Ethanol and its effects on fracture healing and bone mass in male rats

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Operatively induced, standardized tibia fractures in 42 10-week-old male rats were fixed with intramedullary nails. 21 of the rats were fed liquid containing 15% ethanol. 5 weeks after inducing the fracture, the rats were killed and the total body bone mineral density (BMD) was analyzed with the DEXA technique, and the mechanical properties of the fractured and the unfractured tibiae as well as the ipsi- and contralateral femoral shaft and femoral neck were tested. The rats given a liquid containing 15% ethanol were found to have significantly lower total BMD and total calcium than the controls. We also found a significantly lower bending moment and bending stiffness both in the fractured and unfractured tibiae among rats fed on ethanol. The energy absorption until refracture was less in rats fed on ethanol.

Posttraumatic osteopenia was present, as judged by the mechanical tests of the ipsilateral femoral shaft and the femoral neck in all animals. There was no difference in this respect between the animals fed on ethanol and the controls.

We found that ethanol disturbs bone metabolism which reduces the mechanical properties of the tibiae and femora of rats, but the healing process of an induced tibial shaft fracture was not affected.

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The frequency of fractures is higher among alcoholics (Kristensson et al. 1980, Johnell et al. 1985, Crilly et al. 1988). Alcoholics are also more prone to osteoporosis than non-abusers (Nyquist et al. 1995). The pathogenesis of the osteoporosis among chronic alcoholics remains unclear. Earlier studies in rats treated chronically with ethanol have shown a decrease in bone strength—but the fracture repair process has scarcely been addressed (Jänicke-Lorenz and Lorenz 1984, Kusy et al. 1989, Pierce and Perry 1991).

In this study, we assessed whether ethanol has affects bone mass and fracture healing in male rats.

Animals and methods

Animals

42 70-day-old, male Sprague-Dawley rats (Möllegard, Copenhagen) with a mean weight of 378 (355–395) g were randomly divided into two groups. One group (n 21) was fed ad libitum a liquid containing ethanol and glucose and the other group (n 21) was fed a liquid containing glucose. Both groups had free access to laboratory pellets (R3, Lactamin, Vadstena, Sweden). The weight of each animal was recorded at the start, after 8 days and at the end of the experiment. They were housed alone in wire-topped metal cages (420 × 460 mm wide, 150 mm high) in a room with a light-dark cycle of 12:12 h. The humidity in the laboratory was 50–60%. The total study time was 6 weeks. Approval for this study was given by the Swedish Council on Animal Research.

Ethanol

Initially the animals given ethanol were fed an ethanol-glucose liquid of 5% 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. This amount was previously used in the fracture study by Jänicke-Lorenz and Lorenz (1984) and, in a pilot study, was found to give a serum ethanol level of approximately 1‰. A liquid containing an equivalent iso-volumetric amount of glucose was fed to the controls. The volume of liquid and the amount of ethanol (g) consumed by each animal each day was recorded.

Fractures

On day 8 all animals were anesthetized intraperitoneally with chloral hydrate 36 mg/mL–1 mL/100 g body weight (b.w.).

The right tibia in all animals was operatively fractured, under aseptic surgical conditions, according to the method described by Nordsletten et al. (1992, 1994). The animals were operated on according to a blind protocol—i.e., without knowing to which group, ethanol or control, the animals belonged. The lower leg was shaved, and a longitudinal incision was made parallel to the anterior margin of the tibia. A 25 G mandrin was inserted into the medullary canal of the tibia, through the distal patellar ligament and through the proximal tibial crest.

With the mandrin inserted, the tibia was transversely fractured. An 18 G cannula was advanced over the 25 G mandrin until it was jammed in the distal fragment. The cannula was cut flush to the bone with pliers and the wound closed in two layers. All fractures were stable at the end of the operation, and rotation was checked by comparing the alignment of the foot and the thigh.

Postoperatively, the animals were given buprenorphine 0.2 mg/kg b.w. at 12-hour intervals for 2 days. The rats resumed weight bearing within a few days. None developed infection.

1 animal from the ethanol group died during anesthesia at fracture induction. 1 animal in the control group developed a malalignment of the fracture due to insufficient stability of the intramedullary nails on day 7 after fracture induction—this animal was killed.

Bone metabolism

After 6 weeks, the animals were killed. The total body bone mineral density (BMD) (g/cm^2) and total bone calcium (g) were measured by the DEXA technique, using Lunar® DPX small animal software, version 1.0 D. The precision error was calculated at < 2%.

Mechanical testing

After the DEXA measurements, both femora and tibiae were excised and the length, from the trochanteric region to the lateral femoral condyle and from the tibial condyle to the plantar part of the paw, was measured with a micrometer. The tibiae and the femora were stored at –20 °C until mechanical tests could be done. Prior to these tests, the bones were thawed and all soft tissue was removed from the tibiae and femora. Care was taken to leave the callus intact. Intramedullary nails were removed. All soft tissue was removed from the tibiae and femora.

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 center of rotation in the test system. Thereafter the necks were fractured in a combined bending and compression test. Both tibiae were then tested by three-point ventral bending (Nordsletten and Ekeland 1993, Nordsletten et al. 1994).

Table 1. Body weight, weight increase, liquid consumption and the length of the left and right femora and tibiae in rats fed ethanol and their controls, during a 6-week fracture healing study. Mean (SD)

	Ethanol group	Controls
Initial weight, g	375 (10) [a]	380 (6)
Weight after 1 week, g	382 (6) [b]	398 (15)
Weight increase during the first week, g	7 (8) [c]	18 (13)
Weight increase week 1–6, g	58 (16)	68 (18)
Total liquid consumption, mL 0–6 weeks	1120 (75) [b]	1945 (237)
Femoral length, mm		
right	39 (0.5)	39 (0.5)
left	39 (0.5)	39 (1)
Tibial length, mm		
right	45 (1)	44 (1.5)
left	45 (0.5)	45 (0.5)

[a] $p = 0.04$
[b] $p < 0.001$
[c] $p = 0.002$

Load/deflection curves were recorded on line in Work Bench Mac Software (Strawberry Tree Incorporated, Synnyvale, USA). Ultimate bending moment, energy absorption, stiffness and deflection at fracture were read out directly or calculated from the computer readings. Ultimate bending moment was taken as the product of the ultimate load and the moment-arm. Energy absorption was represented by 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 words "strength in relation to the results" were defined according to Burstein et al. (1971).

Statistics

All data are presented as mean (SD) and the data were tested for normality of distribution.The unpaired Students's t-test and Mancova analysis were used for detecting between-group differences. The results of the ipsi- and contralateral femoral shaft, femoral neck and tibial shaft were compared with the paired Students's t-test. $P < 0.05$ was considered significant.

Results

In spite of the randomization, there was a difference in initial weight between the two groups (Table 1).

Table 2. Total body bone mineral density (BMD) (g/cm^2) and total calcium (g) in rats fed a diet containing ethanol for 6 weeks and their controls. Mean (SD)

	Ethanol group	Control
BMD	0.315 (0.006) [a]	0.321 (0.004)
Calcium	4.2 (0.2) [a]	4.5 (0.3)

[a] $p < 0.001$

After 1 week (0–1 week), an initial difference in weight gain between the two groups was noted. The animals fed on ethanol had a smaller weight gain. There was no difference in weight increase from fracture induction to the end of study (1–6 weeks).

The animals fed on ethanol consumed a mean total of ethanol of 128 (115–143) g. The total consumption of liquid was less in the group on the ethanol diet (Table 1). The animals in the two groups showed no difference in the way they moved about or in physical activity. The lengths of the left and right tibiae and femora were similar (Table 1).

The BMD and total calcium content in bone were significantly lower in the ethanol group both before and after correction for weight differences (Table 2).

Bending moment and bending stiffness were significantly lower both in the fractured and unfractured tibiae in the rats on the ethanol diet (Table 3). The energy absorption until refracture was less in the rats fed on ethanol.

When evaluating the posttraumatic mechanical effects of the tibial shaft fracture all animals were considered as one group and the mechanical readings from the ipsi- and contralateral femoral neck, femoral shaft and tibial shaft were analyzed with a paired Students's t-test (Table 3). We found significantly "better" mechanical readings for the femoral neck and the femoral shaft (bending moment, energy absorption, bending stiffness and deflection) in the unfractured limb, more pronounced in the neck region.

The same type of calculation was done groupwise, for the ethanol group and the control group. The same pattern was found—with significantly "better" mechanical readings for the unfractured limb.

The ratios between the ipsi- and contralateral femoral neck, the femoral shaft and the tibial shaft were calculated and compared between the groups. There was no difference in the degree of posttraumatic osteopenia between the animals on the ethanol diet and the controls, as evaluated by mechanical tests.

Table 3. Mechanical results at failure of the fractured tibia, the contralateral unfractured tibia, the femoral shafts and necks ipsi- and contralateral to the healing tibia 5 weeks after fracture. Mean (SD). Differences between the ethanol group (n 20) and control group (n 20) are denoted by letters. Differences between the ipsi- and contralateral sides in the two groups are denoted by roman figures

	Bending moment (Nm × 10^{-2})		Energy absorption (J × 10^{-2})		Stiffness (Nm/° × 10^{-3})		Deflection (°)	
	Ethanol	Controls	Ethanol	Controls	Ethanol	Controls	Ethanol	Controls
Fractured right tibia	39 (11)	49 (15)[a]	5.3 (1.6)	7.5 (3.6)[c]	30 (7.2)	36 (6.4)[d]	14 (2.1)	14 (3.5)
Unfractured left tibia	40 (12)	47 (11)[b]	5.7 (3.2)	6.6 (2.4)	30 (8.2)	35 (6.0)[e]	14 (3.9)	14 (2.6)
Ipsilateral femoral shaft	61 (8.3)[I]	66 (16)[II]	8.8 (2.2)[V]	9.7 (3.5)	41 (3.4)	44 (6.3)[VIII]	16 (2.9)	15 (2.3)
Contralateral femoral shaft	74 (12)	77 (13)	12 (3.2)	12 (4.1)	44 (4.2)	48 (4.0)[f]	17 (2.4)	16 (2.9)
Ipsilateral femoral neck	59 (19)[III]	54 (19)[IV]	16 (6.8)[VI]	14 (5.8)[VII]	29 (7.8)	29 (11)	21 (4.6)[IX]	20 (3.6)[X]
Contralateral femoral neck	71 (24)	80 (22)	23 (8.8)	26 (8.9)	29 (8.1)	32 (8.4)	25 (5.7)	26 (5.2)

[a] Increased bending moment in controls p=0.03
[b] Increased bending moment in controls p=0.04
[c] Increased energy absorption in controls p=0.02
[d] Increased stiffness in controls p=0.02
[e] Increased stiffness in controls p=0.04
[f] Increased stiffness in controls p=0.003
[I] Lower bending moment in the ipsilateral femoral shaft in the ethanol group p<0.001
[II] Lower bending moment in the ipsilateral femoral shaft in controls p=0.01
[III] Lower bending moment in the ipsilateral femoral neck in the ethanol group p=0.04
[IV] Lower bending moment in the ipsilateral femoral neck in controls p<0.001
[V] Lower energy absorption in the ipsilateral femoral shaft in the ethanol group p=0.03
[VI] Lower energy absorption in the ipsilateral femoral neck in the ethanol group p=0.003
[VII] Lower energy absorption in the ipsilateral femoral neck in controls p<0.001
[VIII] Lower stiffness in the ipsilateral femoral shaft in controls p=0.005
[IX] Lower deflection in the ipsilateral femoral neck in the ethanol group p=0.02
[X] Lower deflection in the ipsilateral femoral neck in controls p<0.001

Discussion

Saville and Lieber (1965) noted that chronic alcoholics had a bone mass very similar to that in much older individuals or those who have osteoporosis. The dose-dependency of ethanol-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 and Lieber 1965, Laitinen and Välimäki 1991, Nyquist et al. 1996). In this study of male rats, the daily intake of ethanol was 3.1 (SD 0.2) g/day (corresponding to 7.2 g/kg/day). This amount of ethanol exceeds by far the ingested amount of ethanol consumed by alcoholics in the studies mentioned above. In the present study, we found that a 15% ethanol concentration in the liquid part of the diet caused a significant reduction in BMD, as measured by the DEXA technique, despite a normal weight gain. This indicates that ethanol may have a direct, toxic effect on bone metabolism.

In numerous previous studies, a correlation between osteoporosis and an increased risk of fractures has been found (Peris et al. 1995). It is also well known that chronic alcoholics have a high incidence of fractures (Kristensson et al. 1980, Johnell et al. 1985, Diamond et al. 1990). Disturbances in the healing process of fractures in chronic alcoholics have scarcely been addressed. It is difficult to make statements about the healing tendency in chronic alcoholics because they form a diverse group and their fractures vary greatly. In this paper we present mechanical data on the healing of experimental, standardized fractures in a homogeneous population, the male rat. The rat is the most frequently used animal for studies of fracture healing (Bonnarens and Einhorn 1984, Hou et al. 1991). Most of these experiments have been done on femora (Bonnarens and Einhorn 1984, Huo et al. 1991). The femora, however, are loaded horizontally in ambulation in the rat, which is in contrast to the vertical loading of the weight-bearing long bones in humans. The loading pattern strongly influences the orientation of collagen and minerals and thus the physiological and pathological response of the bone (Boyde and Riggs 1990). The tibia in rats is loaded vertically and, with the intramedullary nailing technique described by Nordsletten et al. (1992, 1994), the tibia can be secured in a perfect alignment after experimentally induced fractures. In our study we found no differences between the two groups in length of the fractured tibia. Load-bearing and/or activity also seem to be of importance when studying defects in bone mineralization after fracture (Saville and Lieber 1965, Turner et al. 1987, 1988, Preedy et al. 1991). Since, in the present study with the given amount of ethanol, there was no difference in the way the animals moved about in their cages, we believe that such confounders between the two groups can be ruled out and that the differences found are directly related to a toxic influence of ethanol on bone metabolism, but not on fracture healing.

This provides us with a fracture model to study the side-effects of ethanol abuse—it has not been done before. Previous reports show that osteopenia develops in the fractured bone and the bone adjacent to a fractured one, both in humans (Finsen et al. 1989) and in rats (Madsen et al. 1995). In humans, this osteopenia persists for a long time (Karlsson et al. 1993). In our study we have shown that the ipsilateral femoral shaft and neck (to the fractured tibia) are weaker than the corresponding contralateral femoral shaft and neck 5 weeks after the fracture. Ethanol had no further influence on the mechanical properties of the femoral shaft and neck. On the other hand, we found that ethanol influenced the mechanical properties both in the fractured and the unfractured tibia to the same extent. The reason for this is somewhat unclear. The normally occurring, posttraumatic bone loss is also associated with an increased metabolism in the bony tissue, leading to loss of cortical thickness (Wendeberg 1961, Nilsson 1970, Nilsson and Obrant 1983, Karlsson et al. 1993, Kirkeby et al. 1993). Both osteoblast and osteoclast activity increase in this situation (Wendeberg 1961, Obrant 1984). As we postulated before, ethanol may have a direct, toxic effect on bone metabolism and thus can account for an increased loss of cortical thickness in the tibia and a reduction in the mechanical properties bilaterally, but ethanol seems to have a limited influence on the healing properties.

In this study of male rats we found that ethanol in itself disturbs bone metabolism, resulting in reduced mechanical properties of long bones, but the healing process 5 weeks after an induced tibia shaft fracture was unaltered. Whether this is the case in humans is yet to be proven, but an earlier study by Nyquist et al. (1997) has indicated that this could be the case.

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