Table 1. Subject characteristics

n	379
Age (years)	63.0 (10.8)
Body weight (kg)	52.1 (7.3)
Body height (cm)	151.6 (6.0)
BMI (kg/m^2)	22.6 (2.8)
K_1 (nmol/l)	3.51 (2.70)
MK-4 (nmol/l)	0.20 (0.31)
MK-7 (nmol/l)	10.0 (15.1)
ucOC (ng/ml)	4.68 (3.15)
iOC (ng/ml)	8.69 (7.13)
25-OH-D (nmol/l)	51.8 (16.3)
iPTH (pmol/l)	4.9 (1.8)
Ca (mmol/l)	2.30 (0.10)
P (mmol/l)	1.12 (0.15)
BAP (U/l)	31.4 (11.2)
NTX (pmol BCE/μmol Cr)	57.3 (25.5)
L_{2-4} BMD (g/cm^2)	0.970 (0.186)
L_{2-4} Z-score	0.178 (1.405)
FN BMD (g/cm^2)[a]	0.750 (0.128)
FN BMD Z-score[a]	0.398 (0.857)

All values are mean (SD)
K_1, phylloquinone; MK, menaquinone; ucOC, undercarboxylated osteocalcin; iOC, intact osteocalcin; 25-OH-D, 25-hydroxyvitamin D; iPTH, intact parathyroid hormone, BAP, bone-derived alkaline phosphatase; NTX, N-terminal telopeptide; BCE, bone collagen equivalent; BMD, bone mineral density; L_{2-4}, lumbar spine$_{2-4}$; FN, femoral neck
[a]FN BMD and FN BMD Z-score were measured in 176 subjects
Plasma and urinary biochemical parameters were within the normal range

Results

Subject characteristics

The subject characteristics are summarized in Table 1. The plasma K_1, MK-4, and MK-7 concentrations (mean ± SD) of the 379 Japanese women were 3.51 ± 2.70, 0.20 ± 0.32, and 10.0 ± 15.1 nmol/l, respectively. Other plasma and urinary biochemical parameters were within the normal range. The location and number of incident fracture were as follows: vertebrae, 35 (9.2%); forearm, 8 (2.1%); femoral neck, 1 (0.3%); and others, 5 (1.3%). Because there were few cases of forearm and femoral neck fractures, the incidence of vertebral fracture was used to evaluate the association between vitamin K status and bone fracture.

Association between plasma vitamin K concentration and incidence of vertebral fracture

Table 2 shows the association between the incidence of vertebral fracture and age, anthropometric parameters, bone metabolic parameters, and plasma vitamin K concentrations. Age ($P < 0.001$) and BAP ($P = 0.011$) were associated positively, and L_{2-4} BMD ($P < 0.001$), K_1 ($P = 0.007$), and log K_1 ($P < 0.001$) were associated negatively with the incidence of vertebral fracture. MK-4 and MK-7 concentrations were not associated with the incidence of vertebral fracture. NTX and log ucOC showed a tendency to be positively associated with the incidence of vertebral fracture, and their P values were almost equal (NTX, $P = 0.089$; log ucOC, $P = 0.088$).

Table 2. Association between incidence of vertebral fracture and age, anthropometric parameters, bone metabolic parameters, and plasma vitamin K concentrations

	β-Coefficient	*P*
Age (years)	0.064	<0.001
BW (kg)	0.028	0.240
BH (cm)	−0.032	0.274
L_{2-4} BMD (g/cm^2)	−3.956	<0.001
NTX (pmol BCE/μmol Cr)	0.012	0.089
BAP (U/l)	0.042	0.011
ucOC (ng/ml)	0.057	0.271
ucOC/iOC	0.145	0.698
Log ucOC	0.487	0.088
Log ucOC/iOC	0.213	0.518
K_1 (nmol/l)	−0.244	0.007
MK-4 (nmol/l)	−0.345	0.602
MK-7 (nmol/l)	−0.005	0.672
Log K_1 (nmol/l)	−0.899	<0.001
Log MK-7 (nmol/l)	−0.057	0.672

Logistic regression analysis was used to test univariate associations of anthropometric or bone metabolic parameters and plasma vitamin K concentrations with incidence of vertebral fracture
Age and BAP were associated positively, and L2–4 BMD, K1, and log K1 were associated negatively with vertebral fracture incidence

Table 3. Relationship between vertebral fracture incidence and age, L_{2-4} BMD, BAP, or plasma vitamin K_1 concentration evaluated by stepwise multiple regression analysis

a. Plausible predictors (age, BAP and log K_1)

	Estimate	r^2	*P*
Age	0.050	0.055	0.017
Log K_1	−0.783	0.033	0.014
BAP	0.040	0.029	0.017

b. Plausible predictors (L_{2-4} BMD, BAP, and log K_1)

	Estimate	r^2	*P*
L_{2-4} BMD	−4.125	0.096	0.001
Log K_1	−0.760	0.033	0.017
BAP	0.036	0.022	0.039

Stepwise multiple linear regression analyses were performed to identify determinants of vertebral fracture incidence
The following plausible predictors were included in the original model: (1) age, BAP, and vitamin K_1 concentration (log K_1), (2) L_{2-4} BMD, BAP, and vitamin K_1 concentration (log K_1)
Variables that correlated strongly with each other, such as age and L_{2-4} BMD, were not entered simultaneously into the original model
Age, L_{2-4} BMD, BAP, and log K_1 concentration were independently associated with vertebral fracture incidence

Stepwise multiple linear regression analyses were performed to explore the determinants of vertebral fracture incidence. In both models, (1) age, BAP, and log K_1 and (2) L_{2-4} BMD, BAP, and log K_1were included in the original model, and age, L_{2-4}, BMD, BAP, and log K_1 concentration were independently associated with the incidence of vertebral fracture (Table 3). Moreover, a Cox proportional hazards model was used to assess the relationship between plasma K_1 concentration and vertebral fracture (Table 4). Hazard ratios and 95% confidence intervals are evaluated by no adjusted model or adjusted model for BMD or BMI. Both plasma K_1 concentration and log K_1 concentration

significantly decreased hazard ratio of vertebral fracture in the no adjusted model and adjusted model for BMD or BMI. Significant association between vitamin K_1 concentration and vertebral fracture was not observed in the age-adjusted model, because age and vitamin K_1 concentration became a strong confounding factor in the Cox proportional hazards model including a time course factor

Vertebral fracture incidence in low and high K_1 groups

Comparison of the incidence of vertebral fracture between the low and high K_1 groups was divided by the median plasma K_1 concentration (2.67 nmol/l) (Table 5). The incidence of vertebral fracture in the low K_1 group (n = 27, 14.4%) was significantly higher than that in the high K_1 group (n = 8, 4.2%), $P < 0.001$. The age of the low K_1 group was significantly higher than that of the high K_1 group. However, no significant difference was observed in L_{2-4} BMD between the two groups. The unadjusted RR for vertebral fractures in the low K_1 group was 3.43 [95% confidence interval (CI), 1.60–7.35) and the age-adjusted RR was 3.58 (95% CI, 3.26–3.93). No significant differences of plasma 25-OH-D (low K_1, 52.8 ± 17.3; high K_1, 51.0 ± 15.3 nmol/l) or PTH (low K_1, 3.3 ± 1.4; high K_1, 3.3 ± 1.2 pmol/l) concentrations were observed between the two groups. Moreover, the inverse prediction values of L_{2-4} BMD at which 25% of subjects would suffer fractures were estimated from logistic regression analysis in the two groups. The predicted L_{2-4} BMD in the low K_1 group was 0.707 (95% CI, 0.053–0.847, P = 0.007), and that in the high K_1 group was 0.578 (95% CI, 0.004–0.711, P = 0.003). These results suggest that subjects with low vitamin K status would suffer fractures at a higher BMD than those with high vitamin K status.

Table 4. Hazard ratio (HR) of vertebral fracture evaluated by Cox proportional hazards model

Variables	HR	95% CI	P	Adjustment
K_1	0.628	0.404–0.899	0.008	No
	0.691	0.453–0.982	0.038	BMD
	0.656	0.415–0.940	0.018	BMI
Log K_1	0.561	0.363–0.867	0.009	No
	0.612	0.397–0.948	0.028	BMD
	0.517	0.332–0.808	0.004	BMI

A Cox proportional hazards model was used to assess the relationship between plasma K_1 concentration and vertebral fracture; hazard ratios (HR) and 95% confidence intervals (CI) are evaluated by no adjusted model or adjusted model for BMD or body mass index (BMI)

Discussion

The associations between dietary vitamin K intake, biochemical indicators of vitamin K status such as plasma K_1 or ucOC concentration, and bone loss and risk of hip fracture were evaluated in several studies [3–10,25]. Low dietary K_1 intake has been reported to be associated with increased hip fracture risk, most notably in postmenopausal women [3,4]. In the Framingham Heart Study, low dietary K_1 intake was not associated with low BMD at either the hip or spine, even though low intake was associated with increased hip fracture risk [3]. However, in the Framingham Heart Study (1996–2000) [6], low plasma K_1 concentration after adjustment for plasma triglyceride concentration was associated with low BMD at the femoral neck among the men and low plasma K_1 concentration was associated with low spine BMD in postmenopausal women. In other studies, low dietary K_1 intake was associated with low BMD in women aged 29–86 years [5], and low plasma K_1 concentration was shown to be associated with low BMD at the spine [26]. The vitamin K concentration in elderly women with hip fractures was reported to be low [7–10]. Although an apparent relationship between vitamin K status and fracture risk has been reported, the relationship between BMD and vitamin K status is still controversial. Therefore, the mechanism(s) responsible for reducing fracture risk with high vitamin K intake or high serum level of vitamin K are not fully understood.

In the present study, the associations between plasma K_1, MK-4, and MK-7 concentrations and incidence of fracture were evaluated in Japanese women. The results showed a significant association between plasma K_1 concentration and incidence of vertebral fracture. Moreover, we could demonstrate that K_1 concentration was associated with vertebral fracture incidence independently of age, L_{2-4} BMD, and BAP. However, vitamin K status and femoral neck or other fractures could not be evaluated in the present population because of the lack of statistical power of these long

Table 5. Relative risk of vertebral fracture incidence in two groups divided by plasma vitamin K_1 concentration

Groups	n	Age	BMD	BAP	Incidence of vertebral fracture	RR (95% CI)	Age-adjusted RR (95% CI)
Low K_1	188	65.3 (12.1)	0.966 (0.195)	31.0 (11.7)	14.4%	3.43 (1.60–7.35)	3.58 (3.26–3.93)
High K_1	191	62.7 (10.1)	0.973 (0.177)	31.8 (10.7)	4.2%	1	1
P		0.020	0.708	0.478	<0.001		

Mean (SD)
Subjects were divided into two groups according to the median of plasma K_1 concentration (2.67 nmol/l)
Student's t test was used to compare the age of the two groups
Crude and age-adjusted relative risks (RRs) for the vertebral fracture incidence are presented with 95% confidence intervals
Crude and age-adjusted RRs for vertebral fracture incidence of the low K_1 group were significantly higher than those of the high K_1 group

bone fractures. In the present study, the numbers of incident femoral neck and forearm fractures were 1 and 8, respectively. A lower prevalence of hip fracture in the Japanese population than in Caucasians was reported [27]. Thus, evaluation of the role of vitamin K in long bone fracture in the Japanese population will require a larger sample size.

In a previous study, it was shown that high serum MK-7 concentration resulting from eating *natto*, which is a high-MK-7-content food, may contribute to the relatively low hip fracture risk in Japanese women [11]. However, in the present study, we did not find that plasma MK-7 concentration was associated with vertebral fracture incidence. It has been reported that MK-7 has equivalent potency regarding γ-carboxylation of OC to K_1 [28–30] and that *natto* intake promotes bone formation in premenopausal woman [12]. The reason why the association between MK-7 concentration and vertebral fracture was weaker than the associations between K_1 concentration and vertebral fracture is not clear. In a previous study [12], the association between the prevalence of femoral neck fracture and the consumption of *natto* was evaluated by comparison of the rate of the fracture between areas with and without the custom of eating *natto*. However, almost all subjects were *natto* eaters in the present study, which may be one of the reasons why no significant association between plasma MK-7 concentration and vertebral fracture incidence was observed. Moreover, a survey of the period or interval of MK-7-rich food intake seems more important than the measurement of serum MK-7 concentration for evaluating the relationship between bone metabolism and MK-7 in Japan. However, unfortunately, a food questionnaire was not employed in the present study, and this will be necessary in future.

Not only the circulating K_1 concentration but also the serum ucOC concentration has been reported to be associated with hip fracture [31–34]. We have reported that circulating K_1 and MK-7 concentrations were negatively correlated with the serum ucOC concentration; however, the level of vitamin K_1 or MK-7 required to reduce the serum ucOC concentration increased with advanced age [35]. In the present study, ucOC concentration or the ratio of ucOC/intactOC did not show a significant association with incident vertebral fracture. Recent studies revealed that vitamin K may play two important roles in bone metabolism, one of which is regulating posttranslational modification of Gla-containing proteins, and the other is regulating the SXR-mediated cellular regulatory system. Recently, Ichikawa et al. [36] reported that collagen accumulation in osteoblastic MG63 cells was enhanced by vitamin K_2 treatment, and the transcription of the extracellular matrix-related gene "*tsukushi*," which is involved in collagen assembly, was regulated by vitamin K_2 via steroid and xenobiotic receptor (SXR). Therefore, vitamin K plays a significant role in bone homeostasis, not only by affecting γ-carboxylation but also by affecting transcriptional regulation of the collagen gene, which may be one of the reasons why the association between ucOC and fracture incidence was weak as compared with that between K_1 and fracture.

In the second analysis, subjects were divided into low and high K_1 groups according to median K_1 concentration (2.67 nmol/l). The low K_1 group showed a higher incidence of vertebral fracture (Table 5). The age of the low K_1 group was also higher than that of the high K_1 group (Table 5). However, both the unadjusted and age-adjusted RRs demonstrated that risk of vertebral fracture was greater in the low K_1 status group. Moreover, L_{2-4} BMD was not different between the two groups, suggesting that K_1 status may be associated with vertebral bone strength, not with L_{2-4} BMD. The inverse predicted value of L_{2-4} BMD at which 25% of the subjects would suffer fractures was significantly higher in the low K_1 group. This finding suggests that subjects with low vitamin K_1 status would easily suffer fractures even with higher L_{2-4} BMD.

In the present study, the average of K_1 concentration was 3.51 nmol/l, and it was two or three times higher than previous reports. Averages of circulating K_1 concentrations in European or U.S. subjects have been reported approximately within the range of 0.7 to 1.7 nmol/l [6,10,37–42]. In other reports of Japanese subjects, 1.58 [26], 1.07 [13], 1.86 [43], and 2.66 [44] nmol/l K_1 concentrations were reported. Average of K_1 concentration in our other epidemiological study of Japanese elderly subjects was 1.71 nmol/l (data have not been published). Precision and accuracy of LC-APCI-MS/MS method used in present study to measure the vitamin K concentration had been confirmed by the HPLC fluorescence determination method [45]. Correlation coefficient and the corresponding P value for K_1 concentration determined by LC-APCI-MS/MS and HPLC fluorescence determination methods were $r = 0.989$ and $P < 0.001$ ($y = 0.841\ x + 0.035$ ng/ml; y, HPLC fluorescence determination method; x, LC-APCI-MS/MS method). From these results, the circulating K_1 concentration of Japanese subjects is considered to be higher than that of European or U.S. subjects, and dietary K_1 intake of Japanese people suggests that the K_1 intake in Japanese may be higher than that in Europe countries of the United States. The reason why the average K_1 concentration in the present study was particularly higher than other studies not only in Europe and the United States but also in Japan was not clear. Subjects were living in a rural area of Nagano. Most subjects have a backyard at their house, and they have the habit of frequently eating the vegetables that they cultivate in their backyard. Thus, although a food questionnaire was not employed in the present study, it is predicted that the dietary K_1 intake of present subjects may be relatively high.

There were some limitations of the present study. The design was a prospective study, but the participants were recruited from a hospital in a rural area of Japan (refer to the paper by Shiraki et al. [46] for the characteristics of this population). Thus, a nationwide prospective survey is required to assess the role of vitamin K in bone fractures conclusively in the near future. Although there were some limitations of the present study, it can be concluded that the incidence of vertebral fractures was associated with the plasma K_1 concentration. Because subjects with low vitamin K_1 status showed increased risk of vertebral fractures

regardless of their L_{2-4} BMD, low vitamin K_1 status may be an indicator of low bone quality.

Acknowledgments We thank Sanko Junyaku Co. Ltd. for measuring the plasma ucOC concentrations. We also thank Yuri Uchino, Yumiko Inoue, Kimi Ueta, and Seiji Hirokawa for technical assistance. None of the authors had any personal or financial conflict of interest. This work was partly supported by a Grant-in-Aid from the Japan Osteoporosis Foundation, a Grant-in-Aid 15590083 from the Ministry of Education, Science, Culture and Sports of Japan, a Grant-in-Aid for Comprehensive Research on Cardiovascular Diseases and the Research on the Dietary Reference Intakes in Japanese from the Ministry of Health, Labor and Welfare of Japan, and the First Project of Advanced Research and High Technology of Kobe Pharmaceutical University.

References

1. Ferland G (1998) The vitamin K-dependent proteins: an update. Nutr Rev 56:223–230
2. Binkley NC, Suttie JW (1995) Vitamin K nutrition and osteoporosis. J Nutr 125:1812–1821
3. Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, Wilson PW, 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
4. Feskanich D, Weber P, Willett WC, Rockett H, Booth SL, Colditz GA (1999) Vitamin K intake and hip fractures in women: a prospective study. Am J Clin Nutr 69:74–79
5. Booth SL, Broe KE, Gagnon DR, Tucker KL, Hannan MT, McLean RR, Dawson-Hughes B, Wilson PW, Cupples LA, Kiel DP (2003) Vitamin K intake and bone mineral density in women and men. Am J Clin Nutr 77:512–516
6. Booth SL, Broe KE, Peterson JW, Cheng DM, Dawson-Hughes B, Gundberg CM, Cupples LA, Wilson PW, Kiel DP (2004) Associations between vitamin K biochemical measures and bone mineral density in men and women. J Clin Endocrinol Metab 89:4904–4909
7. Hart JP, Catterall A, Dodds RA, Klenerman L, Shearer MJ, Bitensky L, Chayen J (1984) Circulating vitamin K_1 levels in fractured neck of femur. Lancet 2:283
8. Hart JP, Shearer MJ, Klenerman L, Catterall A, Reeve J, Sambrook PN, Dodds RA, Bitensky L, Chayen J (1985) Electrochemical detection of depressed circulating levels of vitamin K_1 in osteoporosis. J Clin Endocrinol Metab 60:1268–1269
9. Bitensky L, Hart JP, Catterall A, Hodges SJ, Pilkington MJ, Chayen J (1988) Circulating vitamin K levels in patients with fractures. J Bone Joint Surg Br 70:663–664
10. Hodges SJ, Akesson K, Vergnaud P, Obrant K, Delmas PD (1993) Circulating levels of vitamins K_1 and K_2 decreased in elderly women with hip fracture. J Bone Miner Res 8:1241–1245
11. Kaneki M, Hedges SJ, Hosoi T, Shiraki M, Ouchi Y, Orimo H (2001) Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K_2: possible implications for hip-fracture risk. Nutrition 17:315–321
12. Katsuyama H, Ideguchi S, Fukunaga M, Fukunaga T, Saijoh K, Sunami S (2004) Promotion of bone formation by fermented soybean (Natto) intake in premenopausal women. J Nutr Sci Vitaminol 50:114–120
13. Kawana K, Takahashi M, Hoshino H, Kushida K (2001) Circulating levels of vitamin K_1, menaquinone-4, and menaquinone-7 in healthy elderly Japanese women and patients with vertebral fractures and patients with hip fractures. Endocr Res 27:337–343
14. Shiraishi A, Higashi S, Masaki T, Saito M, Ito M, Ikeda S, Nakamura T (2002) A comparison of alfacalcidol and menatetrenone for the treatment of bone loss in an ovariectomized rat model of osteoporosis. Calcif Tissue Int 71:69–79
15. Mawatari T, Miura H, Higaki H, Moro-Oka T, Kurata K, Murakami T, Iwamoto Y (2000) Effect of vitamin K_2 on three-dimensional trabecular microarchitecture in ovariectomized rats. J Bone Miner Res 15:1810–1817
16. Iwamoto J, Yeh JK, Takeda T (2003) Effect of vitamin K_2 on cortical and cancellous bones in orchidectomized and/or sciatic neurectomized rats. J Bone Miner Res 18:776–783
17. Iwasaki Y, Yamato H, Murayama H, Sato M, Takahashi T, Ezawa I, Kurokawa K, Fukagawa M (2003) Combination use of vitamin K(2) further increases bone volume and ameliorates extremely low turnover bone induced by bisphosphonate therapy in tail-suspension rats. J Bone Miner Metab 21(3):154–160
18. Otomo H, Sakai A, Ikeda S, Tanaka S, Ito M, Phipps RJ, Nakamura T (2004) Regulation of mineral-to-matrix ratio of lumbar trabecular bone in ovariectomized rats treated with risedronate in combination with or without vitamin K_2. J Bone Miner Metab 22: 404–414
19. Matsunaga S, Ito H, Sakou T (1999) The effect of vitamin K and D supplementation on ovariectomy-induced bone loss. Calcif Tissue Int 65:285–289
20. Binkley NC, Krueger DC, Engelke JA, Foley AL, Suttie JW (2000) Vitamin K supplementation reduces serum concentrations of under-gamma-carboxylated osteocalcin in healthy young and elderly adults. Am J Clin Nutr 72:1523–1528
21. Tabb MM, Sun A, Zhou C, Grun F, Errandi J, Romero K, Pham H, Inoue S, Mallick S, Lin M, Forman BM, Blumberg B (2003) Vitamin K_2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. J Biol Chem 278:43919–43927
22. Suhara Y, Kamao M, Tsugawa N, Okano T (2005) Method for the determination of vitamin K homologues in human plasma using high-performance liquid chromatography-tandem mass spectrometry. Anal Chem 77:757–763
23. Shiraki M, Shiraki Y, Aoki C, Hosoi T, Inoue S, Kaneki M, Ouchi Y (1997) Association of bone mineral density with apolipoprotein E phenotype. J Bone Miner Res 12:1438–1445
24. Genant HK, Jergas M, Palermo L, Nevitt M, Valentin RS, Black D, Cummings SR (1996) Comparison of semiquantitative visual and quantitative morphometric assessment of prevalent and incident vertebral fractures in osteoporosis. The study of osteoporotic fractures Research Group. J Bone Miner Res 11:984–996
25. Fukunaga, M, Nakamura T. Shiraki M, Kuroda T, Ohta H, Hosoi T, Orimo H (2004) Absolute height reduction and percent height ratio of the vertebral body in incident fracture in Japanese women. J Bone Miner Metab 22:104–110
26. Kanai T, Takagi T, Masuhiro K, Nakamura M, Iwata M, Saji F (1996) Serum vitamin K level and bone mineral density in postmenopausal women. Int J Gynecol Obstet 56:25–30
27. Hagino H, Yamamoto K, Teshima R, Kishimoto H, Kuranobu K, Nakamura T (1990) The incidence of fractures of the proximal femur and the distal radius in Tottori prefecture, Japan. Arch Orthop Trauma Surg 109:43–44
28. Yamaguchi M, Uchiyama S, Tsukamoto Y (2003) Inhibitory effect of menaquinone-7 (vitamin K_2) on the bone-resorbing factors-induced bone resorption in elderly female rat femoral tissues in vitro. Mol Cell Biochem 245:115–120
29. Tsukamoto Y, Ichise H, Kakuda H, Yamaguchi M (2000) Intake of fermented soybean (natto) increases circulating vitamin K_2 (menaquinone-7) and gamma-carboxylated osteocalcin concentration in normal individuals. J Bone Miner Metab 18:216–222
30. Yamaguchi M, Taguchi H, Gao YH, Igarashi A, Tsukamoto Y (1999) Effect of vitamin K_2 (menaquinone-7) in fermented soybean (natto) on bone loss in ovariectomized rats. J Bone Miner Metab 17:23–29
31. Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K, Delmas PD (1997) Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. J Clin Endocrinol Metab 82:719–724
32. Szulc P, Chapuy MC, Meunier PJ, Delmas PD (1996) Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture: a three year follow-up study. Bone (NY) 18:487–488
33. Szulc P, Arlot M, Chapuy MC, Duboeuf F, Meunier PJ, Delmas PD (1994) Serum undercarboxylated osteocalcin correlates with hip bone mineral density in elderly women. J Bone Miner Res 9: 1591–1595
34. Szulc P, Chapuy MC, Meunier PJ, Delmas PD (1993) Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. J Clin Invest 91:1769–1774
35. Tsugawa N, Shiraki M, Suhara M, Kamao M, Tanaka K, Okano T (2006) Vitamin K status of healthy Japanese women: age-related

vitamin K requirement for γ-carboxylation of osteocalcin. Am J Clin Nutr 83:380–386
36. Ichikawa T, Horie-Inoue K, Ikeda K, Blumberg B, Inoue S (2006) Steroid and xenobiotic receptor SXR mediates vitamin K_2-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells. J Biol Chem 281: 16927–16934
37. Booth SL, Golly I, Sacheck JM, Roubenoff R, Dallal GE, Hamada K, Blumberg JB (2004) Effect of vitamin E supplementation on vitamin K status in adults with normal coagulation status. Am J Clin Nutr 80:143–148
38. Binkley NC, Krueger DC, Engelke JA, Foley AL, Suttie JW (2000) Vitamin K supplementation reduces serum concentrations of under-gamma-carboxylated osteocalcin in healthy young and elderly adults. Am J Clin Nutr 72:1523–1528
39. Binkley NC, Krueger DC, Kawahara TN, Engelke JA, Chappell RJ, Suttie JW (2002) A high phylloquinone intake is required to achieve maximal osteocalcin gamma-carboxylation. Am J Clin Nutr 76:1055–1060
40. Erkkila AT, Lichtenstein AH, Dolnikowski GG, Grusak MA, Jalbert SM, Aquino KA, Peterson JW, Booth SL (2004) Plasma transport of vitamin K in men using deuterium-labeled collard greens. Metabolism 53:215–221
41. Beavan SR, Prentice A, Stirling DM, Dibba B, Yan L, Harrington DJ, Shearer MJ (2005) Ethnic differences in osteocalcin gamma-carboxylation, plasma phylloquinone (vitamin K_1, and apolipoprotein E genotype. Eur J Clin Nutr 59:72–81
42. Martini LA, Booth SL, Saltzman E, do Rosario Dias de Oliveira Latorre M, Wood RJ (2006) Dietary phylloquinone depletion and repletion in postmenopausal women: effects on bone and mineral metabolism. Osteoporos Int 17:929–935
43. Kaneki M, Mizuno Y, Hosoi T, Inoue S, Hoshino S, Akishita M, Akedo Y, Horiki K, Nakamura T, Shiraki M (1995) Serum concentration of vitamin K in elderly women with involutional osteoporosis. Nippon Ronen Igakkai Zasshi 32:195–200
44. Shiraki M, Shiraki Y, Aoki C, Miura M (2000) Vitamin K_2 (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. J Bone Miner Res 15:515–521
45. Kamao M, Suhara Y, Tsugawa N, Okano T (2005) Determination of plasma vitamin K by high-performance liquid chromatography with fluorescence detection using vitamin K analogs as internal standards. J Chromatogr B Anal Technol Biomed Life Sci 816: 41–48
46. Shiraki M, Kuroda T, Nakamura T, Fukunaga M, Hosoi T, Orimo H, Makino K (2006) The sample size required for intervention studies on fracture prevention can be decreased by using a bone resorption marker in the inclusion criteria: prospective study of a subset of the Nagano Cohort, on behalf of the Adequate Treatment of Osteoporosis (A-TOP. Research Group. J Bone Miner Metab 24:219–225

Public Health Nutrition: 12(12), 2366–2370 doi:10.1017/S1368980009005084

Low calcium intake is associated with increased bone resorption in postmenopausal Japanese women: Yokogoshi Study

Kazutoshi Nakamura[1,*], Toshiko Saito[2], Akihiro Yoshihara[3], Miki Ishikawa[1], Yasuo Tsuchiya[1], Rieko Oshiki[4], Ryosaku Kobayashi[4], Keiko Maruyama[5], Keiko Hyodo[6], Mitsue Nashimoto[5], Naoko Tsugawa[7], Toshio Okano[7], Mari Oyama[1] and Masaharu Yamamoto[1]

[1]Department of Community Preventive Medicine, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-dori, Niigata City 951-8510, Japan: [2]Department of Health and Nutrition, Niigata University of Health and Welfare, 1398 Shimami-cho, Niigata City 951-3198, Japan: [3]Department of Oral Health Science, Niigata University Graduate School of Medical and Dental Sciences, 2-5274 Asahimachi-dori, Niigata City 951-8514, Japan: [4]Department of Physical Therapy, Niigata University of Health and Welfare, 1398 Shimami-cho, Niigata City 951-3198, Japan: [5]Department of Nursing, Niigata University of Health and Welfare, 1398 Shimami-cho, Niigata City 951-3198, Japan: [6]Department of Nursing, School of Health Sciences, Niigata University Faculty of Medicine, 2-746 Asahimachi-dori, Niigata City 951-8518, Japan: [7]Department of Hygienic Sciences, Kobe Pharmaceutical University, 4-19-1 Motoyamakita-cho, Higashinada-ku, Kobe City 658-8558, Japan

Submitted 8 July 2008: Accepted 7 January 2009: First published online 12 March 2009

Abstract

Objective: Low Ca intake is common among Japanese women, but its effect on bone metabolism has not been fully elucidated. The aim of the present study was to determine the relationship between Ca intake and serum markers of bone turnover in postmenopausal Japanese women.

Design: A cross-sectional study.

Setting: A community setting.

Subjects: Subjects were 595 home-dwelling postmenopausal Japanese women. Ca intake was assessed by a validated FFQ. Serum type I collagen cross-linked N-telopeptides (NTX) and osteocalcin were measured as markers of bone turnover. The relationships between demographic characteristics, lifestyles, serum Ca, vitamin D and intact serum parathyroid hormone and bone turnover were also assessed.

Results: The average age of the subjects was 64·5 (SD 5·8) years and the mean Ca intake was 527 (SD 160) mg/d. Ca intake was significantly associated with serum NTX ($P = 0{\cdot}0104$), but not with serum osteocalcin. Mean serum NTX concentration in the lowest quartile of Ca intake (<417 mg/d) was significantly higher than in the fourth, referent quartile. Among these Japanese postmenopausal women, very low Ca intake (less than ~400 mg/d) was associated with increased bone resorption but not bone formation.

Conclusions: Increased bone resorption may be one mechanism by which this Ca-depleted population normalizes bone metabolism and prevents osteoporosis.

Keywords
Bone metabolism
Bone resorption
Calcium intake
Postmenopause

Low Ca intake is recognized as a risk factor for osteoporosis and osteoporotic fractures in postmenopausal women. Daily Ca intake of at least 1200 mg is recommended for postmenopausal women[1,2]. However, Ca intake among Japanese women and other East Asian populations is typically well below present recommendations. The National Nutrition Survey of Japan (2003) showed that the average daily Ca intake of peri- and postmenopausal Japanese women was only 562 mg[3]. This is lower than present clinical guidelines and also lower than that of people in many European and North American countries. Nevertheless, the incidence of hip fractures in Japan has been estimated to be less than half that in the USA[4], suggesting that the prevalence of osteoporosis in Japan is also lower. This paradox may partly be explained by the fact that populations consuming Ca-depleted diets exhibit physiological adaptations that maintain normal bone metabolism. However, the relationship between Ca intake and bone

*Corresponding author: Email kazun@med.niigata-u.ac.jp

metabolism in populations with low Ca intake has not been well studied.

Previous studies have demonstrated that a higher rate of bone turnover leads to bone loss, disruption of trabecular networks and reduced connectivity, and that bone turnover markers indicating bone resorption predict subsequent osteoporotic fractures independent of bone mineral density[5]. In fact, high bone remodelling may be a primary cause of osteoporotic bone fragility[6]. Markers of bone turnover can thus be used to assess the relationship between Ca intake and clinically significant aspects of bone metabolism. The aim of the present study was to determine the relationship between Ca intake and two serum markers of bone turnover – serum type I collagen cross-linked N-telopeptides (NTX)[7,8] and osteocalcin[9] – among postmenopausal Japanese women with low Ca intake.

Subjects and methods

Subjects

On 31 March 2006, we targeted all 1310 women aged 55–74 years who lived in the town of Yokogoshi, Japan. Of these women, 667 women agreed to participate and underwent measurement of serum bone turnover markers in the baseline investigation of the Yokogoshi Study, a community-based, epidemiological study on bone health for postmenopausal women[10]. The medical examination was conducted in November 2005. All subjects were non-institutionalized, ambulatory and independent. The following women who had medical histories that may have affected bone metabolism were excluded from analysis: (i) twelve women with a history of bilateral oophorectomy; (ii) seven women who had undergone corticosteroid therapy; and (iii) fifty-three women treated with bisphosphonates, selective oestrogen receptor modulators, active vitamin D analogues, vitamin K (menatetrenone), oestrogen or calcitonin for suspected osteoporosis. Ultimately, 595 of 667 women were analysed. Written informed consent was received from all subjects. The study protocol was approved by the Ethics Committee of Niigata University School of Medicine. Further details of the Yokogoshi Study have been published elsewhere[10].

Biochemical measurements

A fasting blood specimen was drawn during the daytime, at least 6 h following the last ingestion of any food or drink. Each specimen was immediately maintained at 4°C and the serum was obtained within the day of collection by centrifugation at 1613*g* for 10 min.The specimen was subsequently stored at −80°C prior to biochemical analysis. Serum NTX concentration, a marker of bone resorption, was determined by ELISA (Osteomark NTX Serum; Ostex International, Inc., Seattle, WA, USA; reference value: 10·7–24·0 nmol BCE/l), which had an inter-assay CV of 2·8%. Serum osteocalcin concentration, a marker of bone formation, was determined by an immunoradiometric assay (Mitsubishi Kagaku Medical, Inc., Tokyo, Japan; reference value: 3·1–12·7 ng/ml) with an inter-assay CV of 6·6%. Serum vitamin D concentration, measured as 25-hydroxyvitamin D (25(OH)D), was determined by RIA (DiaSorin, Stillwater, MN, USA) with an inter-assay CV of 9·9%. Serum intact parathyroid hormone (PTH) concentration was measured with a two-site immunoradiometric assay (Nichols Institute Diagnostics, San Clemente, CA, USA), which has an inter-assay CV of 1·5%.

Other measurements

Age, medical history, reproductive history, current medication list and lifestyle information were obtained from all patients. Current Ca intake was assessed with a previously validated FFQ for the Japanese diet[11], with the correlation coefficient between values measured by this method and the conventional 3 d diet record being 0·668. Physical activity levels were assessed based on whether subjects engaged in the following activities at least once weekly: (i) light exercise, such as gate ball (or croquet), taking walks, etc., as light activity; and (ii) moderate exercise, such as farm work, gardening, etc., as moderate activity. Body height and weight of the subjects in light underwear were measured to the nearest 1 mm and 100 g, respectively. BMI was calculated by dividing body weight (kg) by the square of body height (m^2).

Statistical analysis

All continuous variables were assessed for normality. Serum NTX and intact PTH concentrations were skewed to higher values, and thus they were transformed logarithmically when conducting statistical tests. Categorical variables, such as 'light exercise' and 'moderate exercise', were coded as 0 for 'no' and 1 for 'yes'. Pearson's product moment correlation coefficients were calculated to evaluate an association between two continuous variables. Student's *t* test was used to test associations between physical activity measures and the two serum bone turnover markers. A stepwise method of multiple regression analysis was used to explore independent variables associated with outcome variables. Candidate predictor variables were significant variables obtained by the bivariate analyses. Analysis of covariance with Dunnett's multiple comparisons was used to compare one reference mean value with other mean values. Computations were performed using the SAS statistical software package release 8·02 (SAS Institute Inc., Cary, NC, USA). A *P* value less than 0·05 was considered statistically significant.

Results

Characteristics of the subjects are shown in Table 1. Regarding physical activity, 569 (95·6%) subjects engaged in light activity and 293 (49·2%) engaged in moderate activity. Serum markers of bone turnover may have an intra-day fluctuation. Nevertheless, there was no

significant difference in mean log-transformed serum NTX concentration between subjects who underwent blood collection in the morning and in the afternoon ($P = 0{\cdot}3234$). The mean serum osteocalcin concentration among samples collected in the afternoon (10·3 mg/ml) was significantly higher ($P = 0{\cdot}0486$) than among those collected in the morning (9·6 mg/ml).

Correlation coefficients between predictor variables and log-transformed serum NTX or osteocalcin concentration are shown in Table 2. Age, weight, BMI, Ca intake and log-transformed serum intact PTH concentration were significantly correlated with log-transformed serum NTX concentration. Years since menopause, weight, BMI and log-transformed serum intact PTH concentration were significantly correlated with serum osteocalcin concentration. None of the physical activity measures was associated with the log-transformed serum NTX or osteocalcin concentration. Additionally, a correlation coefficient between Ca intake and log-transformed serum intact PTH concentration was of borderline significance ($r = -0{\cdot}072$, $P = 0{\cdot}0776$).

Results of the stepwise multiple regression analysis are shown in Table 3. Age, weight, Ca intake and log-transformed serum intact PTH concentration were independently associated with log-transformed serum NTX concentration. BMI and log-transformed serum intact PTH concentration were independently associated with serum osteocalcin concentration.

Table 4 shows mean values of serum NTX and osteocalcin concentration according to quartile relative to the highest quartile of Ca intake. The lowest quartile of Ca intake had significantly lower log-transformed serum NTX concentrations. There was no significant association, however, between Ca intake and serum osteocalcin concentration.

Table 1 Demographic and laboratory characteristics of the study subjects: home-dwelling postmenopausal Japanese women (*n* 595), Yokogoshi Study, 2006

	n	Mean	SD
Age (years)	595	64·5	5·8
Years since menopause	578	14·5	6·9
Height (cm)	595	150·7	5·5
Weight (kg)	595	53·1	8·4
BMI (kg/m^2)	595	23·4	3·5
Total Ca intake* (mg/d)	595	527	160
Serum 25(OH)D (nmol/l)	595	55·5	14·6
Serum intact PTH (pmol/l)	595	4·25	1·40
Serum NTX (nmol BCE/l)	595	21·0	6·5
Serum osteocalcin (ng/ml)	595	9·94	3·95

25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; NTX, type I collagen cross-linked N-telopeptides.
*Ca intake from dietary sources was 518 (SD 146) mg/d.

Discussion

Previous research has shown that increased bone resorption markers are associated with increased fracture risk

Table 2 Correlation coefficients (*r*) between selected variables and serum NTX* and osteocalcin concentrations: home-dwelling postmenopausal Japanese women (*n* 595), Yokogoshi Study, 2006

	Serum NTX*		Serum osteocalcin	
Variable	*r*	*P* value	*r*	*P* value
Age (years)	0·081	0·0482	−0·019	0·6376
Years since menopause	0·004	0·9318	−0·084	0·0442
Height (cm)	−0·023	0·5703	−0·018	0·6574
Weight (kg)	−0·113	0·0060	−0·115	0·0049
BMI (kg/m^2)	−0·105	0·0102	−0·115	0·0051
Ca intake (mg/d)	−0·096	0·0194	−0·020	0·6200
Serum 25(OH)D (nmol/)	0·049	0·2355	−0·052	0·2038
Serum intact PTH* (pmol/l)	0·089	0·0302	0·100	0·0149

NTX, type I collagen cross-linked N-telopeptides; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.
*Logarithmically transformed.

Table 3 Results of a stepwise multiple linear regression analysis with log-transformed serum NTX and osteocalcin concentrations as outcomes: home-dwelling postmenopausal Japanese women (*n* 595), Yokogoshi Study, 2006

Independent variable	Regression coefficient (β)	SE	*P* value
Serum NTX*			
Age (years)	0·00407	0·00193	0·0353
Weight (kg)	−0·00374	0·00132	0·0048
Ca intake (mg/d)	−0·000179	0·0000695	0·0104
Serum intact PTH* (pmol/l)	0·07806	0·0342	0·0230
Serum osteocalcin			
BMI (kg/m^2)	−0·148	0·047	0·0017
Serum intact PTH* (pmol/l)	1·03	0·51	0·0457
Time of blood collection (0, morning; 1, afternoon)	0·726	0·327	0·0269

NTX, type I collagen cross-linked N-telopeptides; PTH, parathyroid hormone.
*Logarithmically transformed.

Table 4 Mean values of serum NTX concentration at each quartile of calcium intake: home-dwelling postmenopausal Japanese women (*n* 595), Yokogoshi Study, 2006

	Serum NTX* (nmol BCE/l)	
	Mean	95 % CI
Ca intake (mg/d)		
Q1 (<417)	22·6†	21·2, 24·0
Q2 (≥417, <525)	20·0	19·1, 20·9
Q3 (≥525, <619)	21·3	20·3, 22·3
Q4 (≥619)	20·2	19·4, 21·1 (reference)
	Adjusted *P* for trend = 0·0135	

NTX, type I collagen cross-linked N-telopeptides; Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile.
*Logarithmically transformed.
†Mean value was significantly different from that of reference group (analysis of covariance and Dunnett's multiple comparisons, with age, weight and log-transformed serum parathyroid hormone as covariates): $P < 0·05$.

independent of bone mineral density[5]. Therefore, the correlates of bone resorption are relevant to assessing the aetiology of osteoporotic fractures. In the present study, we measured serum NTX concentrations as a bone resorption marker. This approach is more robust because serum-based markers of bone turnover show less variability than urine-based markers[12] and serum NTX measurements can assess bone resorption with decreased intra-subject variability[7].

The present study demonstrated that Ca intake was inversely associated with serum NTX concentration. This finding has been reported previously by a small study[13] and is consistent with previous work showing that Ca supplementation reduces bone resorption[14–16]. Increased bone resorption by low Ca intake is hypothesized to be mediated through hyperparathyroidism induced by low Ca intake[16,17]. Our data, however, failed to demonstrate a significant association between Ca intake and intact PTH concentration, but rather showed that serum NTX concentration was associated with Ca intake independent of PTH. This suggests that a different pathophysiological mechanism may be responsible for the effects of low Ca intake on bone metabolism.

An additional key finding of the present study was that mean NTX concentration in the lowest quartile of Ca intake (<417 mg/d) was significantly lower than that in the highest, reference quartile (≥619 mg/d). Interestingly, this finding is in accordance with an epidemiological study demonstrating that the lowest quartile of Ca intake in peri- and postmenopausal Japanese women has a significantly increased risk of vertebral fracture than the highest, reference quartile[18]. Taken together, these data suggest that the lowest levels of Ca intake may be a particularly serious problem in this population.

Average daily Ca intake of most subjects in the present study was only 527 mg, lower than that recommended for peri- and postmenopausal women by the Ministry of Health and Welfare of Japan[19]. This is consistent with the National Nutrition Survey[3], which reported that Ca intake of Japanese persons aged 50–69 years was 568 mg/d on average, much lower than that of people in many European and North American countries. Nevertheless, only a quarter of women with very low Ca intake exhibited increased bone resorption in the present study. Japanese people historically have had a low-Ca diet[20] and therefore they may be physiologically adapted to low Ca through increased Ca absorption.

Although serum osteocalcin concentration is a major predictor of bone mineral density of the elderly[10,21], the strength of the association between serum osteocalcin concentration and fracture occurrence shows considerable variation[5]. In the present study, Ca intake was not associated with serum osteocalcin concentration.

The present study also showed that low body weight or BMI is associated with high bone resorption and formation markers. The inverse association between body weight (or BMI) and bone turnover markers has been reported by others[22–24]. A number of studies have also shown that low body weight is a major predictor of bone mineral density and bone loss of the elderly[21,25,26] and that this association may be mediated by increased bone turnover.

Exercise is known to affect bone turnover markers[27]. However, the present study did not find physical activity to be associated with bone turnover markers. One major reason for this may be that we obtained qualitative, rather than quantitative, data for physical activity, because assessment of physical activity in the elderly is difficult. Physical activity is one of the important determinants of bone health, and future studies should clarify this association in elderly populations.

One limitation of our study is that we measured only serum NTX and osteocalcin concentrations, which reflect only certain aspects of bone turnover or bone quality. Another limitation is that an FFQ is not an ideal method to evaluate Ca intake, although the FFQ used in our study was validated with improved accuracy over other FFQ. These limitations weaken the association of Ca intake with serum NTX concentration seen here.

In summary, the present study showed that very low Ca intake (less than ~400 mg/d) is associated with increased bone resorption in peri- and postmenopausal Japanese women. These results suggest that Ca supplementation programmes should focus on those women at highest risk. On the other hand, a population approach is also of interest because Ca intake in most adults in Japan is lower than current recommendations. Future studies are needed to develop interventions that effectively address low Ca intake among postmenopausal women.

Acknowledgements

The study was supported in part by a Grant-in-Aid for Scientific Research (C) No. 17590537 from the Ministry of Education, Culture, Sports, Science and Technology of Japan; a Grant-in-Aid of the Japan Medical Association

(2005); a grant from the Japan Osteoporosis Foundation (2007); and a grant from The National Dairy Promotion and Research Association of Japan (2007). The authors had no conflict of interest. K.N., A.Y., M.I. and M.Y. designed the study. T.S. was responsible for dietary assessment; K.M., K.H. and M.N. were responsible for demographic information and medical histories; R.O. and R.K. were responsible for physical activity assessment; Y.T., N.T. and T.O. were responsible for biochemical analyses; and M.O. was responsible for statistical analysis. K.N. was a principal author of the paper, and all authors reviewed the paper. We wish to thank the staff of the Health Promotion Division, Niigata City Yokogoshi Branch Office for their help in data collection. We are also indebted to Kyowa Medex Co., Ltd and Toyo Medic Inc. for the determination of serum 25(OH)D and bone mineral density measurements, respectively.

References

1. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine (1997) *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride.* Washington, DC: National Academy Press.
2. Dawson-Hughes B (1998) Vitamin D and calcium: recommended intake for bone health. *Osteoporos Int* **8**, Suppl. 2, S30–S34.
3. Society for Information on Health and Nutrition (2006) *The National Health and Nutrition Survey in Japan, 2003.* Tokyo: Daiichi Shuppan.
4. Fujita T (1994) Osteoporosis in Japan: factors contributing to the low incidence of hip fracture. *Adv Nutr Res* **9**, 89–99.
5. Garnero P (2000) Markers of bone turnover for the prediction of fracture risk. *Osteoporos Int* **11**, Suppl. 6, S55–S65.
6. Heaney RP (2003) Is the paradigm shifting? *Bone* **33**, 457–465.
7. Eastell R, Mallinak N, Weiss S, Ettinger M, Pettinger M, Cain D, Fressland K & Chesnut C III (2000) Biological variability of serum and urinary N-telopeptides of type I collagen in postmenopausal women. *J Bone Miner Res* **15**, 594–598.
8. Chaki O, Yoshikata I, Kikuchi R, Nakayama M, Uchiyama Y, Hirahara F & Gorai I (2000) The predictive value of biochemical markers of bone turnover for bone mineral density in postmenopausal Japanese women. *J Bone Miner Res* **15**, 1537–1544.
9. Delmas PD, Eastell R, Garnero P, Seibel MJ & Stepan J; Committee of Scientific Advisors of the International Osteoporosis Foundation (2000) The use of biochemical markers of bone turnover in osteoporosis. *Osteoporos Int* **11**, Suppl. 6, S2–S17.
10. Nakamura K, Tsugawa N, Saito T *et al.* (2008) Vitamin D status, bone mass, and bone metabolism in home-dwelling postmenopausal Japanese women: Yokogoshi Study. *Bone* **42**, 271–277.
11. Uenishi K, Ishida H & Nakamura K (2008) Development of a simple food frequency questionnaire to estimate intakes of calcium and other nutrients for the prevention and management of osteoporosis. *J Nutr Sci Vitaminol* **54**, 25–29.
12. Machado ABC, Hannon R & Eastell R (1999) Monitoring alendronate therapy for osteoporosis. *J Bone Miner Res* **14**, 602–608.
13. Nakamura K, Hori Y, Nashimoto M, Okuda Y, Miyazaki H, Kasai Y & Yamamoto M (2004) Dietary calcium, sodium, phosphorus, and protein, and bone metabolism in elderly Japanese women: a pilot study using the duplicate portion sampling method. *Nutrition* **20**, 340–345.
14. Shapses SA, Robins SP, Schwartz EI & Chowdhury H (1995) Short-term changes in calcium but not protein intake alter the rate of bone resorption in healthy subjects as assessed by urinary pyridinium cross-link excretion. *J Nutr* **125**, 2814–2821.
15. Scopacasa F, Horowitz M, Wishart JM, Need AG, Morris HA, Wittert G & Nordin BE (1998) Calcium supplementation suppresses bone resorption in early postmenopausal women. *Calcif Tissue Int* **62**, 8–11.
16. Fardellone P, Brazier M, Kamel S, Gueris J, Graulet AM, Lienard J & Sebert JL (1998) Biochemical effects of calcium supplementation in postmenopausal women: influence of dietary calcium intake. *Am J Clin Nutr* **67**, 1273–1278.
17. Riggs BL, O'Fallon WM, Muhs J, O'Connor MK, Kumar R & Melton LJ III (1998) Long-term effects of calcium supplementation on serum parathyroid hormone level, bone turnover, and bone loss in elderly women. *J Bone Miner Res* **13**, 168–174.
18. Nakamura K, Kurahashi N, Ishihara J, Inoue M & Tsugane S; Japan Public Health Centre-based Prospective Study Group (2009) Calcium intake and the 10-year incidence of self-reported vertebral fractures in women and men: the Japan Public Health Centre-based Prospective Study. *Br J Nutr* **101**, 285–294.
19. Ministry of Health, Labour, and Welfare, Japan (2004) *Dietary Reference Intakes for Japanese, 2005.* Tokyo: Ministry of Health, Labour, and Welfare, Japan.
20. Fujita T (1994) Osteoporosis in Japan: factors contributing to the low incidence of hip fracture. *Adv Nutr Res* **9**, 89–99.
21. Nakamura K, Saito T, Nishiwaki T, Ueno K, Nashimoto M, Okuda Y, Tsuchiya Y, Oshiki R, Muto K & Yamamoto M (2006) Correlations between bone mineral density and demographic, lifestyle, and biochemical parameters in community dwelling Japanese women 69 years of age and over. *Osteoporos Int* **17**, 1202–1207.
22. Ravn P, Cizza G, Bjarnason NH, Thompson D, Daley M, Wasnich RD, McClung M, Hosking D, Yates AJ & Christiansen C (1999) Low body mass index is an important risk factor for low bone mass and increased bone loss in early postmenopausal women. Early Postmenopausal Intervention Cohort (EPIC) study group. *J Bone Miner Res* **14**, 1622–1627.
23. Rogers A, Hannon RA & Eastell R (2000) Biochemical markers as predictors of rates of bone loss after menopause. *J Bone Miner Res* **15**, 1398–1404.
24. Papakitsou EF, Margioris AN, Dretakis KE, Trovas G, Zoras U, Lyritis G, Dretakis EK & Stergiopoulos K (2004) Body mass index (BMI) and parameters of bone formation and resorption in postmenopausal women. *Maturitas* **47**, 185–193.
25. Dargent-Molina P, Poitiers F & Breart G; EPIDOS Group (2000) In elderly women weight is the best predictor of a very low bone mineral density: evidence from the EPIDOS study. *Osteoporos Int* **11**, 881–888.
26. Ooms ME, Lips P, Van Lingen A & Valkenburg HA (1993) Determinants of bone mineral density and risk factors for osteoporosis in healthy elderly women. *J Bone Miner Res* **8**, 669–675.
27. Adami S, Gatti D, Viapiana O, Fiore CE, Nuti R, Luisetto G, Ponte M & Rossini M; BONTURNO Study Group (2008) Physical activity and bone turnover markers: a cross-sectional and a longitudinal study. *Calcif Tissue Int* **83**, 388–392.