# Chapter 35
# The Effects of Dietary Taurine Supplementation on Bone Mineral Density in Ovariectomized Rats

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**Abstract** This study was performed to evaluate the effect of a diet rich in taurine (2.0 g/100 g) on bone metabolism in ovariectomized (OVX) rats. All rats were fed deionized water during the experimental period. Bone mineral density (BMD) and bone mineral content (BMC) of spine and femur were measured. Serum and urinary calcium and phosphorus content were determined. The levels serum osteocalcin and alkaline phosphatase (ALP) were used to assess bone formation. The rate of bone resorption was measured by the deoxypyridinoline (DPD) crosslink immunoassay and corrected for creatinine. Urinary Ca and P excretion, serum osteocalcin content, and the crosslink value were not significantly different between the Sham groups. The taurine supplemented, Sham group had higher spinal and femur BMC than those of the untreated control group, but the difference was not statistically significant. However, the taurine supplemented, Sham group had significantly higher spine and femur BMC per weight than those of the untreated control group. Within the OVX group, the taurine supplemented group had a lower crosslink value than the casein group. The taurine supplemented, OVX group had higher femur bone mineral content per weight than those of the control, OVX group, but the difference was not statistically significant. A study examining the long-term effect of taurine supplementation in humans is warranted.

**Abbreviations** *OVX*, ovariectomized; *Sham*, sham operated

## 35.1 Introduction

Osteoporosis is defined as a disease characterized by loss of bone mass, accompanied by microarchitectural deterioration of bone tissue, which leads to an unacceptable increase in the risk of fracture. Osteoporosis is well recognized as a major public health problem. Osteoporosis and low bone mass are currently estimated to be a major public health threat for almost 44 million US men and women aged 50 and older, representing 55% of the population in that age range (Jeri 2005). More than

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40% of postmenopausal women (Chrischilles et al. 1990) and up to 25% of men (Nguyen 1996)will sustain osteoporotic fractures, which will result in substantial expense (Dolan and Torgerson 1998; Stafford et al. 2004) morbidity (Melton 2003) and mortality (Melton 2003; Jalava et al. 2003). The causes of osteoporosis are multiple (Raisz 1999; Heaney 1993). The facts that the organic matrix in bone is mainly composed of protein and that most of the bone mineral content is calcium suggests that the important nutrients for bone health are protein and calcium (Ilich and Kerstetter 2000). Although a great deal of attention has been given to the importance of calcium intake, much less is known about the effects of other nutrients on bone, although recent reports have supported the importance of potassium, magnesium, vitamin K, and fruit and vegetables (Tucker et al. 1999; Booth et al. 2000). Women are at higher risk than men of developing osteoporosis as a result of naturally lower peak bone mass and rapid bone loss after menopause. Estrogen replacement therapy in postmenopausal women reduces the risk of osteoporosis or coronary heart disease (CHD) in part by modulating serum cholesterol. However, estrogen replacement therapy and cholesterol-lowering pharmacologic agents may be accompanied by side effects. On the other hand, taurine is thought to be quite safe and there is little concern about the side effects of excessive intake of taurine (Furukawa et al. 1991).

Taurine could act either directly or indirectly by enhancing growth factor production (Boujendar et al. 2002). According to Boujendar et al. (2002) taurine supplementation of the maternal diet restored normal serum insulin-like growth factor II (IGF-II) expression in islet cells of fetuses of low protein-fed rats (Boujendar et al. 2002). Interest has been expressed in the relation between skeletal maintenance and age-related decreases in serum insulin-like growth factor 1 (IGF-I) concentrations (Jensen et al. 2002). Recombinant IGF-I increases bone formation activity in postmenopausal women (Ghiron et al. 1995; Ebeling et al. 1993; Grinspoon et al. 1995, suggesting that increasing IGF-I levels may help restore bone mass. IGF-I production is markedly affected by the intake of nutrients (Jensen et al. 2002).

The major hormones that regulate tissue growth and metabolism all have a major influence on skeletal growth and remodeling, including the growth hormone-insulin-like growth factor (GH-IGH) system. The GH-IGH system determines body size and regulates the distribution of body fat, lean body mass, and bone modeling and remodeling after epiphyseal closures (Sjogren et al. 1999). GH can stimulate IGF production not only in the liver but also in other target organs, including bone. The GH-IGF system stimulates both resorption and formation (Yakar et al. 2002). Taurine could act either directly or indirectly by enhancing growth factor production (Boujendar et al. 2002). Human growth hormone (hGH) has been shown in some studies to have anabolic effects on bone (Tangpricha et al. 2006) but the effects of taurine on bone are unknown. Taurine is a sulfur-containing amino acid that is best known for its conjugation with bile acids, but it is also involved in the coordination of nerve function, the stabilization of the cell membrane, detoxification, antioxidant reactions and the modulation of osmotic pressure (Huxtable 1992; Sturman 1993). The present study investigated the relationship between taurine content and bone. Influence of diet on postmenopausal bone loss is not well understood. Most work

has focused on calcium and vitamin D or a few isolated nutrients, but little work has focused on taurine. The aim of this study was to determine whether there is an association between taurine supplementation and the indexes of bone health (bone resorption markers and BMD) in the estrogen deficient rat (OVX).

## 35.2 Methods

### *35.2.1 Animals*

Forty female Sprague-Dawley rats (body weight 200±5 g, 9 weeks old age) were randomly divided into two groups. One group was ovariectomized (OVX) while the other group received a sham operation (SHAM). For a 6 week period, each rat group was further divided into control and taurine supplemented (2.0%) dietary groups.

### *35.2.2 Diet*

The dietary supply of vitamins, minerals, and protein was in accordance with the recommended dietary allowances for rats from the American Institute of Nutrition (AIN-93; Reeves et al. 1993) as shown in Table 35.1. All rats were fed an experimental diet and provided deionized water ad libitum for 6 weeks.

**Table 35.1** Composition of experimental diets (g/100 g diet)

| Ingredients | Control | Taurine |
|---|---|---|
| Casein[1] | 20 | 20 |
| Corn starch[2] | 52.9486 | 52.9486 |
| Sucrose | 10 | 10 |
| Soybean oil[3] | 7 | 7 |
| Cellulose[4] | 5 | 5 |
| Min-mix[5] | 3.5 | 3.5 |
| Vit-mix[6] | 1.0 | 1.0 |
| L-cystine | 0.30 | 0.30 |
| Choline[7] | 0.25 | 0.25 |
| Tert-butyl hydroquinone | 0.0014 | 0.0014 |
| Taurine[8] | – | 2.0 |

[1]Lactic Casein, 30mesh, New Zealand Dairy Board, Willington, N.Z.
[2]Corn Starch, Doosan Co. 234-17 Maam-Ri, Bubal-Eup, Inchon-City, Kyunggi-Do.
[3]Soybean oil, CJ CheilJedang Co. Seoul, Korea.
[4]Cellulose, supplied by SIGMA Chemical Company.
[5]Mineral Mixture (AIN-93G), supplied by U.S. CORNING Laboratory Services Company. TEKLAD TEST DIETS, Madison.
[6]Vitamin mixture (AIN-93), supplied by U.S. CORNING Laboratory Services Company. TEKLAD TEST DIETS, Madison, Wisconsin.
[7]Choline, supplied by SIGMA Chemical Company.
[8]Taurine, Dong-A Pharm. Co. Ltd. 434-4 Moknae-dong, Ansan-City, Kyunggi-Do.

### 35.2.3 Bone and Bone Markers Determination

Bone mineral density (BMD) and bone mineral content (BMC) of spine and femur were measured using PIXImus (GE Lunar Co, Wisconsin, USA). Serum alkaline phosphatase activity (ALP), osteocalcin and urinary DPD crosslink values were measured as markers of bone formation and resorption. Bone resorption was calculated by measuring urinary excretion of deoxypyridinoline and bone formation by measuring serum osteocalcin.

### 35.2.4 Statistic Analysis

The statistical significance of differences among the groups was evaluated by two-way ANOVA, using a computer software package (version 9.13, SAS Institute Inc, Cary, NC). Individual comparisons were made by Duncan's multiple range test using the ANOVA. Differences were considered to be significant at $p < 0.05$. Data are expressed as means ± SD.

## 35.3 Results

### 35.3.1 Weight Gain and FER

The results of this study indicate that body weight gain was higher in the OVX groups than in the SHAM groups regardless of diet (Table 35.2). Food intake and the food efficiency ratio were not significantly different between the groups (Table 35.3).

**Table 35.2** The effect of diet on body weight and weight gain in rats

| | Sham | | Ovx | |
|---|---|---|---|---|
| | Control | Taurine | Control | Taurine |
| Initial weight (g) | $204.7 \pm 9.4^{1,a}$ | $201.5 \pm 6.3^{a}$ | $211.5 \pm 6.7^{a}$ | $204.1 \pm 7.6^{a}$ |
| Final weight (g) | $276.6 \pm 21.9^{a}$ | $278.8 \pm 16.7^{a}$ | $317.9 \pm 26.3^{b}$ | $329.2 \pm 22.6^{b}$ |
| Weight gain (g) | $71.9 \pm 13.2^{a}$ | $77.30 \pm 13.9^{a}$ | $106.38 \pm 13.2^{b}$ | $119.10 \pm 11.2^{b}$ |

[1]Mean ± SD
Values with different superscripts within a given row are significantly different at $p < 0.05$ by Duncan's multiple range test.

**Table 35.3** The effect of diet on food intake and food efficiency ratio (FER) in rats

| | Sham | | Ovx | |
|---|---|---|---|---|
| | Control | Taurine | Control | Taurine |
| Food intake (g) | $17.60 \pm 1.09^{1,a}$ | $18.23 \pm 1.09^{a}$ | $18.89 \pm 2.26^{a}$ | $21.09 \pm 1.77^{a}$ |
| FER | $0.09 \pm 0.01^{a}$ | $0.10 \pm 0.01^{a}$ | $0.13 \pm 0.05^{a}$ | $0.14 \pm 0.01^{a}$ |

[1]Mean ±SD
Values with different superscripts within a given row are significantly different at $p < 0.05$ by Duncan's multiple range test.

### 35.3.2 Serum Ca, P, Alkaline Phosphatase and Osteocalcin

Serum calcium and phosphorus content of animals are presented in Table 35.4. Serum calcium and phosphorus content were unaffected by ovariectomy or taurine supplementation.

**Table 35.4** The effect of diet on serum Ca and P in rats

| | Sham | | Ovx | |
|---|---|---|---|---|
| | Control | Taurine | Control | Taurine |
| Ca (mg/dl) | $9.14 \pm 0.45^{1,a}$ | $9.22 \pm 0.18^{a}$ | $9.88 \pm 0.24^{a}$ | $9.78 \pm 0.90^{a}$ |
| P (mg/dl) | $7.74 \pm 0.85^{a}$ | $6.68 \pm 0.19^{a}$ | $7.90 \pm 0.68^{a}$ | $6.86 \pm 1.25^{a}$ |

[1]Mean ± SD
Values with different superscripts within a given row are significantly different at $p < 0.05$ by Duncan's multiple range test.

Serum ALP was significantly higher in the OVX groups than in the SHAM groups. The values for the taurine, OVX group was significantly higher than those of the control, OVX group (Table 35.5).

Bone formation was determined by measuring serum osteocalcin. And the content of serum osteocalcin was not significantly different between the groups (Table 35.5).

**Table 35.5** The effect of diet on serum alkaline phosphatase (ALP) and osteocalcin content of rats

| | Sham | | Ovx | |
|---|---|---|---|---|
| | Control | Taurine | Control | Taurine |
| ALP (u/l) | $169.4 \pm 371.3^{1,a}$ | $260.0 \pm 31.4^{bc}$ | $202.3 \pm 18.6^{ab}$ | $292.8 \pm 46.1^{c}$ |
| Osteocalcin (ng/ml) | $0.31 \pm 0.16^{a}$ | $0.29 \pm 0.11^{a}$ | $0.32 \pm 0.01^{a}$ | $0.38 \pm 0.03^{a}$ |

[1]Mean ± SD
Values with different superscripts within a given row are significantly different at $p < 0.05$ by Duncan's multiple range test.

**Table 35.6** The effect of diet on urine Ca and P in rats

| | Sham | | Ovx | |
|---|---|---|---|---|
| | Control | Taurine | Control | Taurine |
| Ca (mg/day) | $0.27 \pm 0.20^{1,a}$ | $0.31 \pm 0.18^{a}$ | $0.26 \pm 0.13^{a}$ | $0.32 \pm 0.19^{a}$ |
| P(mg/day) | $10.59 \pm 2.53^{a}$ | $5.80 \pm 2.18^{b}$ | $12.13 \pm 4.74^{a}$ | $8.37 \pm 2.49^{ab}$ |

[1]Mean ± SD
Values with different superscripts within a given row are significantly different at $p < 0.05$ by Duncan's multiple range test.

**Table 35.7** The effect of diet on urine deoxypyridinoline, creatinine and crosslink value of rats

| | Sham | | Ovx | |
|---|---|---|---|---|
| | Control | Taurine | Control | Taurine |
| DPD (nM) | $646.5 \pm 311.4^{1,a}$ | $683.2 \pm 381.9^{a}$ | $880.3 \pm 487.5^{a}$ | $993.8 \pm 456.7^{a}$ |
| creatinine (mM) | $4.20 \pm 1.71^{a}$ | $7.60 \pm 7.11^{a}$ | $4.44 \pm 2.09^{a}$ | $8.82 \pm 4.97^{a}$ |
| Crosslink value (nM/mM) | $150.4 \pm 26.8^{a}$ | $109.3 \pm 36.9^{a}$ | $207.8 \pm 73.5^{b}$ | $121.5 \pm 22.7^{a}$ |

Mean ± SD
Values with different superscripts within a given row are significantly different at $p < 0.05$ by Duncan's multiple range test.

**Table 35.8** The effect of diet on spinal BMD, BMC, BMD per weight and BMC per weight of rats

| | Sham | | Ovx | |
|---|---|---|---|---|
| | Control | Taurine | Control | Taurine |
| SBMD($g/cm^2$) | $0.151 \pm 0.013^{1,ab}$ | $0.158 \pm 0.018^{a}$ | $0.136 \pm 0.011^{b}$ | $0.136 \pm 0.008^{b}$ |
| SBMD($g/cm^2$) /Wt(kg) | $0.55 \pm 0.07^{a}$ | $0.57 \pm 0.10^{a}$ | $0.44 \pm 0.10^{b}$ | $0.49 \pm 0.02^{b}$ |
| SBMC($g/cm^2$) | $0.494 \pm 0.066^{a}$ | $0.563 \pm 0.057^{a}$ | $0.440 \pm 0.035^{b}$ | $0.437 \pm 0.046^{b}$ |
| SBMC($g/cm^2$) /Wt(kg) | $1.80 \pm 0.27^{ba}$ | $2.04 \pm 0.32^{a}$ | $1.52 \pm 0.21^{b}$ | $1.69 \pm 0.18^{ab}$ |

[1]Mean ± SD
Values with different superscripts within a given row are significantly different at $p<0.05$ by Duncan's multiple range test.

**Table 35.9** The effect of diet on femur BMD, BMC, femur BMD per weight and BMC per weight of rats

| | Sham | | Ovx | |
|---|---|---|---|---|
| | Control | Taurine | Control | Taurine |
| FBMD($g/cm^2$) | $0.196 \pm 0.007^{1,a}$ | $0.202 \pm 0.011^{a}$ | $0.192 \pm 0.011^{a}$ | $0.195 \pm 0.012^{a}$ |
| FBMD($g/cm^2$) /Wt(kg) | $0.70 \pm 0.06^{a}$ | $0.73 \pm 0.08^{a}$ | $0.60 \pm 0.12^{b}$ | $0.60 \pm 0.03^{b}$ |
| FBMC($g/cm^2$) | $0.388 \pm 0.018^{a}$ | $0.409 \pm 0.027^{a}$ | $0.380 \pm 0.014^{a}$ | $0.399 \pm 0.035^{a}$ |
| FBMC($g/cm^2$) /Wt(kg) | $1.30 \pm 0.10^{ab}$ | $1.52 \pm 0.18^{a}$ | $1.22 \pm 0.15^{b}$ | $1.29 \pm 0.05^{ab}$ |

[1]Mean ± SD
Values with different superscripts within a given row are significantly different at $p < 0.05$ by Duncan's multiple range test.

### *35.3.3 Urine Ca, P, Deoxypyridinoline, Creatinine and Crosslink Value*

Urinary Ca excretion was not significantly different between the experimental groups. But urinary P excretion was significantly decreased in rats fed taurine. Serum calcium and phosphorus content of the SHAM groups were not different from those of the ovarectomized rats (Table 35.6).

Bone resorption was calculated by measuring urinary excretion of deoxypyridinoline, and bone formation by measuring serum osteocalcin. The crosslink value was increased in the ovariectomy group fed the control diet. However, the crosslink value was significantly decreased in the ovarietomized, taurine group (Table 35.7).

### *35.3.4 Spine and Femur BMD, BMC, BMD per Weight and BMC per Weight*

Spine BMD of the ovariectomized groups was significantly lower than that of the SHAM groups. Spine BMD and BMC divided by body weight appears to have a higher BMD (7.5%) and significantly higher BMC (4.5%) in the taurine group, which indicates that taurine has a positive influence on spine bone mineral density and bone mineral content (Table 35.8).

Femur BMC divided by body weight appears to have a larger BMC in the taurine group in ovx rats (Table 35.9).

## 35.4 Discussion

The influence of nutrition on bone health remains largely undefined because most studies have focused on calcium intake. The treatment of osteoporosis remains a major challenge, despite an increasing array of therapeutic agents, including bisphosphonates, hormone replacement therapy, and selective estrogen receptor modulators. Despite widespread use, however, these agents all rely on decreasing osteoclastic absorption of bone. The most potent bone-inducing factors are growth factors, such as bone morphogenetic proteins (Edwards et al. 2000).

Taurine is found in bone tissue, but its function is not fully understood. Therefore, in this study using ovariectomized (OVX) rats we examined the effect of taurine on ovarian hormone deficiency-induced bone loss. Within the OVX group, the taurine supplemented subgroup had a lower crosslink value than the casein subgroup. Thus, taurine supplementation seems to decrease bone resorption. The taurine supplemented subgroup had higher spinal BMC and femur BMC than those of the control, Sham group, although the difference was not statistically significant. However, the taurine supplemented group had significantly higher spinal and femur BMC per weight than those of the control, OVX group. Moreover, spinal BMD (7.5%) and BMC (4.5%) divided by body weight appears to be greater in the taurine group,

indicating that taurine has a positive influence on spinal bone mineral density and bone mineral content. Femur BMC divided by body weight appears to have a larger BMC (5.7%) in the taurine group, indicating that taurine has a positive influence on femur bone mineral content.

The present study suggests that BMD may improve upon taurine supplementation of postmenopausal women, as long as they met the currently recommended intake of calcium and vitamin D. Further research is needed to determine whether a similar association exists in OVX rats consuming a less nutrious diet, such as less calcium intake. Although there is little information on the influence of dietary intake on bone metabolism markers, several theories may help to explain our findings.

## 35.5 Conclusion

Within the OVX group, the taurine supplemented rats tended to have higher femur bone mineral content per weight than those of control rats, although the difference was not statistically significant. Clearly, a study on the long-term effect of taurine supplement in humans is warranted, focusing in part on the effect of taurine on the characteristics of bone.

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## References

Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DG, Cupples LA, Wilson PWF, Ordovas J, Schaefer EJ, Dawson-Hughes B, Kiel DP (2000) Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr 71:1201–1208

Boujendar S, Reusens B, Merezak S, Ahn M T, Arany E, Hill D, Remacle C (2002) Taurine supplementation to a low protein diet during foetal and early postnatal life restores a normal proliferation and apoptosis of rat pancreatic islets. Diabetologia 45:856–866

Chrischilles EA, Butler CD, Davis CS, Wallace RB (1990) A model of lifetime steoporosis impact. Arch Intern Med 151:2026–2032

Dolan P, Torgerson DJ (1998) The cost of treating osteoporotic fractures in the United Kingdom female population. Osteoporos Int 8:611–617

Ebeling PR, Jones JD, O'Fallon WM, Janes CH, Riggs BL. (1993) Short-term effects of recombinant human insulin-like growth factor I on bone turnover in normal women.J Clin Endocrinol Metab 77:1384–1387

Edwards CJ, Hart DJ, Spector TD (2000) Oral ststins and increased bone-mineral density in postmenopausal women. Lancet 355; 2218–2219

Furukawa S, Katto M, Kouyama H, Nishida I, Kikumori M, Taniguchi Y, Toda T., Araki H (1991) Repeated dose toxicity study of intravenous treatment with taurine for 13 weeks and recovery test for 5 weeks in rats. Jap Phar Thera 19:275–306

Ghiron L, Thompson JL, Holloway L (1995) Effects of recombinant insulin-like growth factor-1 and growth hormone on bone turnover in elderly women. J BoneMiner Res 10:1844–1852

Grinspoon SK, Baum HBA, Peterson S, Klibanski A. (1995) Effects of rhIGF-I administration on bone turnover during short-term fasting. J Clin Invest 96:900–906

Heaney RP (1993) Nutritional factors in osteoporosis. Annu Rev Nutr 13:287–316
Huxtable RJ (1992) Physiological actions of taurine. Physiol. Rev 72:101–163
Ilich JZ, Kerstetter JE (2000) Nutrition in bone health revisited: a story beyond calcium.J Am Coll Nutr 19:715–737
Jalava T, Sarna S, Pylkkanen L, Mawer B, Kamichael-Davies JA, Adams J, Francis RM, Robinson J, Euge (2003) Association between vertebral fracture and increased mortality in osteoporotic patients. J Bone Miner Res 18:1254–1260
Jensen C, Holloway L, Block G, Spiller G, Gildengorin G, Gunderson E, Butterfield G, Marcus R (2002) Long-term effects of nutrient intervention on markers of bone remodeling and calciotropic hormones in late-postmenopausal women. Am J Clin Nutr 75:1114–1120
Jeri WN (2005) Supplement: women and micronutrients:Addressing the gap throughout the life cycle: Osteoporosis: the role of micronutrients. Am. J. Clinical Nutrition 81:1232S–1239S
Melton LJ III (2003) Adverse outcomes of osteoporotic fractures in the general population. J Bone Miner Res 18:1139–1141
Nguyen TV, Eisman JA, Kelly PJ, Sambrook PN (1996) Risk factors for osteoporoticfractures in elderly men. Am J Epidemiol 144:255–263
Raisz LJ (1999) Osteoporosis: current approaches and future prospects in diagnosis, pathogenesis and management. J Bone Miner Res 17:79–89
Reeves PG, Nielsen FH, Fahey GC (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN 76 A Rodent Diet. J Nutr 123:1939–1951
Sjogren K, Liu JL, Blad K (1999) Liver-derived insulin-like growth factor I (IGH-I) is the principal source of IGF-Iin blood but is not required for postnatal body growth in mice. Proc Natl Acad Sci USA 96:7088–7092
Stafford RS, Brieling RL, Hersh AL (2004) National trends in osteoporosis visits and osteoporosis treatment . Arch Intern Med 164:1525–1530
Sturman, G. A (1993) Taurine in development. Physiol. Rev 73:119–147
Tangpricha M, Luo C, Fernandez-Estivariz LH, Gu N, Bazargan JM, Klapproth SV, Sitaraman JR, Galloway LM, Leader T R, Ziegler (2006) Growth hormone favorably affects bone turnover and bone mineral density in patients with short bowel syndrome undergoing intestinal rehabilitation. JPEN 30:480–486
Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PWF, Kiel DP (1999) Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr 69:727–736
Yakar S, Rosen CJ, Beamer WG (2002) Circulating levels of IGF-1 directly regulatebone growth and density. J ClinInvest 110:771–781