Role of Mitochondrial Oxidative Stress in Spaceflight-Induced Tissue Degeneration

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Hypothesis

Microgravity and ionizing radiation in the spaceflight environment poses multiple challenges to homeostasis and may contribute to cellular stress. Effects may include increased generation of reactive oxygen species (ROS), DNA damage and repair error, cell cycle arrest, cell senescence or death. Our central hypothesis is that prolonged exposure to the spaceflight environment leads to the excess production of ROS and oxidative damage, culminating in accelerated tissue degeneration. The main goal of this project is to determine the importance of cellular redox defense for physiological adaptations and tissue degeneration in the space environment.

Experiment Design

Hindlimb Unloading Model

Hindlimb unloading (HU) is a ground-based model for musculoskeletal disuse and was utilized in this study to simulate microgravity on the hind limbs of the mice. IACUC pre-approval was obtained before conducting any experiments.

Breeding:

Mice were bred using Harem style breeding where two female WT mice were paired with one mCAT male. A total of 28 females and 14 males were used for breeding. After birth, pups were ear-tagged, weaned, and genotyped. Figure 1. shows the genotyping results of the entire breeding colony.

	mCAT	WT
Female	57	35
Male	30	16

Figure 1. Genotyping results

Experiment:

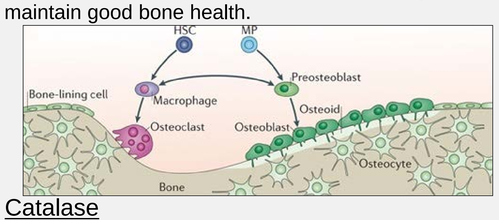
16 week-old female mCAT and wild-type C57BL/6NJ strain mice were subjected to either normal weight bearing (NL) or hindlimb unloading (HU) for 30 days. They were divided into four groups:

Group 1: WT NL (n=12)
Group 2: WT HU (n=12)
Group 3: mCAT NL (n=12)
Group 4: mCAT HU (n=12)

Background

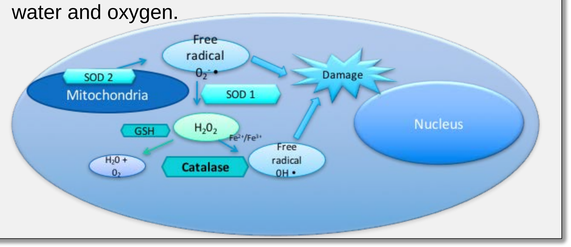
Bone Cells

A balance between the bone forming cells (osteoblasts) and the bone resorbing cells (osteoclasts) in the skeletal tissue is necessary to maintain good bone health.

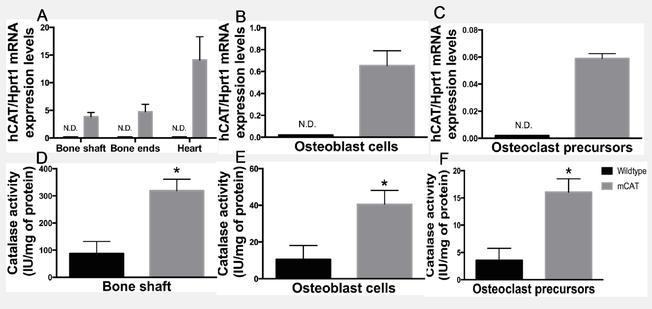


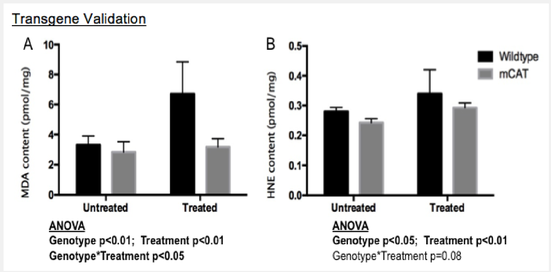
Catalase

When detrimental reactive oxygen species (ROS) are formed in the cell, catalase converts the molecule to water and oxygen.



Transgenic Model Validation





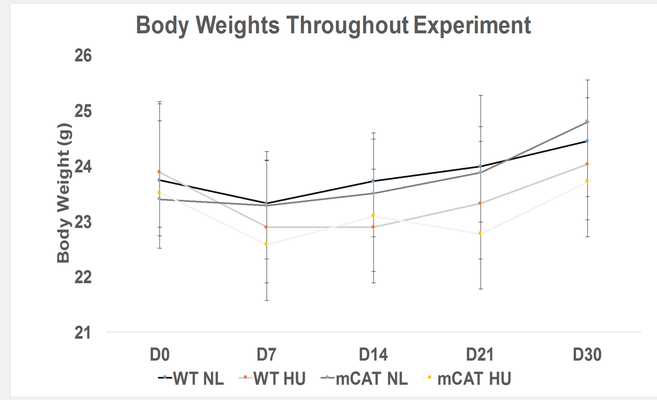
Transgenic mouse model validation:

- mCAT mice expressed mRNA for the human catalase gene in both skeletal tissue (A) and marrow-derived cultures of osteoblast (B) and osteoclast precursors (C). Hearts served as positive controls.
- mCAT mice displayed 3-4-fold greater catalase enzymatic activity compared to WT mice in bone extracts (D), osteoblastic cultures (E) and osteoclast precursors (F), thereby confirming that transgene was enzymatically active.

Oxidative damage measurement:

- Oxidative damage was assessed in mineralized tissue. Malondialdehyde (MDA) and Hydroxynonenal (HNE) levels normalized to total protein content.
- Untreated Control WT and mCAT mice displayed no genotype-dependent differences in oxidative damage whereas treatment increased MDA and HNE content in skeletal tissue of WT but not mCAT mice.

Results

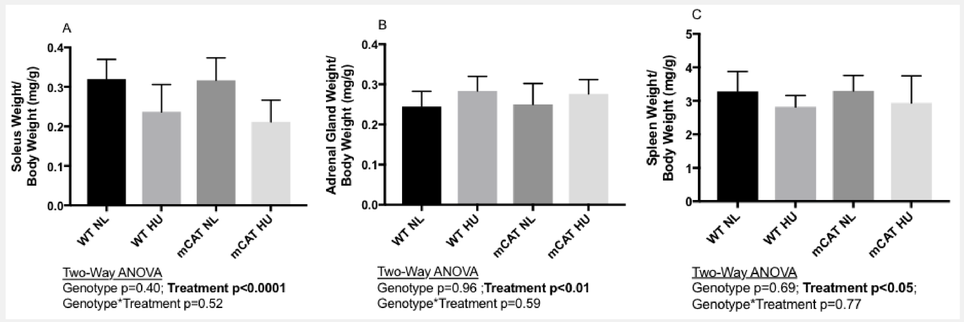


Body Weights were measured every week throughout the experiment. ANOVA testing showed that there were no statistically significant differences in body weight means for any day. Error bars depict the standard deviation for each group.

Approach

To accomplish this, we will use both wildtype (WT) mice and a well-established, genetically-engineered animal model (mCAT mice) which displays extended lifespan (Schriner et al. 2005). The animal model selected to test these ideas is engineered to quench ROS in mitochondria by targeted over-expression of the human catalase gene to the mitochondrial matrix. We showed previously that mCAT mice express the catalase transgene in skeletal tissues, bone forming osteoblasts, and bone resorbing osteoclasts. In addition, mCAT mice also display increased catalase activity in bone. Our findings revealed that exposure of adult, male, C57Bl/6J mice to simulated spaceflight (hindlimb unloading and gamma radiation) led to an increase in markers of oxidative damage (malondialdehyde, 4-hydroxynonenol) in skeletal tissue of WT mice but not mCAT mice. To extend our hypothesis to other, spaceflight-relevant tissues, we are performing a ground-based study simulating 30 days of spaceflight by hindlimb unloading to determine potential protective effects of mitochondrial catalase activity on aging of multiple tissues (cardiovascular, nervous and skeletal).

Results



Animals were sacrificed on D30 of experiment, and tissues were collected for various assays. The right soleus, both adrenal glands, and the spleen were among the collected tissues; they were weighed upon dissection and later flash frozen. Statistical analysis was conducted by normalizing the weight of the tissue(s) to the body mass of the animal on D30.

Two-way ANOVA results showed differences in weight for all three tissues based on treatment of either NL or HU.

Conclusions and Future Goals

mCAT mice over-expressed the transgene in bone tissue and bone cells *ex vivo* (osteoblasts and osteoclasts precursors). Skeletal tissues and cells also showed elevated catalase activity compared to WT.

- **This validates the use of mCAT mice to study the influence of quenching mitochondrial ROS in skeletal tissue.**

Preliminary experimental results show that treatment of 30 day HU contributed to decreased soleus, adrenal, and spleen weights when compared to WT.

- **This shows that HU is an effective method for simulating microgravity and potentially induce oxidative stress.**

Moving forward, we will be conducting oxidative damage analysis on tissues collected from the cardiovascular, nervous, and skeletal system. Many tissues were collected in dissection, and we are sharing eye samples from our studies.

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