Fiscal Year:	FY 2019	**Task Last Updated:**	FY 03/23/2019
PI Name:	Hargens, Alan R. Ph.D.		
Project Title:	Spinal Structure and Function after 90 Days Long-Duration Simulated Space Flight and 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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Comments:			
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Flight Program:			
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Key Personnel Changes/Previous PI:			
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Task Description:	The vertebral bodies and flexible intervertebral discs are important, weight-bearing tissues that have adapted to gravitational stress. Consequently, the absence of gravitational axial loads during exposure to microgravity likely disrupts normal spine physiology. Throughout longer space flight missions, deconditioning of the intervertebral discs and spinal muscles poses a serious injury risk upon re-exposure to upright posture in a gravitational environment. We will use state-of-the-art technologies to quantify morphology, biochemistry, and kinematics of spines (including the vertebrae, intervertebral discs, and spinal muscles) of rats at defined time points as described in the NASA research announcement. After successful completion of our investigation, we will deliver a comprehensive database of simulated microgravity-induced spinal adaptations (type and magnitude). The overarching goal of these proposed studies is to develop a long-duration space flight ground based model of spine function and structure. In addition, this research project will afford the opportunity to examine possible gender differences in spinal structure and function. Our research group is in a unique position to leverage our past rodent space flight experience on STS-131, STS-133, STS-135, and BION M-1 missions and directly compare to this ground based model of simulated microgravity. Moreover, we are also uniquely positioned to compare this 90-days hindlimb unloading model to those changes that occur in our currently funded project to test crew members before and after 6-month International Space Station (ISS) missions. Our project directly addresses Critical Path Roadmap Risks and Questions regarding disc injury (Integrated Research Plan (IRP) Gap-B4)): Is damage to joint structure, intervertebral discs, or ligaments incurred during or following microgravity exposure? Our research will improve understanding of the underlying pathophysiology of spinal deconditioning induced by simulated microgravity, and mechanisms of spinal adaptation following re-exposure to 1-G. Our long-term goal is to prevent such spinal deconditioning with exercise or other physiologic countermeasures. The goal of this research is to comprehensively characterize 90-days simulated space flight and recovery induced changes in spinal tissue morphology, biochemistry, and biomechanics.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	To our knowledge, this study is the first to examine the effects of 90-days simulated space flight on spinal deconditioning in rats and to compare this model of simulated microgravity with actual space flight. The vertebral bodies and flexible intervertebral discs are important, weight-bearing tissues that have adapted to gravitational stress. Our research will aid understanding of spinal deconditioning during simulated microgravity and of the higher incidence of disc prolapse or herniation following re-exposure to 1-G with a long-term view to prevent such spinal deconditioning with exercise or other physiologic countermeasures. This research may aid understanding of spinal deconditioning during inactivity such as after spinal cord injury and bed rest in human patients on Earth.
	INTRODUCTION The vertebral bodies and flexible intervertebral discs are important, weight-bearing tissues that have adapted to gravitational stress. Consequently, the absence of gravitational axial loads during exposure to microgravity likely disrupts normal spine physiology. Throughout longer space flight missions, deconditioning of the intervertebral discs and spinal muscles poses a serious injury risk upon re-exposure to upright posture in a gravitational environment. Hind-limb suspension of rats is accepted as a ground-based model of microgravity. This model involves elevating rodents by the tail to produce a head-down tilt of approximately 30 degrees, effectively unloading the hind limbs. This unloading model reproduces some physiological adaptations to spaceflight such as hind-limb bone and muscle loss, but it's unclear if this model is appropriate for studies of intervertebral discs. However, this rodent model provides an invasive way to test spinal biomechanics, not possible in humans exposed to actual or simulated microgravity. We have previously demonstrated that the caudal (tail) discs from mice exposed to 15 days of space flight had reduced bending strength and range of motion, as well as difference in failure sites using four-point bending tests. Control segments fail near the bone-disc endplate junction, whereas the space flight segments fail in the bone at the growth plate. In this study, we hypothesized that caudal motion segments subjected to hind-limb suspension would behave similarly to those from space flight rodents when compared to controls. Our research group was in a unique position to leverage our past rodent space flight experience on STS-131, STS-133, STS-135, and BION M-1 missions and directly compare to this ground-based model of simulated microgravity. Moreover, we were also uniquely positioned to compare this 90-days hind-limb suspension model to those changes that occur in our currently funded project to test crew members before and after 6-month International Space Station (ISS) missions. Our project directly addresses Critical Path Roadmap Risks and Questions regarding disc injury (IRP Gap-B4): Is damage to joint structure, intervertebral discs, or ligaments incurred during or following microgravity exposure? Our research will improve understanding of the underlying pathophysiology of spinal deconditioning induced by simulated microgravity, and mechanisms of spinal adaptation following re-exposure to 1-G. Our long-term goal is to prevent such spinal deconditioning with exercise or other physiologic countermeasures. The goal of this research is to characterize 90-days simulated space flight and recovery induced changes comprehensively in spinal tissue morphology, biochemistry, and biomechanics. METHODS To investigate the effect of hind-limb suspension, we analyzed the spines and discs of many groups of rats, comparing them to controls and previous flight mice on STS-131. Our joint University of California at San Diego-niversity of California at San Francisco (UCSD-UCSF) team made significant progress to integrate and implement tissue sharing procedures as part of our NASA award (NNX14AP25G). Initially, key team members, Dr. Brandon Macias of UCSD and Dr. Kazuhito Morioka of UCSF traveled to UC-Davis to train and implement rodent calvaria, spine, and tail tissue dissection/collection and storage procedures. Due to the reassignment of the trained NASA dissection team to NASA-Kennedy Space Center, additional training and travel to UC-Davis were required to train new NASA personnel at the UC-Davis site. Our team held an in-service at UCSD to finalize spinal cord expulsion procedures and to verify that this expulsion procedure would not damage vertebral and disc structures. These practice spinal cord procedures, practice tissues dissection, and freezing procedures were successful. Following successful verification of our procedures within out UCSD and UCSF team we worked to integrate and implement them at the UC-Davis site. In addition, our team reviewed and developed a tissue sharing plan within our UCSD/UCSF team. Tissues were centralized at UCSF. Following retrieval of the spinal cord tissue at UCSF, spines were shipped to UCSD for micro-computed tomography analysis of the vertebral bodies. Following completion of micro-computed tomography on tissues from UC-Davis, they were shipped to UCSF for intervertebral disc analysis. Overall this project required timely communication, coordination, and logistical planning to ensure high-quality tissue processing and research results.

Task Progress:

Vertebral Bone Studies

Thirty-seven Long Evans rats (3 months old) were separated into two groups, hindlimb-unloaded (HLU) group and weight-bearing control (WBC) group. Rats in the HLU group were subjected to hindlimb suspension, as reported by Morey-Holton and Globus, for 14 days (n=7, 14D), 90 days (n=8, 90D), and 90 days with 28 days of recovery (n=3, 90D/Recov). The hindlimb suspension was removed during the 28-day recovery period. The WBC group was not subjected to the suspension but was similarly divided into groups of 14D (n=7), 90D (n=9), and 90D/Recov (n=3). NASA-defined protocols were followed in the hindlimb unloading of the rats. After each time point, the groups were sacrificed, and lumbar spines were harvested.

The spines (Th12-S1) were scanned in a Skyscan 1076 Micro-CT scanner (Bruker, Kontich, Belgium) at 9 µm resolution (70kV, 141µA) with a 1.0 mm aluminum filter and hydroxyapatite phantom rods (4mm diameter, 0.25 and 0.75 g/cm3) for BMD (bone mineral density) analysis. Data viewer and CT-Analyzer software (Bruker, Kontich, Belgium) were used to analyze the spines for BMD (g/cm3) and bone morphology parameters, such as percent bone volume (bone volume/tissue volume, BV/TV), trabecular thickness (Tb.Th, mm), and trabecular separation (Tb.Sp, mm). To analyze IVD height, a custom written C++ program calculated the mean disc height distance (DHD) from vertebral endplates isolated in Mimics (Materialize, Leuven, Belgium). All data were analyzed using three or two-way ANOVA with Fisher PLSD post hoc tests ($p<0.05$). Level, group, and time point were treated as independent factors.

RESULTS

On average, intervertebral discs in control rats had a normalized strength of 0.49 ± 0.16 N/mm2 and unloaded had a value of 0.52 ± 0.16 N/mm2 ($p>0.05$). The stiffness and toe displacement of controls were 16.5 ± 6.87 N/mm and 0.67 ± 0.19 mm, respectively, while the unloaded group had a stiffness of 17.7 ± 6.88 N/mm and toe displacement of 0.66 ± 0.19 mm ($p>0.05$). Histological analyses showed consistent failure among all groups at the endplate junction, where the annulus fibers attach to the fibrocartilage of the bony endplate.

Vertebral Bone Results: There were no significant changes among all weight-bearing control (WBC) groups for all levels, groups, and time points.

Bone Mineral Density (BMD) analysis: Group and time significantly affected BMD. BMD of the 14D hindlimb-unloaded (HLU) and 90D HLU groups were significantly lower than those of the 14D WBC and 90D WBC groups, respectively (both, $p<0.05$). BMD of the 90D group was significantly lower than that of the 14D HLU group ($p<0.01$); however, BMD of the 90D/Recov HLU group was significantly higher than that of the 90D HLU and 14D HLU groups ($p<0.01$ for 90D HLU, $p<0.05$ for 14D HLU).

Bone morphological analysis: Similarly, group and time significantly affected BV/TV, Tr. Th, and Tr. Sp. The 14D HLU and the 90D HLU groups had a significantly lower BV/TV than those of corresponding WBC groups (vs. 14D WBC, vs. 90D WBC, respectively, both $p<0.01$). There was no significant progression of BT/TV decrease between the 14D HLU and 90D HLU groups after the initial decrease; importantly, BV/TV of the 90D/Recov HLU group was significantly greater than that of 90D HLU group ($p<0.01$). Tr. Th of the 14D HLU group was significantly lower than that of the 14D WBC and 90D HLU groups ($p<0.01$, respectively). The 90D/Recov HLU group had a significantly higher Tr. Th those of the 14D HLU, 90D HLU, and 90D/Recov WBC groups ($p<0.01$ for all). For Tr. Sp, the 90D HLU group had a significantly higher separation than that of 90D WBC group ($p<0.01$). Tr. Sp of the 90D HLU group was significantly greater than of the 14D HLU group ($p<0.01$); no significant difference was observed between the 90D HLU and the 90D/Recov HLU groups.

Disc height distance (DHD) analysis: Group and time significantly affected DHD. DHD of the 90D HLU group was significantly lower than that of the 90D WBC group ($p<0.01$). DHD differences were not significant in the HLU and WBC groups of the 14D and 90D/Recov time point. The 14D HLU DHD was a significantly higher than that of the 90D HLU and 90D/Recov groups ($p<0.01$ for both). Importantly, there were no significant differences in DHD between the 90D HLU and the 90D/Recov HLU groups.

DISCUSSION

There were no statistically significant differences in biomechanical values or failure mode between control and hind-limb suspended groups. These results suggest that hind-limb suspension may not be a useful model for detecting the effects of unloading on caudal (tail) intervertebral discs of rats. Previous studies of the hind-limb suspension model in our lab document that approximately 50% of the rat's body weight is taken up by tension on the tail. This is not the case in actual microgravity where the intervertebral discs are unloaded except during muscle contractions that are a mild non-gravitation mode of disc loading.

Bone Mineral Density (BMD) analyses of rat vertebral bodies document a significant decrease in the 90D hindlimb-unloaded (HLU) group compared to the 14D HLU group and a significant recovery in BMD in the 90D/Recov group compared to the 90D HLU group. The initial decrease in BMD agrees with previous studies that reveal a similar decrease in mice sent on a 15-day space mission. BMD and bone morphological parameters generally show a significant difference between the 90D HLU and the 90D/Recov HLU groups. However, there are no significant differences between the HLU and WBC groups of the 90D/Recov time point in BMD, BV/TV, Tr. Th, and Tr. Some results suggest that a recovery period can aid in bone recovery to baseline conditions. To examine the extent of bone recovery after hindlimb suspension, future analyses of the bone recovery at different time points is needed.

SIGNIFICANCE

Extended unloading of the hindlimb decreases BMD, bone morphological parameters, and progressive decrease of intervertebral discs height. The 28-day recovery period, where the hindlimb suspension is removed, aids in bone recovery but is ineffective in intervertebral discs height restoration. To our knowledge, this study is the first to examine the effects of 90-days simulated space flight on spinal deconditioning in rats and to compare this model of simulated microgravity with mice exposed to actual space flight. Thus, the vertebral bodies and flexible intervertebral discs are important, weight-bearing tissues that adapt to gravitational stress.

RESEARCH IMPACT/EARTH BENEFIT

Our research will aid understanding of spinal deconditioning during simulated microgravity and of the higher incidence of disc prolapse or herniation following re-exposure to 1-G with a long-term view to prevent such spinal deconditioning

	with exercise or other physiologic countermeasures. This research may aid understanding of spinal deconditioning during inactivity such as after spinal cord injury and bed rest in human patients on Earth. ACKNOWLEDGMENTS: We thank the Animal Resources Center at the University of California, Davis and the Space Biosciences Division at NASA Ames Research Center.
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Awards	Snyder A. "Medical student mentee, American Physiological Society Exercise & Environmental Physiology Section's National Space Biomedical Research Institute's Gravitational Physiology Predoctoral Award, Experimental Biology, Boston, MA, April 2015." Apr-2015
Awards	Siamwala J. "Postdoctoral mentee. American Physiological Society Exercise & Environmental Physiology Section's National Space Biomedical Research Institute Gravitational Physiology Beginning Investigator Award for abstract, Siamwala JH, BR Macias, R Healey, and AR Hargens. 'Cerebral Vascular Changes in Space Mice Calvaria.' Experimental Biology, Boston, MA, April 2015." Apr-2015
Awards	Khieu K. "Kristine Khieu, a UCSD senior in Bioengineering, received the 2017 USRA Frederick A. Tarantino Memorial Scholarship Award. She was selected from among 112 eligible applicants for one of 6 USRA scholarships. October 2017." Oct-2017
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