Young adult vegetarians in Shanghai have comparable bone health to omnivores despite lower serum 25(OH) vitamin D in vegans: a cross-sectional study

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Running title: Bone mineral density in young vegetarians

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ABSTRACT

Background and Objectives: The association between a vegetarian diet and bone mineral density (BMD) remains unclear, particularly in young adults. This study was designed to compare the bone health status of young vegetarians and omnivores in Shanghai, China. **Methods and Study Design:** A total of 246 vegetarians (following a vegan or lacto-ovo-vegetarian diet for at least 1 year) and 246 age- and sex-matched omnivores were recruited among young adult residents of Shanghai, China. The ultrasound bone mineral density analyser CM-200 was employed to measure calcaneus mineral densities, and blood samples were collected to determine serum 25-hydroxyvitamin D status. Intakes of protein, calcium and vitamin D were assessed by the 24-hour dietary recall method. **Results:** The average age of the vegetarians was 32.7±6.5 years, 83.3% of whom were female; 71.3% of the participants had been vegetarians for no more than 5 years. After adjusting for some potential cofounding factors, the serum 25-hydroxyvitamin D concentration of vegans (15.0±13.4 μg/L) was significantly lower than that of omnivores (17.6±8.8 μg/L, $p<0.05$). The protein, calcium and vitamin D intakes of vegetarians were all lower than those of omnivores ($p<0.05$). However, there was no significant difference in calcaneus mineral density between vegetarians and omnivores or between vegans and lacto-ovo vegetarians. **Conclusions:** Serum 25-hydroxyvitamin D concentrations in vegans, but not in lacto-ovo vegetarians, were slightly lower than those in omnivores. However, short-term vegetarian diets did not result in adverse effects on bone mineral density in young Chinese adults.

Key Words: vegetarian, vegan, bone mineral density, serum 25-hydroxyvitamin D, young adults

INTRODUCTION

A vegetarian diet is associated with several factors that may adversely affect bone health, such as an inadequate intake of animal protein, calcium and vitamin D. However, some food components such as magnesium, potassium, isoflavones and phytoestrogens that promote bone health are enriched in a vegetarian diet compared to an omnivore diet.[1,2]

Overall, the association between a vegetarian diet and bone health remains unclear, with conflicting findings from cross-sectional and longitudinal population-based studies published in recent years,[3-14] possibly due to the small sample sizes or characteristics of participants in previous studies. For example, the vegetarian participants of previous studies were mainly elderly,[3-6] Buddhists[5,7,8] and postmenopausal vegetarians,[4,7-11] whose bone mineral densities

(BMDs) may be strongly influenced by declines in physical function and changes in sex hormone levels. Few studies have focused on the bone health of young vegetarian adults, and the impact of a vegetarian diet on the bone mineral density of young adults is unclear.

As our previous research[15] found that a large proportion of young adults in China are adopting a vegetarian diet, it is thus of great importance to investigate the impact of a vegetarian diet on BMD among this population. Hence, this study was designed to examine the association between a vegetarian diet and BMD in young Chinese vegetarian adults.

MATERIALS AND METHODS

Participants and diet classification

Vegetarians were recruited for the study through online advertisements, local vegetarian societies, and vegetarian restaurants in Shanghai, China. The inclusion criteria were as follows: 1) age from 18 to 44 years; 2) Shanghai residence (living in Shanghai for more than half a year); 3) adoption of a vegetarian diet for at least 1 year; 4) no history of pregnancy within the preceding 12 months; and 5) an ability to understand the content of the questionnaires. The following were exclusion criteria: 1) participants with pre-diagnosed bone problems or malformation and 2) participants who had diseases or therapy known to affect bone metabolism. Omnivores were age- (±1 year) and sex-matched with vegetarians and recruited from among the vegetarians' relatives and friends who shared similar lifestyles apart from diet. Other inclusion and exclusion criteria were consistent with those for the vegetarians. All participants provided written informed consent, and they were invited to the Clinical Nutrition Department of Shanghai Xinhua hospital during March 2016 and May 2016 for the investigation. This study was approved by the Institutional Review Board of the Shanghai Jiao Tong University School of Medicine.

Participants completed demographics and personal behaviour questionnaires, including age, sex, education, work intensity, physical activity, alcohol consumption, cigarette smoking, vegetarian diet type (strict vegan, lacto-ovo vegetarian), and vegetarian duration. Participants were defined as vegetarians if they had followed a vegetarian diet at all meals on a daily basis persistently for at least 1 year; otherwise, they were considered omnivores. Among vegetarians, those who did not consume any animal products were defined as "vegans", whereas those who consumed eggs and/or dairy products were defined as "lacto-ovo vegetarians".

Anthropometric and dietary assessments

Anthropometric measurements, including height, body weight, waist circumference, and hip circumference, were obtained. Height was measured using a vertical measuring board at the floor level with no shoes. Participants were weighed on a hospital scale wearing indoor clothing but without shoes. Waist and hip circumference measurements were taken using a tape measure. All measurements were recorded to the nearest 0.1. Body mass index (BMI) was calculated from the measured weight and height as weight/height^2 (kg/m^2); waist-to-hip ratio (WHR) was calculated from the measured waist circumference/hip circumference.

A 24-hour diet recall questionnaire was conducted by a trained dietitian. All participants were required to recall the actual food and drink consumed in the last 24 hours. Oral descriptions, food images, and food models were provided by dietitians in the 24-hour dietary recall method to help the participants recall and estimate their dietary intake. Daily nutrient intakes were calculated from the 24-hour dietary recall data using Nutrition Calculator v2.5 software developed by the Institute for Nutrition and Food Safety of the Chinese Centre for Disease Control and Prevention and Beijing B-win Technology Co. Ltd. The nutrients estimated from this software include the daily intake of calories, protein, lipids, carbohydrate, dietary calcium, phosphate, sodium, potassium, magnesium, vitamin C, vitamin D, vitamin B6, and folate.

BMD measurements

Bone mineral density values (m/sec) were measured in the calcaneus area using the CM-200 ultrasound-bone mineral density analyser (Japan FURUNO Electric Co., Ltd). The CM-200 bone densitometer utilizes ultrasound to measure the speed of sound (SOS) in the calcaneus. The measurement is safe because the densitometer does not use radioactive X-rays. When using the CM-200, gel is applied to the heel, the foot is positioned, the cylinder is aligned, and the START key is pressed. Within 10 seconds, the result is printed from the onboard printer. The CM-200 densitometer contains the Chinese bone database, especially for Chinese males. The precision error (%CV) is less than 0.2% for detecting calcaneus mineral density. The BMD status is reflected by the SOS value (shown in metres per second) and T score, which represents the number of standard deviations from the peak bone mass (taken as aged between 20 and 30 years). The investigators recalibrated the machine before every measurement.

Using World Health Organization criteria, we classified the participants into three groups based on their T scores: scores lower than or equal to −2.5 indicated osteoporosis, scores

between −2.5 and -1 indicated osteopenia, and scores higher than or equal to -1 indicated normal bone density.

Blood sample collection

A venous blood sample from each participant was collected by a professional nurse after 12 hours of fasting. The serum 25(OH)D concentration of each participant was measured at ADICON Clinical Laboratories (Shanghai, China) and determined by Waters Xevo TQD (Q-Tof Premier, Quattro Micro API, ZQ 2000) mass spectrometry.

Statistical analysis

Data were analysed using SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as the mean ± SD, and categorical variables are presented as percentages. To compare differences between the vegetarian group and the omnivore group, paired t tests were performed for continuous variables, and McNemar tests were performed for matched categorical variables. To compare differences between the vegan group and the lacto-ovo-vegetarian group, independent-sample t-tests were applied for continuous variables and χ^2 tests for categorical variables. Differences in BMD values and serum 25-hydroxyvitamin D in vegans, lacto-ovo vegetarians and omnivores were assessed using analysis of covariate (ANCOVA) controlling for age, BMI, WHR, education level, work intensity, cigarette smoking, alcohol consumption, and physical activity. Differences in the extent of bone loss and the rate of former fractures among vegans, lacto-ovo vegetarians, and omnivores were examined by the Wilcoxon rank sum test and chi-squared test (Fisher's exact test), respectively.

Multiple linear regressions were employed to investigate correlations between dietary patterns, vegetarian duration, Buddhist vegetarian and BMD values. Multiple ordered logistic regression analysis was used to evaluate the significance of some possible associated nutrients regarding the extent of bone loss. All p values were calculated based on two-sided tests, and $p<0.05$ or less was considered statistically significant.

RESULTS

A total of 246 vegetarians and 246 omnivores were included in the study. Among the vegetarians, 62 (25.2%) were vegans, and 184 (74.8%) were lacto-ovo vegetarians. Of these, 41 (16.7%) were male, 205 (83.3%) were female, and their average time as a vegetarian was

5.1 years. The average ages of the vegetarians and omnivores were 32.7 and 32.1 years, respectively. Forty-three percent of the vegetarians were Buddhists.

Characteristics of participants

Compared to omnivores, vegetarians had smaller WHR values (0.8±0.1 and 0.8±0.1, $p<0.05$) and lower rates of alcohol consumption (18.3% and 5.7%, $p<0.05$). No difference was observed in baseline characteristics between the vegan and lacto-ovo vegetarian groups ($p>0.05$, Table 1).

Dietary intake

The results for daily nutrient intake among the various diet patterns are presented in Table 2. Protein intake was 36% lower in vegetarians compared to omnivores (45.4±19.8 and 70.0±31.2, g/day, $p<0.001$), and total energy intake among vegetarians was also significantly lower than that of omnivores (1495.2±516.9 and 1764.2±575.6, kcal/day, $p<0.001$). The calcium intake of vegetarians was 15% lower than that of omnivores (448.0±262.7 and 525.9±289.9, mg/day, $p<0.05$), and the average dietary vitamin D intake of vegetarians was significantly lower than that of omnivores (2.6±9.9 and 6.8±23.1, μg/day, $p<0.05$). Compared to omnivores, vegetarians generally had a lower intake of total lipids, phosphorous and vitamin B12. Moreover, comparison of dietary intake between vegans and lacto-ovo vegetarians showed that vegans had higher intake of potassium, magnesium, vitamin C and lower intake of cholesterol than did lacto-ovo vegetarians (all p values<0.05, Table 2).

Bone mineral density and serum 25(OH)D characteristics

Both vegetarians and omnivores had a lower average serum 25(OH)D than the normal range (normal range ≥30 μg/L), with no significant difference between the groups (Table 3). However, based on ANCOVA after controlling for age, BMI, WHR, education, work intensity, cigarette smoking and alcohol consumption, and physical activity, serum 25(OH)D was much lower in vegans, but not in lacto-ovo vegetarians, compared to omnivores (15.0±13.4 and 17.6±8.8, μg/L, $p<0.05$) .

The mean calcaneus mineral density of vegans was lower than that of the lacto-ovo vegetarians and omnivores (see the SOS values in Table 3), but a comparison among the three groups showed no significant difference after controlling for covariates. With regard to the distribution of the extent of bone mineral loss, the prevalence of osteoporosis in vegetarians and omnivores was 1.6% and 4.5%, respectively, but vegetarians were similar to omnivores in

terms of the prevalence of osteopenia or osteoporosis. In addition, there was no significant difference in the rate of fracture among the groups (all p values>0.05).

In multivariable analysis that included vegetarian type, vegetarian duration and Buddhist vegetarian as predictors (Table 4), no significant correlation was observed between either vegetarian type or vegetarian duration and BMD value; no significant difference in BMD value between Buddhist and non-Buddhist was observed either after adjusting for confounders. In multiple ordered logistic regression analysis, low calcium intake was found to slightly increase the risk of osteoporosis (OR: 0.998, 95% CI: 0.996-0.999, p=0.002, not shown in the table).

DISCUSSION

This study found no significant difference in bone mineral density between young vegetarians and omnivores or between young vegans and lacto-ovo vegetarians; furthermore, there was no significant difference in the prevalence of osteoporosis between these groups. This finding was in accordance with a study conducted in the United States,[14] which was the only reported study to investigate BMD values of young vegetarian adults. That study included 28 vegans, 27 lacto-ovo vegetarians, and 27 omnivores, and the BMDs of young vegetarians were similar to those of omnivores in America; however, the sample size was much smaller than that of our study. Moreover, our research provided additional evidence that a vegetarian diet had no adverse effect on BMD in young adults. Some previous studies[6-9, 13] focusing on vegetarians in other age groups have also demonstrated that BMDs of vegetarians and omnivores do not differ, though those studies mainly focused on participants who were elderly or postmenopausal vegetarians.

Regardless, some previous studies have found lower BMDs in vegans than in omnivores. For example, Chiu et al.[4] assessed the lumbar (L2-L4) and femoral neck bone mineral density of 258 postmenopausal female Buddhists in Taiwan and found that long-term (more than 15 years) vegans were at a high risk of having lumbar fractures and femoral neck bone loss. In a study including elderly Chinese female Buddhists who had a vegan diet for more than 30 years, Lau et al[5] reported that the bone mineral density at the hip was lower in vegetarians than in omnivores. Those studies mainly focused on long-term (at least 15-30 years) strict vegetarians. In comparison, lacto-ovo vegetarians were close to 75% of the total vegetarian group in our study. Nonetheless, after controlling for confounding factors, vegetarian type (vegan or lacto-ovo vegetarian) and vegetarian duration were not associated with BMD values for vegetarians in our study. In addition, previous studies mainly focused on elderly or

postmenopausal vegetarians reported that BMD values were strongly influenced by a decline in physical function or a change in sex hormone level. In our study, we focused on young adults with an average age of approximately 32 years old. It has been reported that individuals in this age group are in a period of peak bone mass,[16] and our study indicated that a vegetarian diet might not significantly impact BMD values for the young population at this age. Moreover, we found that most had positive personal behaviours, exercising more and drinking less than omnivores, and these factors are strongly associated with bone health.[17-19] However, in our study, personal behaviours were considered to be confounding factors and were adjusted when comparing BMD values among the diet groups; the finding was that BMD values were not significantly different among diet groups in these young adults.

Although our study found that a vegetarian diet was not a BMD risk factor among young vegetarians, the serum 25(OH)D concentration of vegans was lower than that of omnivores. Overall, intake of protein, calcium and vitamin D was lower than that in omnivores and lower than the recommended nutrient intake (RNI), though we did not find an association between serum 25(OH)D concentration or protein and vitamin D intake and BMD values after adjusting for confounding factors; calcium might have had a modest effect on the rate of bone loss. Some bone health-protective nutrients that are abundant in plants might explain these findings. Carotenoids[20] reportedly contribute to bone resorption via an antioxidant mechanism, and vitamin K[21] is essential for the activation of osteocalcin, which is the most abundant non-collagenous protein in bone. Moreover, soy isoflavones[22] attenuate osteoporotic bone loss by decreasing bone resorption and stimulating bone formation. However, to ensure bone health, we still recommended that young vegetarians, especially individuals who follow a vegan diet, increase their dietary intake of calcium and protein to prevent the possible adverse impact of ageing or insufficient nutrient intake. If possible, vegans could consider following a lacto-ovo vegetarian diet to help ensure that they have sufficient daily nutrient intake from their diet. Furthermore, because a vegetarian diet was not a BMD risk factor for young adults in our study and has a protective effect on obesity and related chronic diseases,[23-25] young adults with obesity or related metabolic disorders may be recommended to choose a vegetarian-style diet.

There were some limitations to the study. First, in terms of the dietary survey, the 24-hour diet recall method cannot reflect the habits of participants in food intake over time, which limits dietary data interpretation. Nonetheless, the 24-hour diet recall questionnaire was administered by trained dietitians, and the questionnaire quality was controlled to the greatest extent possible. Second, our study did not provide detailed food information which would

have allowed the evaluation of the role of individual protective foods (like tofu) or food categories on bone health. Third, the vegetarian participants in our study were convenience samples from voluntary registration online or restaurants, with voluntary registration first, which might constitute bias with regard to representing the true BMD status in the general vegetarian young adult community. However, the omnivores were carefully selected to match for age, sex and similar lifestyle to reduce confounding by known possible predictors of BMD: age and sex. Fourth, the study design was cross-sectional, and any cause-and-effect inference about the relationship between vegetarian diet and bone health was impossible. We intend to follow-up with the participants in our study and design a prospective longitudinal investigation to learn more about the long-term effects of a vegetarian diet on bone health.

Conclusion

Serum 25-hydroxyvitamin D concentrations in vegans, but not in lacto-ovo vegetarians, were slightly lower than those in omnivores. However, short-term vegetarian diets did not have any adverse effects on bone mineral density in young adults.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

All authors have read and approved the final manuscript. The authors have no competing interests.

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Table 1. Characteristics of participants by diet group

Variables	Vegetarians			Omnivores (n=246)
	Vegans (n=62)	Lacto-ovo vegetarians (n=184)	Total vegetarians (n=246)	
Age (y)	33.8±7.1	32.3±6.3	32.7±6.5	32.1±6.5
Vegetarian duration (y)	5.5±5.0	5.0±4.3	5.1±4.5	0
Height (cm)	163.5±8.4	162.3±6.7	162.6±7.2	162.7±7.2
Weight (kg)	55.4±9.3	54.9±7.6	55.0±8.1	55.5±8.6
BMI (kg/m^2)	20.6±2.4	20.8±2.5	20.8±2.5	20.9±2.7
WHR	0.8±0.1	0.8±0.1	0.8±0.1 †	0.8±0.1
Educational level (n, %)				
Elementary & secondary	11 (17.7)	16 (8.7)	27 (11.0)	28 (11.5%)
Vocational	13 (21.0)	28 (15.3)	41 (16.7)	46 (18.9%)
University and above	38 (61.3)	139 (76.0)	177 (72.3)	169 (69.6%)
Work intensity (n, %)				
Light	51 (82.3)	164 (89.1)	214 (87.3)	219 (89.4%)
Middle	9 (14.5)	12 (6.5)	21 (8.6)	17 (6.9%)
Heavy	2 (3.2)	8 (4.3)	10 (4.1)	9 (3.7%)
Exercise time (n, %)				
＜1 h/week	30 (48.4)	83 (45.1)	113 (45.9)	126 (51.2%)
1-4 h/week	21 (33.9)	80 (43.5)	101 (41.1)	103 (41.9%)
＞4 h/week	11 (17.7)	21 (11.4)	32 (13.0)	17 (6.9%)
Cigarette smoker (n, %)	9 (14.5)	15 (8.2)	24 (9.8)	18 (7.3)
Alcohol drinker (n, %)	1 (1.6)	13 (7.1)	14 (5.7)†	45 (18.3)

†Statistical significance in comparison of vegetarian and omnivore groups.

Table 2. Bone health-related nutrient intakes in different diet groups

Variables	Vegetarians			Omnivores (n=246)
	Vegans (n=62)	Lacto-ovo vegetarians (n=184)	Total vegetarians (n=246)	
Energy (kcal/d)	1526.0±552.8	1484.8±505.5	1495.2±516.9†	1764.2±575.6
Protein (g/d)	49.0±22.9	44.1±18.6	45.4±19.8†	70.0±31.2
Lipid (g/d)	37.5±18.4	43.1±21.7	41.7±21.0†	66.3±32.4
Carbohydrate (g/d)	240.0±103.5	223.9±83.1	228.0±88.7	216.5±80.1
Cholesterol (mg/d)	8.3±41.9	162.6±252.7‡	123.7±229.5†	415.0±346.1
Calcium (mg/d)	483.2±306.1	436.1±246.1	448.0±262.7†	525.9±289.9
Phosphorous (mg/d)	836.4±347.4	753.7±322.1	774.5±329.9†	988.6±379.4
Potassium (mg/d)	2123.0±1017.7	1721.5±764.8‡	1822.7±851.6	1960.8±826.0
Sodium (mg/d)	2494.4±1129.7	2718.6±1203.6	2662.1±1187.2	3733.9±1525.5
Magnesium (mg/d)	332.1±165.4	283.7±147.6‡	295.9±153.4	289.4±125.5
Vitamin C (mg/d)	147.4±106.4	109.5±93.1‡	119.1±97.8	105.5±81.6
Vitamin D (μg/d)	1.61±8.7	2.9±10.3	2.6±9.9†	6.8±23.1
Vitamin B-12 (μg/d)	0.1±0.5	0.5±0.7	0.4±0.7†	3.9±7.2

†Statistical significance in comparison of vegetarian and omnivore groups.
‡Statistical significance in comparison of vegan and lacto-ovo-vegetarian groups.

Table 3. Bone health-related factors in different diet groups

Variables	Vegetarians			Omnivores (n=246)
	Vegans (n=62)	Lacto-ovo vegetarians (n=184)	Total vegetarians (n=246)	
Serum 25(OH) VD (ng/ml)	15.0±13.4†	16.4±8.8	16.1±10.1	17.6±8.8
Calcaneus SOS value (m/sec)	1517.6±31.8	1518.8±31.0	1518.5±31.0	1518.6±33.1
Extent of bone mineral loss (n, %)				
Normal	30 (48.4)	102 (55.4)	132 (53.7)	134 (54.5)
Osteopenia	32 (51.6)	78 (42.4)	110 (44.7)	101 (41.0)
Osteoporosis	0	4 (2.2)	4 (1.6)	11 (4.5)
Formerly fracture rate (n, %)	4 (6.5)	20 (10.9)	24	39 (15.9)

† Statistical significance compared to the omnivore group, p=0.047

Table 4. Multiple regression analysis for BMD†

Variables	Model 1	Model 2
Vegetarian types‡		
Vegans	-0.931 (-9.870, 8.008)	-1.529 (-12.441, 9.383)
Lacto-ovo vegetarians	0.287 (-5.844, 6.418)	-0.074 (-8.383, 8.236)
Vegetarian duration	0.278 (0.589, 1.144)	0.088 (-0.816, 0.992)
Buddhist vegetarian	3.227 (-4.641, 11.096)	4.532 (-3.673, 12.738)

†Values represent the β coefficient (95% CI). Model 1, unadjusted regression; model 2, regression with confounding factors adjusted including BMI, WHR, physical activity, alcohol consumption, serum 25-hydroxyvitamin D, daily dietary intake (protein, calcium and vitamin D).
‡Omnivores were the reference group.