Published in final edited form as:

Adv Space Res. 2008 ; 42(12): 1889–1897.

Spaceflight-relevant types of ionizing radiation and cortical bone: Potential LET effect?

Shane A.J. Lloyd^a, Eric R. Bandstra^a, Neil D. Travis^a, Gregory A. Nelson^b, J. Daniel Bourland^c, Michael J. Pecaut^b, Daila S. Gridley^b, Jeffrey S. Willey^a, and Ted A. Bateman^{a,*}

^aDepartment of Bioengineering, Clemson University, 501 Rhodes Engineering Research Center, Clemson, SC 29634, USA

^bDepartment of Radiation Medicine, Loma Linda University and Medical Center, Chan Shun Pavilion, Room A-1010, 11175 Campus Street, Loma Linda, CA 82354, USA

^cDepartment of Radiation Oncology, Wake Forest University Health Sciences, Medical Center Boulevard, Winston-Salem, NC 27157, USA

Abstract

Extended exposure to microgravity conditions results in significant bone loss. Coupled with radiation exposure, this phenomenon may place astronauts at a greater risk for mission-critical fractures. In a previous study, we identified a profound and prolonged loss of trabecular bone (29–39%) in mice following exposure to an acute, 2 Gy dose of radiation simulating both solar and cosmic sources. However, because skeletal strength depends on trabecular and cortical bone, accurate assessment of strength requires analysis of both bone compartments. The objective of the present study was to examine various properties of cortical bone in mice following exposure to multiple types of spaceflight-relevant radiation. Nine-week old, female C57BL/6 mice were sacrificed 110 days after exposure to a single, whole body, 2 Gy dose of gamma, proton, carbon, or iron radiation. Femora were evaluated with biomechanical testing, microcomputed tomography, quantitative histomorphometry, percent mineral content, and micro-hardness analysis. Compared to non-irradiated controls, there were significant differences compared to carbon or iron radiation for only fracture force, medullary area and mineral content. A greater differential effect based on linear energy transfer (LET) level may be present: high-LET (carbon or iron) particle irradiation was associated with a decline in structural properties (maximum force, fracture force, medullary area, and cortical porosity) and mineral composition compared to low-LET radiation (gamma and proton). Bone loss following irradiation appears to be largely specific to trabecular bone and may indicate unique biological microenvironments and microdosimetry conditions. However, the limited time points examined and non-haversian skeletal structure of the mice employed highlight the need for further investigation.

Keywords

Radiation; Spaceflight; Cortical bone; Bone loss; Microcomputed tomography

1. Introduction

Bone loss remains a serious obstacle for the future of long-duration spaceflight. Previous studies have demonstrated that astronauts on 4–6 month missions aboard the International Space Station experience femoral and vertebral bone loss of approximately 0.9–1.6% per month, with losses being associated with declines in estimated bending and compression strength indices at these sites (Lang et al., 2004). Bone loss and the corresponding loss of strength could increase the risk of fractures and pose a risk to mission safety.

The deleterious effects of the space environment include both microgravity and ionizing radiation components. Skeletal unloading and a negative calcium balance have long been understood as a primary cause of spaceflight-induced bone loss (Smith et al., 2005). We have identified a potential role of spaceflight-relevant doses and types radiation in this process (Bandstra et al., 2008; Hamilton et al., 2006; Willey et al., 2008). Radiation sources of particular concern in space include cosmic rays (i.e., heavy ions and protons) and solar particle events (SPEs), which primarily consist of protons (Benton and Benton, 2001; Blakely, 2000; Townsend, 2005). These primary particles are accompanied by various forms of secondary radiation, such as Bremsstrahlung X-rays and neutrons, which are produced as heavy ions strike shielding materials (Cucinotta et al., 2001; Edwards, 2001; Setlow et al., 1996). While cosmic rays result in a relatively continuous low-dose rate exposure, SPEs occur randomly and can deliver a relatively high dose (Benton and Benton, 2001; Blakely, 2000; Stephens et al., 2005).

NASA's current exploration roadmap outlines a return to the Moon in preparation for more ambitious Mars exploration in the following years (Diaz and Elachi, 2005). During lunar missions, unexpected solar flares and coronal mass ejections are of particular concern. Although spacecraft shielding can effectively reduce radiation exposure, the warning provided by surveillance mechanisms may not allow for complete protection during extra-vehicular activities on the lunar surface (Setlow et al., 1996). Even when protected by a mass thickness of 5 g/cm² (approximately 1.9 cm of aluminum, or 5 cm of water), astronauts could receive whole-body radiation doses approaching 2 Gy in rare events, such as those observed during August 1972 and October 1989 (Parsons and Townsend, 2000). Considering the planned 6–8 month duration of lunar outpost mission, a dose of approximately 1 Gy proton is a realistic possibility and should be considered for planning purposes (Cucinotta and Durante, 2006; Cucinotta et al., 2003; Ohnishi and Ohnishi, 2004). These types of events are of less concern on the surface of Mars, where the atmosphere provides some level of protection from SPEs (Reedy and Howe, 1999). However, during long-duration Mars missions there is concern about cumulated radiation exposure from cosmic rays, which is estimated to be on the order of 0.2 Gy per year (Ballarini et al., 2006; Cucinotta and Durante, 2006; National Council on Radiation Protection and Measurements, 2006).

Long bones are composed of dense cortical bone surrounding a spongy network of interconnected trabecular struts embedded in the marrow (Marieb, 2004). The relative importance of cortical and trabecular bone to overall skeletal strength is complex. Both bone types make important contributions to overall strength, the degree to which depends on specific location within the bone. Diaphyseal (mid shaft) strength is entirely comprised of cortical bone, while trabecular bone contributes 11–57% of the mechanical strength in the vertebrae of rats (Ito et al., 2002) and 50–70% to the strength in human vertebrae (Homminga et al., 2001). As a result, specific evaluation of both trabecular and cortical bone compartments following irradiation is required to determine the degree to which overall strength is affected following damage to each.

Previously published studies describe deleterious effects to cortical bone following high-dose X-rays: single doses of 30–50 Gy resulted in substantial reductions in cortical bone mechanical strength (Maeda et al., 1988; Nyaruba et al., 1998; Sugimoto et al., 1991). However, the same doses administered in fractions did not produce the similar reductions (Nyaruba et al., 1998). A recent study from our laboratory demonstrated significant trabecular bone loss following 2 Gy doses of multiple radiation types (Hamilton et al., 2006). Compared to control, trabecular volume fraction was reduced by approximately a third following gamma, proton, and carbon irradiation. Trabecular connectivity density was approximately half of control values following proton, gamma, carbon, or iron irradiation. No difference based on linear energy transfer (LET) was observed for these values, although trabecular thickness was reduced for both carbon and iron radiation types (high-LET) compared to gamma and proton (low-LET) radiation. We have since identified a 13–15% loss of trabecular bone 4 months after exposure to 1 Gy protons, with no long-term loss in mice exposed to 0.5 Gy; the bone changes were limited to trabecular bone with no differences in any cortical bone parameters (Bandstra et al., 2008).

Despite the profound loss of trabecular bone observed previously, a thorough investigation of the impact of multiple spaceflight-relevant types of radiation on cortical bone properties is still required. The purpose of this paper is to examine the effects of various types of spaceflight-relevant radiation types on femoral cortical bone. Knowledge of the effects of moderate dose radiation on cortical bone may allow for the development of targeted countermeasures and more accurate simulations of spaceflight-induced bone loss.

2. Methods

2.1. Animals

Forty-eight female, nine-week old C57BL/6 mice (Charles River Breeding Labs; Wilmington, MA) were utilized in the present study. Animals were acclimatized for 7 days prior to irradiation, with food and water provided ad libitum. The Institutional Animal Care and Use Committee at both Loma Linda University Medical Center (LLUMC; Loma Linda, CA) and Brookhaven National Laboratory (BNL; Upton, NY) approved all procedures.

2.2. Irradiation protocol

Four groups of mice received whole-body irradiation using 60-Cobalt (^{60}Co) gamma rays, linear energy transfer (LET) = 0.23 keV/ μm , $n = 10$; protons ($^1\text{H}^{1+}$, 250 MeV/n), LET = 0.4 keV/ μm , $n = 10$; carbon ($^{12}\text{C}^{6+}$, 290 MeV/n), LET = 13 keV/ μm , $n = 9$; or iron ($^{56}\text{Fe}^{26+}$, 1 GeV/n), LET = 148 keV/ μm , $n = 9$. A control group ($n = 10$) was not irradiated. Low-LET exposures (^{60}Co and protons) were performed at Loma Linda University Medical Center. For ^{60}Co irradiation, a horizontal beam from a retired AECL (Atomic Energy of Canada; Commercial Products Division, Ottawa, Canada) Eldorado therapy unit was used. Protons were delivered in 0.3-s pulses every 2.2 s. High-LET exposures (C and Fe) were performed at the Brookhaven National Laboratory in the NASA Space Radiation Laboratory.

Before irradiation, the animals were placed individually into rectangular polystyrene boxes with air holes (30 × 30 × 85 mm). All irradiations were delivered as 25–40 pulses/min to yield a cumulative 2 Gy with average dose rates ranging from 0.6 to 1.2 Gy/min. Particle radiation (protons, carbon, and iron) was delivered at the entrance plateau region of the beam, at the beginning of the Bragg Peak, such that LET levels were held constant throughout the target volume. Four to six unanesthetized mice were irradiated simultaneously. The irradiation conditions were coordinated between investigators and physicists at the two facilities. Thus, exposures were performed with uniform procedures and fixtures, and euthanasia and tissue harvesting occurred at a similar time post exposure. The mice irradiated at Brookhaven National Laboratory were shipped within 3 days to Loma Linda University for housing and

analysis. A complete summary of the experimental groups and radiation characteristics is outlined in Table 1.

All animals were euthanized with 100% CO₂ at 110 days post irradiation. Hindlimbs were removed immediately following euthanasia and transported at −20 °C to Clemson University (Clemson, SC), where femora were cleaned of all non-osseous tissue. One femur from each animal, required for microCT assays, biomechanical testing, and compositional analysis, was allowed to air-dry for 48 h. The other femur, required for histomorphometry and micro-hardness, was fixed in a 10% neutral buffered formalin solution for 48 h, rinsed with distilled water, and stored in 70% ethanol.

2.3. Microcomputed tomography

Microcomputed tomography (μCT 20, Scanco Medical AG; Bassersdorf, Switzerland) was used to determine maximum, minimum, and polar moments of inertia, as well as cortical porosity and cortical volume. One-hundred and five diaphyseal slices (9 μm each) were scanned, with an interslice distance of 100 μm. The span of the diaphysis scanned was the same as that subjected to later biomechanical testing (9 mm). Scans were initiated at the base of the femoral neck and continued distally in order to isolate the junction of the femoral neck and diaphysis as a reference starting point for offline analysis. A total of 101 slices (approximately 9 mm) were evaluated distal to this established reference point.

2.4. Biomechanical testing

Harvested femora were rehydrated in phosphate buffered saline (0.9% w/v) for 90 min in order to simulate in vivo conditions (Broz et al., 1993). A three-point bending test examined biomechanical properties of the femora at the mid-diaphysis using an Instron 5582 (Instron Corporation; Norwood, MA), with a 50 N load cell (0.05 N resolution) and Bluehill 2 software (Instron Corporation). Femora were loaded to failure using an anvil with a 9 mm span length. Each femur was placed in a standardized position, with condyles facing toward in the anvil. A deflection rate of 5 mm/min was used. Force (N) and deflection (mm) were collected at 10 Hz. Custom software was used to determine the elastic limit (P_e), maximum force (P_m), and force at fracture (P_f) from the measured values. Stiffness (S) was determined based on the acquired force deflection curves.

2.5. Histomorphometry

Fixed femora were allowed to air-dry and were then embedded with non-infiltrating Epo-Kwick epoxy (Buehler; Lake Bluff, IL). The formed disks were sectioned with a low-speed saw (Buehler, 127 × 0.5 mm diamond blade) at the mid-diaphysis of the femur. These sections were wheel-polished to a flat, smooth surface using 600-, 800-, and 1200-grit carbide paper followed by polishing with a cloth impregnated with 6 μm diamond paste. This allowed images of bone cross-sections to be taken at 50× magnification using a Zeiss Axioskop 2 plus microscope (Carl Zeiss MicroImaging; Thornwood, NY) and AxioVision software (Carl Zeiss MicroImaging). SigmaScan Pro 5 (Systat Software; Point Richmond, CA) software was used to analyze the images. The perimeters at the endocortical and periosteal surface were traced and analyzed to determine medullary area (Me.Ar) and total bone area (T.Ar), respectively. Cortical bone area was calculated by subtracting total bone area from medullary area (T.Ar – Me.Ar).

2.6. Compositional analysis

Fractured femora used for biomechanical testing were utilized in order to determine percent mineral content. Proximal and distal epiphyses were separated from the diaphysis and the mass of each was recorded using a calibrated analytical scale (Mettler Toledo UMT2; Columbus,

OH). Each component was then positioned in an Isotemp Muffle furnace (Fisher Scientific; Pittsburgh, PA) at 105 °C for 24 h, after which dry mass (Dry-M) was determined. Femoral components were then reduced to ash at 800 °C for 24 h, after which mineral mass (Min-M) was measured. Organic mass (Org-M) was calculated as Dry-M – Min-M. The percent mineral content (%Min) was calculated as Min-M/Dry-M * 100%.

2.7. Micro-hardness indentation

Micro-hardness indentation analysis was conducted on the same femora utilized for quantitative histomorphometry. Embedded femora were analyzed using a Micromet 5101 micro-hardness indenter (Buehler). Four indentations were performed on the lateral side of each femur. Indentation started 40 µm from the periosteal surface and continued at 40 µm intervals. The length and width of each indentation was measured and recorded. An average value was then calculated from micro-hardness measurements made on each femur. Vicker's method (Callister, 2007) was used to calculate the hardness of each bone in relation to the control group. Vicker's hardness is calculated with respect to the magnification of the ocular at the time of measurement and by the average of the length and width of the diamond shaped indentation produced.

2.8. Statistics

Statistical analysis was performed using SigmaStat v3.5 (Systat Software, Inc.), using a one-way ANOVA with a Fisher LSD post-hoc test for follow-up comparisons. A 95% level of significance (Type I error, $P < 0.05$) was utilized for each of these tests. All values are presented as mean $\pm$ standard error (SE).

3. Results

3.1. Irradiated versus control

Mean animal mass and femoral lengths were not different between any of the irradiated groups and control (Table 3, $P > 0.05$). There were no differences of gamma-or proton-irradiated mice for any parameter compared to control animals, but there were some differences between control and high-LET irradiated mice. For mechanical strength, iron-irradiated mice had a lower fracture force compared to control animals (Fig. 4, $P < 0.05$); with no differences for stiffness and elastic and maximum forces (Table 2). No difference in moment of inertia, cortical volume, cortical area, or micro-hardness was found in irradiated animals versus control (Fig. 5 and Table 3, $P > 0.05$). There was a greater cortical porosity for iron-irradiated mice compared to control at the distal femur (Fig. 1, $P < 0.05$). Medullary area was observed to be different between carbon and control groups (Fig. 2, $P < 0.05$). Compositional analysis (Fig. 3, $P < 0.05$) indicated that both carbon-and iron-irradiated mice had lower percent mineral content compared to control mice for both the epiphyses and whole (total) bone.

3.2. Low- versus high-LET

Significant differences were found between radiation treatment groups, as classified by their relative ionization density (i.e., linear energy transfer, LET). High-LET (carbon and iron) irradiation resulted in significant negative effects for certain bone parameters when compared to low-LET (gamma and proton). Biomechanical testing revealed the mean femora maximum and fracture force following iron irradiation was 12% and 30% lower, respectively, than those from the gamma-irradiated group (Table 2 and Fig. 4, $P < 0.05$). Carbon-irradiated mice also had a 22% lower fracture force compared to gamma-irradiated mice (Fig. 4, $P < 0.05$). Maximum and fracture force in iron-irradiated animals was 12% and 29% lower, respectively, than in proton-irradiated mice (Table 2 and Fig. 4, $P < 0.05$).

Results of microCT analysis revealed that at the distal femur cortical porosity in iron-irradiated mice was 7% and 10% greater than that of gamma- and proton-irradiated groups, respectively (Fig. 1, $P < 0.05$ for both). In addition, quantitative histomorphometry indicated 8% greater medullary area in mice exposed to carbon versus gamma irradiation (Fig. 2, $P < 0.05$). Trends towards increased polar moment of inertia within high-LET group were also observed (Fig. 5, $P > 0.05$).

Compositional analysis (Fig. 3, $P < 0.05$) indicated that carbon-irradiated animals had lower percent mineral content compared to both gamma- and proton-irradiated mice for both the epiphyses and whole (total) bone. Iron-exposed mice also had lower percent mineral composition compared to gamma-irradiated animals for both the epiphyses and whole bone.

4. Discussion

4.1. Possible LET effect on cortical bone

The results of the present study are consistent with a recent examination of only proton irradiation (Bandstra et al., 2008) and suggest that cortical bone is largely spared the severe deleterious effects of irradiation previously described for trabecular bone (Hamilton et al., 2006). Few significant differences in cortical bone parameters were found for radiation groups compared to control in regards to mechanical, structural, or material properties; however, an LET-related effect on the cortical bone compartment may exist. The differences that did exist were with high-LET radiation types (carbon and iron) compared to low-LET species (gamma and proton).

Observed significant differences in cortical parameters for carbon and iron radiation types compared to gamma and proton radiation, suggest that high-LET particles may reduce structural properties and mechanical strength to a greater extent than low-LET particles. Interpretation of these results is difficult. The relatively small, but divergent, effects of the two LET types may have resulted in a net significant difference between the two. These results highlight the need for larger group sizes, which is especially important considering the number of treatments being compared.

MicroCT analysis revealed that the cortical porosity of the iron-irradiated group was significantly greater than both gamma- and proton-irradiated, indicating increased resorption at the proximal and distal portions of the diaphysis. This increase in porosity would tend to reduce structural and mechanical strength, which is consistent with the concomitant decrease in fracture force that was observed for iron-irradiated versus gamma- and proton-irradiated animals.

In order to withstand the same applied loads following increased trabecular resorption, the cross-sectional area of the cortical bone must increase. The finding of increased medullary area in carbon-irradiated mice suggests an expansion of the marrow cavity without a compensatory expansion at the periosteal surface in order to maintain average total cortical area. Such a physiological response would tend to result in a net loss of structural strength. Indeed, the maximum and fracture force in mechanically tested femora isolated from the iron-irradiated mice were significantly lower than those from both gamma and proton-irradiated groups.

A possible LET effect was also suggested by increased polar moment of inertia and significant increases in cortical porosity and area at the distal portion of iron-irradiated femora (Figs. 1 and 5). These findings, combined with the aforementioned mechanical and compositional differences, correspond well to one another. Increased polar moment of inertia would potentially be a compensatory response to increased porosity and the previously described loss of trabecular bone.

4.2. Trabecular specificity

Current literature identifies negative effects of radiation on cortical bone strength, but at doses far higher than experienced in spaceflight (Maeda et al., 1988; Sugimoto et al., 1991; Nyaruba et al., 1998). The specificity of radiation damage to trabecular bone over cortical bone at moderate doses is interesting. The fact that trabecular bone is affected by moderate radiation doses at all may at first be surprising, considering that bone has a radiation tissue-weighting factor (W_T) of 0.01. Bone marrow, however, is quite radiation sensitive, with a W_T of 0.12 (International Commission on Radiological Protection, 1992). A unique property of trabecular bone over cortical bone is that it has a proportionally greater surface area in direct contact with the bone marrow. In addition, trabecular bone is spatially contained within the metabolically active red marrow at the epiphyses of long bones and vertebrae. A possible explanation for the increased loss of trabecular bone is this proximity to marrow ($W_T = 0.12$) and the increased inflammatory activity observed immediately following irradiation (International Commission on Radiological Protection, 1992). The greater material density and presence of larger calcium and phosphorus atoms in bone (Bilezikian et al., 1996) results in more ionization events in the local micro-environment. However, the relative paucity of dividing cells within cortical bone and the presence of radiation insensitive, terminally differentiated osteocytes, means that these ionization events may have little biological effect; a significant reason bone has the lowest tissue-weighting factor (International Commission on Radiological Protection, 1992).

In contrast, the surface of trabecular bone has a much greater potential for radiation effects. An ionizing event within the trabecular bone may impact cells located at this interface between the marrow and solid bone composing the trabecular struts. Radiation interactions with the relatively high density, high atomic number components of both cortical and trabecular bone yield secondary, lower energy electrons that deposit their energy in the bone marrow. This ionization increase in the bone marrow microenvironment may lead to substantial damage to the metabolically active cells it contains, potentially leading to an inflammatory response and non-specific activation of bone-resorbing osteoclasts. Indeed, we have identified a significant increase in osteoclast number within trabecular bone 3 days after exposure to a 2 Gy dose of X-rays (Willey et al., 2008). This proposed mechanism is similar to bone loss associated with rheumatoid arthritis and wear debris osteolysis (Findlay and Haynes, 2005; Holding et al., 2006). This hypothesis is supported by other studies that have identified a clear inflammatory response to damaged marrow following irradiation (Lorimore and Wright, 2003). Because cortical bone has only a relatively small endocortical surface exposed to marrow, it is not as susceptible to the bone-resorbing activity of these activated osteoclasts. Although dose deposition inhomogeneities also occur at the cortical-marrow interface, the magnitude of the effect appears to be lower, possibly due to the spatial orientation of the materials and their interface. The microdosimetric aspects for dose deposition within the trabecular space remain an area of study as a function of ionizing radiation type and energy (Gersh et al., 2007).

4.3. Limitations

There were several important limitations to the experimental design employed in the present study that have influenced our ability to conduct specific analyses. Most important was that skeletal tissue was collected from the mice secondary to the primary systems being investigated in another study. This made control over specific parameters, such as administration of calcein bone labels, impossible. In addition, only one animal sacrifice time point was used (+110 days). Although this single-point schedule allowed for evaluation of the chronic effects of acute radiation exposure, it hindered our ability to observe the early onset and timing of changes following irradiation. Specific responses in the bone marrow, including inflammation and osteoclast activation, likely occurred much earlier (Willey et al., 2008), with the cortical bone already beginning its recovery by the end of the study. Although it is plausible that the bone could have fully remodeled prior to sacrifice, the use of percent mineral content analysis and

micro-hardness indentation assays should have indicated material changes in the presence of active bone remodeling.

Also of note is the fact that mice bones are not vascularized with a haversian system in the same manner as larger mammals, such as rats and humans. As a result, mice are likely to respond differently to radiation. Because the large surface area of the haversian canals resembles the structure of trabecular bone, it is possible that a more substantial cortical response would occur in these animals following irradiation. Moreover, it has been established that radiation damages vasculature (Mitchell and Logan, 1998) and this has the potential to initiate a cortical bone response due to widespread inflammation of the damaged blood vessels within the haversian canals. Furthermore, it should be noted that the C57BL/6 strain of mice utilized in the present study have a relatively low baseline bone mass. It is possible that the observed response following radiation exposure could have been less than that of mouse strains with higher mass.

In future studies, correlation of microdosimetric and cortical parameters in the neck of the femur, where the majority of hip fractures in post-menopausal osteoporosis occur, may provide additional insight into the anatomic and functional consequences of low-dose radiation. Implementation of a time course study, with early time points following irradiation, would allow for a more accurate assessment of the physiological and pathological changes that are occurring.

5. Conclusions

Following microgravity unloading, trabecular and cortical bone compartments are differentially affected (Lang et al., 2004). The net result is an increase in fracture risk both during and after missions (Lang et al., 2006). Ionizing radiation exposure may act in concert to further increase this risk. The results of the present study indicate that cortical bone in mice is relatively resistant to radiation-induced bone loss, considering the large loss of trabecular bone observed in our previous study. While the differences are subtle, high-LET radiation (carbon and iron) may have a greater negative effect on cortical bone compared to low-LET radiation (gamma and proton). Although additional studies with a range of lower radiation doses, microdosimetric assessment, and varying follow-up times are needed to describe the complete physiological response of bone to the space environment, the present study has provided fundamental information to guide future studies on radiation and cortical bone properties.

Acknowledgments

The authors thank Michael F. Moyers for ^{60}Co and proton dose calibrations and Melba L. Andres, Anna L. Smith, and Steve Rightnar for expert technical assistance. Gratitude is also extended to Marcelo Vazquez, Peter Guida, Betsy Sutherland, Laura Thompson, Adele Billups, and the rest of the NASA Space Radiation Laboratory (NSRL) staff; MaryAnn Kershaw, Kerry Bonti and other Brookhaven National Laboratory Animal Care Facility staff; and Adam Rusek and the physics support staff at Brookhaven National Laboratory NSRL.

Support for this work was funded by the following grants to Clemson University: (TAB, JSW, ERB) National Space Biomedical Research Institute (NSBRI) through NASA NCC 9-58, BioServe Space Technologies (NASA NCC8-242), a NASA GSRP Fellowship from Kennedy Space Center (NGT5-50440), and Grant Number R21AR054889 from National Institutes of Health (NIAMS); and to Loma Linda University (GAN) NASA Cooperative Agreement (NCC9-79) Radiation Biology NSCOR.

References

Ballarini F, Battistoni G, Cerutti F, Fasso A, Ferrari A, Gadioli E, Garzelli MV, Mairani A, Ottolenghi A, Paretzke HG, et al. GCR and SPE organ doses in deep space with different shielding: Monte Carlo

- simulations based on the FLUKA code coupled to anthropomorphic phantoms. *Adv. Space Res* 2006;37:1791–1797.
- Bandstra ER, Pecaute 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. *Radiat. Res* 2008;169:607–614. [PubMed: 18494551]
- Benton ER, Benton EV. Space radiation dosimetry in low-Earth orbit and beyond. *Nucl. Instrum. Methods Phys. Res. B* 2001;184:255–294. [PubMed: 11863032]
- Bilezikian, JP.; Raisz, LG.; Rodan, GA. *Principles of Bone Biology*. Academic Press; San Diego: 1996.
- Blakely EA. Biological effects of cosmic radiation: deterministic and stochastic. *Health Phys* 2000;79:495–506. [PubMed: 11045523]
- Broz JJ, Simske SJ, Greenberg AR, Luttgies MW. Effects of rehydration state on the flexural properties of whole mouse long bones. *J. Biomech. Eng* 1993;115:447–449. [PubMed: 8309241]
- Callister, WD. *Materials Science and Engineering: An Introduction*. John Wiley & Sons; New York: 2007.
- Cucinotta FA, Durante M. Cancer risk from exposure to galactic cosmic rays: implications for space exploration by human beings. *Lancet Oncol* 2006;7:431–435. [PubMed: 16648048]
- Cucinotta, FA.; Schimmerling, W.; Wilson, JW.; Peterson, LE.; Badhwar, GD.; Saganti, PB.; Dicello, JF. *Space Radiation Cancer Risk Projections for Exploratory Missions: Uncertainty Reduction and Mitigation*. Johnson Space Center; Houston, TX: 2001.
- Cucinotta FA, Wu H, Shavers MR, George K. Radiation dosimetry and biophysical models of space radiation effects. *Gravit. Space Biol. Bull* 2003;16:11–18. [PubMed: 12959127]
- Diaz, A.; Elachi, C. *A Roadmap for the Robotic and Human Exploration of Mars*, NASA. 2005.
- Edwards AA. RBE of radiations in space and the implications for space travel. *Phys. Med* 2001;17(Suppl 1):147–152. [PubMed: 11771542]
- Findlay DM, Haynes DR. Mechanisms of bone loss in rheumatoid arthritis. *Mod. Rheumatol* 2005;15:232–240. [PubMed: 17029071]
- Gersh JA, Dingfelder M, Toburen LH. Modeling energy deposition in trabecular spongiosa using the monte carlo code penelope. *Health Phys* 2007;93:47–59. [PubMed: 17563492]
- Hamilton SA, Pecaute 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. *J. Appl. Physiol* 2006;101:789–793. [PubMed: 16741258]
- Holding CA, Findlay DM, Stamenkov R, Neale SD, Lucas H, Dharmapathi AS, Callary SA, Shrestha KR, Atkins GJ, Howie DW, Haynes DR. The correlation of RANK, RANKL and TNF α expression with bone loss volume and polyethylene wear debris around hip implants. *Biomaterials* 2006;27:5212–5219. [PubMed: 16806459]
- Homminga J, Weinans H, Gowin W, Felsenberg D, Huiskes R. Osteoporosis changes the amount of vertebral trabecular bone at risk of fracture but not the vertebral load distribution. *Spine* 2001;26:1555–1561. [PubMed: 11462085]
- International Commission on Radiological Protection. 1990 Recommendations of the International Commission on Radiological Protection, user's edition. Published for the International Commission on Radiological Protection by Pergamon Press; Oxford, New York: 1992.
- Ito M, Nishida A, Koga A, Ikeda S, Shiraishi A, Uetani M, Hayashi K, Nakamura T. Contribution of trabecular and cortical components to the mechanical properties of bone and their regulating parameters. *Bone* 2002;31:351–358. [PubMed: 12231406]
- Lang T, LeBlanc A, Evans H, Lu Y, Genant H, Yu A. Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight. *J. Bone Miner. Res* 2004;19:1006–1012. [PubMed: 15125798]
- Lang TF, Leblanc AD, Evans HJ, Lu Y. Adaptation of the proximal femur to skeletal reloading after long-duration spaceflight. *J. Bone Miner. Res* 2006;21:1224–1230. [PubMed: 16869720]
- Lorimore SA, Wright EG. Radiation-induced genomic instability and bystander effects: related inflammatory-type responses to radiation-induced stress and injury? A review. *Int. J. Radiat. Biol* 2003;79:15–25. [PubMed: 12556327]

- Maeda M, Bryant MH, Yamagata M, Li G, Earle JD, Chao EY. Effects of irradiation on cortical bone and their time-related changes. A biomechanical and histomorphological study. *J. Bone Joint Surg. Am* 1988;70:392–399. [PubMed: 3346264]
- Marieb, EN. *Human Anatomy and Physiology*. Pearson Education; New York: 2004.
- Mitchell MJ, Logan PM. Radiation-induced changes in bone. *Radio-graphics* 1998;18:1125–1136. quiz 1242–1243.
- National Council on Radiation Protection and Measurements. *Information Needed to Make Radiation Protection Recommendations for Space Missions Beyond Low-Earth Orbit*. National Council on Radiation Protection and Measurements; Bethesda, MD: 2006.
- Nyaruba MM, Yamamoto I, Kimura H, Morita R. Bone fragility induced by X-ray irradiation in relation to cortical bone-mineral content. *Acta Radiol* 1998;39:43–46. [PubMed: 9498868]
- Ohnishi K, Ohnishi T. The biological effects of space radiation during long stays in space. *Biol. Sci. Space* 2004;18:201–205. [PubMed: 15858386]
- Parsons JL, Townsend LW. Interplanetary crew dose rates for the August 1972 solar particle event. *Radiat. Res* 2000;153:729–733. [PubMed: 10825747]
- Reedy, RC.; Howe, SD. *The Martian Radiation Environment from Orbit and on the Surface.. Workshop on Mars 2001: Integrated Science in Preparation for Sample Return and Human Exploration*; 1999. p. 87
- Setlow, R.; Dicello, JF.; Fry, RJM.; Little, JB.; Preston, RJ.; Smathers, JB.; Ullrich, RL. *Radiation Hazards to Crews of Interplanetary Missions: Biological Issues and Research Strategies*. Space Studies Board, National Research Council, and National Academy Press; Washington, DC: 1996. p. 13-34.
- 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. *J. Bone Miner. Res* 2005;20:208–218. [PubMed: 15647814]
- Stephens DL Jr. Townsend LW, Hoff JL. Interplanetary crew dose estimates for worst case solar particle events based on historical data for the Carrington flare of 1859. *Acta Astronaut* 2005;56:969–974. [PubMed: 15835055]
- Sugimoto M, Takahashi S, Toguchida J, Kotoura Y, Shibamoto Y, Yamamuro T. Changes in bone after high-dose irradiation. Biomechanics and histomorphology. *J. Bone Joint Surg. Br* 1991;73:492–497. [PubMed: 1670456]
- Townsend LW. Implications of the space radiation environment for human exploration in deep space. *Radiat. Prot. Dosimetry* 2005;115:44–50. [PubMed: 16381680]
- Wiley JS, Grilly LG, Howard SH, Pecaue MJ, Obenaus A, Gridley DS, Nelson GA, Bateman TA. Bone architectural and structural properties after $^{56}\text{Fe}^{26+}$ radiation-induced changes in body mass. *Radiat. Res* 2008;170:201–207. [PubMed: 18666808]
- Wiley JS, Lloyd SAJ, Robbins ME, Bourland JD, Norrdin RW, Smith-Sielicki H, Bowman LC, Bateman TA. Early increase in osteoclast number in mice following whole-body irradiation with 2 Gy X-rays. *Radiat. Res* 2008;170:388–392. [PubMed: 18763868]

Fig. 1.

Cortical porosity per slice from mice femora 110 days after whole-body gamma, proton, carbon, or iron irradiation (2 Gy). Slice 1 starts at the proximal portion of the diaphysis and continues distally for 9 mm. At the distal femur a large, significant peak for the iron irradiated group exists compared to all other treatment groups. All other areas have no significant difference ($P > 0.05$).

Fig. 2. Medullary area of the mid diaphysis femur measured in mice 110 days after whole-body gamma, proton, carbon, or iron irradiation (2 Gy). The medullary area after carbon irradiation is significantly increased when compared with gamma irradiation ($P < 0.05$). #Significant difference from carbon-irradiated group ($P < 0.05$). Error bars represent standard error.

Fig. 3.

Percent mineral content of total, epiphysis, and diaphysis components of mouse femora 110 days after whole-body gamma, proton, carbon, or iron irradiation (2 Gy). #Significant difference from carbon-irradiated group ($P < 0.05$), *significant difference from iron irradiated group ($P < 0.05$). Error bars represent standard error.

Fig. 4.

Fracture force of femora isolated from mice 110 days after whole-body gamma, proton, carbon, or iron irradiation (2 Gy). #Significant difference from carbon-irradiated group ($P < 0.05$), *significant difference from iron irradiated group ($P < 0.05$). Error bars represent standard error.

Fig. 5.

Polar moment of inertia per slice from mice femora 110 days after whole-body gamma, proton, carbon, or iron irradiation (2 Gy). Slice 1 starts at the proximal portion of the diaphysis and continues distally for 9 mm. At the distal femur near-significant differences indicate high-LET (iron and carbon) groups may have a greater polar moment of inertia than the low-LET groups (gamma and proton). This may represent a compensatory mechanism to increased cortical porosity and loss of trabecular bone at the distal femur. All other areas have no significant difference ($P > 0.05$).

Table 1

Summary of experimental groups and radiation particle characteristics

Group	Radiation type	Group size	Per particle energy (MeV/n)	Linear energy transfer (keV/μm)	Dose (Gray)
I	Control	10	-	-	0
II	Gamma	10	-	0.23	2
III	Proton	10	250	0.40	2
IV	Carbon	9	290	13	2
V	Iron	9	1000	148	2

Each 2 Gy dose was delivered at a rate of 0.6–2 Gy/min.

Table 2

Biomechanical properties of femora from mice 110 days after whole-body gamma, proton, carbon and iron irradiation (2 Gy)

Irradiation type	Stiffness (N/mm)	Force (N)		
		Elastic	Maximum	Fracture
Control	30.8 ± 2.8	13.3 ± 0.9	16.8 ± 0.4	12.3 ± 0.8 [*]
Gamma	34.6 ± 2.6	15.3 ± 1.1	17.8 ± 0.7 [*]	13.5 ± 1.3 ^{*,#}
Proton	34.8 ± 3.0	15.6 ± 1.7	17.8 ± 1.1 [*]	13.1 ± 1.3 [*]
Carbon	38.7 ± 3.4	14.4 ± 0.9	16.5 ± 0.3	10.5 ± 0.5
Iron	34.7 ± 2.5	13.1 ± 0.9	15.6 ± 0.3	9.4 ± 0.5

[#] Significant difference from carbon-irradiated group ($P < 0.05$).

^{*} Significant difference from iron irradiated group ($P < 0.05$). Data are presented as mean ± standard error.

Cortical bone and animal mass parameters

Table 3

Radiation type	Cortical area (mm ²)	Medullary area (mm ²)	Micro-hardness (no units)	Cortical volume (mm ³)	Mean animal mass at sacrifice (g)
Control	0.94 ± 0.03	0.83 ± 0.02 [#]	75.3 ± 1.9	9.5 ± 0.1	25.2 ± 0.4
Gamma	0.93 ± 0.01	0.82 ± 0.02 [#]	79.9 ± 1.0	9.3 ± 0.2	26.8 ± 0.8
Proton	0.91 ± 0.03	0.86 ± 0.02	76.5 ± 1.7	9.4 ± 0.1	26.6 ± 0.9
Carbon	0.92 ± 0.01	0.90 ± 0.02	75.6 ± 1.3	9.4 ± 0.1	26.7 ± 0.4
Iron	0.93 ± 0.01	0.87 ± 0.01	75.1 ± 2.0	9.3 ± 0.1	26.8 ± 0.7

Cortical area, medullary area, and micro-hardness parameters were obtained from prepared section of mid-diaphysis femur cross-sections. Cortical volume represents the volume of the entire femur diaphysis and was collected by microCT analysis. Femora were collected from mice 110 days after whole-body gamma, proton, carbon and iron irradiation (2 Gy).

[#] Significant difference from carbon-irradiated group (*P* < 0.05). Data are presented as mean ± standard error.