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The Effects of Mitochondrial Catalase Overexpression on Alcohol-Induced Skeletal Toxicity

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Introduction

- According to a 2019 survey by the NIAAA, 69.5% of individuals over the age of 18 had consumed alcohol (ethanol) in the past year, and 25.8% reported engaging in binge drinking within the past month.
- Alcohol downregulates osteoblastogenesis and upregulates osteoclastogenesis and adipogenesis within the skeleton (1).
- Increased concentrations of reactive oxygen species (ROS), including ROS generated by mitochondrial metabolism, are hypothesized to be a mechanism by which alcohol affects bone homeostasis, as suggested by the protective effects of the dietary antioxidant N-acetylcysteine (NAC) (3).
- Overexpression of the hydrogen peroxide-removing enzyme catalase in mitochondria reduces the effects of aging on the skeleton (4).

Hypothesis

Overexpression of catalase in mitochondria counteracts ethanol-mediated skeletal toxicity.

Methods

- MCAT mice contain a transgene for human catalase fused to the leader sequence of ornithine transcarbamylase targeting the protein to mitochondria.
- Gavage Model: WT and MCAT mice were taken through a 4-day gavage protocol with either PBS or 31.5% EtOH at concentrations of 3, 3, 4, and 4.5 g/kg bodyweight. First and fourth day weights were used to calculate weight change throughout the protocol (5).
- RNA was isolated from femoral shafts and femoral bone marrow. Concentrations were determined by absorbance at 260 nm. RNA quality was determined by Agilent RNA Screentape System.
- QRT-PCR analysis determined the effects on gene expression for markers of osteoblastogenesis (Col1a1, Smpd3), osteoclastogenesis (Calcr, RANKL), and adipogenesis (Fabp2, Ppar-γ, and Ebf-1). QRT-PCR also confirmed that human mCAT transgene was properly overexpressed in mCAT mice and not present in controls (5).
- Serum concentration of procollagen 1a1 was determined by ELISA (5).

Femoral Shaft QRT-PCR

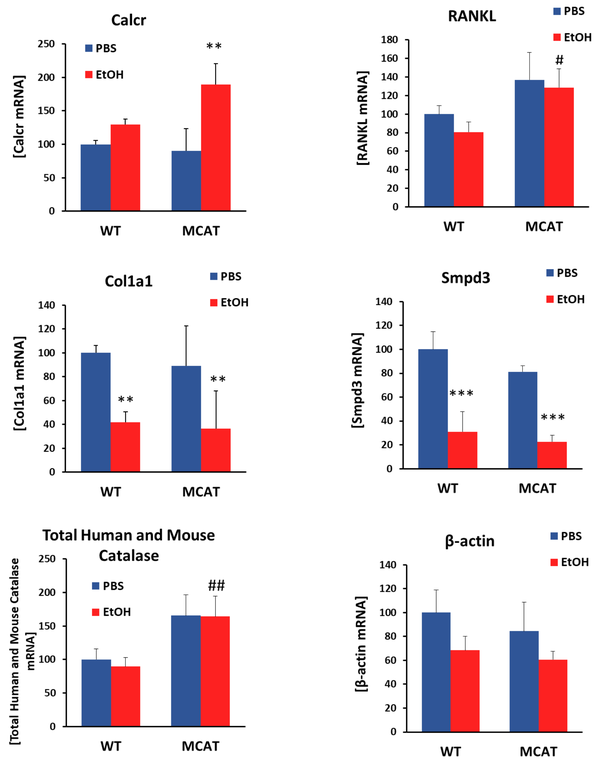


Figure 1: QRT-PCR analysis of the femural shaft demonstrates that increased concentrations of mitochondrial catalase did not result in protection from alcohol's down regulation of expression of osteoblast markers (Smpd3 and Col1a1) or upregulation of osteoclastogenesis markers (Calcr and RANKL). β-actin was used as a house-keeping gene. QRT-PCR confirmed expression of human catalase only in MCAT mice (data not shown) and the overexpression of total amount of catalase mRNA. **, ***: P < 0.01, 0.001 vs. PBS. #, ##: P < 0.05, 0.01 vs. WT.

Femoral Marrow QRT-PCR

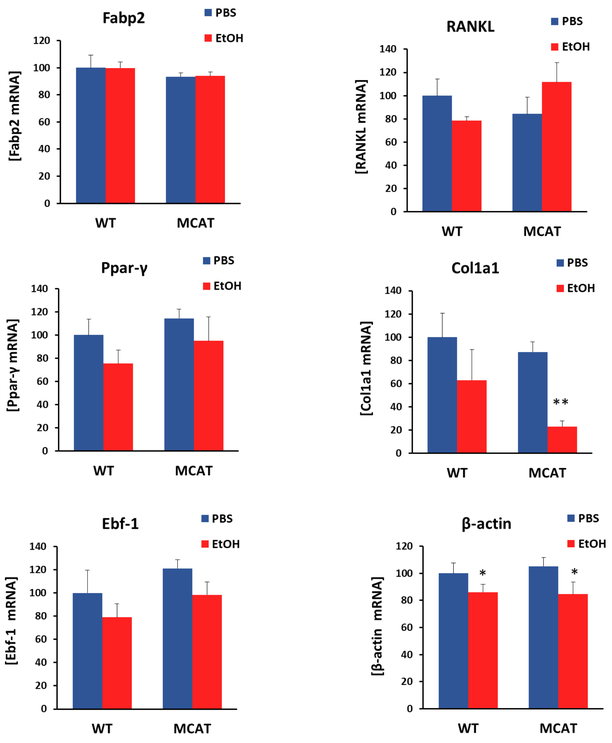
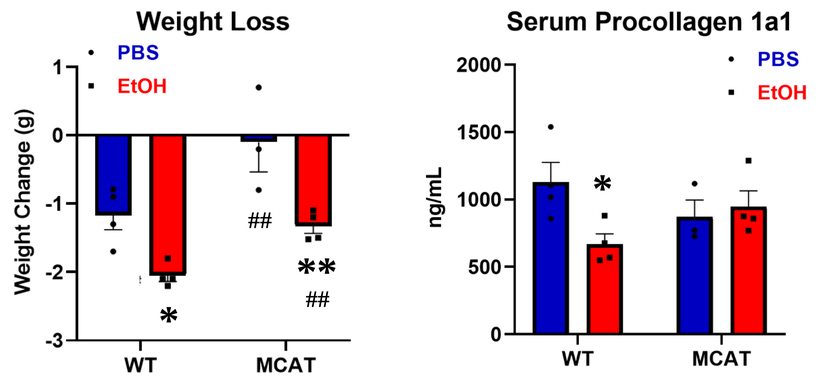


Figure 2: QRT-PCR analysis of femur bone marrow demonstrates that binge ethanol and overexpression of mCAT did not have an effect on the expression of adipogenic markers (Fabp2, Ppar-γ, and Ebf-1). The house-keeping gene β-actin was slightly downregulated in both WT and MCAT mice. Ethanol-mediated downregulation of Col1a1 expression was not prevented in MCAT mice. *, **; P < 0.05. 0.01 vs. PBS.

Weight and Serum Analysis



Genotype X Treatment interaction P = 0.046

Figure 3: The weight change of each mouse was recorded as the difference between the weight of the first and fourth days of the binge model. Ethanol-treated mice lose more weight than mice gavaged with PBS, but MCAT mice have less weight loss than WT mice. Serum ELISA analysis to determine the concentration of procollagen 1a1 in circulation showed that mCAT overexpression is protective against the characteristic depletion of procollagen 1a1. *, **: P < 0.05, 0.01 vs PBS. ##: P < 0.01 vs. WT.

Conclusion and Discussion

- Overexpression of mitochondrial catalase protected against ethanol-mediated downregulation of a serum bone formation marker procollagen 1a1.
- Overexpression of mitochondrial catalase protected against systemic weight loss during gavage.
- Unexpectedly, overexpression of mitochondrial catalase did not prevent ethanol-mediated downregulation of gene expression for Col1a1 and Smpd3 or ethanol-mediated upregulation of gene expression for Calcr in femoral bone.
- The 4-day ethanol gavage exposure did not upregulate adipogenesis genes in bone marrow.
- Further experiments with more animals are needed, as the small numbers within the groups limit statistical impact (n= 3,4,4,4).
- Further investigation into post translational effects that may mediate alcohol effects on matrix protein secretion in bone are needed.
- Redox status of the cell may be another hypothesis to investigate with regard to the mechanism of ethanol skeletal toxicity since NAC is both a ROS scavenger and a glutathione precursor and GSH/GSSG ratios have previously been suggested to impact osteoblastogenesis (6).

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References: 1. Ronis M. et al., Research Outreach 105: 48-52, 2018. 2. Mercer KE et al. Alc. Clin. Exp. Res. 38: 672-682, 2014. 3. Chen, J-R et al. J. Pharmacol. Exp. Ther. 336: 734-742, 2011. 4. Ucer et al. JBMR, 32: 560-74, 2017. 5. Denys et al. Toxicol. Sci, 185: 232-245, 2022. 6. Romagnoli et al. FEBS Journal 280: 867-79, 2013.