Osteoporosis profoundly affects the aged and exposure to high doses of radiation can cause bone loss resembling osteoporosis, although the influence of low doses of radiation ($\leq$10cGy) is not known. Furthermore, the adverse effects of radiation may be exacerbated by other skeletal challenges, such as those posed by aging and physical inactivity. Our central hypothesis is that radiation modulates subsequent skeletal degeneration via oxidative pathways. We performed *in vivo* and *in vitro* experiments to address two main objectives. First, determine if low dose, low LET radiation causes radio-adaptive changes in bone metabolism mediated by oxidative stress and second, determine if low doses of either low LET or high LET radiation modulate skeletal health during aging via oxidative mechanisms. We used C57Bl/6J mice as an animal model to address these aims experimentally.

To evaluate the dose-dependence of exposure to low LET radiation and possible adaptive responses, male or female mice were irradiated (TBI, ^{137}Cs, 90 cGy/min, 0.1-200 cGy) at 8-10 wk old, a time when cancellous bone mass begins a steady, age-related decline. Controls were sham-irradiated (IR) and experimental groups were exposed to either a single dose or a fractionated regimen ("priming dose") with or without a high "challenge dose" (100-200 cGy) and tissues were harvested 2 or 3 wk later. Acute high dose exposure (100-200 cGy) but not low exposure (0.1-15 cGy), reduced trabecular bone mass in mice. If combined with a high dose (200 cGy), priming with low dose radiation (10cGy, X5 fractions) further compromised skeletal microarchitecture as measured by 3D microcomputed tomography, although the effect was modest. Thus, although low doses had no observable effect on cancellous tissue structure, our results indicate that fractionated, low dose irradiation when combined with a challenge dose, exerted adverse, rather than beneficial, effects on bone microarchitecture.

To evaluate the time-dependence of bone loss with age, male mice were irradiated and tissues recovered at day 0 (basal controls), 1 and 4 months later. Irradiation did not affect body mass at any dose or time post-IR so the changes in bone structure observed were not due to reduced weight. In sham-IR control mice, indices of trabecular microarchitecture, including trabecular number (TbN), and connectivity density (CnD), did not change between 0 and 1 mo. post-irradiation ($p>0.05$). Cancellous tissue subsequently underwent an age-related decline between 1 and 4 mo. in TbN (-17%) and CnD (-41%) ($p<0.05$). In 10cGy-IR mice, bone loss did not differ from 0cGy-controls at any time. In 100cGy-IR mice, TbN and CnD declined faster between 0 and 1 mo. post-IR (-20% and -36%, respectively; $p<0.05$, ANCOVA for slope differences between groups) relative to age-matched controls, and then remained constant between 1 and 4 mo. post-IR. Thus, a low dose of gamma radiation (10cGy) did not cause bone loss relative to age-matched controls, although a higher dose (100cGy) accelerated detrimental changes in microarchitecture to a rate that was comparable to later skeletal aging in control mice.

The differentiated progeny of both the haematopoietic and mesenchymal cell lineages, which are responsible for bone turnover throughout life, may contribute to adverse skeletal effects of radiation. Total body irradiation

increases the number of osteoclasts and surfaces of trabeculae covered by osteoclasts. To determine if low dose radiation can stimulate the formation of osteoclasts and resorptive activity isolated from the heterogeneous bone marrow compartment, non-adherent bone marrow cells isolated from mice were treated with macrophage colony-stimulating factor (M-CSF; 30 ng/ml) and receptor activator of nuclear factor kappa-B ligand (RANKL; 60 ng/ml), exposed to 10-200cGy of gamma radiation, then cultured for 2-5 days. Osteoclastogenesis was assessed by counting multi-nucleated, tartrate resistant acid phosphatase-positive (TRAP+) cells, by measuring the pits formed on mineralized substrata as an assay for resorptive activity, and by analyzing mRNA expression levels of osteoclast-related genes by RT-PCR. To assess osteoblast differentiation, adherent marrow cells were grown with the addition of ascorbate (50 microg/ml) and beta-glycerophosphosphate (10 mM), and were irradiated 2 days after plating. Osteoblastogenesis was assessed 15 days after irradiation by quantifying alizarin-red mineralized nodule formation and expression levels of osteoblast-related genes. Both low dose (10 cGy) and high dose (200 cGy) radiation increased the number of TRAP+ multinucleated osteoclasts (47% and 66%) and appeared to increase the size but not number of resorptive pits formed in mineralized matrices. Preliminary results indicated irradiation increased expression levels of differentiation and fusion-related genes which were evident at an earlier time point (day 2) than sham-irradiated controls (day 5). In osteoblast cultures, high dose (200cGy) radiation reduced mineralized nodule formation (61% decrease), compared to the sham-irradiated controls although lower doses (10cGy) had no effect. In sum, our results indicate that even low dose (10cGy) ionizing radiation stimulated osteoclastogenesis whereas osteoblastogenesis was not affected at these low doses. Acceleration of osteoclast differentiation and stimulation of resorptive activity provides a plausible explanation for the increased number of osteoclasts lining trabecular surfaces and the bone loss that is observed acutely following irradiation in vivo.

Portions of the skeleton that typically bear weight on Earth are quite sensitive to mechanical loading and/or physical activity; astronauts, patients on bedrest, and often the aged are subjected to musculoskeletal disuse and prolonged periods of inactivity. Using a rodent model (hindlimb unloading) that simulates weightlessness and bedrest, we showed previously that unloading when combined with exposure to high LET radiation causes an increase in radiation sensitivity of bone marrow-derived osteoprogenitors as well as a further decrement in biomechanical properties (ultimate force in compression). We recently extended these observations using both the hindlimb unloading model in mice, and exposure of Newts (*Pleurodeles waltl*) to spaceflight (a novel organ culture system). Our findings lend support to the hypothesis that mechanical unloading can influence the behavior of both haematopoietic and mesenchymal skeletal cell populations in response to irradiation. Changes in mechanical loading are likely to mediate these effects by eliciting changes in the oxidative milieu within the marrow and mineralized compartments of bone.