Fiscal Year:	FY 2016	**Task Last Updated:**	FY 10/12/2015
PI Name:	Bloomfield, Susan A. Ph.D.		
Project Title:	Sclerostin's Role in Regulating Bone Formation during Long-term Simulated Microgravity and Subsequent Recovery		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Biomedical countermeasures		
Joint Agency Name:		**TechPort:**	No
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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Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2013 HERO NNJ13ZSA002N-Crew Health (FLAGSHIP & NSBRI)
Start Date:	10/06/2014	**End Date:**	08/31/2017
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	1	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	2	**Monitoring Center:**	NASA JSC
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Flight Program:			
Flight Assignment:	NOTE: Extended to 8/31/2017 per NSSC information (Ed., 1/24/17) NOTE: Extended to 10/05/2016 per NSSC information (Ed., 10/28/15)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):			
Grant/Contract No.:	NNX15AB05G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	The tissue sharing opportunity outlined in Appendix A, Item B ("Cerebral Spinal Fluid Production/Absorption....") offers an exciting chance to study the evolution of changes over 90 days of hindlimb suspension (HLS) in a rodent model. Few laboratories have the capability or expertise to carry out HLS for such a long period; the impact of this experiment will be multiplied many-fold with piggyback projects making strategic use of other tissues harvested from these animals. This proposal focuses on tracking the evolution of changes in a protein important to bone integrity called sclerostin over the course of 90 days of unloading and then during the 90-day recovery phase. Importantly, we will also track alterations in the key physiological function sclerostin regulates in bone: osteoblast activity resulting in the formation of new bone. Sclerostin is produced by osteocytes, the bone cells embedded in bone matrix and the key sensors of loading/unloading; it works to inhibit the Wnt-Beta catenin signaling pathway in osteoblasts, which normally stimulates bone formation activity. Most studies to date document an increase in osteocyte's sclerostin expression with unloading, which provides the mechanism for the suppressed bone formation seen with HLS on Earth and, presumably, with microgravity exposure. A recent study examining the impact of a novel therapeutic agent (sclerostin antibody) on mice flown on STS-135 yielded very positive findings, suggesting that manipulating sclerostin expression could be an important therapeutic tool to augment the usual exercise countermeasures employed by astronauts. Hence it becomes critical, before any such systemic therapy is considered, to understand clearly the relationship between sclerostin expression and the functionally important outcome (bone formation activity) in multiple bone sites. Because there is evidence that Sost, the gene encoding the protein sclerostin, is expressed differently in mid-shaft vs metaphyseal bone, we will assess sclerostin expression and histomorphometric measures of bone formation in 3 different bone compartments for each bone: mid-shaft cortical bone, cancellous bone of the metaphysis and the metaphyseal cortical shell. We propose to first study these outcomes in paired tibiae or femurs (unloaded bone) and in normally loaded humeri. With the availability of female mice in the parent study's 2nd specific aim, we can then assess if there are gender differences in this response. Recently published data on spaceflown mice document an increase in bone volume in calvarial bone, raising the intriguing possibility that fluid shifts during spaceflight may increase fluid pressures in the rodent brain compartment, providing a mechanical loading of sorts to the skull. Hence, a third specific aim of our proposal, pending verification that calvarial bone can be made available, will assess sclerostin expression and bone formation variables in this unique site to test this hypothesis. At the end of one year's intensive effort, then, we will have gained a much more clear picture of how this important regulatory molecule is altered by unloading and whether sclerostin antibody does present a viable therapeutic tool for maintaining bone integrity on long-duration missions. In addition, we will gain important fundamental knowledge about the time course of sclerostin expression and its relationship to bone formation rate with alterations in mechanical loading.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Phase III clinical trials testing the efficacy of sclerostin antibody (Scl-AB) are in progress, focusing on the value of this agent in reversing aging-related bone loss and osteoporosis. It will be very useful to have data on a physiologically relevant mammalian model (skeletally mature rats) yielding information on the efficacy of Scl-AB for bone loss due to prolonged disuse. This applies to individuals subjected to prolonged bed rest (complicated orthopedic injuries, frail elderly with severe illness) or to conditions like spinal cord injury or even stroke (if significant muscle paralysis is involved). Additionally, this study will provide a sex comparison, so we will have preliminary clues as to whether women might respond similarly as do men to this potent anabolic treatment.
Task Progress:	The first major accomplishment was the acquisition, installation and training with an upgraded OsteoMeasure image analysis system in the Bloomfield Bone Biology Laboratory, completed by November 15, 2014. The improved resolution of the digital camera and a digitizing screen that allows tracing right on the screen itself has resulted in improved speed on analyses of histomorphometry and of immunostaining sections. Both of these methods are central to the purpose of this project. We received our first shipment of bones from the parent protocol at UC-Davis in early April of 2015 (n ~ 43) from young adult male rats, including samples from animals sacrificed after 7, 14, 28, and 90 days of hindlimb unloading, as well as after 28 days of weight-bearing recovery. Our revised protocol submitted in August, 2014, after negotiations with Dr. Peter Norsk and the parent protocol Principal Investigator (PI) (C Fuller), described proposed work we would perform on the tibial bone samples targeted to our laboratory for analysis. Our first task was to run ex vivo pQCT scans to quantitate bone structural changes on both the metaphysis, a site very sensitive to disuse, and the mid-shaft bone. Outcomes here include volumetric bone mineral density (vBMD), bone mineral content, and cross-sectional geometry (e.g., bone area, cortical thickness, marrow area). Once these were complete, the proximal half of one tibia was prepared histologically for embedding in hard plastic in order for us to perform standard static histomorphometry on the proximal tibial metaphysis. Quantitating the extent of surfaces covered by newly formed bone matrix (osteoid) and by osteoclasts (bone resorbing cells) provides useful information on the balance between bone forming and bone resorbing activity. [Because the parent protocol PI was reticent to add more procedures to an already complicated protocol, there was no injection of fluorochrome labels in these rats during the experiments at UC-Davis, which disallows determination of bone formation rate.] Summary of results: To date, 2-month old male rats exposed to hindlimb unloading (HLU) demonstrate the expected reductions in vBMD at the proximal tibia metaphysis, as compared to age-matched controls at both 14 and 90 days of HLU. The trend of bone loss appears to remain consistent from 7 days through to recovery (28 days of weight bearing recovery following 90 days of HU). Our histomorphometry results thus far are not consistent with these structural changes in bone described by the pQCT data. Despite the bone loss, there is a paradoxical increase in osteoid surface (newly formed bone matrix yet to be mineralized) at both 14 days and 90 days with the suggestion of similar trends at both 28 days HLU and 28 days of recovery. Likewise, there is a decrease in osteoclast surface (a measure of bone resorption) in HLU at 14 days with no different between HLU and CC at 90 days. Limitations of our current work with this study include the low animal numbers at all time points except 14 days and 90 days, making it difficult to draw firm conclusions of the time course of HLU changes. These preliminary data will be presented at the NASA Human Research Program (HRP) Investigator Workshop in February 2016.
Bibliography Type:	Description: (Last Updated: 05/28/2021)