Skeletal Toxicity Associated with Chronic Ethanol Exposure in a Rat Model Using Total Enteral Nutrition

ELIZABETH C. BROWN, DANIEL S. PERRIEN, TERRY W. FLETCHER, DAVID J. IRBY, JAMES ARONSON, GUAN G. GAO, WILLIAM J. HOGUE, ROBERT A. SKINNER, LARRY J. SUVA, MARTIN J. J. RONIS, REZA HAKKAK, THOMAS M. BADGER, and CHARLES K. LUMPKIN JR.


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ABSTRACT
Chronic alcohol abuse decreases bone mass, inhibits osteoblast differentiation and function, increases fracture incidence, and delays fracture healing. Four studies were designed to use intragastric ethanol delivery as part of a total enteral nutrition (TEN) system to determine the negative systemic effects of chronic ethanol on 1) the rat skeleton and 2) local rapid bone formation during limb lengthening (distraction osteogenesis, DO). In study 1, three-point bending tests demonstrated that after 75 days of ethanol exposure, the tibiae had significantly lower load to failure versus control diet (p = 0.0006) or ad libitum chow-fed rats (p = 0.0029). Study 2 examined alcohol’s effects on the density and cross-sectional area of the proximal tibial metaphysis using peripheral quantitative computed tomography and found that after 25 days of ethanol exposure the trabecular volumetric bone mineral density (p = 0.011) and cortical cross-sectional area (p = 0.011) were lower compared with controls. In study 3, a comparison of distracted tibial radiographs and histological sections demonstrated ethanol-related decreases in both gap mineralization (p = 0.03) and bone column formation (p = 0.01). Histological comparisons in study 4 reproduced the ethanol-related deficits in new bone formation during DO (p = 0.001). These results indicate that the TEN system is a viable model to study ethanol’s effects on the skeleton and that chronic ethanol delivery via TEN decreases trabecular bone density, cortical area, and mature bone strength. Also, the DO studies demonstrate, for the first time, that chronic ethanol inhibits rapid bone formation during limb lengthening.

Alcohol abuse may be the nation’s number one health problem in terms of estimated costs of $117 billion annually, most in lost productivity (Holden, 1987). Numerous clinical studies have shown that alcohol abuse is correlated with osteoporosis, decreased bone mass, and risk of fractures with or without liver involvement (Purohit, 1997). Ethanol’s effects on the skeleton have been examined through studies using biomechanical, histomorphological, molecular, and mineral quantitative techniques. Studies have demonstrated inhibition of bone growth (Sampson et al., 1997), reduced mineral density (Turner, 2000), and reduced bone strength (Hogan et al., 2001). The skeletal changes that result from alcohol consumption seem to be independent of liver damage or calcitropic hormone levels, and there is much evidence that ethanol acts directly on osteoblast activity (Turner et al., 2001).

To date, the majority of ethanol studies in animals have used the Lieber-DeCarli liquid diet protocol or modifications thereof. Issues concerning restricted nutrition and consumption patterns may limit data interpretation with these models. An alternative, which provides complete dietary support, is total enteral nutrition (TEN), which is an intragastric dietary infusion model similar to that developed by French and Tsukamoto (Tsukamoto et al., 1984). Numerous studies have established the efficacy of an intragastric ethanol/diet delivery system in rodents to study ethanol’s effects on various metabolic and endocrine systems (Tsukamoto et al., 1984; Ronis et al., 1991; Badger et al., 1993a,c). To our knowledge, the application of such a model to study ethanol’s effects on the skeleton has not been reported.

Distraction osteogenesis (DO) is a unique clinical method of bone formation and can be considered a variant of fracture healing that stretches the biological repair process to its natural limits. The method was developed by Ilizarov and has been used both experimentally and clinically (Ilizarov, 1990; Liu et al., 1999; Rowe et al., 1999). DO is induced by slowly pulling apart the edges of an intentionally introduced

ABBREVIATIONS: TEN, total enteral nutrition; DO, distraction osteogenesis; EtOH, ethanol; UEC, urinary ethanol content; NBF, neutral buffered formalin; BMD, volumetric bone mineral density; pQCT, peripheral quantitative computed tomography.

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bone fracture (osteotomy) with an external fixator to permit rapid formation of new bone in the slowly expanding gap. The DO process creates large areas of intramembranous bone formation while spatially and temporally separating the stages of osteoblastogenesis from osteoclastogenesis. This type of bone formation seems analogous to the periosteal bone formation during normal fracture healing. These characteristics create an excellent opportunity for the study of osteoblastogenesis in a variety of experimental models (Lumpkin et al., 1996; Ueng et al., 1999; Aronson et al., 2001a; Ronis et al., 2001). It has been demonstrated that the histological pattern of bone formation by DO in dogs, rabbits, rats, and mice is analogous to that in humans (Aronson, 1994a; Aronson et al., 1997; Tay et al., 1998; Isefuku et al., 2000). In addition, the efficacy of DO during intragastric dietary delivery without ethanol has been established (Lumpkin et al., 1996). These studies suggest that a rat model of DO would be useful for the investigation of ethanol-associated alterations in bone formation. As noted above, alcoholism increases the risk of fracture, interferes with bone homeostasis and repair, and has been shown to impair fracture healing. A salient finding of a recent review is that patients with alcohol-related bone disease display a marked impairment in bone formation (Purohit, 1997). Therefore, we hypothesized that the rat model of DO in conjunction with TEN/EtOH, should allow one to demonstrate and study the negative effects of ethanol exposure on large-scale intramembranous bone formation (Lumpkin et al., 1996; Aronson et al., 1997). Thus, two studies were designed to focus on the inhibitory effects of ethanol on local rapid bone formation during DO in the context of complete nutrition.

To summarize, four studies were initiated with two ends in mind. Two studies were designed to determine whether chronic ethanol delivered with the TEN system would result in negative systemic skeletal effects. These studies examined the effects of chronic dietary ethanol infusion on extrinsic tibial bending strength, volumetric bone mineral density, and cross-sectional area. The second set of studies was designed to determine whether chronic dietary ethanol infusion impairs bone formation during the DO process.

**Materials and Methods**

**Animals**

The following protocol was used in all studies. Virus-free adult (350–400 g), 3-month-old, male Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). They were housed in individual cages in temperature-controlled (22°C) and humidity-controlled (50%) rooms having a 12-h light/dark cycle with lights on at 6:00 AM. All animals were handled for 1 week before surgery. After acclimation, all rats, excluding those assigned to the chow group in study 1 (see below), underwent surgical placement of intragastric cannulae for infusion of liquid diet. The control and EtOH groups were acclimated to the diet over the 1st week after surgery. After surgical recovery and acclimation to the liquid diet, the animals were assigned to the respective treatment groups for each study (again, with the exception of the chow group in study 1). Mean body weights were equivalent in all groups (± 4 g) within the individual studies. All animals were weighed at regular intervals throughout the experiments to monitor weight gain in each group. These studies were approved by the Institutional Animal Care and Use Committee.

**Procedures for Placement of Intragastric Cannulae and Infusion of Liquid Diet**

Care has been taken to develop the surgical procedures and the postsurgical systems with the idea of reducing stress to a minimum and assuring that the cannulated rat is a fully standardized and physiologically competent model to study endocrinological and metabolic systems (Tsukamoto et al., 1984; Ronis et al., 1991; Badger et al., 1993a,c; Lumpkin et al., 1996). Surgical placement of the intragastric cannula was performed as previously described (Lumpkin et al., 1996). Briefly, sodium pentobarbital (Nembutal; 50 mg/kg i.p.; Abbott Laboratories, Chicago, IL) was used for anesthesia and reflexes were monitored such that any purposeful movement in response to pain stimuli was abolished. Breathing and skin color were monitored throughout. The stomach was exposed and the interface between the cardiac and pyloric regions located. A small hole was made with a scalpel point, and the cannula (0.025-inch i.d. by 0.047-inch o.d.) was inserted and secured with a suture. The free end of the cannula was threaded subcutaneously to the headpiece (Badger et al., 1993a). Immediately after cannulation, the anesthetized animal was placed in a Kopf rat stereotaxic device. The dorsal aspect of the parietal bones was exposed and cleaned, and five small jeweler’s screws were inserted to provide a foundation for anchoring the cannula to the skull with acrylic dental cement. The swiveled spring tethering device was attached, and its weight was supported from the top of the cage (Badger et al., 1993c). In studies 1 and 2, animals were placed on solid pellets for no longer than 4 h after recovery from anesthesia and water was available ad libitum throughout the study. The 1st day after surgery, the rats were infused with 50% of the National Research Council requirements and slowly increased to 100% by the 3rd day. The control diet was formulated as described previously and met the National Research Council requirements for rodent nutrition (Lumpkin et al., 1996). The TEN diets used peptides and small soluble proteins from hydrolyzed whey supplemented with amino acids obtained from common nutritional companies as outlined in Badger et al. (1993c). Calories in the control diet were distributed as follows: protein, 16%; carbohydrate, 74%; and lipids, 10%. In diet containing ethanol, carbohydrates were replaced by ethanol to keep the diet isocaloric to the control. The diets were prepared on a daily basis using sterile techniques. The diet was infused at a rate of 187 kcal/kg0.75/day in a continuous and steady manner over 23 h of each day. Infusion lines were flushed with hot sterilized tap water once daily and replaced as needed to prevent accumulation of sediment. In addition, the cannula of each rat was flushed three times daily with warm sterilized tap water.

In studies 3 and 4, urine ethanol concentrations (UECs), which accurately reflect blood ethanol concentrations, were measured daily for the EtOH rats (Badger et al., 1993a,b). Briefly, each afternoon, urine collection bottles were replaced with clean bottles containing 1 ml of toluene (to prevent evaporation of ethanol) and 1 ml of 1 M H2SO4 (to prevent growth of bacteria). The following morning, 1 ml of urine was collected from each bottle, thus providing a sample representative of the average concentration of ethanol in the urine excreted over a 16-h period of each day. The UEC of each sample was determined using a GL5 Analox analyzer (Analox Instruments Limited, London, UK) according to the manufacturer’s directions.

Rats placed in the EtOH groups were initially given 8 to 10 g/kg/day EtOH and gradually acclimated to and maintained on the maximum tolerated dose of 12–14 g/kg/day EtOH. The operational definition of maximum tolerated dose is the highest dose above which the animals exhibit either loss of response to stimulation or absence of a righting reflex (when placed in a dorsal recumbent position). Therefore, when greater than 25% of the animals exhibit the above-mentioned characteristics then the dose is lowered one step and not exceeded throughout the study. This maximum dose varies from one group of animals to the next and also increases with prolonged exposure to EtOH due to acquisition of tolerance. For
unexplained reasons, batches of rats of the same strain and from the same supplier (Harlan) tolerate different daily doses of ethanol. Thus, it was necessary to increase the EtOH dose over the duration of an individual experiment and vary the maximum EtOH dose between experiments (i.e., longer studies required higher maximum doses of EtOH; see details below). Chow rats (study 1 only) were fed Teklad 22/5 Rodent Diet (W) 8640 (Teklad Premier, Madison, WI).

**DO Surgical Technique (Studies 3 and 4)**

Stainless steel ring fixators were surgically applied, during the first surgery (described above), to the left tibia as described previously (Lumpkin et al., 1996; Aronson et al., 1997). This protocol results in a standard alignment of the fixator to the normal curvature of the tibia. The rats were given 0.1 mg/kg Buprenex for analgesia and returned to their cages for observation during recovery from anesthesia. After acclimation to TEN and increasing exposure to ethanol, the second procedure (left tibial osteotomy) was performed, under isoflurane anesthesia and followed by 0.1 mg/kg Buprenex analgesia (Aronson et al., 1997).

**Distraction**

Distraction was initiated at 0.2-mm b.i.d. (0.4 mm/day) for the respective distraction period on either the first morning after osteotomy (1-day latency) or the 6th day after osteotomy (6-day latency). Immediately after the distraction period, rats were euthanized under anesthesia, and both the operated and contralateral tibiae were surgically removed by disarticulation at the knee and ankle. The soft tissues were dissected away, and the distracted tibiae with fixators intact, were placed in 10% neutral buffered formalin (NBF) for 48 h. Tibiae were removed from the fixators using a manual saw. Radiographs of the specimens were taken, and each tibia was then returned to 10% NBF for subsequent histological processing as described below.

**Experimental Design**

**Study 1.** The following study was designed to investigate the effects of long-term EtOH exposure on extrinsic tibial bending strength. Sixteen male Sprague-Dawley rats were divided into three groups: control (n = 4), EtOH (n = 5), and chow (n = 7). The EtOH group received increasing doses of ethanol (8–14 g/kg/day) over a 4-week period. The EtOH levels were maintained at 14 g/kg/day for the remainder of the 75-day study. At the time of sacrifice, tibiae were dissected as described previously (Aronson et al., 1997) and frozen for three-point bending analyses (Aronson et al., 2001b).

**Study 2.** To examine the effect of short-term EtOH on volumetric bone mineral density (BMD) and cross-sectional area in the mature rat tibia, nine male Sprague-Dawley rats were randomly assigned to control (n = 5) and EtOH (n = 4) groups. Both groups were maintained on their respective diets for 25 days. Rats in the EtOH group received increasing doses of ethanol up to 10 g/kg/day. At sacrifice, left tibiae were harvested as in study 1 and stored in 10% NBF. The excised tibiae were scanned by peripheral quantitative computerized tomography (pQCT).

**Study 3.** Two studies were designed to test the effects of short-term EtOH on bone formation via DO. The first of these used 20 male Sprague-Dawley rats that were randomly divided into two groups of equal numbers (control and EtOH). External fixators were placed on the left tibia at the time of intragastric cannulation. At this time the tibiae were not fractured. The EtOH group received increasing doses of EtOH (8–10.5 g/kg/day) over an 11-day period before undergoing left tibial fractures. UECs were measured daily (Badger et al., 1993a). Dietary infusion was resumed no sooner than 2 h after recovery from anesthesia. To mimic the adult clinical situation, distraction began 6 days after surgery (6-day latency) and continued for 14 days at 0.2 mm b.i.d. During the latency and distraction periods, ethanol levels were increased incrementally from 10.5 to 12 g/kg/day.

**Three-Point Bending Procedures.** For three-point bending analyses, tibiae from study 1 were tested to failure in the mid-sagittal plane with a Bionix 858 materials testing machine (MTS Systems, Eden Prairie, MN), operated at a constant rate of 0.25 mm/s. Tibiae were centered on two lower roller points (spanning 26 mm) with the anterior aspect facing down. The load was applied from above, transverse to the long axis of the bone, by a single (third) roller contacting the posterior surface midway between the two lower supports. The roller points in this system contact the bone over a space of 2 mm. Previous results obtained using this method demonstrated small variances associated with this technique, and in fact the variances were small enough to allow the use of n = 3 with appropriate statistical power (Aronson et al., 2001b). Load to failure was recorded and bending moment calculated.

**pQCT Analysis.** The excised tibiae from study 2 were scanned by a pQCT X-ray machine (XCT Research SA, Norland Medical Systems, Fort Atkins, WI) with software version 5.40. A 0.26-mm-thick cross section of the tibia was taken 6 mm distal to the proximal end with a voxel size of 0.10 mm. Volumetric density, cross-sectional area, and mineral content (density × area) were determined for trabecular and cortical bone (Ke et al., 2001). A threshold of 570 mg/cm³ was used to distinguish cortical bone and a threshold of 214 mg/cm³ was used to distinguish cancellous bone throughout the experiment. Using these threshold settings, we determined that the ex vivo precision of volumetric content, density, and area of total bone, trabecular, and cortical regions ranged from 0.99 to 3.48% with repositioning.

**Single Beam Radiography and Analysis by Video Microscopy.** As previously described, a Xerox Micro 50 closed system radiography unit (Xerox, Pasadena, CA) was used at 40 kV (3 mA) for 20 s with Kodak X-OMAT film to radiograph the specimens (Aronson et al., 2001a). Comparison of distraction gap radiodensities was made by videomicroscopy using Media Grabber 2.0 video capture board (Raster Ops, Santa Clara, CA) and NIH Image Analysis 1.49 (NIH, Bethesda, MD; http://rsb.info.nih.gov/nih-image/index.html). The measured distraction gap area was outlined from the outside corners of the two proximal and the two distal cortices forming a quadrilateral region of interest. Mean pixel density of the defined distraction gap was measured using NIH Image on an arbitrary 256-point scale. Gaps were also measured to ensure that all were of comparable length.

Measurement techniques were assessed for aging and fixation artifacts to understand and possibly control for limitations in the methods (Aronson et al., 2001a). The distraction gaps were measured to account for soft tissue shrinkage. The method used for gap analysis in these studies entailed removal of the bone segment from the external fixator for high-resolution radiography without magnification and to allow for orthogonal radiographic views. In specimens with incomplete bony bridging, as seen in all the rats during active distraction, the soft tissue segments of the gap retracted equally after fixator removal. As previously documented, this reduced the overall gap area but not the mineral density or the bone matrix structures (Aronson et al., 2001a).

**Histology.** After radiography, the distracted tibiae were decalcified in 5% formic acid. The specimens were paraffin-embedded and cut into 5- to 7-μm longitudinal (coronal) sections on a microtome (Leitz 1512; Leitz, Wetzlar, Germany) for staining (hematoxylin and eosin) (Skinner and Nicholas, 1990; Skinner et al., 1997). The sections chosen for analysis were selected to represent a central or near
central gap location. This was accomplished by choosing slides that contained all four full-thickness cortices with intact marrow spaces on both the proximal and distal ends of the distraction gap. All organized osteoid/sinusoid columns were defined as new bone. We defined periosteal new bone as that outside of each cortex and endosteal new bone as that within the cortices (Aronson et al., 2001a). In several specimens, progressive sections were taken end to end from one peripheral cortex to the opposite side cortex in 50-μm increments to judge the reproducibility of our mid-coronal sampling technique.

Histologically, three zones of bone formation are defined within this distraction gap: 1) the fibrous interzone located in the middle of the gap and filled with fibroblastic cells within parallel collagen bundles; 2) the primary matrix front located on both borders of the fibrous interzone and where cell proliferation and new osteoid deposition occurs; and 3) the zone of microcolumn formation where the osteoblasts become embedded in parallel mineralized bone columns, which are often separated by new sinusoids (Aronson et al., 1997).

For quantitation of histology, the slides were recorded on video and analyzed using NIH Image Analysis 1.49 under low-power (1.25× objective) microscopic magnification. Again, the distraction gap area was outlined from the outside corners of the two proximal and the two distal cortices forming a quadrilateral region of interest, and the area of that region (gap area) was recorded. Both the proximal and distal endosteal new bone matrix, which is easily distinguished from the central fibrous tissue at the primary matrix front, was outlined and the area recorded (bone matrix area). The percentage of new bone area within the distraction gap (percentage of new bone) was calculated by dividing bone matrix area by gap area. These procedures were performed as described previously (Aronson et al., 1997; Skinner et al., 1997).

**Statistical Methods.** The results of study 1 were analyzed by one-way analysis of variance using the Tukey post hoc test. Analysis of data from studies 2, 3, and 4 were performed using the Student’s t test, with the exception of cortical mineral content and trabecular area from study 2. The data for cortical mineral content failed the equal variance test and data for trabecular area failed normality. Therefore, the Mann-Whitney rank sum test was used to analyze these data sets. Differences in mean values between groups were considered significant if p < 0.05.

**Results**

In all studies the rats showed a average weight loss of 13 g over the first 3 days after placement of the intragastric cannulae. After this initial weight loss all animals consistently gained weight over the remainder of each study, although weight gains were slightly slowed in all groups in studies 3 and 4 for the first 3 days after the DO surgical procedure. In all four studies, weight gains for control rats were slightly faster than either EtOH or ad libitum chow, but these differences were well within the previously published and accepted range for the TEN system (Badger et al., 1993c). Furthermore, growth curves for the EtOH group were similar to the standard curves published by Harlan. Although ethanol was infused at a constant rate, the UECs, which directly correlate to blood alcohol concentration (Badger et al., 1993b), of all EtOH rats varied with a pulsatile pattern from near 0 to 550 mg/dl over a 6-day cycle as established previously (Badger et al., 1993a,b).

**Study 1: 75-day Ethanol Exposure.** The comparison of three-point bending strength values (demonstrating extrinsic whole bone strength) for the left tibiae taken at sacrifice revealed significant differences, as determined by one-way ANOVA and Tukey post hoc test, in mean load to failure for control (n = 4) versus EtOH (n = 5) (a, p = 0.0006) and chow (n = 7) versus EtOH (b, p = 0.0029) groups. The results are expressed as mean ± S.E.M.

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Ethanol</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabecular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volumetric density (mg/cm³)</td>
<td>125.0 ± 5.1</td>
<td>94.5 ± 7.7</td>
<td>0.011</td>
</tr>
<tr>
<td>Area (cm²)</td>
<td>8.17 (7.72, 8.30)</td>
<td>7.65 (6.92, 7.93)</td>
<td>0.286</td>
</tr>
<tr>
<td>Mineral content (mg/cm)</td>
<td>996.1 ± 61.6</td>
<td>703.7 ± 75.3</td>
<td>0.019</td>
</tr>
<tr>
<td>Cortical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volumetric density (mg/cm³)</td>
<td>1066.8 ± 10.8</td>
<td>1041.9 ± 18.5</td>
<td>0.260</td>
</tr>
<tr>
<td>Area (cm²)</td>
<td>6.50 ± 0.18</td>
<td>5.72 ± 0.11</td>
<td>0.011</td>
</tr>
<tr>
<td>Mineral content (mg/cm)</td>
<td>6891.9 (6583.9, 7376.9)</td>
<td>5949.0 (5884.3, 6070.3)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Fig. 1. Three-point bending analysis of tibial strength. Comparison of three-point bending strength values for the left tibiae taken at sacrifice revealed significant differences, as determined by one-way ANOVA and Tukey post hoc test, in mean load to failure for control (n = 4) versus EtOH (a, p = 0.0006) and chow (n = 7) versus EtOH (b, p = 0.0029) groups. The results are expressed as mean ± S.E.M.
Studies 3/4: 30-Day Ethanol Exposure. A comparison of distracted tibial radiographs from study 3 revealed that the mean radiodensity of the distraction gap in ethanol infused rats was significantly lower than that in control animals (control = 72.49 ± 6.97; EtOH = 49.53 ± 6.48; p = 0.03; Fig. 2A). Histological analyses of tibiae from both study 3 (control = 56 ± 6%; EtOH = 32 ± 7%; p ≤ 0.02) and study 4 (control = 57.7 ± 2.7%; EtOH = 39 ± 3.5%; p = 0.001) verified inhibition of new bone formation (measured as a percentage of the distraction gap) in the ethanol-exposed rats (Fig. 2B). Representative histological sections from study 3 are shown in Fig. 3.

Discussion

This is the first report using intragastric dietary ethanol delivery to 1) determine and measure chronic ethanol’s negative systemic effects on the rat skeleton and 2) demonstrate chronic ethanol’s effects on local intramembranous bone formation during DO. Studies have shown that alcohol abuse is correlated with osteoporosis, decreased bone mass, and an increased risk of fracture both clinically and in other animal models, but these studies were limited in their ability to control for nutritional variables. In addition, the mechanisms by which alcohol abuse increases the risk of osteoporosis and fracture and inhibits repair remain unclear, and some studies indicate that nutrition may be a confounding factor (Weinberg et al., 1990; Bourrin et al., 2000; Banu et al., 2001). The rat TEN model used herein allows one to study the effects of ethanol on skeletal dynamics in the context of complete nutrition, thereby isolating ethanol’s effects from those of restricted nutrition.

The results of study 1 show that chronic ethanol exposure dramatically decreases the strength of the intact mature tibia over a 75-day exposure period. Such results are consistent with other studies using the traditional ad libitum/pair-fed alcohol diets in male rats (Peng et al., 1988; Wezeman et
al., 1999). The technique used herein tests only extrinsic (whole bone) properties, thus it cannot be determined from these data whether these effects were due to differences in tissue quality or cross-sectional geometry. However, a number of studies have found ethanol-related deficiencies in various geometric and/or intrinsic properties in both male and female rats (Peng et al., 1988; Hogan et al., 1999; Wezeman et al., 1999; Hogan et al., 2001). Therefore, a more detailed study of bone mechanical properties using the TEN system is needed to determine whether the difference in whole bone strength seen herein is due to the same underlying changes in geometric and/or intrinsic properties observed in the Lieber-DeCarli system.

The results of study 2 show that the reduction of ethanol exposure to 25 days produces significant decreases in the volumetric BMD of trabeculae and the cross-sectional area of the cortex in the tibial metaphysis. Interestingly, these results suggest the possibility of differential effects on trabecular and cortical bone. The presence of significantly lower trabecular volumetric density without a significant ethanol-related difference in trabecular area might suggest that alcohol consumption affects the quality of the trabeculae without significantly lowering number or thickness. In contrast, the surrounding cortex displayed an ethanol-related decline in cross-sectional area without a corresponding change in volumetric BMD. This is in agreement with other studies that examined cortical width (Dyer et al., 1998; Sampson, 1998; Hogan et al., 1999; Wezeman et al., 1999). However, this is in contrast to histomorphometric studies that have demonstrated lower trabecular volume, width, and connectivity in alcohol-consuming animals (Sampson et al., 1997; Sampson, 1998; Wezeman et al., 1999; Dai et al., 2000; Turner, 2000; Hogan et al., 2001). This apparent discrepancy may be due to the resolution limits of the pQCT, which is unable to discern individual trabeculae (volume averaging), and/or to the reduced time of ethanol exposure in study 2. It should be noted that analysis of bone mass by densitometry (pQCT) correlates closely to histomorphometry, although densitometry is not as subject to sampling error (Roggia et al., 2001). Therefore, a more definitive histomorphometric or micro CT analysis might be useful in determining the exact effects that intrauterine ethanol infusion has on the trabeculae. Finally, the data on cortical area suggest that the lower tibial load to failure in ethanol-infused rats seen in study 1 was due, at least in part, to changes in the geometric properties of the cortex, which has been observed in studies using the traditional pair-fed protocol (Hogan et al., 1999, 2001).

Taken together, the results of studies 1 and 2 suggest that chronic ethanol consumption has dramatic deleterious effects on the skeleton in the context of full nutritional support.

The results of studies 3 and 4 demonstrate that chronic ethanol exposure decreases the amount of regenerate bone formed during the distraction period of DO with either a 1- or 6-day latency, the latter being common in adult clinical protocols. To our knowledge, this is the first report to examine the effects of ethanol on DO. This indicates that ethanol exposure inhibits rapid intramembranous bone formation by osteoblasts in the DO/TEN model. These results are consistent with clinical studies that demonstrated alcohol-related delays in fracture healing (Nyquist et al., 1998), as well as both in vitro and in vivo studies that demonstrated ethanol’s ability to inhibit osteoblast proliferation and function (Sampson, 1998; Wezeman et al., 1999; Maran et al., 2001). Furthermore, the inhibition of bone formation seen here suggests that the decreases in bone strength and trabecular bone volume, seen in studies 1 and 2, may result from the inhibition of osteoblast activity either with or without the aid of increased osteoclast activity (Dai et al., 2000). During the distraction phase of DO, the rat model provides large areas of regenerate bone formation characterized by specific stages of osteoblastogenesis and spatially separating processes of bone formation and resorption (Aronson et al., 1997). Therefore, the combination of the DO and TEN models may facilitate studies examining the effects of chronic ethanol exposure throughout the time course of bone formation and resorption.

It should be noted that for the DO studies, the ethanol was given before and during the distraction period. Future studies will be needed to determine the relative impact of ethanol exposure before and/or concomitant with DO.

It has been reported that ethanol exposure may also have deleterious effects on the vasculature and disrupt angiogenesis (Kraszpulski et al., 2000). Because angiogenesis is coupled with and necessary for rapid bone formation during DO, we speculate that alcohol consumption may have an indirect effect on bone formation in DO through the inhibition of angiogenesis (Aronson, 1994b; Rowe et al., 1999). Although a body of existing data suggests that ethanol has direct effects on osteoblast function, future studies should be designed to address the potential of angiogenic delays.

Collectively, these results demonstrate that chronic ethanol exposure, using the TEN model, decreases tibial bending strength, trabecular volumetric BMD, and cortical cross-sectional area, as well as inhibits rapid bone formation during DO. These observations are consistent with the results of numerous clinical and animal studies using traditional models. This suggests that the TEN system is a viable model to study ethanol’s effects. An advantage of the TEN model is that it could be used to test possible interactions between nutrition and ethanol. This model may also help test preclinical intervention protocols and facilitate the cellular and molecular studies necessary to elucidate the effects of alcohol on the skeleton. The use of intrauterine infusion can overcome limitations associated with normal feeding patterns (which may not accurately reflect the drinking pattern in certain situations) and alcohol aversion (which limits consumption of both alcohol and calories) in traditional pair-fed liquid diet protocols. In fact, a recent review noted a need for models that better replicate the drinking patterns of moderate versus binge drinking adolescents and adults (Turner, 2000). The results from future studies using this model should provide information on the basic biological mechanisms of both ethanol’s effects on osteogenesis in vivo and ethanol’s contributions to deleterious changes in bone density, tissue composition, and geometry associated with osteoporosis.

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References


Address correspondence to: Dr. C. K. Lumpkin Jr., Arkansas Children’s Hospital Research Institute, Slot 512-20B, 1120 Marshall St., Little Rock, AR 72202. E-mail: lumpkinc@uams.edu