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Twelve months of voluntary heavy alcohol consumption in male rhesus macaques suppresses intracortical bone remodeling☆

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ABSTRACT

Chronic heavy alcohol consumption is a risk factor for cortical bone fractures in males. The increase in fracture risk may be due, in part, to reduced bone quality. Intracortical (osteonal) bone remodeling is the principle mechanism for maintaining cortical bone quality. However, it is not clear how alcohol abuse impacts intracortical bone remodeling. This study investigated the effects of long-duration heavy alcohol consumption on intracortical bone remodeling in a non-human primate model. Following a 4-month induction period, male rhesus macaques (*Macaca mulatta*, n = 21) were allowed to voluntarily self-administer water or alcohol (4% ethanol w/v) for 22 h/d, 7 d/wk for 12 months. Control monkeys (n = 13) received water and an isocaloric maltose-dextrin solution. Tetracycline hydrochloride was administered orally 17 and 3 days prior to sacrifice for determination of active mineralization sites. Animals in the alcohol group consumed 2.7 ± 0.2 g alcohol/kg/d (mean ± SE) during the 12 months of self-administration, resulting in a mean daily blood alcohol concentration of 77 ± 9 mg/dl from samples taken at 7 h after the start of a daily session. However, blood alcohol concentration varied widely from day to day, with peak levels exceeding 250 mg/dl, modeling a binge-drinking pattern of alcohol consumption. The skeletal response to alcohol was determined by densitometry, microcomputed tomography and histomorphometry. Significant differences in tibial bone mineral content, bone mineral density, and cortical bone architecture (cross-sectional volume, cortical volume, marrow volume, cortical thickness, and polar moment of inertia) in the tibial diaphysis were not detected with treatment. However, cortical porosity was lower (1.8 ± 0.5 % *versus* 0.6 ± 0.1 %, p = 0.021) and labeled osteon density was lower (0.41 ± 0.2/mm^2 *versus* 0.04 ± 0.01/mm^2, p < 0.003) in alcohol-consuming monkeys compared to controls, indicating a reduced rate of intracortical bone remodeling. In concordance, plasma CTx was lower (2.5 ± 0.3 ng/ml *versus* 1.7 ± 0.1 ng/ml, p = 0.028) in the alcohol group. These results suggest that chronic heavy alcohol consumption may negatively impact bone health, in part, by suppressing intracortical bone remodeling.

Introduction

Chronic heavy alcohol consumption is associated with an increase in all cause fracture risk [1–5]. A low bone mineral density (BMD) is often observed in chronic heavy alcohol consumers and when detected is generally associated with decreased bone formation [6–14]. In contrast, the precise role of bone resorption in the etiology of this condition is less clear [6,7,11,12,15–18]. Because alcohol consumption often results in an overall reduction in the rate of bone remodeling, bone loss may occur when the reduction in bone formation exceeds the reduction in bone resorption [19–21].

Although alcoholics often exhibit reduced BMD, the specific effects of alcohol on the human skeleton are poorly defined. This is due, in part, to complications associated with co-morbidities, such as smoking, poor diet and alcohol-induced disease [22]. In addition, alcohol intervention studies are exceedingly difficult to perform in humans. As a result, most intervention studies have been conducted using experimental animal models, with rodents being the mainstay for assessing the specific effects of alcohol on bone metabolism [23–25]. To date, rodent studies have focused on the effects of alcohol on cortical bone accrual and cancellous bone turnover. However, because intracortical (osteonal) bone remodeling is absent in small rodents, these animals are not ideal for evaluation of the effects of alcohol on cortical bone turnover [26].

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In humans, cortical bone comprises the great majority (~80%) of bone mass and plays a fundamental role in the mechanical function of the skeleton. Cortical bone fractures are common in young adult and middle aged males and are more prevalent in chronic heavy alcohol consumers [26,27]. Interestingly, heavy alcohol consumers are more likely to sustain low trauma fractures than non-heavy consumers [27]. Because the ability of a bone to resist fracture depends not only on bone mass, but also on factors such as architecture and intrinsic properties of the bone material [28], a deficit in cortical bone quality caused by detrimental changes in architecture and material properties can increase fracture risk independent of reduced BMD [29–31]. In this regard, intracortical remodeling is essential for maintenance of cortical bone quality [32].

Intracortical bone remodeling is initiated by osteoclast-mediated resorption on a quiescent bone surface to form a cutting cone, which is followed spatially and temporally by a closing cone lined by osteoblasts. In a completed osteon, bone fills most of the cavity that was created by the action of osteoclasts, leaving only a Haversian canal [33]. An increase in the overall rate of bone remodeling, or a remodeling imbalance where bone formation fails to keep pace with bone resorption, can result in increased cortical bone porosity and decreased BMD [34]. The effect of heavy alcohol consumption on intracortical bone remodeling rate and balance is unknown. The purpose of this study was to evaluate the specific impact of alcohol on intracortical bone remodeling and cortical porosity in a non-human primate model for chronic heavy alcohol consumption. The study focused on late adolescent to young adult male monkeys to model the human population that is most prone to chronic heavy alcohol consumption [35].

Materials and methods

Animals

The study population was comprised of a total of 34 late adolescent/young adult (5.4 ± 0.1 years old at initiation of alcohol protocol) male rhesus macaques (*Macaca mulatta*). Epiphyseal closure in rhesus macaque males is complete by 6.5 years of age [36]. Animals were pooled from 3 cohorts of monkeys selected from a colony born and reared in captivity at the Oregon National Primate Research Center at Oregon Health and Sciences University. The first cohort was sent to necropsy in May 2010 (n = 5 control, 8 alcohol), the second in March 2012 (n = 4 control, 8 alcohol), and the third in July 2012 (n = 4 control, 5 alcohol). All of the animals from each cohort were subjected to the same experimental design (described below). Throughout the study, monkeys were housed individually under constant temperature (20–22 °C) and humidity (65%) and an 11-h light cycle (light 0700–1800 h) in a room allowing visual, auditory, and olfactory contact with other monkeys. Body weights were recorded weekly throughout the study. Alcohol intake and blood alcohol concentration data for some of these subjects have been published elsewhere [37,38].

Experimental design

Induction phase

The experimental design is described in detail elsewhere [39]. Briefly, monkeys were trained to self-administer food and beverage (using an operant panel integrated into the side of their cage) and to submit their leg for blood sampling. Monkeys in the treatment group were then induced to drink increasing volumes of an alcohol solution (4% w/v ethanol mixed in deionized water) in a step-wise fashion over 4 consecutive 30-day periods for a total of 120 days. To induce drinking, a 1 g flavored food pellet was delivered every 5 min and water was the only fluid available. After water consumption became associated with the delivery of the pellet, the monkeys underwent a 30-day session where water was the only drinking fluid provided. During the second 30-day interval, animals drank a predetermined volume of alcohol solution corresponding to 0.5 g/kg/d alcohol, followed by volumes of alcohol corresponding to 1.0 and 1.5 g/kg/d during the third and fourth 30-day intervals, respectively. Monkeys were allowed to drink only 4% w/v alcohol until the required dose of alcohol was reached (e.g., 0.5 g/kg/d), at which point animals were allowed to drink only water. This step-wise increase in alcohol induction was done to circumvent alcohol taste aversion and increase the opportunity to associate drinking alcohol with its intoxicating effects.

Voluntary drinking phase

Following the 120-day induction, monkeys in the alcohol group (n = 21) were given simultaneous access to both water and alcohol (4% w/v) and allowed to voluntarily self-administer alcohol and/or water for 22 h/d (1100–0900 h each day), 7 d/wk for 12 months. Control animals (n = 13) were allowed to self-administer a volume of maltose-dextrin solution isocaloric to the mean volume of alcohol consumed by the alcohol group. Consumption was recorded daily by using weighing scales (Ohaus Corp., Parsippay, NJ) to measure the change in the mass of containers dispensing the solutions.

Blood samples were collected every 5 days from the saphenous vein of monkeys in the alcohol group just before the lights were turned off (between 1800 and 1900 h), which corresponded to 7 h into the 22 h sessions. Blood samples were sealed in airtight vials containing 0.5 ml of distilled water and 0.02 ml of 10% isopropanol (internal standard), and stored at −4 °C until analysis.

Food consisted of 1 g banana-flavored pellets (carbohydrate, 63%; fat, 4%; protein, 22%; PJ Noyes, Lancaster, NH). Over the 12-month duration of the experiment, the monkeys were required to eat their daily allotment of food in no fewer than 3 "meals," with at least 2 hours between each meal. A meal was defined by the proportion of daily food allotted to each monkey and the pace of the animal to obtain the food. The meal ended if one-third of the daily food allotment was obtained at a time, or if the monkey took longer than 2 minutes to obtain a pellet.

Fluorochrome tetracycline hydrochloride (20 mg/kg) was administered orally (17 and 3 days) prior to sacrifice for determination of active mineralization sites and rates of bone formation. At necropsy, the right tibia with attached fibula was harvested from each animal, placed into 70% alcohol, and stored at 4 °C until analysis.

Blood alcohol concentrations

Blood alcohol concentrations were assayed in plasma using a gas chromatograph (Hewlett-Packard 5890 Series II, Avondale, PA) equipped with a headspace autosampler, flame ionization detector, and a Hewlett Packard 3392A integrator.

Blood marker of bone resorption

Carboxyterminal cross-linking telopeptide of type 1 collagen (CTx), a marker of bone resorption, was measured in samples collected at necropsy using Serum CrossLaps ELISA (Immunodiagnostic Systems, Fountain Hills, AZ).

Blood vitamin D

25-Hydroxyvitamin D was measured in plasma samples collected at necropsy using an electrochemiluminescence binding assay (Roche Diagnostics, Indianapolis, IN).

Dual-energy X-ray absorptiometry

Bone mineral content (BMC, g), bone area (cm^2), and areal BMD (g/cm^2) in tibia/fibula (the tibia and fibula of each animal were analyzed together) were determined *post mortem* using a dual-energy X-ray