Fiscal Year:	FY 2018	**Task Last Updated:**	FY 03/28/2018
PI Name:	Anbar, Ariel Ph.D.		
Project Title:	Stable Calcium Isotopes in Urine as a Biomarker of Bone Mineral Balance in Spaceflight		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Biomedical countermeasures		
Joint Agency Name:		**TechPort:**	Yes
Human Research Program Elements:	(1) **HHC**:Human Health Countermeasures		
Human Research Program Risks:	(1) **Bone Fracture**:Risk of Bone Fracture due to Spaceflight-induced Changes to Bone (2) **Osteo**:Risk Of Early Onset Osteoporosis Due To Spaceflight		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
PI Email:	anbar@asu.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	480-965-0767
Organization Name:	Arizona State University		
PI Address 1:	School of Earth & Space Exploration		
PI Address 2:	Bateman Physical Sciences Bldg, Box 871404		
PI Web Page:			
City:	Tempe	**State:**	AZ
Zip Code:	85287	**Congressional District:**	9
Comments:			
Project Type:	Flight	**Solicitation / Funding Source:**	2012 Crew Health NNJ12ZSA002N
Start Date:	12/01/2013	**End Date:**	11/30/2017
No. of Post Docs:		**No. of PhD Degrees:**	
No. of PhD Candidates:	1	**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA JSC
Contact Monitor:	Ploeger, Stephanne	**Contact Phone:**	
Contact Email:	stephanne.l.ploeger22@nasa.gov		
Flight Program:	ISS		
Flight Assignment:	NOTE: End date changed to 11/30/2017 per PI and NSSC information (Ed., 9/6/16) NOTE: End date changed to 11/30/2016 per PI and NSSC information (Ed., 12/14/15)		
Key Personnel Changes/Previous PI:	Final report: none		
COI Name (Institution):	Gordon, Gwyneth Ph.D. (School of Earth & Space Exploration, Arizona State University) Skulan, Joseph Ph.D. (Elemental Biomarkers, LLC) Romaniello, Stephen Ph.D. (School of Earth and Space Exploration, Arizona State University)		
Grant/Contract No.:	NNX14AB78G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Long duration human spaceflight leads to loss of bone mass. As a consequence, there is a need for techniques to sensitively detect changes in the net rate of bone formation or resorption (i.e., changes in "bone mineral balance") and to assess the effectiveness of countermeasures. We have documented, in bed rest experiments, that measurements of the Ca isotope composition of urine using mass spectrometry can be used to monitor rapid changes in net bone mineral balance that are not directly observable by other means (1, 2). We propose to extend these experiments to the International Space Station (ISS), in order to demonstrate the utility of Ca isotopes as a tool for monitoring bone mineral balance and countermeasures to bone resorption in space. This proposal builds on a successful existing collaboration between researchers at Arizona State University (ASU) and Johnson Space Center (JSC) to study and apply the Ca isotope method as a bone biomarker (2, 3). The proposed project paves the way for future development of capability to measure Ca isotopes in-flight, to monitor bone health during exploration-class space missions where in situ evaluation of countermeasure effectiveness will be required to assure crew health and safety. The project will also have broad clinical application for Earth-based populations. Our proposal falls under the Spaceflight Biochemical Profile Human Research Program (HRP) research emphasis, and addresses Integrated Research Plan (IRP) Gap N3: How do nutritional status/nutrition requirements change during spaceflight? References: (1). Morgan JL, Skulan JL, Gordon GW, Romaniello SJ, Smith SM, Anbar AD (2012). Rapidly assessing changes in bone mineral balance using natural stable calcium isotopes. Proc. Natl. Acad. Sci. USA 109, 9989-9994; (2). Skulan J, Bullen T, Anbar AD, Puzas JE, Shackelford L, LeBlanc A, Smith SM (2007). Natural calcium isotopic composition of urine as a marker of bone mineral balance. Clin. Chem. 53,1155-1158; (3). Morgan JLL, Skulan JL, Gordon GW, Romaniello SJ, Smith SM and Anbar AD (2011). High-precision measurement of variations in calcium isotope ratios in urine by multiple collector inductively coupled plasma mass spectrometry. Anal. Chem. 83, 6956–6962.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Our research has demonstrated that changes in the natural Ca isotope composition of blood and urine reflect changes in net bone mineral balance (BMB) within days of the onset of disruption of BMB. By contrast, changes in BMB require months or years to produce changes in bone mineral density large enough to be detected by radiological techniques such as dual-energy x-ray absorptiometry (DXA), which currently are the only clinically practical methods of determining BMB. By permitting rapid measurements of changes in BMB, Ca isotopes allow disruptions in BMB to be detected before they have caused significant skeletal damage, and the effectiveness of countermeasures to abnormal bone loss or gain to be quickly evaluated in individual people. The usefulness of this technique extends beyond measuring bone loss in spaceflight to the detection and evaluation of treatment for any disease involving disruption in BMB, including osteopenia/osteoporosis, cancer, and Paget's disease. For example, we currently are exploring the application of the Ca isotope technique to the early detection of osteolytic lesions in multiple myeloma. The potential usefulness of Ca isotopes supports their widespread clinical application. We are exploring the possibility of using laser fluorescence, rather than conventional mass spectrometry, to build small, compact Ca isotope measurement instruments suitable to both spaceflight and clinical use. Beyond the numerous potential clinical applications of Ca isotopes per se, our research on Ca isotopes has been a driving force behind international research into biomedical application of other isotope and elemental systems, including Fe, Zn, and Cu. Collectively, these efforts hold the promise of the development of an entirely new and powerful class of disease biomarkers.
	Our results confirm that Ca isotopes act as an accurate, near-real time monitor in spaceflight and allow us to address the five primary questions posed at the beginning of the study. Question 1: Is the observed pattern of decreasing d 44/42Ca values with bone loss in previous bed rest studies similar to the pattern in astronauts in spaceflight? In our previous bed rest studies (Morgan et al., 2012), urinary d44/42Ca begins to fall on day 4 of bed rest and drops to values significantly lower than baseline (-0.2‰, $p < 0.001$) by day 15. Data from the ISS indicates that urinary d44/42C in Advanced Resistive Exercise (ARED) and interim Resistive Exercise Device (iRED) treatment groups decreased significantly by flight day (FD) 15 (~-0.26‰) comparable in magnitude and timing to the bed rest studies. Question 2: Is the magnitude of bone loss calculated from Ca isotopes measurements and a mass balance model supported by the DEXA data from before and after spaceflight? Our previously developed mass balance model indicates that a shift in Ca isotopes of -0.26‰ correlates to a loss of ~100 mg of Ca per day. This is consistent with loss rates calculated from Ca isotope data in bed rest studies. Morgan et al. (2012) report that bed rest subjects lost 0.25 ± 0.07% of whole body bone mass between days 7-30, which is consistent with previously reported rates of bone loss in bed rest (e.g., 0.19 ± 0.07%/month), and with rates of bone loss measured by DXA in the bed rest subjects. For the ISS subjects, the magnitude of bone loss calculated from Ca isotope data is consistent within error with bone loss indicated by DXA data from before and after spaceflight. For the cohorts losing bone (ARED and iRED), rates of bone lose were ~2-4% over 120-215 days in space, consistent with loss rates calculated by the mass balance model and by DXA. Question 3: How does the high temporal resolution Ca isotope data compare to protein biomarker data (NTX, BSAP) measured in the same samples? Ca isotopes offer high temporal resolution comparable to protein biomarkers. Previous studies indicate that protein biomarkers and Ca isotopes respond to skeletal unloading by day 9 of bed rest. Ca isotopes offer the same high temporal resolution tracking during spaceflight that they do during bed rest: data from the ISS shows both Ca isotopes and protein biomarkers responding to skeletal unloading due to microgravity by FD 15. Question 4: What does the Ca isotope data say about the relative effectiveness of exercise (iRED, ARED) and pharmaceutical countermeasures (bisphosphonates)?

Task Progress:	All crewmembers underwent a 30-day adjustment period at the beginning of flight, during which they performed no resistive exercise. Treatment with ARED and iRED is therefore considered to start at FD 30. The dramatic decrease in d44/42Ca in the exercise-only groups during FD 0-30 reflects untreated bone loss due to skeletal unloading. The ARED+Alendronate group received Alendronate several months before flight. Thus, their baseline and all in-flight measurements reflect a state of inhibited bone turnover. The differences between d44/42Ca measurements in the three treatment groups is striking: crewmembers treated with ARED+Alendronate maintained near constant d44/42Ca throughout spaceflight, while those treated with exercise alone showed a more variable response, but overall had lower d44/42Ca during spaceflight relative to pre-spaceflight baselines. This suggests that ARED+Alendronate effectively maintained BMB in all crewmembers. Direct comparison of the effectiveness of ARED+Alendronate with exercise alone is not possible: The ARED+Alendronate group was protected from bone loss throughout spaceflight, while the exercise groups were unprotected for the first 30 days of spaceflight, resulting in measurable bone loss over that period. For most crewmembers, exercise alone was not sufficient to completely restore preflight BMB before the end of the mission. Ca isotope values from the ARED and iRED groups diverge after the start of exercise on FD 30. The differences between the groups are not statistically significant, which is unsurprising given the large inter-personal variation of baseline d44/42Ca values. However, the ARED group shows a trend of increasing d44/42Ca over time, while mean d44/42Ca of the iRED changes insignificantly (+0.17‰ for ARED vs +0.03‰ for iRED), suggesting that ARED provided better protection from bone loss than iRED. In the exercise only groups, Ca isotope data reveal considerable inter-personal variation in BMB during spaceflight, which results in large standard deviations in d44/42Ca group means. Some crewmembers in each exercise group recovered to near neutral BMB during spaceflight, while others remained in negative BMB despite exercise. Individual differences in response of BMB to exercise could be caused by differences in the type and intensity of exercise performed by crewmembers, and/or by inherent physiological differences. The relative importance of these two possible causes of inter-personal differences cannot be resolved with available evidence. Question 5: Can the Ca isotope data be used to tailor personalized treatment plans for astronauts? The behavior of BMB in spaceflight is highly dynamic, and the degree of skeletal response to microgravity is not universal. Ca isotopes provide information on the dynamics of BMB that cannot be gained from biochemical markers or DXA. By revealing previously inaccessible dynamics of individual bone metabolism in spaceflight, d44/42Ca monitoring may be useful for guiding individualized bone loss countermeasures. Trends in d44/42Ca values in 24-hour pooled urine samples from 30 ISS crewmembers suggest that ARED+Alendronate effectively maintained BMB in all crewmembers, while exercise alone did not always maintain BMB. Further, the data reveal what may be an interpersonal differential response to type of exercise intervention. The range of responses underlines the importance of individualized monitoring of net BMB, and of creating individualized treatment plans. Reference Morgan JL, Skulan JL, Gordon GW, Romaniello SJ, Smith SM, Anbar AD (2012). Rapidly assessing changes in bone mineral balance using natural stable calcium isotopes. Proc. Natl. Acad. Sci. USA 109, 9989-9994
Bibliography Type:	Description: (Last Updated: 10/09/2019)
Abstracts for Journals and Proceedings	Gordon GW, Romaniello SJ, Skulan JL, Smith SM, Zwart S, Letcher A, Anbar AD. "Monitoring bone mineral balance in spaceflight using natural calcium isotopes in astronaut urine." Presented at the 12th International Symposium on Applied Isotope Geochemistry (AIG-12), Copper Mountain, Colorado, September 17-22, 2017. 12th International Symposium on Applied Isotope Geochemistry (AIG-12), Copper Mountain, Colorado, September 17-22, 2017. , Sep-2017
Abstracts for Journals and Proceedings	Skulan J, Smith S, Anbar AD, Gordon GW, Romaniello SJ, Zwart S. "Calcium isotopes measure change in bone mineral balance during spaceflight." Presented at the 22017 NASA Human Research Program Investigators’ Workshop, Galveston, TX, January 23-26, 2017. 2017 NASA Human Research Program Investigators’ Workshop, Galveston, TX, January 23-26, 2017. , Jan-2017
Abstracts for Journals and Proceedings	Gordon GW, Romaniello SR, Skulan JL, Smith SM, Zwart S, Zheng W, Letcher AJ, Anbar, AD. "Natural calcium isotopes provide rapid and precise monitoring of bone mineral balance changes in microgravity." 2018 NASA Human Research Program Investigators’ Workshop, Galveston, TX, January 22-25, 2018. 2018 NASA Human Research Program Investigators’ Workshop, Galveston, TX, January 22-25, 2018. , Jan-2018
Abstracts for Journals and Proceedings	Gordon GW, Romaniello SR, Skulan JL, Smith SM, Zwart S, Anbar AD. "Stable Calcium Isotopes in Urine as a Biomarker of Bone Mineral Balance in Spaceflight." 2016 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 8-11, 2016. 2016 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 8-11, 2016. , Feb-2016
Abstracts for Journals and Proceedings	Skulan JL, Gordon GW, Anbar AD, Zwart S, Smith SM. "Bone Mineral Balance in Astronaut Urine Before, During and After Spaceflight as Inferred from Ca Isotope Variations." 2015 NASA Human Research Program Investigators’ Workshop, Galveston, TX, January 13-15, 2015. 2015 NASA Human Research Program Investigators’ Workshop, Galveston, TX, January 13-15, 2015. , Jan-2015
Articles in Other Journals or Periodicals	Skulan JL, Gordon GW, Romaniello S, Zheng W, Letcher AL, Smith SM, Zwart S, Anbar AD. "Dynamics of bone metabolism during extended spaceflight revealed by Ca isotope analysis." Science Advances, in preparation for submission as of March 2018. [Ed. note Oct 2019--not found online] , Mar-2018