Fiscal Year:	FY 2010	**Task Last Updated:**	FY 02/22/2011
PI Name:	Hogan, Harry Ph.D.		
Project Title:	Contributors to Long-Term Recovery of Bone Strength following Exposure to Microgravity		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
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:**	2007 Crew Health NNJ07ZSA002N
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No. of Post Docs:	0	**No. of PhD Degrees:**	0
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No. of Master's Candidates:	2	**No. of Bachelor's Degrees:**	1
No. of Bachelor's Candidates:	3	**Monitoring Center:**	NASA JSC
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Flight Program:			
Flight Assignment:	NOTE: New end date is 5/19/2012 per NSSC information (Ed., 5/31/2011)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Bloomfield, Susan (Texas A&M University) Martinez, Daniel (University of Houston)		
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Task Description:

The project uses the adult male hindlimb unloaded (HU) animal model with three specific aims and associated experiments. The first aim addresses the observed "discordant recovery dynamic" reported for astronaut data (Lang et al., JBMR 21:1224, 2006) and will characterize bone mass, bone mineral density (BMD), and bone strength relationships after HU and during various periods of recovery. Additional outcome measures include bone biochemistry and gene expression. A major emphasis is to compare detailed dynamics between the animal model and astronaut data. The animal model also permits direct comparison of calculated/estimated bone strengths with measured strengths. The second aim examines multiple mission scenarios and will use HU, recovery for a period, and then a second HU exposure. The third aim will also follow the two-exposure procotol but with exercise (both aerobic and resistive) added during the recovery period.
The cross-cutting area, or element, of the Bioastronautics Critical Path Roadmap (CRP) that this research project addresses is Human Health & Countermeasures (HHC). The specific health risk is the Risk of Accelerated Osteoporosis as identified in the Bioastronautics Roadmap (Risk No. 1, Bone Loss, p. 19 of NASA/SP–2004–6113) and the Human Research Program (HRP) Integrated Research Plan (Risk 14.0). The Gaps addressed, as defined in the HRP-IRP, are: B1 (Is bone strength completely recovered with recovery of BMD) ; B10 (Time-course of bone degradation during missions)

The 2007 NASA Research Announcement (NNJ07ZSA002N) to which the proposal for this project responded included the following specific solicitation wording for Gap B1: "There are preliminary indications that overall bone quality/strength does not recover at the same rate that bone mineral density recovers after spaceflight. It is not known if there is a long term health effect related to this discordant recovery dynamic." Research proposals are solicited that directly address this relationship. The specific topic solicited is: Novel research that defines the precise relationship between long term recovery of bone mineral density and bone strength/quality, including the effects of multiple spaceflights."

The research products to be generated from this project will mainly take the form of new knowledge about bone recovery from simulated microgravity and the response to a second exposure of simulated microgravity (following recovery from the first). In particular, insight will be gained into the interrelationships between three different, but crucial, types of bone parameters: bone mass, bone mineral density (BMD), and bone quality. Bone mass is characterized by size, shape, geometry, and bone mineral content (BMC) outcomes. Bone mineral density (BMD) has its typical meaning of the amount of mineral per unit volume, and it is thus a characteristic of bone properties at the tissue level (i.e., normalized for amount). The most basic and fundamental measure of bone quality is bone strength, and this will be measured directly with comprehensive mechanical testing of bone from multiple sites following each experiment. We will also quantify pertinent properties of the organic matrix (the unmineralized portion of bone), which is another important contributor to bone quality and forms the scaffold on which mineral nucleation occurs. Specifically, collagen content, gene expression, and cross-link maturity will be assessed. Very few of these outcome measures are available from human studies (bed-rest or flight-based). The results will define more completely the consequences of discordant recovery dynamics on long term skeletal health. The experiments will also quantify the risks of previous exposure to microgravity, plus recovery, on a second exposure to microgravity.

Three sets of experiments will be conducted to address three specific aims. In all cases, adult male Sprague-Dawley rats (6-mos.-old) will be used, and the period of initial HU will be 28 days. Recovery will be characterized in two ways: (a) by comparing to age-matched, ambulatory cage control animals (no HU, but same age); and (b) by comparing to values at the end of the initial 28 days of HU (day 0 of recovery). The major outcome variables to be examined are bone mass (size, geometry, BMC), bone mineral density (total, cortical, cancellous BMD), and bone quality (strength, plus measures of tissue-level organic matrix). Tissue-level organic matrix assays will quantify collagen content, cross-link maturity, and gene expression. These will be assessed for: (i) cortical bone in the mid-diaphysis (tibia and femur); (ii) mixed cortical and cancellous bone in the metaphysis (proximal tibia and distal femur); (iii) mixed cortical and cancellous bone in the femoral neck. Using these anatomic sites permits evaluation of the response of both cortical and cancellous bone, both separately and combined (integrally).

The main goal of Experiment 1 is to determine the extent of loss and the time course of recovery in bone outcomes following an initial period of HU (28 days). Bone mass and density outcome variables are derived from pQCT (peripherel quantitative computed tomography) scanning, which is done both in vivo (longitudinally) and ex vivo (at each time endpoint). In vivo scans are limited to the tibia midshaft and proximal metaphysis, but ex vivo scans will be made for a full slate of anatomic sites to provide an even more thorough approach. Specifically, ex vivo pQCT results will be generated at the following locations:

* Left Tibia ~ midshaft (primarily cortical bone)

~ proximal metaphysis (below knee, mixed cortical & cancellous)

* Left Femur ~ midshaft (primarily cortical bone)

~ distal metaphysis (above knee, mixed cortical & cancellous)

~ femoral neck (mixed cortical & cancellous)

A distinct advantage of animal studies is that bone strength can be measured directly using harvested bones and machined specimens. The specific sites, type of test, and bone tissue are:

* Left Tibia ~ 3-point bending of midshaft (primarily cortical bone)

~ RPC or ICC of proximal metaphysis (primarily cancellous bone)

* Left Femur ~ 3-point bending of midshaft (primarily cortical bone)

~ RPC or ICC of proximal metaphysis (primarily cancellous bone)

~ femoral neck (mixed cortical & cancellous bone)

* Right Femur ~ femoral neck (mixed cortical & cancellous bone)

Bone quality and underlying mechanisms will be studied and characterized through biochemical assays and gene expression quantification. This work will be done by Dr. Martinez at the University of Houston. The properties of the organic matrix will be assessed using remnants from mechanical testing specimens. The following outcome measures are

planned:

* Collagen Concentration ~ hydroxyproline (Hyp) by HPLC

* Collage Cross-Links ~ non-reducible hydroxylysylpyridinoline (HP) by HPLC. Six genes of interest have been identified for characterization using qRT-PCR (quantitative Reverse Transcription Polymerase Chain Reaction). These genes are:

* Col1a2 and Col3a1 ~ Extra Cellular Matrix (ECM) collagen genes

* MMP2 or 13 ~ ECM resorption marker genes

* Osteocalcin ~ mineral homeostasis and osteogenic marker

* Runx2 and Osterix ~ mechanical loading/unloading transcription factors

The aim of Experiment 2 is to determine the precise relationships between bone mass, BMD, and bone strength after a second exposure to 28 days of HU, following an initial 28 days of HU plus a recovery period. Two recovery periods interposed between HU exposures will be examined, 28 and 56 days. The recovery periods may be modified, however, based upon results from Experiment 1. Experiment 3 will also follow the two-exposure procotol but with exercise (both aerobic and resistive) added during the recovery period. Data will be analyzed to characterize and compare the effects of resistance training and treadmill running during recovery from 28 days of HU on the relationships between bone mass, BMD, and bone strength.

Bone losses observed in hindlimb unloaded adult rats parallel those in humans subjected to long-duration ISS or Mir flights; hence, this ground-based animal model is highly relevant to human astronauts. The new knowledge gained from the proposed studies will provide a better understanding of the factors affecting long term astronaut health in response to periods of exposure to microgravity. The results will provide direct, quantitative, and objective evidence for better defining the risks of space travel on long term crew member health. The results will also help define which factors are most critical to monitor in assessing recovery of bone health following single or multiple missions. From the last set of experiments, exercise countermeasure efficacy will be better understood by comparing resistance training and treadmill running when used during recovery from an initial exposure to microgravity. Results will characterize the relative effectiveness of these two types of exercise in reducing the risks of initial exposure on a second exposure to microgravity.

Rationale for HRP Directed Research:

Research Impact/Earth Benefits:

Results from this project will provide fundamental understanding of the way bone responds to mechanical unloading and how it recovers when mechanical loads are restored. Insights gained should be applicable to the clinically relevant case of aging adults with reduced activity levels. In addition, many of the same basic mechanisms overlap considerably with the broader health problem of osteoporosis and increased fracture risk in aging humans. It is widely known that bone mineral density (BMD) is not an accurate predictor of fracture incidence despite its wide use as a screening tool for osteoporosis. The findings of the research being conducted in this project will help define the relationships between BMD and other important factors, such as bone mineral content (BMC, i.e., bone mass), bone tissue quality, and most importantly bone strength. Further, the project will identify which anatomic sites in the rat provide the closest correspondence to bone loss and recovery characteristics in humans (astronauts in this case). These results should bolster the utility and robustness of rodent animal models and linking their findings to clinical cases. Finally, the project will compare and contrast the effects of aerobic and resistive exercise in restoring skeletal integrity during recovery from mechanical unloading. This information should be directly applicable to corresponding efforts aimed at using exercise to combat age-related losses from osteoporosis or related pathologies.

Task Progress:

[Editor's note 2/10/2011: No Task Book report received. Progress section and Bibliography compiled from PI's Annual Technical Report.]
As Year 2 ends and Year 3 begins, data analysis and interpretation of results from animal Experiments 1 and 2 remains the highest priority and continues in earnest. The in vivo animal protocols for Experiment 3 will begin in late summer or early autumn.

Several trends and conclusions are apparent from results analyzed thus far:

1. The results that most closely match astronaut data for bone mass (BMC) and density (vBMD) outcome variables are from the in vivo scans of the proximal tibia metaphysis. Loss and recovery dynamics for ex vivo scans of the distal femur metaphysis and proximal tibia metaphysis do not match as well.

2. For 'estimated' strengths, results from the ex vivo scans of the proximal femur metaphysis best match astronaut data.

3. Based on the limited results to date for mechanical testing of the femoral neck, the 'estimated' strength parameters Neck Compressive Strength Index (NCSI) and Neck Bending Strength Index (NBSI) do not predict actual measured strength values very well. Further, measured strength values to not mimick astronaut results for estimated strengths.

4. In analyzing gene expression in the tibia and femur, patterns are differentially expressed in different bones and within the same bone at different anatomic sites after HU. Also, initial reloading of 28d after HU demonstrated the greatest change in collective gene expression compared to longer durations of recovery from HU.

Another important development during Year 2 was consideration of additional analyses that could significantly leverage results from the studies. These possibilities arose from the Investigator Review Meeting held in November 2009, and also from the NASA HRP Workshop in February 2010. The specific idea is to have microCT analyses done for the metaphysis region of either the tibia or femur. This will be done on bones slated for mechanical testing, but no adverse effect is expected because microCT is non-invasive and non-destructive. Results will provide important insights into the microarchitecure of cancellous bone as well as additional density and mass outcomes.

Bibliography Type:	Description: (Last Updated: 01/11/2021)
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