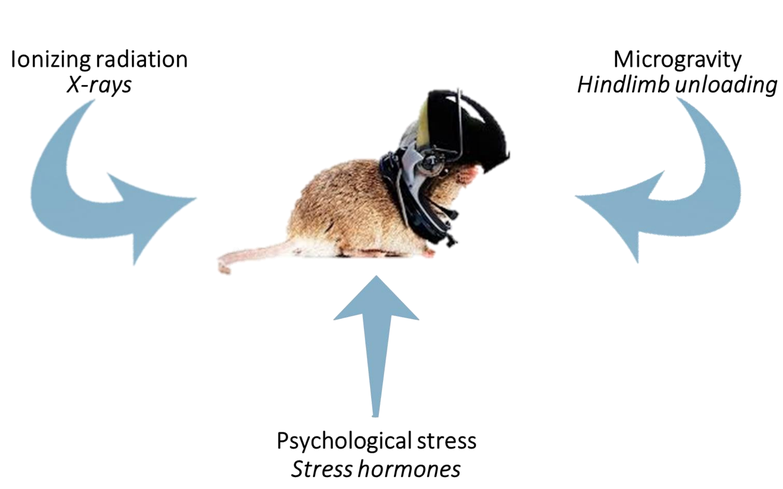
Unloading-Induced Cortical Bone Loss is Exacerbated by Low-Dose Irradiation During a Simulated Deep Space Exploration Mission

Antoine Farley[1], Vasily Gnyubkin[1], Arnaud Vanden-Bossche[1], Norbert Laroche[1], Mieke Neefs[2], Sarah Baatout[2], Bjorn Baselet[2], Laurence Vico[1], Carmelo Mastrandrea[1]
[1]Université de Lyon, Saint-Priest-en-Jarez, France
[2]Belgian Nuclear Research Centre, SCK CEN, Mol, Belgium
E-mail: bjorn.baselet@sckcen.be

Introduction

Spaceflight challenges the human body with a unique set of environmental conditions that are unmatched anywhere on Earth. The three main space stressors are **ionizing radiation, microgravity** and **psychological stress**. It is well known that the lack of mechanical stimulation in microgravity leads to a loss of bone, most prominently in the load-bearing bones of the lower limbs. This loss becomes problematic upon return (fractional) gravitational environments as they predispose astronauts to elevated fracture,

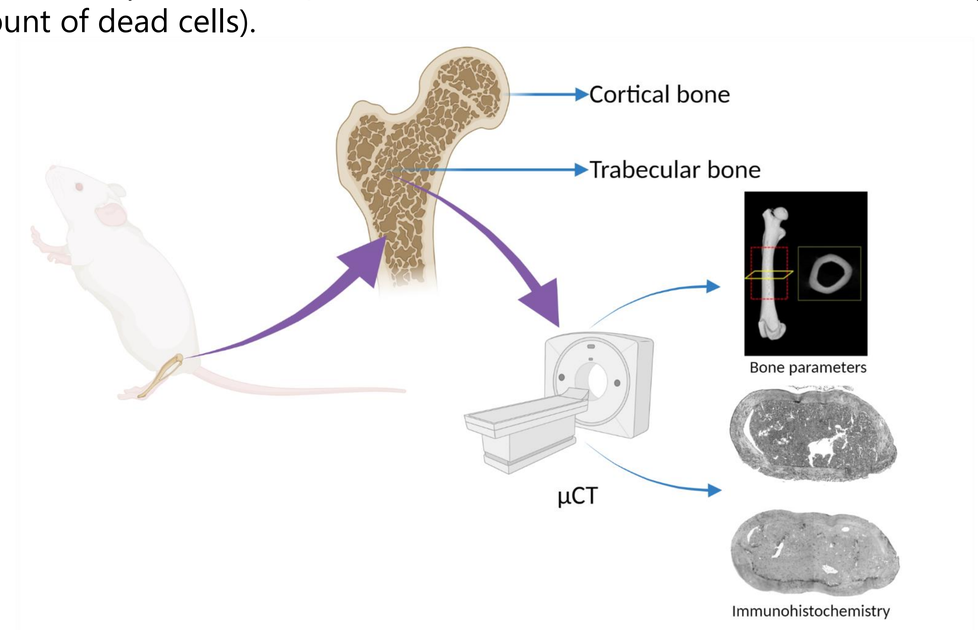


Objective

In order to try to limit these changes and thereby reduce potential deleterious consequences for the skeleton, a more complete understanding of the processes occurring within the bone that lead to these changes is required. Specifically, little is known on the **concomitant effect of low dose radiation and microgravity exposure on bone loss.**

Methods

Hind-limb unloading (HLU) studies, a well-researched rodent model used on Earth which is developed to mimic the microgravity aspects of spaceflight, were performed in mice. To this end we divided 27 male mice in 3 groups: control (**CONT**), hind-limb unloading (**HLU**), and hind-limb unloading plus irradiation (**HLUIR**). In total the hind-limbs of the HLU and HLUIR group were **unloaded for 14 days**. HLUIR mice were irradiated on day 7 after unloading with **25 ± 0.7 mGy X-rays**, an estimated galactic cosmic rays (GCR) equivalent dose for a 14-day exposure to the deep space GCR environment. After 14 days of HLU, all mice were euthanized and bones of their hind-limbs (femur and tibia) were used for a **combination of bone imaging assays** to look at a set of bone parameters (such as bone volume, thickness, bone density and amount of dead cells).



Results

First, we looked at the trabecular (or spongy) bone compartment. Following the 14-day HLU protocol, trabecular bone parameters (mass, bone formation, bone resorption) in the femur and tibia were significantly lower in the HLU and HLUIR groups as compared with CONT. However, we found **no differences in trabecular bone parameters between the HLU and HLUIR groups**. In other words, trabecular bone loss in mice during HLU appears to be unaffected by exposure to a low dose (25 mGy X-rays) of ionizing radiation.

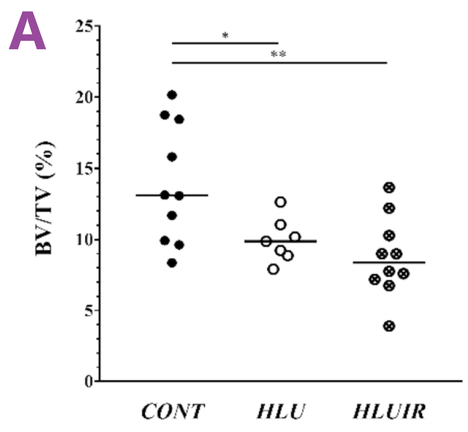


Figure 1: Femoral trabecular bone volume [BV/TV] **(A)** and trabecular thickness [Tb,Th] **(B)** were lower in the HLU and HLUIR groups when compared to CONT. (*p < 0.05, **p < 0.01, ***p < 0.001)

Secondly, we looked at the cortical (or hard/compact) bone compartment. While there was no difference in medullary area and volumetric bone mineral density, we could identify **significant differences in cortical thickness and cortical bone area when comparing either the HLU and CONT animals with the HLUIR animals**. Our data indicates in other words that the combination of low-dose irradiation and HLU in mice led to significant losses in cortical bone.

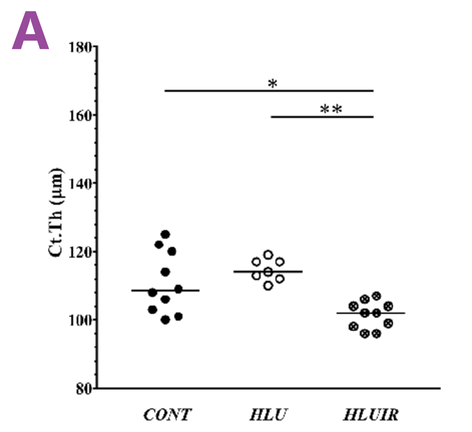


Figure 2: Cortical thickness [Ct.Th] **(A)** and cortical bone area [Ct.Ar] **(B)** were lower in the HLUIR group when compared to CONT and HLU group. (*p < 0.05, **p < 0.01)

Lastly, to determine whether the increased cortical bone loss in HLUIR mice was due to osteocyte (cell that maintains bone tissue) death, we performed immunochemical stainings making use of either Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining combined or DAPI. We found **no evidence for increased osteocyte death when comparing HLU or HLUIR mice with CONT animals**. Hence, the loss of cortical bone in our HLUIR group occurred in the absence of identifiable osteocyte death.

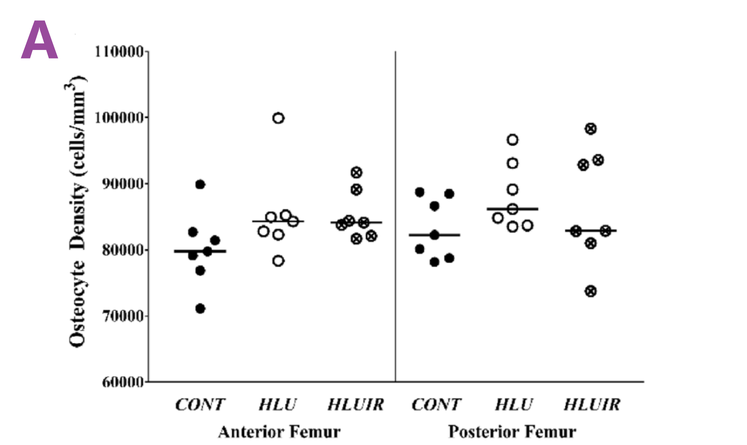


Figure 3: Osteocyte density (DAPI-positive occupied lacunae/analyzed volume) **(A)** and the number of dead (TUNEL positive) osteocytes **(B)** identified by yellow arrowheads **(C)**.

Conclusion

The space environment is known to induce bone loss in astronauts. However, little is known on the concomitant effect of low dose radiation and microgravity exposure on bone. We identified **noticeable differences in the cortical (hard/compact) bone**, with irradiated animals showing significantly lower cortical bone thickness and cross-sectional bone area driven by elevations in osteoclastic resorption (bone break down). Our results indicate that even the low-dose radiation exposure of these missions may negatively influence bone physiology beyond that of induced by microgravity alone, and that these differences may be most apparent in the cortical bone compartment.

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