Fiscal Year:	FY 2010	**Task Last Updated:**	FY 05/21/2010
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
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2007 Crew Health NNJ07ZSA002N
Start Date:	06/01/2008	**End Date:**	05/31/2012
No. of Post Docs:	1	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	1	**Monitoring Center:**	NSBRI
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:	Note change in Element, Risk, Gap to align with IRP Rev C, per JSC HRP (Ed., 5/25/2011)		
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 ARC)		
Grant/Contract No.:	NCC 9-58-MA01604		
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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 healing of fibular fractures in rats during actual spaceflight or under simulated hypogravity conditions is deemed to be impaired. This application seeks to continue funding of NSBRI BL00405 which found that fibular osteotomy healing in hind limb unloaded (HLU) rats was delayed leading to a significant number of non-unions, and was associated with a substantially reduced number of marrow-derived osteoprogenitor cells providing a partial explanation for impaired healing. 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, (d) colony forming unit assessments of marrow-derived osteoprogenitor cell numbers, and (e) measurements of osteoinductive, chondrogenic and angiogenic factor expression during early healing periods. In its first year, NSBRI MA01604 has determined visually using 3D micro-CT imaging that closed femoral fractures in HLU rats exhibit a smaller hard callus healing response when compared to weight bearing (WB) counterparts. In addition, this project has determined that HLU results in the regression of major blood vessels within the hind limb within the initial 2-weeks of HLU. Associated with this vessel regression was a noticeable drop in blood flow rates within the femoral artery. Altogether, these findings suggest that healing of closed femoral fractures in HLU rats is altered as compared to WB healing. The extent of HLU closed femoral hard callus healing is of greater magnitude as compared to 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 after 10-weeks of healing indicated sound mechanical properties for both HLU and WB calluses, though the HLU calluses were substantially more brittle than their WB counterparts. Thus, closed femoral fracture healing is not impaired and these findings are in stark contrast to those found for HLU open fibular fractures. Assessments for early fracture healing gene expression levels (osteogenic & angiogenic factors, and osteoprogenitor cell numbers) and callus tissue histology in the closed femoral fracture model in HLU versus WB adult rats are ongoing and final outcomes are expected in June 2010.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Findings to date from NSBRI MA01604 suggest that closed femoral fracture healing in HLU rats produces a smaller callus size, 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. The impact of this 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. The nature of these alterations seems to result in a somewhat delayed healing response for closed femoral fractures, but a more severe non-union response for open fibular fractures. The implication of these findings 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. Previous data obtained from our prior award period suggested that treatments with bone anabolic therapies seem to partially counteract the impairment of bone healing under simulated spaceflight conditions. Our current award will explore additional potential countermeasures in the third year and may also offer potential treatments for augmenting bone healing in Earth-bound, non-weight bearing patients.
Task Progress:	Cleveland Clinic (Aim 2) (1) 34 WB femora contained desired mid-femoral diaphyseal breaks of simple transverse or bending wedge patterns. 31 HLU femora contained desired mid-femoral diaphyseal breaks of simple transverse or bending wedge patterns. All others failed to fracture, were not localized to the mid-femoral region, or were of incorrect fracture pattern (fragmented or oblique). All unbroken femora will be utilized as controls for any gene expression changes due to medullary reaming and soft tissue injuries associated with the blunt impact of the 3-point bend device. (2) All callus tissues from 1-wk & 2-wk femoral fracture healing have been harvested and snap-frozen in liquid nitrogen. RNA harvest protocol from callus tissue has been optimized to increase the yield & purity of RNA harvested. (3) Quantitative PCR primer sets were redesigned to increase the specificity of amplification, and now span intron:exon junctions with at least one primer from each pair spanning an exon:exon junction. Efficacy of these primers is currently being assessed utilizing a real-time quantitative PCR instrument (ABI 7500). Gene expression analyses to be completed in June 2010. NASA Ames Research Center (Aim 2) (1) NASA-ARC arm of MA01604 aims to measure & evaluate the tissue histology within early phases of fracture healing. (2) Study included 4 groups, HLU and normal weight-bearing - 7 days and 14 days post-fracture. Animals (total 79 rats) were received, acclimated to cages and placed in HLU (standardized NASA analog developed by E. Holton) or WB groups. (3) Four weeks after initial HLU, animals underwent bilateral femoral fracture protocol. High resolution x-ray images of the femoral area were taken to verify complete fracture, confirm pin placement, & identify location of fracture. (4) All procedures were performed at ARC with the support of a team from the CCF Midura lab. This ensured that procedures were performed the same way at ARC as normally done at CCF. Also, this approach allowed us to complete the entire study in one experimental run, with significant savings of time and resources. (5) At 7-

	& 14-days post fracture, animals were euthanized. Blood was collected in heparinized tubes from all animals, including some baseline rats that were part of the original cohort received from the vendor, but housed in standard cages throughout the study. Plasma collected from the animals was stored for future analysis of stress markers. (6) Femora were collected and fixed for histology, and then sent to CCF for micro-CT imaging. Femoral samples were scanned and then decalcified at CCF prior to return to NASA-ARC. (7) Tissue embedding and staining procedures were established. Embedding, sectioning and staining of samples is currently in progress. Histomorphometry will be performed to measure the callus tissue composition, and completed in June 2010. Additional sections will be returned to CCF for immunohistological analysis.
Bibliography Type:	Description: (Last Updated: 03/01/2017)