Mitochondrial oxidative stress: importance for skeletal structure and responses to simulated spaceflight

Presented by Joshua Alwood:

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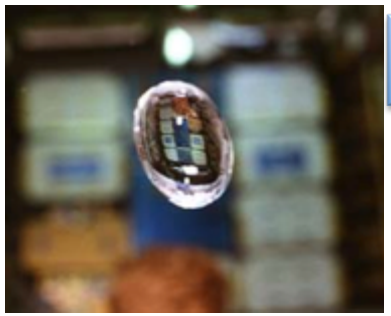
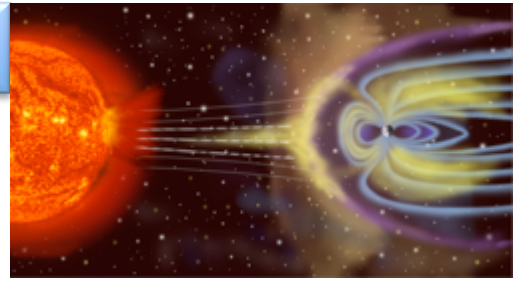
Spaceflight conditions lead to bone loss



Microgravity

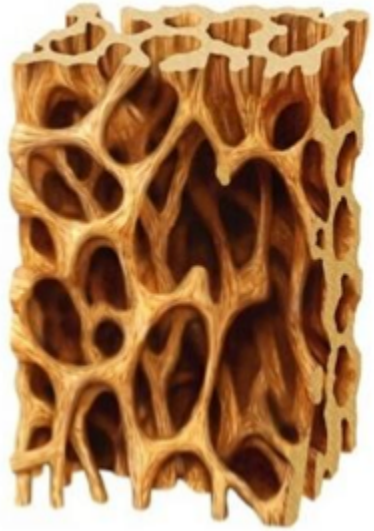
Space conditions



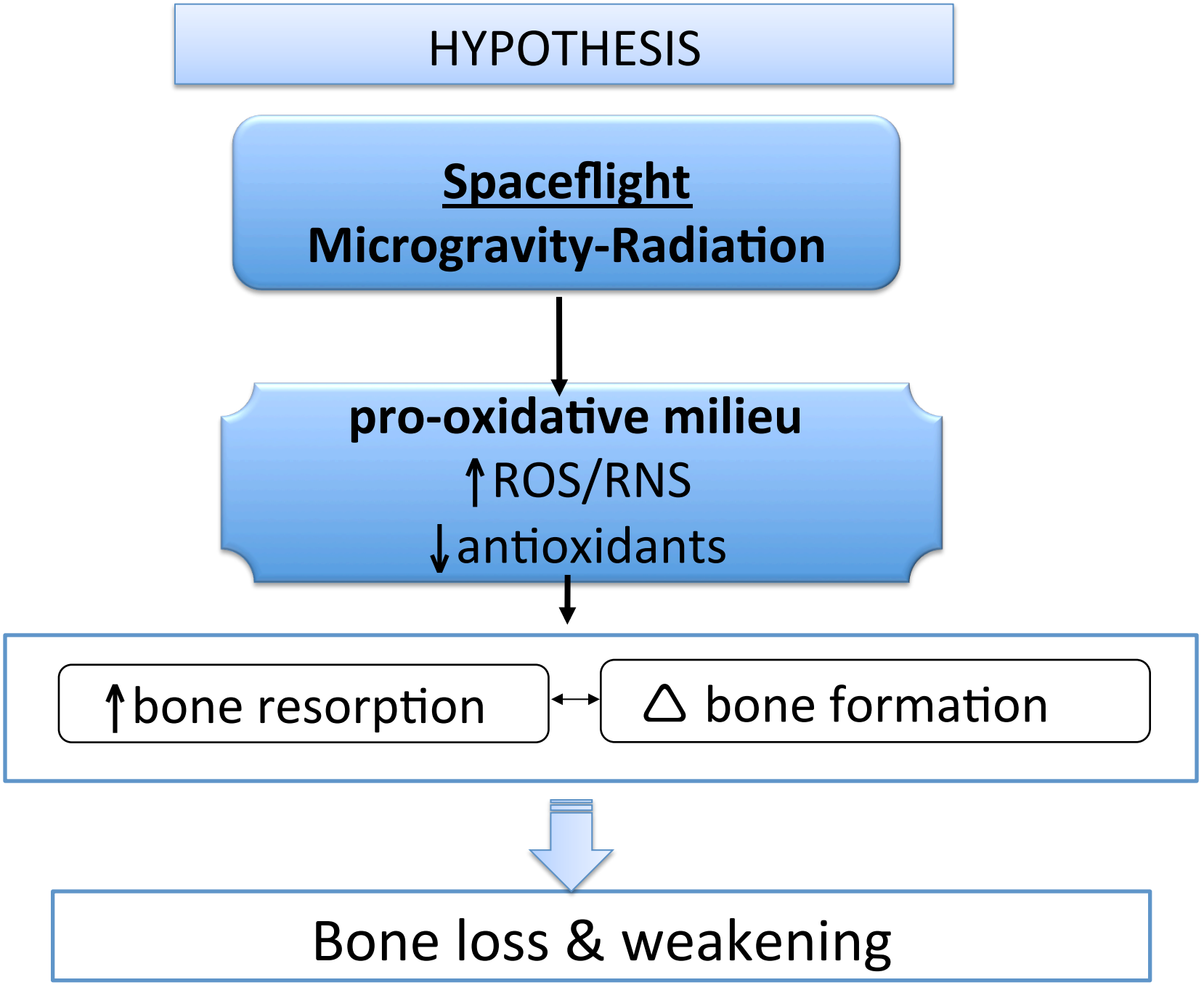
Space Radiation

results

Healthy bone



Osteoporotic bone


HYPOTHESIS
Spaceflight
Microgravity-Radiation
pro-oxidative milieu
↑ROS/RNS
↓antioxidants
↑bone resorption
↔
△ bone formation
Bone loss & weakening

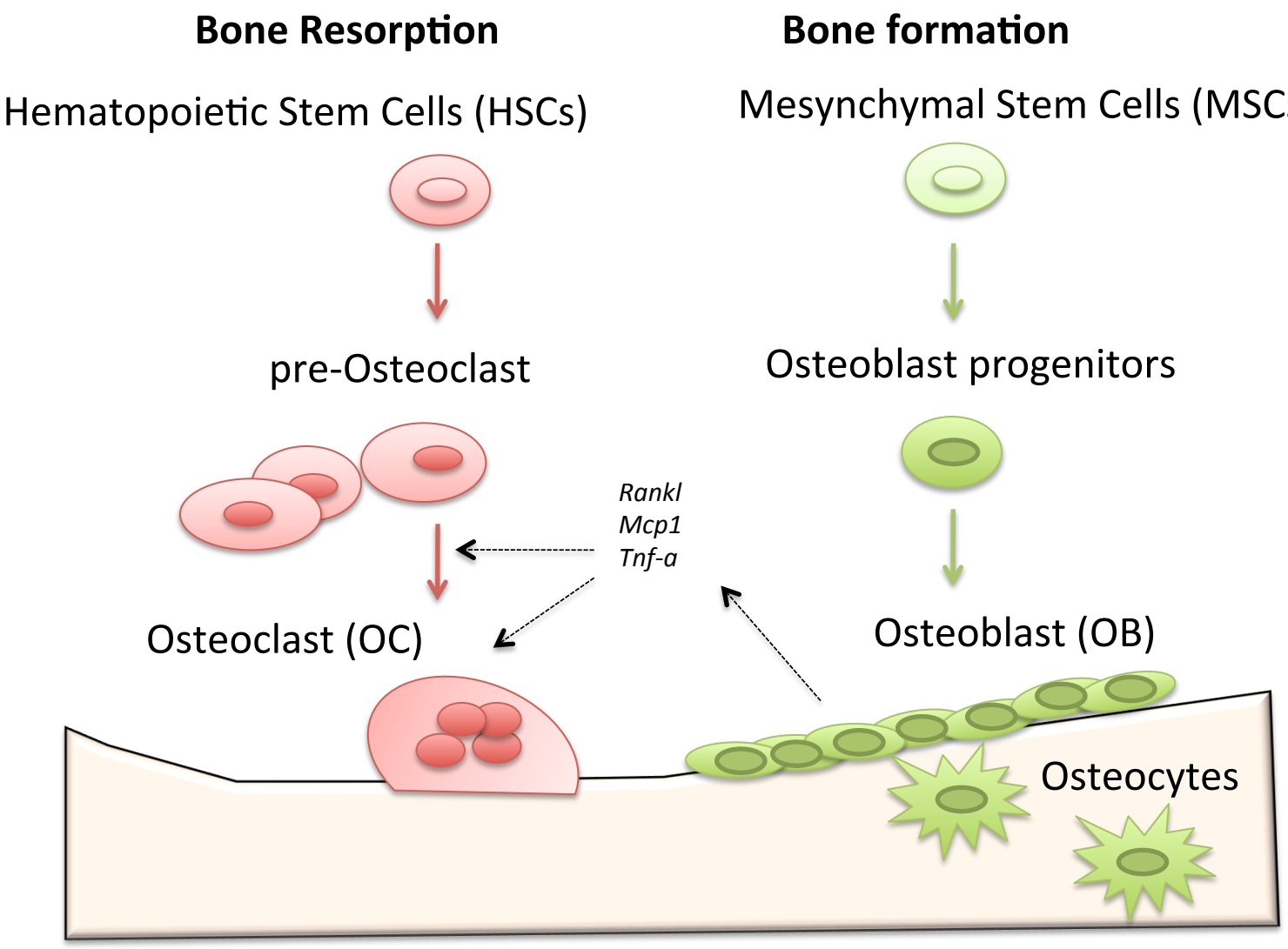
Hypothesis & Experimental Approach

- **Hypothesis:** Excess reactive oxidative species (ROS) alters the tight balance between osteoclast and osteoblast activities, leading to accelerated skeletal remodeling and culminating in bone loss, resembling aging.

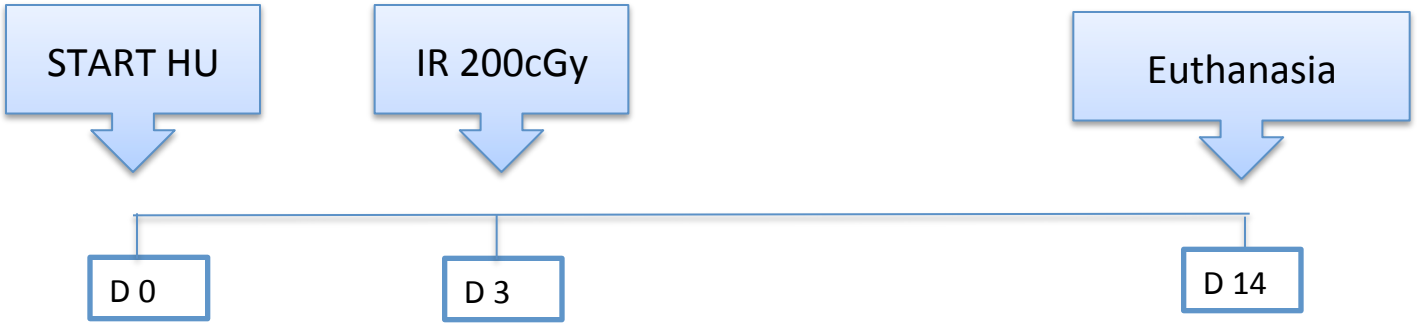
- mCAT mouse transgenic model: over-express human catalase gene targeted to mitochondria, the major organelle contributing free radicals:
  - -improved longevity
  - -reduced cardiovascular degeneration and other age-related disease
  - -radioprotection in the brain
  - -interference with osteoclast maturation and bone resorption (conditional expression)

- Use mCAT transgenic and wildtype mice to test role of ROS:
  - Intrinsic: compare phenotype of mCAT vs WT mice (untreated)
  - Extrinsic: compared responses to treatment of 'simulated spaceflight', known to lead to excess ROS

Bone remodeling:
Balance between bone resorption and bone formation



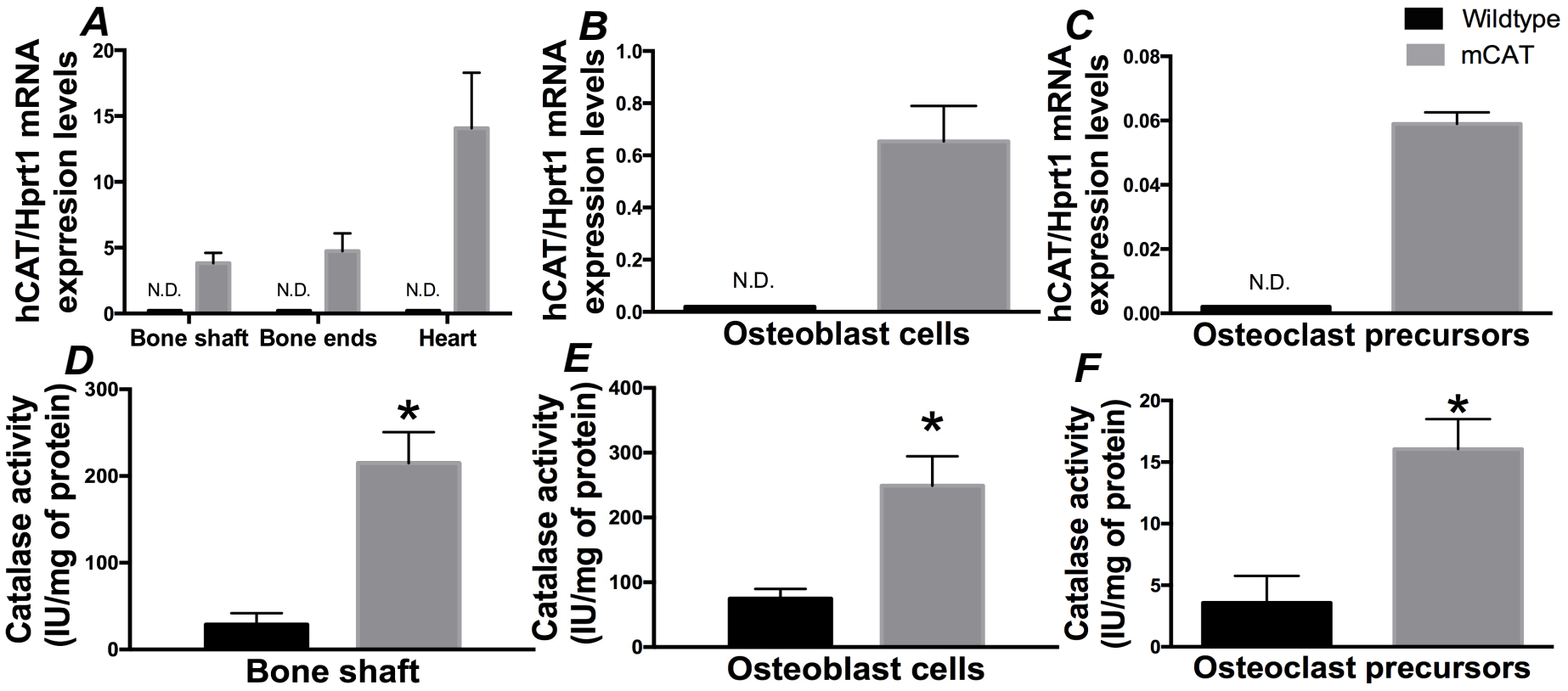
Experiment designs



Experimental details:

- Male, 16wk old (n=7-9/group)
- Mice strains: WT (C56BL/6NJ) and mCAT littermates
- Total Body Irradiation: ^{137}Cs 200 cGy (0.83 Gy/min)
- Hindlimb-Unloading on day 0, IR on day 3 and tissue harvest on day 14

mCAT model mice validation (1) : transgene expression in bone and bone cells



• mCAT mice expressed the transgene in both skeletal tissue (A) and marrow-derived cultures of osteoblast (B) and osteoclast precursors (C)

• mCAT mice has 3-4-fold greater catalase enzymatic activity compared to WT mice in bone (D), osteoblastic cultures (E) and osteoclast precursors (F)

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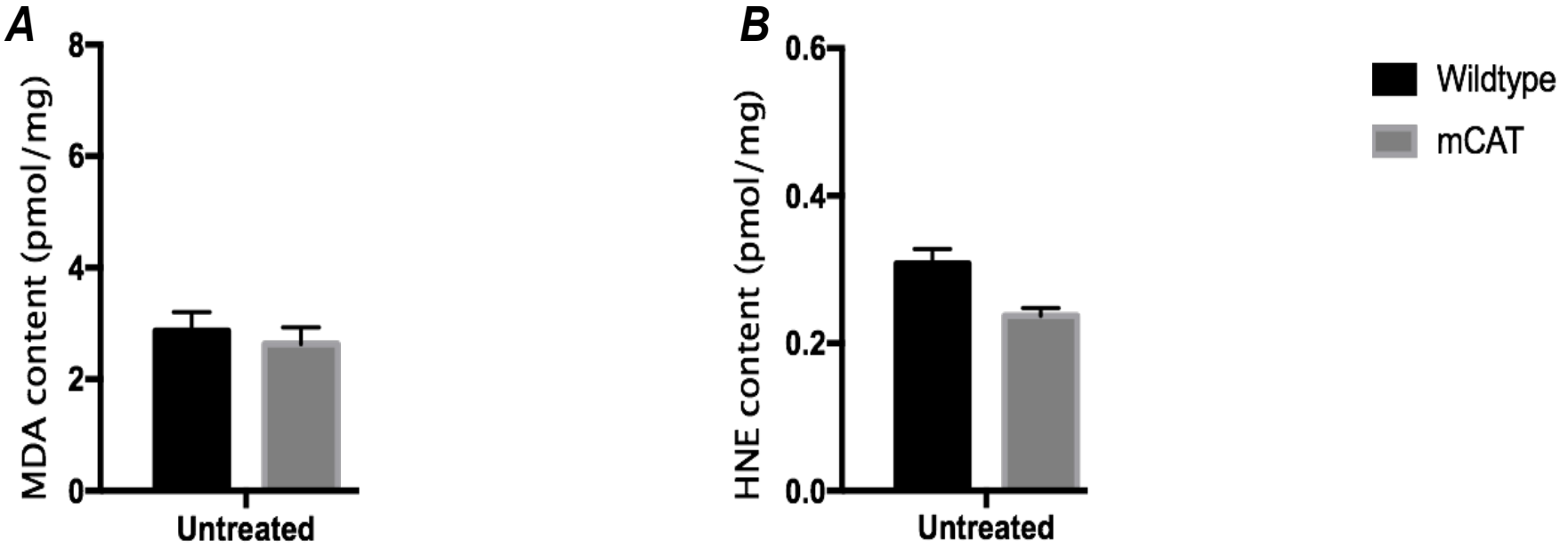
mCAT model mice validation : mCAT mice similar levels of oxidative damage in bone



•Oxidative damage was assessed in mineralized tissue using malondialdehyde (A, MDA) levels and 4-Hydroxynonenal (B, HNE)

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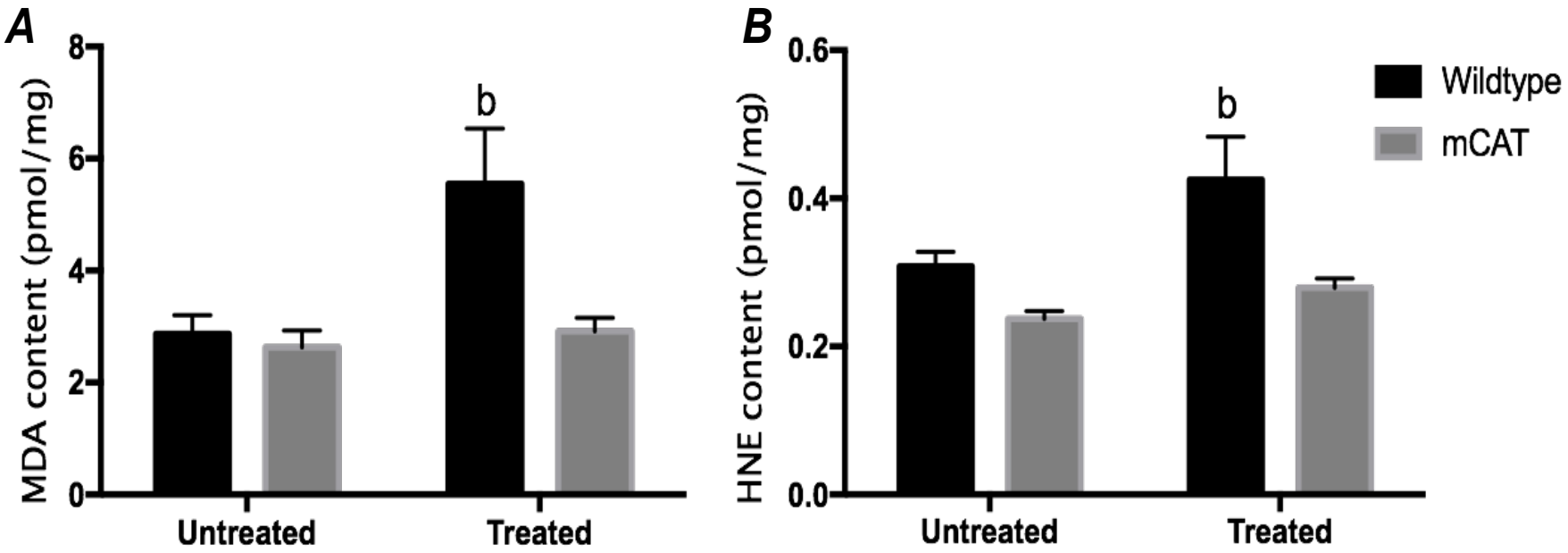
mCAT model mice validation:
Treatment increased oxidative damage in WT but not mCAT mice



a: p<0.017 between WT untreated and mCAT untreated
b: p<0.017 between WT untreated and treated
c: p<0.017 between mCAT untreated and treated

•Oxidative damage was assessed in mineralized tissue using malondialdehyde (A, MDA) levels and 4-Hydroxynonenal (B, HNE)

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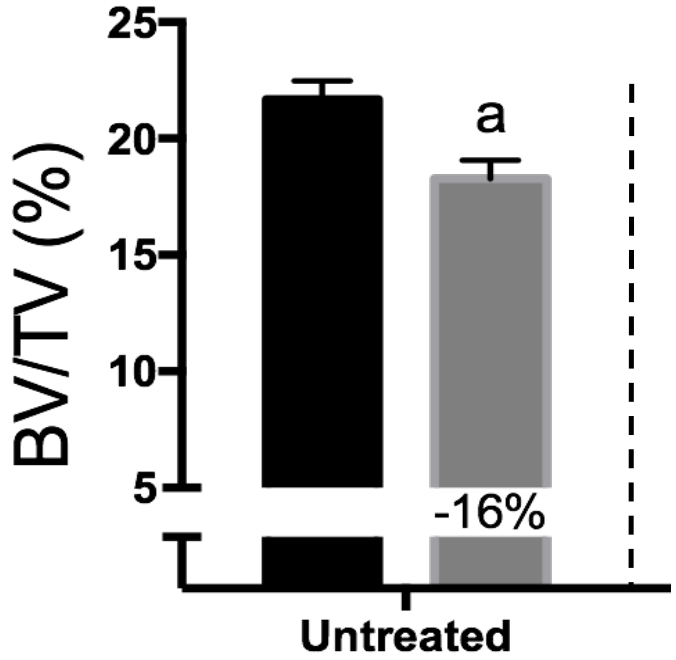
Skeletal structure: Cancellous
Phenotype of WT vs mCAT: reduced bone volume



a: $p<0.017$ between WT untreated and mCAT untreated
b: $p<0.017$ between WT untreated and treated
c: $p<0.017$ between mCAT untreated and treated

Untreated mCAT mice show lower bone compared to WT mice in percent bone volume (BV/TV, -16%) and trabecular numbers (Tb.N, -18%, not shown)

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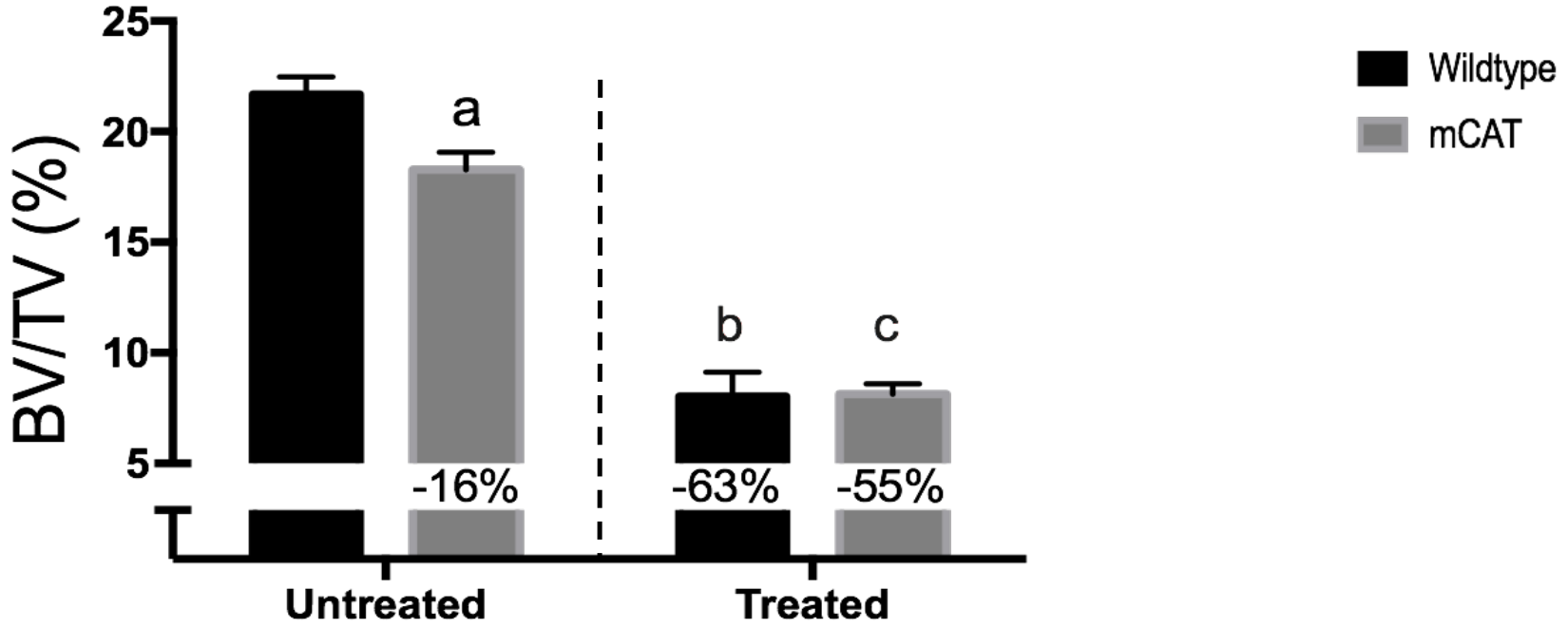
Skeletal structure: Cancellous
Treatment effect: Bone loss in both WT and mCAT mice



a: p<0.017 between WT untreated and mCAT untreated
b: p<0.017 between WT untreated and treated
c: p<0.017 between mCAT untreated and treated

Both the WT and mCAT mice showed significant cancellous bone loss after.

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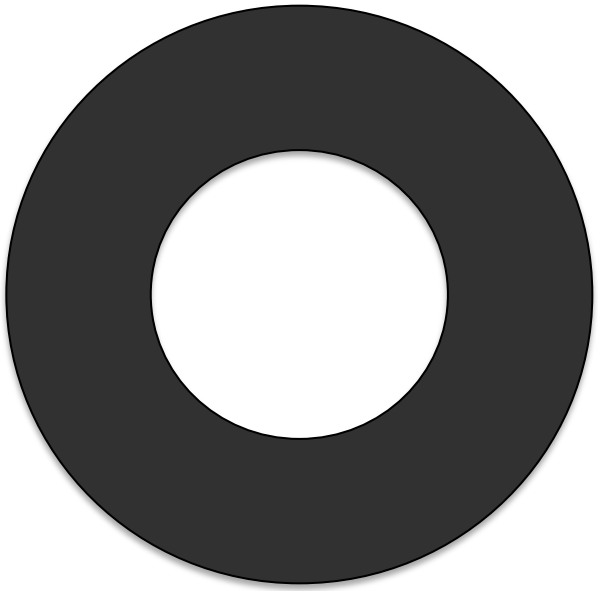
Cortical structure: mCAT bones were smaller than WT bones (despite same body weights)

Untreated WT mice



Untreated mCAT mice

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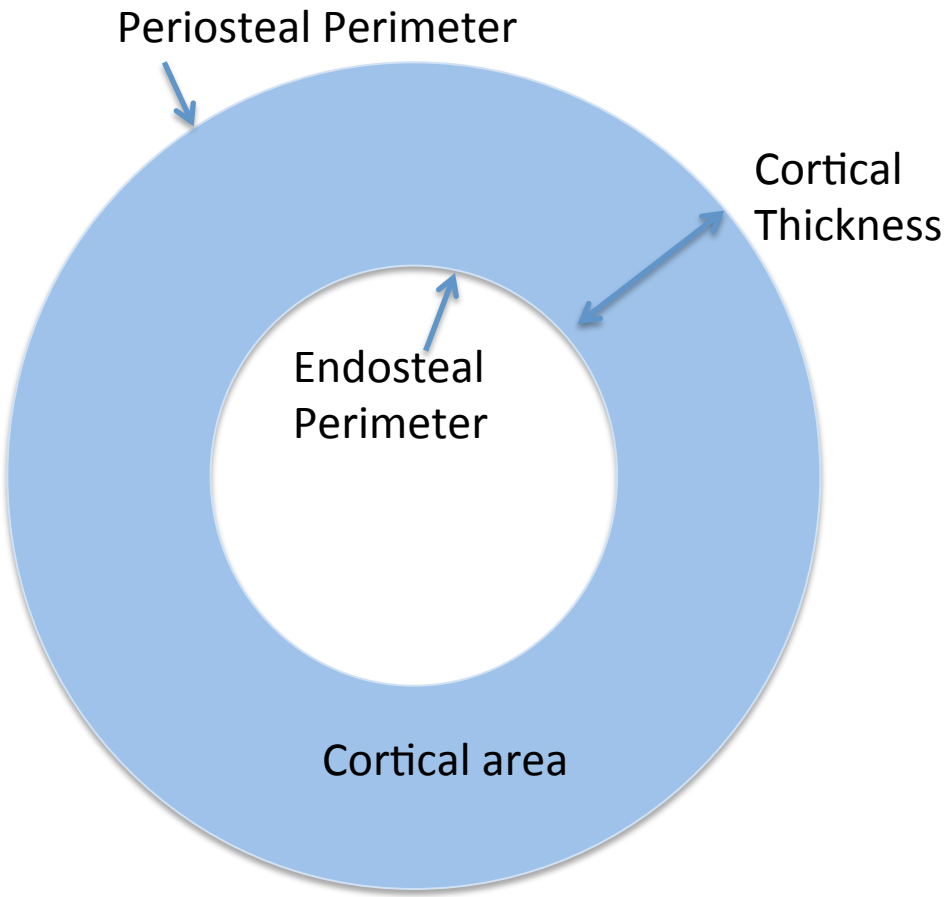
Skeletal structure: Cortical



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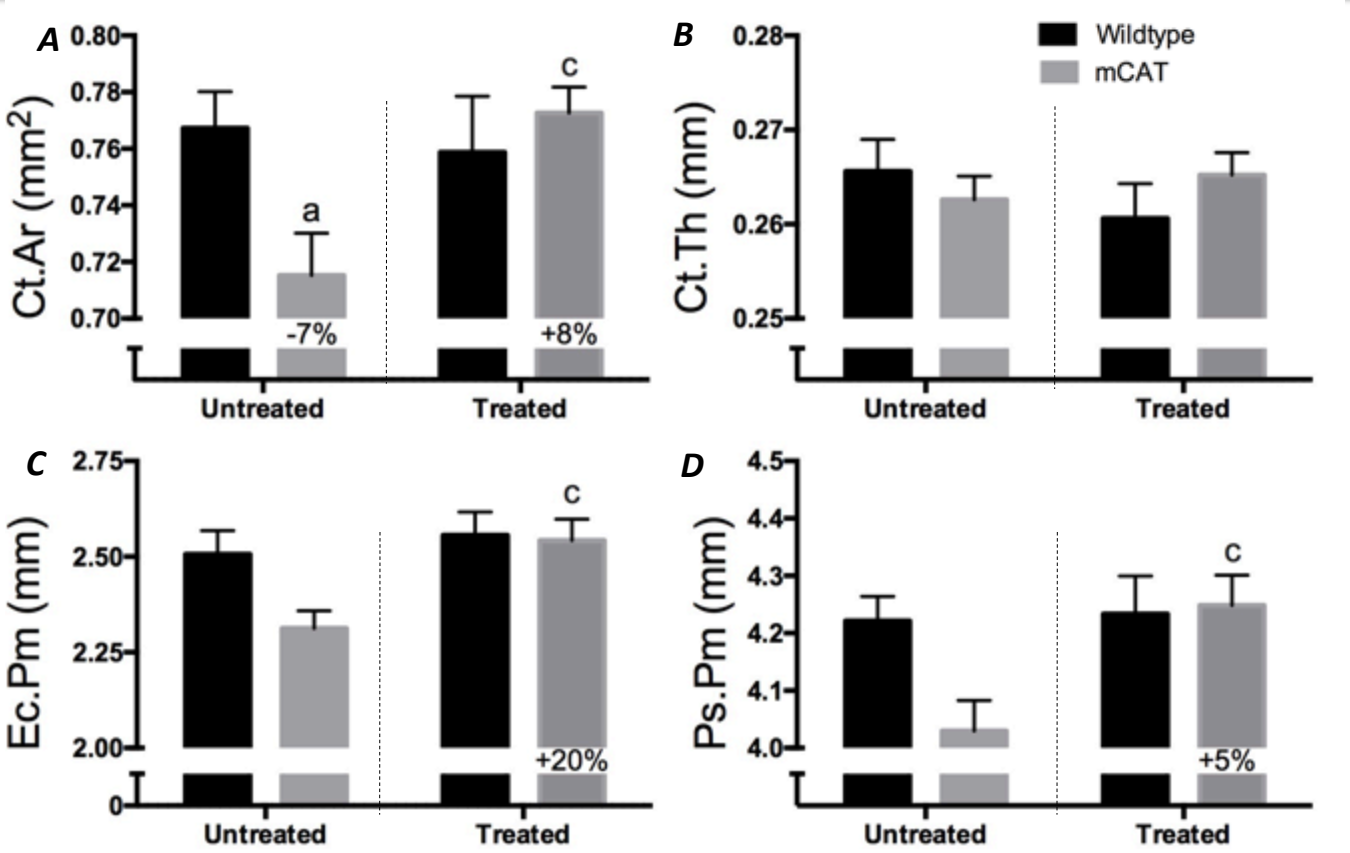
Skeletal structure: Cortical
Genotype WT vs mCAT: radial expansion in mCAT mice



a: $p<0.017$ between WT untreated and mCAT untreated
b: $p<0.017$ between WT untreated and treated
c: $p<0.017$ between mCAT untreated and treated

Interestingly, the mCAT mice displayed radial growth in cortical bone after treatment

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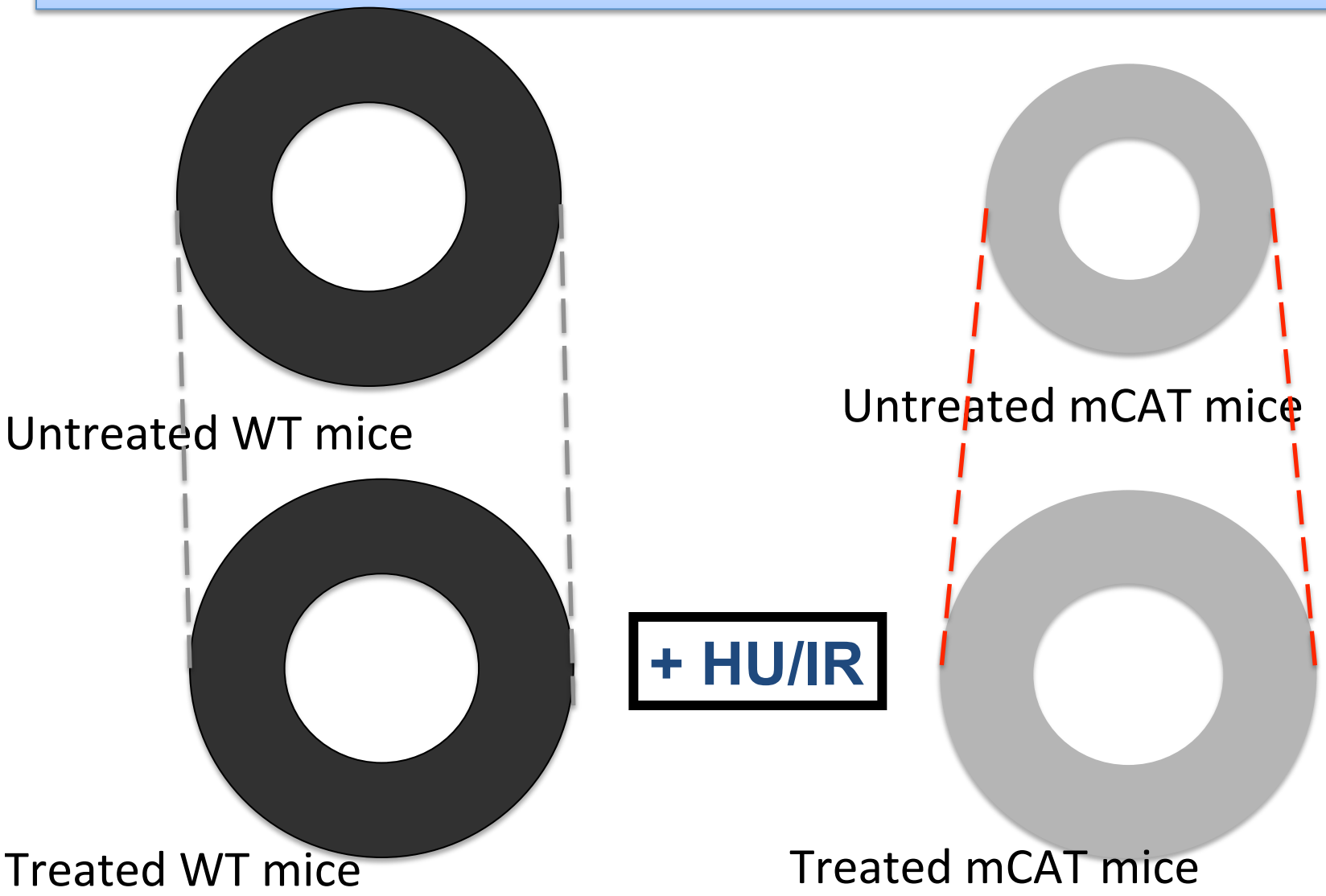
Treatment triggers rapid radial expansion in cortical bone of mCAT, but not WT mice.



Note: Radial expansion also occurs during periods of either rapid modeling (skeletal growth) and during skeletal aging (compensatory response)

Summary & Conclusions

- mCAT mice overexpress transgene in both osteoblast and osteoclast lineage cells, providing useful animal model to determine:
  - importance of normal mitochondrial ROS and bone phenotype (intrinsic)
  - quenching excessive ROS and oxidative damage (extrinsic)
- Long bones (not vertebrae) from mCAT mice are smaller than WT mice, despite comparable body weights
  - endogenous mitochondrial ROS is important for normal bone remodeling and skeletal structure
- Overexpression of catalase in mitochondria disrupts cortical remodeling responses to the challenges of simulated spaceflight;
- - we speculate that quenching endogenous mitochondrial ROS removes a 'brake' on remodeling activity and allows radial expansion during treatment

Acknowledgements

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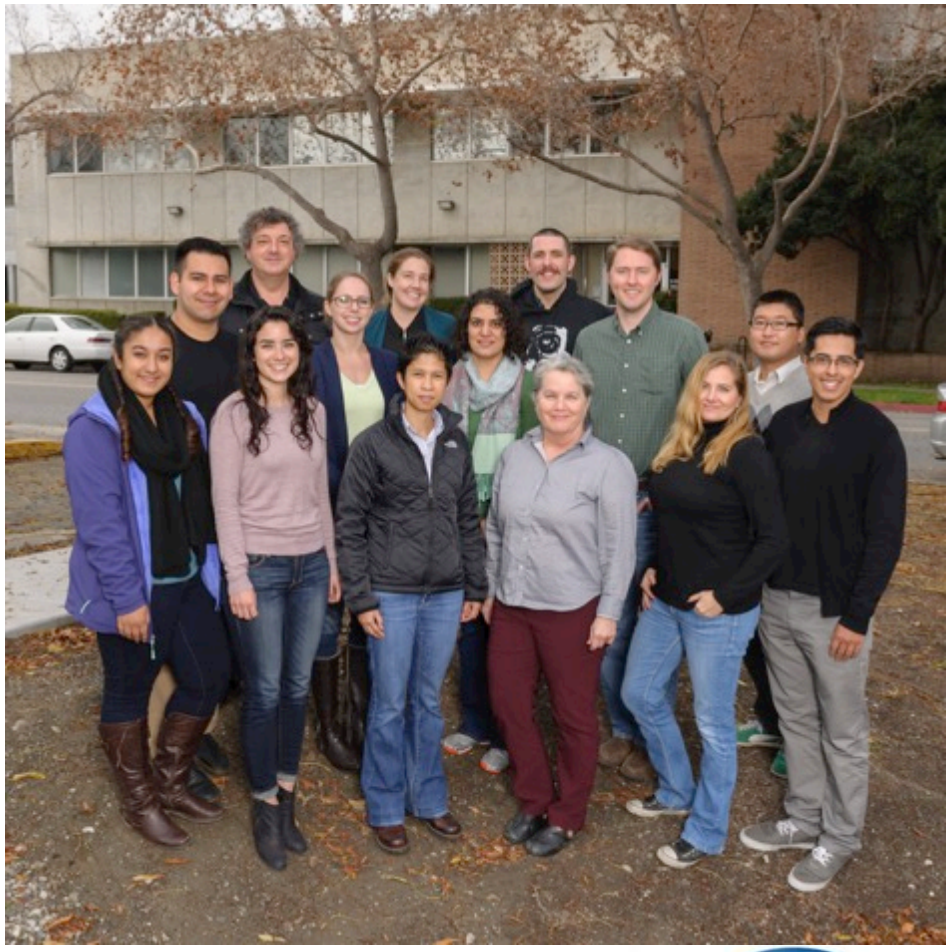

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