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High Vitamin C Intake Is Associated with Lower 4-Year Bone Loss in Elderly Men[1,2]

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Abstract

Vitamin C is essential for collagen formation and normal bone development. We evaluated associations of total, supplemental, and dietary vitamin C intake with bone mineral density (BMD) at the hip [femoral neck, trochanter], spine, and radial shaft and 4-y BMD change in elderly participants from the Framingham Osteoporosis Study. Energy-adjusted vitamin C intakes were estimated from the Willett FFQ in 1988–89. Mean BMD and 4-y BMD change was estimated, for men and women, by tertile/category of vitamin C intake, adjusting for covariates. We tested for interaction with smoking, calcium, and vitamin E intake. Among 334 men and 540 women, the mean age was 75 y and mean vitamin D intake was 8.25 μg/d (women) and 8.05 μg/d (men). We observed negative associations between total and supplemental vitamin C intake and trochanter-BMD among current male smokers (P-trend = 0.01). Among male nonsmokers, total vitamin C intake was positively associated with femoral neck BMD (P-trend = 0.04). Higher total vitamin C intake was associated with less femoral neck and trochanter-BMD loss in men with low calcium (all P-trend $\leq$ 0.03) or vitamin E intakes (all P-trend = 0.03). Higher dietary vitamin C intake tended to be associated with lower femoral neck-BMD loss (P-trend = 0.09). These associations were attenuated but retained borderline significance (P-trend $<$ 0.1) after adjusting for potassium intake (a marker of fruit and vegetable intake), suggesting that vitamin C effects may not be separated from other protective factors in fruit and vegetables. Null associations were observed among women. These results suggest a possible protective role of vitamin C for bone health in older men. J. Nutr. 138: 1931–1938, 2008.

Introduction

It has been estimated that almost 10 million Americans have osteoporosis (1) and low bone mass is a major public health threat for almost 44 million people in the U.S. population aged ≥50 y. Studies have consistently shown that higher fruit and vegetable intake has positive effects on bone mineral status (2–9) and that fruit- and vegetable-specific antioxidants, such as vitamin C, may decrease oxidative stress (10–12) arising from reactive oxygen intermediates that may be involved in the bone-resorptive process (13–15). Therefore, vitamin C might help in preventing osteoporosis (16). In addition, bone matrix contains over 90% of protein as collagen (17) and it is well established that vitamin C is an essential cofactor for collagen formation and synthesis of hydroxyproline and hydroxylysine required for the formation of stable triple helixes (18). Furthermore, animal studies have demonstrated that experimental deficiency of vitamin C leads to impaired bone mass, cartilage, and connective tissue (19,20).

Several epidemiologic studies have examined the association of vitamin C in relation to bone mineral density (BMD) (21–26). However, results from these studies have been mixed, indicating a complex association involving interaction of both total and dietary vitamin C with nutritional and nonnutritional factors. Because vitamin C supplements may be used more frequently by individuals with other risk factors for bone loss (e.g. smoking), we sought to examine the effects of vitamin C from both dietary intakes as well as from total vitamin C intakes including supplements. Therefore, in this study, we examined associations between vitamin C intake (total, supplemental, and dietary intake) and cross-sectional, as well as longitudinal, changes in BMD at the hip, spine, and radial shaft in men and women in the Framingham Osteoporosis Study. Furthermore, we examined these associations for effect modification by smoking status, total calcium and vitamin E intake, and current estrogen use (in women).

Methods

The Framingham Osteoporosis Study is an ancillary study of the Framingham Heart Study, a population-based cohort study, which began

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in 1948 to examine risk factors for heart disease. The original subjects (5209 men and women aged 28–62 y) were selected as a population-based random sample of two-thirds of the households in Framingham, Massachusetts, and have been examined biennially for >50 y (27). Of the 1402 surviving subjects from the original cohort, 1164 cohort members participated in the Framingham Osteoporosis Study, when BMD measurements were made at the 20th biennial examination (**Fig. 1**). We excluded subjects with missing FFQ ($n = 333$), with incomplete FFQ (based on the criteria of >12 food items left blank in the FFQ), or with energy intakes <2.51 or >16.74 MJ (<600 or >4000 kcal/d) ($n = 92$) at the 20th exam. We further excluded subjects with missing BMD measurements (111 subjects for femoral neck BMD, 123 for trochanter BMD, 91 for radial shaft BMD, and 288 for lumbar spine BMD) and 24 participants were excluded due to missing covariate information (BMI, physical activity index, multivitamin use, smoking status, or estrogen use). There were no study participants taking medications during our baseline examination in 1988–89 that were known to influence bone metabolism other than estrogen use in some women. The final analytic sample for cross-sectional analyses included 334 men and 540 women with a complete FFQ and BMD measurement at exam 20. Longitudinal analyses were confined to 213 men and 393 women who had FFQ at the 20th examination and BMD measurement at examinations 20 and 22. All participants gave informed consent for their participation in the study. The Institutional Review Board for Human Research at Boston University, Hebrew Rehabilitation Center, and Tufts University approved this study.

Assessment of BMD. BMD (g/cm^2) was measured in the original cohort in 1988–89 (exam 20) and 1992–93 (exam 22), at the femur, spine, and radius as previously described (28,29). BMD of the proximal right femur (femoral neck, trochanter) and lumbar spine (average L2 to L4) were measured in g/cm^2 using a Lunar DP3 dual-photon absorptiometer at baseline. At the 4-y follow-up exam, BMD was measured using dual X-ray absorptiometry (DPX-L, Lunar Radiation).

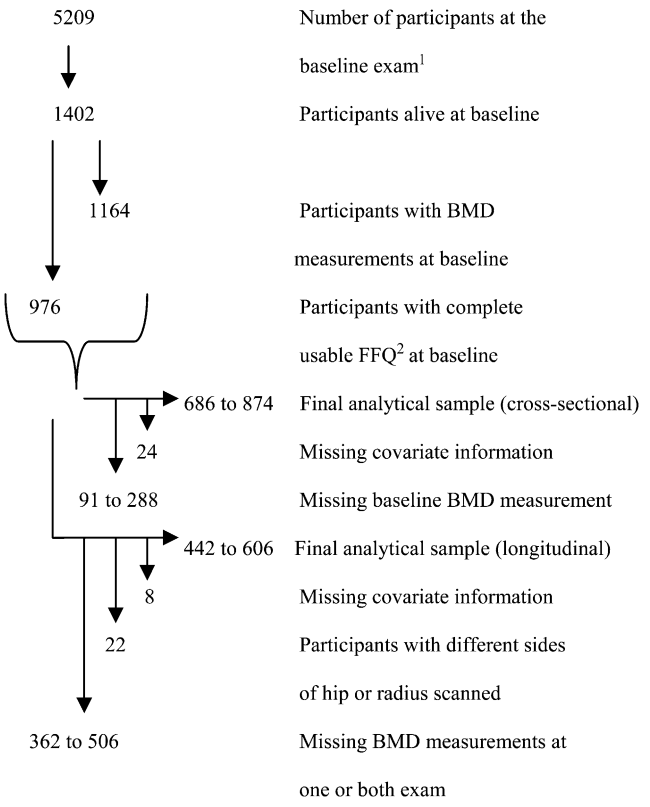

FIGURE 1 Flowchart showing total number of subjects enrolled in the Framingham Heart Study and the final number of subjects included in the analyses. 1, Framingham Heart Study; 2, FFQ.

There were strong correlations between measures taken with dual photon and dual X-ray absorptiometry, but due to a small but consistent shift in BMD values between the 2 methods, femoral BMD were adjusted for a change from dual-photon absorptiometer to dual X-ray absorptiometry technology using published corrections (30). BMD at the radial shaft was measured in g/cm^2 with a Lunar SP2 single-photon absorptiometer (Lunar Radiation) at both examinations.

Assessment of dietary intake. Usual dietary intake was assessed in 1988–89 with a semiquantitative, 126-item Willett FFQ (31). Questionnaires were mailed to the study participants. They were asked to complete them, based on their intake over the previous year, and to bring them to the examination where they were reviewed with participants by clinic staff. This FFQ has been validated for many nutrients and in several populations against multiple diet records and blood measures (32,33). Intake of vitamin C (mg/d), total calcium (mg/d), vitamin D (μg/d), vitamin E (mg tocopherol equivalents (TE)/d), caffeine (mg/d), alcohol (g/d), potassium (mg/d), and total energy (MJ) was assessed using the food list section of the FFQ. Based on their alcohol intake, subjects were categorized as nondrinker, moderate drinker (<13.2 g/d of alcohol for women and <26.4 g/d of alcohol for men), or heavy drinker (≥13.2 g/d of alcohol for women and ≥26.4 g/d of alcohol for men) based on cut-offs recommended by dietary guidelines for Americans (34). Intake of multivitamin supplements, as recorded on the supplement section of the FFQ, was coded as a yes-no variable. Intake of vitamin C from supplements was categorized into groups based on the estimated average requirements for vitamin C (35) (group 1, nonsupplement users; group 2, intake <75 mg/d among women and <90 mg/d among men; group 3, intake ≥75 mg/d among women and ≥90 mg/d among men).

Potentially confounding factors. Previous studies on this cohort have reported several risk factors for osteoporosis, including age (y), female sex (29), BMI (36), smoking (37), caffeine (38), alcohol (39), current estrogen use in women (29), low physical activity (40), and low intake of calcium and vitamin D (41,42). BMI (kg/m^2), a known risk factor for osteoporosis, was calculated in the Framingham Study from measurements of height at exam 1 (1948–1949), measured without shoes, in inches, and measurements of weight taken at the 20th examination in pounds (converted to kilograms) with a standardized balance-beam scale. Because BMI is a measure of relative weight designed to be independent of height, we included both BMI and height at exam 1 (converted to meters) in our equations to adjust for ponderosity and body stature, which may be related to dietary intake and BMD (4).

Smoking status was assessed via questionnaire in 1988–89 as current cigarette smoker (smoked regularly in the past year), former smoker, or never smoked. Physical activity was measured with the Framingham physical activity index as an estimated measure of energy expenditure calculated from questions about number of hours spent in heavy, moderate, light, or sedentary activity and number of hours spent sleeping during a typical day (4). The physical activity index at the 1986–87 exam (exam 19) was used for the subjects who had a missing physical activity index at the 1988–89 exam (exam 20). Women were divided into 2 groups: those currently using estrogen who had been using continuously for >1 y and those who had never used estrogen, had used it previously, or had used it only for a short time, because there is evidence that past use does not sustain bone benefits (43). A previous study by our group supported the hypothesis that alkaline-producing dietary components such as potassium, present in fruits and vegetables, play a beneficial role in bone health (6). Therefore, final models were adjusted for potassium intake to examine if the association of vitamin C intake with BMD was independent of this measure of dietary quality. Previous research has indicated that there are seasonal changes in BMD in New England (44). Therefore, for the cross-sectional analyses, we created a categorical variable for the time of BMD measurement: July, August, and September were coded as summer; October, November, and December as fall; January, February, and March as winter; and April, May, and June as spring.

Statistical analysis. Total and dietary vitamin C intakes were adjusted for total energy intake using the residual method (45). Logarithmic

transformation was applied to total vitamin C intake and square root transformation was applied to the dietary vitamin C intake, to achieve normality, before creating residuals.

Mean BMD measures were estimated for men and women combined and separately by tertiles of total and dietary vitamin C intake, and by defined categories of supplemental vitamin C, using the General Linear Models procedure in SAS. We regressed each of the 4 BMD measures onto the continuous and categorical measures of energy-adjusted total vitamin C intake, adjusting for potential confounders and covariates. We also regressed baseline BMD on intake of vitamin C from supplements as well as from diet in the same model. Analyses were adjusted for multiple comparisons using Dunnett's adjustment. Test of trend across tertiles was conducted using the median measure for each of the tertiles. We regressed change in BMD (BMD at exam 22 − BMD at exam 20) onto the continuous and categorical energy-adjusted total vitamin C and then the dietary vitamin C and supplemental vitamin C intake variables, adjusting for potential confounders and covariates.

All models were adjusted for age, BMI, height at exam 1, total energy intake, physical activity index, smoking, and intake of total calcium, total vitamin D, caffeine, and alcohol, multivitamin use, season of BMD measurement (for cross-sectional analyses on BMD only), and estrogen use (in women). Models for supplemental vitamin C were also adjusted for dietary vitamin C intake. All analyses were performed using SAS statistical software (SAS Institute, version 9.1, 2001). A nominal 2-sided *P*-value <0.05 was considered significant for all the analyses.

We tested the associations for effect modification by sex, smoking status, total calcium intake, total vitamin E intake, and estrogen use. Previous studies identified smoking status, estrogen use (21), total calcium intake (22), and total vitamin E (46) as important effect modifiers. Because we were testing multiple interactions, only those that were significant at $P < 0.01$ were examined further, to avoid false positives.

Results

Subject characteristics. Women represented two-thirds (62%) of the study sample. The mean age of men and women was 75 y and mean BMI was 24.9 (for women) and 26.2 (for men) (**Table 1**). Approximately one-third of women and men reported education beyond high school. One-half of the women and two-thirds of the men reported alcohol use, whereas more than one-half of the women and three-fourths of the men reported ever having smoked cigarettes. Most women (95%) reported no estrogen use. Approximately one-fourth of men and women reported multivitamin supplement use. The mean intake of calcium was 831 mg/d in women and 763 mg/d in men.

Cross-sectional analyses of BMD. We observed several statistically significant 3-way interactions between vitamin C (total, supplemental, and dietary), sex, and smoking status (*P* ranged from 0.003 to 0.02 at 3 of the 4 bone sites examined). Therefore, we continued with separate analyses for men and women. Sex-specific analyses were further tested for interaction with total calcium and vitamin E intake, smoking status, and current estrogen use.

Among men who never smoked, total vitamin C intake was positively associated with femoral neck BMD (*P*-trend = 0.04) (data not shown). In contrast, total vitamin C (**Table 2**) and supplemental vitamin C intake (**Table 3**) were negatively associated with trochanter BMD among current smokers (*P*-trend = 0.01 for total and supplemental vitamin C intake). No cross-sectional associations were observed between vitamin C intake and BMD among women ($P > 0.05$).

4-y change in BMD. Interactions were observed for dietary vitamin C and sex (*P* for interaction = 0.05) and for dietary vitamin C and total calcium intake (*P* for interaction = 0.009 at radial shaft). We therefore continued with our analyses separately for men and women. Among men, an interaction was observed for total vitamin C and calcium intake (low calcium group: intake < median intake of 661 mg/d, high calcium group: intake > median intake) (*P* for interaction = 0.001 at the radial shaft) as well as for total vitamin C and vitamin E intake (low vitamin E group: intake < median intake of 7.7 mg TE/d, high vitamin E group: intake > median intake) (*P* for interaction = 0.003 at the radial shaft). No significant interactions were

TABLE 1 Characteristics of study participants of the Framingham original cohort at the 20th examination[1]

Descriptive variables	Men[2]	Women[2]
Age, *y*	75.3 ± 5.0	75.5 ± 5.0
BMI, *kg/m²*	26.2 ± 3.9	24.9 ± 4.7
Physical activity score	33.7 ± 6.1	33.1 ± 4.9
Education group, %		
No high school diploma	32.9	29.1
High school diploma	33.4	38.2
Higher education	33.7	32.7
Current cigarette smokers, %	9.1	11.8
Alcohol use, %		
Nondrinkers	35.3	48.5
Moderate drinkers	44.4	34.4
Heavy drinkers	20.3	17.1
Multivitamin supplement users, %	21.5	27.6
Current estrogen use, %		
Current user	—	4.8
Former user	—	1.4
Nonuser	—	93.8
Season of BMD measurement, %		
Winter	27.1	28.4
Spring	28.0	28.1
Summer	24.6	21.3
Fall	20.3	22.2
Intake		
Total energy, *MJ/d*	7.8 ± 2.6	7.0 ± 2.3
Dietary vitamin C, *mg/d*	140.8 ± 73	158.2 ± 83
Supplemental vitamin C, *mg/d*	81.8 ± 235	95.1 ± 248
Total vitamin C,[3] *mg/d*	222.7 ± 259	253.3 ± 267
Total vitamin E,[3] *mg TE/d*	48.2 ± 122	50.3 ± 125
Total calcuim,[3] *mg/d*	762.7 ± 389	831.2 ± 452
Total vitamin D,[3] *μg/d*	8.0 ± 6.9	8.2 ± 6.3
Fruit and vegetables,[4] *servings/d*	4.9 ± 2.5	5.7 ± 2.8
BMD at baseline,[5] *g/cm²*		
Femoral neck	0.878 ± 0.146	0.720 ± 0.114
Trochanter	0.847 ± 0.150	0.624 ± 0.127
Radial shaft	0.719 ± 0.085	0.512 ± 0.091
Lumbar spine	1.330 ± 0.227	1.070 ± 0.189
4-y change in BMD,[6] *g/cm²*		
Femoral neck	−0.015 ± 0.064	−0.026 ± 0.052
Trochanter	0.002 ± 0.083	−0.022 ± 0.060
Radial shaft	−0.027 ± 0.043	−0.026 ± 0.041
Lumbar spine	−0.0006 ± 0.109	−0.044 ± 0.094

[1] Values are means ± SD, or %.
[2] Sample sizes varied from 362 to 374 for men and from 584 to 602 for women.
[3] Total nutrient intake = dietary intake + intake from supplements.
[4] One serving of fruit or vegetables = 1 cup raw leafy vegetable (30g), 1/2 cup cut-up raw or cooked vegetable (91g), 1/2 cup fruit or vegetable juice (124g), 1 medium fruit (138g), 1/4 cup dried fruit (36g), 1/2 cup fresh, frozen, or canned fruit (115g).
[5] Sample sizes varied from 259 to 340 for men and from 429 to 545 for women.
[6] Sample sizes varied from 160 to 216 for men and from 288 to 398 for women.

TABLE 2 BMD at femoral neck and trochanter sites by tertiles of total vitamin C intake in men stratified by smoking status[1]

Site of BMD *(g/cm²)* measurement	*n*	Tertiles of total vitamin C intake[2] 1st tertile	2nd tertile	3rd tertile	*P*-trend
Nonsmokers		Median = 106 mg/d	132 mg/d	314 mg/d	
Femoral neck	85	0.86 ± 0.04 [2]	0.85 ± 0.03	0.94 ± 0.03	0.04
Trochanter	84	0.82 ± 0.05	0.84 ± 0.04	0.88 ± 0.04	0.32
Former smokers		Median = 90 mg/d	146 mg/d	249 mg/d	
Femoral neck	209	0.87 ± 0.03	0.88 ± 0.03	0.87 ± 0.03	0.97
Trochanter	203	0.84 ± 0.03	0.84 ± 0.03	0.84 ± 0.03	0.89
Current smokers		Median = 80 mg/d	146 mg/d	242 mg/d	
Femoral neck	30	0.89 ± 0.07	0.91 ± 0.07	0.78 ± 0.07	0.24
Trochanter	29	0.94 ± 0.07	0.84 ± 0.08	0.68 ± 0.07*	0.01

[1] Values are means ± SE. *Different from tertile 1, $P < 0.05$.
[2] Models adjusted for multivitamin use (yes/no), age at exam 20 (y), BMI (kg/m^2), height at exam 1 (m), total energy intake (MJ/d), physical activity index at exam 20, alcohol intake (non drinker; moderate drinker: <26.4 g/d of alcohol; heavy drinker: ≥26.4 g/d of alcohol), total vitamin D (μg/d) and calcium (mg/d), caffeine (mg/d), and season of BMD measurement at exam 20. The primary predictor was energy-adjusted residuals added to a constant, where the constant equals the nutrient intake for the mean energy intake of the study population. Logarithmic transformation was applied to the nutrient intake before creating residuals.

observed with smoking status among men or women or with estrogen use among women at $P > 0.01$.

Among men with low calcium intake, higher total vitamin C intake was associated with less BMD loss at the femoral neck (P-trend = 0.03) and trochanter (P-trend = 0.02) (**Fig. 2**). Similarly, among men with low total vitamin E intake, higher total vitamin C intake was associated with less BMD loss at the femoral neck (P-trend = 0.03) and trochanter (P-trend = 0.03) (**Fig. 3**). In contrast, no associations were observed among men with high calcium intake, men with high vitamin E intake, or among women. No significant longitudinal associations were observed for supplemental vitamin C intake among men or women ($P > 0.05$).

Among men, significant associations were observed for dietary vitamin C (adjusting for supplemental intake of vitamin C) and BMD at multiple bone sites. Higher dietary vitamin C intake was associated with less BMD loss at the trochanter (P-trend = 0.05) and lumbar spine (P-trend = 0.04) and tended to be associated with less BMD loss at the femoral neck (P-trend = 0.09) (**Fig. 4**). However, no associations were observed among women ($P > 0.05$). When these analyses were repeated among nonsupplement users, we observed an interaction of dietary vitamin C intake with total vitamin E intake (P for interaction = 0.0006 at the radial shaft). Also, higher dietary vitamin C intake tended to be associated with less BMD loss at the trochanter (P-trend = 0.08) among men with low total vitamin E intake (data not shown). However, no associations were observed at other bone sites among men with high vitamin E intakes or among women.

After adjustment for potassium intake, the negative cross-sectional association between total and supplemental vitamin C intake and trochanter BMD in current smokers did not change. However, the positive association of total vitamin C among those who never smoked lost significance ($P > 0.10$). In lon-

TABLE 3 BMD at femoral neck and trochanter BMD by tertiles of supplemental vitamin C intake in men stratified by smoking status[1,2]

Site of BMD *(g/cm²)* measurement	*n*	Categories of supplemental vitamin C intake[3] 1st tertile	2nd tertile	3rd tertile	*P*-trend
Nonsmokers		Median = 0 mg/d	60 mg/d	200 mg/d	
Femoral neck	85	0.93 ± 0.05	0.94 ± 0.07	0.92 ± 0.04	0.44
Trochanter	84	0.80 ± 0.06	0.90 ± 0.08	0.87 ± 0.05	0.82
Former smokers		Median = 0 mg/d	60 mg/d	250 mg/d	
Femoral neck	209	0.87 ± 0.04	0.90 ± 0.04	0.86 ± 0.04	0.40
Trochanter	203	0.84 ± 0.04	0.87 ± 0.05	0.84 ± 0.04	0.64
Current smokers		Median = 0 mg/d	60 mg/d	200 mg/d	
Femoral neck	30	0.80 ± 0.10	0.89 ± 0.13	0.71 ± 0.09	0.20
Trochanter	29	0.76 ± 0.11	0.91 ± 0.12	0.56 ± 0.08	0.01

[1] Values are means ± SE.
[2] Models adjusted for dietary vitamin C intake (mg/d), multivitamin use (yes/no), age at exam 20 (y), BMI (kg /m^2), height at exam 1 (m), total energy intake (MJ/d), physical activity index at exam 20, alcohol intake (nondrinker; moderate drinker: <26.4 g/d of alcohol; heavy drinker: ≥26.4 g/d of alcohol), total vitamin D (μg/d) and calcium (mg/d), caffeine (mg/d), and season of BMD measurement at exam 20.
[3] Categories of vitamin C intake from supplements were defined as: group 1: nonsupplement users; group 2: intake < 75 mg/d among women and <90 mg/d among men; group 3: intake ≥75 mg/d among women and ≥90 mg/d among men.

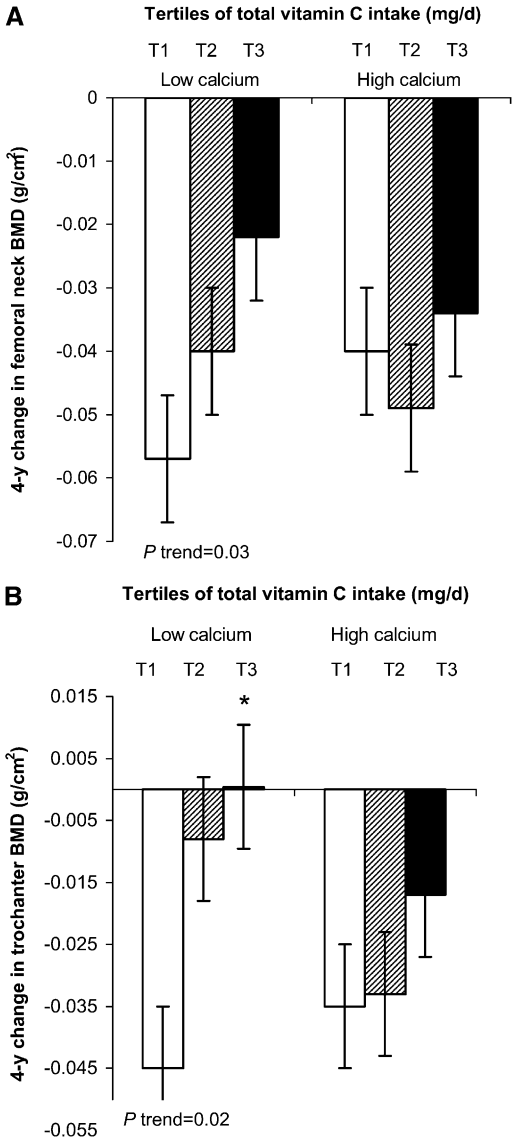

FIGURE 2 Adjusted mean 4-y changes in femoral neck (*A*), trochanter BMD (*B*) by tertiles of total vitamin C intake among men stratified by total calcium intake. Low calcium group: total calcium intake < median intake (661 mg/d); high calcium group: total calcium intake > median intake. Models were adjusted for age at exam 20 (y), BMI (kg/m^2), height at exam 1 (m), total energy intake (MJ/d), physical activity index at exam 20, alcohol intake (none/moderate: <26.4 g/d of alcohol; high: ≥26.4 g/d of alcohol), smoking (never/former/current smokers), and intake of total vitamin D (μg/d), caffeine (mg/d), and multivitamin use (yes/no). Values are means ± SE, $n = 201$ (femoral neck) or 193 (trochanter). Analysis was based on a general linear model with Dunnett's adjustment for multiple comparisons. *Different from T1, $P < 0.05$.

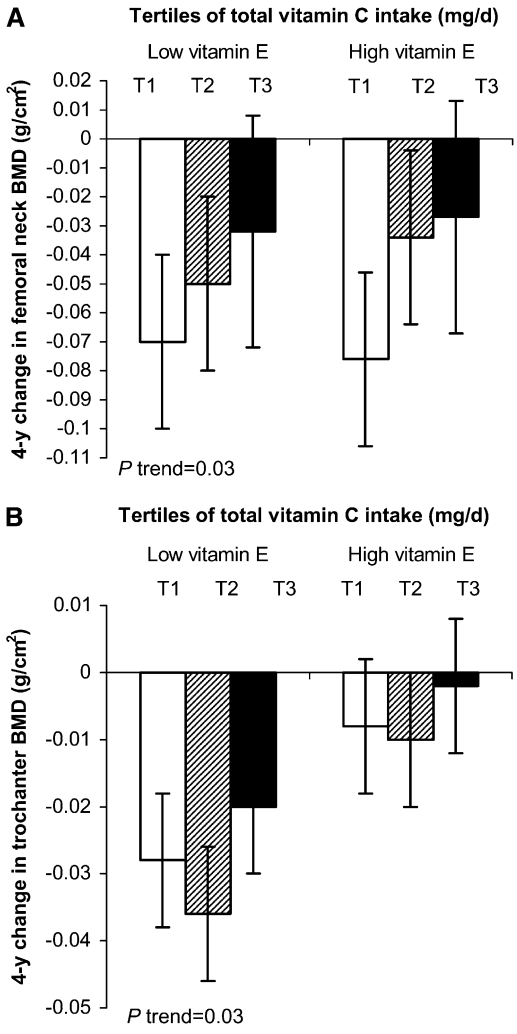

FIGURE 3 Adjusted mean 4-y changes in femoral neck (*A*) and trochanter BMD (*B*) by tertiles of total vitamin C intake among men stratified by total vitamin E intake. Low vitamin E group: total vitamin E intake < median intake (7.7 mg TE/d); high vitamin E group: total vitamin E intake > median intake. Models were adjusted for age at exam 20 (y), BMI (kg/m^2), height at exam 1 (m), total energy intake (MJ/d), physical activity index at exam 20, alcohol intake (none/moderate: <26.4 g/d of alcohol; high: ≥26.4 g/d of alcohol), smoking (never/former/current smokers), and intake of total calcium and vitamin D (μg/d), caffeine (mg/d), and multivitamin use (yes/no). Values are means ± SE, $n = 201$ (femoral neck) or 193 (trochanter). Analysis was based on a general linear model with Dunnett's adjustment for multiple comparisons.

gitudinal analyses, the protective association of total vitamin C in men with low calcium intake was attenuated (P-trend = 0.07 for femoral neck and 0.06 for trochanter BMD). The protective association of total vitamin C in men with low total vitamin E intake was attenuated for trochanter BMD (P-trend = 0.06) but did not change for femoral neck BMD. Similarly, the marginal protective association of dietary vitamin C in men did not change for femoral neck BMD but lost significance at other bone sites ($P > 0.10$ for trochanter and lumbar spine BMD). Among nonsupplement-using men with low vitamin E intakes, the protective association of dietary vitamin C lost significance after adjustment for potassium intake ($P > 0.10$ for trochanter).

Discussion

We observed an unexpected negative cross-sectional association of total vitamin C and supplemental vitamin C intake with trochanter BMD among men who were current smokers. In contrast, dietary vitamin C appeared to be protective against 4-y losses in BMD among men, whereas total vitamin C appeared to be protective only among men with low calcium or vitamin E intakes. These protective associations were attenuated after adjustment for potassium intake.

Studies have consistently shown that higher fruit and vegetable intakes have positive effects on bone mineral status

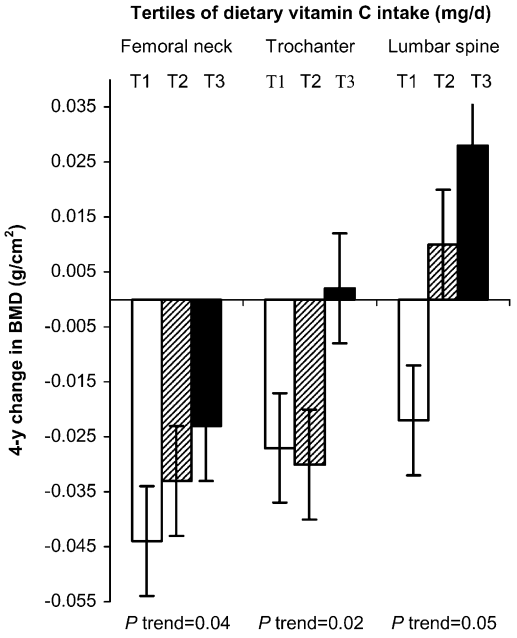

FIGURE 4 Adjusted mean 4-y changes in femoral neck (*A*) and trochanter BMD (*B*) and lumbar spine (*C*) by tertiles of dietary vitamin C intake among men. Models adjusted for intake of vitamin C from supplements (category 1 = nonsupplement users; category 2 = supplemental vitamin C intake > 0 mg/d but <90 mg/d; category 3 = intake ≥90 mg/d), multivitamin use (yes/no), age at exam 20 (y), BMI (kg/m^2), height at exam 1 (m), total energy intake (MJ/d), physical activity index at exam 20, alcohol intake (none/moderate: <26.4 g/d of alcohol; high: ≥26.4 g/d of alcohol), smoking (never/former/current smokers), and intake of total calcium (mg/d), vitamin D (μg/d), and caffeine (mg/d). Values are means ± SE, $n = 201$ (femoral neck), 193 (trochanter), and 158 (lumbar spine). Analysis was based on a general linear model with Dunnett's adjustment for multiple comparisons.

(2–7). Maggio et al. (24) observed that antioxidant defenses are markedly decreased in osteoporotic women. New et al. (9) reported lower mean pyridinoline excretion (a marker for bone resorption) with higher intakes of total vitamin C compared with low intakes ($P < 0.02$). Fruit- and vegetable-specific antioxidants may, therefore, play a role in osteoporosis prevention or treatment (47). Hence, more studies are required to clarify the role of fruit- and vegetable-specific antioxidants such as vitamin C in the prevention of osteoporosis.

Observational studies of vitamin C and bone have reported mixed results. Studies on vitamin C intake from supplements have provided some evidence of a protective role in bone health. However, this association has appeared to be complex, due to interactions with estrogen use and calcium intake. For example, 1 population-based study ($n = 994$ postmenopausal women) reported that vitamin C supplement users had higher BMD (3%) at the radius, femoral neck, and total hip ($P < 0.05$) than nonusers (25). They also reported that women with both estrogen and vitamin C intake from supplements had higher BMD at all sites and women taking supplemental vitamin C plus calcium and estrogen had the highest BMD. Another study (23) reported that longer duration of vitamin C supplement use was associated with higher BMD in women who had not used estrogen replacement therapy (P-trend = 0.02) and among women aged 55–64 y (P-trend = 0.01). In the latter group, women who used vitamin C supplements for ≥10 y had higher BMD than nonusers in the same age group ($P = 0.02$). In contrast, negative cross-sectional associations existed for supplemental vitamin C intake among men who were current smokers, but there were no significant longitudinal associations for supplemental vitamin C in men or women.

In contrast to our findings that vitamin C appeared protective only in those with low calcium or vitamin E intake, the Postmenopausal Estrogen/Progestin Interventions Trial found that dietary vitamin C was associated with BMD at the femoral neck ($P = 0.002$) and total hip ($P = 0.01$) only in subjects with higher calcium intake (22). The Women's Health Initiative Study (26) reported no significant association between dietary or total vitamin C and BMD. However, in contrast with our lack of interaction, they reported a beneficial effect of current hormone therapy on BMD at the femoral neck ($P = 0.004$), total body ($P < 0.04$), spine ($P = 0.03$), and total hip ($P = 0.02$) that was stronger with higher intakes of total vitamin C (interaction $P < 0.01$). Similarly, Leveille et al. (23) found no evidence of association of dietary or total vitamin C intake with BMD. We observed a positive association between total vitamin C and BMD among those who never smoked. However, we found an unexpected negative association between vitamin C intake (total and supplemental) and BMD among male smokers. It is possible that these observations reflect reverse causation, because smokers, with concomitant low BMD, consciously increase their vitamin C intake in the hopes of mitigating some of smoking's negative effects. It is important to note that the level of dietary intake of vitamin C among subjects in our study was similar to the intake level reported by Hall and Greendale in the Postmenopausal Estrogen/Progestin Interventions Trial study (22) as well as by Zhang et al. (48) in the Utah Study of Nutrition and Bone Health, a statewide, population-based, case-control study. The level of dietary intake of vitamin C in our study was higher than the levels reported in 2 other studies (a study of Swedish women aged 40–76 y and female participants from the Women's Health Initiative) (26,46). For total vitamin C intake, the level among subjects in our study was ~29% higher than the intake level reported by the Utah Study of Nutrition and Bone Health (48) but was ~44% less than the levels reported by the Women's Health Initiative (26). Additionally, the intake of fruit and vegetables among men in our study were slightly lower than those reported for men (aged 51–70 y) in the NHANES (1999–2000). However, the intake of fruit and vegetables among women in our study and in the NHANES Survey (1999–2000) was comparable (49).

Together, these results suggest that effects of vitamin C on bone appear to be complex and that they may interact with smoking (21,48), estrogen use/hormonal therapy after menopause (21,25,26), calcium intake (22,25), and vitamin E intake (46). We found significant interactions between vitamin C intake (total, supplemental, and dietary), sex, and smoking status in cross-sectional analyses. Significant positive associations were observed for total vitamin C among men who never smoked, with negative associations for total vitamin C among men who currently smoked. However, these interactions did not appear in the longitudinal analyses. Rather, the longitudinal analyses revealed protective effects of total vitamin C intake on 4-y change in femoral neck BMD as well as trochanter BMD, which was most evident in men with low calcium or vitamin E intakes. We also observed a greater protective effect of dietary relative to supplemental vitamin C against bone loss in men. Not surprisingly, adjustment for potassium intake, as a marker of fruit and vegetable intake and dietary quality, led to attenuation of most of these results, suggesting that whatever positive effects of vitamin C we observe may not be able to be separated from those of a good quality diet.

There are, however, physiologic reasons to expect protective effects from vitamin C. Oxidative stress may increase bone resorption through activation of nuclear factor-κB protein, which is a crucial mediator of tumor necrosis factor-α and osteoclastogenetic activity (50–53). Vitamin C is capable of reducing oxidative stress and therefore may affect bone health by inhibiting bone resorption. Furthermore, bone matrix contains over 90% of protein as collagen (17) and vitamin C is an essential cofactor for collagen formation and for the synthesis of hydroxyproline and hydroxylysine required for the formation of stable triple helixes (18).

The current study is unique in that it used data from a population-based cohort that included men as well as women and has both cross-sectional and longitudinal analyses. However, as in any observational study, residual confounding may occur despite control for several major potential confounders.

In summary, we did not observe any significant effects of vitamin C intake on BMD in women. In men, we observed effects only in interaction with smoking, suggesting that smokers with low BMD are using more vitamin C supplements. Consistent with previous findings of protective effects of fruit and vegetables, we observed protective associations between vitamin C intake and 4-y change in femoral neck BMD as well as trochanter BMD among men with low calcium or vitamin E intakes. The dietary rather than the supplement component of vitamin C intakes appeared most strongly protective against bone loss. Additional adjustment for dietary quality (adjustment for potassium intake) weakened these associations further, suggesting that possible effects of vitamin C may not be able to be separated from other protective factors in fruit and vegetables.

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