Osteoporos Int (2002) 13:200–204

Osteoporosis International

Original Article

Soy Product Intake and Serum Isoflavonoid and Estradiol Concentrations in Relation to Bone Mineral Density in Postmenopausal Japanese Women

C. Nagata[1], H. Shimizu[1], R. Takami[2], M. Hayashi[2], N. Takeda[3] and K. Yasuda[3]

[1]Department of Public Health, Gifu University School of Medicine; [2]Internal Medicine of Matsunami General Hospital; and [3]The Third Department of Internal Medicine, Gifu University School of Medicine, Gifu, Japan

Abstract. To evaluate soy intake and serum concentrations of estradiol and isoflavonoids and their relationship to bone mineral density (BMD) and serum bone-specific alkaline phosphatase (bone ALP) activity, we conducted a cross-sectional study of 87 postmenopausal Japanese women. Soy product and isoflavone intake from soy products and intake of nutrients were assessed with a semiquantitative food-frequency questionnaire. BMD (mg/cm^2) was measured by single-energy X-ray absorptiometry at the site of the calcaneus. Serum estradiol (E_2) and the sex hormone-binding globulin (SHBG) were measured by radioimmunoassay. Serum genistein and daidzein concentrations were measured by a high-performance liquid chromatography MS/MS method. A statistically significant correlation was observed between the ratio of E_2 to SHBG and BMD (Spearman $r = 0.38$, $p = 0.0003$) after controlling for age, body mass index, smoking status, age at menarche, and intake of vegetable fat, vitamin C and salt. Soy product and isoflavone intake and serum isoflavones were not significantly correlated with BMD after controlling for the covariates. Serum ALP was not significantly correlated with soy product and isoflavone intake, the E_2/SHBG ratio or serum isoflavones. The present study supports the association of BMD with serum estradiol; however, it does not support the association of BMD with soy or isoflavone intake or serum isoflavone levels.

Correspondence and offprint requests to: Chisato Nagata, Department of Public Health, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500-8705, Japan. Tel. +81 58 267 2247. Fax: +81 58 265 9020. e-mail: chisato@cc.gifu-u.ac.jp

Keywords: Bone mineral density; Bone-specific alkaline phosphatase; Estradiol; Isoflavonoids; Postmenopausal Japanese women; Soybeans

Introduction

It is generally recognized that estrogen deficiency following menopause plays a major role in osteoporosis. The isoflavones are structurally similar to estradiol [1]. Hence, the estrogenicity of isoflavones may lead to reduced bone loss. The Japanese consume a diet that is very rich in the isoflavones, genistein and daidzein, which are found in soybeans and soy products. Some researchers assume that the lower incidence of osteoporosis in Japanese women is attributable to a high consumption of soy foods [2].

The synthetic isoflavone derivative, ipriflavone, has been suggested to reduce bone loss [3]. A number of clinical trials to test the efficacy of ipriflavone on bone mass have been conducted but the data are conflicting. There are insufficient data about the effect of naturally occurring isoflavones on bone health. Some well-designed animal studies demonstrated that dietary soy or genistein prevented bone loss [4,5]. In human studies, a relative low average and variation in soy intake may have precluded a cross-sectional assessment of this relationship in non-Asian countries. To our knowledge, five studies have previously addressed the relationship cross-sectionally between dietary soy and bone mineral density (BMD) and all of them were conducted in Japan. The results were inconsistent. Yukawa et al. [6] found no association between soy product intake estimated from 3-

day diet records and BMD at the lumbar spine L3 in women aged 65–79 years. Kimira et al. [7] also reported no association between isoflavone intake estimated from 3-day diet records and BMD in women aged 28–71 years. Tsuchida et al. [8] observed significantly higher (by 4.5%) BMD of the second metacarpal bone among women aged 40–49 years who had soybeans 2 or more times per week compared with those who had them 0 or 1 times, although the dietary questionnaire for this study was not validated. Horiuchi et al. [9] found a significantly positive correlation (r = 0.23) between soy protein intake estimated from 3-day diet records and Z-scores of BMD at the lumbar spine (L2–L4) in postmenopausal women aged 52–83 years. Somekawa et al. [10] reported a significantly positive correlation ($r = 0.16$) between isoflavone intake and BMD at the lumbar spine (L2–L4) in postmenopausal women aged 44–80 years. None of these studies included measurements of endogenous estrogen status. The endogenous estrogen level may affect the relationship between soy intake and BMD. Similarly, soy intake may affect the relationship between endogenous estrogen level and BMD.

The present study examined the relationships of soy intake and the serum estrogen level with parameters of bone health, BMD, and a bone formation marker, serum bone-specific alkaline phosphatase (bone ALP) activity. We included the measurement of serum isoflavones and isoflavone metabolites (genistein, daidzein and equol). Serum isoflavonoid data should be useful with regard to the bioavailability of isoflavones and to further infer the mechanism for potential biological effects of dietary soy on bone health. We used a validated semiquantitative food-frequency questionnaire to estimate the intake of soy products and other dietary components and took account the potential confounding effects of various dietary and lifestyle factors.

Subjects and Methods

Subjects for this study were participants in a health check-up program provided by a general hospital in Gifu, Japan between September, 1996 and August, 1997. A total of 403 women agreed to participate in the present study and completed a self-administered questionnaire (the response rate was 96.4%). To obtain complete data, a nurse epidemiologist interviewed those who returned the questionnaire with incomplete information.

The questionnaire sought information about demographic, smoking and drinking habits, diet, exercise, and menstrual and reproductive histories. Diet was assessed by a semiquantitative food-frequency questionnaire. The women were asked to indicate the average frequency with which they had consumed 169 food items during the year prior to the study and the usual portion size of each item. We included nine food items for soy products (miso soup, tofu, deep-fried tofu, fried bean curd, dried bean curd, fermented soy beans, houba-miso, soymilk and boiled soy beans). The total intake of soy products was calculated as the sum of these nine food items. The intake of foods and nutrients was estimated from the frequency of ingestion and portion size using the Japanese Standard Tables of Food Composition, 4th edition, published by the Science and Technology Agency of Japan. Isoflavone intake from soy products was also estimated using isoflavone concentration data provided by Wakai et al. [11]. Detailed information on the questionnaire including its validity and reproducibility, has been provided elsewhere [12]. For example, the Spearman correlation coefficients comparing estimates of soy product intake from this questionnaire with the estimates from 12 daily diet records kept over a 1-year period were 0.71.

Exercise was assessed by asking the average hours per week spent performing various kinds of activities during the past year. The details, including its validity, are described elsewhere [13].

BMD, expressed in g/cm^2, was measured by single-energy X-ray absorptiometry at the calcaneus. The measurement was obtained with a commercial instrument (OsteoAnalyzer, Siemens-Osteon, HA; precision $< 1.0\%$).

A fasting blood sample was collected from each subject in the morning. The samples were stored at 80 °C until assayed. Serum isoflavonoids were measured using the high-pressure liquid chromatography (HPLC)-MS/MS method [14]. This method allows the detection of the isoflavonoids in human serum with improved selectivity, sensitivity and precision. For serum isoflavonoid measurement, β-glucuronidase/sulfatase (2000 units of β-glucuronidase and 1000 units of sulfatase) was added to 0.2–0.5ml of serum. The aglicones of the isoflavones and their metabolites were recovered by diethyl ether extraction. The diethyl ether extracts were evaporated to dryness under nitrogen and redissolved in acetonitrile prior to HPLC-MS/MS analysis. Isoflavonoids in serum extracts were separated using an HPLC method and analyzed by negative-ion multiple-reaction ion- monitoring mass spectrometry using an electorospray interface. The intra-assay coefficients of variations (CVs) were 4.3% for genistein, 4.3% for daidzein and 4.6% for equol. The inter-assay CVs were 4.9% for genistein, 5.6% for daidzein and 6.7% for equol. Serum estradiol (E_2) and sex hormone-binding globulin (SHBG) were measured by radioimmunoassay. Serum E_2 was determined after extraction using a kit purchased from Diagnostic Products Japan, Chiba, Japan. Serum SHBG was determined using a kit purchased from Pharmacia & Upjohn, Tokyo, Japan. Serum bone ALP was determined by enzyme immunoassay method. The intra-assay CVs were 10.9% for E_2. 7.8% for SHBG and 6.8% for bone ALP. The inter-assay CVs were 17.6% for E_2, 7.1% for SHBG and 7.3% for bone ALP. All samples were analyzed at SRL, Tokyo, Japan.

Postmenopausal (defined as cessation of menses for 12 or more months) women were selected for the present analysis. We excluded women who were taking hormone replacement therapy or other hormones (n = 2) and who had a history of breast cancer (n = 3), ovarian cancer ($n = 1$), bone tumor (n = 1) or endogenous diseases such

as diabetes mellitus ($n = 3$) and thyroid disease ($n = 3$). Of the 90 eligible women, 87 had sufficient sera available for hormone assays.

Spearman correlation coefficients were used to examine the associations of study variables with BMD and serum bone ALP. Intake of soy product and the individual nutrient were log-transformed and adjusted for total energy using the method proposed by Willett [15]. Potentially confounding variables were controlled by first regressing the confounders to the variables of interest and then calculating the partial coefficients between the residuals. We examined a number of potential confounders, including age, weight, height, body mass index, years since menopause, surgical removal of ovaries, hysterectomy, age at menarche, number of births or pregnancies, age at first and last births, lactation, smoking status, age when regular menstrual cycle started, exercise, intake of alcohol and caffeine, intake of macro- and micronutrients, and use of antihypertensive drugs and vitamin supplements. Because serum equol was not detected in 45 women, we used the minimum value (i.e., 2.06 nmol/l) for them in the analysis.

Results

Characteristics of the study subjects are presented in Table 1.

Serum E_2 concentration and the ratio of E_2 to SHBG were significantly correlated with BMD after controlling for age (Table 2). Serum E_2 and the E_2/SHBG ratio were inversely correlated with serum bone ALP, but the correlations were not statistically significant. There were no significant correlations between soy product and isoflavone intake and the bone health parameters after controlling for age. None of the serum isoflavonoids was significantly correlated with BMD and bone ALP after controlling for age.

The other variables which were significantly correlated with BMD after controlling for age were as follows: age at menarche ($r = –0.27$, $p = 0.01$), weight ($r = 0.24$, $p = 0.03$), body mass index (BMI) ($r = 0.25$, $p = 0.02$), intake of vegetable fat ($r = –0.23$, $p = 0.03$), vitamin C ($r = 0.23$, $p = 0.04$) and salt ($r = –0.22$, $p = 0.04$) and smoking status (age-adjusted BMD values were 314.4, 403.6 and 352.7 g/cm^2 in current, former and never-smokers, respectively; $F = 3.14$, $p = 0.048$). Serum bone ALP was inversely marginally correlated with age at menarche ($r = 0.23$, $p = 0.04$) and carbohydrate intake ($r = 0.27$, $p = 0.01$) after controlling for age.

The correlation between serum E_2 and BMD decreased to 0.12 ($p = 0.30$) after additional adjustment for BMI, age at menarche, smoking status, and intake of vitamin C, vegetable fat and salt. The E_2/SHBG ratio was still significantly correlated with BMD after the adjustment ($r = 0.40$, $p = 0.0002$). The correlations of serum bone ALP with serum E_2 and the E_2/SHBG ratio were not altered after additional adjustment for age at menarche and carbohydrate intake. The correlations between soy product and isoflavone intake and BMD or serum bone ALP were close to null. There were no significant correlations between serum isoflavonoid concentrations and the bone health parameters after controlling for the covariates.

The correlation coefficients between the sum of the measured serum isoflavonoid concentrations and the bone health parameters were almost the same whether equol was included or not.

The correlations between E_2/SHBG and BMD were not altered substantially after a further adjustment for isoflavone intake or serum total isoflavonoids ($r = 0.37$). Similarly, the correlations of BMD with soy product and isoflavone intake and serum isoflavonoids were not altered after an additional adjustment for the E_2/SHBG ratio.

Some of women who had had a surgical menopause still had high estrogen levels because their ovaries were

Table 1. Selected characteristics of 87 postmenopausal Japanese women

Variable	Mean±SD	(range)
Age (years)	54.2±6.5	(38–68)
Years since menopause	6.7±5.3	(1.0–23.0)
Height (cm)	154.4±4.7	(144.2–168.0)
Weight (kg)	54.2±7.0	(35.0–79.0)
Body mass index (kg/m^2)	22.9±2.7	(17.5–31.3)
Age at menarche (years)	13.9±1.7	(10–20)
No. of births	2.1±0.8	(0–4)
Exercise (METs · hours/day)	2.1±2.8	(0–12.6)
Alcohol (ml/day)	7.3±13.5	(0-74)
Smoking (%)		
Current	9.2	
Past	5.7	
Nutrient and food intake (per day)		
Energy (kcal)	2198±738	(903–4205)
Soy products (g)	62.4±41.2	(4.9–221)
Isoflavones from soy products (mg)	32.0±17.2	(2.5–79.1)
Protein (g)	92.8±35.1	(35.3–178)
Fat (g)	62.4±25.5	(21.8–30.8)
Crude fiber (g)	6.0±2.9	(1.9–15.5)
Calcium (mg)	859±384	(273–1968)
Vitamin C (mg)	176±115	(29.0–616)
Phosphorus (mg)	1490±552	(542–2855)
Iron (mg)	14.5±6.2	(6.1–33.8)
Sodium (mg)	5618±2230	(2050–11461)
Potassium (mg)	4051±1803	(1697–9996)
Salt (g)	14.0±5.6	(5.1–28.5)
Bone parameters		
Bone mass density (mg/cm^2)	352±70.3	(175–547)
Serum bone ALP activity (IU/l)	24.1±7.7	(10.2–46.3)
Serum hormone concentration		
Estradiol (pmol/l)	76.0±127.9	(19.6–727)
Serum SHB (nmol/l)	79.1±33.0	(28–180)
Ratio of estradiol to SHBG	1.1±1.8	(0.16–10.3)
Serum isoflavonoid concentration		
Genistein (nmol/l)	413.9±643.9	(3.7–4085)
Daidzein (nmol/l)	162.0±203.9	(3.1–906)
Equol[a] (nmol/l)	39.5±96.9	(2.06–716)

METs, metabolic equivalents; ALP, alkaline phosphatase; SHBG, sex hormone-binding globulin.
[a]2.06 nmol/l was allotted to 45 women with an undetectable level of equol (<2.06 nmol/l).

Table 2. Partial correlation coefficients for serum estradiol and soy intake with bone health parameters in 87 postmenopausal Japanese women

	Bone mass density		Serum bone ALP	
	Age-adjusted	Adjusted[a]	Age-Adjusted	Adjusted[b]
Serum estradiol	0.22*	0.12	–0.07	–0.04
Serum SHBG	–0.38**	–0.38**	0.11	–0.09
Estradiol/SHBG	0.43**	0.38**	–0.12	0.04
Soy product intake[c]	–0.04	–0.07	–0.13	–0.03
Isoflavone intake[c]	–0.04	–0.02	–0.12	–0.02
Serum isoflavonoids[d,e]	–0.05	–0.11	0.18	0.16
Serum genistein	–0.05	–0.10	0.16	0.16
Serum daidzein	–0.04	–0.13	0.21	0.20
Serum equol[c]	0.19	0.11	–0.15	–0.11

*$p < 0.05$; **$p < 0.01$.
[a]Adjusted for age, body mass index, age at menarche, smoking status, and intake of vegetable fat, vitamin C and salt.
[b]Adjusted for age, age at menarche and carbohydrate intake.
[c]Energy-adjusted.
[d]Genistein, daidzein plus equol.
[e]2.06 nmol/l was allotted to 45 women with an undetectable level of equol (<2.06 nmol/l).

intact. When we restricted the analysis to 61 women who had a natural menopause, the mean ± SD of serum E_2 concentrations was 44.0 ± 19.1 pmol/l (range 20.0–131 pmol/l). The findings on E_2, the E_2/SHBG ratio, isoflavonoids and soy intake in relation to BMD were not substantially altered; the correlation between E_2/SHBG ratio and BMD remained statistically significant ($r = 0.30$, $p = 0.03$) after controlling for the covariates.

Three women reported a history of osteoporosis but had never received any pharmacologic treatment, including supplementation of flavonoids. Exclusion of these women did not alter the results of the present study. For example, the correlations of BMD with the E_2/SHBG ratio and serum isoflavonoids were 0.40 and −0.14, respectively, after controlling for the covariates. We could not confirm whether flavonoid supplements had been used by women without a history of osteoporosis.

Discussion

We observed that the E_2/SHBG ratio, which may reflect the bioavailable estrogen level, was significantly associated with BMD. However, none of the serum isoflavone levels, or soy product and isoflavone intake, was associated with BMD, in spite of isoflavone's known estrogenicity.

A recent large randomized, double-masked, placebo-controlled 4-year study failed to show any effect of ipriflavone (600 mg/day) on BMD and fracture rates in postmenopausal women with osteoporosis [16].

Previously, two intervention studies examined the effect of dietary soy on BMD [17,18]. In a study reported by Potter et al. [17], diets with 90 mg, 56 mg, and 0 mg of soy isoflavones per day over 6 months increased BMD of the lumbar spine (L1–L4) in postmenopausal women by 2.2%, –0.2% and –0.6%, respectively. Only the diet with the highest isoflavone level showed a moderate but statistically significant increase in BMD compared with a control diet (with 0 mg isoflavone). In our study, the estimates of soy product and isoflavone intake may be underestimated by our questionnaire because, in the validity test, the soy product and isoflavone intake estimated from the 12 daily diet records over 1 year were about 25% higher than the estimates from the questionnaire. Even considering this bias, most of our women subjects may have had less than 90 mg of isoflavones per day. Their intake of isoflavones and, probably, serum isoflavonoid levels may have been too low to exhibit an estrogenic effect on bone. It is also possible that soy intake when peak bone mass was attained is the determinant of current BMD. Serum isoflavone levels cannot be a long-term markers of soy exposure. No long-term biomarkers are currently available. A dietary history approach to measure the more distant past would be important.

The magnitude of correlation between isoflavone intake and BMD in the present study was similar to that reported by Kimira et al. ($r = –0.09$) [7]. The association between isoflavone intake and BMD was not very strong even among the studies that reported positive associations [8–10]. Somekawa et al. [10] reported a relatively large association compared with those in the other studies: BMD was greater by 8% in women with a high intake of isoflavones (>65 mg/day; the mean is about 81 mg/day) compared with women with a low intake (<35 mg/day; the mean is about 29 mg/day). Our results were only obtained at the level of the calcaneus. Different compartments of the skeleton may have a different response to soy or isoflavone intake. A discrepancy in the results between the present study and those that reported significant positive associations [8–10] may be due to differences in the bone site used for BMD measurement, BMD measurement method, dietary assessment, range of soy or isoflavone intake

among study subjects, and selection of factors used for statistical adjustments.

Serum equol was not detectable in about half the women. The use of the minimum value for them might have affected the real association between serum equol and BMD or serum bone ALP. When we compared BMD and serum bone ALP between women with detectable and undetectable equol levels, there was a marginally significant difference in the mean BMD (356.3 vs 334.4, $p = 0.07$) between the two groups of women after controlling for age and the other covariates. We cannot rule out the possibility that equol, a metabolite of daidzein, may be positively associated with BMD.

It is generally recognized that estrogen deficiency plays a role in the genesis of postmenopausal bone loss. Estrogen therapy has been shown to reduce the rate of bone loss [13,18]. However, the contribution of an endogenous estrogen level after menopause to bone loss has not been studied in depth. The results from the studies evaluating the relationship between serum estrogen concentrations and BMD have been inconsistent. We observed a significant correlation between the E_2/SHBG ratio and BMD. Some previous studies also found a significant positive association between serum E_2 and BMD in postmenopausal women [19,20], but others did not [21,22].

The serum E_2/SHBG ratio and soy product intake or serum isoflavones did not confound each other in relation to BMD. The effect of currently circulating bioavailable estradiol on BMD may be not attenuated by soy intake or serum isoflavone levels.

Serum bone-specific ALP has been considered to be a marker for bone formation and to be associated with future bone loss. Estrogen administration suppresses the rise in the ALP level in postmenopausal women. In the present study, the E_2/SHBG ratio was inversely correlated with the serum ALP level, but the correlation was weak. Soy intake was not associated with serum bone ALP. Dietary soybean did not suppress the total or bone-specific ALP level in the ovariectomized rat model of osteoporosis [4,5].

Although the present study has limitations reflecting the nature of a cross-sectional study and the problems inherent in epidemiologic methods assessing nutritional status, the results support the association of BMD with endogenous estrogen levels; however, they do not support the association with soy or isoflavone intake and serum isoflavonoid levels.

Acknowledgements. This study was supported in part by grants 10670347 and 11470106 from the Ministry of Education, Science, Sports, and Culture, Japan.

References

1. Kurzer MS, Xu Xia. Dietary phytoestrogens. Annu Rev Nutr 1997;17:353–81.
2. Barrett J. Phytoestrogens: friends or foes? Environ Health Perspect 1996;104:478–82.
3. Gennari C. Ipriflavone: background. Calcif Tissue Int 1997; 61:S1–4.
4. Arjmandi BH, Alekel L, Hollis BW, et al. Dietary soybean protein prevents bone loss in an ovariectomized rat model of osteoporosis. J Nutr 1996;126:161–7.
5. Harrison E, Adjei A, Ameho C, Yamamoto S, Kono S. The effect of soybean protein on bone loss in a rat model of postmenopausal osteoporosis. J Nutr Sci Vitaminol 1998;44:257–68.
6. Yukawa H, Suzuki T, Shibata H, Amano H, Haga H, Yasumura S. Relationship between nutrient intake and bone mineral density in an urban community of healthy elderly women [Abstract]. Jpn J Public Health 1998;45:968–78.
7. Kimira M, Arai Y, Shimori K, Watanabe S. Japanese intake of flavonoids and isoflavonoids from foods. J Epidemiol 1998;8:168–75.
8. Tsuchida K, Mizushima S, Toba M, Soda K. Dietary soybeans intake and bone mineral density among 995 middle-aged women in Yokohama. J Epidemiol 1999;9:14–9.
9. Horiuchi T, Onouchi T, Takahashi M, Ito H, Orimo H. Effect of soy protein on bone metabolism in postmenopausal Japanese women. Osteoporos Int 2000;11:721–4.
10. Somekawa Y, Chiguchi M, Ishibashi T, Aso T. Soy intake related to menopausal symptoms, serum lipids, and bone mineral density in postmenopausal Japanese women. Obstet Gynecol 2001;97:109–15.
11. Wakai K, Egami I, Kato K, Kawakamura T, Tamakoshi A, Lin Y, et al. Dietary intake and sources of isoflavones among Japanese. Nutr Cancer 1999;33:139–45.
12. Shimizu H, Ohwaki A, Kurisu Y, et al. Validity and reproducibility of a quantitative food frequency questionnaire for a cohort study in Japan. Jpn J Clin Oncol 1999;29:38–44.
13. Suzuki I, Kawakami N, Shimizu H. Reliability and validity of a questionnaire for assessment of energy expenditure and physical activity in epidemiological studies. J Epidemiol 1998;8:152–9.
14. Coward L, Kirk M, Albin N, Barnes S. Analysis of plasma isoflavones by reversed-phase HPLC–multiple reaction ion monitoring–mass spectrometry. Clin Chim Acta 1996;247:121–42.
15. Willett W. Implications of total energy intake for epidemiological analyses. In: Willett W, editor. Nutritional epidemiology. Oxford; Oxford University Press, 1990:245–71.
16. Alexanderson P, Toussaint A, Christiansen C, et al. Ipriflavone in the treatment of postmenopausal osteoporosis: a randomized controlled trial. JAMA 2001;285:1482–8.
17. Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman Jr JW. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am J Clin Nutr 1998;68(Suppl):S1375–9.
18. Alkel DL, St Germain A, Peterson CT, Hanson KB, Stewart JW, Toda T. Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. Am J Clin Nutr 2000;72:844–52.
19. Murphy S, Khaw KT, Sneyd MJ, Compston JE. Endogenous sex hormones and bone mineral density among community-based postmenopausal women. Postgrad Med J 1992;68:908–13.
20. Hall GM, Perry LA, Spector TD. Depressed levels of dehydroepiandrosterone sulphate in postmenopausal women with rheumatoid arthritis but no relation with axial bone density. Ann Rheum Dis 1993;52:211–4.
21. Shono N, Kugino K, Yoshida S, Nakayama M, Ueno H, Nishizumi M. Bone mineral density by ultrasonic measurement in pre- and postmenopausal women: relationship with sex hormones and nutritional states. Jpn J Hyg 1997;51:755–62.
22. Akatsu T. Sex steroids, bone turnover and bone mineral density in pre-, peri-, and postmenopausal women. Environ Health Prev Med 1998:3:123–9.

Received for publication 13 August 2001
Accepted in revised form 3 October 2001