Effect of Long-Term Alcohol Administration on Bone Metabolism in Rats

Asako YAMAMOTO, Akiko SEKINO, Mikie TAJIMA, Van Chuyen NGUYEN and Ikuko EZAWA*

Department of Food and Nutrition, Japan Women's University, Bunkyo-ku, Tokyo 112, Japan
(Received December 9, 1996)

Summary The purpose of this study was to investigate the effects of different degrees of alcohol ingestion on bone strength and mineral density. Three different groups of growing female rats were administered different doses of an alcohol-water solution for a period of 6 months. These three groups were divided into: 1) the control group, which was only given water; 2) the moderate group, which was given 5% ethanol solution for only 2 h per day; and 3) the excess group, which was given only 5% ethanol solution for 163 days. This ethanol consumption induced no detrimental effect on biochemical parameters including liver function. The moderate group showed significantly higher (p<0.05) levels of proximal metaphysis as compared to the control group, while there was no difference between the excess group and the control group. Similarly, in comparison to the control group, the moderate group exhibited a significant increase (p<0.001) in bone mechanical strength, while the excess group showed either the same or decreased bone stiffness. These results indicate that alcohol intake has both beneficial and hindering effects on the skeleton, depending on the concentration and frequency of ethanol intake.

Key Words ethanol, bone mineral density, mechanical strength

Alcoholism is recognized as one risk factor for osteoporosis (1,2), which can result in a high incidence of fractures (3). The effects of alcohol on the increase of bone resorption and inhibition of bone formation have been observed in histologic studies (2-4) and in vitro studies (5,6).

It has been suggested that the etiology of bone disease is multifactorial, which may have induced a malnourishing effect due to caloric overload from alcohol (7). Intestinal calcium absorption is inhibited as well (8). The changes of calcitropic

*To whom correspondence should be addressed.

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMD, bone mineral density.
hormones such as vitamin D and parathyroid hormone, inferior liver function, alteration of gonadal function and hypocortisolism associated with alcohol intake may also produce chronic bone disorders (9).

On the other hand, several cross-sectional studies have recently reported that moderate social drinking may be associated with higher bone mineral density (10–13). In addition to those confounding factors, other covariates such as age, smoking, obesity and exercise obscure the protective effect of alcohol on bone mineral density. It has been found that few experimental studies administer lower concentrations of ethanol. The purpose of this study was to further investigate the association of social drinking and bone density by studying the long-term effects of different levels of ethanol consumption on bone mineral density and fragility in rats.

**MATERIALS AND METHODS**

*Experimental animals and protocols.* Fifteen female Wistar rats, 4 weeks old, were divided into three groups: the control group, which was given water; the overdrinking (excess) group, which was given only 5% ethanol ad libitum; and the moderate drinking (moderate) group, which was given 5% ethanol instead of water for just 2 h per day on weekdays for 163 days. The latter group resembled the ethanol consumption of a man with a 60 kg body weight who drinks one bottle of beer (633 mL) per day. All the rats were allowed free access to food containing 1.2% calcium and 1.1% phosphate, and were housed in a room maintained at 23 ± 1°C on a 12 h/12 h light-dark cycle. After 163 days of the experiment, all rats were fasted overnight. On the following day, blood samples were taken under ether anesthesia from the abdominal aorta and serum was separated by centrifugation. Tibiae and femurs were excised, and adherent soft tissues were removed and used for bone mineral analysis and biomechanical testing.

*Biochemical determinations.* The levels of aspartate aminotransferase (AST) (14), alanine aminotransferase (ALT) (15), triglyceride (16), total protein (17), alkaline phosphatase (18), calcium (19) and phosphorus in the serum (20) were measured.

*Bone mechanical properties.* The three-point bending test was performed on the femur using a Dynagraph (DYN-1255, Iio Electric, Tokyo, Japan) as reported previously (21). The center of the femur, under the condition of 1.0 cm sample space, was fractured by applying perpendicular pressure at a speed of 100 mm/min.

*Bone mineral analysis.* The bone mineral density (BMD) of the right tibia was measured by dual energy X-ray absorptiometry (DXA, QDR-2000, Hologic, Walthams, MA, USA) as reported previously (22). A detector collimator with a single slit was applied on the X-ray generator and the scan was performed in ultra-high-resolution mode (rat mode, Version 2.0 software). The BMD of the 1/3 proximal tibia to the epiphysis was examined as trabecular bone and the 2/3 proximal to the tibiofibular as cortical bone.

*Statistical methods.* Student's t-test was used to analyze the differences be-
between the control, moderate and excess groups; $p < 0.05$ was considered to be statistically significant.

RESULTS

As shown in Table 1, the control group was significantly superior to the other two groups in both body weight gain and food intake. The food efficiency for the moderate group was less efficient than the control group, while the excess group was higher in efficiency than the control group. The moderate and excess groups were both lower than the control group in AST and ALT. Although the phosphorus quantity of the moderate group was lower than that of the control group, both were in the normal range. The other parameters also showed that all biochemical measurements were at normal levels. The results of BMD measurement of tibia are shown in Fig. 1. The moderate group had higher BMD levels than the control group, which was due to the high levels of proximal metaphysis, while there was no difference between the excess group and the control group. The mechanical properties of bone are shown in Fig. 2, where the moderate group was significantly greater than the control group, while the excess group showed no difference in comparison to the control group.

The alcohol intake of the moderate and excess groups resembled the ethanol consumption of 1 and 12 bottles of beer (633 mL) per day, respectively, by a man with a 60 kg body weight. The total intake of water and alcohol were similar in the three groups.

| Table 1. Body weight gain, food intake, food efficiency and biochemical parameters. |
|---|---|---|---|
| | Control $n=5$ | Moderate $n=5$ | Excess $n=5$ |
| Body weight gain (g/day) | 1.31±0.03 | 1.16±0.06** | 1.16±0.05* |
| Food intake (g/day) | 15.5±0.3 | 14.6±0.5* | 13.0±0.4*** |
| Food efficiency$^1$ | 0.085±0.003 | 0.078±0.002** | 0.089±0.003* |
| Biochemical values in serum | | | |
| Aspartate aminotransferase (IU/L) | 64.0±5.5 | 54.0±2.0** | 52.8±1.3** |
| Alanine aminotransferase (IU/L) | 36.4±6.2 | 24.4±2.9** | 26.2±2.6** |
| Triglyceride (mg/dL) | 45.0±8.0 | 43.0±8.5 | 57.2±14.6 |
| Total protein (g/dL) | 6.06±0.10 | 6.00±0.10 | 6.14±0.12 |
| Alkaline phosphatase (IU/L) | 100.6±12.0 | 91.8±6.4 | 112.4±8.2 |
| Calcium (mg/dL) | 9.02±0.10 | 9.00±0.10 | 9.10±0.15 |
| Phosphorus (mg/dL) | 5.22±0.32 | 4.86±0.09* | 5.16±0.20 |

$^1$Food efficiency: Body weight gain/Food intake.

All values expressed as mean±SE.

Significantly different from control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).
Fig. 1. Bone mineral density of the tibia. The bone mineral density (BMD) of the right tibial bone was measured by dual X-ray absorptiometry (DXA). The proximal metaphysis (trabecular bone) is the upper 1/3 from the tibiofibular junction. The diaphysis (cortical bone) is the middle 2/3 between the proximal epiphysis and the tibiofibular junction. All values are expressed as mean±SE. Significantly different from the control group (*p<0.05, **p<0.01, ***p<0.001).

DISCUSSION

Alcoholics usually incur high bone mineral losses, leading to a high incidence of bone fractures. This reduction in bone mass has been found in the iliac crest, femoral neck and calcaneus of human (2), which are all predominantly trabecular bones. In this study, the moderate group showed higher mechanical bone strength mainly due to higher BMD in the cortical bone area. The tibial diaphyses as models of cortical bone had higher BMD, and so showed a significantly higher level of proximal metaphysis, a site rich with trabecular bone which metabolized more rapidly than cortical bone (22).

The calorifacient effect of alcohol constituted 2.5% and 15.6% of the caloric intake in the moderate and excess groups, respectively. Alcohol intake depresses
food consumption because of a high-energy content (7). We observed a decrease in body weight gain for the ethanol-treated rats, which might have been caused by depressed food intake. Consequently, we obtained varied food efficiency, which showed that the moderate group was less and the excess group was more than the control group. The moderate group was superior to the other groups in terms of BMD and bone mechanical properties regardless of depressed food intake, while the excess group showed either the same or lower parameters than the control group. It is suggested that the metabolic rate changes during the process of bone formation. Bone mineral losses associated with ethanol may result from lower nutrient density due to dietary deficiencies as reported by Krawitt, which showed alcohol inhibition of intestinal calcium transport (8) and hypocalciuria as reported by Baran et al. (23). Alcohol impairs the absorption of essential nutrients besides calcium (9). Alcohol ingestion may alter mineral homeostasis observable in bone reinforcement by enhancing the absorption of nutrients.

Several authors have reported higher BMD in moderate social drinkers (10–13), however they failed to indicate any important factors. There are a few studies which indicate dose-dependent variations of alcohol effects on bone cells in vitro (6). These results are potential to contribute to the interpretation of the differential function induced by some extent of alcohol consumption. Hormonal changes including parathyroid hormone and vitamin D metabolites (9) are affected by alcohol intake, which induces a biphasic effect on bone metabolism.
The effect of lower concentrations of ethanol consumption for a longer period is out of the scope of this report. This study confirmed the beneficial effect of social drinking on bone mineral density and bone fragility in vivo, which is based on conditions similar to the living environment that was used. The factors and mechanisms of bone alterations related to alcohol are multifactorial. Hence, an assessment on the effect of alcohol remains for further investigation, which should include alterations in mineral homeostasis and liver function.

REFERENCES

1) Seeman E, Melton LJ III, O'Fallon WM, Riggs BL. 1993. Risk factors for spinal osteoporosis in men. *Am J Med* 75: 977–983.

2) Bikle DD, Genant HK, Cann C, Recker RR, Halloran BP, Striwler GJ. 1985. Bone disease in alcohol abuse. *Ann Intern Med* 103: 42–48.

3) Johnell O, Nilsson BE, Wiklund PE. 1982. Bone morphometry in alcoholics. *Clin Orthop* 165: 253–258.

4) Crilly RG, Anderson C, Hogan D, Delaquerriere-Richardson L. 1988. Bone histomorphometry, bone mass, and related parameters in alcoholic males. *Calcif Tissue Int* 43: 269–276.

5) Farley FR, Fitzsimmons R, Taylar AK, Jorch UM, Lan KHM. 1985. Direct effects of ethanol on bone resorption and formation in vitro. *Arch Biochem Biophys* 238: 305–314.

6) Cheung RCY, Gray C, Boyde A, Jones SJ. 1995. Effects of ethanol on bone cells in vitro resulting in increased resorption. *Bone* 16: 143–147.

7) Forsander OA. 1994. Hypothesis: Factors involved in the mechanisms regulating food intake affect alcohol consumption. *Alc Alcohol* 29: 503–512.

8) Krawitt EL. 1973. Ethanol inhibits intestinal calcium transport in rats. *Nature* 243: 88–89.

9) Rico H. 1990. Alcohol and bone disease. *Alc Alcohol* 25: 345–352.

10) Angus RM, Sambrook PN, Pocock NA, Eisman JA. 1988. Dietary intake and bone mineral density. *Bone Miner* 4: 265–277.

11) Hansen MA, Overgaard K, Riis BJ, Christiansen C. 1991. Potential risk factors for development of postmenopausal osteoporosis examined over a 12-year period. *Osteoporosis Internatl* 1: 95–102.

12) Holbrook TL, Barrett-Connor E. 1993. A prospective study of alcohol consumption and bone mineral density. *Br Med J* 306: 1506–1509.

13) Felson DT, Zhang Y, Hannan MT, Kannel WB, Kiel DP. 1995. Alcohol intake and bone mineral density in elderly men and women. *Am J Epidemiol* 142: 485–492.

14) Karman A, Wróblewski F, Ladue JS. 1955. Transaminase activity in human blood. *J Clin Invest* 34: 126.

15) Wróblewski F, Ladue JS. 1956. Serum glutamic pyruvic transaminase in cardiac and hepatic disease. *Proc Soc Exp Biol Med* 91: 569.

16) Eggstein M, Kreutz FH. 1966. Eine neue Bestimmung der neutrafetleim Blutserum und Gewebe. *Klin Wschr* 44: 262.

17) Gornal AG, Bardawill CJ, Dabid MM. 1944. Determination of serum proteins by *J Nutr Sci Vitaminol*
means of the biuret reaction. *J Biol Chem* **177**: 751–766.
18) Bessey OT, Lowry OH, Broch MJ. 1946. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *J Biol Chem* **164**: 321–329.
19) Harold V, Connertry MD, Analis RBBS. 1966. Determination of calcium by means of orthocresolphthalin complexone. *Am J Clin Pathol* **45**: 290–296.
20) Fiske CH, Subbarow Y. 1925. The colorimetric determination of phosphorus. *J Biol Chem* **66**: 375–400.
21) Ezawa I, Arai F. 1982. The effect of voluntary exercise on calcium utilization in growing rats. *J Home Econ Jpn* **33**: 614–618.
22) Omi N, Morikawa N, Hoshina A, Ezawa I. 1992. The effect of spiny lobster shell powder on bone metabolism in ovariectomized osteoporotic model rats. *Jpn Soc Food Nutr* **45**: 271–276.
23) Baran DT, Teitelbaum SL, Bergfeld MA, Parker G, Cruvant EM, Avioli LV. 1980. Effect of alcohol ingestion on bone and mineral metabolism in rats. *Am J Physiol* **238**: E507–510.