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

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.