6–1 month prior to launch), ii) immediate postflight (5–30 days after return), and iii) later postflight (approximately 1 year after return). All DXA scans were performed on a single Hologic QDR Discovery whole body densitometer located in the Bone & Mineral Laboratory at the NASA Johnson Space Center (JSC). Precision measurements for the JSC Bone & Mineral Lab (Least Significant Change, LSC, 95% confidence limit) for each skeletal region of interest during the period of the study were: total hip, 2.1% (0.021 g/cm^2); trochanter, 3.0% (0.022 g/cm^2); femoral neck 3.9% (0.031 g/cm^2); lumbar spine, 2.3% (0.023 g/cm^2). A single scan operator performed all scans for a given crewmember to ensure consistency between serial measurements. At each scan session, scans of the left and right hip and the lumbar spine were performed. For the Bis + ARED group only, pre-flight scans were performed in duplicate and averaged; all other scan sessions, including those for the ARED group, consisted of single scans. Scans were analyzed using the acquisition and analysis procedures recommended by the manufacturer, with the exception of the hip scans which were analyzed using procedures established for earlier spaceflight and bed rest studies. In brief, the lateral margin of the Region of Interest box was manually placed adjacent to the lateral cortex of the greater trochanter and the distal border was a set number of lines from the distal margin of the lesser trochanter [11]. Calibration of the Hologic densitometer was verified by regular scanning of a calibration phantom (weekly and on the day of testing), with scans analyzed using the manufacturer's automated software.

2.4.2. QCT

The percentage change in vBMD of hip trabecular bone (total proximal femur) is the primary endpoint to assess ARED protection from bone loss in space as previously reported for the combined Bis + ARED countermeasure [10]. Quantitative Computed Tomography (QCT) scans of the proximal femur were performed in all subjects at time points similar to those used for the medically-required DXA tests: i) once prior to launch, ii) ~1 wk after return, and iii) at approximately 1 year after return. QCT scans were performed using a clinical QCT scanner at hospitals local to JSC [10]. The precision values for the QCT measurements of vBMD (95% confidence limits for Least Significant Change) were femoral neck 12.5% (trabecular) 4.5% (cortical); trochanter 1.7% (trabecular) 3.9% (cortical); total hip 2.3% (trabecular) 3.4% (cortical) [12,13].

The scans performed on the bisphosphonate-treated subjects were based on 2.5-mm sections, 80 kVp, and 280 mA acquisition protocol. As previously reported [10], there was a change in QCT scanner at the local hospital which occurred during the mission of the fourth Bis + ARED subject affecting the pre- to post-flight serial scanning of this crewmember. As described in reference [12], images were cross-calibrated using a European Hip Phantom (EHP, QRM, Erlangen, Germany) scanned on both the old and new CT systems. All of the ARED-only subjects were imaged on the new CT system and, in order to improve image quality, the protocol was modified to 140 kVp 50 mA with 2.5 mm sections, with these settings and scanner used for all three visits. With respect to the pre-ARED group, there was a scanner change during acquisition of the R + 1 yr data. For this group, BMD values were cross-calibrated using the Image Analysis Torso phantom scanned at each subject visit per manufacturer's recommendations. However, EHP measurements were not available for this earlier study for cross-calibration of FEM measurements as reported [12], and thus we decided not to report R + 1 year FEM measures for the pre-ARED group. Images from all three studies were analyzed using the QCT software version employed previously [12].

All QCT scans were analyzed at the University of California at San Francisco to determine trabecular, cortical and integral vBMD, using methods previously applied to the Pre-ARED subjects [5]. QCT images were further analyzed to estimate bone strength using FE modeling, for loading conditions simulating single-limb stance loading or a fall onto the posterolateral aspect of the greater trochanter, using non-linear modeling described previously [14]. The precision values for FE estimates of hip strength (95% Confidence Limits for Least Significant Change) were 9.7% for Non-linear Stance (NLS) and 9.9% for Non-linear Fall (NLF) [13].

2.5. Bone biochemistry

Blood and urine specimens were obtained pre-, in-, and postflight to measure changes in levels of bone-related biochemical markers, as described previously [2,9]. Biochemical assays performed are listed in Table 4. Urine specimens were pools of either 48-hour collections (preflight, landing day, 30 days postflight) or 24-hr collections (on flight days 15, 30, 60, 120, 180). Blood samples were collected, after an overnight fast, at the same time points for urine collections, with the exception of landing day (R + 0). In this report, results from flight days (FD) 15 and 30 were averaged for a value denoted as early flight; results obtained at around 60 days were designated as mid- flight; and results from FD 120 and 180 days were averaged for a value denoted as late flight. All 17 subjects had at least 1 urine collection for each of the early, mid and late flight time periods. One of the 7 Bis + ARED subjects and 3 of the 10 ARED subjects did not participate in in-flight blood measurements.

Biochemistry data were acquired from the assays performed across several years. Some preflight and postflight assays are performed realtime after collection as part of scheduled medical testing by a clinical laboratory at JSC accredited by the College of American Pathologists. Assays of urine and blood specimens collected for research studies were assayed by the NASA Nutritional Biochemistry Lab at JSC which uses an ISO 9001 quality management system for quality assurance. All specimens (which included preflight, inflight, and postflight specimens) were assayed in one batch after flight per each crewmember. Batched assays were not always feasible with blood specimens due to limited volume and thus some pre/postflight results were shared from medical testing. Samples were analyzed in batch by return from flight order, and there was no attempt to batch assays according to group.

The results from biochemical assessments were previously reported under a different thesis [2,9]. Osteocalcin was measured on all subjects; however, the assay changed partway through testing of the ARED group and will not be reported here. Osteocalcin results from all Bis + ARED subjects were reported previously [10]. The changes in whole body (WB) body mass (Table 3) were evaluated to compare food intake between the ARED and Bis + ARED groups as a possible confounder. Biochemical assays and energy intakes were not included in the densitometry study of crewmembers in the Pre-ARED group [5].

2.6. Statistics

Data from subjects representing the Pre-ARED (n = 18) and Bis + ARED (n = 7) groups, have been previously reported [10]. Data from 10 ARED subjects described in this paper have not been reported and include study-specific data derived from QCT hip scans and from biochemical analyses of bone metabolism. Generalized linear mixed effects models were used to assess pre- to post-mission changes within and between groups (Pre-ARED, ARED and Bis + ARED). Subject-specific random intercepts with robust standard errors were used to reflect the repeated measures within subjects while allowing for heteroscedasticity of the error terms. A fixed effect was included for the interaction between time (pre-, post-), and group. When the interaction effect was identified to be statistically significant, pairwise comparisons were conducted to assess for pre- to post- differences within groups, and differences in change from baseline (pre-) between the groups. All residual plots were evaluated and displayed no large deviations from normality. Further, to determine if there were influential points driving inference results, analyses were completed with and without potential outliers. No results substantially changed and inferences were consistent with and without the removal of potential outliers. All analyses were conducted using SAS v9.4 using the GLIMMIX procedure. A significant difference was considered at $p < 0.05$. Although graphics display change in terms of percent from baseline, all analyses were conducted on the raw scale. The means depicted are the average of these percent changes across individuals and do not represent the model-based mean predictions as these were not generated for percent change.

3. Results

3.1. Subjects

As previously reported, 3 subjects of the Bis + ARED group terminated study participation with 2 of the 3 subjects withdrawing due to gastric issues; none of the subsequent analyses included data from these 3 subjects. Following spaceflight, photographs of tablet blister cards, for the 7 subjects who completed the study, indicated that on-average 98% of the alendronate pills were dispensed [10].

Comparison of demographic data was conducted (Table 2) with no significant difference between groups as detected by 1-way ANOVA.

The total volume of lower body resistive exercise (normalized to body mass of crewmember) was not significantly different between the Bis + ARED and the ARED groups for total dead lifts and squats per flight day (82.2 ± 14.4 Bis + ARED, 88.8 ± 47.6 ARED, NS), for maximum deadlift or squat load (1.77 + 0.25 Bis + ARED, 1.82 ± 0.39 ARED, NS) or for the change in deadlift + squat exercise over the course of the flight (increase from baseline in total weight lifted or maximum weight lifted, data not shown). Likewise, no significant difference between groups was confirmed by ANOVA for change in lean body mass of the whole body and of both legs (Table 3).

3.2. DXA

DXA scans were serially performed at the following time periods (group mean days ± SD) for measurements of Preflight, Postflight and Recovery aBMD: i) 96 ± 44 d before flight, ii) 9 ± 2 d immediately after return, and then iii) 383 ± 45 d approximately 1-year after return). DXA results for the hip and spine (Fig. 1) are plotted as percentage change from pre-flight for the total hip, trochanter, and femoral neck and for the lumbar spine. Within-group comparisons detected statistically significant reductions for all regions of the hip and for the lumbar spine in the Pre-ARED group; significant reductions in mean values were also evident in the ARED group, with the exception of femoral neck. All mean pre vs. post changes in the Bis + ARED group were non-significant for all hip regions but a significant increase from preflight was detected for the lumbar spine. (Two of the 7 subjects in Bis + ARED had post-flight increases of 5% and 11% which are beyond measurement error for the lumbar spine). Between-group comparisons determined that the mean % decline from preflight in the Pre-ARED group was significantly more negative compared to the mean % decline in ARED group for the total hip and femoral neck, but not for the trochanter and lumbar spine. The percentage changes in the Bis + ARED group were significantly different from the ARED group for the total hip (less negative), trochanter (less negative) and the lumbar spine (positive change) but not for femoral neck.

3.3. QCT

Mean QCT changes for the hip trabecular bone are presented in Fig. 2, with trabecular vBMD results plotted as percentage change from pre-flight for the total hip, trochanter, and femoral neck. Statistical analyses for within-group differences showed significant pre vs. post differences for all hip regions in both the Pre-ARED and ARED groups, whereas the Bis + ARED group showed no change (NS) in preflight vs. postflight means. Between-group analyses determined significant attenuation of bone loss in the ARED group compared to the Pre-ARED group for total hip and trochanter, but not the femoral neck. The mean change from preflight was more negative in the ARED group than in the Bis + ARED group for all hip regions but the between-group difference was statistically significant for the femoral neck ($p < 0.05$) and nearly significant for total hip ($p = 0.055$).

Corresponding changes from preflight in cortical vBMD for the 3 hip regions were also similarly plotted and evaluated for the total hip, trochanter and the femoral neck (Fig. 3). Within-group analyses determined that the Pre-ARED group had modest but statistically significant decreases in cortical vBMD in all hip regions. Significant reductions were evident in the ARED group for the total hip and trochanter, but not the femoral neck. There were no changes in the Bis + ARED group for cortical vBMD. Between-group changes were not statistically significant for either Pre-ARED vs. ARED or for Bis + ARED vs. ARED.

The efficacy of countermeasures was also assessed using FE estimates of hip bone strength which integrate the collective changes in 3-

Table 2
Demographic preflight data by study group (mean ± SD).

	Age (years)	Flight duration (days)	Height (cm)	Body mass (kg)	Body mass index (kg/m^2)	Lean mass (kg)	Fat mass (kg)
Pre-ARED n = 18	45 ± 4	173 ± 23	174 ± 5	74 ± 5	25 ± 1	53 ± 5	18 ± 2
Bis + ARED n = 7	50 ± 4	164 ± 15	174 ± 6	76 ± 13	25 ± 3	54 ± 10	18 ± 3
ARED n = 10	47 ± 5	154 ± 24	177 ± 5	78 ± 12	25 ± 3	58 ± 9	17 ± 4

Table 3
Change in whole body (WB) and leg lean mass (group mean ± SD) during spaceflight.

		WB mass			WB lean			Leg lean		
		Pre (kg)	Post (kg)	% change	Pre (kg)	Post (kg)	% change	Pre (kg)	Post (kg)	% change
Bis + ARED	Mean	75.6	73.5	−2.9	54.5	54.3	−0.36	18.6	18.3	−1.64
n = 7	SD	12.5	12.7	1.9	9.7	9.8	0.6	3.8	3.9	2.0
ARED	Mean	78.0	77.7	−0.4	58.2	58.4	0.35	19.0	18.7	−1.70
n = 10	SD	11.8	12.2	3.1	9.5	9.9	2.8	3.1	3.1	3.0
Pre-ARED	Mean	73.7	72.3	−2.0	53.3	52.8	−0.92	17.8	17.4	−2.7
n = 9	SD	4.8	5.7	3.2	5.4	6.0	2.2	1.9	2.2	3.9

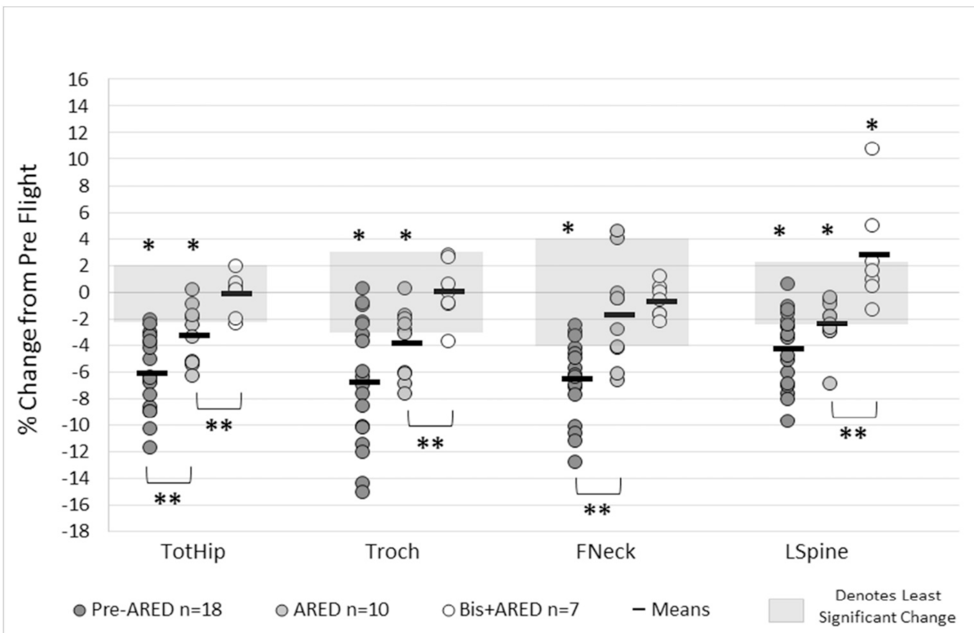

Fig. 1. Changes in DXA areal BMD (aBMD) after spaceflight. DXA measurements of aBMD for total hip (TotHip), trochanter (Troch), femoral neck (FNeck) and lumbar spine (LSpine) were acquired before and after spaceflight, and the percentage change from preflight to postflight over the entire spaceflight is plotted by group for skeletal regions of interest. Significant delta changes are denoted for within-group comparisons (*p < 0.05, pre vs. post) and for between-group comparisons (**p < 0.05 below line comparing Pre-ARED vs. ARED and ARED vs. Bis + ARED). The measurement error ("least significant change") for the DXA measurements at JSC are denoted by shaded areas.

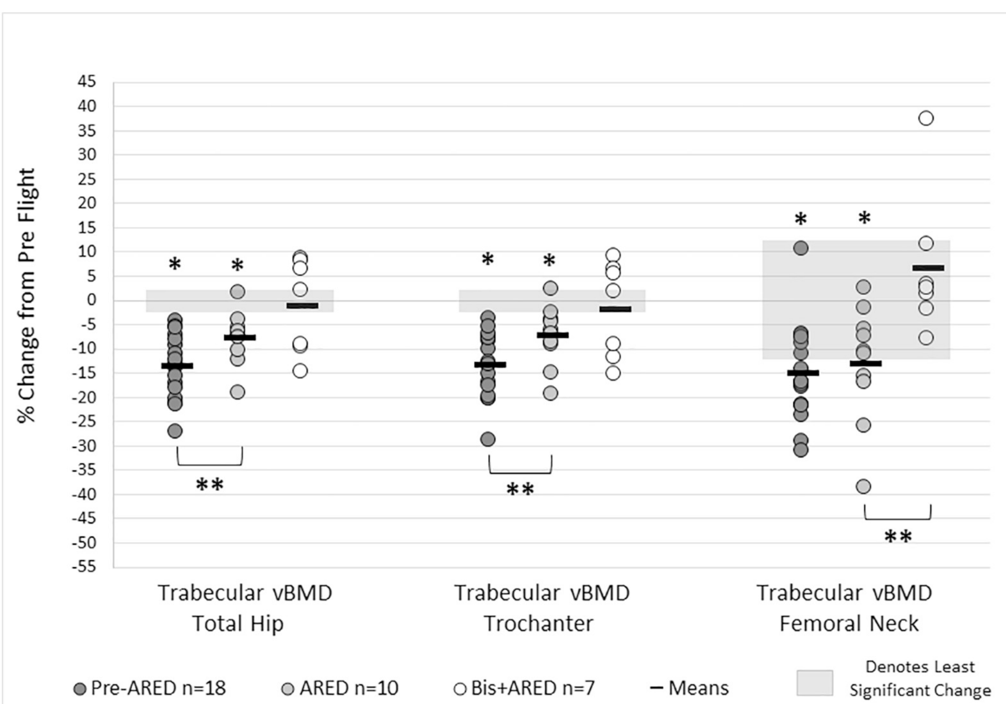

Fig. 2. Changes in QCT trabecular vBMD after spaceflight for the hip and its sub-regions. QCT data are similarly derived and plotted as described for Fig. 1. The QCT measurement error for trabecular vBMD is denoted by shaded areas [12,13]. The effectiveness of in-flight countermeasures (Pre-ARED, ARED and Bis + ARED) was assessed for significant mitigation of group mean declines from preflight. Significant delta changes are denoted for within-group comparisons (*p < 0.05, pre vs. post) and for between-group comparisons (**p < 0.05 below line comparing Pre-ARED vs. ARED and ARED vs. Bis + ARED).

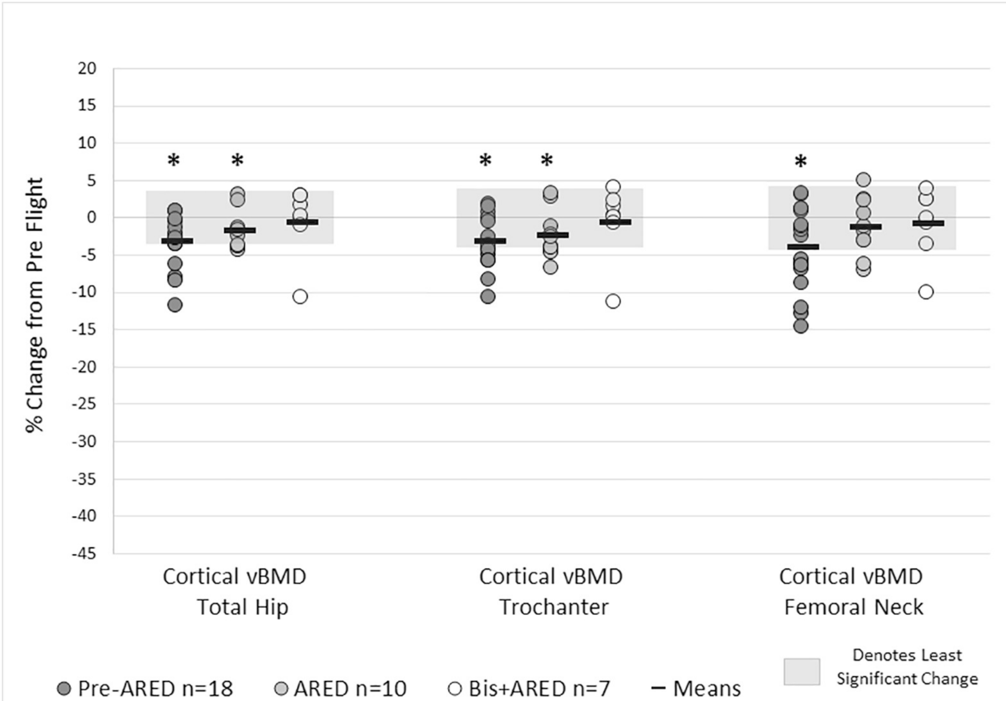

Fig. 3. Changes in QCT cortical vBMD after spaceflight for the hip and its subregions. QCT data are similarly derived and plotted as described for Fig. 1. The measurement error for the QCT measurements of cortical bone vBMD is denoted by shaded areas [12,13]. The effectiveness of in-flight countermeasures (Pre-ARED, ARED and Bis + ARED) was assessed for significant mitigation of group mean declines from preflight. Significant delta changes are denoted for within-group comparisons (*p < 0.05, pre vs. post); there were no significant differences for between-group comparisons.

dimensional bone geometry and the distribution of mineral density. The percentage change from preflight for FE estimates of hip strength are plotted for NLS and NLF loading (Fig. 4). Within-group analyses detected statistically significant reductions in mean FE estimates of hip strength for both NLS and NLF loading in the Pre-ARED group and in NLF loading in the ARED group. There were no statistically significant declines in mean values, for either NLS or NLF loading, detected for the Bis + ARED group. Between-group analyses determined that these declines in the Pre-ARED group were also significantly different (more negative) compared to declines in the ARED group.

3.4. Assessments of total hip 1 year after return to earth

The recovery of deficits induced by spaceflight was assessed with postflight evaluations of DXA aBMD, trabecular vBMD, cortical vBMD and FE estimates of hip strength (fall and stance loading) conducted at approximately 1 year (R + 1 yr) after return. Group means changes immediately postflight (approximately R + 1 wk) were previously discussed (Figs. 1–4). Fig. 5 displays the data to assess recovery for total hip only although significant differences for trochanter and femoral neck are noted. Between-group differences in group mean changes from preflight (Pre-ARED, ARED and Bis + ARED) had been analyzed to

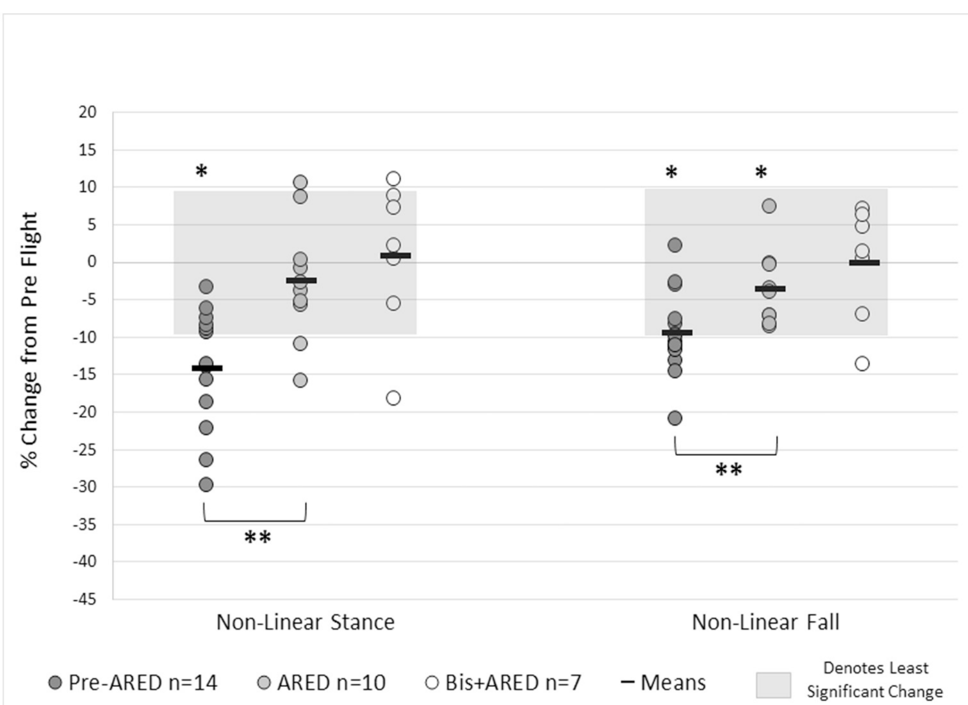

Fig. 4. Changes in QCT finite element estimates of hip strength after spaceflight. Finite element models were generated from QCT scan data acquired before and after spaceflight and analyzed to estimate hip strength for two loading scenarios (Non-linear Stance and Non-linear Fall). Data points are similarly derived and plotted as described for Fig. 1. The measurement error for estimation of hip strength is denoted by shaded areas. The effectiveness of in-flight countermeasures (Pre-ARED, ARED and Bis + ARED) was assessed for significant mitigation of group mean declines from preflight. Significant delta changes are denoted for within-group comparisons (*p < 0.05, pre vs. post) and for between-group comparisons (** p < 0.05 below line comparing Pre-ARED vs. ARED and ARED vs. Bis + ARED).

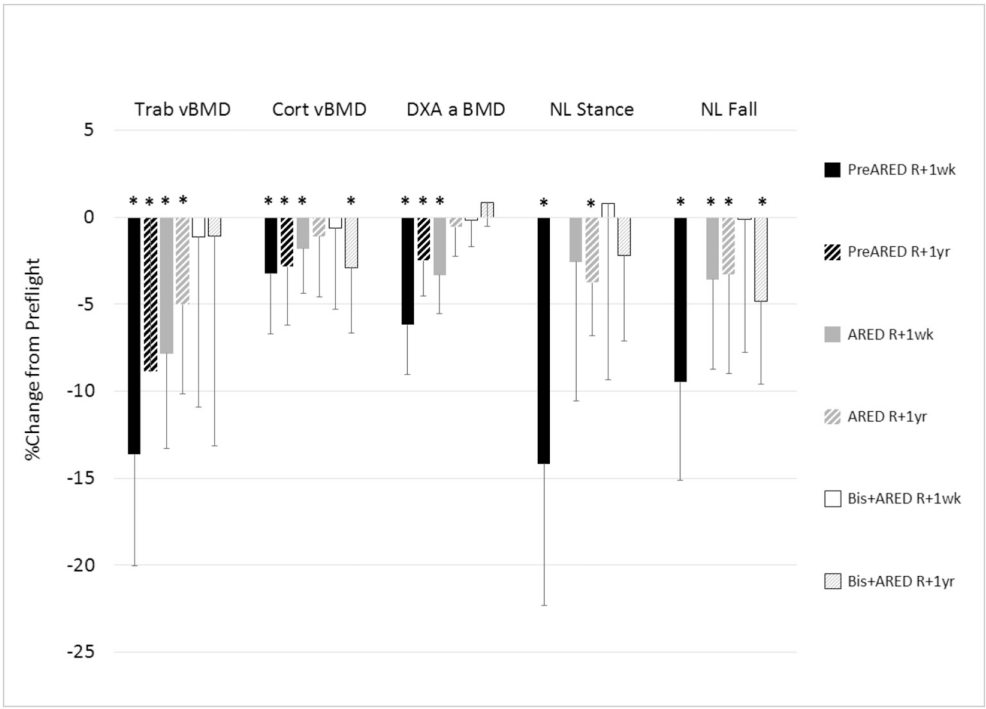

Fig. 5. Changes in areal and volumetric BMDs and FE strength estimates for total hip at 1 year postflight. Postflight changes (group mean ± SD) in trabecular bone (Trab vBMD), in cortical bone (Cort vBMD), in DXA-measured areal BMD (DXA aBMD) and in estimates of hip strength by FE modeling for non-linear stance (NL Stance) and non-linear fall (NL Fall) loadings were evaluated one year after return (R + 1 yr). Postflight changes immediately after return (R + 1wk) are included to provide a context to compare changes during the year back on Earth. * denotes significance at $p < 0.05$, for pre vs. post deltas within group; there were no significant differences between groups in pre vs. post deltas at R + 1 yr. Statistics performed on absolute values.

associate in-flight countermeasures with the restoration of the hip:

i) Trabecular vBMD - at R + 1 yr no group mean changes from preflight (NS, within-group difference) were detected in the Bis + ARED group for all regions of the hip (total hip in Fig. 5, trochanter and femoral neck, data not shown). In contrast, significant deficits from preflight were evident in Pre-ARED and ARED groups for all regions of the hip (as mentioned earlier in Results section). Between-group analyses at R + 1 yr detected significant difference between ARED and Bis + ARED (positive change) for the femoral neck (data not shown);
ii) Cortical vBMD: at R + 1 yr significant deficits were evident in the Pre-ARED group for all hip sites (including trochanter and femoral neck, data not shown); no significant differences from preflight were detected in the ARED group for all hip sites; and significant declines from preflight were detected in the Bis + ARED group for all hip regions. Between group comparisons at R + 1 yr determined no significant differences between Pre-ARED vs. ARED and Bis + ARED vs. ARED groups;
iii) DXA aBMD: within-group analyses at R + 1 yr detected significant declines in the Pre-ARED group for total hip and hip regions (trochanter and femoral neck, data not shown) and for the lumbar spine (data not shown). For the ARED and Bis + ARED groups, no significant difference from preflight was noted. In the Bis + ARED group, there were significant increases from preflight noted for trochanter and lumbar spine, but the increase for total hip was not statistically significant. Between-group analyses for R + 1 yr timepoint indicated that the Bis + ARED group (vs. ARED) also had a significant change from preflight (positive) in the total hip (Fig. 5) and lumbar spine (data not shown).
iv) FE estimates of hip strength (fall and stance loading): within-group analyses at R + 1 yr detected deficits in the ARED group for NLS and NLF, and in the Bis + ARED group for NLF only. FE estimates at R + 1 yr for the Pre-ARED group appeared spurious and inconsistent presumably due to the change in the QCT scanner (data not shown). No significant differences were detected for between group analyses.

4. Biomarkers of bone turnover

Results of serum and urinary measures related to bone turnover are shown below in Table 4 and in Fig. 6a and b for the ARED and Bis + ARED groups only. Within-group analyses (i.e., preflight to timepoint change) conducted on markers of bone resorption – Urinary N-telopeptide (NTX) (Fig. 6a) and serum CTX-β (Table 4) – detected significantly elevated levels during spaceflight in the ARED subjects which were also significantly greater compared to levels in Bis + ARED subjects at corresponding time points. In the Bis + ARED group, the urinary biomarkers for bone resorption (NTX – Fig. 6a and urine Ca – Table 4) were unchanged from preflight during spaceflight but were slightly reduced in late spaceflight (120–180 day window) and after spaceflight (R + 30 d and R + 1 yr). The serum biomarker for bone resorption (CTX-β, Table 4) was also elevated in the ARED group during spaceflight while in the Bis + ARED group it remained stable with no significant change from preflight throughout early, mid and late spaceflight. Between-group comparisons indicated that CTX-β was significantly lower in the Bis + ARED group compared to the ARED group at early and late flight time points. There were no changes in whole body mass (WB) between the ARED and Bis + ARED groups (Table 3).

For Bone Specific Alkaline Phosphatase (BSAP) (U/liter), a biomarker of bone formation (Fig. 6b), the within-group analyses indicated a transient decrease relative to preflight in early spaceflight (15–30 days on orbit) which trended upward (NS) during spaceflight until becoming significantly elevated at R + 30 d in the ARED subjects. A reduction in BSAP levels, relative to preflight, was also observed at R + 1 yr in the ARED group. In the Bis + ARED subjects, there was a small, transient decrease in BSAP at mid-flight (60–90 days in flight), but BSAP was otherwise unchanged from pre-flight. Between-group analyses (of changes from preflight) detected significantly greater changes for BSAP levels in the ARED group at the mid-spaceflight and R + 1 yr time point.

The mean value for total non-corrected serum Ca (Table 4) was unchanged from pre-flight in the ARED group in early, mid- and late flight. There was a decreasing, but sub-clinical, trend in total serum Ca in the Bis + ARED group during flight (pre-flight mean 9.24 mg/dL to 8.77 mg/dL late flight) and all values were significantly different from the ARED group. The normal range of serum calcium in the adult is 8.6–10.0 mg/dL (Mayo Medical Laboratories). A Bis + ARED subject

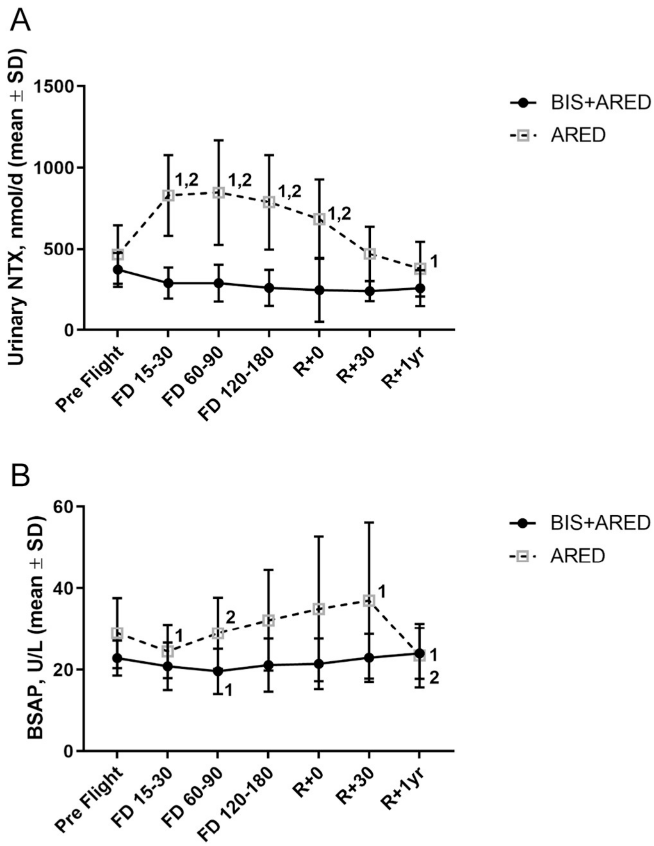

Fig. 6. a. Temporal changes in urinary N-telopeptide (NTX) as a biomarker for bone resorption. The methods and procedures for assaying urine specimens (24- or 48-h pools were as previously reported [10]. [1] denotes significant Within-group mean delta change in assay results between preflight and the inflight or postflight time-point of specimen collection, $p < 0.05$. [2] denotes significant Between-group comparison (Bis + ARED vs. ARED) of delta change from preflight assay at specific inflight or postflight time-points.
b. Temporal changes in serum Bone-specific Alkaline Phosphatase (BSAP). As described in Fig. 6a, Blood specimens were collected before, during and after spaceflight using methods and procedures previously reported [10]. [1] denotes significant Within-group mean delta change in assay results between preflight and the inflight or postflight time-point of specimen collection, $p < 0.05$. [2] denotes significant Between-group comparison (Bis + ARED vs. ARED) of delta change from preflight assay at specific inflight or postflight time-points, $p < 0.05$.

had a serum calcium 7.5 mg/dL on flight day15 which also corresponded with an averaged vitamin D intake of 229 IU during this early flight period No ARED subjects had serum Ca values below 8.8 mg/dL at any time point.

Within-group analyses determined that mean 25-OH Vitamin D (Table 4) was largely unchanged; crewmembers in both the Bis + ARED and ARED groups showed modest decreases toward the end of flight through R + 30 time points. Mean 1, 25 Di-hydroxyvitamin D was unchanged in the Bis + ARED subjects for all time points during spaceflight and reduced in ARED subjects at the early and mid-flight time points. These fluctuations in both groups (ARED and Bis + ARED) are considered sub-clinical and group mean values were no different (NS) from preflight by R + 1 yr for both 25-OH Vitamin D and 1, 25 Di-hydroxyvitamin D. Mean serum PTH was increased relative to preflight at the early and late-flight time point and at R + 30 in Bis + ARED subjects, whereas between-group comparisons indicated that the mean PTH values in the Bis + ARED group were significantly higher at the early flight but significantly lower at R + 1 yr vs. ARED group. Mean serum PTH in ARED subjects showed no changes during spaceflight except for an increase from preflight at R + 30 and R + 1 yr.

During spaceflight there was a relatively smaller increase in the bone formation marker (Fig. 6b) in either the ARED or Bis + ARED groups while the bone resorption marker (Fig. 6a) is elevated in the ARED group.

5. Discussion

The purpose of this extended investigation of skeletal outcomes after spaceflight was to delineate the effects of resistive exercise from the combined benefits of a bisphosphonate and resistive exercise in astronauts during spaceflight. It was previously reported that resistive exercise on the ARED during ~6 month spaceflight aboard the ISS can maintain the group mean values of preflight aBMDs in the astronauts (n = 5–7) [2]. In contrast, this study of ARED only, as an in-flight countermeasure for bone loss, detected a significant group mean decline in aBMD from preflight (Fig. 1). Group mean comparisons in both studies documented improved postflight aBMD outcomes (less negative) in some of the hip regions in ARED relative to Pre-ARED (Fig. 1) (or to IRED [2]). Notably, the Pre-ARED group reported herein included data from ISS Russian cosmonauts. As mentioned, since we cannot confirm IRED use by cosmonauts those data might not have been fully representative of IRED effects [5]. Additionally, some of the ARED-only astronauts (n = 5) as reported in the earlier publication [2] flew for < 100 days (total range 48–215 days) or exercised on the ARED for less than half of the entire spaceflight during the transition between IRED to ARED (data not shown). There were, however, no subjects shared between the two investigations of ARED skeletal effects (n = 10 this report and n = 5–7 in the previous report [2]) suggesting that the earlier ARED analysis [2] was underpowered to detect bone loss especially by DXA which cannot specifically detect losses in trabecular bone.

As previously reported, exercise on ARED only (i.e., without an anti-resorptive drug) did not suppress the elevation of NTX during spaceflight [2] suggesting that the attenuated deficits in aBMD reported for these astronauts (n = 5–7) were not solely through the suppression of bone resorption. We similarly reported elevated group mean levels of bone resorption biomarkers (NTX and CTX) during spaceflight in ARED only group (Fig. 6a and Table 4), which did not increase in the crewmembers using alendronate in addition to the exercise program. The inability to detect differences in group means of whole body mass suggests that food intake was comparable between the ARED exercisers irrespective of alendronate intake. ARED alone, additionally, did not prevent significant losses in Preflight bone mass in all parameters. The ARED group generated a less negative group mean decline of bone mineral density following spaceflight although significant losses in bone mass were still evident in individual subjects (Figs. 1–3). ARED alone did not maintain as many, or more, astronauts at their preflight bone densitometry values (i.e., within Least Significant Change for imaging test) as in combination with the alendronate (Figs. 1–3).

In combination, resistive exercise and an anti-resorptive agent might have additive effects by working independently on different compartments of the hip (cortical and trabecular bones, respectively): i) ARED activation of osteoblasts and lining cells on the periosteal surface – where strain is increased as mass is distributed furthest from the neutral axis of cortical bone [15] and ii) alendronate suppression of osteoclast-mediated bone resorption in trabecular and endocortical bone surfaces and with intra- cortical Haversian remodeling. This activation is nicely documented in animal models where mechanical loading of the rodent ulna increases fluorochrome labeling of mineralizing fronts on the periosteal vs. the endocortical surface of the ulna [16]. However, we have no data from this study to substantiate this bone modeling response.

The metabolic effects of both countermeasures during spaceflight were similar to those of previous bed rest studies [11,17], which are long-established analogs for the skeletal effects of spaceflight [18]. A protective effect of alendronate, without exercise, in subjects with bone loss induced by prolonged bed rest has been previously reported [11].

Table 4
Serum and Urine Markers of Bone Metabolism. Biochemical assays of bone turnover biomarkers and of mediators of calcium homeostasis were conducted in astronaut specimens acquired before, during and after spaceflight. [1]$p < 0.05$, Comparison of within-group means, pre vs. flight time point (Early/Mid/Late); [2]$p < 0.05$, Between-group comparison of mean deltas between pre- and flight time point (comparison to ARED); [†]N = 6 Bis + ARED, N = 7 ARED for serum assays only.

		Pre-flight	Early flight[†] (FD 15-30)	Mid-flight[†] (FD 60-90)	Late flight[†] (FD 120-180)	R + 0 d	R + 30 d	R + 1 yr
Urinary Ca, mg/d	Bis + ARED	268 ± 98	195 ± 85[1,2]	210 ± 94[1,2]	210 ± 85[1,2]	200 ± 93	207 ± 95[1]	244 ± 112
	ARED	221 ± 52	320 ± 81[1]	277 ± 121[1]	240 ± 87	222 ± 86	187 ± 86[1]	199 ± 54[1]
CTX-β, ng/mL	Bis + ARED	0.35 ± 0.14	0.36 ± 0.19[2]	0.31 ± 0.22[2]	0.36 ± 0.26	0.16 ± 0.10[1,2]	0.33 ± 0.16	0.39 ± 0.25
	ARED	0.64 ± 0.25	0.97 ± 0.22[1]	0.85 ± 0.24[1]	0.73 ± 0.25[1]	0.70 ± 0.43	0.68 ± 0.21	0.55 ± 0.19
Total serum Ca, mg/dL	Bis + ARED	9.24 ± 0.36	8.74 ± 0.46[1,2]	8.92 ± 0.28[1,2]	8.77 ± 0.13[1,2]	9.16 ± 0.34	9.19 ± 0.12	9.10 ± 2.4[1]
	ARED	9.32 ± 0.31	9.51 ± 0.26	9.46 ± 0.26	9.48 ± 0.23	9.24 ± 0.39	9.32 ± 0.27	9.30 ± 0.27
Albumin-corrected serum Ca, mg/dL	Bis + ARED	9.32 ± 0.32	8.87 ± 0.40[2]	9.06 ± 0.26[2]	8.91 ± 0.12[1,2]	9.24 ± 0.40	9.25 ± 0.10	9.11 ± 0.22[1,2]
	ARED	9.32 ± 0.31	9.55 ± 0.28	9.51 ± 0.24	9.52 ± 0.25	9.34 ± 0.22	9.36 ± 0.29	9.35 ± 0.24
25-OH vitamin D, nmol/L	Bis + ARED	89.7 ± 21.2	87.3 ± 23.8	84.5 ± 25.6	73.4 ± 13.7[1]	67.9 ± 15.4[1]	70.6 ± 15[1]	92.5 ± 33
	ARED	91.7 ± 17.8	83.2 ± 11.2	79.2 ± 12.5[1]	75.8 ± 12.9[1]	72.8 ± 13.0[1]	82.7 ± 17.5[1]	81.2 ± 23
1,25-$(OH)_2$ vitamin D, pmol/L	Bis + ARED	120 ± 30	135 ± 72	120 ± 52	100 ± 52	187 ± 62[1]	125 ± 54	105 ± 38
	ARED	171 ± 75	106 ± 40[1]	104 ± 44[1]	123 ± 59	170 ± 56	152 ± 46	146 ± 49
PTH, pg/mL	Bis + ARED	24.5 ± 6.7	31.9 ± 9.3[1,2]	30.4 ± 7.22	33.4 ± 9.2[1]	28.4 ± 11.1	28.2 ± 7.4[1]	23.4 ± 10.9[2]
	ARED	23.9 ± 7.5	22.7 ± 2.7	25.7 ± 4.9	28.3 ± 9.0	24.2 ± 8.3	31.8 ± 10.7[1]	31.6 ± 9.0[1]

However, the combination of these two specific countermeasures (alendronate and ARED exercise) was not tested in this or other bed rest studies although the combination of a bisphosphonate (Etidronate) and exercise has been investigated with 120 days of bed rest [19]. Bisphosphonates are similarly effective in preventing bone loss due to spinal cord injury [20,21]. Additionally, combined exercise and a bisphosphonate have been documented to protect against bone loss induced in rats by ovariectomy [22], in test subjects by prolonged bed rest [23], and in females after the onset of menopause [24].

Clearly, postflight bone densitometry was significantly improved with ARED on-orbit, presumably due to the higher maximum resistive forces and greater fidelity in mimicking exercise with free weights [3]. The attenuated change observed with ARED only (vs. Pre-ARED) may not be due to a mitigation of bone resorption (per elevated NTX levels) but may be a net positive effect of stimulated cortical bone formation displacing cortical bone outward from the periosteal surface [25]. In a cohort-based study, radial bone growth appears to accompany declines in bone mass observed with cortical porosity, loss in trabecular vBMD and endocortical resorption [26]. A similar adaptive response is observed in younger-aged ISS crewmembers after spaceflight with re-ambulation (along with any post-mission rehabilitation) in 1 g during the first year after return to Earth [4]. It is, however, more difficult to detect radial growth of cortical bone with QCT densitometry of in vivo bones in humans based upon a voxel by voxel basis threshold for bone mineral content.

Likewise, the continued decline of cortical vBMD in the Bis + ARED group with re-ambulation on Earth (R + 1 yr), which coincided with the reduction in NLF at R + 1 yr, was an unexpected finding (Fig. 5). It seems unlikely that the decline in cortical vBMD was due to bone breakdown because of the presumed anti-resorptive activity of alendronate uptake in bone matrix [27]. Consequently, we cannot provide a reasonable interpretation for these observations based upon the indirect measures by densitometry and biochemical assays. However, it is notable that the within group postflight decline in cortical BMD (at R + 1 yr) was not evident in the ARED only group.

The reported changes in FE-computed whole bone hip strength reflect the net effect of the combined changes in cortical bone and trabecular bone while accounting for the three-dimensional distribution of these changes. Eliminating alendronate from the ARED exercise regimen did not statistically influence reductions in FE estimates of hip strength (Fig. 4), indicating that the contribution of cortical vBMD, relative to trabecular vBMD, was dominant over the preservation of FE-estimated hip strength (NLS and NLF). Although changes in cortical vBMD may at first appear to drive the changes in NLS and NLF, this relationship is not generalizable. For example, in the Pre-ARED group, mean Total Hip cortical vBMD decreased by 3% during spaceflight (Fig. 3), compared with a 13% reduction in trabecular vBMD (Fig. 2). These combined changes reduced NLS by about 14% and NLF by about 9%, more than triple the change in cortical bone (Fig. 5). This illustration should serve as a reminder that BMD is a measure that has been used epidemiologically to evaluate bone health, primarily on Earth, while NLS and NLF are biomechanical measures that are associated with hip fracture through the laws of physics [28].

There are additional anti-resorptive therapies, such as Denosumab, which have proven clinical efficacy for suppressing elevated bone turnover markers in women with postmenopausal osteoporosis [29]. Its approval for the prevention of bone loss or the onset of osteoporosis, and its efficacy in a ground-based analog for spaceflight (e.g., prolonged bed rest), would need to be established before it could be tested as an in-flight countermeasure in the astronaut cohort [30]. Moreover, the elevation of bone resorption in astronauts occurs only during spaceflight eliminating the need for anti-resorption therapy after spaceflight. Consequently, the increased bone resorption and fracture risk reported with the discontinuation of Denosumab [31] reduces its attraction as an inflight bone loss therapy for younger-aged astronauts (35–55 years of age) due to the need for continued osteoporosis treatment after return to Earth.

There are several limitations to this flight study. The number of participants was low and it was not possible to conduct a flight study with investigators blinded to alendronate-treated astronauts. Likewise, it was also not possible to study the effects of alendronate therapy in the absence of exercise during spaceflight because exercise performance is a medical requirement. Hence, we could not determine whether the combined effects of Bis and of ARED were additive or synergistic. Central QCT measures do not have sufficient resolution to evaluate changes in trabecular bone microarchitecture. Additionally, there is large data variability evident in all the groups presented.

In summary, it has been suggested that the rapid bone loss that occurs during spaceflight represents a risk to astronaut health, including the risk for irreversible changes that weaken skeletal integrity [7]. Our data document that the sole use of ARED in space ameliorates but does not prevent bone loss (especially in trabecular bone). The loss of cortical bone in the Bis + ARED group during the first year after return to Earth is notable and requires further investigation. In spite of this observation, we should not discount that alendronate is an effective countermeasure for bone loss during long-duration spaceflight and would provide protection in the event that resistive exercise hardware should fail, especially during the transit to Mars. These findings suggest that the use of an anti-resorptive to prevent bone loss during long duration spaceflight may be beneficial, particularly since it is not possible to predict which individuals will lose bone. A bisphosphonate with a documented safety profile, long-acting efficacy, and easy administration (e.g., an IV bisphosphonate such as zoledronic acid, which can be administered preflight as a single IV infusion) may be particularly attractive.

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Declaration of competing interest

None.

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