Aging and spaceflight: catalase targeted to mitochondria alters skeletal structure and responses to musculoskeletal disuse

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INTRODUCTION

- Spaceflight factors such as weightlessness and radiation result in decreased bone mass, density, and strength (Smith et al., 2012)
- Radiation can increase oxidative damage through the production of excess ROS, which in turn may be a potential mechanism for bone loss
- Some tissue functional and structural deficits caused by spaceflight and its analogs resemble certain aspects of aging
- Transgenic mice overexpressing a human catalase transgene (mCAT mice) targeted to the mitochondria display improved longevity and reduced age-related diseases compared to wild type controls (Schriner et al. 2005, Dai et al. 2010).

PURPOSE OF THE STUDY

Understand the role of cellular redox defenses in physiological adaptation and tissue degeneration during spaceflight or prolonged bedrest on Earth.

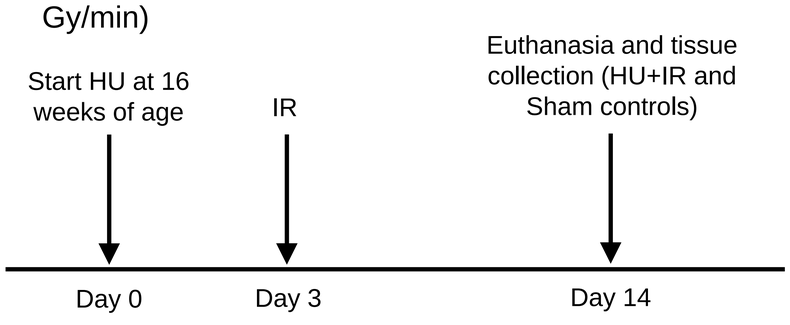
HYPOTHESIS

Quenching ROS production in the mitochondria will protect from bone loss induced by spaceflight and its analogs

STUDY DESIGN

Groups and treatments

- *Animals:* C57BL/6NJ male mice carrying a human catalase transgene targeted to the mitochondria (mCAT), and wildtype (WT) littermates
- *Treatment:* Combination of hindlimb unloading (HU) to simulate weightlessness and a single dose 2 Gy total body exposure to ionizing radiation (IR, ^{137}Cs, 0.83 Gy/min)



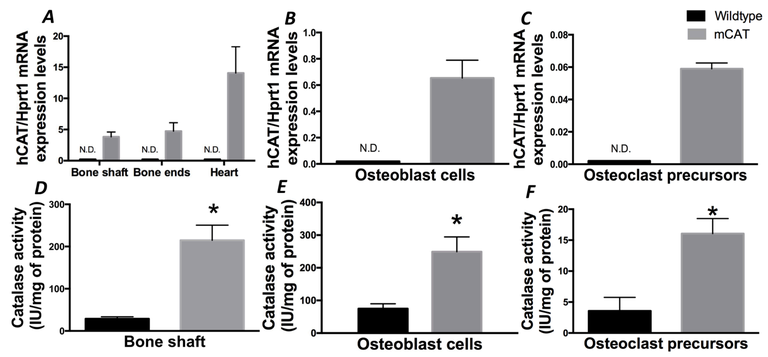
SAMPLING AND ANALYSIS

Assays

- Microcomputed tomography (microCT) imaging of cortical and cancellous compartments of tibiae at a resolution of 12.1 μm and 6.7 μm respectively.
- Oxidative damage assays: Femurs flushed of bone marrow cells were used for lysis and measurement of MDA and HNE using ELISAs
- Ex-vivo cell culture: Bone marrow cells were harvested from bones and grown in osteogenic media for either osteoblasts or osteoclasts.

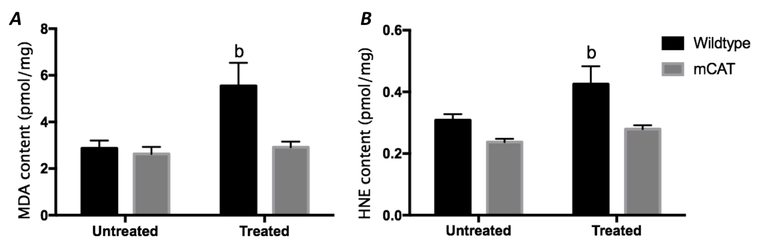
RESULTS

The human catalase transgene is expressed in skeletal tissue and constituent cells of mCAT mice



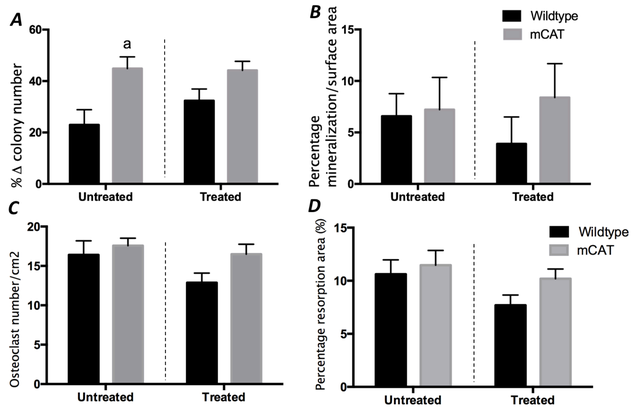
Gene expression of human catalase transgene normalized to housekeeping gene Hprt1 (A-C). ELISA-based assessment of catalase activity (D-F). ND: not detected, *Significant at p<0.05 by Student's t-test (b-f). Values shown are means +/- SEM, n=8-9 per group.

mCAT mice are protected from the increases in levels of oxidative damage markers caused by combined HU and IR



Malondialdehyde (A) and HNE (B) content in bone from wild type and mCAT mice exposed to combined HU and IR (Treated) and untreated controls. Values shown are means +/- SEM (n=4-5) [b]p<0.05 by 1-way ANOVA accepted as significant

Human catalase transgene targeted to mitochondria prevents the decline in osteoprogenitor number after combined HU and IR



Marrow-derived osteoblast (A and B) and osteoclast (C and D) progenitors from wild type and mCAT animals were cultured ex vivo. Osteoblast colony expansion (% change in colony numbers between day 7 and day 9, A). Extent of mineralization in osteoblast cultures (B). Osteoclast numbers were quantified as multinucleated, *TRAP+ cells (C) and osteoclast activities measured by resorption pit area (D). Values shown are mean +/- SEM (n=7-9), [a] indicates P<0.05 comparing mCAT/Untreated to WT/Untreated

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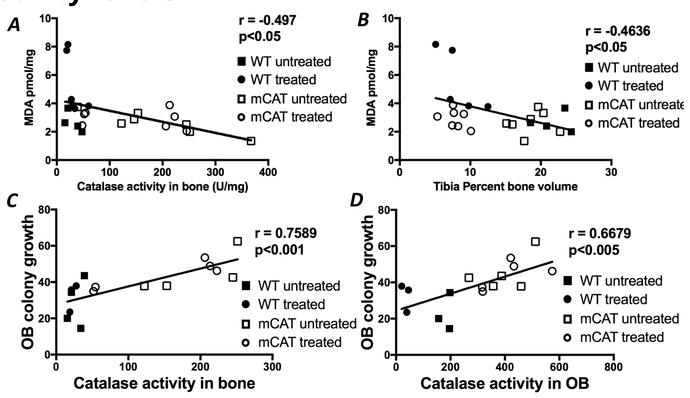
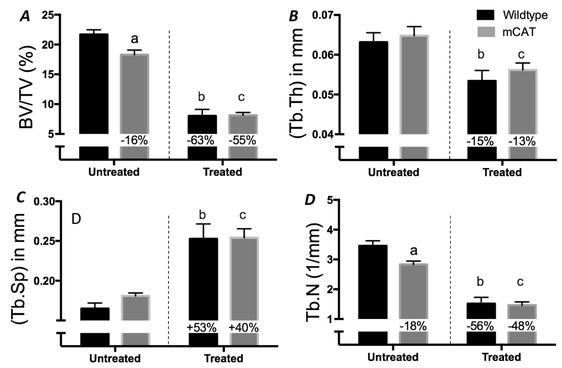
Ex vivo colony growth correlates with catalase activity levels



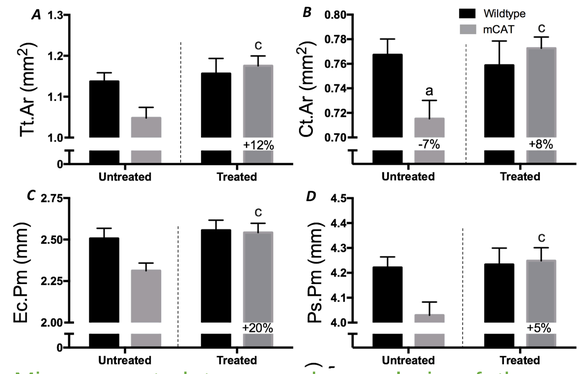
High turnover bone show diminished size in mCAT mice; no genotype-dependent difference in cancellous bone loss caused by treatment



Legend:
BV/TV: % bone volume
Tb.Th: trabecular thickness
Tb.Sp: trabecular separation
Tb.N: Trabecular number

Microcomputed tomography analysis of the cancellous bone included BV/TV% (A), Tb.Th (B), Tb.Sp (C), Tb.N(D). Values shown are mean +/- SEM (n=7-9), By 1-factor ANOVA P<0.05 by 1- comparing mCAT/Untreated to WT/Untreated [b]; indicates P<0.05 comparing WT/Treated to WT/Untreated; [c] indicates P<0.05 comparing mCAT/Treated to mCAT/Untreated.

Slow turnover (cortical) bone was smaller in untreated mCAT mice; treatment of mCAT mice showed a rapid stimulation of radial expansion



Legend:
Tt.Ar.: Total area
Ct.Ar.: Cortical area
Ec.Pm: Endocortical perimeter
Ps.Pm: Periosteal perimeter

Microcomputed tomography analysis of the cortical bone included Tt.Ar (A), Ct.Ar (B), Ec.Pm (C), Ps.Pm (D). Values shown are mean +/- SEM (n=7-9), By 1-factor ANOVA [c] indicates P<0.05 comparing mCAT/Treated to mCAT/Untreated

CONCLUSION

- Quenching of mitochondrial ROS by over-expression of catalase stimulates growth but not differentiation of osteoprogenitors *ex vivo*
- Mitochondrial ROS signaling is important for the acquisition of adult bone structure.
- Transgene expression leads to changes in cortical structure following treatment that are similar to those that occur during aging, indicative of a role for mitochondrial ROS in coordinating skeletal remodeling in response to challenge