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“Development of an Acetate Infusion Model to Determine the Role of Acetate in Alcohol-Mediated Bone Loss”

Background: High alcohol (ethanol) consumption is a well-established risk factor for bone loss. Ethanol impairs bone homeostasis, characterized by decreased bone formation and increased bone resorption. We previously developed a binge ethanol gavage model in which mice received 3, 3, 4, and 4.5 g ethanol per kg body weight on four consecutive days. Relative to mice gavaged with PBS, ethanol suppressed osteoblastic gene expression, including genes for key structural proteins and enzymes, such as type I collagen (Col1a1 and Col1a2) and sphingomyelin phosphodiesterase 3 (Smpd3), while simultaneously upregulating genes involved in osteoclast differentiation, including genes for the Calcitonin Receptor (Calcr), Osteoclast Stimulatory Transmembrane Protein (Ocstamp), and Receptor activator of nuclear factor kappa-B ligand (RANKL). Recent studies suggest that acetate, a primary metabolite of ethanol metabolism in the liver, could be a potential mediator of these effects. Intraperitoneal injections of acetate to mice led to increased expression of osteoclast differentiation markers Calcr and Ocstamp in the femoral shaft, suggesting that acetate promotes osteoclast differentiation.

Hypothesis: The increased acetate concentration in the circulation is the primary mechanism for osteoclast activation after binge ethanol exposure.

Aim: A drawback of previous acetate delivery by gavage or intraperitoneal injection is that they cause a short-lived, lasting less than 3 hours, peak in the concentration of circulating acetate, whereas binge ethanol increases acetate for more than 6 hours. We aimed to model circulating acetate after ethanol exposure by continuous infusion of acetate.

Methods: Mice were implanted with a cannula in the external jugular vein. The infusion line was threaded to the back, exiting between the shoulder blades. The infusion line was flushed daily with heparinized saline. 3 days after surgery, sodium acetate was infused at a rate of 67 μmol acetate/min/kg body weight for 6 hours. Control mice received isovolumetric amounts of saline. We measured acetate in serum and gene expression in the femoral shaft by quantitative RT-PCR.

Results: The average concentration of serum acetate after acetate infusion for 6 hours was 2.8 mM, which was significantly higher than after 4 days of ethanol gavage (1.2 mM). Mice subjected to infusions 3 days after surgery had markedly reduced expression of genes involved in osteoblast function (Col1a1, Col1a2, and Smpd3) suggestive of post-operative stress inhibiting bone formation. A single acetate infusion at 67 μmol acetate/min/kg body weight for 6 hours did not clearly regulate the expression of genes involved in osteoclast differentiation or osteoblast function. A longer recovery period, a slower acetate infusion rate, and infusions for 4 days should be implemented for future experiments.