Change in Mouse Bone Turnover in Response to Microgravity on RR-1

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Background

Mechanical unloading during spaceflight is known to adversely affect mammalian physiology. During our previous short-duration experiments using the Animal Enclosure Modules on Shuttle flights and our recent long-duration experiments on the BionM1 mission, we have observed significant bone loss in both the pelvis and the femur of space flown animals. Specifically, after 15 days of microgravity on STS-131, proximal femurs displayed a 17% decrease in bone volume fraction and 12% decrease in trabecular thickness (**Figure 1**). Furthermore, during 30 days of spaceflight on BionM1, we observed significant bone loss (31%) but perhaps more importantly, we observed the onset of an accelerated aging-like phenotype and osteoarthritic disease state (**Figure 2**).

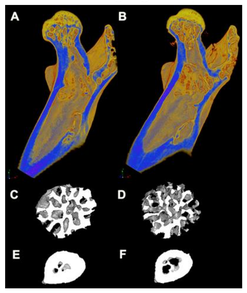


Figure 1. Proximal femur reconstruction from mice flown on 15-day STS-131 mission (B,D,F,) compared to ground controls (A,C,E). BV loss of 17% in flight samples.

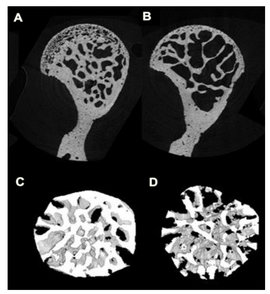


Figure 2. Proximal femur from 30-day BionM1 mission (B,D) compared to ground controls (A,C). Bone Volume (BV) loss of 31% after 30 days of spaceflight.

As bone is a dynamic tissue undergoing constant remodeling and repair from stem cell precursors in the bone marrow compartment (**Figure 3**), we hypothesized that these dramatic alterations in bone tissue may be a consequence of the inhibition of stem cell based tissue regeneration.

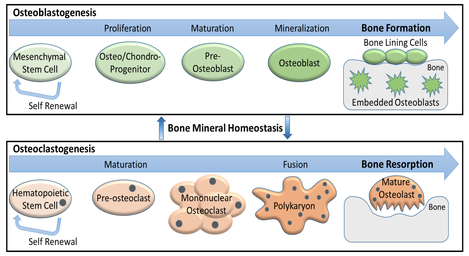


Figure 3. Bone mineral homeostasis is a balance between bone formation by osteoblasts and bone resorption by osteoclasts.

Molecular analysis of key markers of proliferation and differentiation within the bone marrow population of the short-duration spaceflight animals revealed that mechanical unloading in microgravity resulted in an inhibition of differentiation of mesenchymal and hematopoietic stem cells in the bone marrow compartment (**Figure 4**). Furthermore, analysis of the bone tissue revealed significant expression of a potent cell cycle arrest molecule, CDKN1a/p21, that was localized to proliferating osteoprogenitors on the surface of the bone and proliferating chondro-precursors in the cartilage of the femoral head, potentially indicating a molecular mechanism responsible for these changes.

Overall, these results indicate that stem cells within the bone tissue fail to repair and regenerate tissues in a normal manner, leading to drastic tissue alterations in response to microgravity.

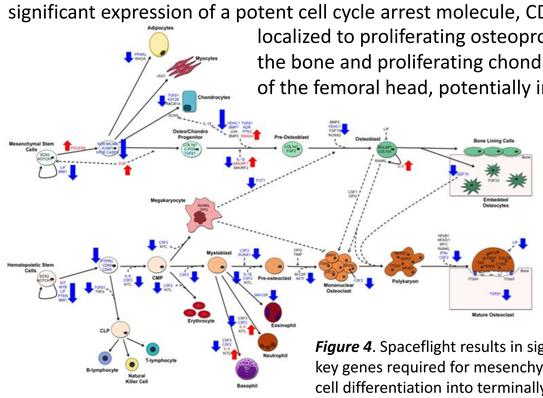


Figure 4. Spaceflight results in significant down-regulation of key genes required for mesenchymal and hematopoietic stem cell differentiation into terminally differentiated lineages.

Hypothesis

We hypothesize that long-duration spaceflight will cause a greater change in key bone morphometric parameters than short-duration spaceflight, possibly caused by the effects of alterations to stem cell functions within the marrow compartment.

Methods

The Rodent Research Hardware System provides the capability to investigate the effects of long-duration experiments on the International Space Station. During the Rodent Research-1 mission in 2014, ten 16-week old female C57Bl/6J mice were exposed to 37 days of microgravity. Two mice from each group were euthanized and dissection partially on orbit. Eight mice were euthanized and frozen for dissection on the ground.

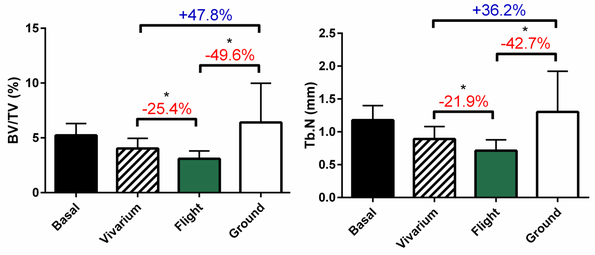
Figure 6. (Above) NASA's Rodent Habitat. Photo Credit: NASA/Dominic Hart.

Group	Housing	Timeline
Basal Controls (n=10)	Standard mouse cages	Partially dissected shortly after launch.
Vivarium Controls (n=10)	Standard mouse cages	Processed to match flight animal timeline.
Flight Animals (n=10)	Rodent Research Hardware	37 days of microgravity on the ISS.
Ground Controls (n=10)	Rodent Research Hardware	Processed to match flight animal timeline.

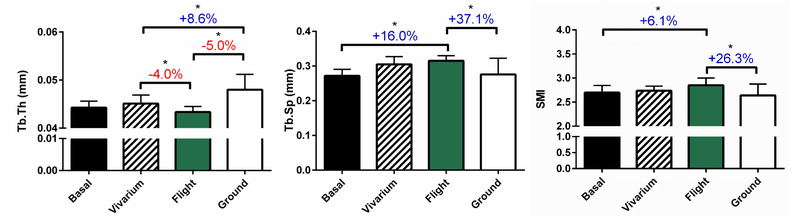
Rodent Research-1 marked the longest duration rodent study conducted in NASA facilities.

Results

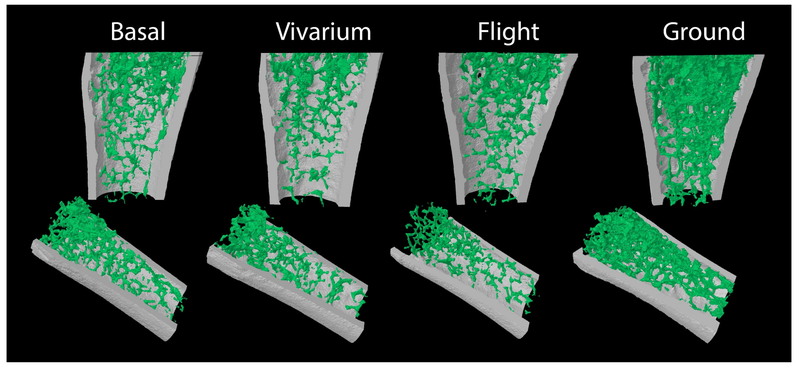
MicroCT of the Trabecular Bone of the Distal Femur



Spaceflight mice exhibit a significant decrease in trabecular bone volume, up to **49.6%**, compared to all control groups. This decrease is mainly attributed to a decrease in trabecular number.



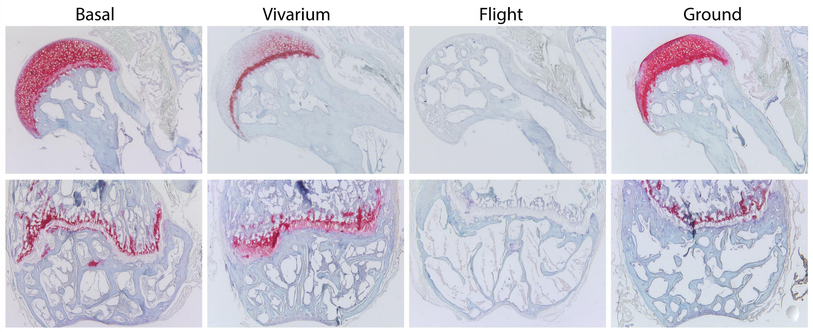
Ground controls display significantly increased bone volume (**+47.8%**) compared to vivarium controls. This indicates that the RR hardware has a significant affect on bone morphometry.



Results

Safranin-O Staining of the Distal and Proximal Femur

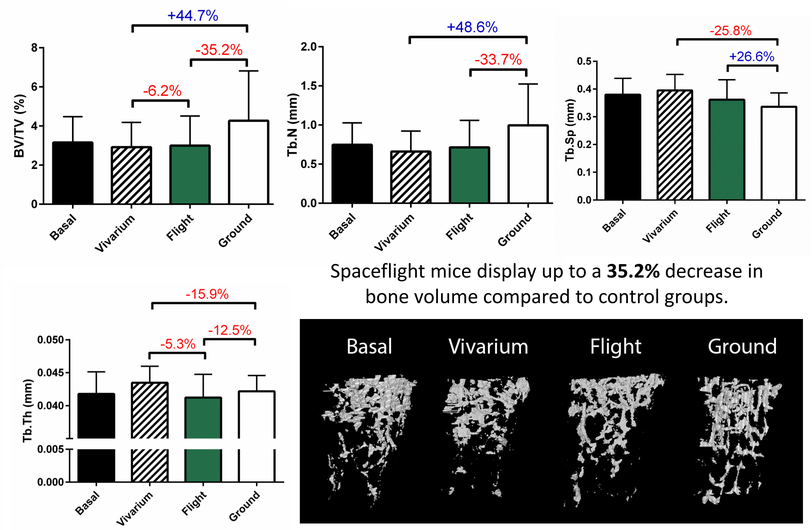
Histological staining can expose differences in cartilage thickness and chondrocyte number.



Femoral head cartilage and chondrocytes forming the distal femur growth plate stain red.

Spaceflight bones display almost a complete loss of cartilage in the femoral head and distal femur.

MicroCT of the Trabecular Bone of the Proximal Tibia



Spaceflight mice display up to a **35.2%** decrease in bone volume compared to control groups.

Conclusion

- Long duration spaceflight has detrimental effects on bone volume. Flight animals display a dramatic loss of trabecular bone volume in the distal femur and proximal tibia.
- Ground control animals housed in the Rodent Habitat exhibit an increase in bone volume compared to vivarium controls in traditional housing.
- The Rodent Research hardware has a significant affect on bone morphometry.
- Similar microgravity and housing effects are seen in the femoral head.
- Staining reveals an extensive loss of cartilage at the femoral head and a potential change in chondrocyte staining potential in the distal femur of flight animals.
- Investigations are currently underway to understand if stem cell-related mechanisms are the cause of this dramatic bone loss.
- This research will help elucidate the complex mechanisms underlying bone tissue maintenance and stem cell regeneration.

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