Fiscal Year:	FY 2013	**Task Last Updated:**	FY 06/06/2013
PI Name:	Midura, Ronald J Ph.D.		
Project Title:	Extent, Causes, and Countermeasures of Impaired Fracture Healing in Hypogravity		
Division Name:	Human Research		
Program/Discipline:	NSBRI		
Program/Discipline--Element/Subdiscipline:	NSBRI--Musculoskeletal Alterations Team		
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		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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City:	Cleveland	**State:**	OH
Zip Code:	44195	**Congressional District:**	11
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Project Type:	Ground	**Solicitation / Funding Source:**	2007 Crew Health NNJ07ZSA002N
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No. of Bachelor's Candidates:	0	**Monitoring Center:**	NSBRI
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
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Key Personnel Changes/Previous PI:			
COI Name (Institution):	Cavanagh, Peter (University of Washington) Muschler, George (The Cleveland Clinic Foundation) Warden, Stuart (Indiana University Purdue University at Indianapolis) Burr, David (Indiana University School of Medicine) Hill, Esther (Lockheed Martin Mission Services) Globus, Ruth (NASA Ames Research Center)		
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Task Description:

Lunar missions will expose astronauts to continuous hypogravity and bouts of strenuous physical exertion. Accidental fractures during missions could present a commander with a potentially life threatening situation and a serious reduction in team effectiveness. Thus, the scope and extent of bone healing in a space environment needs to be investigated, as well as the development of countermeasures to augment bone healing responses. The current work seeks to extend the findings from a previously funded project, NSBRI BL00405, which found that fibular osteotomy healing in hind limb unloaded (HLU) rats was delayed leading to a significant number of non-unions. Also, bone anabolic drugs decreased the incidence of fibular non-unions and improved the number of osteoprogenitor cells. Altogether, this suggests that fracture healing in space is not Earth normal and provides the rationale to further investigate whether impairment of fibular fracture healing would extend to more clinically relevant closed femoral fractures. Our global hypothesis is that long duration hypogravity impairs fracture healing. Our objectives are: (1) Determine the scope and extent of femoral fracture healing impairment, (2) Determine the underlying biological causes of the impairment, (3) Develop countermeasures to prevent fracture healing impairment, and (4) Determine whether current Earth-based clinical procedures will reverse severely delayed fracture healing situations resulting from hypogravity. HLU rats will undergo closed femoral fractures and healing will be assessed using (a) micro-CT bone imaging to evaluate hard callus structure, (b) hard callus strength via torsion testing, (c) callus tissue composition using histomorphometry, and (d) measurements of osteoinductive, chondrogenic and angiogenic factor expression during early healing periods.
In its first year, NSBRI MA01604 has determined by micro-CT imaging and histological analyses that closed femoral fractures in HLU rats exhibit a smaller hard callus healing response compared to weight bearing (WB) counterparts. This suggests that healing of closed femoral fractures in HLU rats is altered. Previously in our project NSBRI BL00405, we determined that an extended period of HLU impaired open fibular fracture healing in rats resulting in a non-union rate of >50% after 6-weeks of healing. However the extent of hard callus healing in HLU closed femoral fractures is of greater magnitude as compared to that exhibited by HLU open fibular fractures.

In its second year, NSBRI MA01604 has confirmed the initial findings that closed femoral fractures in HLU rats exhibit substantially smaller hard callus volumes (40-60% smaller than WB ones) even after 10-weeks of healing. Yet, torsion testing assessments of HLU vs. WB hard calluses indicated sound mechanical properties for both HLU and WB calluses, though the HLU calluses were more brittle. Histological assessments at 10-weeks indicate that the content within the HLU calluses is ~40% mineralizing tissue and ~20% soft/fibrous tissue. Assessments of gene expression and tissue alterations for early fracture healing timepoints (1 and 2 weeks post-fracture) correlating to the chrondrogenic phase (soft tissue callus formation) and the beginnings of the endochondral ossification phases (hard tissue callus formation) of fracture healing are complete. This analysis highlights a delay in endochondral ossification due to lagging chondrocyte hypertrophy, in effect delaying subsequent steps of the healing process such as angiogenic vessel infiltration and mineral deposition. Safranin O histological staining results of proteoglycan deposition within the fracture callus from WB and HLU rats suggested similar amounts of hyaline cartilage tissue in each test group. These histological findings are in agreement with those of gene expression findings whereby aggrecan mRNA levels were similar between groups at 1- and 2-weeks post-fracture. This prolonged chondrocyte maturation step exemplified by delayed hypertrophy, reduced osteo-inductive factor expression, and reduced pro-angiogenic factor expression likely leads to a postponement of the requisite vascularization of HLU callus tissue and its subsequent mineralization. While after a full 10 weeks of healing, it is apparent that HLU fractures heal, the data also indicate that the healing process itself may be altered as compared to fractures from WB rats. In fact, fractures from HLU rats are mechanically sound compared to fractures from WB rats. Yet the explanation for this adaptive healing response in HLU callus is not identified currently.

In its third and fourth years, NSBRI MA01604 utilized pharmaceuticals and biophysical stimulation in attempts to augment fracture healing in rat femora. Both PTH and LIPUS therapies have been shown to enhance the endochondral healing response of femoral fractures under normal gravity conditions. Scl-Ab therapy has also been shown to demonstrate a potent bone-building capacity in rats exhibiting estrogen-depletion osteoporosis and to enhance bone healing responses. Our observations indicate that closed femoral fracture healing response of the treated HLU rats parallels that of the ground based studies and all treatments appear to augment the fracture healing response by the fifth week of healing. Mechanical strength of the hard callus via torsion testing and histo-morphometry of the tissue composition at the fracture site assessments continue to be quantified and characterized. However our overall observations from the raw data indicate that the fracture healing process in treated rats as compared to untreated HLU rats has resulted in calluses with an increased mineralizing tissue content and increased mechanical properties.

Rationale for HRP Directed Research:

Research Impact/Earth Benefits:

Findings to date from NSBRI MA01604 show that closed femoral fracture healing in HLU rats (1) is delayed in the early stages of fracture healing, (2) demonstrate a slower rate of hard callus formation and a lower maximum callus volume, (3) but appears to provide adequate mechanical strength across the fracture site by 10-weeks of healing time. These findings are in contrast to those uncovered in NSBRI BL00405 whereby open fibular fractures produced smaller callus size that did not provide adequate mechanical strength across the fracture site and a 50% non-union rate. The nature of these alterations seems to be as a result of a delayed healing response for closed femoral fractures, but a more severe non-union response for open fibular fractures. The impact of this research and our previous research for NASA is that the rate of fracture healing and the integrity of the fracture callus seem to be altered under chronic simulated spaceflight conditions. Previous data obtained from our prior award period (NSBRI BL00405) suggested that treatments with a bone anabolic therapy (parathyroid hormone (PTH) peptide) seemed to partially counteract the impairment of bone healing under simulated spaceflight conditions. Our current award explored additional potential countermeasures in the third year and found that the two systemically delivered bone-anabolic drugs, PTH peptide and sclerostin monoclonal antibody (Scl-Ab)), and the anabolic biophysical modality low intensity pulsed ultrasound (LIPUS) augmented the fracture healing response as evidenced by increased rates of hard callus formation and larger maximum callus volumes by week 5 of fracture healing. The impact of this research for NASA is that these particular countermeasure approaches are practical and could potentially be used during a space mission to augment fracture healing. Further these countermeasures may also offer potential treatments for augmenting bone healing in Earth-bound, non-weight bearing patients. The overall implication of these findings for NASA is that select bone trauma repair responses on long duration space missions might be compromised in astronauts, and presents a potential threat to mission effectiveness and astronaut health. The research impact of our findings from MA01604 and those from our prior award BL00405, for Earth based medical practice, would suggest that an extended period of unloading and a cephalic fluid shift out of normally weight bearing lower extremity bones may manifest a delayed or an impaired bone healing response. This information may have relevance towards a better understanding of the underlying causes of impaired bone healing in

	patients experiencing paralysis, chronic immobility or extended bed rest. Further potential countermeasures to augment bone healing responses have been identified.
Task Progress:	IUPUI Aim 1: Hard tissue histology and histomorphometry of femoral fracture callus tissue from WB and HLU rats following a ten week healing period was completed in July of 2011. Specifically, the analysis of fracture callus tissue utilizes toludine blue and von Kossa stains to visualize and quantify amounts of cartilage and mineralized tissue, respectively. Results were presented by Dr. Kathleen Hill (Dr. David Burr's group, IUPUI) at the 41st International Sun Valley Workshop. Cleveland Clinic Aim 2: The results from this aim were completed and compiled for publication with our collaborators at NASA Ames Research Center (Drs. Ruth Globus and Esther Hill). The manuscript was submitted for review to Acta Astronautica in November 2011 and accepted in April 2012. It is currently in press but available on-line. Cleveland Clinic and IUPUI Aim 3: All animal trials utilizing the pharmacologic countermeasures (injections of PTH peptide or anti-sclerostin antibody), and the use of an anabolic biophysical modality (LIPUS) have been completed. All the in-vivo micro-CT and X-ray volumes have been analyzed and results reported. Since the completion of these analyses, programming code has been created for another study that allows us to go back and determine extent of hard callus bridging (percent union value) on a per time point basis. We anticipate using this code to give a better visualization of union in 3D versus the 2D data currently reported. All mechanical testing has been completed and the raw data collected. Data has been reviewed and we are currently finalizing the analyses of the data. Ex-vivo micro-CT callus contents will also be determined and correlated to our mechanical results. Similarly as above, a percent union analysis will be completed on the end-point volumes and correlated to our mechanical results. Histological quantification of callus content for the Aim 3 animals is still underway. Data analyses for these specimens will be the same as those presented for Aim 1. Specifically the quantification of fracture callus tissue will utilize the same histological techniques as in Aim 1. We anticipate finalizing data analyses over the next 6-9 months. The results from this aim will then be compiled for publication with our collaborators at IUPUI (Drs. David Burr and Stuart Warden).
Bibliography Type:	Description: (Last Updated: 03/01/2017)
Articles in Peer-reviewed Journals	Androjna C, McCabe NP, Cavanagh PR, Midura RJ. "Effects of spaceflight and skeletal unloading on bone fracture healing." Clinical Reviews in Bone & Mineral Metabolism. 2012 Jun;10(2):61-70. http://dx.doi.org/10.1007/s12018-011-9080-z , Jun-2012
Articles in Peer-reviewed Journals	McCabe NP, Androjna C, Hill EL, Globus RK, Midura RJ. "Simulated microgravity alters the expression of key genes involved in fracture healing." Acta Astronautica. In Press, Corrected Proof. Available online 25 April 2012. http://dx.doi.org/10.1016/j.actaastro.2012.04.016 , Apr-2012