Fiscal Year:	FY 2010	**Task Last Updated:**	FY 03/26/2010
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:**	05/19/2012
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
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Flight Program:			
Flight Assignment:	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 Loris (University of Wisconsin-Madison Geology Museum) Smith, Scott M (Human Adaptation and Countermeasures Division) Bullen, Thomas (USGS)		
Grant/Contract No.:	NNX08AQ36G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	We propose to develop 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 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 proposal team recently demonstrated the promise of this method in a published pilot study in which we measured calcium isotopes in a small suite of urine samples from a bed rest study. Here, we propose an expanded examination of bed rest samples, involving a larger number of subjects, measurements of blood and dietary samples as well as urine, and daily or even sub-daily sampling. This research would address critical questions unresolved by the pilot study.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	We are developing a technique that uses analyses of natural variations in the calcium isotope composition of urine, blood and other biological materials to measure changes in bone mineral balance. The focus of this research is 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 may provide a way of detecting incipient bone loss before it has produced any measurable change in bone mineral density, and long before it has progressed to osteopenia or osteoporosis. Because soft tissue Ca isotope composition changes very rapidly in response to changes in bone mineral balance, our technique also may be used to rapidly assess the effectiveness of treatments designed to alter bone mineral balance, greatly accelerating the pace of discovery of new treatments for metabolic bone diseases such as osteoporosis.
Task Progress:	Project efforts over the past year have progressed on two fronts.
	1. The sampling originally intended for year 1 was postponed to the end of year 2 because of unforeseen events at the bed rest facility. During year 2 we developed a sampling protocol that has been incorporated into a 12 subject, 30-day bed rest study. The 30-day bed rest study began October 2009. Samples from two patients in this study arrived at ASU in January 2010 and are currently being processed for Ca isotope analysis. The results from these initial samples are expected by mid April 2010. We expect all remaining patient samples to be delivered to us by the end of 2010. As we are analyzing the samples as they arrive, we expect the bulk of the analyses to be completed by April 2011.
	This sampling protocol will generate approximately 1750 urine, blood and food samples, and is by far the most detailed investigation of Ca isotopes ever undertaken in any field. It will provide the data needed to address the most important questions in order for Ca isotopic analysis to become an effective tool for measuring bone mineral balance in space flight. The core of the sampling protocol focuses on two periods of intensive sampling, which consists of per void sampling of urine and sub daily sampling of blood during times when bone mineral balance can be expected to change rapidly. These rapid changes should occur during the first week of bed rest and the first week of recovery. Data from these samples will allow us to determine how quickly changes in skeletal loading produce detectable changes in urinary and blood Ca isotope composition, and to begin to estimate how these changes vary between individuals. The data from these intensive sampling sections will allow us to resolve diurnal changes and other variations in urinary and blood Ca isotope composition. By measuring the isotopic composition of all dietary items containing significant Ca and obtaining the detailed records of what each subject ate, we will be able to reconstruct the isotopic composition of each subjects' dietary Ca on a meal by meal basis. This will allow us to disentangle the dietary signal in urinary and blood Ca isotope composition from the endogenous physiological signal that reflects changes in bone mineral balance.
	The importance of intensive sampling is highlighted by new data from a study that our lab conducted (in collaboration with the University of Wisconsin National Primate Research Center) in parallel with our bed rest study. This new study investigated the ability of Ca isotopes to detect bone loss caused by estrogen depletion in female rhesus macaques. Although not a formal part of our NASA project, the rhesus study provided us with urine and blood samples that allowed us to perfect sample preparation and purification techniques for use on human samples. Our pilot study of Ca isotopes in bed rest (Skulan et al, 2007) demonstrated that changes in bone mineral balance induce changes in urinary Ca isotope composition by the fourth week of bed rest, which was the earliest bed rest sample point in that study. The results of the rhesus study indicate that changes in Ca isotope composition can occur very rapidly, within one day of a bone loss stimulating event and that these changes most likely reflect changes in bone mineral balance.
	2. During year 2 we moved MC-ICP-MS analysis of biological samples from a theoretical possibility to a practical technique.
	As explained in our original documents, application of Ca isotopes to human samples requires a high-throughput analytical techniques. High-throughput is required because the changes being monitored happen rapidly, requiring the analysis of large numbers of samples in order to determine optimum sampling strategies for application to space flight. Previously, analyses of Ca isotopes has been done primarily using thermal ionization mass spectrometry (TIMS). TIMS analyses are slow, labor-intensive and cannot easily be automated. We are instead using multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS). This method intrinsically allows 5 – 10 times higher sample throughput than TIMS. It also offers the potential for an automated process for Ca.
	A key stumbling block to the use of MC-ICP-MS for such analyses of biological samples are interferences arising from chemical impurities in the samples. This is much more or a problem for MC-ICP-MS than for TIMS because, unlike TIMS, MC-ICP-MS simultaneously and completely analyzes all elements present in a sample. Early in year 2 analysis of rhesus urine and blood samples demonstrated that published techniques for purifying geological samples (the only

kind of samples previously analyzed) for MC-ICP-MS analysis were not sufficient for biological samples, which continued to have unacceptably large interferences after purification.

Over the course year 2 we identified the sources of these interferences and developed methods of removing them. We now are able to process and analyze samples from the bed rest study as they arrive, and are on target to finish these analyses in year 3.

Bibliography Type:	Description: (Last Updated: 10/09/2019)