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Binge alcohol treatment of adolescent rats followed by alcohol abstinence is associated with site-specific differences in bone loss and incomplete recovery of bone mass and strength

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Abstract

We previously demonstrated that alcohol-fed adolescent rats exhibit reductions in lumbar spine bone mineral density (BMD) and vertebral body height, suggesting that chronic alcohol consumption has negative consequences for skeletal development during adolescence. Binge alcohol consumption is common in adolescents and young adults, yet little is known about its consequences on skeletal integrity or the attainment of peak bone mass. We used a previously validated binge alcohol exposure model to test the hypothesis that binge alcohol treatment of adolescent rats would be associated with distinct temporal and site-specific bone loss profiles, with incomplete recovery from bone loss following a period of alcohol abstinence. Seventy-two male adolescent Sprague-Dawley rats were assigned to one of six treatment groups ($n = 12$/group) receiving binge alcohol (3 g/kg) or saline intraperitoneal, 3 consecutive days (acute binge), 4 consecutive weekly (3-day) binge cycles (chronic binge), or 4 weekly binge cycles followed by a 30-day abstinence period without alcohol or saline injections (chronic binge with abstinence). Cancellous BMD was determined by quantitative computed tomography and compressive strength determined by biomechanical testing. Serum testosterone and osteocalcin levels were measured by enzyme-linked immunosorbent assay. Tibial cancellous BMD was significantly reduced by 25% ($P < .05$) after both acute and chronic binge alcohol treatment and vertebral cancellous BMD was significantly reduced by 15% ($P < .05$) after chronic binge exposure. Vertebral compressive strength was also significantly decreased by 31% ($P < .05$) after chronic binge alcohol treatment. Tibial cancellous BMD returned to control levels after the 30-day alcohol abstinence period, but vertebral cancellous BMD remained 15% below control values ($P < .05$) 30 days after termination of binge alcohol exposures. Serum osteocalcin levels were significantly decreased following acute binge alcohol exposure ($P < .05$). These results show that binge alcohol exposure can produce both short- and long-term skeletal damage in the adolescent rat. These data might have relevance to peak bone mass attainment and future risk of skeletal disease in adolescents and young adults who engage in repeated binge-drinking episodes.

Keywords: Binge alcohol; Adolescent rat; Peak bone mass; Osteoporosis; Fracture

Introduction

Because bone mass is lost throughout adult life as part of the aging process, a major risk factor for the development of osteoporosis is the failure to obtain a normal peak bone mass during early adulthood (Schettler and Gustafson, 2004). Multiple studies have shown that alcohol abuse is an important risk factor for osteopenia (for a review, see Chakkalakal, 2005). Excessive alcohol consumption continues to be a major public health problem in the United States with dangerous drinking practices such as binge drinking common in the adolescent and young adult populations (Windle, 2003). Binge drinking exposes young people to a variety of risks; from a bone health perspective this behavior is especially troubling because it tends to occur during the period when peak bone mass is attained (Abrams, 2003). Despite this connection, little is known about how binge alcohol consumption damages the adolescent skeleton and affects the attainment of peak bone mass. Approximately 25% of young men and 20% of women aged 18–30 years report engaging in at least one binge-drinking episode (defined as six or more drinks per occasion) each month according to a recent survey (Grossberg et al., 2004). A subset of young adults with a history of

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binge-drinking behavior report engaging in binge-drinking episodes five or more times per month, suggesting that multiple binge-drinking episodes may be common in the young adult population (Grossberg et al., 2004). These data are in agreement with another study of young adult drinking behavior, which suggests that problem drinking behaviors beginning during adolescence (aged 17–20 years) tend to continue into the early adult years (aged 30–31 years) (McCarty et al., 2004) encompassing the most critical developmental periods for accruement of bone mass (Abrams, 2003).

Despite the evidence that a significant proportion of the adolescent and young adult populations tend to consume alcohol in a binge pattern, most studies of the effects of alcohol exposure on adolescent skeletal development in rodents have used chronic alcohol feeding models. These studies have shown that alcohol inhibits bone growth, decreases bone mineral density (BMD), and negatively impacts cancellous bone architecture in adolescent rats (Hogan et al., 1997; Sampson et al., 1996, 1997). Biomechanical properties of bone from growing rats are also negatively affected by chronic alcohol exposure (Hogan et al., 1997). These studies also suggest that compared to adult bone, more cortical bone than cancellous bone is lost in adolescent rats exposed to alcohol, reflective of the fact that bone growth rather than bone remodeling is the primary skeletal activity in the developing skeleton.

An important question regarding the effects of alcohol on adolescent bone is whether the developing skeleton can overcome damage caused by alcohol, or does alcohol consumption cause lasting deficits in bone mass or strength? A prior investigation demonstrated that a period of recovery following chronic alcohol consumption by adolescent rats normalizes most bone metabolism parameters affected by alcohol, but cancellous bone area (BATA) remained significantly decreased after a 60-day alcohol recovery period (Wezeman et al., 1999). Chronic alcohol consumption by adolescent rats also significantly lowers both vertebral BMD and vertebral body height in male and female animals compared to pair-fed controls (Wezeman et al., 2003), suggesting that chronic alcohol consumption by adolescent animals negatively affects bone growth and mass, and that these deficiencies may persist well past the active drinking period. Blood alcohol concentrations (BACs) associated with binge alcohol administration may be an important outcome determinant for the effects of patterned alcohol on the adolescent skeleton as demonstrated by a previous binge alcohol study examining the effect of moderate binge alcohol exposure (BAC: 43–106 mg/dL) in young rats. This study demonstrated an unexpected beneficial effect of moderate alcohol consumption on skeletal growth and BMD following a 2-day/week alcohol binge, and no bone-related effects of 5-day/week binge alcohol administration (Sampson et al., 1999).

In the present study, we use a model of binge alcohol exposure previously associated with aggressive bone loss in adult male and female rats (Callaci et al., 2004, 2006; Wezeman et al., 2007) for the first time in adolescent animals to address the questions raised here regarding the short- and long-term effects of binge alcohol exposure on developing skeletal health. Our hypothesis was that binge alcohol treatment of adolescent rats would cause distinct temporal and site-specific decreases in normal bone mass acquisition, with incomplete recovery observed following a period of alcohol abstinence. The results presented here are part of a larger effort currently underway using adolescent animals to identify unique mechanisms responsible for short- and long-term bone damage caused by binge alcohol consumption prior to attainment of peak bone mass.

Materials and methods

Binge alcohol model

This investigation received approval from the Loyola University Institutional Animal Care and Use Committee. Adolescent male Sprague-Dawley rats were obtained at 7 weeks of age (175–199-g range), (Harlan, Indianapolis, IN). Prior to treatment, animals were randomly assigned to one of six treatment groups with an n of 12 animals per group, chosen based on a power analysis performed using preliminary data (not shown). For animals receiving 1 week of treatment, the groups were saline control (C1) and binge alcohol treated (A1). The 1-week binge alcohol group will also be referred to in the text as acute binge alcohol-treated rats. For animals receiving 4 weeks of treatment, the treatment groups were saline control (C4) and binge alcohol treated (A4). The 4-week binge alcohol group is also referred to in the text as chronic binge alcohol-treated rats. To test the ability of rats to recover from binge alcohol treatment effects on bone, two additional groups of rats were treated with saline or alcohol for 4 weeks as previously mentioned and then held for an additional 30 days without treatment prior to euthanasia. These groups are designated as saline-control abstinent (C4-Abs) and binge-alcohol abstinent (A4-Abs). Animals were housed in pairs, with paired animals always assigned to the same treatment group. Animals were weighed prior to the initiation of treatment and twice weekly throughout the study period.

Alcohol was administered by a single daily intraperitoneal (ip) injection of a 20% (vol/vol) ethanol/saline solution at a dose of 3 g/kg, chosen to achieve peak BALs of approximately 300 mg/dL (Nation et al., 1993). Control animals were given an ip injection of an equal volume of sterile isotonic saline at the time of alcohol group injections. A once daily alcohol treatment regimen was chosen to avoid alcohol withdrawal symptoms that can occur when high doses of alcohol are administered twice daily (Penland et al., 2001). Alcohol or saline injections were given starting at 9:00 am, 3 days/week. No ip injections were given during the remaining 4 days each week. Twenty-four hours after