Astronauts lose bone structure during long-duration spaceflight. These changes are due, in part, to insufficient bone formation by the osteoblast cells. Little is known about the role that small (~22 nucleotide), non-coding micro-RNAs (miRNAs) play in the osteoblast response to microgravity. We hypothesize that osteoblast-lineage cells alter their miRNA status during microgravity exposure, contributing to impaired bone formation during weightlessness. To simulate weightlessness, female mice (C57BL/6, Charles River, 10 weeks of age, n = 6) were hindlimb unloaded for 12 days. Age-matched and normally ambulating mice served as controls (n=6). To assess the expression of miRNAs in skeletal tissue, the right and left tibia of the mice were collected ex vivo and cleaned of soft-tissue and marrow. Total RNA was collected from tibial bone and relative abundance was measured for miRNAs of interest using quantitative real time PCR array looking at 372 unique and well-characterized mature miRNAs using the delta-delta Ct method. Transcripts of interest were normalized to an average of 6 reference RNAs. Preliminary results show that hindlimb unloading decreased the expression of 14 miRNAs to less than 1.4-2.9X control levels and increased the expression of 5 miRNAs relative to the control mice greater than 1.2-1.5X (p<0.05, respectively). Using the miRSystem we assessed overlapping target genes predicted to be regulated by multiple members of the 19 differentially expressed miRNAs as well as in silico predicted targets of our individual miRNAs. Our miRSystem results indicated that a number of our differentially expressed miRNAs were regulators of genes related to the Wnt-Beta Catenin pathway—a known regulator of bone health—and, interestingly, the estrogen-mediated cell-cycle regulation pathway, which may indicate that simulated weightlessness induced systemic hormonal changes that contributed to bone loss. We plan to follow up these findings by measuring gene expression of miRNA-regulated genes within these two pathways with the aim of furthering our understanding of the function of miRNAs in the skeletal response to spaceflight.

**Methods**

- **Experiment Design**
  - 12 female mice
  - 10 weeks of age
  - 6 sham, 6 hindlimb unloaded
- **Hindlimb Unloading (HU)**
  - For 12 days to simulate weightlessness
  - Dissections on day 12

**Sample Collection and Data Analysis**

- qPCR C1 values above 33 were regarded as ‘Not Detected’
- All p-values are calculated via a two-tailed T-test

**miRNA Expression Results 12 Days HU**

The P Value column shows the significance of the change in miRNA expression relative to controls. The values are calculated using the delta Ct method.

**Conclusions**

- We successfully confirmed the first part of our hypothesis that Ob-lineage cells alter their miRNA status as a result of HU
- Future work will be directed towards the second half of the hypothesis looking at connecting the changes we see in miRNA to bone loss as a result of HU, and possibly the reproductive system

**Future Directions**

- *In vitro* work studying our differentially expressed miRNA
  - Knockouts, and addition of mimics followed by qPCR and Western blotting
- *In vivo* study corroborating *in vitro* work utilizing miRNA knockouts and utilizing RNaseq