CONTINUOUS, LOW DOSE IRRADIATION RESULTS IN NO DETRIMENT TO BONE

AFTER 4, 12, AND 28 DAYS

A Thesis

by

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ABSTRACT

As the focus shifts to potential Lunar and Mars missions, there are many aspects of the space environment that are still unknown, one of those being radiation exposure. There are many radiation studies examining the effects of acute, high dose radiation on bone; however, it has been discovered that the total exposure astronauts will experience will be much lower than the doses these studies have utilized. The purpose of this study was to determine the effect of a continuous, low-dose irradiation exposure on bone at various time points. Sixty-six male C57Bl/6 mice, aged 47-49 weeks, were placed in the continuous radiation field for either four, twelve, or twenty-eight days. The radiation field was created by locating four activated ^{60}Co wires emitting gamma radiation around a mouse rack in a lead-shielded room. At a dose rate of 6.49 mGy/day, the total cumulative dose was approximately 25.6 mGy for four days, 77.88 mGy for 12 days, and 181.72 mGy for 28 days. Fluorochrome calcein labels were injected to obtain bone formation rate post termination. DXA scans were performed prior to radiation exposure and again just before termination to determine bone mineral density and body composition. At termination, tibiae and femurs were collected from all animals for histomorphometry assessments. Also, osteocyte apoptosis was quantified in cancellous bone via staining for Annexin V. Continuous radiation exposure at this low dose rate did not have any impact on total body bone mineral density, lean mass, fat mass, and percent fat at any time point. There were also no differences in cortical or cancellous bone formation rate at any time point in radiation-exposed mice vs. control animals. Also, bone volume and trabecular microarchitecture were not affected by radiation at any time point. Osteoid and osteoclast surfaces were not different after radiation at any time point, nor were there any differences in osteocyte apoptosis at any time point examined. These results are quite different from those seen after acute, high-dose radiation exposures, after which decreased bone

volume, depressed bone formation rate, and increased osteoclast surface are often observed. This data begins to elucidate a time course of continuous low dose radiation impacts on bone, similar to radiation that might be experienced during transit to Mars and alludes to the potential that it may not be deleterious to bone health, as previously assumed.

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Finally, a thank you to my family for their support throughout my education. To my husband who has been my cheerleader throughout my degree, and who has constantly listened to me talk about radiation and bone for the past two years. Thank you all for helping to reach the finish line!

CONTRIBUTORS AND FUNDING SOURCES

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Bone Biology

There are two different types of bone, cortical and cancellous. Cortical bone makes approximately 80% of the human skeleton (1). It is the hard, outer shell of bone that provides the supportive and protective function of the skeleton (1). Cancellous bone is the lattice-like structure encased in cortical bone located at the ends of long bones and in the vertebral bodies. With more surface area and a more rapid turnover rate than cortical bone, cancellous bone typically responds to changes in mechanical loading and hormonal effects more quickly than cortical bone. Therefore, it is important to examine both types of bone since they respond differently to stressors placed on the body.

Bone is a dynamic tissue that has the ability to respond and adapt to many situations. The variety of cells making up bone contribute to its ability to respond to environmental changes. Osteoblasts are derived from mesenchymal precursor cells and are responsible for the bone formation component of bone turnover. Bone formation takes place in two stages, matrix formation followed by mineralization (1). Osteoblasts create osteoid, which is the organic component of bone matrix, that is then mineralized by the deposition of calcium phosphate crystals and hydroxyapatite which, in humans, starts about 15 days later (1, 2). Since there is a lag between matrix formation and mineralization, osteoid remains visible on the bone surfaces for several weeks, and this can be measured via bone histomorphometry to determine newly laid bone matrix (1). Osteoblasts can also help to regulate the differentiation and activity of osteoclasts, by releasing macrophage-colony stimulating factor (M-CSF) and RANKL (2). Osteoclasts are multinucleated cells that attach to the bone surface and are responsible for the bone resorption component of the bone turnover equation.

They create a sealed space between the cell and bone matrix, transporting protons into the sealed space to decrease the pH, and creating an acidic environment that solubilizes the bone mineral (3).

The activity of these bone cells is further coordinated by osteocytes, which help to orchestrate bone formation and resorption in response to many physiological and environmental changes. Osteocytes are osteoblasts that become embedded in the bone matrix and then change their phenotype, developing dozens of dendritic cell extensions (4). They compose over 90% of bone cells and could survive for decades within a bone that has a slow turnover rate (5,6). Osteocytes are able to communicate with each other, cells on the bone surface and in the marrow cavity through the vast network of dendritic processes (7). Through these dendrites they are able to sense and respond to mechanical load placed on bone. Osteocytes are able to regulate mineral metabolism through the release of a variety of molecules (8). Also, they express Dkk1 and sclerostin, which are negative regulators of the Wnt/B-catenin pathway, inhibiting osteoblast activity (8). A major recruiter of osteoclasts is osteocyte apoptosis due to the release of RANKL (8). Osteocyte apoptosis can be induced by glucocorticoids, various cytokines, mechanical overload, decrease in estrogen, hypoxia, immobilization, and nutrient limitation (5). Osteocyte apoptosis is necessary to maintain healthy bone turnover; however, if there is excessive osteocyte apoptosis, high bone resorption can result in bone loss (Figure 1.1).
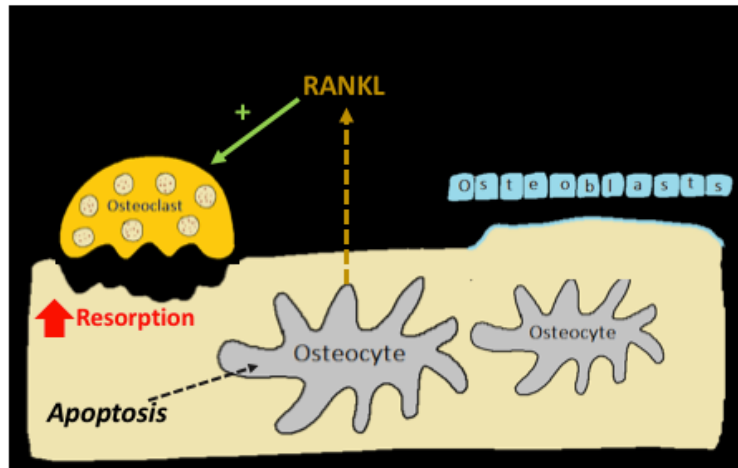
Figure 1.1: Depiction of osteocyte apoptosis and the effect on osteoclasts (used with permission from 9)

There are dramatic changes in bone cell activity and bone structure as an animal or human age. This specific study uses older male mice; therefore, it is important to take into account what aging does to bone. As an animal grows bone mass increases rapidly, until approximately 16-20 weeks of age in which they reach skeletal maturity, after which bone mass can begin to slightly decline with age. Bone loss due to aging differs between cortical and cancellous bone: typically, trabecular number decreases rather than thickness in cancellous bone and cortical porosity is increased (8,10). Also, with age the presence of reactive oxygen species (ROS) is increased in the bone microenvironment, which can contribute osteocyte apoptosis and increase osteoclasts resulting in an increase in resorption (11).

Spaceflight Environment

The spaceflight environment poses a number of health risks to astronauts including microgravity and radiation exposure. We have limited information about the health effects of the radiation sources and doses relevant to spaceflight. The major sources of radiation in deep space

are solar particle events (SPE) and galactic cosmic radiation (GCR). SPE's result in large fluxes of energetic particles, but, they are predictable and easy to shield against (12).

The GCR environment consists of ~98% protons and heavier ions, 88% of which are hydrogen, 10% helium, and remaining 2% heavy ions, and ~2% electrons and positrons (12). GCR is not easily shielded against and results in a continuous dose rate as low as 180 to 225 mGy/day (13). The total mission radiation dose for a round trip Mars surface mission will result in approximately 1.01 Gy of exposure, whereas a Lunar mission results in a GCR exposure of 380 mGy, during a solar minimum (13, 14). Therefore, GCR poses the greatest to human health. Importantly, there is limited information available on the effects exposure to continuous, low-dose radiation, particularly for bone.

Whole Body Radiation Effects

Earth-based radiation exposure is most known and commonly used in the treatment of cancer. Radiation doses for radiotherapy are delivered in fractionated doses ranging from 2-20 Gy/fractionation, whereas acute studies can deliver as much as 60 Gy in a single dose, which results in extremely different biological effects (15). There are a variety of effects that can be seen throughout the body. After 16 Gy of radiation to the heart in 8-12-week-old male mice, microvascular density was significantly decreased, which was accompanied by significant endothelial damage (16). This damage was not restored after treatment with bone marrow-derived endothelial progenitor cells (16). This is a great concern since this vasculature is not found to be restored following irradiation, leading to decreased blood flow and damage to the heart (17). It has also been found that there is both a rapid burst in free radicals and reactive oxygen species (ROS) immediately following irradiation and prolonged increases in ROS and reactive nitrogen species (RNS) for several days post-irradiation (18). This increase in oxidative species can result in DNA

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damage and gene mutations in cells. DNA double strand breaks is the most common DNA damage seen following ionizing radiation, and they pose the greatest risk to cell cycle survival (19). It has been found that DNA damage rises linearly with dose, suggesting that there is a dose dependent relationship with the effects of radiation (20).

Results of studies examining lower doses of radiation more relevant to the space environment demonstrate a potentially different response then acute, high doses. When 10 cGy of low-dose, low-LET gamma is delivered over 48 hours to normal AG1522 and GM1603 human skin fibroblasts, the number of micronuclei, which result from un-rejoined DNA double strand breaks, was significantly decreased (21). The researchers hypothesized that this decrease could be due to an increase in antioxidant activity seen at this dose-rate (21). A similar effect has been observed in immune function following low-dose, low-LET radiation exposure. Comparing the immune response of an acute 1.6 Gy/min dose to a continuous low-dose-rate gamma irradiation at 1.2mGy/hour for three weeks in five week old female mice results in vastly different responses (22). After acute irradiation the number of abnormal immune cells increased, while those that had been exposed to continuous low-dose irradiation, no abnormal immune cells were detected (22). In a study examining the impact of various doses of gamma radiation on splenocytes in 6-8 week old female mice, they found that at a low dose (0.01 Gy) decreased apoptosis in splenocyte subpopulations (specifically natural killer cells and dendritic cells), whereas 2 Gy of radiation resulted in increased apoptosis in all splenocyte subpopulations (23). These data suggests that the physiological response to low-dose radiation may be different than that of high doses.

Radiation Effect on Bone

Most radiation studies to date examining bone outcomes utilized higher, acute doses of radiation. These studies demonstrate bone loss due to radiation, especially trabecular bone loss.

With doses as low as 2 Gy, there is a 29% loss in trabecular bone volume, due to a decrease in trabecular number and an increase in trabecular spacing (24). After the delivery of an acute dose of 2 Gy X-ray radiation to 20 week old male mice, the presence of early stage apoptotic osteocytes increases in the trabecular bone compartment of the irradiated limb (25). This possibly contributed to the observed increase in osteoclast number and subsequent decrease in trabecular bone volume after irradiation (25). In another study using 2 Gy of gamma radiation on 17-week-old male mice, there was an increase in osteoclast number (26). Also, with 2 Gy of X-ray radiation on 13-week-old female mice, not only was there an increase in osteoclast number but also an increase of eroded surface (27). This is concerning since osteoclasts are the bone resorbing cells, and if this increase in osteoclasts is not matched by an increase in osteoblast activity, then net bone loss can occur (Figure 1.2). Also, reactive oxygen species increase in the bone marrow cavity following gamma radiation exposure, causing apoptosis of marrow cells which can be a contributing factor in the increase of osteoclasts (26). The results of these studies, however, are much different than those observed following even lower doses of radiation exposures.
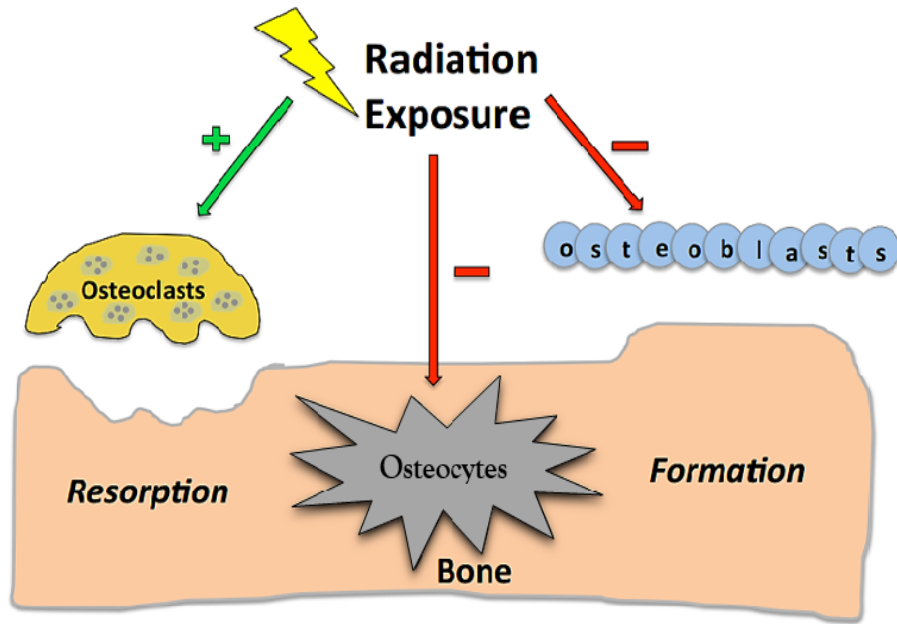
Figure 1.2: Depiction of the bone response to radiation exposure. Following radiation exposure there is an increase in osteoclasts, and a decrease in osteoblasts and osteocytes (used with permission from 28).

A full year after an acute, non-targeted dose of 4.4 cGy delivered to 19-week-old male mice, a 56% increase in trabecular bone volume, 16% increase in trabecular number, and a 17% decrease in trabecular spacing in the distal femur is seen (29). These results are opposite than those seen at higher, acute doses and suggest to a positive effect on bone at lower doses of radiation. Exposing 14-week-old female mice to a dose rate of 8.5 cGy/day for 20 days, resulting in a total dose of 1.7 Gy, produces no change in trabecular bone volume or trabecular microarchitecture properties (30). Also, there was no increase in osteoclasts or osteoblasts following this continuous radiation exposure (30). The results of these two studies paint a different picture of the effects of continuous, low dose radiation on bone, suggesting it might not be as detrimental as higher doses appear to be. Nonetheless, these studies still do not reach the prolonged low dose rate expected during long-duration space missions that we aimed to mimic in our radiation field.

Significance

There are no published data describing a time course of the effects of continuous, low dose irradiation (CIRR) on bone; therefore, my goal was to examine total body and site-specific changes in bone at different durations of living in a CIRR field. The time points of 4, 12, and 28 days were chosen to establish a possible mechanism for how bone is being affected by the CIRR field. Additionally, I aimed to study this effect in middle-aged mice to better represent the human population that would likely embark on deep space exploration missions. An understanding of the effects of low dose, continuous radiation is critical for discerning the risks astronauts could be exposed to outside the protection of Earth's orbit.

I hypothesized that continuous low-dose irradiation would not have an effect on cortical bone formation rate; however, I hypothesized there will be a decrease in cancellous bone formation rate (BFR) and therefore a decrease in cancellous bone volume. Finally, I expect there will also be an increase in cancellous osteoclast surface and the prevalence of apoptotic osteocytes following irradiation. Specifically, there would be the greatest increase of apoptotic osteocytes at the 4 day time point. I hypothesized changes in BFR and osteoclast surface would be most different at day 12, while changes in bone volume would not be apparent until 28 days of radiation exposure.

CHAPTER II

MATERIALS AND METHODS

Animals: Sixty-six male wild type C57Bl/6 mice, aged 47-49 weeks, were obtained from an in-house breeding colony and housed in a 12-hour light/dark cycle in an institutionally approved animal facility. After a four-week acclimation to a purified rodent chow (AIN93G; Research Diets, Inc, New Brunswick NJ), animals were randomly assigned to either the CIRR treatment (RAD) or to a non-irradiated control (CON) group. Animals were terminated at one of three time points: after four, twelve, or twenty-eight days of living in the radiation field. Each group had an n of 10-12 animals. DXA whole body scans were performed on the same day as the start of radiation and just prior to termination. Fluorochrome calcein labels (Sigma Aldrich, St Louis, MO) were injected intraperitoneally prior to entering the radiation field for animals assigned to the four day time point, and at 6 and 2 days prior to termination in mice assigned to the remaining time points. At the completion of each duration of CIRR, animals were anesthetized via inhaled isoflurane, euthanized via thoracotomy, and tissues were collected for analyses. All procedures were approved by the Texas A&M Institutional Animal Care and Use Committee (IACUC).

Radiation Field: The CIRR field was set up in a dedicated animal facility room, formerly used as the x-ray procedure room; lead-shielded walls prevented any leakage of gamma radiation to adjacent rooms or hallways. The radiation for the field was created by activating ^{60}Co wires, so that they would emit gamma radiation. Four wires were placed in the corners to surround the rack containing animal cages to create an equivalent exposure for all animals in the field (Figure 2.1). The cart also contained standard dosimeters, 20 placed at various points on the cart, which measured the dose rate of the radiation in the field. The animals were removed from the field for

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approximately five minutes per day for health checks. The animals in the field experienced approximately a dose of 6.49 mGy/day, which was a total of 25.6 mGy for four days, 77.88 mGy for 12 days, and 181.72 mGy for 28 days.
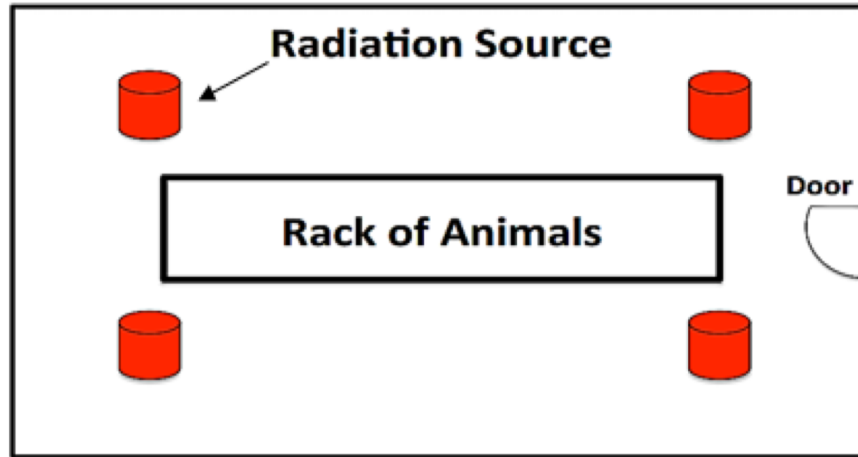

Figure 2.1: Continuous Radiation Field Layout (with permission from R. Bokhari)

DXA: Dual-energy x-ray absorptiometry (DXA) (GE Lunar Prodigy; Madison, Wisconsin) with small animal software was used to measure whole body bone mineral density, lean and fat mass in animals prior to the start radiation and the day before termination. Animals were anesthetized via inhaled isoflurane for the duration of the scan (~5 minutes) and positioned prone on the scanner bed. The whole-body bone mineral density (BMD) value reported does not include the skull and tail.

Dynamic histomorphometry assessment: Excised, undemineralized distal femurs saved for histomorphometry were stored in 10% formalin solution for 24 hours and then switched to 70% ethanol at 4°C. Samples were then serially dehydrated and embedded in methyl methacrylate (JT Baker, Avantor Performance Materials, Center Valley, PA). Thin sections (4 and 8 microns) were cut on a motorized Leica RM2255 microtome and mounted on slides. Image analysis was

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performed using an epifluorescent microscope and interfaced with an OsteoMeasure Analysis System, software V3.3 (OsteoMetrics, Inc., Atlanta, GA, USA), interfaced with an Olympus DP73 camera.   For cancellous dynamic analysis, frontal 8 μm thick slices of the distal femur were assessed at 200X magnification, 150 um from the growth plate and a 2.4 mm region of interest, for single- (sLS) and double- labeled surfaces (dLS) and inter-label width (the mean distance between two labels) at all doubly-labelled sites using standard methods (31).   From these primary data, % mineralized surface/total surface and mineral apposition rate (MAR, um/day) are calculated.   Percent mineralized surface is calculated as $[(sLS/2) + dLS]$/total surface.   For the four-day time point, only single-labeled surface (%sLS/BS) could be assessed, as the mice were only given one injection of flurochrome label on day "zero" at the start of radiation.   Bone formation rate (BFR) was calculated as %MS/BS x MAR and expressed as $um^3/um^2/d$. For cortical bone analysis, 100 μm cross-sectional slices of the midshaft tibia were cut on a Buehler saw and then were assessed for %MS/BS and MAR, enabling calculation of BFR at the periosteal and endocortical surfaces.   For samples containing no double label, disallowing a direct measure of MAR to use for calculating BFR, an imputed value of 0.02 (um/d) for cancellous and 0.02 (um/d) for the endocortical surface and 0.06 (um/d) for the periosteal surface of cortical bone was used. These imputed values were the lowest observed values for MAR on that bone surface across all samples .

Static cancellous histomorphometry assessment: For cancellous bone analysis 4 μm frontal sections of the distal femur were Von Kossa stained with a tetrachrome counterstain and assessed for % osteoclast surface, osteoid surface and cancellous bone volume (%BV/TV) and trabecular microarchitecture assessment (trabecular number, thickness and separation) at 400X using

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standard methods in the same region of interest as specified above for the fluorochrome label analyses (29).

Immunohistochemical staining for apoptosis marker Annexin V: Contralateral distal femurs for immunohistochemistry fixed in 10% formalin solution for 24 hours and then switched to 70% ethanol at 4°C. Whole femurs were decalcified in a formic acid/sodium citrate solution for approximately 7 days, paraffin embedded, and sectioned to 4 μm thickness. Sections were immunostained for Annexin V to quantify apoptotic osteocytes, using an avidin-biotion method as previously described, with an antibody dilution of 1:200 (32). Cells were counterstained with methyl green. Ten fields of view of the distal femur cancellous region, 150 um from the growth plate, were analyzed. All osteocyte counts were performed using OsteoMeasure Analysis System software V3.3 (OsteoMetrics, Inc., Atlanta, GA, USA) interfaced with an Olympus DP73 camera.

Statistical Analysis: All data sets were tested for normality using normality plots with tests. Independent t-tests were used to compare CON and RAD animals' values at each time point, for normally distributed data; if data were not normally distributed, nonparametric Independent Samples Kruskal-Wallis tests were used to compare group means. Although a 2X3 factorial could have been used to test for changes over time as well, I chose to focus on the impact of radiation within each time point with t-tests or the non-parametric equivalent, to maximize statistical power of these comparisons. Statistical significance was accepted at p<0.05. All statistical analyses were completed with SPSS (IBM, Armonk, NY).

CHAPTER III

RESULTS

Animals: After four, twelve, and twenty-eight days, there were no significant differences in body weight across time points when pooling animals across groups (4 day p=0.672, 12 day p=0.748, 28 day p=0.364; Table 3.1). The animals maintained normal eating and grooming patterns and appeared to be in good health; there were no animal deaths during the protocol.

Average Body Weight	4 Day	12 Day	28 Day
CON	41.59 +/- 5.03	42.82 +/- 4.40	43.47 +/- 3.19
RAD	40.72+/-3.65	43.06+/-3.20	41.36+/-6.29

Table 3.1: Average body weights at the end of the study.

Radiation resulted in no differences in body composition at all time points compared to age-matched controls: There were no differences between means for total body bone mineral density (BMD) in CIRR-exposed mice when compared to age matched controls (4 day p=0.387, 12 day p=0.847, 28 day p=0.924; Figure 3.1A). There were also no differences in lean mass (4 day p=0.387, 12 day p=0.328, 28 day p=0.416; Figure 3.1B), fat mass (4 day p=0.867, 12 day p=0.133, 28 day p=0.809; Figure 3.1C), and percent fat (4 day p=0.744, 12 day p=0.088, 28 day p=0.715; Figure 3.1D) between radiation and CON mice.
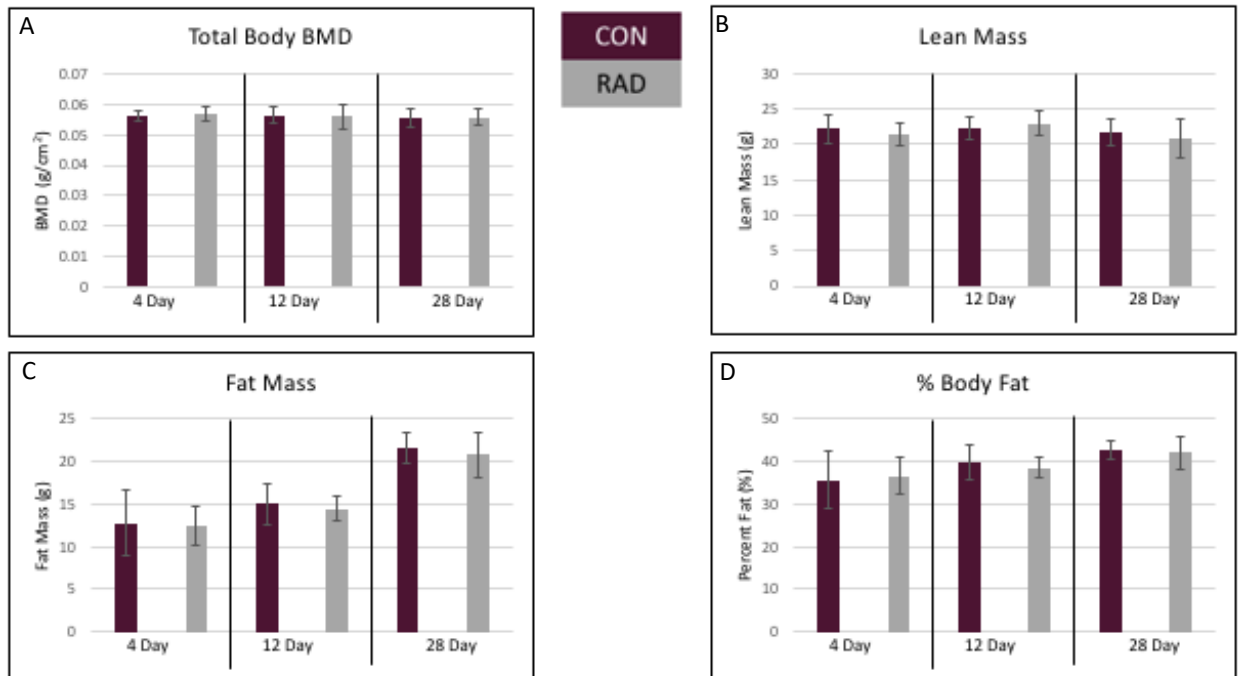
Figure 3.1: Total body BMD and body composition measured via DXA (dual-energy X-ray absorptiometry). Effects of CIRR exposure on (A) total body BMD (B) total body lean mass (C) total body fat mass (D) % body fat. There were no significant differences between RAD and CON for any variable across all time points. DXA=

Cancellous microarchitecture remained unaltered at each time point of CIRR: There were

no significant differences in cancellous bone volume (%BV/TV) at any time point (4 day p=0.343,

12 day p=0.723, 28 day p=0.189; Figure 3.2A) in CIRR-exposed mice compared to CON. There

were also no significant differences in trabecular thickness (4 day p=0.981, 12 day p=0.063, 28

day p=0.497; Figure 3.2B), trabecular separation (4 day p=0.601, 12 day p=0.931, 28 day p=0.612;

Figure 3.2C), or trabecular number (4 day p=0.635, 12 day p=0.795, 28 day p=0.441; Figure 3.2D)
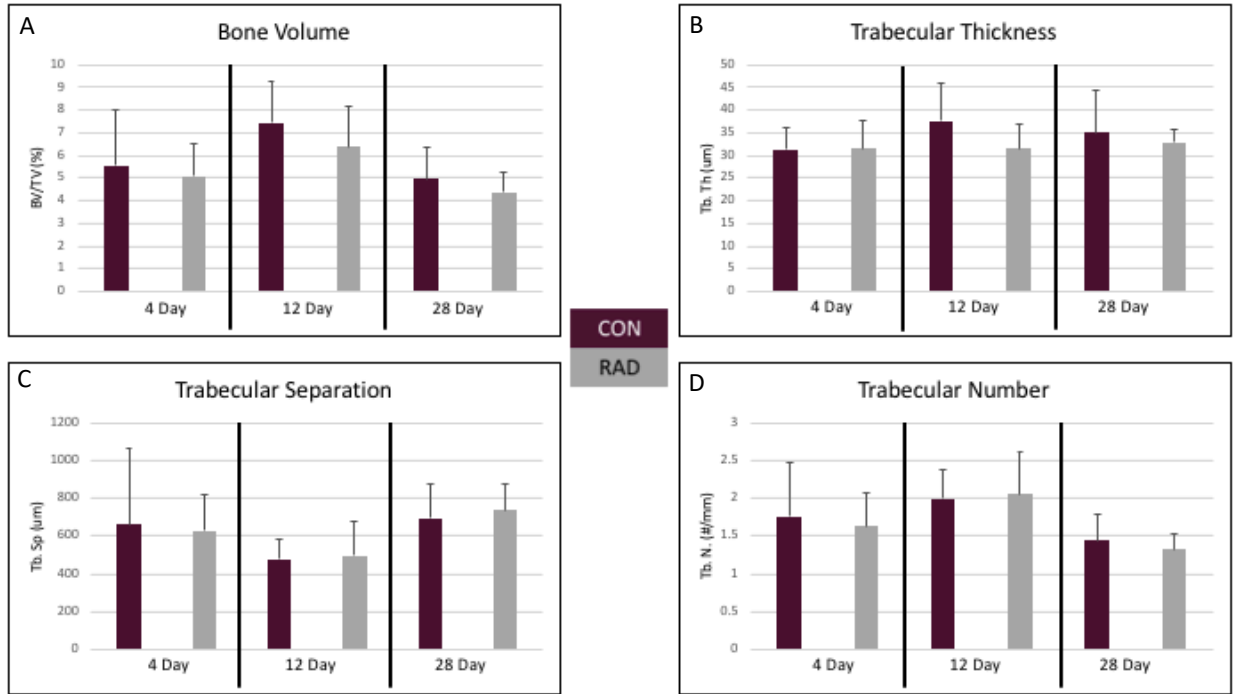
compared to CON.

Figure 3.2: Cancellous bone volume and microarchitecture at the distal femur. Effects of CIRR exposure on (A) BV/TV (B) trabecular thickness (C) trabecular separation (D) trabecular number. There were no significant differences between RAD and CON for any variable across all time points. BV/TV= bone volume/total volume

Cortical bone formation rate was not different from age-matched controls due to radiation exposure: After four days of radiation there were no differences seen in % single-labeled surface (%sLS/BS) at the periosteal (p=0.259; Table 3.2) or endocortical surfaces (p=0.724). There were no differences seen in periosteal bone formation rate (BFR) (12 day p=0.279, 28 day p=0.666; Figure 3.3A) and endocortical BFR (12 day p=0.798, 28 day p=0.796; Figure 3.3B) following irradiation.

	4 Day CON	4 Day Rad
Periosteal %sLS/BS	0.48 +/- 0.46	0.38 +/- 0.85
Endocortical %sLS/BS	4.75 +/- 2.34	4.31 +/- 2.21

Table 3.2: Cortical %sLS/BS in the midshaft tibia. No significant differences between CON and RAD in the periosteal and endocortical sites at the 4 day time point.
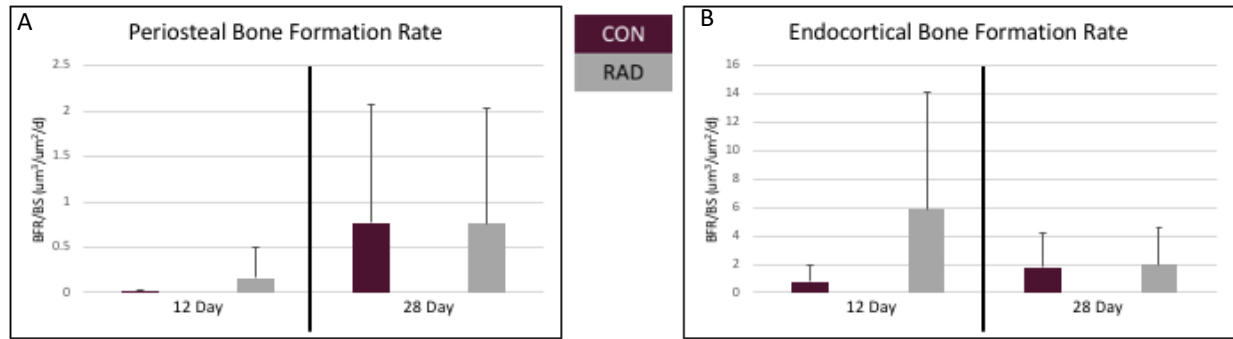
Figure 3.3: Cortical BFR at the midshaft tibia. Effects of CIRR exposure on (A) periosteal BFR (B) endocortical BFR. There were no significant differences between RAD and CON for any variable across all time points. BFR/BS= bone formation rate

CIRR-exposed mice had no alterations in cancellous bone formation rate compared to age-matched controls: After four days of radiation there were no differences seen in %sLS/BS in cancellous bone (CON= 2.34 +/- 1.21, RAD=3.28 +/- 2.19, p=0.244) compared to age-matched controls. There were also no differences seen in %MS/BS (12 day p=0.384, 28 day p=0.189; Figure 3.4A), mineral apposition rate (MAR) (12 day p=0.451, 28 day p=0.867; Figure3.4B), and BFR (12 day p=0.442, 28 day p=0.867; Figure3.4C) following 28 days of CIRR.
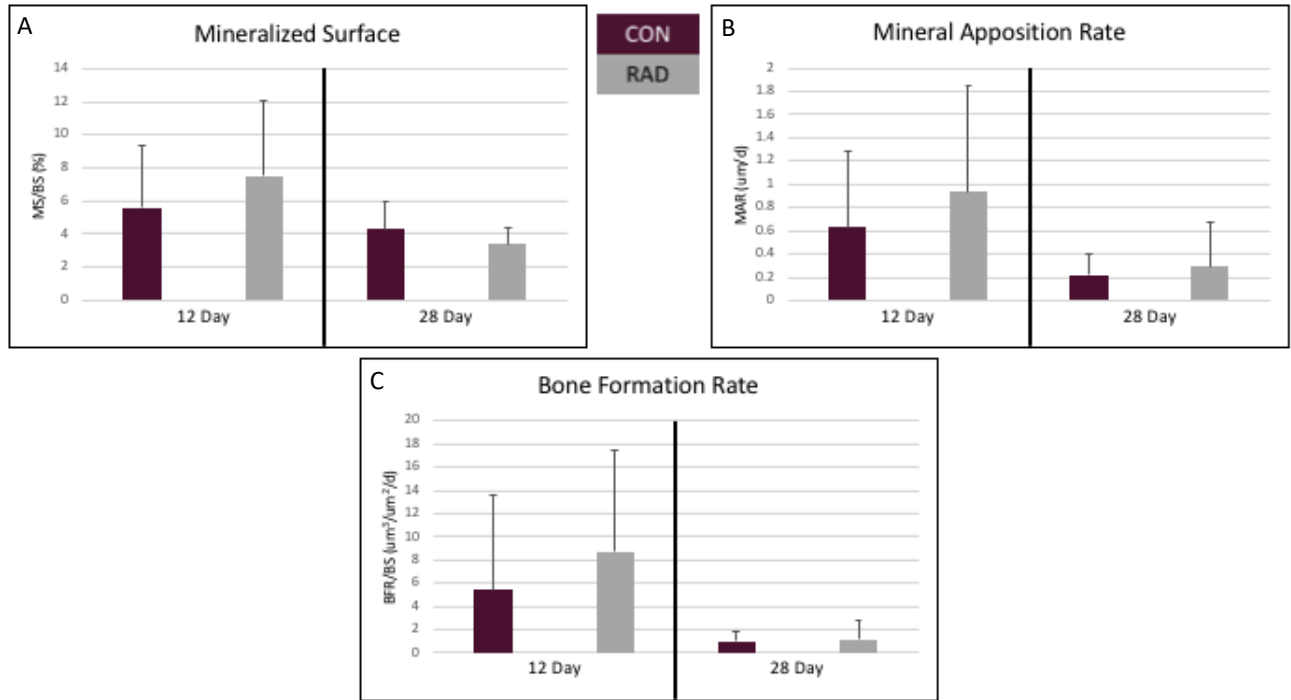
Figure 3.4: Cancellous BFR at the distal femur. Effects of CIRR exposure on (A) % MS/BS (B) MAR (C) BFR. There were no significant differences between RAD and CON for any variable across all time points. MS/BS= mineralized bone surface; MAR= mineral apposition rate; BFR/BS= bone formation rate

Cancellous osteoclast and osteoid surfaces were not different between radiation and CON mice at any time point: There were no significant differences detected in osteoclast surface (%Oc.S/BS) (4 day p=0.109, 12 day p=0.796, 28 day p=0.299; Figure 3.5A) in CIRR-exposed mice compared to age-matched controls. After irradiation, there were also no differences in cancellous osteoid surface (%OS/BS) (4 day p=0.601, 12 day p=1.000, 28 day p=0.536; Figure 3.5B) as compared to age matched controls.
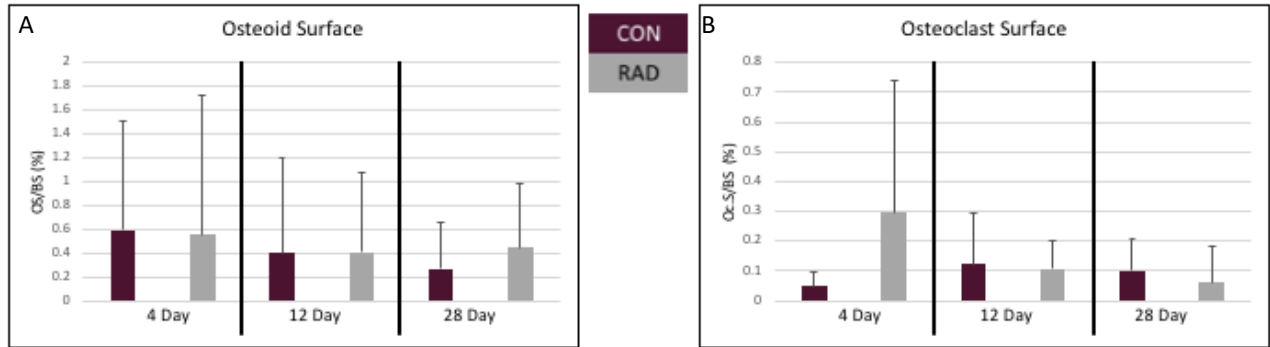
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Figure 3.5: Indices of bone formation and resorption activity in distal femur. Effects of CIRR exposure on (A) % osteoid surface (B) % osteoclast surface. There were no significant differences between RAD and CON for any variable across all time points. OS/BS= osteoid surface; Oc.S/BS= osteoclast surface

Osteocyte apoptosis, measured by % annexin V+ osteocytes, was not different between radiation-exposed and CON mice: Following 28 days of CIRR there were no significant differences in the % Annexin V positive osteocytes in the cancellous bone of the distal femur at any time point (4 day p=0.950, 12 day p=0.330, 28 day p=0.279; Figure 3.6).
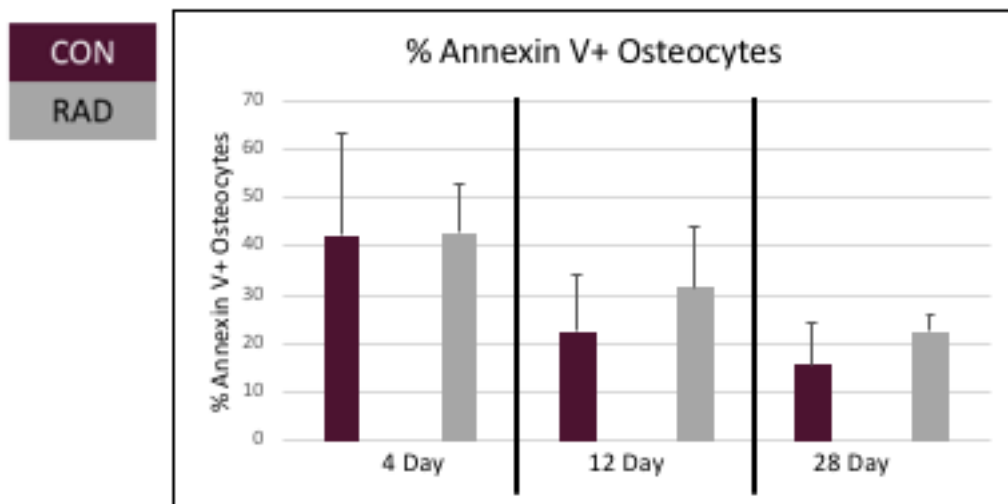

Figure 3.6: Immunohistochemistry staining results for Annexin V positive osteocytes in the distal femur cancellous bone. %Annexin V+ cells were not different between CON and RAD at any time point.

CHAPTER IV

CONCLUSIONS

The primary findings of this study are that continuous, low dose gamma radiation (CIRR) does not negatively impact bone outcomes in older (astronaut age-appropriate) male mice. Specifically, at 3 time points during a 28-day exposure to CIRR, total bone mass, cortical and cancellous bone formation rate, cancellous bone volume and microarchitecture, osteoid and osteoclast surface, and prevalence of osteocyte apoptosis were not detrimentally impacted. This is contrary to what has been previously observed in bone in studies examining higher, doses delivered in one acute exposure

Although there are many factors that can influence astronaut's health during spaceflight, the focus of this study has been on simulating the continuous low dose radiation they will be exposed to during a long duration mission to the moon or Mars. The majority of published literature looking at the effect on radiation on bone has focused on the effect of acute, high dose radiation, usually greater than 2 Gy. Although the energies and ions focused on in these studies examining higher acute doses are relevant to the most damaging parts of the GCR spectrum, their short-term delivery may render their results to not be biologically representative of the radiation astronauts on long duration missions outside Earth's orbit could experience. Only recently has interest in the biological effects of spaceflight driven radiation researchers to examine low, continuous doses of radiation and, to date, very few published studies exist in this arena.

Previous studies examining higher acute doses typically demonstrate that radiation leads to an increase in osteoclast activity following radiation, triggering rapid resorption and bone loss. Three days following a 2 Gy dose of X-ray radiation delivered acutely to 13-week-old mice, there

is a 44% increase in the number of osteoclasts present following radiation, along with a 213% increase in osteoclast surface. (27). After 2 Gy of gamma radiation and hind limb unloading in four-month-old male mice, tissues harvested three days post radiation, there was a 46% increase in osteoclast surface in the tibiae due to radiation (26). This increase is similar to the increase observed following two weeks of hind limb unloading (26). Also, after just three days a 16% decrement in cancellous bone volume in the tibiae, determined by micro-CT, occurs. (26). Also, in this particular study radiation had no effect on cancellous bone formation rate, suggesting that the loss in bone volume was due to increased resorption by osteoclasts, rather than reduced BFR (26). Results from my study indicate no changes in osteoclast surface in continuous low dose radiation

The current knowledge base of radiation literature on bone points to a decrease in bone formation rate due to radiation exposure. Following a single-limb exposure to an acute 2 Gy dose of X-ray radiation in 20-week-old mice, with the other limb serving as a contralateral control, there is a 22% decrease in bone volume one-week post irradiation, as determined by microcomputed tomography (25). This radiation exposure also leads to a significant decrease in bone formation rate compared to control animals, this is due primarily to reductions in mineralizing surface (25). This means that there are fewer osteoblast teams being recruited to the bone surface resulting in a decrease of formation activity and contributing to this decline of bone volume seen in this acute, high dose radiation setting.

Osteocytes are key regulatory cells of bone tissue orchestrating changes in bone formation and bone resorption. In the study mentioned previously, in which one limb was irradiated with 2 Gy of X-ray radiation, detection of fragmented DNA (TUNEL+) osteocytes was used to quantify osteocytes in the early stage of apoptosis one week following irradiation (25). It was found that

TUNEL+ osteocytes were increased in the trabecular compartment of bone following irradiation (25). They also quantified empty lacunae in trabecular bone, which is an indicator of osteocyte cell death, and found that there were no differences between the irradiated limbs and control (25). As osteocytes go through apoptosis they release RANKL, which is a primary stimulator of in osteoclast formation (8). This study found a significant increase in osteoclast number, via TRAP staining, following irradiation (25). This shows that osteocyte apoptosis is associated with a rise in osteoclasts in the area of that osteocyte apoptosis and results in greater resorption, leading to bone loss at that site. Therefore, osteocyte proteins and osteocyte apoptosis could be drivers of the increase in bone resorption seen in high doses of radiation. In my study, osteocyte apoptosis, as measured by staining for annexin V, was not affected by CIRR at short term or chronic time points during a continuous period of radiation exposure. Additionally, there were no increases in cancellous osteoclast surfaces, consistent with no differences in prevalence of osteocyte apoptosis. Therefore, it can be concluded that the continuous low dose radiation to which the older male mice were exposed did not lead to increases in osteocyte apoptosis as seen at higher, acute doses.

Fractionated doses have been studied to mimic the effects that could be seen after exposure to a continuous exposure. However, when three fractionated doses of 0.17 Gy or an acute 0.5 Gy dose of high-LET Si^{28} radiation were delivered to four-month-old female mice, detriments to bone were seen with both dosing regimens (33). Bone volume was decreased in the fractionated and acute dose group compared to sham irradiated, along with significant declines in trabecular number (33). The periosteal and endocortical MS/BS were lower than sham after the fractionated and acute dose (33). These results show that even at what is still considered low doses in the field, albeit considerably higher than the doses in our study, either fractionated or acute, bone loss occurs. My study examined the impact of delivering a low dose continuously and discovered this was not

detrimental for any of my outcome measures of bone mass or microarchitecture. However, it is important to consider that my study used a low-LET radiation, which acts differently within the body than a high-LET radiation. This highlights the importance of future studies examining the impact of different LET radiation doses on bone, as there could be biological differences not only due to the amount of radiation and the rate of delivery, but also the energy of the ionizing radiation.

One other published study examined the effect on low-dose continuous radiation and found similar results to ours. Fourteen-week-old female mice were exposed to a daily dose of 8.5 cGy of gamma for twenty days, resulting in a cumulative dose of 1.7 Gy (30). In these young female mice, some in hindlimb unloading to induce disuse, radiation was found to have no independent effect on cortical and cancellous bone volume and trabecular microarchitecture (30). Also, there were no differences in osteoblast or osteoclast surfaces after twenty days of continuous radiation (30). The authors concluded that the primary effect on bone was disuse-induced bone loss from hindlimb unloading, with no detectable synergistic effects of radiation (30). The dose used in Yu et. al was 10-fold higher than the dose delivered to the animals in our study over twenty-eight days (at the longest time point) at approximately 181.72 mGy/day. Combined these two studies demonstrate that continuous low dose radiation, even doses 10-fold different, appear to have no measureable impact on bone structure or bone turnover, indicating this continuous, low dose-rate radiation may not be as harmful as previously believed.

Another unpublished study performed in our CIRR field, examined the effects of 70 days of this low-dose radiation on twenty male mice (36-41 weeks old) Ten were exposed to approximately 7.14 mGy/day, resulting in a cumulative dose of 0.5 Gy after 70 days. In these mice radiation resulted in an increase in whole body BMD and lean mass, with no effect on fat mass (see Appendix B). These animals were on a modified AIN-76A Diet with 420 gm of sucrose, 60

gm of cellulose, and therefore could not be directly compared to the animals in this study. However, it suggests that CIRR exposure does impact on bone and lean mass at higher, accumulated dose and/or at a later time point that was not examined in this study.

Limitations of this study include examining only bone tissue and not the other factors that could impact on bone cell activity and bone mass. For example, one area of research not addressed in this study is the impact of continuous low dose radiation on immune functioning. Published literature demonstrates an effect of low-dose radiation on immune markers (22). Alterations in immune function can have a significant impact on bone and, even though this study is not seeing a change in bone volume, changes in the immune system could be occurring and, eventually, these changes could impact bone. The use of gamma radiation, as opposed to high energy particles such as iron as a radiation source, can only approximate the effects that could be seen after GCR exposure. This study examined the effects of this continuous low-dose irradiation in older, male mice; younger mice could exhibit a different response due to the fact that they are still growing and have a higher rate of bone turnover. However, using these "middle-aged" mice is also a major strength of this study, since they are closer to typical astronaut age. Also, it is important to examine the effects on female mice, to determine if there are sex-specific differences. It would also be beneficial to look at later time points to ensure that prolonged exposure to this type of radiation does not have a long-term negative impact.

In summary, these results demonstrate that CIRR is not having a detrimental effect on bone microarchitecture nor on osteoblast/osteoclast activity in the first month of exposure. The findings are similar to the one other published study that examined the effects of continuous, low-dose radiation and/or hind limb unloading on bone, which demonstrated no detrimental effect of CIRR on bone and that the major cause of reduced bone mass was the disuse resulting from hind limb

unloading. These results suggest that the radiation experienced from the GCR spectrum in space may not be as detrimental to bone as previously thought.

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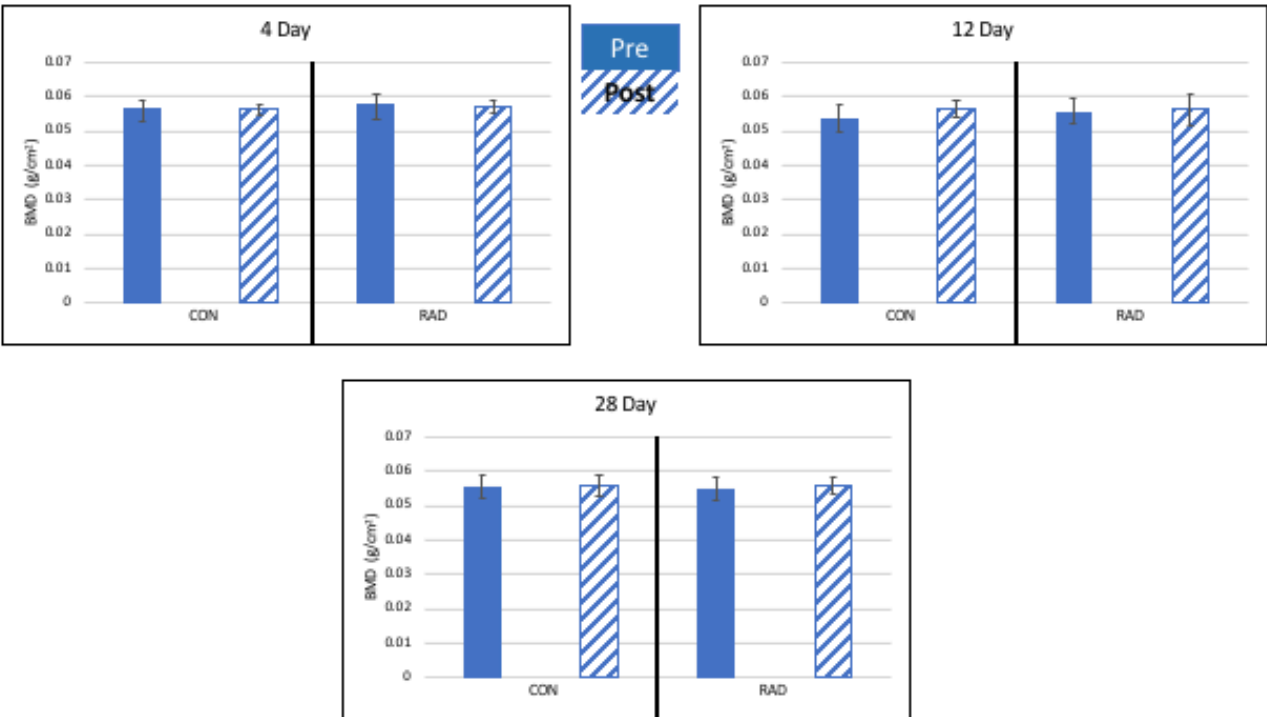
Total body BMD values measured via DXA before the start of CIRR (pre) and after the completion of CIRR (post) at each time point in 47-49-week-old male mice. There was no significant differences between pre and post at each time point and between each treatment (either CON or RAD).
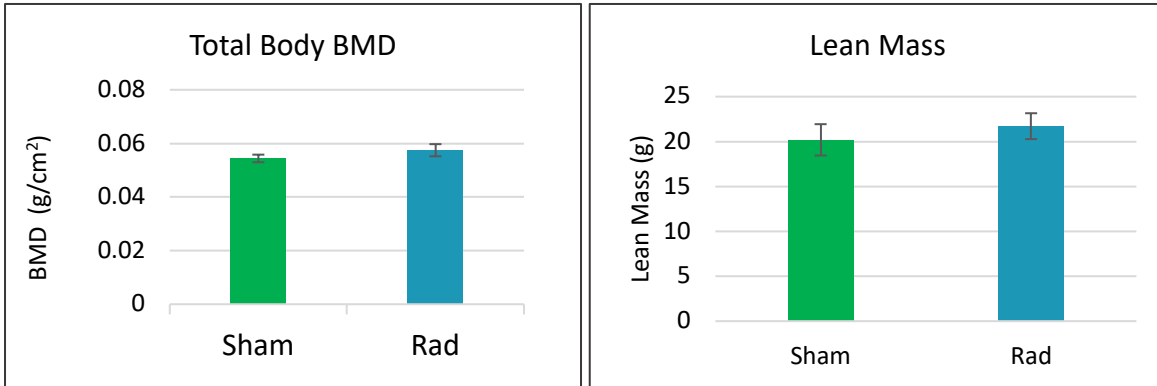
In-Vivo Total Body DXA after 70 days of CIRR exposure. Twenty male mice (36-41 weeks old) were used and a cumulative dose of 0.5 Gy was experienced by the RAD group. Seventy days of CIRR revealed that RAD lead to a 5.6% increase in total body BMD and a 7.5% increase in lean mass compared to SHAM