Aging Enhances Ethanol-Mediated Bone Toxicity in Male and Female Mice

Epiphany N. Baker[1], Kim B. Pedersen[1], Mary Bordelon[1], Martin J. J. Ronis[1]
Dept. of Pharmacology; Louisiana State University Health Sciences Center; New Orleans, LA

Abstract

BACKGROUND: Alcohol consumption and aging are factors that can lead to osteopenia. How these factors interact is poorly understood. To investigate these relationships, a binge ethanol exposure model was used for both female and male mice to assess the changes in bone formation and resorption across age and sex.

HYPOTHESIS: Aging enhances ethanol-mediated bone toxicity by repressing the activity of osteoblasts and amplifying the activity of osteoclasts.

AIMS: To investigate how binge ethanol exposure and aging impact bone formation and resorption in females and males.

METHODS: Dmp1-Cre TdTomato mice with the florescent marker TdTomato expressed in osteoblasts and osteocytes and control mice without Cre expression were used. Data was collected by the extraction of femoral shafts, lumbar vertebrae, and serum from 12-week-old and 78-week-old males and females who were gavaged for 4 consecutive days with 3, 3, 4, and 4.5 g of ethanol/kg of body weight or with PBS (control). The mice were sacrificed 6 hours after the last gavage and bones were collected. Gene expression and protein measurement in the femoral shaft and lumbar vertebrae was determined by qRT-PCR and Western blots. Bone turnover markers in serum were determined by ELISA.

RESULTS: Ethanol and aging both significantly decreased serum levels of Procollagen Type I (P1NP) in males and females ($P < 0.001$), indicating reduced bone formation. Serum levels of CTX-1 increased significantly with aging ($P < 0.001$), reflecting enhanced bone resorption. In addition, ethanol increases another serum bone resorption marker (TRACP-5b) in 78-week-old male and female mice, indicating increased osteoclast activity. In the femoral shaft, ethanol and aging increased the expression of genes involved in osteoclast activation, Calcitonin receptor (Calcr) and RANKL ($P < 0.01$) with a larger induction of RANKL mRNA in 78-week-old than 12-week-old mice ($P = 0.003$ for ethanol-aging interaction). Osteoblast-associated genes, Collagen Type I Alpha 2 Chain (Col1a2) and Sphingomyelin phosphodiesterase 3 (Smpd3), were downregulated by ethanol ($P < 0.05$), with further reduction in 78-week-old ethanol-treated mice ($P <0.05$). Aging did not decrease the expression of TdTomato mRNA, indicating no loss of osteoblasts and osteocytes. In the lumbar vertebrae, there was a trend of increased soluble RANKL protein (relative to β-Actin) in 78-week-old ethanol-treated females ($P = 0.09$), but no significant changes in full-length RANKL. Surprisingly, the levels of Cathepsin K (CTSK), an osteoclast protease, and Pro-CTSK were significantly higher in 12-week-old females than in 78-week-old females ($P = 0.03$ and 0.01, respectively).

CONCLUSIONS: Binge ethanol exposure and aging independently and synergistically disrupt bone remodeling by inhibiting bone formation, indicated by a downregulation of P1NP, Col1a2 mRNA and Smpd3 mRNA, and enhancing bone resorption, indicated by an upregulation in CTX-1, RANKL mRNA and Calcr mRNA in both sexes. However, the expression of CTSK was diminished in the aging female mice.

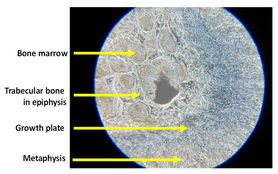
Objective

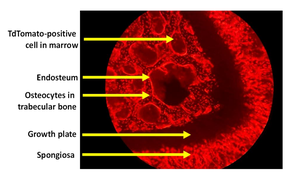
- To assess how aging in mice affects the skeletal response to binge alcohol.

Methods

Mouse strain

- Dmp1-Cre TdTomato mice with red fluorescent osteoblasts and osteocytes (below).
- TdTomato control mice.





A decalcified section of the proximal femur from a male Dmp1-Cre TdTomato mouse at 8 weeks of age was imaged by phase contrast and red fluorescence microscopy (1).

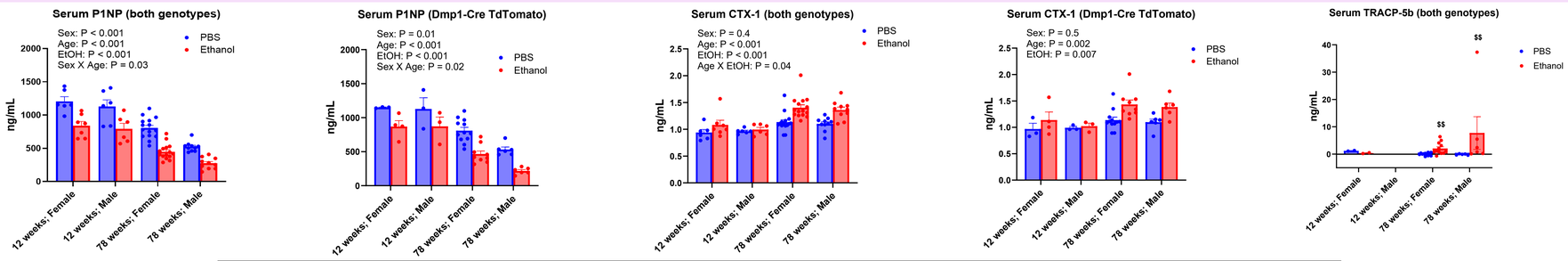
Ethanol exposure

- 12-week-old and 78-week-old male and female mice were gavaged on 4 consecutive days with 3, 3, 4, and 4.5 g ethanol/kg body weight or with isovolumetric PBS (2).
- Mice were sacrificed 6 hours after final gavage with collection of serum and bones.

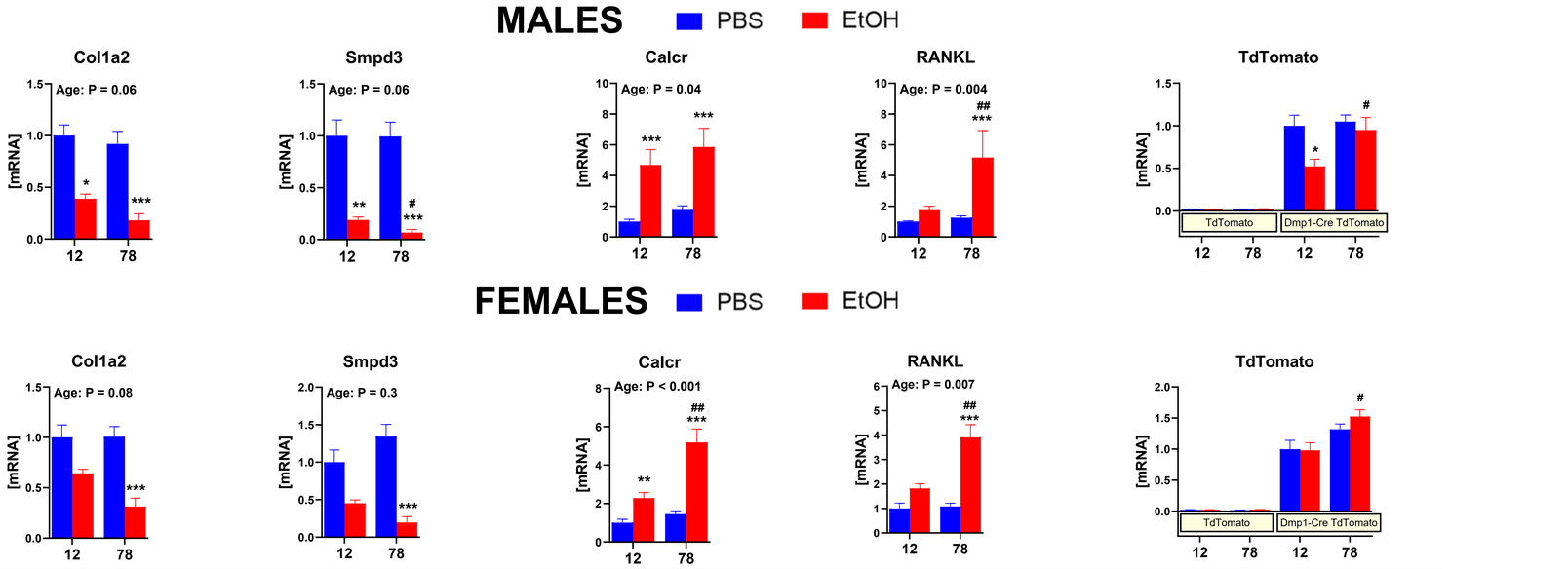
Analyses

- Bone turnover markers were determined in serum and vertebrae by ELISA.
- Gene expression in the femoral shaft and vertebrae was determined by qRT-PCR.
- Protein expression in lumbar vertebrae was determined by Western blots.

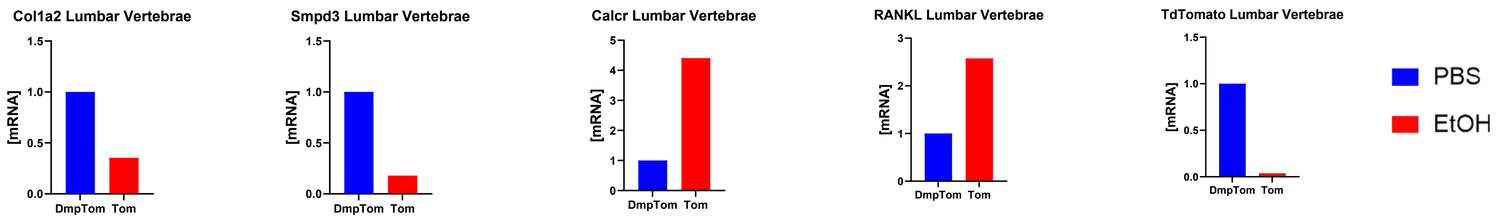
Results



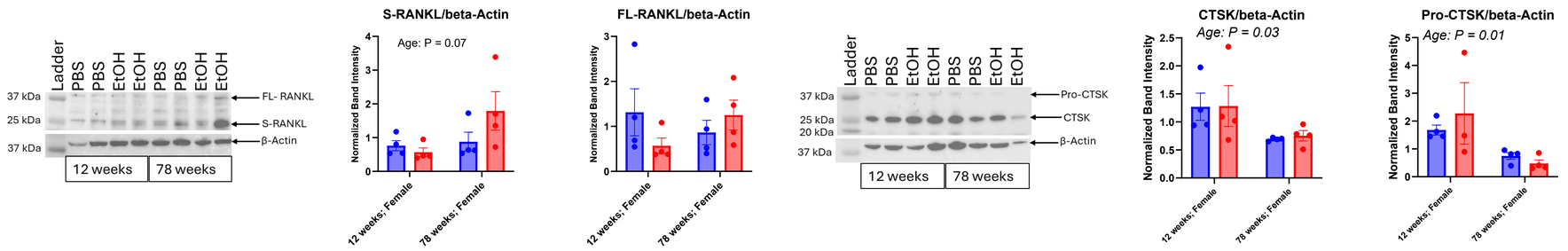
- A serum marker for bone synthesis, Procollagen I α (P1NP), and serum markers for bone resorption, CTX-1 and TRACP-5b, were measured in serum by ELISA.
- Test probabilities for main effects and significant interactions based on two-way ANOVA are listed in each panel. $$: $P < 0.01$ vs. PBS in Mann-Whitney test.



- Gene expression in the femoral shaft was determined by qRT-PCR. Collagen 1a2 (Col1a2) and Sphingomyelin phosphodiesterase 3 (Smpd3) are markers for osteoblast function. Calcitonin receptor (Calcr) and Receptor activator of nuclear factor kappa-B ligand (RANKL) are markers of osteoclast differentiation (1). Except for TdTomato mRNA, data for the two genotypes are combined.
- Data were analyzed by 2-way ANOVA for each sex. Main effects of age are listed in panels. *, **, ***: $P < 0.05$, 0.01, 0.001 vs. PBS. #, ##: $P < 0.05$, 0.01 vs. 12 weeks.



- Lumbar vertebrae RNA was isolated from a Dmp1-Cre TdTomato (DmpTom) mouse and from a TdTomato mouse (Tom) gavaged with PBS and ethanol (EtOH), respectively. Both mice were 78-week-old females. Gene expression by qRT-PCR shows the same pattern as for the femoral shaft.



- Protein expression in the lumbar vertebrae from female mice was determined by Western blots. RANKL is a signal for osteoclast activation. Cathepsin K (CTSK) is a lysosomal protease in osteoclasts. Membrane-bound full-length RANKL (FL-RANKL), soluble RANKL (S-RANKL), the inactive Pro-form of CTSK (Pro-CTSK) and CTSK proteins were quantified relative to beta-Actin.
- Data were analyzed by 2-way ANOVA. Main effects of age are listed in panels.

References

1. Pedersen, K., Watt, J., Maimone, C., Hang, H., Denys, A., Schroder, K. et al. (2023). Alcohol Clin Exp Res (Hoboken) 47, 2233-2247
2. Denys, A., Pedersen, K. B., Watt, J., Norman, A. R., Osborn, M. L., Chen, J.-R. et al. (2021). Toxicological Sciences 185, 232-245

Conclusions

- Aging exacerbates ethanol effects on bone formation and resorption markers.
- Aging exacerbates the inhibitory effect by ethanol of gene expression of osteoblast markers Col1a2 and Smpd3.
- Aging exacerbates the stimulatory effect by ethanol of gene expression of osteoclast differentiation markers RANKL and Calcr.
- Expression of TdTomato mRNA in Dmp1-Cre TdTomato is not reduced by aging, suggesting no overall loss of osteoblasts/osteocytes.
- Soluble RANKL may be increased in 78-week-old females.
- Relative to beta-Actin, CTSK and Pro-CTSK protein concentrations are reduced in 78-week-old females.

This research project was supported by Award Number: DBI-2051440 through the National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Program, Award Number R37 AA018282 (MJR).