SIMULATED GALACTIC COSMIC RADIATION EXPOSURE IMPAIRS MOUSE VERTEBRAL BONE ADAPTATIONS TO EXERCISE DURING RECOVERY FROM PARTIAL WEIGHTBEARING

An Undergraduate Research Scholars Thesis

by

KATHERINE ANNE ELMER

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

Approved by Research Advisor: Susan A. Bloomfield, PhD, FACSM

May 2013

Major: Kinesiology

TABLE OF CONTENTS

Page

ABSTRACT 1

DEDICATION 3

ACKNOWLEDGEMENTS 4

NOMENCLATURE 5

CHAPTER

I INTRODUCTION 6

II METHODS 9

- Experimental design 9
- Simulated galactic cosmic radiation 10
- Partial weightbearing 11
- Resistance exercise 12
- Animal terminations 14
- Micro-computed tomography of L4 vertebrae 15
- Compression test of L4 vertebrae 16
- Ash weight: L5 vertebrae 16
- Statistical analyses 17

III RESULTS 18

IV CONCLUSIONS 23

- Affected populations 26
- Final conclusions 27

REFERENCES 28

ABSTRACT

Simulated Galactic Cosmic Radiation Exposure Impairs Mouse Vertebral Bone Adaptations to Exercise During Recovery from Partial Weightbearing. (May 2013)

Katherine Anne Elmer
Department of
Health and Kinesiology
Texas A&M University

Research Advisor: Dr. Susan A. Bloomfield
Department of
Health and Kinesiology
Graduate Faculty of Nutrition & Food Science
Texas A&M University

Partial weightbearing that simulates Lunar gravity (one-sixth of Earth's gravitational force) results in a loss of bone volume. High-energy radiation like that found in galactic cosmic radiation exposure also negatively affects the skeleton. Because resistance training is the most effective exercise mode to counteract disuse-induced bone loss, this experiment combined low-dose, high-energy simulated galactic cosmic radiation (GCR) exposure, followed by a period of partial weightbearing (PWB), and then a period of climb training resistance exercise or normal cage activity during recovery. *Ex vivo* micro-computed tomography (μCT) scans were performed by Matthew Allen, PhD at the Indiana University School of Medicine to quantify cancellous bone microarchitecture in the 4^{th} lumbar vertebral body before biomechanical compression tests were performed at Texas A&M University. Ash weights were calculated on the fifth lumbar vertebrae. Means for cancellous bone volume (%BV/TV), trabecular thickness (Tb.Th.), trabecular number (Tb.N.), ash weight, and maximum stress from Day 42 of the experiment were

compared to Day 21 means using unpaired *t*-tests to determine the changes occurring through the recovery period. These change scores were then analyzed using a two-way ANOVA to determine differences across experimental groups. Exercise had no significant effect on ΔBV/TV or ΔTb.Th., but ΔBV/TV and ΔTb.Th. were significantly lower in RAD groups than in SHAM groups ($p<0.001$). Additionally, Ex SHAM bones showed gains in cancellous bone mass and trabecular thickness during the recovery period. SHAM groups increased in trabecular Tb.Th. during recovery, while the trabeculae of RAD bones became thinner. ΔTb.N. was significantly higher in exercised groups than non-exercised groups ($p<0.05$), but no significant differences in ΔTb.N. were shown between RAD and SHAM groups. Ash weights showed significant differences in bone mineral content between Ex RAD and Ex SHAM groups. While maximum stress data did not show significant changes during recovery, the trends mirror those seen in other tests of bone integrity. These data suggest that GCR exposure diminishes the ability of bone to respond to exercise during recovery form a period of reduced weightbearing.

DEDICATION

This undergraduate thesis is submitted in honor of the incredible men and women who have devoted their lives to the exploration of the final frontier, and to all those who support them. It is my privilege to dedicate this small project to the astronauts who bravely venture out into unexplored territories, to the scientists and physicians that log countless hours of crucial work behind the scenes, and to the administrative entities and governing bodies who make space investigation possible. This is for you. In addition to the extensive contributions you all have made to the scientific community, the vast influence of your work has been, and will continue to be felt for many years to come. Your stories have inspired generations of young people to believe in themselves, trusting that their greatest aspirations just might become a reality. That in itself is something to be proud of. I hope that at some point in my lifetime, I can make even a sliver of that kind of impact on another human being. Thank you so much for your courage, your commitment, and your contributions. You really are some of the truest American heroes.

ACKNOWLEDGMENTS

This study was funded by the National Space Biomedical Research Institute via NASA Cooperative Agreement NCC 9-58. The author would like to gratefully acknowledge the help of Drs. Adam Rusek and Peter Guida at NASA's Space Research Laboratory at Brookhaven National Laboratory for assistance with the radiation exposures, and Dr. Matt Allen at Indiana University School of Medicine for performing the μCT scans on the fourth lumbar vertebrae.

The author would like to thank her advisor, Dr. Sue Bloomfield, for making such an incredible investment in her, especially at such a young age. The author is very appreciative of everything.

The author would like to acknowledge the wonderful assistance provided by her mentor and friend, Ray Boudreaux. Were it not for you, this project never would have been completed. Thank you for sharing your vast statistics knowledge, for all of your help with compression testing, for your constant availability, and for listening to *countless* presentation run-throughs.

The author would like to extend her extreme gratitude to the peers who provided support through this process: Corinne Metzger, Kaleigh Camp, Evelyn Yuen, Dr. Brandon Macias, Yasaman Shirazi, and Michael Hedges. The author would like to thank you for immediately welcoming her in, for calming her nerves during stressful times, and most importantly, for your friendship.

Finally, the author would like to thank Stephen Franklin for his infinite and invaluable support.

NOMENCLATURE

GCR	low-dose, high-intensity galactic cosmic radiation (0.5 Gy ^{56}Fe)
PWB	partial weightbearing (1/6th of Earth's gravitational pull)
resistance exercise	weightbearing exercise (climb training protocol)
NSBRI	National Space Biomedical Research Institute
BNL	Brookhaven National Laboratory, Upton, NY 11973
Experimental Groups:	
-SHAM21	PWB group not exposed to GCR, terminated Day 21
-RAD21	PWB group exposed to GCR, terminated Day 21
-No Ex SHAM	PWB group not exposed to GCR, allowed to recover normally, terminated Day 42
-Ex SHAM	PWB group not exposed to GCR and then resistance exercised during recovery, terminated Day 42
-No Ex RAD	PWB group exposed to GCR and then allowed to recover normally, terminated Day 42
-Ex RAD	PWB group exposed to GRC and then resistance exercised during recovery, terminated Day 42

CHAPTER I

INTRODUCTION

In order to ensure the lifelong health and safety of our nation's best and brightest astronauts, it is essential to understand the physiological mechanisms involved in spaceflight-induced bone loss. More importantly, it is necessary that we understand and implement methods to prevent this degeneration and mitigate its harmful effects once astronauts return to Earth.

During spaceflight, astronauts are exposed to numerous environmental stimuli. The environmental alterations accompanying spaceflight are believed to be the cause of physiological changes observed in human astronauts upon their return to Earth. Spaceflight-induced bone loss, associated with increased bone resorption and decreased intestinal calcium absorption, is one of the major biomedical challenges for human long-term missions in space (14). Aside from obvious gravitational differences inevitably encountered when leaving the Earth's atmosphere, astronauts also endure chronic exposure to galactic cosmic radiation (GCR) while on missions. Galactic cosmic radiation is a low-dose, high-energy radiation that has a profoundly negative influence on bone mass, microarchitecture, and strength (3,6). Heavy iron particles are the most biologically damaging component of galactic cosmic radiation (16), which is of great concern due to the recent drive to implement longer-duration Lunar and Martian spaceflight missions. When low doses of GCR are combined with musculoskeletal disuse, the loss of mechanical competence in mouse lumbar spine bones is worsened (1). Although we have knowledge of the effects of simulated spaceflight on spinal bone, little information exists on vertebral bone adaptations to climb training exercise during recovery from these treatments.

While it is difficult to perform experimental analyses on astronauts themselves due to a very limited sample size, the use of appropriate animal models is an appropriate alternative (13). In order to better understand how to prepare astronauts for their future endeavors, this study utilized an animal model to simulate a spaceflight mission. This experiment involved an initial exposure to heavy iron particles in an accelerator beam at Brookhaven National Laboratory, which mimics the effects of galactic cosmic radiation that astronauts encounter during spaceflight. This was followed, after shipment of the research animals to Texas A&M, by a 21-day period of partial weightbearing, equivalent to simulated lunar gravity. A 21-day recovery period followed, where half of the animals took part in a resistance exercise regimen using a climb training model.

Low back pain is one of the most frequently reported medical complaints from astronauts during spaceflight, with sixty-eight percent of astronauts reportedly affected (19). The commonly accepted cause of this discomfort is due to spinal lengthening associated with a stretch of the intervertebral disk space (11), but of primary interest in this study are the physiological changes that are occurring to the bones in the vertebrae of those astronauts. Resistance exercise is widely accepted as a useful and necessary tool to mitigate spaceflight-induced bone loss and its resulting complications. Currently, astronauts utilize treadmills, cycle ergometers, and the Advanced Resistive Exercise Device (ARED) on the International Space Station, and are encouraged to participate in resistance exercise protocols to regain some of their musculoskeletal losses after returning to Earth.

The ultimate goal of this experiment is to simulate an astronaut's spaceflight experience and analyze resulting changes in bone integrity. This includes simulated galactic cosmic radiation exposure and lunar gravity, followed by a return to Earth coupled with resistance training exercise after landing, a protocol included in the current astronaut rehabilitation regimen. From that simulation, we will determine whether or not exposure to GCR prior to a period of PWB will affect lumbar vertebral bone's ability to respond to exercise during recovery. We hypothesized that exercise would be osteogenic during recovery from a period of PWB in non-irradiated animals. Furthermore, radiation exposure just preceding the PWB period was expected to impair the bone's ability to recover with exercise. This study is the first of its kind to examine the results of exercise on recovery from exposure to simulated galactic cosmic radiation and partial weightbearing.

CHAPTER II

METHODS

Experimental design

Young adult (4 months old) female BALB/c mice were randomly assigned to one of six experimental groups: SHAM21, RAD21, No Ex SHAM, Ex SHAM, No Ex RAD, and Ex RAD. The experimental design is illustrated in Figure 1. Animals were initially assigned to gravitational environment groups. From there, animals were further divided into irradiated or non-irradiated groups. Mice allocated to the irradiated groups (RAD) underwent simulated galactic cosmic radiation, or 0.5 Gy ^{56}Fe, 1 GeV. Groups not exposed to radiation (SHAM) underwent all of the same handling and procedures as the RAD group to ensure similar stress levels across all animals. SHAM21 and RAD21 animals were terminated on Day 21 to provide an initial point from which to make comparisons about changes in bone integrity during the period of recovery. Finally, animals were then allowed to recover from their 21-day period of PWB in one of two groups: normal, or “resting” recovery (No Ex) or resistance training exercise during recovery (Ex). The recovery period lasted an additional 21 days, for an experiment total of 42 days. On Day 42, all remaining animals were terminated and their tissues harvested. This study specifically analyzed the portion of Figure 1 outlined in the black box. This allowed for the determination of what was occurring between Days 21 and 42, during the period of recovery from reduced weightbearing. All animal procedures herein were approved by the Texas A&M Institutional Animal Care and Use Committee (IACUC).

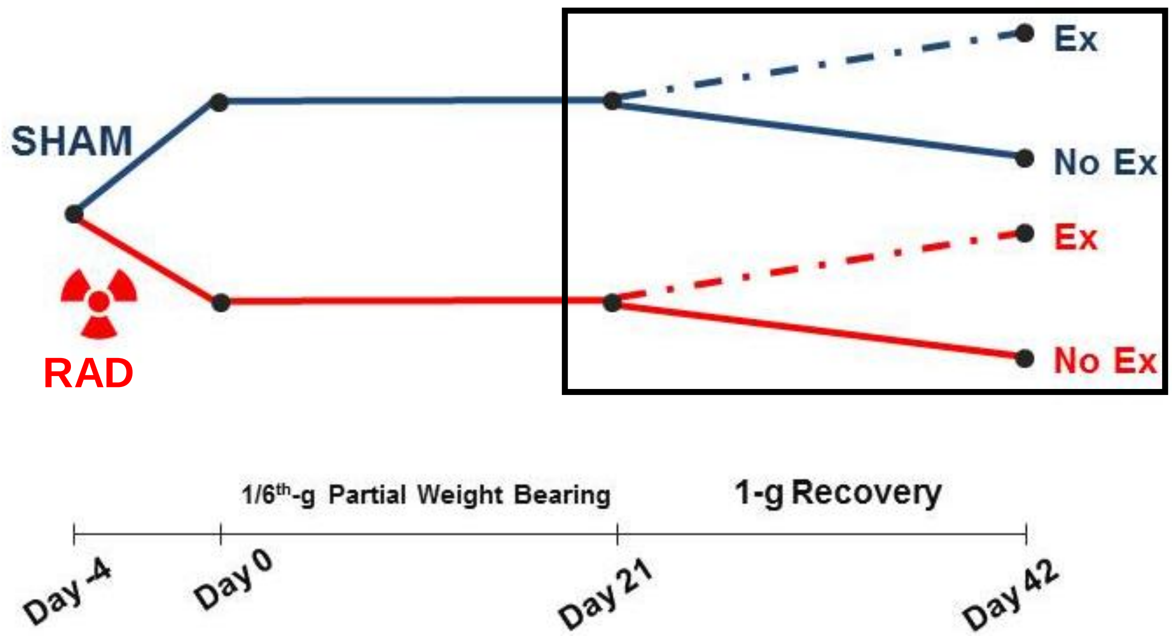


Figure 1. Experimental design detailing initial GCR/SHAM exposure followed by a 21-day period of PWB and concluding in exercise or rest during a 21-day recovery period. Diagram courtesy of Mr. Ramon Boudreaux (4).

Simulated galactic cosmic radiation

Mice were delivered directly from the vendor to the Brookhaven National Laboratory (BNL) Animal Facility and allowed to acclimate for 3 weeks. On the day of radiation exposure, mice were transported one mile to NASA's Space Radiation Laboratory (NSRL) on the BNL campus. All mice were placed in ventilated 50-mL centrifuge tubes. RAD groups were shuttled into the beam room to receive radiation exposure, while SHAM groups underwent all procedures except transportation into the beam room and the actual exposure. This SHAM procedure ensured that skeletal adaptations were not due to increased animal stress levels. Whole-body exposure of the conscious mice to 0.5 Gy of ^{56}Fe at 1 GeV was performed in order to take into account all possible tissue effects. These include those direct effects on hindlimb and forelimb bones and possible indirect systemic effects. The radiation exposure lasted approximately two minutes, and mice were restrained in the centrifuge tubes for 20 minutes or less. These procedures were performed by Dr. Bloomfield, assisted by her graduate students Brandon Macias and Ramon Boudreaux.

Partial weightbearing

Upon their arrival at the Texas A&M Comparative Medicine Program's (CMP) main facility (LARR) from BNL, all mice were allowed to acclimate in single housing for 2 days. The mice assigned to PWB groups (SHAM21, RAD21, No Ex SHAM, Ex SHAM, No Ex RAD, Ex RAD) were placed into shoulder and tail harnesses developed by Wagner *et al.* (17) under isofluorane anesthesia, as shown in Figure 2.b . PWB mice were then transferred to plastic Lucite 13" square cages (Figure 2.a.) and allowed to continue their 21 days of partial gravity. The partial weight suspension rodent model has been shown effective in providing a full-body partial-gravity experience (15). PWB animals were titrated to one-sixth of their body mass so that the load placed on the animal simulated gravity present in a Lunar environment. This titration was accomplished by taking the true weight of the animal, dividing that value by six, and adjusting the spring portion of the harness up or down until the scale read the adjusted measurement. Tissues of animals terminated on Day 21 were harvested to use as a means of comparing bone integrity from Day 21 to Day 42 of the study. This allowed for a determination of the effect of bone to respond to exercise during a period of recovery from simulated galactic cosmic radiation and reduced weightbearing.

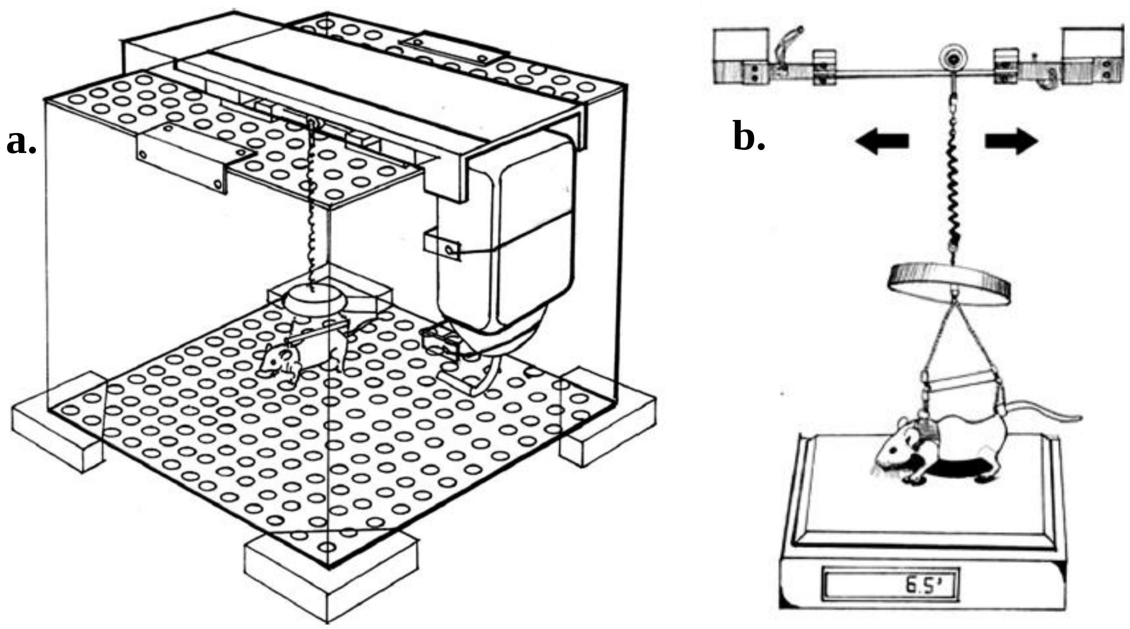


Figure 2. a.) PWB cage where animals underwent 21 days of reduced weightbearing. b.) partial weight suspension model showing the titration of adjusted animal weights. Both figures courtesy of Dr. Ericka Wagner (19).

Resistance exercise

All animals previously exposed to PWB were removed from their harnesses and allowed to return to normal cage activity on Day 21. On Day 22, animals assigned to the exercise group began a climb training regimen during which they climbed a one-meter wire mesh screen positioned at an 85° incline, as shown in Figure 3.a. Mice began their training regimen by climbing up the tower 50 times with no attached weight, and ended their final session by completing 5 climbs with 60% of their body mass taped to their tails and 31 climbs with 75% of their body mass taped to their tails. The complete climb schedule is outlined in Table 1**.** As the training sessions progressed, the number of climbs completed decreased, but the amount of additional weight taped to the mice tails increased. This ensured the mice would be perform the

Table 1. Climb training model of resistance exercise.

Day	**Training Regimen (# climbs performed / % body mass lifted)**
22	50 / 100%
23	25 / 100% + 25 / 125%
24	25 / 100% + 25 / 125%
25	**Rest**
26	5 / 100% + 45 / 125%
27	5 / 100% + 45 / 125%
28	5 / 100% + 45 / 125%
29	**Rest**
30	5 / 125% + 37 / 150%
31	5 / 125% + 37 / 150%
32	5 / 125% + 37 / 150%
33	**Rest**
34	5 / 150% + 34 / 160%
35	5 / 150% + 34 / 160%
36	5 / 150% + 34 / 160%
37	**Rest**
38	5 / 160% + 31 / 175%
39	5 / 160% + 31 / 175%
40	5 / 160% + 31 / 175%

same amount of work (weight lifted times distance traveled) during each training session for the duration of the protocol. Mice were trained once a day for three days, followed by one day of rest. This cycle was completed five times, for a total of 15 climbing sessions.

Animal terminations

Calcein injections to label mineralizing bone were given on days -9, -8, and -3, -2 before the terminations on Days 21 and 42. On termination days the animals were anesthetized with a cocktail of ketaset and dexdomitor (3:2 ket:med) then euthanized by decapitation, and tissues collected. The spine (from the sacrum to the attachments of the ribs) was dissected out of each animal, wrapped in gauze that had been soaked in phosphate-buffered saline solution (PBS), placed into a vial, and then covered with additional PBS. Samples were stored in -30°C until individual vertebrae were to be isolated. On the day that the dissections took place, the entire spine was allowed to thaw, and the fourth (L4) and fifth (L5) lumbar vertebrae were dissected out, re-wrapped in PBS-soaked gauze, and transferred to individual vials. L4 vertebrae were sent to University of Indiana School of Medicine (Indianapolis) for μCT analysis. L5 vertebrae were saved for determination of ash weight, which yielded the weight of the bone mineral remaining after all cellular matter was burned off. Upon their arrival back from Indianapolis, L4 vertebrae underwent mechanical compression tests to determine stress and strain, failure load, stiffness, and total deflection. The combination of compression test data, μCT analyses, and ash weights provide comprehensive data to aid in the determination of overall integrity of the bones.

Micro-computed tomography of L4 vertebrae

Matthew R. Allen, PhD and the Department of Anatomy & Cell Biology at Indiana University School of Medicine in Indianapolis performed micro-computed tomography (μCT) scans of the fourth lumbar vertebrae (Figure 3.b). Scans were taken using a piXarray 100 Digital Specimen Radiography System (Bioptics, Inc., Tucson, AZ). Using a 60-kV source voltage with a 6-μm pixel size, images were acquired at 24 kV with an integration time of 400 ms. Structural parameters, including cancellous bone volume, trabecular thickness, and trabecular number, were measured by Skyscan™ CT-analyzer software (SkyScan 1172; SkyScan, Kontich, Belgium).

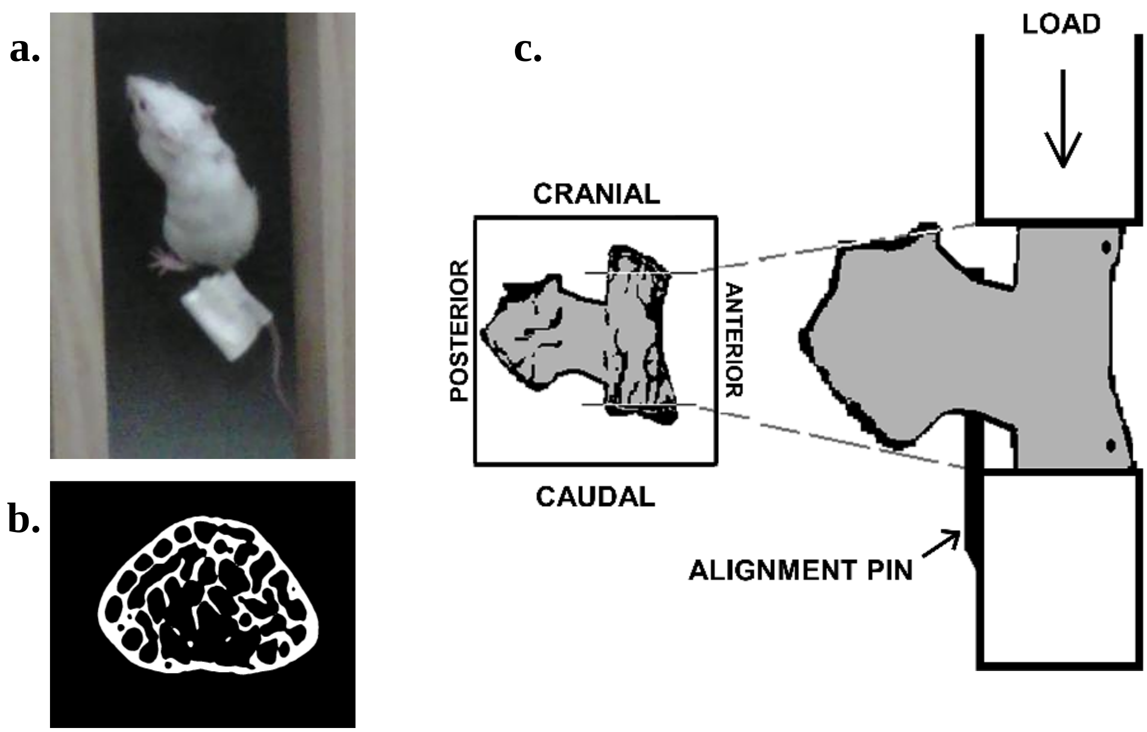


Figure 3. a.) climb training resistance exercise model shown with added weight. b.) cephalic (top) view of fourth vertebral body cancellous bone microarchitecture taken during μCT scanning. Image courtesy of Dr. Matt Allen. c.) biomechanical compression test of fourth lumbar vertebral body. Image courtesy of Dr. Tommasini (15).

Compression test of L4 vertebrae

After being μCT scanned, the fourth lumbar vertebrae returned to Texas A&M to be biomechanically tested. According to the methods of Tommasini *et al*. (15), bone samples were minimally shaved flat on cephalic and caudal endplates with a scalpel blade to achieve parallel testing surfaces before mechanical testing. Axial compression by a 3-mm diameter platen at a speed of 0.05mm/s was applied to the caudal surface of the vertebral body only (see Figure 3.c). To keep the spines from slipping during the test, a thin layer of epoxy was applied to the platens and an alignment pin was attached to the lower platen and placed through the vertebral foramen, as shown in Figure 3.c. Stiffness, maximum force, and maximum stress were determined. Stiffness is defined as the slope of the initial linear region of the force-displacement curve. Maximum stress was calculated by taking the ratio of the ultimate force and vertebral body cross-sectional area.

Ash weight: L5 vertebrae

The cleaned L5 vertebrae were thawed and then, after assessing wet weight of the entire vertebra on a weighing scale (Denver Instrument, SI-234) to the nearest 0.1mg , were baked in an ashing oven for 16 hours at 100°C to slowly burn off all water. A second “dry weight” was assessed on the same electronic scale. Bones were then baked another 16 hours at 600°C to burn off all organic material. This left behind the hydroxyapatite, or bone mineral, with a final ash weight determined after the specimens cooled. The weight of the bone mineral was taken as a percentage of the dry weight to normalize for any differences between bone sample sizes. It’s important to note that this test includes the vertebral body plus all the spinous processes (posterior and lateral) attached to the vertebral body, so the “sampling region” is different from that described for the compression testing and μCT.

Statistical analyses

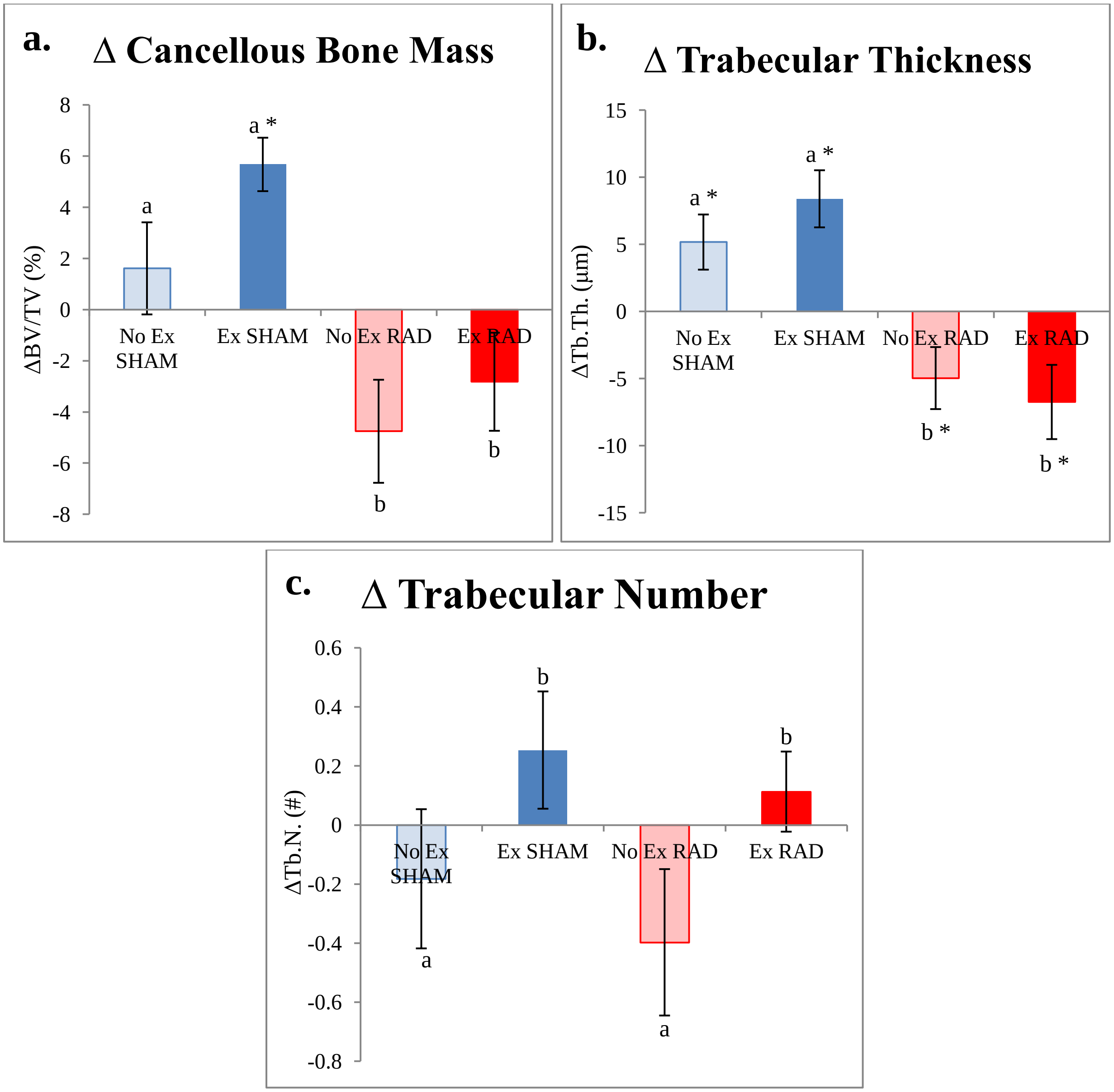
Means, standard deviations, and standard error measures were calculated for all animal groups. Change scores were determined by calculating the difference between Day 42 and Day 21 means. Δ Cancellous Bone Mass, ΔTrabecular Thickness, ΔTrabecular Number, Δ Ash Weight, and Δ Maximum Stress were calculated, and all analyses were performed on change scores. This allowed for a determination of what, if any, bone transformations were taking place during the period of recovery from RAD and PWB. A two-way ANOVA was used to test for overall differences between groups. Post hoc analyses were performed using the Student-Newman-Keuls test. Where appropriate, unpaired *t*-tests were used to determine differences between mean values at Day 21 (end of PWB) and Day 42 means (end of recovery period). P values <0.05 were considered significant.

CHAPTER III

RESULTS

Graphs shown indicate delta scores quantitating change in absolute values between Day 21 (end of PWB) and Day 42 (end of study) to analyze the ability of bone to respond to exercise during a 3-week period of recovery from partial weightbearing and to assess whether radiation exposure impairs that response. Bars sharing the same letter are not significantly different ($p<0.05$); asterisks indicate significant difference in Day 42 values compared to Day 21 means ($p<0.05$), which implies a change in bone integrity during recovery. Red bars indicate irradiated groups, while blue are groups not exposed to radiation. Darker-colored bars were resistance exercised animals, while lighter bars represent animals allowed normal cage activity during recovery. Graphs 1.a, 1.b., and 1.c show results from μCT data, while Graph 2.a shows ash weight results and Graph 2.b shows mechanical compression test data.

Comparisons across animal groups allows for determinations to be made on the effects of different experimental manipulations in the study. For example, comparing RAD groups to SHAM groups and Ex groups to No Ex groups allows for a determination of any main effects of radiation and exercise, respectively, on the bone's ability to recover from reduced weightbearing. When comparing Ex SHAM to No Ex SHAM, we see the differences in how bone responds to either rest or exercise during recovery from reduced weightbearing. Comparing No Ex RAD to No Ex SHAM illustrates the effect of simulated galactic cosmic radiation exposure on the ability of bone to recover during rest, while comparing Ex RAD to Ex SHAM allows us to see how that radiation exposure may or may not blunt the osteogenic response of bone to resistance exercise.



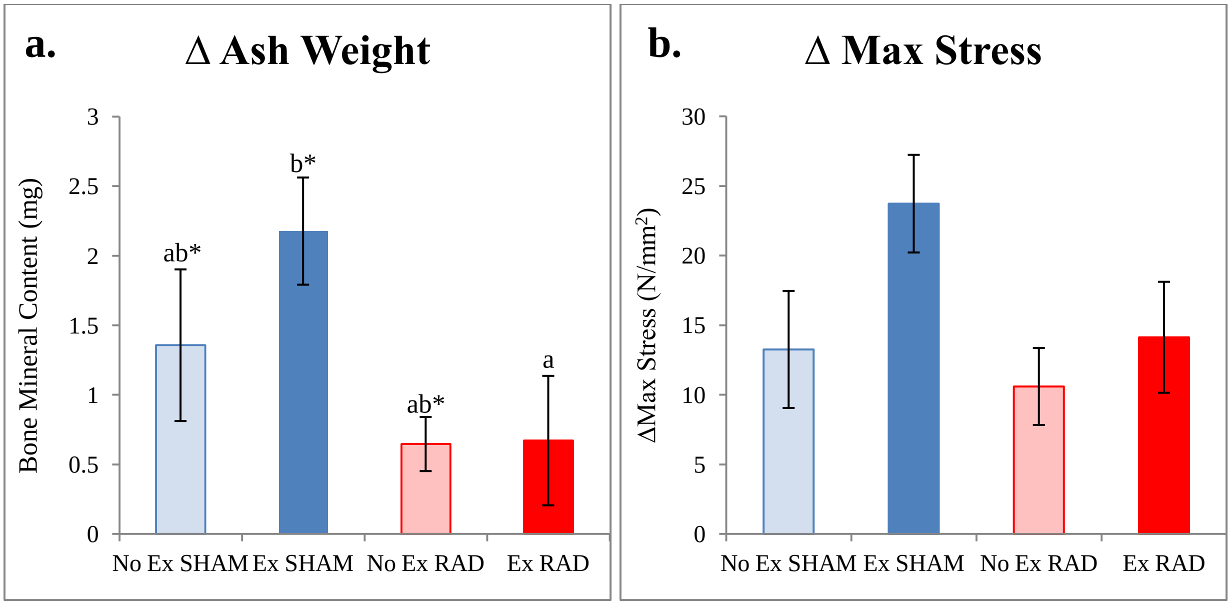
Graph 1. a.) change in cancellous bone mass over 21 day period of recovery from radiation and partial weightbearing. b.) change in bone thickness of trabeculae (bone struts) over 21 day period of recovery. c.) change in number of trabeculae (bone struts) during recovery period.

Graph 1.a shows the change in cancellous bone mass (ΔBV/TV) over the recovery period, and is measured as a percentage of bone volume divided by total tissue volume. When analyzing ΔBV/TV, we see a main effect of radiation, with significant differences between RAD and

SHAM groups. Additionally, exercised animals not exposed to radiation (Ex SHAM) significantly increased their cancellous bone mass during the 21-day recovery period.

Graph 1.b shows changes in the thickness of the trabeculae, or the strut-like projections that compose the cancellous bone. Thicker columns indicate that osteoblasts have been laying down new bone mineral, while thinner columns indicate osteoclasts resorbing bone mineral. Similar to ΔBV/TV, we see a main effect of radiation on measures of trabecular thickness (ΔTb.Th.), with significant differences between RAD and SHAM groups. Additionally, all four experimental groups show changes in Tb.Th. between days 21 and 42. Both SHAM groups exhibit an increase in thickness, while those mice in both RAD groups show a decrease in thickness during recovery from partial weightbearing.

Graph 1.c details changes in the overall number of the trabeculae (ΔTb.N.) comprising the cancellous vertebral bodies. While no significant increases or decerases in Tb.N. were found across the recovery period, we do see an apparent main effect of resistance exercise. ΔTb.N. is significantly higher in Ex SHAM and Ex RAD than in No Ex SHAM and No Ex RAD.



Graph 2. a.) change in ash weight (bone mineral weight) over 21 day period of recovery from radiation and partial weightbearing. b.) change in maximum stress (maximum force to compression normalized by cross-sectional area) during recovery period.

Ash weight (Graph 2.a) is a true measure of bone mineral content by weight. Hence, the higher the ash weight, the higher the bone mineral content. Unlike ΔBV/TV, the region of interest defined in ash weight testing includes the entire L5 vertebra. This means that the ash weight quantifies cortical bone as well as cancellous bone mineral. Here we see significant differences between Ex SHAM and Ex RAD groups, showing that radiation exposure affects bone mineral content from exercise during recovery from simulated galactic cosmic radiation and partial weightbearing. All groups except Ex RAD significantly increased in bone mineral content over the 21-day period of recovery. This apparent lack of recovery is likely due to the high variability seen in the Ex RAD bones’ ash weight.

Data from the mechanical compression test performed on the fourth lumbar vertebrae after μCT scans are illustrated in Graph 2.b. The maximum force withstood by the vertebral body was analyzed, and normalized to the cross-sectional area of the vertebral body to yield maximum stress. As seen in similar studies using compression testing of the lumbar vertebrae of mice (1,15), the variability in this test was high, even after taking extreme care to ensure surfaces were parallel and bone samples were held secure. While no significant differences were shown in maximum stress values, the trend closely mirrors that of ash weights.

Animals responded well to the partial weightbearing model, and performed soundly during climb training. Although the bone samples were quite small in size, L4 and L5 dissections were easy to replicate. Shaving the endplates parallel before compression testing resulted in bones that were all very similar in height, showing consistency across the study. This project was very time-intensive and took a lot of patience and finesse to work with such small samples, but I am incredibly pleased with the results.

CHAPTER IV

CONCLUSIONS

From the data collected, many conclusions can be drawn about what structural changes occur within the microarchitecture of the fourth and fifth lumbar vertebrae during recovery from radiation and partial weightbearing. Additionally, we can infer what those changes mean, and what implications they have for affected populations.

It is well-known that weightbearing exercise leads to increased bone health in both animal (7) and human models (8). However, the main effect of radiation on cancellous bone mass and trabecular thickness seen in this study tells us that simulated galactic cosmic radiation exposure appears to inhibit the bone's ability to respond to exercise. BV/TV does not recover and furthermore, the non-significant decrease in BV/TV suggests that bone loss might even be continuing during recovery. RAD groups did not show an increase in cancellous bone bass during recovery, like that which was seen in exercise SHAM groups. Moreover, RAD groups showed significant declines in trabecular thickness during this period. This means that not only does radiation lead to decreased gains in bone integrity during recovery; it actually results in significant absolute losses.

On the other hand, there was not a main effect of radiation on Tb.N. These results do not directly align with the story told by other data values. However, conclusions can be drawn about the implications of these data. There were significant differences between EX and No EX groups. One possible explanation for the different responses to exercise in microarchitecture could be the different sensitivities of Tb.N. and Tb.Th. to exercise (8). Another possible explanation is also a limitation to the use of trabecular number to determine bone microarchitecture. Tb.N. is calculated by counting the number of trabeculae, or strut-like projections, composing the cancellous bone mass. When osteoblasts, or bone-builders, lay down new bone, they can act in a way that forms more of those complete trabeculae, thereby increasing Tb.N. When osteoclasts, or bone-breakers, resorb bone mineral, they can break down entire struts, which would lead to a decreased Tb.N. However, if the osteoclasts only resorb the central section of a trabeculae, leaving two portions of the strut on both sides, the count for Tb.N. actually increases, even though the bone microarchitecture is compromised. I believe that this is the case in the RAD groups. Ex RAD and No Ex RAD showed similar responses in ΔBV/TV and ΔTb.Th., but not ΔTb.N. It is possible that the osteoclasts completely eroded more full struts in the No Ex group, and in doing so, decreased Tb.N. The Ex group may have had more partial cancellous bone resorption, which would increase Tb.N. This partial resorption is less detrimental to the microarchitecture of the bone than full resorption, so that could signify that exercise is attempting to mitigate those deleterious radiation effects.

Significant differences in the ash weights of exercised animals also support the idea that simulated galactic cosmic radiation exposure is detrimental to bone health. Ex SHAM animals show increased bone mineral content during the recovery period that is significantly greater than the bone mineral content of Ex RAD mice. These findings are reinforced by the trends shown in results from maximum stress tests. It is important to note that variability intrinsic to mechanical compression testing likely influenced the statistical significance of these values, but the trends shown are of value. It is apparent that simulated galactic cosmic radiation exposure inhibits the ability of bone to respond to exercise during recovery from radiation and partial weightbearing, and is shown by impaired cancellous bone mass, trabecular thickness, and bone mineral content determined by ash weight.

Little information exists about the affects of partial weightbearing on the lumbar vertebrae of mice. Furthermore, few studies have looked at the response of mice lumbar vertebrae following acute radiation exposure. The structural and functional changes shown by the fourth and fifth lumbar vertebrae in this study show that vertebral bone does in fact respond to both simulated galactic cosmic radiation and resistance exercise. These initial findings suggest that much more information can be gained from the analysis of the lumbar vertebrae of mice using the partial weightbearing model.

Partial weightbearing using a partial weight suspension system is a relatively new rodent model, but has been shown to decrease measures of trabecular bone morphology, including cancellous bone volume and trabecular thickness (17,5). This study focused only on the recovery period immediately following partial weightbearing, so no absolute conclusions can be directly made about the effects of partial weightbearing on bone. However, some of the changes in microarchitecture appear to extend well into the recovery period, implying that PWB exposure is detrimental to bone health.

Although the exact mechanisms of radiation-induced declines in cancellous bone integrity caused by GCR exposure remain unknown, bone cell damage is thought to contribute to these declines (10). The suspected mechanism by which these bone cells are damaged is radiation-induced inflammation. Exposure to radiation activates the body's inflammatory response, which leads to the release of cytokines, which then act on bone cells in a variety of ways. Three of the most influential players in this inflammatory process are the cytokines interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α). While the metabolic pathways leading to resulting losses in cancellous bone mass are not fully known, it is believed that they operate by manipulating the balance of bone formation and bone resorption (18).

Simulated galactic cosmic radiation has been shown to cause long-term effects on trabecular bone (3, 6). These deleterious radiation-induced effects, including decreased trabecular bone mass, trabecular number, and trabecular thickness, align with our findings that radiation exposure is deleterious to cancellous bone.

When reduced weightbearing and radiation exposure are combined, the resulting reductions in bone's mechanical integrity are exacerbated. Declines in the mechanical properties of cancellous bone are worse than what is seen in either PWB or GCR exposure alone (1). Similarly, osteoblast cell suppression leading to decreased bone formation and stimulated osteoclast cell activity leading to increased resorption are greatest in irradiated animals in a reduced weightbearing environment (16). As this study is novel in its utilization of resistance exercise as a means to counteract these biomechanical declines, no data exists to compare our results to.

Affected populations

While these results are more applicable to astronauts on both long- and short-duration spaceflight missions, many additional populations are also affected. Most directly, trans-arctic airline crews are directly exposed to elevated levels of galactic cosmic radiation on long-duration flights across the North Pole (2,12). This radiation is the same that astronauts are exposed to on their missions. Airline pilots also remain seated during these lengthy flights. Although their exposure lasts a period of hours instead of days or weeks like in space, the frequency of trips is something that should be approached with caution and, indeed, is regulated by the airline industry, limiting crews to predetermined yearly levels of exposure.

To a lesser extent, x-ray technicians and nuclear power plant workers are also exposed to occupational radiation. While it is a different type of radiation (much lower energy) than that found in space, high enough doses might have similar effects to that observed with the low-dose, high-energy radiation used in this project. This study also has implications for populations that have not been exposed to simulated galactic cosmic radiation. If we focus on results from the sham-exposed groups, we can analyze the results of exercise or rest during recovery from a partially loaded environment. Exercised SHAM animals showed a significant increase in cancellous bone mass, trabecular thickness, and ash weight during the recovery period. This speaks to the efficacy of resistance exercise as a means of counteracting bone loss resulting from reduced weightbearing. This includes patients on extended bed rest, those suffering spinal cord injuries, and even those choosing to participate in a largely sedentary lifestyle.

Final conclusions

In this novel study investigating the response of bone integrity to radiation and partial weightbearing, the osteogenic response of vertebral bone to exercise appears to be blunted for up to six weeks following an acute dose of radiation and reduced weightbearing.

REFERENCES

1. Alwood JS, Yumoto K, Mojarrab R, Limoli CL, Almeida EAC, Searby ND, Globus RK. Heavy iron irradiation and unloading effects on mouse lumbar vertebral microarchitecture, mechanical properties and tissue stress. Bone. 47; 248-255.

2. Bailey S. Air crew radiation exposure – an overview. Nuclear News. 2000. (1)32-40

3. Bandstra ER, Pecaut MJ, Anderson ER, Willey JS, De Carlo F, Stock SR, Gridley DS, Nelson GA, Levine HG, Bateman TA. Long-term dose response of trabecular bone in mice to proton radiation. Radiation Research. 2008. 169(6)**:**607-14.

4. Boudreaux RD, Metzger CE, Camp, KT, Yuen EP, Macias BR, Allen MR, Braby LA, Hogan HH, Bloomfield SA. Skeletal recovery from partial weightbearing may be altered in mice after radiation exposure [Abstract]. 2013. NASA Human Resource Program Investigators' Workshop.

5. Ellman R, Spatz J, Cloutier A, Palme R, Christiansen B, Bouxsein ML. Partial reductions in mechanical loading yield proportional changes in bone density, bone architecture, and muscle mass. Journal of Bone Mineral Research. 2012. doi:[10.1002/jbmr.1814].

6. Hamilton SA, Pecaut MJ, Gridley DS, Travis ND, Bandstra ER, Willey JS, Nelson GA, Bateman TA. A murine model for bone loss from therapeutic and space-relevant sources of radiation. Journal of Applied Physiology. 2006. 101(3)**:**789-93.

7. Huang TH, Lin SC, Chang FL, Hsieh SS, Liu SH, Yang RS. Effects of different exercise modes on mineralization, structure, and biomechanical properties of growing bone. Journal of Applied Physiology. 2003. 95(1):300-7.

8. Ju YI, Sone T, Ohnaru K, Choi HJ, Fukunaga M. Differential effects of jump versus running exercise on trabecular architecture during remobilization after suspension-induced osteopenia in growing rats. Journal of Applied Physiology. 2012. 112(5)**:**766-72.

9. Kohrt WM, Bloomfield SA, Little KD, Nelson ME, Yingling VR. Physical activity and bone health. Medicine & Science in Sport & Exercise. 2004. 1985-1996.

10. Kondo H, Yumoto K, Alwood JS, Mojarrab R, Wang A, Almeida EA, Searby ND, Limoli CL, Globus RK 2010 Oxidative stress and gamma radiation-induced cancellous bone loss with musculoskeletal disuse. Journal of Applied Physiology 108(1)**:**152-61.

11. Lee SH, Hargens AR, Fredericson M, Lang P. Lumbar spine disc heights and curvature: upright posture vs. supine compression harness. Aviation Space, Environmental Medicine. 2003; 74(5):512-516.

12. Lim MK. Cosmic rays: are air crews at risk? Occupational Environmental Medicine. 2002. (59): 428-433.

13. Morey-Horton ER, Globus RK. Hindlimb unloading rodent model: technical aspects. Journal of Applied Physiology. 2002. 92:1367-1377.

14. Smith SM, Wastney ME, O'Brien KO, Morukov BV, Larina IM, Abrams SA, Davis-Street JE, Oganov V, Shackelford LC. Bone markers, calcium metabolism, and calcium kinetics during extended-duration space flight on the mir space station. Journal of Bone Mineral Research. 2005. 20: 208-218.

15. Tommasini SM, Morgan TG, van der Meulen MCH, Jepsen KJ. Genetic variation in structure–function relationships for the inbred mouse lumbar vertebral body. Journal of Bone Mineral Research 2005. 20:817–827

16. Vazquez ME, Neurobiological problems in long-term deep spaceflights. Advanced Space Research. 1998. 22, 171–183.

17. Wagner EB, Granzella NP, Saito H, Newman DJ, Young LR, Bouxsein ML. Partial weight suspension: a novel murine model for investigating adaptation to reduced musculoskeletal loading. Journal of Applied Physiology. 2010. 109:350-357.

18. Willey JS, Lloyd SA, Nelson GA, Bateman TA 2011 Ionizing Radiation and Bone Loss: Space Exploration and Clinical Therapy Applications. Clinical Reviews in Bone and Mineral Metabolism. 9(1)**:**54-62.

19. Wing PC, Tsang IK, Susak L. Back pain and spinal changes in microgravity. Orthopaedic Clinic North America. 1991:255-262.