EFFECTS OF ALCOHOL ON BONE MINERAL AND MECHANICAL PROPERTIES OF BONE IN MALE RATS

FREDRIK NYQUIST*, HENRIK DÜPPE1, KARL J. OBRANT1, LENNART BONDESON2 and LARS NORDSLETten3

Departments of 1Orthopaedics and 2Pathology, Malmö University Hospital, SE-205 02 Malmö, Sweden and 3Surgical Research, Rikshospitalet, The National Hospital, University of Oslo, Norway

(Received 16 February 2001; in revised form 20 June 2001; accepted 30 July 2001)

Abstract — The effect of ethanol on bone mineral is poorly understood. In this study we have investigated whether ethanol affects bone mineral content (BMC), bone mineral density (BMD) measured by dual energy X-ray absorptiometry, and the mechanical properties of the tibia and femora in male Sprague–Dawley rats without histopathological signs of liver disease or nutritional deficit. Thirty-five male rats were fed a liquid diet containing 15% ethanol and glucose. An equivalent iso-volumetric amount of glucose-containing liquid was fed to the controls (n = 35). After an initial difference in weight, we found no difference in weight gain from week 1 to week 6. All animals were killed at 6 weeks. We found no evidence of ethanol-induced liver disease in a histopathological evaluation. The BMD and BMC were found to be lower in the ethanol group. No differences between the groups were found in the mechanical properties or in the length and size of the femora. We suggest that alcohol may have a toxic effect on bone in male rats known not to suffer from any histopathological hepatic lesions.

INTRODUCTION

The cause of osteoporosis is a multifactorial entity in which alcohol consumption is known to play a part (Rico, 1990; Moniz, 1994). The concept of how alcohol affects bone metabolism is still not fully elucidated. Earlier studies have shown that, in animals, a chronic alcohol intake can cause defects in mineralization (Saville, 1965; Turner et al., 1987, 1988). In rats treated with alcohol, Peng et al. (1982, 1988, 1991) found a decreased bone strength. Kusy et al. (1989) also found that the femora of rats fed an alcohol liquid for 4 weeks not only was weaker, but also could absorb less energy. In the above-mentioned studies, and in other published rat model studies, the histopathological absence of liver disease has not fully been evaluated, and thus the direct effect of alcohol on bone mineral and bone strength could have been overestimated. The purpose of the present study was therefore to assess, in an in vivo model, whether ethanol has any short-term effects on bone mineral, measured by dual energy X-ray absorptiometry (DEXA), or the mechanical properties of bone in male rats.

MATERIALS AND METHODS

Animals

Seventy, 70-day-old, male Sprague–Dawley rats (Møllegard, Copenhagen, Denmark) with a mean weight of 389 g (range 355–425) were randomly divided into two groups. One group (n = 35) were fed ad libitum a liquid containing ethanol and glucose and the other group (n = 35) were fed a liquid containing only glucose. Both groups had free access to laboratory pellets (R3; Lactamin, Västena, Sweden) (1.1% calcium, 0.8% phosphorus; 315 kcal/100 g, each pellet 3 g). The amount of consumed pellets was determined and recorded each day.

The weight of each animal was recorded at the start, after 8 days and at the end of the experiment. The animals were housed alone in wire-topped metal cages (420 × 260 mm wide, 150 mm high) in a room with a 12-h light/12-h dark cycle, with a relative humidity of 50–60%. The total study time was 6 weeks. The animals’ nocturnal activity was observed and recorded by the laboratory technicians. The daily amount of liquid consumed by each animal was recorded. The experiments conformed to the Swedish Council of Animal Research code for the care and use of animals for experimental purposes.

Ethanol administration

Initially the group of animals being given ethanol was fed an ethanol–glucose liquid of 5% (v/v) ethanol and 260 g glucose in 890 ml water. The amount of ethanol was successively increased to 10% and finally to 15% on day 8. The same amount of alcohol (%) has previously been used in several studies (Jänicke-Lorenz and Lorenz, 1984; Pierce and Perry, 1991). The serum ethanol level was measured once a week throughout the study period. A liquid containing an equivalent iso-volumetric amount of glucose was fed to the controls. The volume of the liquid as well as the amount of ethanol (g) consumed by each animal each day was recorded.

Bone measurements

At our bone metabolic research laboratory the total bone mineral area density (BMD), expressed in g/cm², total bone content (BMC) expressed as gram (g) and the total bone calcium (g) of each animal were measured by the dual-energy X-ray absorptiometry technique after the animals were killed, using the Lunar® DPX small animal software, version 1.0 D. The total body content was measured. The precision was calculated to <2%. The measurements were also corrected for weight differences that existed between the groups.

Liver histopathology

The animals were killed after 6 weeks and livers from all animals were then carefully excised, after the DEXA measurement, and their weights recorded. The liver was frozen, sectioned and stained with Oil Red. A histopathological examination was then performed at the Department of Pathology, Malmö
University Hospital, Malmö, Sweden. The investigator looked for an increase in the amount of fat, increased inflammatory activity and fibrosis. This was a ‘blind’ study, as the investigator did not know which group of animals the liver had been taken from.

**Mechanical testing**

After the DEXA measurement, the right femora and tibia were excised and the size and length of the femora were measured, from the trochanteric region to the lateral femoral condyle, with a micrometer. This was done to detect any differences in bone growth during the study period. The tibia and femora were then stored at −20°C until mechanical testing. Before the mechanical testing, the bones were thawed and cleared of soft tissues.

The femoral shaft and the neck were fractured in a hydraulic testing device using a loading rate of 0.095 radians/s. First, the shaft was fractured 19 mm above the knee joint in a three-point ventral bending test, the fulcrum being the centre of rotation in the test system. Thereafter the necks were fractured in a combined bending and compression test. The tibia was then tested in three-point ventral bending (Nordsletten and Ekeland, 1993; Nordsletten et al., 1994).

Load–deflection curves were recorded on line in Work Bench Mac Software (Strawberry Tree Incorporated, Sunnyvale, CA, USA). Ultimate bending moment, energy absorption, stiffness and deflection at fracture were read out directly or calculated from the computer readings. The ultimate bending moment was taken as the product of the ultimate load and the moment-arm. Energy absorption was the area under the load–deflection curve. Bending stiffness was defined as the slope of the linear elastic part of the curve, and was read directly from the computer. Deflection was the distance on the x-axis from the point of intersection of the linear portion of the load–deflection curve to the point of failure. The term strength in relation to the results was defined according to Burstein et al. (1971). The coefficient of variation (CV) of the apparatus, for testing steel rods to 45° deflection, is ~1%. The precision is therefore high but the CV in the present study (resected rat bones) was estimated to be 15%. This is mainly due to the biological variation in the bone (Nordsletten and Ekeland, 1993).

**Statistics**

All data are presented as means ± SD. Statistical analyses were done using the Macintosh Statistica software. The data followed a normal distribution and the unpaired Student’s t-test and multiple analysis of covariance (MANCOVA) were used for detecting between-group differences. A significance level of $P < 0.05$ was adopted.

**RESULTS**

The results in Table 1 show that, in spite of the randomization into two groups, there was a difference in initial weight between the groups. After an initial difference in weight gain over the first week, there was no significant difference in weight gain after the 6 week experimental period. No significant differences in the amount of pellets consumed were noted between the groups (average daily consumption of 20 g pellets/day in each group).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ethanol group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD (g/cm²)</td>
<td>0.315 ± 0.006*</td>
<td>0.321 ± 0.006</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>11.2 ± 0.6b</td>
<td>11.9 ± 0.7</td>
</tr>
<tr>
<td>Calcium (g)</td>
<td>4.2 ± 0.2c</td>
<td>4.5 ± 0.3</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

*Lower BMD in the ethanol-fed rats ($P < 0.001$); b lower BMC in the ethanol-fed rats ($P < 0.001$); c lower total calcium in the ethanol-fed rats ($P < 0.001$).
ALCOHOL, BONE AND RATS

Table 3. Mechanical results at failure of the right tibia, right femoral shaft and neck in male rats fed an ethanol-containing diet for 6 weeks and their controls

<table>
<thead>
<tr>
<th></th>
<th>Bending moment (Nm x 10^-2)</th>
<th>Energy absorption (J x 10^-3)</th>
<th>Stiffness (Nm/° x 10^-3)</th>
<th>Deflection (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Control</td>
<td>Ethanol</td>
<td>Control</td>
</tr>
<tr>
<td>Right tibia</td>
<td>51.8 ± 9.1</td>
<td>50.7 ± 9.6</td>
<td>7.1 ± 1.9</td>
<td>6.1 ± 2.1</td>
</tr>
<tr>
<td>Right femoral shaft</td>
<td>79.0 ± 12.4</td>
<td>75.9 ± 15.3</td>
<td>11.3 ± 2.9</td>
<td>10.5 ± 3.0</td>
</tr>
<tr>
<td>Right femoral neck</td>
<td>90.2 ± 22.7</td>
<td>88.4 ± 26.0</td>
<td>26.7 ± 7.9</td>
<td>25.9 ± 9.6</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

DISCUSSION

The dose-dependency of alcohol-induced derangements of bone and mineral metabolism is unknown. In male chronic alcoholics, a daily consumption of ethanol exceeding 120 g/day (corresponding to 1.7 g/kg/day) has been reported to have a negative impact on bone mineral density and biochemical markers of bone metabolism (Saville, 1965; Laitinen and Välimäki, 1991; Nyquist et al., 1996). It has also been shown by Laitinen et al. (1991) that prolonged moderate ethanol intake of 60 g/day impairs osteoblastic function, leading to lowered serum levels of osteocalcin. It is not always possible to extrapolate results from human studies to animals: in this study among male rats, the daily consumption of ethanol was 3.4 ± 0.2 g/day (corresponding to 7.9 g/kg/day). This amount of ethanol exceeds by far the ingested amount of ethanol consumed by the alcoholics in the studies mentioned above and is at the same level as previous animal studies (Jänicke-Lorenz and Lorenz, 1984; Pierce and Perry, 1991). The aim of the present study was to investigate if ethanol would have any effects on BMD and bone strength. We were interested in a possible direct effect on bone tissue of high intake of ethanol for a short period of time, which is why we chose a 15% ethanol concentration in a liquid diet.

Additionally, confounding variables, such as dietary deficiencies and liver damage, are known to interfere with bone metabolism. Thus, in previous studies (Lalor et al., 1986; Diamond et al., 1989; Sampson et al., 1996; Hogan et al., 1997) the impact of alcohol itself may have been over-estimated. In this study, we looked at the short-term effects of high ethanol doses over a time span which is sub-optimal for high ethanol doses over a time span which is sub-optimal for everyday life (Nordsletten and Ekeland, 1993). In dissected bones, bending moment is considered the most appropriate measure of bone strength (Hayes, 1991). Neither of these entities showed any alteration in this study. There was a slight, but not significant, decrease in bending stiffness among rats fed an ethanol liquid. Our findings in this study are consistent with the consensus in the literature on ethanol-induced osteopenia (Bikle et al., 1985; Klein, 1997; Nyquist et al., 1999), but it appears that inhibition of new bone formation during bone remodelling was less affected than the inhibition that occurs during bone healing (Nyquist et al., 1999), and therefore the mechanical properties of bone were accordingly less affected. Another explanation could be that bone metabolism in Sprague–Dawley male rats is more resistant to ethanol than earlier suggested by Hogan et al. (1997) and Sampson et al. (1997) in studies performed on female Sprague–Dawley rats. To elucidate this, further studies are needed.

The ideal situation would have been to have a pretreatment bone mass value; this would have even further strengthened our suggestion that ethanol has a toxic effect on bone. However, in this study, we were unable to measure the pretreatment bone mass value, since DEXA measurements, using the Lunar® DPX small animal software, is a time-consuming procedure (>60 min) and therefore the animals have to be anaesthetized during the whole measurement. To anaesthetize the animals for such a long period of time would certainly have caused some death in both groups and there would also have been a potential risk for hepatic lesions.

The influence of ethanol on bone was, presently, studied with DEXA measurement and biomechanical testing. In the DEXA measurements, we found a decrease in BMD and
BMC, but were unable to detect any negative impact of ethanol on the biomechanical characteristics of male rat femora and tibiae. The DEXA technique is a more sensitive measure, with a lower degree of variation, so that small changes in bone quality are easier to detect with such a technique. Perhaps the differences in the mechanical properties of bone would have been more evident in a study performed over a longer period of time.

In the present study, we found ethanol to have a negative impact on bone mineral content, but the mechanical properties were not influenced. Although it is not always possible to extrapolate data from animal studies, our data could possibly suggest that the direct toxic effect of ethanol accounts to some extent for the well-known increased risk of fractures in alcoholics. Other side-effects of ethanol, such as repetitive trauma associated with drunkenness, malnutrition and liver disease may be the dominant cause for the high fracture incidence among abusers.

Acknowledgements — Financial support was obtained from the Swedish Medical Research Council, the Lund University Research Funds, the Swedish Council for Planning and Coordination of Research, and the Greta and Johan Kock Foundation.

REFERENCES


