Femoral Head Bone Loss Following Short and Long-Duration Spaceflight

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Spaceflight mechanically unloads the body and causes physiological effects at all levels of biological organization.

<table>
<thead>
<tr>
<th>Organism</th>
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<th>Cells</th>
<th>Molecules</th>
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<td>p21/CDKN1a, p53</td>
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- Nausea, Vestibular Effects
- Cardiovascular Deconditioning, Calcium Imbalance, Reduced Immunity
- Bone and Muscle Tissue Loss, Anemia, Poor Healing
- Proliferation, Differentiation, Migration

Can some of these effects be explained by microgravity effects on stem cells?
Stem cell proliferation and differentiation occurs during growth and development resulting in the formation of functional adult tissues

A portion of these stem cells remain partially differentiated (multipotent) in specific tissues during adult life to enable the process of tissue regeneration, repair and maintenance to occur. This process is thought to be dependent on mechanical load.
Gravity Tissue Regeneration Hypothesis:
Mechanical unloading in microgravity may inhibit the proliferation and/or differentiation of somatic/adult stem cell required for normal tissue repair and regeneration

Normal tissue health requires constant tissue regeneration and repair by tissue-specific somatic stem cells

Mechanical stimulation promotes bone, muscle, and immune tissue repair and regeneration mediated by somatic stem cell proliferation and differentiation
Can some of the tissue, cell and molecular effects of microgravity be linked to stem cell-based tissue regenerative processes?

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Bone as a model of tissue regeneration

The mouse hindlimb musculoskeletal system is used as an in-vivo model to study tissue degeneration in conditions of mechanical unloading in microgravity, and disuse on earth.

Bone loss is mediated by active osteoclast-mediated degeneration as well as osteoprogenitor regenerative deficits.
BionM1 and RR1 provided unique opportunities to study long-duration spaceflight.

We hypothesized that the bone loss and inhibition of stem cell based tissue regeneration observed during short-duration shuttle missions would continue during long-duration spaceflight and result in significant alterations in tissue structure.
BionM1 vs. RR1

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<tr>
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<th>BionM1</th>
<th>RR1</th>
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<tbody>
<tr>
<td><strong>Age</strong></td>
<td>16 weeks old</td>
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</tr>
<tr>
<td><strong>Gender</strong></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td><strong>Flight period</strong></td>
<td>30 days</td>
<td>37 days</td>
</tr>
<tr>
<td><strong>Ground control</strong></td>
<td>Asynchronous (3 months)</td>
<td>Asynchronous (3 days)</td>
</tr>
<tr>
<td><strong>Live animal return</strong></td>
<td>Yes (12 h delay)</td>
<td>No</td>
</tr>
<tr>
<td><strong>Dissection</strong></td>
<td>Biospecimen Sharing Program</td>
<td>Frozen Carcass</td>
</tr>
<tr>
<td><strong>Tissues collected</strong></td>
<td>Pelvis, proximal femur, bone marrow cells</td>
<td>Pelvis, femur, tibia</td>
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**Analysis Methods**
- MicroCT, histology, gene expression analysis, stem cell proliferation and differentiation, motility/migration assays
- MicroCT, histology, mechanical strength testing, gene expression analysis
Male mice exhibit significant bone loss during 30 days of spaceflight.

Habitat: p<0.0001
Gravity: p=0.0003
Habitat*Gravity: p<0.0001

Habitat: p=0.5083
Gravity: p<0.0001
Habitat*Gravity: p<0.0001

Habitat: p<0.0001
Gravity: p<0.0001
Habitat*Gravity: p=0.0006

Hardware: p=0.0004
Spaceflight: p=0.0492
Hardware*Spaceflight: p=0.0004
Female mice also exhibit significant bone loss during spaceflight, albeit to a lesser extent.
Cortical bone loss in the femoral neck of male mice

**Ground Control**

**Spaceflight**

![Graphs showing bone density and diameter changes between Ground Control and Spaceflight conditions.](Image)

**Habitat:** p<0.001

**Gravity:** p=0.007

**Habitat*Gravity:** p=0.0119
Cortical bone loss in the femoral neck of female mice

Ground Control

Spaceflight

10/28/16

ASGSR, 2016
In addition to bone loss, BionM1 mice exhibit significant alterations in the tissue structure of the femoral head.

600 nm resolution microcomputed tomography images of the femoral head from BionM1

Spaceflight phenotype resembles that of aging but how accelerated is this?
Bone loss in male mice during normal ageing

30 days of spaceflight causes more drastic bone loss than what normally occurs during 5 months of aging with normal ambulation.
In addition to accelerated aging, fissures on the surface of the femoral head resemble that which occurs during the development of osteoarthritis.
Osteo-chondro matrix – Saffranin-O staining

Pritzker et al. (2006) OsteoArthritis and Cartilage, 14, p. 13-29
Saffranin-O staining shows the extent of cartilage degradation following long-duration spaceflight.
Flight samples appear to exhibit similar characteristics to stage 4/5 osteoarthritis.

BionM1 and RR1 spaceflight samples appear similar to Stage 4/5 osteoarthritis.

RR mice also exhibit similar phenotype in the distal femur – see Meg Cheng-Campbell’s poster #248.
Stem cell senescence and inhibition of cell regeneration is thought to be a major contributor of osteoarthritis. Mechanical loading is required for maintenance of stem cell health and tissue regeneration and we have shown that spaceflight results in an inhibition of stem cell based tissue regeneration.

Could mechanical unloading in spaceflight result in early onset OA? Could this help us to elucidate the mechanisms of OA induction on Earth and develop countermeasures?
Spaceflight causes an **alteration in tissue regeneration** - increased stem cell pluripotency and inhibition of differentiation

Under conditions of reduced mechanical load e.g. physical inactivity, mechanical disuse conditions and spaceflight, **differentiation of somatic stem cells** may be **inhibited**, possibly resulting in serious regenerative health effects

Mice exposed to spaceflight in the BionM1 hardware, exhibit significant bone loss and furthermore exhibit signs of **accelerated aging** and early onset **osteoarthritis**

Mice exposed to spaceflight in the Rodent Research hardware also exhibit bone loss - but not to the same extent as BionM1

Although there were differences in these experiments (gender, orbit etc.), it is possible that the **mechanical stimulation** gained from running on the 3D grid during RR1 partially **mitigates** the accelerated **aging** and early onset **osteoarthritis** phenotype seen in the BionM1 mice

Mechanical unloading during spaceflight could provide critical insight into the **mechanisms of OA** and potential treatments
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Poster #248