Fiscal Year:	FY 2013	**Task Last Updated:**	FY 07/08/2013
PI Name:	Anbar, Ariel Ph.D.		
Project Title:	Rapid Measurements of Bone Loss Using Tracer-less Calcium isotope Analysis of Blood and Urine		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
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:	Ground	**Solicitation / Funding Source:**	2007 Crew Health NNJ07ZSA002N
Start Date:	05/20/2008	**End Date:**	03/19/2013
No. of Post Docs:	1	**No. of PhD Degrees:**	1
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	1
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA JSC
Contact Monitor:	Maher, Jacilyn	**Contact Phone:**	
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Flight Program:			
Flight Assignment:	NOTE: Risk/Gap changes per IRP Rev E (Ed., 3/27/14) NOTE: Received extension to 3/19/2013 per NSSC (Ed., 5/8/2012) NOTE: Received extension to 5/19/2012 per C. Guidry/JSC and NSSC [Ed. 3/2/2011]		
Key Personnel Changes/Previous PI:	None		
COI Name (Institution):	Skulan, Jospeh (University of Wisconsin-Madison Geology Museum) Smith, Scott (Human Adaptation and Countermeasures Division) Bullen, Thomas (United States Geological Survey)		
Grant/Contract No.:	NNX08AQ36G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Our research was aimed at developing a method to rapidly detect changes in bone mineral balance by measuring the natural (i.e., tracer-less) isotope composition of calcium in blood and/or urine. This method would provide a way to detect incipient bone loss before changes in bone density are detectable by conventional X-Ray methods. The resorption of bone when astronauts are exposed to microgravity is a major challenge for NASA's plans for human exploration of the Moon and Mars. Our proposed technique would be immediately valuable in ground-based studies of countermeasure strategies, accelerating the pace of discovery of countermeasures to bone loss. In the long run, flight-qualified versions of mass spectrometric or other systems for Ca isotope characterization could accompany astronauts on long-duration missions. Precise measurements of the calcium isotope composition in blood or urine provide information about bone mineral balance because the isotopic composition of calcium in human soft tissues is naturally affected by the relative rates of bone formation and resorption. Specifically, lighter calcium isotopes are preferentially incorporated into bone during formation. Because of the short residence time of calcium in soft tissues, calcium isotope ratios should change rapidly in response to changes in bone gain or loss. These changes, while small, can be measured by multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS) or thermal ionization mass spectrometry (TIMS). The research expanded on a pilot study in which we measured calcium isotopes in a small suite of urine samples from a bed rest study. The expanded study involved a larger number of bed rest subjects, measurements of blood and dietary samples as well as urine, and a much denser sample schedule, including sub-daily urine and blood sampling during critical early and late phases of the bed rest and post bed rest periods. This research allowed us to answer critical questions unresolved by the pilot study. Most importantly, we were able to determine how long after the onset of bed rest bone loss can be detected with Ca isotopes, and to begin to quantify the relationship between changes in Ca isotope ratios and changes in bone mineral balance (BMB).
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	We have developed a technique that uses analyses of natural variations in the calcium isotope composition of urine to measure changes in bone mineral balance. This research focused on detecting bone loss resulting from skeletal unloading in the microgravity of space, but our technique is equally applicable wherever disruptions in bone mineral balance are an issue. Ca isotope analysis provides a way of detecting incipient bone loss before it can be detected by radiographic techniques. Apart from early detection of osteoporosis, preliminary investigations of Ca isotopes in multiple myeloma patients indicate that the technique is well suited to the early detection of bone lesions in cancers where skeletal involvement is prominent. Ca isotope analysis is non-invasive and imposes no radiological hazard, making it well suited to continuous monitoring of the bone mineral status of cancer patients.
Task Progress:	The resorption of bone when astronauts are exposed to microgravity is a major challenge for humans engaged in long-term space travel. The goal of our research is to develop a Ca isotope assay of urine, blood, or other soft tissues and soft tissue proxies that allows rapid, quantitative measures of the changes in bone mineral balance (BMB) that lead to bone loss, providing key information that other techniques, for example dual energy X-ray absorptiometry (DEXA) and biochemical markers of bone metabolism, cannot provide. Such a Ca isotope technique would facilitate quick evaluation of countermeasures to bone loss in space. In the long run, flight-qualified versions of mass spectrometric or other systems required for Ca isotope measurement could accompany astronauts on long-duration missions. The final outcome will be better monitoring of human health in space through more rapid and accurate measurement of changes in BMB. Prior to the start of the research described in this report, a relationship between BMB and soft tissue Ca isotope composition had been hypothesized (Skulan and DePaolo, 1999) and confirmed in a pilot study using archived urine samples from a previous 17-week bed rest study (Skulan et al., 2007--see below). These preliminary studies raised several questions that were addressed in the NASA-funded research described here: 1. How soon after the start of bed rest does a bone loss signal appear in urine and blood? In the pilot study, the bone loss signal in urine was apparent at 28 days after the start of bed rest, which was the first bed rest sample point. Because the residence time of Ca in soft tissues is short (hours to days) we predicted that the bone loss signal should appear far earlier than this. 2. What factors other than BMB can affect soft tissue Ca isotope composition? In particular, how does the renal Ca isotope fractionation observed in earlier studies affect the ability of soft tissue Ca isotope composition to track BMB? 3. Can soft tissue Ca isotope composition be quantitatively related to BMB? The centerpiece of our research was a suite of blood, urine, and diet samples collected in a 30 day, 12 subject bed rest study. Samples were analyzed for Ca isotope composition the University of Arizona’s W.M. Keck Foundation Laboratory for Environmental Biogeochemistry. With that aid of a mathematical model, data generated by the study allowed us to answer each of the three initial questions. How soon after the start of bed rest does a bone loss signal appear in urine and blood? All-subject average urinary Ca isotopes begin to show bone loss on the third day of bed rest. This signal persists throughout the bed rest and recovery periods and becomes statistically significant by day 9 of bed rest. Ca isotopes in urine reflect the onset of negative BMB at least ten times faster than radiological techniques like DEXA, which can detect changes in bone mass that require several months of bed rest to produce. What factors other than BMB can affect soft tissue Ca isotope composition? Several factors other than BMB possibly could affect soft tissue Ca isotope composition. Extraneous factors like changes in dietary Ca isotope composition and differences in geographic and dietary history between subjects can be adequately controlled for under the strict conditions of bed rest studies by normalizing each subjects Ca isotope composition to his or her individual average pre-bed rest value. The former factor—changes in dietary d44/42Ca—is harder to control for, but based on our data does not seem to obscure the bone loss signal under the carefully controlled experimental conditions of bed rest, in which all subjects are fed the same menu on the same ten day rotation. The mass of Ca in soft tissues is large enough to dampen meal-to-meal variations in d44/42Ca of absorbed Ca. This damping effect is enhanced in 24-hour pooled urine samples, which for that reason are ideal for Ca isotope analysis. Internal, physiological processes other than bone remodeling possibly also could affect soft tissue Ca isotope composition. Two processes in particular—the production of bile salts in the liver and excretion of Ca by the

	kidney—involve a mass of Ca large enough to potentially affect the Ca isotope composition of the whole body. We have investigated the effects of hepatic and renal Ca isotope fractionation using the quantitative model developed and calibrated to results of this and previous research on Ca isotope fractionation. Based on results of this model, we conclude that neither hepatic nor renal fractionation interfere with the interpretation of Ca isotope data from bed rest studies in terms of changes in BMB. Can soft tissue Ca isotope composition be quantitatively related to BMB? The mathematical model combined with data collected in this and previous studies allow us to estimate the average rate of bone loss during the 30 day bed rest period at ˜0.4%/month. This agrees well with rates of bone loss measured by DEXA in longer bed rest studies, which are about 1% over three months. Future plans Research on the Ca isotope technique is proceeding on three fronts. 1. Validation of the Ca isotope technique in spaceflight, using urine and blood and other biological samples collected from crew members on the International Space Station. 2. Development of a flight-ready instrument for measuring Ca isotopes in spaceflight. 3. Development of Earth-based clinical applications of the Ca isotope technique. The technique holds particular promise in the detection and monitoring of skeletal involvement in cancer. In particular, we are exploring potential applications to the early detection of skeletal involvement in multiple myeloma and other cancers.
Bibliography Type:	Description: (Last Updated: 10/09/2019)
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