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ORIGINAL ARTICLE

Favorable effect of dietary vitamin C on bone mineral density in postmenopausal women (KNHANES IV, 2009): discrepancies regarding skeletal sites, age, and vitamin D status

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Abstract
Summary Dietary vitamin C intake showed significant positive associations with BMD in postmenopausal women, especially with vitamin D deficiency.
Introduction Although there is a positive role of vitamin C in osteoblastogenesis, debate remains about the contribution of vitamin C to bone mineral density (BMD) in humans.
Methods Data were derived from the Fourth Korean National Health and Nutrition Examination Survey. Dietary information was assessed using a 24-h dietary recall questionnaire. BMD was measured by dual-energy X-ray absorptiometry at the lumbar and hip.
Results A total of 1,196 postmenopausal women aged 50 years and older were stratified into tertiles by daily dietary vitamin C intake. After adjusting for traditional confounders, dietary vitamin C intake tertile was significantly positively associated with BMD at all sites (R=0.513 for lumbar spine (LS) and R=0.657 for femoral neck (FN), P<0.05 for each). The subjects with osteoporosis had significantly lower dietary vitamin C intake than did subjects without osteoporosis (74.4±66.2 vs 94.1±78.6 mg/day for LS and 65.5±56.6 vs 94.3±79.2 mg/day for FN, respectively, P<0.001). The multiple-adjusted odds ratio for osteoporosis for dietary vitamin C <100 mg/day was 1.790 (95 % CI 1.333–2.405, P<0.001). However, the significant association between vitamin C intake and BMD was only observed in subjects with vitamin D deficiency and aged 50–59 years or >70 years.
Conclusion Dietary vitamin C intake was positively associated with BMD in postmenopausal women, and inadequate vitamin C intake could increase the risk of osteoporosis.

Keywords Bone density · Menopause · Osteoporosis · Vitamin C · Vitamin D

Introduction

Osteoporosis is a major public health problem worldwide because of the increased risk of fragility fracture and chronic disability later in life. The lifetime risk of osteoporotic fractures has been estimated as 40 % for white women aged 50 years in the USA [1, 2]. The incidence of hip fractures is increasing worldwide by 1–3 % per year and is expected to total 8.2 million in 2050 [3].

Several pathological mechanisms are involved in age-related bone loss. Menopause, in particular, is the most important predisposing factor for bone loss because of the rapid decline in the production of estrogen, a hormone that inhibits bone resorption in premenopausal women [4]. Oxidative stress is also thought to contribute to bone loss during aging in both women and men [5]. Accumulating data suggests that oxidative stress could induce bone loss by osteoclast

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activation [6] and osteoblast suppression [7]. Vitamin C is a well-known antioxidant and acts as a scavenger of superoxide anion and hydrogen peroxide [8, 9]. Administration of vitamin C in vivo prevented bone loss with decreased reactive oxygen species [10–12]. Some studies of humans also showed that high plasma levels or dietary intake of antioxidants is associated with increased bone mineral density [13–15]. In addition, vitamin C is a cofactor in collagen formation and hydroxylation of lysine and proline [16], which are important for bone maintenance [17]. This nutrient also stimulates in vitro osteoblast differentiation and alkaline phosphatase activity, thereby contributing to bone formation [18, 19]. In addition, some animal studies also showed the importance of vitamin C in skeletal development and homeostasis [20, 21]. Given the favorable roles of vitamin C on bone cells and its capacity as an antioxidant, we reasoned that dietary vitamin C might play a protective role against postmenopausal bone loss in humans.

Several epidemiological studies have reported a conflicting relationship between dietary vitamin C intake and bone mineral density (BMD) in postmenopausal women [22–27, 14]. The Women's Health Initiative Study reported no independent association between dietary vitamin C and BMD [28]. By contrast, some studies have reported a positive relationship between dietary vitamin C and BMD, but this was significant only in women with a high calcium intake [24, 29, 30], those taking estrogen therapy [29], or smokers [31, 32]. Vitamin C is well tolerated and is easily taken from food and from supplement. Definite information about the effects of vitamin C on bone is important for establishing practical strategies for preventing bone loss in postmenopausal women.

The primary aim of this study was to investigate the association between dietary vitamin C intake and bone loss in postmenopausal women using the data from the Korea National Health and Nutrition Examination Survey IV (KNHANES IV). We also investigated whether the effect of dietary vitamin C on bone could be related to vitamin D status or age.

Subjects and methods

Subjects

This cross-sectional study was based on public domain data from KNHANES IV performed in 2009. KNHANES has been conducted periodically for noninstitutionalized Koreans by the Korea Centers for Disease Control and Prevention since 1998. The data contain a health interview, nutritional survey, and health examination. Multistage probability design was used to select the household units [33]. The survey was conducted on subjects aged 19 years and older who were not pregnant and had been chosen from a randomly selected representative household through household interviews and by direct standardized physical examinations in mobile examination centers. All participants provided written informed consent. The institutional review board of the Korea Centers for Disease Control and Prevention approved the study protocol. We acquired data for 5,690 women and 4,843 men from the second year (2009) of the KNHANES IV. Among them, 2,073 women aged 50 years and older were included. Subjects were excluded if they were premenopausal (n=138), took osteoporosis medication (n=214), had renal insufficiency (serum creatinine (Cr) concentration ≥1.4 mg/dL, n=210), used estrogen or progesterone (n=211), or had missing data about dietary vitamin C intake (n=104). A total of 1,196 postmenopausal women were finally included in the analysis.

Dietary assessment

Nutrient intakes, including total calorie and calcium intake, were assessed using a 24-h dietary recall questionnaire administered by a trained dietician. The results were calculated using the Food Composition Table provided by the National Rural Resources Development Institute (seventh revision).

BMD measurement

BMD (g/cm^2) was measured at the lumbar spine (LS; L1–L4), femoral neck (FN), and total hip (TH) using dual-energy X-ray absorptiometry (DXA, QDR4500A; Hologic Inc., Bedford, MA, USA). The DXA equipment was housed in the mobile examination centers. The left hip was scanned routinely, but in participants with a left hip fracture or device, the right hip was scanned. Subjects with T-score ≤−2.5 at each skeletal site were defined as having osteoporosis at the corresponding site. The DXA data were analyzed using Hologic Discovery software version 12.1 in its default configuration.

Laboratory measurements

Participants fasted for 8 h, and blood samples were collected during the survey. The samples were immediately refrigerated and transported in cold storage to the Central Testing Institute in Seoul Korea (NeoDin Medical Institute, Seoul, Korea). All samples were analyzed within 24 h after transportation. Serum 25-hydroxyvitamin D [25(OH)D] concentration was measured with a radioimmunoassay (RIA, DiaSorin Inc., Stillwater, MN, USA) using a gamma counter (1470 Wizard; PerkinElmer, Turku, Finland). The interassay coefficient of variation (CV) was 2.8 to 6.2 %. The concentration of 25(OH)D was measured in the same institute for all samples to minimize analytical variation. We converted 25(OH)D concentration from nanogram per milliliter to nanomole per liter by multiplying the concentration by 2.49. Serum-intact parathyroid hormone (PTH) concentration was measured using a chemiluminescence assay (DiaSorin Inc., Stillwater, MN,

USA). The CV of intact PTH concentration was 6.2 %. Serum Cr concentration was obtained using a kinetic compensated Jaffe assay (Roche Diagnostics Ltd., Lewis, UK).

Statistical analysis

The total sample was stratified into tertiles according to dietary vitamin C intake. Characteristics of the participants, including dietary intake and fracture history, were compared between vitamin C tertiles using one-way analysis of variance (ANOVA) and chi-square test. The differences between groups were evaluated further with Tukey's post hoc analysis. To minimize the effects of confounding variables, the differences in BMD at each site between tertiles of dietary vitamin C intake were estimated using analysis of covariance (ANCOVA) after adjusting for age, body mass index (BMI), total energy intake, smoking, monthly income, and 25(OH)D concentration. Monthly household income was categorized into quartiles: lowest (<USD 500), medium-lowest (USD 500–1,000), medium-highest (USD 1,000–3,000), and highest (≥USD 3,000). Subjects with lower and higher T-scores at each site were compared using an independent *t* test. To analyze dietary vitamin C intake, ANCOVA was used after adjusting for age, BMI, serum 25(OH)D concentration, smoking, monthly income, and total energy intake.

To investigate the effect of potential confounding factors on BMD, age and 25(OH)D concentration were stratified further. Age was stratified as 50–59, 60–69, and ≥70 years. For dietary vitamin C intake, we used binary categories divided by 100 mg/day for the recommended dietary allowance (RDA) of vitamin C in Korea [34] instead of the tertile categories. After adjusting for age, BMI, serum 25(OH)D concentration, smoking, monthly income, and total energy intake, the BMD at each site was compared between groups with high or low dietary vitamin C intake grouped according to 25(OH)D concentration (<50 and ≥50 nmol/L). The prevalence of osteoporosis after adjusting for BMI, serum 25(OH)D concentration, smoking, monthly income, and total energy intake was evaluated for groups with high or low dietary vitamin C intake according to age (50–59, 60–69, and ≥70 years).

Results

Baseline characteristics of the study participants

A total of 1,196 postmenopausal women aged ≥50 years were included; their mean age was 65.2±9.0 years, BMI was 24.3±3.3 kg/m^2, and mean vitamin C intake was 86.8±74.8 mg/day. The subjects' characteristics grouped by tertiles of dietary vitamin C intake are shown in Table 1. The lowest tertile was defined as dietary vitamin C intake <46.9 mg/day, the middle as ≥46.9 and <92.6 mg/day, and the highest as ≥92.6 mg/day. The study participants in the first tertile were older than those in the second and third tertiles (68.2±9.2, 64.6±8.9, and 62.7±8.1 years, respectively, $P<0.001$). Women in the first tertile of vitamin C intake had the lowest total energy intake (1,194.1±474.5, 1,526.2±649.3, and 1,754.5±599.5 kcal/day, respectively, $P<0.001$) and dietary calcium intake (287.2±689.2, 388.1±227.9, and 475.6±270.3 mg/day, $P<0.001$). Subjects with lower vitamin C intake were more likely to have a history of smoking ($P=0.002$). BMI, serum 25(OH)D concentration, and socioeconomic status evaluated by monthly household income did not differ between tertiles of dietary vitamin C intake.

BMDs at each site according to the dietary vitamin C intake tertiles

Figure 1 shows the BMD at each skeletal site according to the dietary vitamin C intake tertiles. The BMD at the three sites LS, FN, and TH correlated positively with vitamin C intake tertiles. The significance was maintained after further adjusting for age, BMI, serum 25(OH)D concentration, smoking, monthly income, and total energy intake ($P<0.05$) (Fig. 1). The association was stronger at FN and TH than at LS (coefficient of multiple correlation, $R=0.513$ for LS, 0.657 for FN, and 0.653 for TH, $P<0.05$ for each) (Fig. 1). The fracture rate did not differ between groups probably because of the low prevalence of fracture (<2 %, *data not shown*).

Dietary vitamin C intake in the osteoporosis and nonosteoporosis groups

Next, we compared the clinical factors related to BMD and dietary vitamin C intake between the osteoporosis group and the nonosteoporosis group (Table 2). In the osteoporosis group, those with a T-score ≤–2.5 at each skeletal site were older and had lower BMI compared with those with a T-score >−2.5 at those sites. The PTH concentrations were higher in the osteoporosis groups at LS and FN, and 25(OH)D concentration did not differ between the groups. However, PTH concentrations did not show any significant differences between the groups, and only 25(OH)D level at TH was lower in the osteoporosis group after adjusting for age (41.8±14.9 nmol/L vs 45.6±16.4 nmol/L, $P=0.035$). Household income was higher in the osteoporosis group at all skeletal sites ($P<0.05$, respectively). Total energy intake was lower in osteoporosis groups at all skeletal site, but it was not significantly different after adjusting for age. Dietary calcium intake was lower only at LS, and the statistical difference was maintained even after multiple adjustment for covariates (321.3±233.9 vs 417.0±533.8, $P<0.001$).

Dietary vitamin C intake was lower in the osteoporosis group than in the nonosteoporosis group at all sites (74.4±66.2 vs 94.1±78.6 mg/day at LS, 65.5±56.6 vs 94.3±

Table 1 Subjects' characteristics by tertiles of dietary vitamin C intake

Variables	Study participants (n=1,196)	Dietary vitamin C intake category			P value
		First tertile (n=399) (<46.9 mg/day)	Second tertile (n=399) (≥46.9, <92.6 mg/day)	Third tertile (n=398) (≥92.6 mg/day)	
Age (years)[a]	65.2±9.0	68.2±9.2*,**	64.6±8.9***	62.7±8.1	<0.001
BMI (kg/m^2)[a]	24.3±3.3	24.2±3.5	24.5±3.2	24.3±3.0	0.460
Waist circumference (cm)[a]	82.8±9.5	83.0±10.2	83.3±9.4	82.2±8.7	0.249
Serum 25(OH)D (nmol/L)[a]	45.5±16.6	44.2±16.1	46.6±17.5	45.7±16.0	0.131
Serum PTH (pg/mL)[a]	70.4±33.2	74.6±32.6**	69.2±29.7	67.3±36.6	0.006
Smoking status[b]					
Never	1,091 (91.2 %)	348 (87.2 %)	369 (92.5 %)	374 (94.0 %)	
Ex-	60 (5.0 %)	34 (8.5 %)	15 (3.8 %)	11 (2.8 %)	
Current	45 (3.8 %)	17 (4.3 %)	15 (3.8 %)	13 (3.3 %)	0.002
Monthly household income[b]					
Lowest	150 (13.0 %)	48 (12.5 %)	59 (15.3 %)	43 (11.0 %)	
Medium-lowest	241 (20.8 %)	75 (19.6 %)	75 (19.5 %)	91 (23.3 %)	
Medium-highest	286 (24.7 %)	100 (26.1 %)	80 (20.8 %)	106 (27.2 %)	
Highest	481 (41.5 %)	160 (41.8 %)	171 (44.4 %)	150 (38.5 %)	0.126
Intake[a]					
Total energy intake (kcal/day)	1,491.4±622.7	1,194.1±474.5*,**	1,526.2±649.3**	1,754.5±599.5	<0.001
Dietary calcium (mg/day)	383.6±453.5	287.2±689.2*,**	388.1±227.9***	475.6±270.3	<0.001
Dietary vitamin C (mg/day)	86.8±74.8	25.5±12.3*,**	67.9±13.2**	167.1±77.0	<0.001

BMI body mass index, *25(OH)D* 25-hydroxyvitamin D, *PTH* parathyroid hormone

*P<0.01 vs second tertile; **P<0.01 vs third tertile; ***P<0.05 vs third tertile

[a] Continuous variables such as age, BMI, waist circumference, serum concentrations of 25(OH)D, PTH, and dietary intake were analyzed using ANOVA and are shown as mean±SD

[b] Categorical variables such as smoking status, and monthly household income were analyzed using the chi-square test and are shown as number and percentage in parenthesis

79.2 mg/day at FN, 46.3±38.2 vs 89.2±76.0 mg/day at TH, P<0.001 at each site). After adjusting for covariates, age, BMI, serum 25(OH)D concentration, smoking, monthly income, and total energy intake, dietary vitamin C intake was significantly lower at FN and showed lower trend at TH (65.9±57.1 vs 94.5±79.0 mg/day at FN, 47.8±39.1 vs

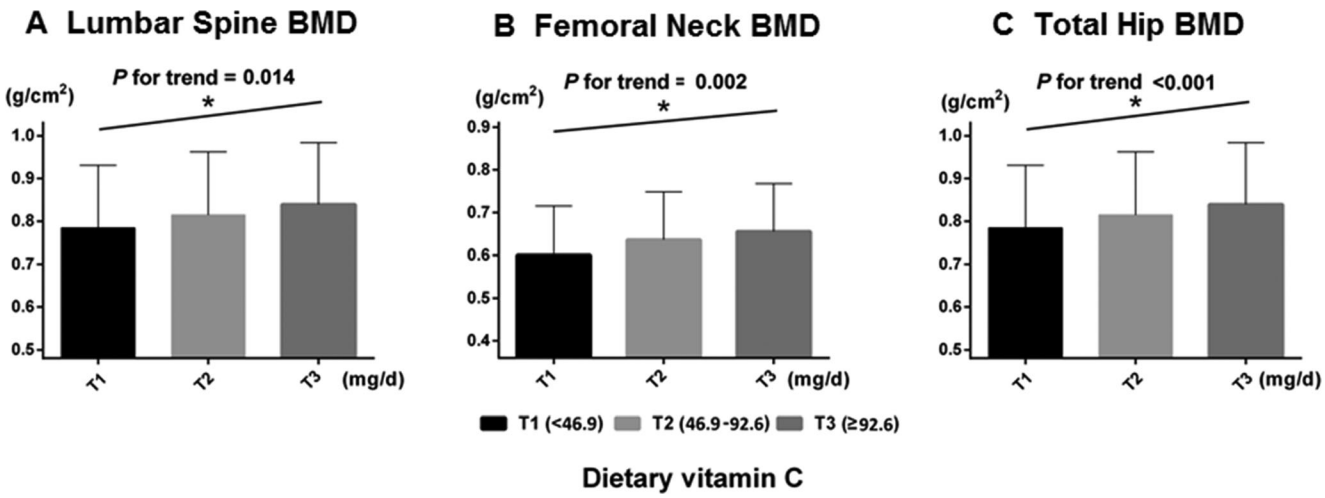

Fig. 1 Mean (± SE) BMD at each site by tertiles of dietary vitamin C (<46.9, 46.9–92.6, or ≥92.6 mg/day) in postmenopausal women; KNHANES IV, 2009. The P values for trend were produced by the general linear regression model after adjusting for age, body mass index, serum 25-hydroxyvitamin D concentration, smoking, monthly income, and total energy intake. *BMD* bone mineral density

Table 2 Comparison of BMD-related factors between the osteoporosis and nonosteoporosis groups according to the T-score at each site

Variables	T-score at lumbar spine			T-score at femoral neck			T-score at total hip		
	≤−2.5 (*n*=379)	>−2.5 (*n*=775)	*P* value	≤−2.5 (*n*=288)	>−2.5 (*n*=887)	*P* value	≤−2.5 (*n*=52)	>−2.5 (*n*=1,123)	*P* value
Age (years)[a]	69.7±8.1	62.7±8.5	<0.001	72.1±7.8	62.8±8.1	<0.001	74.7±8.2	64.6±8.7	<0.001
BMI (kg/m^2)[a]	23.1±3.1	25.0±3.2	<0.001	23.0±3.3	24.8±3.1	<0.001	21.6±3.3	24.5±3.2	<0.001
BMD (g/cm^2)[a]	0.639±0.063	0.857±0.104	<0.001	0.676±0.112	0.821±0.127	<0.001	0.610±0.095	0.793±0.135	<0.001
PTH (pg/mL)[a]	73.3±33.3	68.9±33.9	0.038 (0.805*)	76.3±33.8	68.4±32.9	<0.001 (0.439*)	72.9±28.6	70.2±33.5	0.170 (0.351*)
Serum 25(OH)D (nmol/L)[a]	46.0±17.3	45.3±16.4	0.512 (0.875*)	45.6±17.1	45.3±17.1	0.743 (0.599*)	41.8±14.9	45.6±16.4	0.060 (0.035*)
T-score[a]	−3.19±0.55	−1.29±0.91	<0.001	−2.87±0.97	−1.61±1.11	<0.001	−3.44±0.83	−1.85±1.17	<0.001
Monthly household income[b]									
Lowest	42 (11.4 %)	104 (13.8 %)		30 (10.8 %)	120 (13.9 %)		0 (0 %)	150 (13.7 %)	
Medium-lowest	59 (16.0 %)	173 (23.0 %)		32 (11.6 %)	205 (23.7 %)		7 (14.6 %)	230 (21.0 %)	
Medium-highest	105 (28.5 %)	174 (23.2 %)		88 (31.8 %)	198 (22.9 %)		4 (8.3 %)	282 (25.8 %)	
Highest	163 (44.2 %)	300 (39.9 %)	0.012	127 (45.8 %)	342 (39.5 %)	<0.001	37 (77.1 %)	432 (39.5 %)	<0.001
Intake[a]									
Total energy intake (kcal/day)	1,409.8±565.2	1,541.1±651.0	0.001 (0.311*)	1,347.0±551.6	1,544.4±640.4	<0.001 (0.056*)	1,226.1±474.5	1,508.6±628.9	0.001 (0.088*)
Dietary calcium (mg/day)	321.3±233.9	417.0±533.8	<0.001 (0.048**)	361.6±805.5	392.5±255.9	0.318 (0.256**)	272.8±218.0	390.1±463.8	0.070 (0.555**)
Dietary vitamin C (mg/day)	74.4±66.2	94.1±78.6	<0.001 (0.098**)	65.5±56.6	94.3±79.2	<0.001 (0.043**)	46.3±38.2	89.2±76.0	<0.001 (0.054**)

BMI body mass index, *BMD* bone mineral density, *25(OH)D*, 25-hydroxyvitamin D

**P* value by ANCOVA, after adjusting for age; ***P* value by ANCOVA, after adjusting for age, BMI, serum 25(OH)D concentration, smoking, monthly income, and total energy intake

[a] Continuous variables such as age, BMI, BMD, serum concentrations of PTH and 25(OH)D, T score, and dietary intake were analyzed using ANOVA and are shown as mean±SD

[b] Monthly household income was analyzed using the chi-square test and is shown as number and percentage in parenthesis

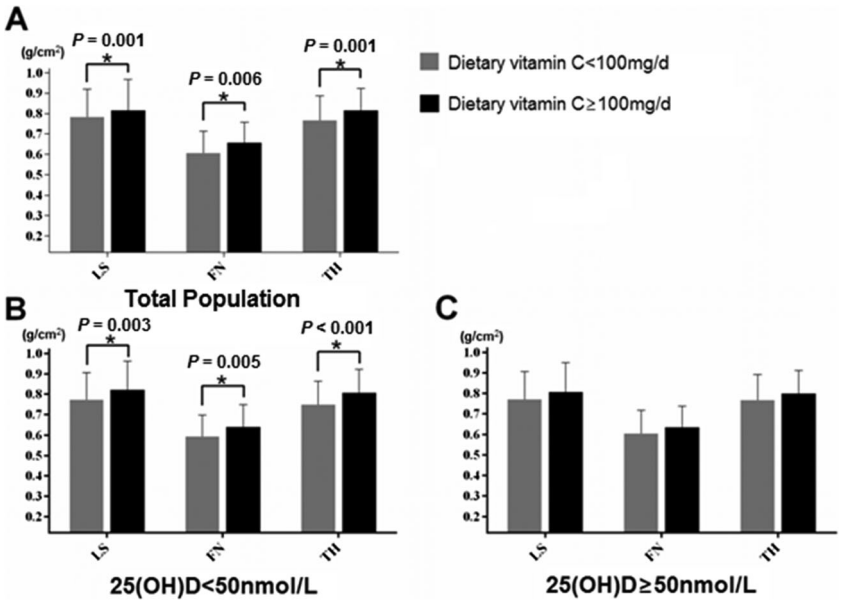

Fig. 2 Mean (± SE) BMD at each site in the high and low dietary vitamin C intake groups presented according to serum 25(OH)D concentration. Dietary vitamin C intake was divided in two groups: higher and lower than the Korean RDA of 100 mg/day. The *P* values for trend were produced from the general linear regression model after adjusting for age, body mass index, serum 25-hydroxyvitamin D concentration, smoking, monthly income, and total energy intake. *LS* lumbar spine, *FN* femoral neck, *TH* total hip

89.3±76.0 mg/day at TH, P=0.043 for FN and P=0.054 for TH).

Associations between vitamin C intake and BMD according to vitamin D status

The study subjects were further stratified into two groups according to serum 25(OH)D concentrations (<50 and ≥50 nmol/L), and the relationship between dietary vitamin C intake and BMD was analyzed in each group (Fig. 2). In the vitamin D-deficient group (25(OH)D <50 nmol/L), after adjusting for age, BMI, serum 25(OH)D concentration, smoking, monthly income, and total energy intake, BMD at all sites was higher in subjects with dietary vitamin C intake of ≥100 mg/day than in those with <100 mg/day. However, in the vitamin D-nondeficient group serum (25(OH)D concentration ≥50 nmol/L), the adjusted BMD at all sites did not differ significantly between dietary vitamin C groups.

Relationship between dietary vitamin C intake and BMD according to age

We also evaluated whether the favorable effects of dietary vitamin C on BMD differed between age groups. The subjects were stratified into three age groups (50–59, 60–69, and ≥70 years) (Table 3). The multiple-adjusted odds ratio for osteoporosis for dietary vitamin C <100 mg/day was 1.790 (95 % CI 1.333–2.405, P<0.001) compared with higher vitamin C intake. Women aged 50–59 years had the highest adjusted odds ratio for lower vitamin C intake (2.756, 95 % CI 1.335–5.686, P=0.006). In subjects aged ≥70 years, the adjusted odds ratio for osteoporosis for lower vitamin C intake was 1.888 (95 % CI 1.079–3.303, P=0.026). Interestingly, in

Table 3 Prevalence of osteoporosis in study subjects categorized by high dietary vitamin C intake and by age[a]

Age	Number	Vit C ≥100 (%)	Vit C <100 (%)	Odds ratio	95 % Confidence interval	*P* value
All	1,155 (1,196)[b]	107 (30.1)[c]	364 (45.5)	1.790[d]	1.333–2.405	<0.001
≥50, <60 years	358 (365)	12 (8.3)	37 (17.4)	2.756	1.335–5.686	0.006
≥60, <70 years	418 (432)	124 (43.1)	55 (42.3)	1.038	0.641–1.681	0.880
≥70 years	379 (399)	40 (50.0)	203 (67.9)	1.888	1.079–3.303	0.026

[a] Subjects were classified by age into groups 50–59 years, 60–69 years, and ≥70 years

[b] Number of participants included in the analysis and number in each age group in parenthesis

[c] Number of participants included in the analysis for each category of age and vitamin C and percentage in parenthesis

[d] Multiple-adjusted odds ratios and 95 % confidence intervals are shown after adjusting for body mass index, serum 25-hydroxyvitamin D concentration, smoking, monthly income, and total energy intake

women in their 60s, the adjusted odds ratio for osteoporosis was not increased with lower vitamin C intake.

Discussion

This study showed that dietary vitamin C intake was positively associated with BMD at all sites measured in postmenopausal women. These relationships remained significant after adjusting for confounding factors in the multiple logistic regression. Subjects with lower dietary vitamin C intake (<100 mg/day) had a higher odds ratio for osteoporosis than those with higher vitamin C intake. However, the protective association between higher vitamin C intake and BMD was observed only in subjects with a deficient serum 25(OH)D concentration (<50 nmol/L). Age-stratified analysis revealed a significant association between vitamin C intake and BMD among women aged 50–59 years and ≥70 years, but not in women aged 60–69 years.

Although several observational studies have shown positive associations between dietary vitamin C and BMD, the results have not been consistent [22, 24–26, 14]. A 17-year longitudinal study reported a protective association between higher vitamin C intake and fracture, but this was attenuated after adjusting for potassium intake [27]. Other studies that failed to find a general association between vitamin C intake and BMD showed partial positive relationships only in the groups with higher dietary [23] or supplementary [29] calcium intake. This inconsistency might reflect the fact that dietary nutrients can be related to other parameters, including age, sex, and ethnicity, and that these parameters can also affect bone health directly or indirectly.

Vitamin C can alter bone resorption by osteoclasts [12] and is required for osteoblast differentiation [35]. Several experimental studies have shown favorable roles of vitamin C in skeletogenesis. Park et al. showed that vitamin C-deficient mice had high expression levels of receptor activator of nuclear factor κ-B ligand (RANKL), which plays a key role in osteoclast differentiation, and peroxisome proliferator-activated receptor gamma (PPARγ), which might promote the transition of osteoblasts to adipocytes [21]. Zhu et al. reported that vitamin C prevents bone loss in ovariectomized mice by activating the Runx2 promoter, a potent stimulator of osteoblast differentiation [20].

Oxidative stress activates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) proteins, which are important for osteoclastogenesis. Fruits and vegetables are abundant sources of antioxidant vitamins such as vitamin C and carotenoids. Previous studies have shown that a higher intake of fruit and vegetables has a positive effect on BMD [36–38]. Maggio et al. reported that plasma antioxidant concentrations are markedly decreased in older osteoporotic women [13]. Based on these findings, vitamin C may have a positive association with bone density through its antioxidant activities by scavenging single oxygen and peroxyl radicals [39, 40].

In our study, dietary vitamin C intake was positively associated with multiple factor-adjusted BMD only in the group with deficient serum 25(OH)D concentration but not in the group with repleted 25(OH)D concentration (≥50 nmol/L). Low serum 25(OH)D concentration increases the release of PTH, which stimulates bone resorption and bone loss [41]. Vitamin C has been reported to reduce bone resorption by inhibiting osteoclastogenesis and inducing osteoclast apoptosis, especially in high bone-turnover states [12, 42]. Moreover, previous study reported significant inverse relationship between plasma vitamin C level and serum PTH concentration, indicating that higher vitamin C concentrations could suppress PTH elevation [43]. Based on those reports and our findings, vitamin C might have fortified the preventive effects with low serum 25(OH)D status by attenuating high bone turnover.

We observed the strong association in women aged 50–59 years, but this positive association was diminished in women aged 60–69 years and reappeared—albeit weakly—in women ≥70 years. The reasons of this finding are not clear, but we suggest that the differences in the rate of bone loss between those age groups could affect the negative influence of lower vitamin C intake on BMD. The rate of bone loss differs according to postmenopausal status; bone loss initiates and accelerates in transmenopausal period up to 5–10 years after menopause, but the rate then slows [44, 45]. Therefore, we cautiously suggest that the negative effect of lower dietary vitamin C intake on bone might be most influential in vulnerable postmenopausal period of bone metabolism and could explain why the risk of lower vitamin C intake for developing osteoporosis was highest in women aged 50–59 years. However, further studies are needed to clarify such age differences in the effects of vitamin C intake on bone metabolism.

This study has several limitations. First, this was a cross-sectional study and it cannot indicate any direct causal relationships or identify associated mechanisms. Second, we could not obtain data about the blood levels of other oxidants such as flavonoid and serum vitamin C concentration. Third, because of the low incidence of fractures, our study does not have enough power to investigate the association between dietary vitamin C intake and fracture.

To our knowledge, we are the first to investigate the association between dietary vitamin C intake and BMD in relation to serum 25(OH)D concentration and age. This study population had a lower serum 25(OH)D concentration compared with the US and other Caucasian population [46–48] possibly because of the limited exposure to sunlight or inadequate vitamin D intake [49]. Positive associations between dietary vitamin C and multiple factor-adjusted BMD were evident in the group with lower serum 25(OH)D concentration compared with other studies. Based on this, we propose that

sufficient dietary vitamin C intakes should be encouraged in postmenopausal women, who are at high risk of bone loss. Moreover, further longitudinal studies are needed to clarify the protective role of dietary vitamin C on future BMD loss.

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Conflicts of interest None.

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