Alcohol overuse: cutting to the bone

Building on several decades of research, Dr Martin Ronis, a Professor at the Louisiana State University Health Sciences Center-New Orleans explores the effects of alcohol on bone turnover. His current research aims to better understand the molecular basis for the toxic effects of alcohol, particularly in women, with a view to uncovering vital therapeutic targets to prevent bone loss. As alcohol-induced bone loss shares many features in common with bone loss during menopause and ageing, his work provides fundamental insights into common pathways underlying the regulation of bone growth and turnover.

**Better Bones**

This is where the research of Dr Martin Ronis, Professor in the Department of Pharmacology and Experimental Therapeutics at the Louisiana State University Health Sciences Center—New Orleans comes in. His research explores the effects of alcohol on bone turnover. He is particularly interested in the relationship between inhibition of bone formation and stimulated bone resorption associated with alcohol abuse in young women and the increased risk of osteoporosis – resulting from a failure to attain PBM. His research involves multidisciplinary basic sciences, including whole animal physiology, nutrition and endocrinology, pathology and molecular biological techniques. Ultimately, Prof Ronis aims to better understand the molecular basis for the toxic actions of alcohol, which might uncover new therapeutic targets to prevent bone loss. Prof Ronis’ current work builds on a long history of alcohol research with Prof Ronis’ current work aims to unpick in fine detail, the ROS signalling mechanisms in bone after excess ethanol consumption.

**Ethanol Effects in Pregnancy**

In a study published in 2006, Prof Ronis directly tested the effect of ethanol in pregnancy and cycling rats. The team measured the effect of alcohol on skeletal parameters including tibial bone mineral density (TBMD), bone mineral content and bone mineral area (BMA). A dose-dependent skeletal toxicity following alcohol exposure was observed, demonstrated by decreases in TBMD and BMA. Their data suggested that ethanol-induced bone loss in pregnant rats is mainly due to inhibited bone formation. In striking contrast, bone loss in non-pregnant rats was higher than in that observed in pregnant rats and occurred through a different mechanism, concomitant with additional increases in bone resorption and a decrease in circulating osteocalcal. 

**Protective Estradiol Levels**

Exploring the pregnancy-protective effect of alcohol further, Prof Ronis and his team demonstrated the role of osteocalcin – the process whereby osteoclasts are developed. Osteons are the cells responsible for breaking down bone, resulting in bone resorption. Prof Ronis showed that osteodiol levels block alcohol-stimulated oxidative stress, which in turn promotes RANKL (a receptor activator of nuclear factor κB ligand), a substance needed for osteoclast formation – inducing osteocalcin. This finding has significant implications for the use of contraceptives containing osteocalcin for women with a history of alcohol use. Prof Ronis also showed that anabolic bone rebuilding is completely blocked by alcohol in post-ordination female rats. This has important implications for post-lactating women who resume drinking after stopping breast feeding, suggesting that they may be at increased risk of osteoporosis with age.

The role of oxidative stress

In a study involving bone remodelling during specific physiologic states (pregnancy, lactation and menopause), Prof Ronis went on to explore the underlying molecular mechanisms further. In an elegant series of experiments, the team showed that ethanol inhibits bone formation via a shift in the differentiation of bone marrow mesenchymal stem cells. Rather than forming osteoblasts (bone cell forming cells), these stem cells instead form adipocytes, which are adipose tissue cells that store fat.

**Overview of pathways underlying ethanol effects on bone turnover.**

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Ethanol Effect</th>
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<tr>
<td>ROS – reactive oxygen species; TNF – tumor necrosis factor</td>
<td>Reduced ROS levels and TNF production in the knockout mice.</td>
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<tr>
<td>TRAP – tartarate-resistant acid phosphatase</td>
<td>Increased TRAP activity in wild-type (WT) mice as a result of reduced stem cell formation.</td>
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<tr>
<td>E2/NAC – estrogen and N-acetylcysteine</td>
<td>Enhanced E2/NAC levels as a result of reduced TNF production.</td>
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<tr>
<td>E2 – estradiol; E2 – estradiol; NAC – N-acetylcysteine; RANKL – receptor activator of nuclear factor κB ligand; 1,25 – 1,25-dihydroxyvitamin D; TRAP – tartarate-resistant acid phosphatase.</td>
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They demonstrated that oxidative stress was an important mechanism for this. The team then went on to show that the production of excess reactive oxygen species (ROS) production by NADPH oxidase (NOX) enzymes was responsible for the effects of ethanol on bone turnover. Experiments of bone loss in female rats and mice fed ethanol was blocked by dietary antioxidants including N-acetylcysteine (NAC), vitamin E and also DPI an inhibitor of NADPH oxidase (NOX) enzymes.

Interestingly, Prof Ronis found that the effects of ethanol to inhibit osteoblastogenesis (the production of osteoblast cells that form bone) and stimulate bone marrow adipose cell formation were not blocked in experimental genetic mice that lacked an active form of NOX1/2 enzymes. Since a major component of ethanol pathology in bone is the generation of excess ROS, this led the team to hypothesise that another source of ROS mediates this effect. The researchers subsequently explored the contribution of ROS-generating NADPH oxidase-4 (NOX4) in ethanol-induced ROS in driving ethanol-induced suppression of bone formation.

CURRENT RESEARCH

Using a new mouse model of excess alcohol consumption, Prof Ronis' current work aims to unpick in fine detail the ROS signalling in bone after ethanol exposure. Excitingly, recent data also suggests that a certain level of ROS signalling is necessary for normal stem cell self-renewal and bone development during ageing. To test whether NOX4 is responsible for mediating the effects of ethanol on bone formation, ex-vivo (experiments on tissue removed from the body) bone marrow cultures from genetic mice that lack NOX4 in selected bone cell types (osteoblast precursors, osteocytes, osteoclasts) will be studied down on the mechanisms involved.

The teams’ recent findings suggest that dietary antioxidants prevent alcohol-induced osteopenia as a result of blocking excess hydrogen peroxide. 

Hydrogen peroxide is a form of ROS produced as the result of the activation of NADPH oxidase enzymes following the metabolism (breakdown) of ethanol. Importantly, since alcohol-induced bone loss shares many features in common with bone loss during menopause and ageing, molecular studies of alcohol actions on hydrogen peroxide downstream effects in bone may well provide fundamental insights into common molecular pathways underlying the regulation of normal bone growth and turnover.

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Bio
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Research Objectives

The goal of Dr Ronis research is to better understand the molecular basis for the toxic actions of alcohol, particularly in women, with a view to uncovering therapeutic targets. In particular, the results from research aim to link the deleterious effects of alcohol consumption on bone in women of reproductive age with increased risk of osteoporosis, a major cause of morbidity and mortality in post-menopausal women.

References


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