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Monitoring bone mineral balance in spaceflight using natural calcium isotopes in astronaut urine

Gwyneth W. Gordon[a], Stephen J. Romaniello[a], Joseph L. Skulan[b], Scott M. Smith[c], Sara Zwart[d], Alice Letcher[a], Ariel D. Anbar[a,e]

[a]School of Earth & Space Exploration, Arizona State University, 550 E Tyler Mall, Tempe, AZ 85287-1404
[b]Elemental Biomarkers, LLC, 7820 Caribou Ct, Vernon, WI 53593
[c]Biomedical Research and Environmental Sciences Division, NASA Johnson Space Center, Houston, TX, 77058
[d]University of Texas Medical Branch, 301 University Boulevard, Galveston, TX, 77555
[e]School of Molecular Sciences, Arizona State University, 551 E University Drive, Tempe, AZ 85287

Abstract

Bone loss due to exposure to microgravity during long-term spaceflight is a serious health concern for astronauts. X-ray densitometry (DEXA) shows cumulative change in bone mass, but requires significant bone loss to have already occurred and has only limited ability to determine dynamic changes in bone health. Likewise, bone formation and resorption biomarkers monitor changes in formation or resorption rates, but are neither quantitative nor reliable at indicating changes in overall net bone mineral balance (BMB). Calcium isotopes in blood and urine reflect changes in BMB because isotopically light calcium is preferentially partitioned into bone from blood, while bone resorption has no net isotope fractionation. We present $\delta^{44/42}$Ca isotope data from 30 International Space Station (ISS) crewmembers undergoing different exercise and pharmaceutical interventions against bone loss. Exercise-only crewmembers on average lost bone, particularly in the first 30 days of spaceflight. Individuals who did heavier resistance exercise maintained BMB better than those who did lower resistance exercise, particularly as the length of the mission increased. All the crewmembers who did heavier resistance exercise and also took alendronate, a drug that suppresses bone metabolism, were able to maintain BMB throughout the mission. Although on average, exercise-only crewmembers lost BMB, some individuals were more resilient to bone loss. Hence, Ca isotopes hold promise for evaluating the effectiveness of bone loss countermeasures in individual crewmembers before significant bone loss occurs, and potentially of informing clinical decisions about if and when to implement pharmaceutical countermeasures.

Introduction

The technique of determining BMB from calcium isotopes stems from an asymmetry in bone formation and resorption mechanisms. When bone forms, it preferentially takes up the lighter isotopes of calcium. When bone resorbs, it dissolves small pockets of bone in bulk, with no associated isotope fractionation. The calcium in soft tissue (serum and urine) reflects the complement of this process. Bone formation results in isotopically heavier serum as the light isotopes are taken up in new bone. Bone resorption results in isotopically lighter serum as calcium from bone is dissolved into blood. Ca isotope modelling of BMB is an inherently quantitative tool.

Bed rest is a ground-based analogue of space flight, limiting the gravitational loading needed to maintain bone health. Previously measured calcium isotopes in urine and serum of bed-rest subjects (Skulan et al 2007, Morgan et al 2012, Channon et al 2015) demonstrated that subjects began losing bone within ten days of the onset of bed rest, and remained in negative bone mineral balance throughout the study period. During these studies, we developed a mathematical mass balance model to quantitatively estimate the amount of bone loss or gained from the changes in calcium isotope composition.

Methods

Twenty-four hour urine samples (n=11 to 15 per subject) were collected from astronauts (n=30) up to two years prior, during, and after extended stays on the International Space Station. Samples included men (n=22) and women (n=8), and mean age at launch was 45 years old (range 39-56). Mean time in space was 160 days (range 58-215 days). DEXA measurements were made less than two years prior to launch and within 30 days after return to Earth. Urine samples were measured previously for biomarkers of bone

formation (BSAP, bone-specific alkaline phosphatase), bone resorption (NTx, N-telopeptides of Type I collagen), and calcium concentration (Smith et al 2012, Smith et al 2014, Smith et al 2015).

Urinary samples for calcium isotope analysis were acid digested to remove organic matter, purified to remove matrix elements using an automated analytical exchange protocol (Romaniello et al 2015) and analyzed on a Neptune multi-collector ICP-MS. Initially, samples were analyzed by using sample-standard bracketing (Morgan et al 2011). Later, a double spike (^{43}Ca-^{46}Ca) was added to correct for instrumental mass fractionation, and most samples were subsequently reprocessed and reanalyzed to improve data quality. Data is expressed in delta notation relative to SRM 915a.

Prior to flight, crewmembers were divided into three intervention groups: interim resistive exercise device (iRED), advanced resistive exercise device (ARED, permitted greater resistive loads than iRED), and ARED plus administration of alendronate. Alendronate, a bisphosphonate, is a long-acting drug that hinders resorption of bone and are frequently prescribed for osteoporosis patients.

Results

In order to compare the changes in BMB directly between crewmembers, values were corrected to the individual's average baseline prior to spaceflight. Both exercise groups showed significantly lower $\delta^{44/42}$Ca values inflight compared to preflight values (Figure 1). Crewmembers who took alendronate as well as exercised showed small increases in $\delta^{44/42}$Ca values, indicating a positive bone mineral balance status. This was similar to the overall DEXA results, which showed that the iRED crewmembers lost the most bone mineral content, and the alendronate plus ARED group lost the least amount of bone. The bone formation marker BSAP and resorption marker NTx were elevated in both ARED and iRED crewmembers compared to the alendronate group.

A mass balance model constructed from the known sources, sinks, and fractionation factors in the human body provided a quantitative estimate of net bone mineral balance consistent with that from DEXA measurements.

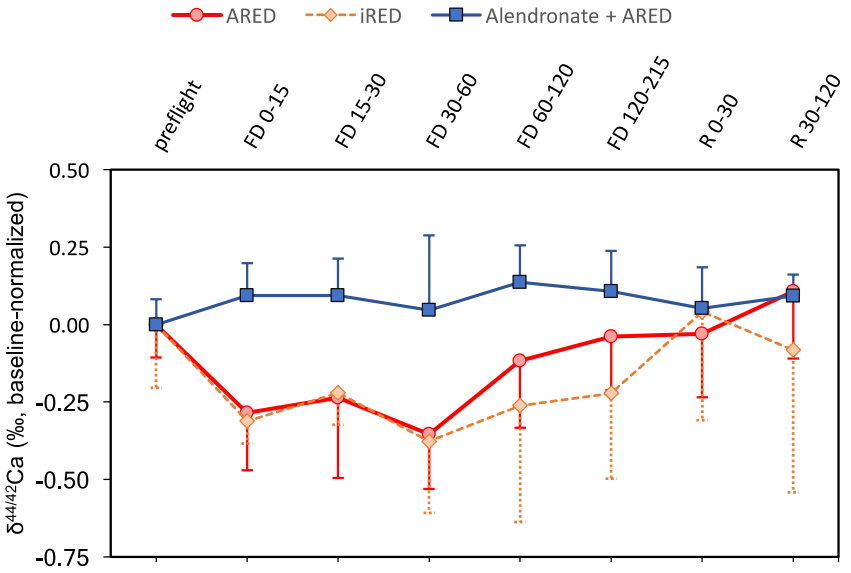

Fig. 1. Change in $\delta^{44/42}$Ca from baseline for crewmember study groups, with 1 SD error bars.

Discussion

In addition to replicating the results of DEXA measurements, $\delta^{44/42}$Ca isotopes provided a high temporal-resolution, dynamic picture of changing bone mineral balance in astronauts. Exercise-only crewmembers

lost the largest amount of bone during the first thirty days of spaceflight. In order to reduce injury during adaptation to microgravity, crewmembers did minimal exercise during early spaceflight, coincident with the greatest rate of bone loss. Crewmembers taking alendronate, however, showed no evidence of bone loss even during the period of minimal exercise.

Another important finding emerging from this study is the interpersonal differences between crewmembers. Although group averages showed that crewmembers who underwent ARED lost bone mineral, some individuals lost little or no bone, while others showed sharp decreases. Crewmembers who lost significant bone mineral balance did not correlate with sex, age, or body mass index (BMI). Additional research may be able to determine which individuals are resilient against bone loss in microgravity and may make better astronaut candidates for long-term spaceflight.

This study demonstrates that calcium isotopes in urine provide non-intrusive quantitative information on dynamic bone mineral balance in spaceflight. Crewmembers on longer-term spaceflight missions, such as required for trips to Mars, may show additional delayed bone loss. Inflight measurement of Ca isotopes would allow exercise and pharmaceutical prescriptions individualized for each crewmember. Although current Earth-bound measurement techniques are not suitable for weight and energy restrictions of spacecraft, we are actively pursuing development of alternative measurement modalities.

This technique has many human health applications, from monitoring bone involvement during metastatic cancer progression to determining nutritional requirements during normal development. The mass balance techniques of naturally-occurring isotopes are fundamentally different from genomic and proteomic techniques dominating current medical research. Such radically different outlooks on disease can provide profound and innovative insights into conditions that desperately need such revolutionary perspectives.

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