Fiscal Year:	FY 2011	**Task Last Updated:**	FY 06/08/2011
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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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No. of Bachelor's Candidates:	1	**Monitoring Center:**	NSBRI
Contact Monitor:		**Contact Phone:**	
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 12/31/2012 per NSBRI (Ed., 5/1/2012) Note change in Element, Risk, Gap to align with IRP Rev C, per JSC HRP (Ed., 5/25/2011)		
Key Personnel Changes/Previous PI:			
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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, (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 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 year, NSBRI MA01604 has utilized pharmaceuticals and biophysical stimulation in attempts to augment fracture healing in rat femora. Both WB and HLU rats were given intermittent parathyroid hormone (PTH) injections to stimulate fracture healing and fractures were monitored longitudinally by micro-CT at multiple post-fracture time points. Analysis of this data is ongoing. Low intensity pulsed ultrasound (LIPUS) was also utilized to augment fracture healing in HLU rats. Preliminary findings indicate that fracture callus bridging occurs more rapidly in response to LIPUS than to sham treated femoral fractures. In addition to these findings, further analysis of fracture callus after 10 weeks of non-pharmaceutical or -biophysical stimulated healing by histomorphometrics confirms micro-CT data indicating that total callus volumes are decreased in HLU fractures and that HLU callus tissue contains reduced amounts of cartilagenous and fibrous tissues. Use of anti-sclerostin as a pharmaceutical method to augment healing has not been completed as yet due to delays in obtaining the antibody. We anticipate continuing this study as part of year 4 work.
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:	CC Aim 1: Mechanical testing of non-manipulated HLU & WB rat femora (no break, no titanium rod) has begun to determine what mechanical alterations, if any, occur as a result of intramedullary insertion of the titanium rod. Data is being compared to unfractured femora containing titanium rods. Aim 2: (1) Real-time qPCR of genes whose products are key players in the fracture healing process has been completed. This includes genes for the chondrogenic factors type 2 Collagen & aggrecan; genes for angiogenic factors Vascular Endothelial Growth Factor A (VEGF-A) & basic fibroblast growth factor (FGF-2); genes for the osteogenic factors bone morphogenetic proteins-2 & -7 (BMP-2 & BMP-7), as well as, genes for the osteogenic markers alkaline phosphatase, osteocalcin, & bone sialoprotein. (2) Gene expression analysis of femoral callus fracture tissue at 1- & 2-weeks post trauma has been completed. Quantification was completed utilizing Linear Regression of Efficiency with normalization to ribosomal 18S. Aim 3: Pharmacologic counter measures using intermittent parathyroid hormone (PTH) injections were conducted. Callus formation following femoral fracture was longitudinally monitored by micro-CT over a 10 week period. NASA ARC Aim 2: Tissue histology within the early phases of fracture healing was evaluated. This study included 4 groups, HLU & normal WB at 7- or 14 days post-fracture. Tissue sections from formalin fixed, TCA decalcified, paraffin embedded femora were assessed histologically by safrannin O/fast green. Callus volumes and hyaline cartilage content were quantified. IUPUI Aim 1: Hard tissue histology and histomorphometry of femoral fracture callus tissue following a ten week healing period will be completed in June 2011. Analysis of fracture callus tissue utilizes toludine blue and von Kossa/McNeal stains to visualize cartilage and mineralized tissue, respectively. Aim 3: One complete trial utilizing Low Intesity Pulsed UltraSound (LIPUS) as a modality to augment fracture healing in WB and HLU rats (n=12 per group) has been completed. A second trial was completed to investigate aspects of prerodding duration on fracture site stabilization. Groups were as follows: 1) fracture 4 weeks following prerodding (n=8), 2) fracture two weeks following prerodding (n=8), 3) fracture immediately following rodding, 4) rodded control (rodded for 4 weeks, no fracture; n=8), 5) cage control (no rod, no fracture; n=5). A third trial consisting of 20 animals with a healing time of 12 weeks is underway. Quantification of callus bridging for the trial 1 will be completed in June 2011. U of W Aim 1: The method for creating Finite Element (FE) mesh models to describe mechanical properties of healing femora has been streamlined. The FE model will be validated against specimens that have undergone torsional testing to enable model use in future studies to assess mechanical integrity without having to implement destructive testing.
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