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ORIGINAL ARTICLE

Vegetarianism, bone loss, fracture and vitamin D: a longitudinal study in Asian vegans and non-vegans

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Background/Objectives: The effect of vegan diet on bone loss has not been studied. The aim of this study was to examine the association between veganism and bone loss in postmenopausal women.
Subjects/Methods: The study was designed as a prospective longitudinal investigation with 210 women, including 105 vegans and 105 omnivores. Femoral neck (FN) bone mineral density (BMD) was measured in 2008 and 2010 by dual-energy X-ray absorptiometry (Hologic QDR4500). The incidence of vertebral fracture was ascertained by X-ray report. Serum levels of C-terminal telopeptide of type I collagen (βCTX) and N-terminal propeptide of type I procollagen (PINP) were measured by Roche Elecsys assays. Serum concentration of 25-hydroxyvitamin D and parathyroid hormone were measured by electrochemiluminescence.
Results: Among the 210 women who initially participated in the study in 2008, 181 women had completed the study and 29 women were lost to follow-up. The rate of loss in FN BMD was -1.91 ± 3.45%/year in omnivores and -0.86 ± 3.81%/year ($P = 0.08$) in vegans. Lower body weight, higher intakes of animal protein and lipid, and corticosteroid use were associated with greater rate of bone loss. The 2-year incidence of fracture was 5.7% ($n = 5/88$) in vegans, which was not significantly different from omnivores (5.4%, $n = 6/93$). There were no significant differences in βCTX and PINP between vegans and omnivores. The prevalence of vitamin D insufficiency in vegans was higher than in omnivores (73% versus 46%; $P = 0.0003$).
Conclusions: Vegan diet did not have adverse effect on bone loss and fracture. Corticosteroid use and high intakes of animal protein and animal lipid were negatively associated with bone loss.
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Introduction

Vegetarianism is increasingly popular in western societies. Recent estimates suggest that between 3 and 5% of the population (Gottfredson *et al*., 2005; Vinnari *et al*., 2009) is on vegetarian diets, and this proportion is increasing with time. In Asia, although there is no official statistics, the number of vegetarians is believed to be higher than in western countries. Vegetarianism is seen as a healthy lifestyle, because it has been suggested that individuals on vegetarian diets have lower risk of chronic diseases and lower risk of mortality than the general population, although the difference in mortality between vegetarian and non-vegetarian groups is a controversial issue (Chang-Claude *et al*., 2005; Key *et al*., 2009a, b).

Bone health among vegetarians has been a concern for some time. Although bone mineral density (BMD) in vegetarians, particularly vegans, is lower than non-vegetarians

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(Ho-Pham *et al*., 2009a); the risk of fracture in vegetarians is not different from that in non-vegetarians (Appleby *et al*., 2007). In a previous study, we have shown that Buddhist nuns strictly on vegan diet had similar BMD with non-vegetarians, despite the former group having much lower calcium intakes than the latter (Ho-Pham *et al*., 2009b). BMD in postmenopausal women is the sum of peak bone mass (achieved between the age of 20 and 30 years) and subsequent age-related bone loss (Riggs *et al*., 1998). However, there have been no longitudinal studies to assess the rate of bone loss among vegetarians. Bone loss is resulted from an imbalance between two opposing processes of bone formation and bone resorption, but few studies have examined the association between bone turnover markers and bone loss in vegetarians.

In this prospective study, we sought to assess the rate of bone loss and fracture risk, and their associations with bone turnover markers and vitamin D status in a group of vegans and omnivores.

Materials and methods

Study design and setting

The study setting was Ho Chi Minh City (formerly Saigon), a major city and an economic hub of Vietnam. The study was designed as a longitudinal investigation, which involved 20 monasteries and temples within the City. The temples were randomly selected from 286 temples and monasteries that are listed by a local Buddhist association. We sent letters of invitation to each monastery or temple to invite nuns over 50 years of age to participate in the study. In the next step, we obtained the electoral roll in each ward surrounding the temple, and then randomly selected households where there are female residents aged 50 years or above. We sent a letter of invitation to female members of the selected households. The women received free health check-up and bone density measurement, but they did not receive any financial incentive. The study protocol and procedures were approved by the scientific committee of the Pham Ngoc Thach University of Medicine, and written consent was obtained from all participants.

On average, between 5 and 6 nuns of each temple/monastery participated in the study. The nuns are strictly vegans because they are from the school of Mahayana Buddhism. Their diets do not include any product of animal or seafood origin. None of the participants had any diseases deemed to affect osteoporosis (such as hyperthyroidism, hyperparathyroidism, renal failure, malabsorption syndrome, alcoholism, chronic colitis, multiple myeloma, leukemia and chronic arthritis) or previous use of therapies that interfere with bone metabolism (for example, heparin, warfarin, thyroxin and estrogen). Each individual was examined twice: at baseline and follow-up visit. The baseline measurement was taken place in April and August 2008. The follow-up visit took place between April and July 2010.

Data collection

Clinical data including blood pressure, pulse and reproductive history (that is, parity, age of menarche and age of menopause) and medical history (that is, previous fracture, previous and current use of pharmacological therapies) were obtained by a standardized questionnaire. Blood pressure and pulse were measured by the investigators (for example, doctors) using a mercury sphygmomanometer. Blood pressure was taken after participants had been in a quiet room for 5 min and was measured three times. The average BP was used in the analysis. Heart rate was measured for one full minute with a stopwatch after blood pressure readings.

The questionnaire also solicited data on physical activity and lifestyle factors. Questions were asked concerning the average number of hours per day spent in each of five levels of activity based on similar questionnaire used. The five activities were: basal activity (sleeping or lying down), sedentary (sitting or standing), light (casual walking), moderate (gardening or carpentry) and heavy (lifting or heavy gardening). A weighting or intensity factor based on the approximate oxygen consumption needed for each level of activity was multiplied by the number of hours engaged in each activity, such as basal activity 1, sedentary 1.1, light 1.5, moderate 2.4 and heavy 5. The resulting products for all activities were then summed to yield an index of total physical activity. The women were asked to report their past and current cigarette smoking, alcohol use and coffee drinking history. Anthropometric parameters, including age, weight and standing height, were obtained. Body weight was measured by using an electronic balance with indoor clothing without shoes. Height was determined without shoes on a portable stadiometer with mandible plane parallel to the floor.

Nutrient analysis

Nutritional analysis was done at baseline. The participants were asked to fill-in a structured questionnaire for collecting data concerning 2-day dietary habits. The questionnaire includes eight broad food items, including rice, fish, red meat, white meat, egg, dairy-based food, vegetable and fruits. We used models, spoons and glasses of various sizes to help participants estimate their food intakes. The data were then entered into 'Eiyokun', a computer software specifically designed for analyzing nutritional components in Vietnamese food. The software was developed and validated by the Vietnam National Institute of Nutrition (Ho Chi Minh City, Vietnam). The nutrient estimates from this program include the amount of calories, animal and vegetable protein intakes, animal and vegetable lipids, carbohydrate, dietary calcium intake, phosphate, sodium, potassium and magnesium.

Fracture ascertainment

Vertebral fracture was assessed from X-ray report. Standard lateral and anterio–posterior lumbar spine (LS) radiographs

were taken with a 101.6-cm tube-to-film distance and were centered at L2. X-rays were taken at baseline and follow-up visits. Fracture was ascertained by the Genant's semiquantitative method with two independent readers (LTH-P and NDN; Genant *et al.*, 1993). The agreement between two readers was 89.6%. The semiquantitative criteria were also used to determine the severity of vertebral fractures (mild, moderate or severe). A fracture was considered mild (grade 1) if a reduction of 20–25% in vertebral anterior, middle and/or posterior height; moderate (grade 2) if a reduction of 25–40% in height; and severe (grade 3) if a reduction of $>40\%$ in height was observed.

BMD measurement

BMD at the LS, femoral neck (FN) and whole body was measured at baseline (2008) and subsequent visit (2010). The measurements were performed by the same dual-energy X-ray absorptiometry and the same technologist. The measurement was done with a dual-energy X-ray absorptiometry densitometer (Hologic QDR 4500, Hologic, Bedford, MA, USA). The precision error (% coefficient of variation (CV)) in our hospital was 2% for LS and 1.8% for FN BMD and 1.5% for whole-body BMD. The densitometer was standardized by standard phantom every time before measurement.

BMD was expressed in g/cm^2 or in *T*-score, which represents the number of standard deviations from the peak bone mass (taken as aged between 20 and 30 years). As there was a lack of population reference in BMD in Vietnam, we chose the Thai reference database for determining the *T*-score (Limpaphayom *et al.*, 2001). Using the World Health Organization criteria (Kanis, 2002), we classified women into two groups based on the *T*-score: those with osteoporosis if their *T*-scores were $\leqslant -2.5$, and those without osteoporosis if their *T*-scores were >-2.5.

Biochemical analysis

Blood samples were taken in the morning (0700–1200 hours) after a 12-h overnight fast, centrifuged at 20 °C and stored at −80 °C before assay. Fasting serum was obtained for total calcium, creatinine, liver enzymes, N-terminal propeptide of type I procollagen (PINP), C-terminal telopeptide of type I collagen (βCTX), parathyroid hormone and 25-hydroxyvitamin D (25(OH)D). Serum levels of βCTX were measured by the automated Roche beta crosslaps assay (Roche Diagnostics, Basel, Switzerland). Serum levels of PINP were also measured by the Roche Elecsys autoanalyzer, with intraassay CV of 1.2–4.1% and interassay CV of 3.7–5%. Concentrations of 25(OH)D and parathyroid hormone in serum were measured by the methods of electrochemiluminescence immunoassay and analyzed by Roche Elecsys 10100/201 system (Roche Diagnosis Elecsys, Roche Diagnostics). The sensitivity of the assay is 1.5 ng/ml, with an intraassay CV of 5.59% at 15.9 ng/ml and 11.62% at 58.9 ng/ml. The interassay CV at these two levels was 8.99 and 11.94%, respectively. All laboratory analyses were carried out in batches, with all samples from a single subject run in one assay. The same batch of the respective assay was used for all measurements.

Data analysis

Statistical analysis was aimed at testing the hypotheses of association between veganism and (a) bone loss and (b) fracture risk. To test the first hypothesis, the rate of change in BMD was calculated for each individual. Let BMD0 and BMD1 represent baseline and follow-up BMD measurements, respectively, the rate of change in BMD (denoted by ΔBMD) was determined by the formula: $\Delta\text{BMD} = (\text{BMD1} - \text{BMD0})/\text{BMD0} \times 100$. The analysis of covariance model was used to test for the difference in ΔBMD between vegans and non-vegans, with covariates being BMD0, age, body weight, bone turnover markers, vitamin D and nutritional factors. Given many covariates, the number of 'candidate models' can be very large. We used the Bayesian model average (Hoeting, 1999) to search for most parsimonious models. A logarithmic transformation was applied to the skewed data before the formal analysis of covariance. The association between a covariate and bone loss was assessed in terms of raw regression coefficient and standardized regression coefficient. The standardized regression coefficient allows for a direct comparison of importance between covariates, because it measures the change in the rate of bone loss per one standard deviation increase in a covariate.

The logistic regression model was used to test the second hypothesis (that is, association between veganism and vertebral fracture risk). In this model, the incidence of new vertebral fractures was modelled as a function of the study group (vegans versus omnivores), previous fracture, corticosteroid use, vitamin D, age and BMD. We did not attempt to fit a multivariable logistic regression model to the data, because the number of fracture is low relative to the number of parameters. All statistical analyses were performed with R Statistical Environment (Harrel, 2001; R Development Core Team, 2007).

Results

At baseline, 210 women (105 vegans and 105 omnivores) participated in the study. Two years later, 181 women (88 vegans and 93 omnivores) remained in the study. Thus, 29 women (13%) had dropped out from the study. The reasons for dropout were: death (five), not interested (nine), immobility and/or lack of transportation (five), emigration (four) and lost to follow-up (five). Compared with the analysis cohort, the lost-to-follow-up group was older (66 versus 61 years; $P=0.014$) and had lower LS BMD (0.70 and 0.77 g/cm^2; $P=0.014$; Table 1). There were no significant differences in other clinical characteristics between the two groups. For the analysis cohort ($n=181$), there were no significant differences in age, weight, height and BMD

between vegans and omnivores. However, compared with the omnivores, vegans had significantly lower dietary calcium intakes, total protein and lipid (Table 2).

Baseline profile: BMD, bone markers, lipids and vitamin D

Although BMD among vegans was slightly lower than omnivores, none of the differences were statistically significant (Table 3). Results of lipid analysis showed that mean total cholesterol levels were ~10% ($P=0.006$) lower in vegans compared with omnivores. However, there was no significant difference in triglyceride and leptin between vegans and omnivores.

There were also no significant differences in βCTX and PINP levels between vegans and omnivores. Vegans had 18% ($P<0.0001$) lower serum levels of 25(OH)D than omnivores.

Table 1 Baseline characteristics of participants stratified by follow-up status

	Complete follow-up (n = 181)	*Lost to follow-up (n = 29)*	P-*value*
Vegans (*n*, %)	88 (83.8)	17 (16.2)	
Omnivores (*n*, %)	93 (88.6)	12 (11.4)	
Age (years)	61 (9.2)	66 (10.4)	0.014
Lumbar spine BMD (g/cm^2)	0.77 (0.15)	0.70 (0.13)	0.019
Femoral neck BMD (g/cm^2)	0.63 (0.11)	0.59 (0.10)	0.062
Whole-body BMD (g/cm^2)	0.90 (0.11)	0.86 (0.12)	0.074

Abbreviation: BMD, bone mineral density.
Values represent mean (s.d.).

As expected, parathyroid hormone in vegans was higher than in omnivores, but the difference did not reach statistical significance ($P=0.09$). Using the criteria of 25(OH)D < 30 ng/ml (Holick, 2007), the prevalence of vitamin D insufficiency was 73% in vegans and 46% in omnivores ($P=0.0003$). Using the criteria of 25(OH)-D < 20 ng/ml (Holick, 2007), the prevalence of vitamin D deficiency was 27% in vegans, fourfold higher than omnivores (6.5%; $P=0.0002$; Table 4).

Rate of change in BMD

There was a site-dependent change in BMD. FN BMD decreased in vegans (mean ± s.d., −0.86 ± 3.81%/year), which was slightly lower than the decrease in omnivores (−1.91 ± 3.45%/year; $P=0.08$). LS BMD in vegans showed a slight increase (0.85 ± 4.94%/year), which was not significantly different from the rate observed in omnivores (0.89 ± 3.22%/year; $P=0.078$; Table 5).

Analysis by age revealed that the decline in FN BMD increased with advancing age, such that those aged 70+ years had a greater rate of bone loss (−1.03%/year) compared with those aged between 60 and 69 years (−0.54%/year). For LS BMD, the rate of change was also age dependent: −0.07%/year among those aged 50–59 years; 1%/year among those aged 60–69 years and 1.15%/year among those aged 70+ years.

At the LS, the rate of change in BMD was significantly related to age, lean mass, vegetable protein, animal fat and use of corticosteroid (Table 6). At the FN, the rate of change

Table 2 Baseline characteristics of participants stratified by follow-up status

	Vegans (n = 88)	*Omnivores (n = 93)*	P-*value*
Age (years)	60 (9)	61 (9)	0.617
Duration of vegan diet (years)[a]	34 (20, 43)	0	
Age at menopause (years)	48 (4.8)	49 (4.9)	0.103
Age at menarche (years)	15 (2.0)	15.0 (2.1)	0.439
Parity[b]	0.6 (1.7)	3.0 (2.0)	<0.001
Weight (kg)	53 (9)	54 (6)	0.556
Height (cm)	148 (6)	150 (5)	0.164
Body mass index (kg/m^2)	24 (3)	24 (3)	0.885
Lean mass (kg)	32.3 (4.7)	32.5 (3.4)	0.721
Fat mass (kg)	18.7 (5.2)	19.3 (4.4)	0.425
Percent body fat	34.6 (5.7)	35.6 (6.4)	0.300
Systolic blood pressure (mm Hg)	124 (19)	121 (915)	0.373
Diastolic blood pressure (mm Hg)	77 (10)	77 (9)	0.990
Pulse rate	77 (8)	76 (7)	0.174
Calcium intakes (mg/day)[a]	300 (182, 432)	590 (420, 763)	<0.0001
Total protein (mg/day)[a]	36 (28, 53)	62 (53, 73)	0.015
Total lipid (mg/day)[a]	21 (15, 32)	35 (28, 46)	<0.0001
Total calories (cal/day)[a]	1093 (870, 1286)	1429 (1246, 1726)	0.0005
Morning exercise (*n*, %)	67 (76.1)	75 (80.6)	0.461
Coffee drinking (*n*, %)	26 (29.5)	44 (47.3)	0.025
Alcohol use (*n*, %)	0	9 (9.7)	0.011

[a]Mean (25th and 75th percentile). For variables 'morning exercise', 'coffee drinking' and 'alcohol use', values are number of women and percentage of total sample size for each group.
[b]Twenty nuns were previously married and had children before becoming nun.
Values represent means (s.d.).

Table 3 Baseline BMD, vitamin D, PTH, lipids and hormones

	Vegans (n = 88)	*Omnivores (n = 93)*	*Difference (95% CI)*	P-*value*
Lumbar spine BMD (g/cm^2)	0.77 (0.14)	0.79 (0.13)	−0.02 (−0.06, 0.03)	0.401
Femoral neck BMD (g/cm^2)	0.62 (0.14)	0.64 (0.13)	−0.02 (−0.06, 0.02)	0.203
25(OH)D (ng/ml)	26.1 (7.4)	31.6 (6.9)	−5.6 (−7.6, −3.4)	<0.0001
PTH (ng/l)	45.8 (19.4)	40.5 (21.9)	5.3 (−0.8, 11.4)	0.089
Creatinine (μmol/l)	0.81 (0.13)	0.83 (0.13)	−0.015 (−0.053, 0.022)	0.429
Glucose (mmol/l)	4.94 (1.62)	4.79 (0.89)	0.14 (−0.24, 0.52)	0.455
Triglyceride (mmol/l)	2.20 (1.44)	1.87 (0.86)	0.33 (−0.02, 0.68)	0.062
C-reactive protein (mg/l)	3.23 (5.71)	2.52 (3.13)	0.71 (−0.63, 2.05)	0.301
Total cholesterol (mmol/l)	5.10 (1.07)	5.55 (1.10)	−0.45 (−0.77, −0.13)	0.006
Serum calcium (mmol/l)	1.51 (0.57)	1.22 (0.29)	0.29 (0.16, 0.43)	<0.0001
Leptin (ng/ml)	17.9 (15.4)	16.0 (10.7)	1.9 (−2.0, 5.7)	0.333
Serum βCTX (pg/ml)	486 (251)	476 (226)	10 (−59, 80)	0.774
PINP (ng/ml)	58.5 (25.4)	56.6 (32.8)	1.9 (−6.7, 10.6)	0.657

Abbreviations: BMD, bone mineral density; CI, confidence interval; PINP, amino-terminal propeptide of type I procollagen; PTH, parathyroid hormone; serum βCTX, serum carboxy-terminal telopeptide of type I collagen; 25(OH)D, 25-hydroxyvitamin D.
Values represent mean (s.d.).

Table 4 Prevalence of vitamin status in vegetarians and non-vegetarians

25(OH)D level (ng/ml)	*Vegans (n = 88)*	*Omnivores (n = 93)*	P-*value*
⩽20	24 (27.3)	6 (6.5)	0.0002
⩽25	41 (46.6)	15 (16.1)	<0.0001
⩽30	64 (72.7)	43 (46.2)	0.0003
⩽50	88 (100.0)	91 (97.9)	0.167

Abbreviation: 25(OH)D, 25-hydroxyvitamin D.
Values shown are number of women and group-specific percentage (in parentheses).

in BMD was associated with advancing age, lean mass and fat mass, animal fat and the animal protein: vegetable protein. These factors collectively accounted for 9% and 13% variance of change in LS and FN BMD, respectively.

Incidence of fractures

During the 2-year follow-up period, 10 women (5 vegans and 5 omnivores) had sustained a new vertebral fracture. There was no significant difference in the incidence of vertebral fracture between vegans and omnivores. Further analyses revealed that a personal history of previous fracture was associated with an increased risk of subsequent fracture. However, corticosteroid use, vitamin D deficiency and osteoporosis were not associated with increased fracture risk (Table 7).

Discussion

Bone health in vegetarians has been a subject of concern for some time, because on average they have lower BMD than non-vegetarians (Craig, 2009; Ho-Pham *et al*., 2009a), and lower BMD is a risk factor for fragility fracture (Kanis, 2002; Nguyen *et al*., 2005a, 2007b). However, there has been no longitudinal study to assess the association between vegetarianism and bone loss, which is also a risk factor for fragility fracture (Nguyen *et al*., 2005b). In this prospective study, we have shown that the rate of FN bone loss in vegans was not different from that in non-vegetarians. There was also no significant difference in fracture incidence between the two groups. These findings reaffirm the view that vegetarianism does not exert adverse effects on bone health.

Postmenopausal bone loss is a universal phenomenon. In Caucasian populations the rate of FN bone loss varied between 0.7 and 2%/year (Ensrud *et al*., 1995; Jones *et al*., 1994). Data from the present study showed that the rate of bone loss was ~1%/year, consistent with previous findings in Caucasian women (Nguyen *et al*., 2007a). However, we found that the loss of BMD was mainly observed in the hip, not at the LS. Actually, LS BMD tended to increase with advancing age, and this finding is also consistent with previous data in Caucasian women (Jones *et al*., 1994; Nguyen *et al*., 2005b). Although we are unsure of the differential changes in BMD, it is highly possible that the increase in LS BMD was artificially induced by osteophytosis (Jones *et al*., 1995), which is commonly found in individuals with osteoarthritis. However, we did not assess osteophytosis in our study, and as a result it is not possible to make inference on the magnitude of osteophytosis effect on change in LS BMD. Nevertheless, the present result confirms that BMD at the LS is not an ideal measure for the diagnosis of osteoporosis in postmenopausal women.

We found no significant effects on dietary calcium and vitamin D on bone loss. Dietary calcium intake in the present study's participants was relatively low, but it did not have adverse effect on bone loss. Indeed, the average dietary calcium intake among vegans was only 375 mg/day, much lower than the intakes observed in non-vegetarians (683 mg/day). In both groups, the dietary calcium intake was well below the recommended level of 1000 mg/day. Nevertheless, the low levels of dietary calcium did not have any adverse effect on either BMD or bone loss in both vegetarians and omnivores. Disturbingly, almost 3/4 vegans had 25(OH)D

Table 5 Rate of change (%/year) in BMD classified by group

	Vegans (n = 88)	*Omnivores (n = 93)*	*Difference (95% CI)*	P-*value*
Age (years)	61.7 (9.5)	61.6 (9.6)	0.08 (−2.53, 2.68)	0.954
Lumbar spine BMD (g/cm^2)	0.85 (4.94)	0.89 (3.22)	−0.04 (−1.36, 1.29)	0.958
Femoral neck BMD (g/cm^2)	−0.86 (3.81)	−1.91 (3.45)	1.04 (−0.12, 2.21)	0.080
Whole-body BMD (g/cm^2)	1.16 (2.38)	1.81 (3.56)	−0.64 (−1.63, 0.34)	0.197

Abbreviations: BMD, bone mineral density; CI, confidence interval.

Table 6 Determinants of changes in BMD: regression analysis

	Regression coefficient (s.e.)	*Standardized coefficient*	P-*value*
Lumbar spine BMD (g/cm^2)			
Age (years)	0.105 (0.034)	0.235	0.002
Lean mass (kg)	0.195 (0.074)	0.192	0.009
Use of corticosteroid (yes)	−1.879 (0.813)	−0.166	0.022
Vegetable protein (mg/day)	−0.075 (0.035)	−0.223	0.036
Vegetable fat (mg/day)	0.142 (0.045)	0.417	0.002
Femoral neck BMD (g/cm^2)			
Age (years)	−0.072 (0.038)	−0.137	0.051
Lean mass (kg)	0.277 (0.089)	0.234	0.002
Fat mass (kg)	0.183 (0.075)	0.182	0.016
Animal fat (kg)	−0.065 (0.030)	−0.170	0.028
Animal protein: vegetable protein ratio	−0.244 (0.094)	−0.192	0.01

Abbreviation: BMD, bone mineral density.
R^2 for lumbar spine BMD: 0.15 and femoral neck BMD: 0.18.

Table 7 Risk factors for new vertebral fractures

Risk factor	*Number of fracture/total*	*Incidence rate (%)*	*RR (95% CI)*
Group			
Omnivores	5/93	5.4	1.0
Vegans	5/88	5.7	1.02 (0.54, 1.92)
Previous fracture			
No	3/143	2.1	1.0
Yes	7/38	18.4	8.78 (2.38, 32.4)
Corticosteroid use			
No	7/153	4.6	1.0
Yes	3/28	10.7	2.34 (0.64, 8.52)
Vitamin D deficiency			
No	9/151	6.0	1.0
Yes	1/30	3.3	0.56 (0.01, 4.25)
Age group (years)			
50–59	4/97	4.1	1.0
60–69	3/45	6.7	1.62 (0.38, 6.92)
70+	3/39	7.7	1.87 (0.44, 7.95)
Osteoporosis			
No	7/154	4.5	1.0
Yes	3/27	11.1	2.76 (0.76, 10.0)

Abbreviations: CI, confidence interval; RR, relative risk.

levels at the level of insufficiency and more than a quarter at the level of deficiency. Although these prevalence rates were significantly higher than non-vegetarians, the difference did not seem to translate into adverse effect on bone density or bone loss. Indeed, we found no significant correlation between 25(OH)D and BMD or changes in BMD. Although the null association could be attributed to sample size, measurement errors of both BMD and 25(OH)D, and the duration of follow-up, the finding suggests that vitamin D may have modest effect, if any, on the rate of bone loss in postmenopausal women.

The rates of bone loss were highly variable among individuals, and the factors considered in this study explained a modest proportion of the variance. Apart from age, body composition measures (that is, lean mass and fat mass) had a 'positive' effect on bone loss, which seems to suggest that maintaining a stable body weight during postmenopausal period can be protective against bone loss (Nguyen *et al*., 1998, 2000). Interestingly, we found that animal lipid and the ratio of animal protein to vegetable protein had a significant effect on bone loss at the FN. Previous studies observed that higher intakes of animal protein had lower risk of fracture, but other studies found that individuals with greater intakes of animal proteins had a greater rate of bone loss (Sellmeyer *et al*., 2001) and increased risk of fracture (Feskanich *et al*., 1996). On the other hand, in the elderly, supplementation of protein may have a protective effect against hip fracture (Munger *et al*., 1999; Tylavsky and Anderson, 1988). Thus, our results when considered in relation to the existing literature suggest that higher intakes of animal proteins may have negative effect on bone health. This finding is consistent with the hypothesis that animal

protein produces a large amount of endogenous acid, which leads to increase bone resorption (Barzel and Massey, 1998) and increased bone loss.

Fracture is an ultimate outcome of osteoporosis. In this study, we found no significant difference in fracture incidence between vegans and omnivores. A previously larger study (Appleby *et al.*, 2007) found that although vegans had a slightly higher risk of fragility fracture than omnivores (relative risk 1.3), vegeterians as a group did not have greater risk of fracture than omnivores. It should be noted that in this study we considered only morphometric vertebral fracture, not any type of fracture. Moreover, the present study's sample size is modest and the duration of follow-up is relatively short, which might not be adequate to delineate a real effect of vegans on fracture risk.

Bone is a net result of two opposing processes of formation and resorption. Bone resorption and formation could be assessed by beta-cross-laps and PINP, respectively. In this study, we found that there was no significant difference in the markers between vegans and omnivores, and that there was no significant association between the markers and the rate of bone loss. Moreover, neither marker was significantly related to fracture risk. Taken together, these data again suggest that vegan diet does not exert negative effect on either bone resorption or bone formation.

The present study's findings must be interpreted within the context of strengths and weaknesses of the study. The prospective design of this study allows a better quantification of bone loss than cross-sectional studies. The dual-energy X-ray absorptiometry technology for measuring BMD is considered a gold standard for assessing skeletal health. However, the study could be biased toward the healthy group, as those who dropped out or lost to follow-up tended to have lower BMD than the group with complete follow-up. It can thus be argued that the present study may represent an underestimation of the rate of bone loss. The duration of follow-up (for example, 2 years) may be adequate for assessing change in BMD, but it may not be sufficient for assessing fracture risk largely because of the rarity of fracture in the general population. Moreover, most participants were from urban areas, which may not represent the true rate of bone loss in the general community.

In summary, this prospective study found no significant difference in the rate of bone loss between vegans and omnivores. Although vegans had a higher prevalence of vitamin D deficiency and lower dietary calcium intakes than omnivores, the two factors were not associated with bone loss. A high intake of animal protein and lipid may increase the rate of bone loss among postmenopausal women.

Conflict of interest

The authors declare no conflict of interest.

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