Fiscal Year:	FY 2018	**Task Last Updated:**	FY 03/23/2018
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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Zip Code:	77843-4375	**Congressional District:**	17
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2013 HERO NNJ13ZSA002N-Crew Health (FLAGSHIP & NSBRI)
Start Date:	10/06/2014	**End Date:**	11/06/2017
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:**	1
No. of Bachelor's Candidates:	2	**Monitoring Center:**	NASA JSC
Contact Monitor:	Norsk, Peter	**Contact Phone:**	
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Flight Program:			
Flight Assignment:	NOTE: Extended to 11/06/2017 per NSSC information (Ed., 12/7/17) 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 sex 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.
	The parent protocol at University of California (UC)-Davis apparently experienced many delays. Shipments have been received as recently as August 2017. This Principal Investigator (PI) requested and received two no-cost extensions in order to complete these analyses, given the delays in tissue availability. This allowed for analyses to extend beyond the young male samples to include young female and older male samples. There must have been a decision at UC-Davis to modify time points at which rats were sacrificed (or allocation of time points for tissue-sharing opportunities), since we never received samples from 4-d hindlimb unloading (HU) nor 4-d Recovery groups. We did receive samples from 14-d HU and 14-d Recovery animals. Further, there were many time points for which we had only 2-4 specimens, so decided to strategically focus our analyses on those time points for which we received at least 6 samples in matching groups. This was an unexpected, but welcome outcome; originally, we were cautioned to not expect more than 3 samples/timepoint/group. The only timepoint for which we received adequate numbers of samples from the female cohorts was at 14 d HU; hence, we were able to complete statistical analyses of sex differences for that one time point. These were the planned analyses; some are still in progress : A. Peripheral quantitative computed tomography (for BMD, bone geometry) a. Young males at 7, 14, 28, and 90 d of HU and 14, 28, and 90 d of recovery from HU b. Old males at 14 and 90 d HU and 14 and 90 d of recovery from HU c. Young females at 14 d HU B. Static histomorphometry for % osteoid (new bone; index of formation activity) and for % osteoclast (bone resorbing) surfaces; cancellous bone volume and microarchitecture a. Young males at 7, 14, 28, and 90 d of HU and 14, 28, and 90 d of recovery from HU b. Old males at 14 d and 90 d of HU and 14 and 90 d of recovery from HU (still in progress) c. Young females at 14 d HU C. Immunohistochemistry staining for osteocyte sclerostin a. Young males at 14 and 90 d of HU and 14 and 90 d of weightbearing (WB) recovery from HU b. Old males at 14 d and 90 d of HU and 14 and 90 d of weightbearing recovery from HU (still in progress) c. Young females at 14 d HU

Task Progress:	Summary of key findings (organized under original hypotheses): H1: Increases in Scl-IHC in osteocytes of cortical mid-shaft bone will be observed by 4 days of HLS in mid-shaft cortical bone, preceding a decline in formation indices. These increases will remain high throughout the period of HLS. H2: Scl-IHC in metaphyseal bone osteocytes (cortical shell and cancellous core) will follow a similar pattern as in mid-shaft osteocytes, but increases will be delayed till 7 days of HLS or later. Information in NASA Human Research Program (HRP) meeting 2016 poster: At 14 d of HU there was no detectable elevation of % sclerostin-positive osteocytes in any of 3 bone compartments analyzed (mid-shaft cortical bone, cortical shell and cancellous core of femur metaphysis) in young male rats. An unusual 3-fold elevation of % osteoid surface (our one bone formation index) was observed at this time point. We did observe a small decline over 14 d HU in total/integral and cancellous volumetric bone mineral density (vBMD) at the proximal tibia metaphysis (by pQCT measures). [NASA HRP 2016 poster:] We did observe ~50% increase in %sclerostin-positive osteocytes at 90 d HU but only in cancellous bone compartment. At this same time point, % osteoid surface remained elevated above weightbearing controls. The same % deficit in total/integral and cancellous volumetric bone mineral density (vBMD) at the proximal tibia metaphysis that was observed after 14 d HU was observed at 90 d HU, suggesting a plateauing of bone loss in the latter stages of this prolonged unloading period. H3: During the initial weightbearing recovery period, Scl-IHC will decline relative to peak HLS values in all bone compartments, preceding a local increase in formation indices. Scl-IHC and indices of bone formation will approach aging control values after 90 days of recovery. Answering this hypothesis awaits completion of sclerostin protein immunostaining osteocytes in recovery samples. H4: Relative changes in Scl-IHC and indices of bone formation, and the timing thereof, during HLS and recovery in young female rats will not be different from those observed in young male rats. Information in NASA HRP 2017 poster: There were no differences in the prevalence of %sclerostin-positive osteocytes between male and female rats after 14 d HU. However, females did not exhibit the increase in % osteoid surface as observed in males at this time point (no change vs. WB controls); they also exhibited a 25% increase in %osteoclast surface. Interestingly, pQCT scans could not detect declines in total/integral and cancellous vBMD at the proximal tibia metaphysis in these female rats as was observed in males. Histological (and likely more precise evaluation) of cancellous bone volume revealed a significant decline in females but not males. H5: Relative changes in Scl-IHC and indices of bone formation will be smaller in these older male rats (9-mo-old) with unloading and a return to weightbearing, but the relationship between magnitude of change in Scl-IHC and bone formation will not be altered by aging. Answering this hypothesis awaits completion of sclerostin immunostaining in older male rat samples. Information in Texas Chapter of American College of Sports Medicine (ACSM) 2017 poster: Older (9-month-old) male rats do not experience the significant declines (12-26%) in total/integral and cancellous vBMD at the proximal tibia, nor the decline in cancellous bone volume and trabecular number (by histomorphometry) at the proximal tibia metaphysis that were observed in young (3-month-old) rats after 14 days of hindlimb unloading. This could result, in part, from the fact that older male weightbearing controls have much lower values for these parameters than do the young controls, suggesting there is a lower inherent limit to these values.
Bibliography Type:	Description: (Last Updated: 05/28/2021)
Abstracts for Journals and Proceedings	Metzger CE, Bloomfield SA. "Altered osteocyte sclerostin with 90 days of hindlimb unloading." Poster Presentation at 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	Metzger CE, Bloomfield SA. "Altered osteocyte sclerostin with 90 days of hindlimb unloading." Poster Presentation at 2017 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	Anderson AM, Metzger CE, Bloomfield SA. "Disuse–induced bone loss is impacted by age." 2017 meeting of the Texas Chapter of the American College of Sports Medicine, Waco, TX, February 16-17, 2017. 2017 meeting of the Texas Chapter of the American College of Sports Medicine, Waco, TX, February 16-17, 2017. , Feb-2017