Oxidative stress and gamma radiation-induced cancellous bone loss with musculoskeletal disuse

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Kondo H, Yumoto K, Alwood JS, Mojarrab R, Wang A, Almeida EA, Searby ND, Limoli CL, Globus RK. Oxidative stress and gamma radiation-induced cancellous bone loss with musculoskeletal disuse. J Appl Physiol 108: 152–161, 2010. First published October 29, 2009; doi:10.1152/japplphysiol.00294.2009.—Exposure of astronauts in space to radiation during weightlessness may contribute to subsequent bone loss. Gamma irradiation of postpubertal mice rapidly increases the number of bone-resorbing osteoclasts and causes bone loss in cancellous tissue; similar changes occur in skeletal diseases associated with oxidative stress. Therefore, we hypothesized that increased oxidative stress mediates radiation-induced bone loss and that musculoskeletal disuse changes the sensitivity of cancellous tissue to radiation exposure. Musculoskeletal disuse by hindlimb unloading (1 or 2 wk) or total body gamma irradiation (1 or 2 Gy of 137Cs) of 4-mo-old, male C57BL/6 mice each decreased cancellous bone volume fraction in the proximal tibia and lumbar vertebrae. The extent of radiation-induced acute cancellous bone loss in tibiae and lumbar vertebrae was similar in normally loaded and hindlimb-unloaded mice. Similarly, osteoclast surface in the tibia increased 46% as a result of irradiation, 47% as a result of hindlimb unloading, and 64% as a result of irradiation + hindlimb unloading compared with normally loaded mice. Irradiation, but not hindlimb unloading, reduced viability and increased apoptosis of marrow cells and caused oxidative damage to lipids within mineralized tissue. Irradiation also stimulated generation of reactive oxygen species in marrow cells. Furthermore, injection of α-lipoic acid, an antioxidant, mitigated the acute bone loss caused by irradiation. Together, these results showed that disuse and gamma irradiation, alone or in combination, caused a similar degree of acute cancellous bone loss and shared a common cellular mechanism of increased bone resorption. Further, irradiation, but not disuse, may increase the number of osteoclasts and the extent of acute bone loss via increased reactive oxygen species production and ensuing oxidative damage, implying different molecular mechanisms. The finding that α-lipoic acid protected cancellous tissue from the detrimental effects of irradiation has potential relevance to astronauts and radiotherapy patients.

spacelife; osteopenia; hindlimb unloading; α-lipoic acid

SPACE IS A UNIQUE ENVIRONMENT that challenges the skeletal health of astronauts. Long-duration spaceflight causes a negative calcium balance and a decline in bone density selectively in tissues that are normally loaded on Earth, posing an increased risk for fracture (45). On entry into space, musculoskeletal disuse and a cephalad fluid shift in microgravity trigger rapid physiological adaptations (26). In addition to the risks imposed by reduced gravity, astronauts are exposed to radiation at low doses and various dose rates and energies as a result of background galactic cosmic radiation and occasional solar particle events (17). Musculoskeletal disuse and radiation exposure each can cause degeneration of connective tissues, including bone (20, 52). Whether the weightless environment of space affects tissue responses to space radiation is unknown. In an early spaceflight experiment, rats were exposed to a relatively high dose (800 rad) of 137Cs during flight (Cosmos 690; 20.5 days of spaceflight), and results were compared with ground-based simulation controls and a previous flight without an onboard radiation source (Cosmos 605) (41). Spaceflight reportedly worsened some of the adverse effects of radiation; however, because this conclusion relied heavily on qualitative and histological analyses, rather than functional tests, further research is needed. Better insight into the cellular and molecular mechanisms for bone loss in space may yield new strategies for early intervention to prevent subsequent bone loss and osteoporosis.

Limited access to the spaceflight environment and the importance of understanding physiological mechanisms underlying disuse motivated the development of a ground-based animal model to simulate certain aspects of weightlessness (36, 53). Hindlimb unloading removes weight bearing from the hindquarters of rats or mice and causes a cephalad fluid shift. Hindlimb unloading reduces bone mass (osteopenia), bone strength, and bone formation by osteoblasts and, in some cases, can increase the numbers of osteoclasts in unloaded bones (8, 9, 11, 15, 25, 42, 46). Hindlimb unloading of mice recapitulates many of the cardiovascular, hormonal, immunosuppressive, and musculoskeletal changes observed in astronauts and humans subjected to bed rest, which is a spaceflight analog for humans and, thus, provides a well-characterized model for studying physiological adaptations to the weightless environment of space (2).

Although radiation at high doses can cause substantial degeneration of various tissues, including bone marrow, little is known about how the complex spectrum of space radiation may affect bone. Space radiation cannot be precisely replicated on Earth, although recent insight has been gained by exposure of mice to a single ~2-Gy dose of total body irradiation (3, 16, 23, 56, 57); a total ~2-Gy dose corresponds to the dose to which an astronaut would be exposed on a lengthy mission outside the Earth’s magnetosphere and to a typical single dose of fractionated therapeutic radiation. The dose rate used in these published reports exceeds that of space, although a test of different radiation sources that contribute to galactic cosmic radiation (protons and heavy ions) reveals that total body irradiation causes a cancellous bone loss similar to that observed following exposure to X-rays or gamma sources (16; Yumoto et al., unpublished observations). In efforts to simulate

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the low-linear energy transfer (LET), high-energy (>100-
meV) proton component of the space radiation environment, 
we chose to use gamma rays emitted from the decay of 137Cs. 
These low-LET photons elicit radiobiological effects similar to 
those elicited by higher-energy protons and provide the means 
to compare the effects of higher-LET heavy ions in the galactic 
cosmic radiation. Thus gamma irradiation provides a suitable 
experimental model to investigate the influence of space-
relevant doses of radiation on bone.

Reactive oxygen species (ROS) and reactive nitrogen spe-
cies (RNS) may mediate tissue degeneration in age-related 
diseases, such as osteoporosis, and after insults, such as radia-
tion exposure (30). In addition, spaceflight followed by return 
to Earth can lead to oxidative damage in blood and various soft 
tissues (18, 48, 49). ROS can directly stimulate bone resorption 
by osteoclasts (7, 28), and the bone loss due to aging and 
estrogen deficiency may be attributed, at least in part, to 
oxidative stress (1, 6, 34). The release of ROS/RNS can cause 
oxidative damage, which is associated with excess bone re-
sorption by osteoclasts over bone formation by osteoblasts, 
reduced viability of the putative mechanosensory cells (i.e., 
osteocytes), and osteoporosis (1). Furthermore, treatment with 
the potent antioxidant α-lipoic acid (α-LA) can prevent inflam-
mation-induced bone loss (13). The generation of excess ROS/ 
RNS and resulting oxidative damage within skeletal tissues 
during spaceflight may contribute to later bone loss and weak-
ening during recovery.

We showed previously that gamma irradiation leads to a 
rapid decrease in cancellous fractional bone volume [bone 
volume (BV) as a fraction of total volume (TV)] in postpuber-
tal mice (23) and an increase in bone-resorbing osteoclasts (23, 
57). We also showed that in vitro irradiation of osteogenic cells 
from the bone marrow leads to increased generation of ROS (22). 
These findings support the following hypotheses: 1) irradiation 
causes oxidative stress and bone loss, and 2) acute radiation 
damage may be worsened by concomitant musculoskeletal 
disuse. To begin to test these possibilities, we subjected mice 
to hindlimb unloading followed by irradiation with 137Cs and 
evaluated parameters relevant to cancellous microarchitecture, 
cell dynamics, and oxidative stress. We treated animals with 
α-LA to determine its effectiveness in ameliorating acute 
radiation-induced bone loss. Our results show that hindlimb 
unloading and irradiation each acutely stimulated osteoclastic 
bone resorption and bone loss and that α-LA inhibited the 
acute osteopenic effects of gamma irradiation.

**METHODS**

**Animals.** Male C57BL/6J mice (Jackson West, Bar Harbor, ME) at 
17 wk of age were used for all experiments. Mice were randomized by 
weight, assigned to groups (n = 6/group), and acclimatized to cages 
for 2 days before initiation of the experiments. Mice were housed in 
an animal room under controlled conditions (24 ± 2°C, 55 ± 5% 
humidity, 12:12-h light-dark cycle). Food and water were available ad 
libitum. Mice were maintained in standard cages with the same 
footprint as custom-designed hindlimb-unloading cages. Mice were 
hindlimb unloaded by tail traction according to previously described 
methods (35). We measured body weights throughout the experiments 
to monitor the animals’ health. The Ames Research Center Institu-
tional Animal Care and Use Committee approved all procedures.

**Experimental protocols.** The experimental conditions for two sepa-
rate hindlimb-unloading experiments (Fig. 1) were selected on the 
basis of our previous results showing dose and time dependence of 
radiation-induced cancellous bone loss (23) and separate hindlimb-
unloading studies (40). Two different durations of hindlimb unloading 
and doses of radiation were used, with the goal of capturing interac-
tion effects of hindlimb unloading and irradiation, should this occur. 
Our previous time-dependent, radiation dose-response experiments 
revealed that a longer time was required to elicit significant changes 
with 1 Gy than with 2 Gy (23). In planning the hindlimb-unloading 
experiments, we reasoned that hindlimb unloading may accelerate 
and/or worsen the effects of irradiation, not knowing a priori the 
effects hindlimb unloading might have on the radiation response. 
Therefore, we tested the more effective (i.e., higher) dose in the 
short-term experiment (2 Gy, 3 days), as well as the lower dose in the 
longer-term experiment (1 Gy, 10 days). Our rationale for irradiating 
mice during ongoing disuse was that this regimen reflects a likely 
spaceflight scenario.

In experiment 1, mice were hindlimb unloaded or normally loaded, 
4 days later they were exposed to 2 Gy of 137Cs or sham-irradiated, 
and after an additional 3 days they were euthanized and tissues were 
harvested (Fig. 1). Mice were injected with saline (1 mg/kg sc) 3 
days and 1 day before tissue harvest for measurements of bone 
formation. In experiment 2, mice were hindlimb unloaded or normally 
loaded, 4 days later they were exposed to 1 Gy of 137Cs or sham-
irradiated, and after an additional 10 days they were euthanized and 
tissues were harvested. In experiment 3, normally loaded mice were 
irradiated with 1 or 2 Gy of 137Cs 10 days or 3 days before tissue 
harvest, respectively. In experiment 4, normally loaded mice were 
irradiated with 2 Gy of 137Cs or sham-irradiated and injected subcu-
taneously twice daily with α-LA (25 mg/kg body wt) or vehicle, and tissues were harvested 3 days later.

**Gamma irradiation.** Mice were subjected to uniform whole body 
irradiation using a 137Cs irradiator (Mark I, JL Shepherd) equipped
with a turntable at a dose rate of 0.92 Gy/min. The animals were positioned in a ventilated animal holding chamber within the irradiator, which provided exposures within 95% of the selected dose.

Sample collection and handling. After the mice were euthanized, bones were harvested from the carcass. Left tibiae were fixed in 70% ethanol and stored at room temperature until analysis. Lumbar vertebrae were removed as a unit and wrapped with saline-soaked gauze and then stored at −20°C. Muscle was removed from the tibia, and the vertebrae were thawed at 4°C overnight before micro-CT scanning at room temperature.

Micro-CT. Tibiae were subjected to three-dimensional (3-D) micro-CT analysis using a Viva CT 40 (Scanco Medical, Bassersdorf, Switzerland), as previously described in detail (23). Briefly, we evaluated several images from control mice to establish nominal segmentation values, which were selected as a best average match to gray-scale images to capture bone structure without excessive porosity. The segmentation values were held fixed for all 3-D trabecular evaluations throughout the study. The proximal metaphyses were scanned at maximum resolution (voxel size = 10.5 μm) into a single stack consisting of 210 slices. Cancellous tissue was analyzed in a region 0.2–1.2 mm distal to the growth plate. The first lumbar vertebral body was scanned at maximum resolution into a single stack consisting of 360 slices (3.8 mm) transverse to the cranio-caudal axis. A 2.1-mm region was analyzed, beginning 0.5 mm proximal to the caudal end cap and extending proximally toward the cranial end cap. Data are based on calculations for total area, bone volume, and BV/TV. BV/TV describes the fraction of total volume within the selected region of interest that is occupied by bone itself, not marrow space, and values are generally proportional to mechanical properties of the tissue.

Cancellous histomorphometry. The left tibiae were fixed in 4% paraformaldehyde overnight and then decalcified in 20% EDTA for 2 wk. For quantification of osteoclasts, decalcified bones were embedded in paraffin, and 5-μm-thick sagittal sections were cut and stained with paraformaldehyde overnight and then decalcified in 20% EDTA for 2 wk and stained with osteocalcin antibody and counterstained with hematoxylin. TRAP-positive multinucleated osteoclasts were measured, and extending distally 0.7 mm. TRAP-positive cells that also contained more than two nuclei were scored as osteoclasts, and cell numbers were normalized to the total length of bone surface (BS) within the region of interest. For quantification of the cancellous surfaces occupied by osteoclasts, the lengths of bone surfaces occupied by TRAP-positive multinucleated osteoclasts were measured, summed, and normalized to BS within the region of interest. For measurements of bone formation rate (BFR) in mineralized sections, left femora were embedded in polymethylmethacrylate, and 0.5-mm sections were processed for fluorescent detection of calcein. Sections were analyzed by fluorescent confocal microscopy using a Zeiss 510 confocal microscope with postimaging software to optimize signal detection. Surface labeling of bone sections using calcein is visualized on a 2.1-mm region was analyzed, beginning 0.5 mm proximal to the caudal end cap and extending proximally toward the cranial end cap. Data are based on calculations for total area, bone volume, and BV/TV. BV/TV describes the fraction of total volume within the selected region of interest that is occupied by bone itself, not marrow space, and values are generally proportional to mechanical properties of the tissue.

Influence of hindlimb unloading and irradiation on body weight. The health of the mice was monitored by twice-daily weighing and observation of the animals. Body weights at the time of tissue harvest did not differ significantly between groups (Table 1), although the mice that were both hindlimb unloaded (1 wk) and irradiated (2 Gy) weighed 8% less than normally loaded controls at the time of sample recovery. Irradiation (2 Gy) and hindlimb unloading (1 or 2 wk) each caused splenic atrophy, as expected (54, 55), with no greater effect in combination (data not shown). Thus the combination of hindlimb unloading and gamma irradiation was well tolerated by the mice.

Influence of hindlimb unloading and irradiation on cancellous microarchitecture. Mice were exposed to two different experimental regimens on the basis of our previous findings.
that irradiation caused time- and dose-dependent cancellous bone loss (23). Micro-CT revealed similar structural changes after irradiation and hindlimb unloading, with less bone and more void space (marrow) evident in 3-D reconstructions of the tissue (Fig. 2A). At 3 days after 2-Gy irradiation, a significant decrement in cancellous BV/TV of tibiae (Fig. 2, A and B) and lumbar vertebrae (Fig. 2D) was observed; in normally loaded mice, irradiation (2 Gy) caused a 16% decrement in tibiae compared with sham-irradiated controls and a 5% decrement in lumbar vertebrae. The lower dose (1 Gy) after 10 days (Fig. 2C) caused a similar decline in the tibia, but not vertebrae (Fig. 2E), which was consistent with our previous results (23). Similarly, hindlimb unloading for 7 or 14 days caused cancellous bone loss in tibiae and lumbar vertebrae. There were no interaction effects in BV/TV by two-factor ANOVA, indicating that the response to irradiation was not significantly different due to loading condition. Thus acute effects of irradiation in normally loaded or hindlimb-unloaded mice were similar in both experiments, and no additional effect was observed when the two factors were combined, suggesting that cellular pathways mediating acute bone loss may be shared.

Influence of hindlimb unloading and irradiation on bone cells, marrow cells, and oxidative damage to mineralized tissue. Hindlimb unloading for 1 wk caused a 47% increase in cancellous bone surface covered with TRAP-positive osteo-

Fig. 2. Hindlimb unloading and irradiation effects on cancellous bone volume in axial and appendicular bones. Mice were hindlimb unloaded or normally loaded for 7 days and irradiated with 2 Gy of $^{137}$Cs 3 days before tissue harvest (experiment 1; A, B, and D) or hindlimb unloaded for 14 days and irradiated with 1 Gy of $^{137}$Cs 10 days before tissue harvest (experiment 2; C and E). Cancellous tissue within the proximal tibiae (A, B and C) or the centra of the 1st lumbar vertebrae (D and E) were analyzed by micro-CT. Hindlimb unloading and irradiation each reduced cancellous fractional bone volume [bone volume/total volume (BV/TV)], and there was no additional effect when hindlimb unloading was combined with irradiation. Values are means ± SD. Data were analyzed statistically by 2-factor ANOVA, with irradiation and loading condition (IR × load) as factors. $P < 0.05$ was accepted as significant.
clasts (OcS/BS) and a 33% increase in the numbers of osteoclasts per bone surface (N.Oc/BS; Fig. 3, A and B). Irradiation at 2 Gy also increased OcS/BS and N.Oc/BS to an extent similar to that observed after hindlimb unloading. No further effects were found when mice were subjected to hindlimb unloading and irradiation. In contrast, irradiation had no effect on BFR, whereas hindlimb unloading of sham-irradiated mice reduced BFR 17% relative to normally loaded controls, as expected (Fig. 3E). The decline in BFR due to unloading in sham-irradiated mice was due to a reduction in MAR (reflecting osteoblast activity; Fig. 3C), but not MS/BS (reflecting osteoblast number; Fig. 3D). The lack of change in BFR due to irradiation was due to a combination of reduced MS/BS and a trend \( P < 0.0586 \) toward increased MAR; thus irradiation did not significantly affect BFR (product of MAR and MS/BS). These results indicate that the rapid cancellous bone loss caused by irradiation was due primarily to increased bone resorption by osteoclasts, rather than reduced BFR.

Given the marked radiosensitivity of bone marrow that contains progenitors for both mesenchymal-derived osteoblasts

![Fig. 3. Hindlimb unloading and irradiation effects on osteoclasts and bone formation in situ. Mice were hindlimb unloaded or normally loaded for 7 days and irradiated with \(^{137}\)Cs (2 Gy) 3 days before tissue harvest (experiment 1). Proximal tibiae were analyzed for number of osteoclasts normalized to bone surface (OcS/BS, A), number of osteoclasts normalized to total length of bone surfaces within region of interest (N.Oc/BS, B), mineral apposition rate (MAR, C), mineralizing surface as a fraction of bone surface (MS/BS, D), and bone formation rate (BFR, E). Hindlimb unloading and irradiation each increased numbers and bone surfaces covered by osteoclasts, and there was no additional effect when hindlimb unloading was combined with irradiation. Hindlimb unloading alone inhibited BFR as a result of inhibition of MAR (reflecting osteoblast activity). Although irradiation reduced MS/BS (reflecting osteoblast number), it had no effect on BFR. Values are means ± SD. Data were analyzed statistically by 2-factor ANOVA, with irradiation and loading condition as factors. \( P \leq 0.05 \) was accepted as significant.](http://jap.physiology.org/)
and hematopoietic-derived osteoclasts, we investigated viability and apoptosis in marrow cells freshly extracted from femora. Irradiation (2 Gy) markedly reduced marrow cell viability (−58%) and increased the fraction of cells that were undergoing apoptosis within 3 days, whereas hindlimb unloading for 7 days had no such effects (Fig. 4, A and B).

To determine whether irradiation and/or hindlimb unloading caused oxidative damage to the mineralized compartment of bone, which may be related to the observed loss of marrow cell viability, we assayed mineralized tissue of femora for lipid peroxidation by measuring the levels of MDA and 4-HNE. Irradiation with 2 Gy of normally loaded mice caused a modest increase in lipid peroxidation after 3 days of exposure, whereas hindlimb unloading for 1 wk had no effect (Fig. 5C).

**Gamma irradiation and oxidative stress.** To explore further the cellular and molecular changes triggered by gamma radiation, we irradiated mice with 1 or 2 Gy and recovered samples 3 or 10 days later (experiment 3 in Fig. 1). Irradiation markedly reduced marrow cell viability within 3 days, as previously observed (Fig. 4), but only with the higher 2-Gy dose (Fig. 5A). This effect was transient, inasmuch as viability returned to near-control levels by day 10 (Fig. 5A). The early drop in viability coincided with a transient increase in the fraction of apoptotic cells detected at day 3 (Fig. 5B). To test for the generation of ROS after irradiation, we loaded marrow cells with the fluorogenic dye precursor CM-H2DCFDA and conducted flow cytometric analysis. Relatively low doses of gamma rays were sufficient to increase ROS levels 3 and 10 days after irradiation (Fig. 5C). Lipid peroxidation levels in the mineralized tissue increased significantly at 1 and 2 Gy 10 days after irradiation (Fig. 5D), indicative of persistent and cumulative oxidative damage. Immunoreactivity for MDA and 4-HNE was evident throughout marrow and mineralized tissue and appeared qualitatively more intense in samples from irradiated mice than controls (Fig. 5, E and F).

In summary, irradiation and hindlimb unloading caused cancellous bone loss and increased osteoclast number. Only hindlimb unloading inhibited BFR, whereas only irradiation decreased marrow cell viability, increased apoptosis of marrow cells, and caused oxidative damage to lipids in mineralized tissue. In no case did the combination of hindlimb unloading and irradiation exert a greater effect than either factor alone.

**α-LA mitigated the adverse effects of gamma irradiation on mineralized tissue and marrow cell viability.** To test whether treatment with an antioxidant may prevent radiation-induced skeletal damage, we treated mice with α-LA during and after irradiation. Mice irradiated 3 days before tissue harvest showed the expected decrement in BV/TV (Fig. 6A), as previously observed (see Fig. 2, A, B, and D, and Ref. 23). Injection of α-LA alone did not significantly affect BV/TV in sham-irradiated mice but did reduce bone loss caused by irradiation (Fig. 6A). Analysis of freshly extracted bone marrow cells showed that α-LA partially, but significantly, ameliorated the radiation-induced decline in marrow cell viability (Fig. 6B).

**DISCUSSION**

We investigated the short-term effects of musculoskeletal disuse and gamma irradiation, reasoning that probing early changes in bone cell function that lead to tissue loss will yield insight into interactions, mechanisms, and potential therapeutic interventions. Some of the skeletal responses to disuse and gamma irradiation, including the degree of cancellous bone loss and the increase in osteoclasts, were similar, even when treatments were combined. These findings imply that musculoskeletal disuse and irradiation share cellular pathways leading to acute bone loss, namely, bone resorption by osteoclasts. There were, however, notable differences in the skeletal responses to the two stimuli. Hindlimb unloading reduced cancellous BFR, although irradiation did not. In contrast, irradiation caused oxidative changes in bone marrow cells and min-
eralized tissue that were not observed with hindlimb unloading. Together, these results support the proposal that although increased bone resorption by osteoclasts was responsible for the acute cancellous bone loss due to hindlimb unloading or irradiation, only gamma irradiation did so by causing oxidative stress.

Acute cancellous bone loss induced by hindlimb unloading or irradiation was observed in appendicular (tibiae) and axial (vertebrae) bones, as previously reported (23), although the extent of acute bone loss was greater in the tibiae than in the vertebrae. Similarly, cancellous bone loss is evident in axial and appendicular bones of astronauts during long-duration missions (27), presumably due to insufficient mechanical stimulation by weight bearing. The degree of gamma irradiation-induced osteopenia was similar in hindlimb-unloaded and normally loaded mice in this short-duration study, consistent with results obtained using a heavy-particle radiation source (56Fe; Yumoto et al., unpublished observations). These findings do not preclude the possibility that disuse influences skeletal responses to radiation in the long term.

Hindlimb unloading and irradiation each increased the numbers and resorbing surface of osteoclasts in cancellous tissue, and these effects were not additive. Although hindlimb unloading inhibited BFR as expected, irradiation did not. These results, together with earlier findings of a net loss of bone in postpubertal mice within 3 days of exposure to gamma radiation (23), show that acute osteopenia was caused predominantly by increased bone resorption, rather than decreased bone formation. Similarly, astronauts show evidence that bone resorption increases in flight, whereas bone formation does not decrease, on the basis of calcium kinetic studies and biomarker levels (47). The lack of an additive effect of gamma irradiation and hindlimb unloading on osteoclasts may be due to each stimulus saturating a key biochemical pathway, leading to
increased osteoclast activity and/or depletion of the pool of osteoclastogenic cells. Further studies are needed to address this issue.

Analysis of bone marrow cell and mineralized tissue responses to irradiation and hindlimb unloading revealed differences in cell viability and oxidative damage. Irradiation, but not hindlimb unloading, decreased viability and increased apoptosis of marrow cells and increased lipid peroxidation within mineralized tissue, as measured by MDA and 4-HNE levels. Furthermore, radiation increased ROS generation by marrow cells. These results are consistent with well-established damaging effects of radiation on other tissues (14). The influence of gamma irradiation on ROS generation was transient and relatively modest (increase of 20–28% relative to controls 3 days after irradiation), whereas radiation-induced oxidative damage to lipids measured by MDA and 4-HNE content accumulated further over time (10 days). Interestingly, immunohistochemical analysis showed that mineralized tissue and bone marrow cells stained positively for MDA adducts. Proteolipids participate in mineralization of the extracellular matrix (5); therefore, the immunopositive material evident in mineralized tissue may be oxidized lipid from extracellular matrix and cell membranes. Lipid peroxidation of chondrocytes in experimental models of osteoarthritis is linked to increased matrix degradation via oxidation of collagen (51), and similarly, the radiation-induced increase in skeletal MDA observed in this study may lead to further degradation of cancellous tissue by osteoclasts.

Our finding that α-LA mitigated radiation-induced cancellous bone loss supports the hypothesis that the generation of ROS in marrow and bone cells leads to cumulative oxidative damage, recruitment and activation of osteoclasts, and resorption of bone. Treatment with α-LA improved the viability of bone marrow cells following irradiation, as observed after other cytotoxic chemotherapeutic treatments, e.g., cyclophosphamide (44). Injection of α-LA in vivo protects connective tissue in experimental models of arthritis (29) and inflammatory bone disease (13, 19). In vitro, α-LA inhibits osteoclast differentiation (19, 21, 29) and protects a bone marrow cell line from DNA damage and apoptosis induced by TNF-α or H2O2 (4). In fact, α-LA has pleiotropic functions, including regulation of cellular redox and cytokine and insulin signaling (24, 43). However, our results, together with the findings of others, suggest that the potent antioxidant activity of α-LA mediates its radioprotective effects (32, 33).

Although ground-based models have certain limitations, results from these experiments shed new light on the skeletal effects of unloading and irradiation in animal models subjected to conditions designed to simulate the space environment, (10, 16). In contrast to the space environment, hindlimb unloading removes weight bearing from the hindquarters, whereas in space the entire skeleton is unloaded (36). Furthermore, radiation in space consists of a complex mixture of radiation types (high-energy particles, protons, and secondary particles) with far lower dose rates than the single exposure to gamma rays applied in this study at the higher dose rate (38). Nonetheless, insight into skeletal physiology gained from ground-based studies such as this improves our understanding of the causes and progression of bone loss, which has relevance to human health in space and on Earth.

In conclusion, musculoskeletal disuse and a single dose of gamma irradiation triggered rapid increases in osteoclast numbers and trabecular surfaces covered by osteoclasts and also a loss of cancellous tissue in skeletally mature, male mice; these responses were not additive in the short term. Gamma irradiation caused oxidative damage within bone, and treatment with an antioxidant mitigated the damage. Thus treatment with antioxidants (12) may serve as a useful approach to protect bone from the adverse effects of radiation.

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DISCLOSURES

No conflicts of interest are declared by the authors.

Fig. 6. α-LA effects on radiation-induced changes in cancellous bone volume and marrow cell viability. Mice were injected twice daily with α-LA and irradiated with 0 or 2 Gy of 137Cs (experiment 4). After 3 days, tissues were harvested and analyzed by micro-CT for cancellous architecture of the proximal tibia (A) and viable marrow cells by flow cytometry (B). Treatment with α-LA inhibited radiation-induced bone loss and decline in marrow cell viability. Values are means ± SD. Data were analyzed statistically by 2-factor ANOVA, with α-LA treatment and irradiation (IR × α-LA) as factors. P ≤ 0.05 was accepted as significant. Bars show significant differences by Tukey-Kramer post hoc given the significant interaction effects.
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