Fiscal Year:	FY 2016	**Task Last Updated:**	FY 03/08/2016
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		
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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:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:	1	**Monitoring Center:**	NASA JSC
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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:			
COI Name (Institution):	Gordon, Gwyneth Ph.D. (Arizona State University) Skulan, Joseph Ph.D. (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 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.
Task Progress:	The primary goal of this project was to demonstrate whether the relationship between bone mineral balance (BMB) and changes in the natural isotope composition of blood and urine observed in Earth-based bed rest studies could also be observed in crewmembers in spaceflight, providing the basis for inflight measurements of BMB and evaluation of effectiveness of bone loss countermeasures in individual crewmembers. We have achieved this goal. Although not all of our analyses are completed, analysis of archived urine samples from 32 crewmembers from previous ISS missions clearly shows the same pattern of change in Ca isotope composition observed in bed rest and predicted in spaceflight. More specifically, the 44Ca:42Ca ratio in urine (expressed as d44/42Ca) typically drops to below each crewmember's individual average pre-flight value shortly after spaceflight begins, remains low during spaceflight, and returns to pre-flight values upon return to Earth. This is the pattern expected in crewmembers transitioning to more negative BMB in microgravity, and a confirmation of our ability to detect this change using Ca isotopes. A great deal more information can be extracted from the Ca isotope data we have gathered, a task that we have begun and will be our primary activity during the remainder of the project. Changes in Ca isotope composition are revealing details of the dynamics of BMB on previously inaccessible timescales, and offer new insights into bone biology. In particular, while on Ca isotopes show what on average crewmembers lose bone during spaceflight, our data reveal striking differences between the responses of individual crewmembers. Some of these differences are related to countermeasures. For example, crewmembers treated with both bisphosphonate and Advanced Resistive Exercise (ARED) uniformly showed not bone loss, while the response of crewmembers treated with exercise alone was more variable: most lost bone, some did not. Variations in response to treatment do not appear to be related to age, sex, or BMI (body mass index). The cause of these variations is of great interest, and we hope that further analysis will shed light on this and other questions. We do anticipate, however, that the answers to many questions will require more data from future projects. A secondary goal of our project was to increase the speed with which samples could be processed and analyzed. This goal is, of course, a moving target. Processing speed can always be increased, but we have made substantial improvements over where we were at the start of the project. We designed and validated an automated column chromatography procedure for faster and cleaner sample purification prior to analysis, and have validated a new microwave digestion system for improved sample digestion is now 80% complete. In addition, we are in the process of developing an improved mass spectrometric analytical procedure using a 46Ca-43Ca double spike, which should improve data quality, accuracy, and precision.

	Finally, we have begun exploring new technology to measure Ca isotope ratios using laser fluorescence rather than conventional mass spectrometry. Conventional mass spectrometers are not suited to either inflight or widespread clinical use. Such instruments are large, difficult or impossible to miniaturize, complex, and expensive, problems that could be overcome by using laser fluorescence. Whether a practical laser fluorescence device can be built remains to be seen, but preliminary results are promising, and efforts to build small and simple devices for measuring other isotopes (C and O) have been successful.
Bibliography Type:	Description: (Last Updated: 10/09/2019)
Abstracts for Journals and Proceedings	Anbar A, Gordon G, Skulan J, Smith S, Fonseca R. "Developing a clinically useful calcium isotope biomarker." 25th V. M. Goldschmidt Conference, Prague, Czech Republic, August 16-21, 2015. Abstracts of the 25th Goldschmidt Conference, 2015. , Aug-2015
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Abstracts for Journals and Proceedings	Smith HB, Kim H, Romaniello SJ, Field P, Anbar AD. "A robust and fullyautomated chromatographic method for the quantitative purification of Ca and Sr for isotopic analysis." Presented at the Fall Meeting 2014, American Geophysical Union, San Francisco, CA, Dec 15-19 2014. EOS, Transactions American Geophysical Union. 2014 Dec 9;95(49):abstract #PP51C-1143. , Dec-2014
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Patents	US 9,261,519. Patent, February 2016. Feb-2016 Anbar A, Skulan J, Gordon G, Morgan J. "Isotopic biomarkers for rapid assessment of bone mineral balance in biomedical applications."