Cross-sectional and prospective associations between dietary and plasma vitamin C, heel bone ultrasound, and fracture risk in men and women in the European Prospective Investigation into Cancer in Norfolk cohort[1]

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ABSTRACT

Background: Vitamin C sufficiency may help prevent osteoporosis and fractures by mediating osteoclastogenesis, osteoblastogenesis, and bone collagen synthesis.

Objective: We determined whether dietary intakes and plasma concentrations of vitamin C were associated with a heel ultrasound and hip and spine fracture risks in older men and women.

Design: Participants were recruited from the European Prospective Investigation into Cancer in Norfolk study with 7-d diet diary estimates of vitamin C intake and plasma concentrations. A random subset (4000 of 25,639 subjects) was available for the cross-sectional (ultrasound) study of broadband ultrasound attenuation (BUA) and velocity of sound (VOS), which were determined during the second health examination. The prospective (fracture) study was a case-cohort sample of all participants with a fracture up to March 2009 and the random subset (n = 5319). ANCOVA-determined associations between quintiles of vitamin C intake and plasma status with adjusted BUA and VOS and adjusted Prentice-weighted Cox proportional HRs were calculated for fracture risk.

Results: Women were 58% of the population (39–79 y old), and the median follow-up was 12.6 y (range: 0–16 y). Positive associations across all quintiles of vitamin C intake but not plasma status were significant for VOS in men (β = 2.47 m/s, P = 0.008) and BUA in women (β = 0.82 dB/MHz, P = 0.004). Vitamin C intake was not associated with fracture risk, but there was an inverse association with plasma concentrations in men, with quintile 4 having significantly lower risks of hip fractures (HR: 0.35; 95% CI: 0.16, 0.80) and spine fractures (HR: 0.26; 95% CI: 0.10, 0.69) than quintile 1.

Conclusions: Higher vitamin C intake was significantly associated with higher heel ultrasound measures in men and women, and higher plasma vitamin C concentrations were significantly associated with reduced fracture risk in men only. Our findings that vitamin C intake and status were inconsistently associated with bone health variables suggest that additional research is warranted. *Am J Clin Nutr* 2015;102:1416–24.

Keywords: ascorbic acid, collagen, fracture risk, osteoporosis, vitamin C

INTRODUCTION

Osteoporosis is an increasingly prevalent public health burden, particularly in the elderly population (1, 2), and currently affects >70 million people in the United States, Europe, and Japan (3). Each year, osteoporosis accounts for ∼8.9 million fractures worldwide, most commonly at the hip, spine, and wrists (4). Hip fractures, in particular, are associated with an immense economic burden of ∼£2.3 billion/y (equivalent to ∼3.5 billion US$/y) in the United Kingdom (5). Because of the increase in the ageing population (2), predictions have indicated a steep increase in fracture incidence over the next decades (6). Consequently, strategies for the prevention of osteoporosis are crucial for limiting its clinical and financial burden. Modifiable factors involved in the cause of osteoporosis, including diet, are of great interest for expanding our currently limited knowledge of bone health and for improving the dietary recommendations (7). Vitamin C, a micronutrient predominantly found in fruits and vegetables, is crucial for the initiation of collagen synthesis in osteoblasts (8). Studies have also suggested that vitamin C may mediate osteoclastogenesis and osteoblastogenesis (9–12); hence, a deficiency of vitamin C may lead to lower bone density and a subsequent increase in fracture risk.

To date, a number of epidemiologic studies have reported positive associations between vitamin C intake from foods and supplements and higher bone density (13–16) as well as inverse associations with bone loss and risk of fractures (17–19). However, a recent review of the literature (20) highlighted that published studies have predominantly used food-frequency questionnaires (FFQs)[6] to estimate vitamin C intake (13, 15, 18, 19, 21–25) despite evidence suggesting that misclassification is lower when food diaries are used (26, 27). Moreover, data on blood concentrations of vitamin C are scarce (14, 17). These

[1] Supported by a PhD studentship funded by the postgraduate funding office of the Faculty of Medicine and Health Sciences, University of East Anglia. The European Prospective Investigation into Cancer in Norfolk study received grants from the Medical Research Council (G9502233) and Cancer Research UK (SP2024-0201 and SP2024-0204).

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[6] Abbreviations used: BMD, bone mineral density; BUA, broadband ultrasound attenuation; FFQ, food-frequency questionnaire; HRT, hormone replacement therapy; VOS, velocity of sound; 7dDD, 7-d diet diary.

Received March 28, 2015. Accepted for publication September 23, 2015.
First published online November 4, 2015; doi: 10.3945/ajcn.115.111971.

data have the benefit of eliminating human recall error and accounting for biological processes such as absorption and saturation in contrast with estimations of dietary intake (28). Epidemiologic evidence is particularly scarce for men despite fractures becoming an increasing health problem in both sexes (6). The review also showed that there is only limited data from British populations, and of previous studies, all had studied <1000 participants (13, 17, 22). Finally, studies that have investigated dietary vitamin C intake with fracture risk as the clinical endpoint of osteoporosis have currently only been reported in 2 US studies that showed contradictory results, possibly because of differences in study designs, sample sizes, and dietary assessments (14, 19).

Therefore, the aims of the current study were to investigate *1*) cross-sectional associations between, first, vitamin C intake measured from foods, and, second, plasma vitamin C concentrations with heel ultrasound measures (the ultrasound study); and *2*) prospective associations between dietary intake and plasma status with hip and spine fracture risks (the fracture study) in British men and women. We hypothesized that higher vitamin C intakes or plasma status would be associated with greater heel ultrasound and reduced fracture risk.

METHODS

Study population

The study population was registered in the European Prospective Investigation into Cancer in Norfolk study, which is a prospective cohort study of 25,639 mainly Caucasian men and women aged 39–79 y at baseline who had been recruited from 35 general practices in innercity, suburban, and rural areas of Norfolk. The current study had a cross-sectional component (ultrasound study) as well as a prospective component (fracture study). The cross-sectional ultrasound study was based on a random subcohort of 4000 participants (1759 men and 2241 women) who had attended the initial health check in 1993–1997. The longitudinal case-cohort fracture study included all participants from the full cohort who had experienced a fracture up to 31 March 2009 (n = 1502) and the same random subset of 4000 participants, which, after accounting for the overlap, resulted in 5319 participants. All participants gave their informed consent at the beginning of the original cohort study and are currently being followed up. The study was approved by the Norwich District Health Authority Ethics Committee and was conducted according to the Declaration of Helsinki.

Nondietary-exposure assessments

At recruitment in 1993–1997, participants completed detailed questionnaires on their health and lifestyle, including age, family history of osteoporosis, medication use, menopausal status in women (premenopausal, perimenopausal <1 y, perimenopausal for 1–5 y, and postmenopausal) and hormone replacement therapy (HRT) in women (current, former, and never) (29). In our population, current smokers compared with former and never smokers had significantly lower vitamin C intake and plasma vitamin C concentrations. Therefore, we adjusted for smoking history (current, former, and never) on the basis of participant responses to the questions "Have you ever smoked as much as one cigarette a day for as long as a year?" and "Do you smoke cigarettes now?". Physical activity was assessed from both work and leisure activities with the use of a validated European Prospective Investigation into Cancer questionnaire, and participants were grouped into active, moderately active, moderately inactive, and inactive categories (30). Weight and height measurements were recorded by trained nurses with participants wearing light clothing and without shoes during the baseline clinic visit. BMI (in kg/m^2) was calculated as weight measured to the nearest 0.2 kg divided by the square of height, with height measured to the nearest 0.1 cm. The exact protocol was repeated at the second health check in 1997–2000. The fracture study used data from the baseline nondietary-exposure assessments, whereas the ultrasound study was based on nondietary data from the second health check.

Dietary exposure assessment

Data for vitamin C intake from foods, hereafter referred to as dietary intake, were estimated from a 7-d diet diary (7dDD) taken at baseline for both the ultrasound and fracture studies. The A5 booklet contained 17 color-print photographs of foods to aid portion-size estimations, 4 pages for recording the foods and drinks consumed each day, a recipe notation, a checklist of commonly consumed foods, and a short questionnaire regarding the types of milk, bread, and spread consumed (31). The first day was an interviewed 24-h diet recall completed onsite by a trained nurse, and participants were requested to complete the remaining 6 d at home. Previous validation studies in this cohort have shown that the 7dDD was more comparable to a weighed food record with respect to estimating dietary vitamin C intake (r = 0.70, $P < 0.05$) than was an FFQ or a 24-h recall (both: r = 0.54, $P < 0.05$) (26, 27), hence providing a relatively accurate estimate of vitamin C intake. The in-house data-entry program Data into Nutrients for Epidemiologic Research (DINER), which is based on the United Kingdom food-composition database, had previously been designed for the dietary analysis and contains >11,000 food items and almost 600 portions (32). Entered data were checked and cleaned for a statistical analysis by trained nutritionists (31). Supplement data were entered into the vitamin and mineral supplement database (ViMiS) from which we obtained vitamin C, vitamin D, and calcium-supplement use (33).

Blood measurements

In both the ultrasound study and the fracture study, data for plasma vitamin C concentrations were available from nonfasting blood samples taken at baseline. Blood was drawn into vacuum tubes that contained citrate and were refrigerated overnight at 4–7°C in dark boxes before being centrifuged at 2100 × g for 15 min the next day (34). Plasma was stored at −70°C after its stabilization with a standardized volume of metaphosphoric acid. Plasma vitamin C concentrations were estimated ≤1 wk of blood sampling with the use of a fluorometric assay (35). The CV was 6.2% and 2.7% at the lower and upper ends of the range, respectively.

Follow-up measurements

The cohort was followed up after the initial recruitment in 1993–1997. Heel ultrasound was measured in 2341 of 4000

participants of the random subcohort as part of the second clinic visit (1997–2000) with a CUBA Clinical Ultrasonometer (McCue Ultrasonics) (36), and these data were used in the ultrasound study. The ultrasound measurements estimated the structural organization of bone from broadband ultrasound attenuation (BUA; in dB/MHz) and bone stiffness from velocity of sound (VOS; in m/s). Measurements were performed at least twice on each heel, and the mean values for BUA and VOS were calculated and used for the analysis. Higher values for both variables were indicative of bone with greater resistance to fracturing independent of bone mineral density (BMD) (37). The 5 machines were calibrated daily with their physical phantom. Monthly calibrations included the use of a roving phantom and measurements of the heel bone of one of the operators. The CV was 3.5%.

For the fracture study, data for fractures were available up to 31 March 2009 and were identified from the East Norfolk health authority database (38), which records all local hospital contacts with the use of the National Health Service number. We focused on investigating hip and spine fractures as the most common and economically relevant fracture sites (4, 5).

Statistical analyses

Descriptive statistics were determined for all continuous variables (means ± SDs) separately for the ultrasound study and the fracture study, and percentage frequencies were calculated for binary and categorical variables. Sex-specific differences in demographics were examined with the use of chi-square and unpaired *t* tests.

For the ultrasound study, we excluded participants who did not attend the second health check and, thus, did not have heel-ultrasound measurements (n = 1659; 41%). Participants were also excluded if they had missing covariate information on BMI, HRT, and menopausal status (for women) that were recorded during the second health check (n = 9; 0.2%) or a missing baseline 7dDD (n = 4; 0.1%). Furthermore, one person with a disproportionally high vitamin C intake from foods (>600 mg/d, which was >2 SDs above the mean) was also excluded. A total of 2327 participants (968 men and 1359 women) remained. Investigations of plasma vitamin C status were further confined to 2080 participants (885 men and 1195 women) because of availability. We used a larger data set for the investigations of dietary intake and only excluded subjects with missing plasma measurements in the respective analyses to increase subject numbers. Pearson correlation coefficients were calculated to determine the relations between dietary intake and plasma concentrations of vitamin C with BUA and VOS. Associations between vitamin C and heel ultrasound were determined by means of linear regression with multivariate adjustment. Mean ± SEM BUA and VOS values were stratified by sex-specific quintiles of vitamin C intake or plasma vitamin C concentrations and were adjusted for age, family history of osteoporosis, BMI, smoking, physical activity, steroid medication, menopausal status (in women), HRT (in women), total energy intake, dietary calcium intake, and the use of calcium- or vitamin D–containing supplements. Differences in adjusted BUA and VOS means between the upper 2 quintiles of vitamin C intake or plasma vitamin C concentrations referent to the lowest quintile were determined. A test for a linear trend gave an indication of the strength of the associations. To determine whether alcohol intake, which is a strong risk factor for fractures in men, would affect our findings, we also performed an analysis that included alcohol consumption in the covariate model.

For the fracture study in the case-cohort subset (n = 5319), we excluded 5% of participants with a missing baseline 7dDD (n = 285), very low energy intakes (<400 kcal/d, n = 1; >2 SDs below the mean), or very high dietary vitamin C intakes (>600 mg/d, n = 1; >2 SDs above the mean). Participants with missing covariate information including BMI and HRT use (in women) were also excluded from the study (n = 21; 0.4%). Because of the nature of the case-cohort design, participants were excluded from the study if they were not part of the random subcohort and had sustained a fracture at a site other than the hip or spine. Thus, a total of 4510 participants (1902 men and 2608 women) remained in the fracture study. For the analyses of site-specific fractures, the cohort sample sizes were very similar at 4367 participants for hip fractures (1842 men and 2525 women) and 4142 participants for spine fractures (1808 men and 2334 women). After the exclusion of subjects with missing plasma vitamin C concentrations (n = 534; 12% for the hip-fracture investigations; n = 499, 12% for the spine-fracture investigations), associations between plasma vitamin C status and hip and spine fracture risks were determined in 3833 participants (1650 men and 2183 women) and in 3643 participants (1619 men and 2024 women), respectively. Again, we only excluded subjects with missing plasma measurements in the respective analyses to increase subject numbers in our investigations of dietary intake. Mean vitamin C intake and plasma vitamin C concentrations were calculated for participants who had and those who had not experienced a fracture stratified by sex and the fracture sites hip and spine. An unpaired *t* test was used to test for differences in vitamin C intake and plasma status between the 2 groups. Prentice-weighted Cox proportional HRs with 95% CIs were calculated to determine prospective associations of vitamin C and fracture risk. This modified Cox regression took account of the overlap of the fracture cases with the random subcohort in the current prospective case-cohort study (39). HRs for hip and spine fracture were regressed on quintiles of vitamin C intake or plasma vitamin C concentrations after multivariate adjustment as previously mentioned. Differences between quintiles 4 and 1 as well as between quintiles 5 and 1 of vitamin C were determined, and the linearity of the associations was indicated with the use of *P*-trend values. Because alcohol intake is a strong risk factor for fractures in men, we also performed an analysis that included alcohol consumption to determine whether this variable would influence our findings.

For both the ultrasound and fracture studies, we investigated dietary vitamin C intake from foods as well as combined intake from foods and supplements (total intake). In the interest of space, only the results for dietary intake are shown here.

All statistical analyses were performed with the use of STATA 11 software (StataCorp, 2009, Statistical Software: Release 11; StataCorp LP). $P < 0.05$ was considered statistically significant.

RESULTS

Descriptive statistics in the ultrasound study

In the ultrasound study of 2327 participants (women: 58%), men were significantly older than women, and mean BMI was

also significantly higher in men than in women (**Table 1**). Women compared with men had significantly higher mean baseline plasma vitamin C concentrations (60.3 ± 19.9 and 48.7 ± 17.0 μmol/L, respectively; $P < 0.001$), although mean baseline dietary vitamin C intake did not differ between sexes (93.7 ± 49.6 mg/d in women and 90.7 ± 52.0 mg/d in men; $P > 0.05$). Men compared with women had significantly higher BUA and VOS.

Descriptive statistics in the fracture study

In the fracture study of 4510 participants (women: 58%), the mean ± SD age at baseline was 60 ± 10 y in both sexes, but mean baseline BMI was significantly higher in men than in women (Table 1). Women compared with men had significantly higher mean dietary vitamin C intake (89.3 ± 50.0 and 85.6 ± 51.4 mg/d, respectively; $P = 0.015$) and mean plasma vitamin C concentrations (58.2 ± 20.1 and 46.4 ± 18.2 μmol/L, respectively; $P < 0.001$) at baseline. The range in plasma vitamin C concentrations by quintiles was 3–31 μmol/L in men and 4–43 μmol/L in women in the lowest quintile, which indicated inadequate plasma status, and 53–61 μmol/L in men and 64–73 μmol/L in women in quintile 4, which indicated adequate plasma concentrations. The range in plasma vitamin C in the top quintile showed the largest variance (62–132 μmol/L in men and 74–170 μmol/L in women). In this case-cohort, the total number of fractures was 451 at the hip and 202 at the spine after a median follow-up of 12.6 y (range: 0–16.1 y). Of these fractures, 57 hip fractures (1.4%) and 33 spine fractures (0.8%) occurred in the random subcohort of 4000 participants. Hip fractures were more common in women than in men, but there were no sex differences in spine fractures.

TABLE 1
Characteristics of EPIC-Norfolk men and women in the ultrasound study ($n = 2327$) and in the fracture study ($n = 4510$)[1]

	Ultrasound study			Fracture study		
	Men ($n = 968$)	Women ($n = 1359$)	P	Men ($n = 1902$)	Women ($n = 2608$)	P
Age, y	63.0 ± 9.3[2]	61.7 ± 9.2	<0.001	59.8 ± 9.5	59.7 ± 9.6	0.66
BMI, kg/m^2	26.9 ± 3.4	26.5 ± 4.4	0.041	26.5 ± 3.3	26.2 ± 4.3	0.012
Energy intake, kcal/d	2262 ± 478	1732 ± 374	<0.001	2235 ± 508	1686 ± 385	<0.001
Dietary calcium intake, mg/d	925 ± 282	782 ± 247	<0.001	913 ± 294	760 ± 252	<0.001
Dietary vitamin C intake, mg/d	90.7 ± 52.0	93.7 ± 49.6	0.17	85.6 ± 51.4	89.3 ± 50.0	0.015
Plasma vitamin C,[3] μmol/L	48.7 ± 17.0	60.3 ± 19.9	<0.001	46.4 ± 18.2	58.2 ± 20.1	<0.001
BUA, dB/MHz	89.6 ± 17.4	72.1 ± 16.6	<0.001	—	—	—
VOS, m/s	1645 ± 40	1624 ± 40	<0.001	—	—	—
Hip fractures,[4] % (n)	—	—	—	5.9 (112)	13.0 (339)	<0.001
Spine fractures,[4] % (n)	—	—	—	4.1 (78)	4.8 (124)	0.30
Menopausal status, % (n)			NA			NA
Premenopausal	—	6.2 (85)		—	15.5 (403)	
Perimenopausal (<1 y)	—	3.5 (47)		—	4.6 (121)	
Perimenopausal (1–5 y)	—	18.1 (246)		—	16.4 (427)	
Postmenopausal	—	72.2 (981)		—	63.5 (1657)	
HRT, % (n)			NA			NA
Current user	—	21.2 (288)		—	17.2 (450)	
Former user	—	16.3 (222)		—	12.0 (312)	
Never user	—	62.5 (849)		—	70.8 (1846)	
Smoking history, % (n)			<0.001			<0.001
Current smoker	7.8 (76)	9.8 (133)		12.1 (230)	12.6 (328)	
Former smoker	55.6 (538)	31.6 (430)		55.3 (1051)	32.2 (839)	
Never smoked	36.6 (354)	58.6 (796)		32.6 (621)	55.2 (1441)	
Physical activity, % (n)			<0.001			<0.001
Inactive	28.4 (275)	25.1 (341)		31.7 (603)	32.4 (844)	
Moderately inactive	24.4 (236)	34.0 (462)		24.0 (456)	32.2 (840)	
Moderately active	25.6 (248)	24.5 (333)		22.2 (422)	21.0 (549)	
Active	21.6 (209)	16.4 (223)		22.1 (421)	14.4 (375)	
Family history of osteoporosis, % (n)	3.2 (31)	6.1 (83)	0.001	3.0 (57)	5.6 (145)	<0.001
Steroid medication, % (n)	4.4 (43)	5.2 (71)	0.39	3.6 (68)	4.1 (106)	0.40
Vitamin C–containing supplements, % (n)	10.0 (97)	17.9 (243)	<0.001	9.0 (172)	16.1 (420)	<0.001
Calcium-containing supplements, % (n)	1.5 (14)	7.2 (98)	<0.001	1.3 (24)	5.7 (149)	<0.001
Vitamin D–containing supplements, % (n)	23.6 (228)	34.7 (471)	<0.001	22.1 (421)	31.5 (822)	<0.001

[1]In the ultrasound study, dietary and plasma data were from the first health check, whereas the heel ultrasound measurements and the covariates were from the second health check. In the fracture study, the intake, plasma, and covariate data were all available from the first health check. P values for differences in demographic variables between men and women were calculated with the use of unpaired t tests. Chi-square tests were used to determine P values for differences between groups. BUA, broadband ultrasound attenuation; EPIC-Norfolk, European Prospective Investigation into Cancer in Norfolk; HRT, hormone replacement therapy; NA, not applicable; VOS, velocity of sound.

[2]Mean ± SD (all such values).

[3]$n = 885$ men and 1195 women in the ultrasound study; $n = 1703$ men and 2257 women in the fracture study.

[4]Fractures in the case-cohort sample after a median follow-up time of 12.6 y.

Results from the ultrasound study

In the ultrasound study, dietary vitamin C intake did not correlate with heel ultrasound in univariate analyses in men, but a weak correlation was shown with BUA in women ($r = 0.07$, $P < 0.05$). There were no correlations between plasma vitamin C and both heel-ultrasound measures in either sex. The results from the multivariate-adjusted linear regression analyses showed that BUA was not associated with dietary vitamin C intake in men (**Figure 1**A). However in women, BUA increased significantly across all quintiles of intake even after adjustment for age, family history of osteoporosis, BMI, smoking, physical activity, the use of steroids, menopausal status, HRT, energy intake, dietary calcium intake, and the use of calcium- and vitamin D–containing supplements ($\beta = 0.82$ dB/MHz, P-trend = 0.004; Figure 1B). Adjusted BUA was also significantly higher for women in quintiles 4 (3.7%) and 5 (5.8%) of vitamin C intake than for women in quintile 1 of vitamin C intake [$\beta = 2.59$ dB/MHz ($P = 0.038$) and 4.06 dB/MHz ($P = 0.001$), respectively]. There was a positive linear association between quintiles of dietary vitamin C intake and the heel ultrasound variable VOS in men ($\beta = 2.40$ m/s, P-trend = 0.010), even after adjustment for all covariates (Figure 1C). VOS was also significantly 0.6% higher in quintile 4 ($\beta = 9.15$ m/s, $P = 0.026$) and 0.5% higher in quintile 5 ($\beta = 8.65$ m/s, $P = 0.038$) of dietary vitamin C intake than in men in quintile 1 of dietary vitamin C intake. In contrast with men, dietary vitamin C intake was not significantly associated with VOS in women (Figure 1D). Multivariate-adjusted linear regression analyses showed no significant associations between plasma vitamin C concentrations and heel ultrasound in either sex (Figure 1).

Analyses of total vitamin C intake (dietary and supplemental intake combined) did not differ substantially from those of diet alone (data not shown). There was a significant linear association between quintiles of total intake and VOS in men ($\beta = 2.00$ m/s; P-trend = 0.034), and with BUA in women ($\beta = 0.71$ dB/MHz, P-trend = 0.014). Moreover, the addition of alcohol intake to our analysis did not markedly attenuate the magnitude or direction of associations with BUA and VOS (data not shown).

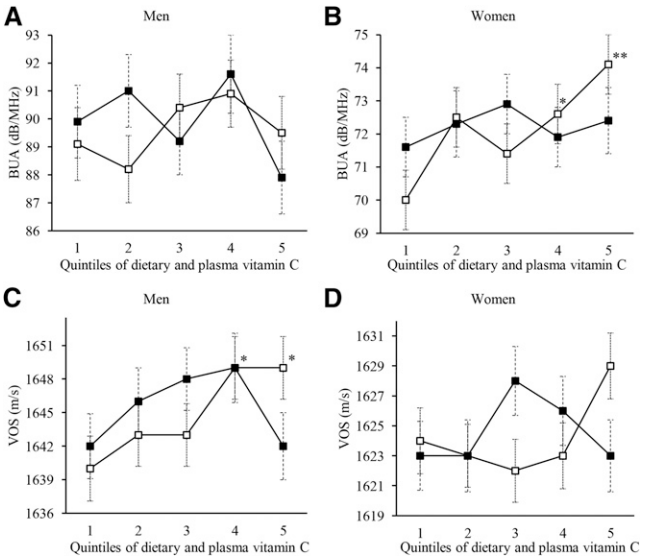

FIGURE 1 Mean ± SEM associations between quintiles of dietary vitamin C intake (open squares; $n = 2327$) and plasma vitamin C concentrations (closed squares; $n = 2080$) with BUA (dB/MHz) in men (A) and women (B) and VOS (m/s) in men (C) and women (D). The range of dietary vitamin C intake in each quintile was as follows—for men: quintile 1, 0–49 mg/d; quintile 2, 50–68 mg/d; quintile 3, 69–92 mg/d; quintile 4, 93–128 mg/d; and quintile 5, 129–471; for women: quintile 1, 0–52 mg/d; quintile 2, 53–73 mg/d; quintile 3, 74–96 mg/d; quintile 4, 97–128 mg/d; and quintile 5, 129–336 mg/d. The range of plasma vitamin C concentrations in each quintile was as follows—for men: quintile 1, 5–35 μmol/L; quintile 2, 36–45 μmol/L; quintile 3, 46–54 μmol/L; quintile 4, 55–62 μmol/L; and quintile 5, 63–106 μmol/L; for women: quintile 1, 4–46 μmol/L; quintile 2, 47–56 μmol/L; quintile 3, 57–64 μmol/L; quintile 4, 65–74 μmol/L; and quintile 5, 75–170 μmol/L. Quintiles for intake do not contain the same participants as the quintiles for plasma. Differences in mean ± SEM calcaneal BUA and VOS between the upper 2 quintiles referent to the lowest quintile of vitamin C were determined with the use of a linear regression analysis with adjustment for age, family history of osteoporosis, BMI, smoking, physical activity, steroid medication, menopausal status (in women), hormone replacement therapy (in women), total energy intake, dietary calcium intake, and the use of calcium- or vitamin D–containing supplements. *,**Compared with quintile 1: *$P < 0.05$, **$P < 0.01$. BUA, broadband ultrasound attenuation; VOS, velocity of sound.

Results from the fracture study

In the fracture study, results from the unpaired t tests showed that mean dietary vitamin C intake measured at baseline did not differ significantly between participants who did and those who did not experience a fracture at the hip or spine during the median 12.6 y follow-up.

Moreover, in women, mean plasma vitamin C concentrations measured at baseline did not differ significantly between women with and those without a fracture. However, baseline plasma concentrations were significantly lower in men who experienced a hip or spine fracture during the follow-up than in men without a fracture (41.2 ± 18.5 and 46.9 ± 18.0 μmol/L, respectively; $P < 0.001$).

We calculated Cox proportional HRs for fracture risk at the hip and spine separately. We showed that vitamin C intake from foods was not associated with fractures at the hip (**Table 2**) or the spine (**Table 3**) in either sex even after adjustment for age, family history of osteoporosis, BMI, smoking, physical activity, use of a steroid medication, menopausal status and HRT in women only, energy intake, dietary calcium intake and the use of calcium- and vitamin D–containing supplements. For plasma vitamin C status, there was a linear inverse relation between quintiles of plasma vitamin C and hip fractures (HR: 0.82; P-trend = 0.016) (**Table 4**) in men after multivariate adjustment. Moreover, men in quintile 4 of plasma vitamin C had a significantly lower risk of hip fracture (HR: 0.35; 95% CI: 0.16, 0.80; $P = 0.012$) (**Table 5**) and spine fracture (HR: 0.26; 95% CI: 0.10, 0.69; $P = 0.007$) than did men in the lowest quintile. Plasma vitamin C concentrations were not significantly associated with fractures in women. In the fracture study, the addition of alcohol intake to the covariate model did not markedly affect our findings.

DISCUSSION

To our knowledge, this study provides the first prospective investigations of plasma vitamin C and fracture risk and also adds to the scarcity of data on vitamin C and bone health in both men and women (17–19). We showed significant positive associations between vitamin C intake and heel ultrasound in the ultrasound

TABLE 2
Hip fracture risk after a median follow-up of 12.6 y stratified by quintiles of dietary vitamin C intake in 1842 men and 2525 women aged 39–79 y at baseline[1]

	Men					Women				
Quintile	Vitamin C intake, mg/d	*n*	Hip fracture, *n*	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[2]	Vitamin C intake, mg/d	*n*	Hip fracture, *n*	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[3]
1	0–45	369	30	1.00 (referent)	1.00 (referent)	0–48	505	71	1.00 (referent)	1.00 (referent)
2	46–62	368	21	0.67 (0.36, 1.23)	0.68 (0.36, 1.29)	49–67	505	67	1.09 (0.74, 1.62)	1.15 (0.77, 1.71)
3	63–86	369	24	0.77 (0.43, 1.38)	0.77 (0.41, 1.46)	68–91	505	68	1.08 (0.73, 1.60)	1.20 (0.80, 1.79)
4	87–119	368	18	0.54 (0.29, 1.01)	0.55 (0.28, 1.09)	92–124	505	60	0.93 (0.63, 1.39)	1.02 (0.67, 1.53)
5	120–471	368	19	0.61 (0.33, 1.14)	0.64 (0.34, 1.23)	125–405	505	73	1.26 (0.85, 1.85)	1.33 (0.88, 2.00)
P-trend	—	—	—	0.09	0.15	—	—	—	0.49	0.34

[1]Hip fracture risk was not associated with vitamin C intake in men and women. Hip fracture risk was calculated from Prentice-weighted Cox proportional HRs regressed on quintiles of vitamin C intake to determine differences between the upper quintiles referent to quintile 1 and *P*-trend values.
[2]Adjusted for age, family history of osteoporosis, BMI, smoking, physical activity, steroid medication, energy intake, dietary calcium intake, and calcium- and vitamin D–containing supplements.
[3]Adjusted for age, family history of osteoporosis, BMI, smoking, physical activity, steroid medication, energy intake, dietary calcium intake, calcium- and vitamin D–containing supplements, menopausal status, and hormone replacement therapy.

study and inverse associations between plasma vitamin C and fracture risk in the fracture study in men only. In the ultrasound study, there was a significant positive linear relation between vitamin C intake and VOS in men and BUA in women. Moreover, in men, mean intakes of 107 and 173 compared with 37 mg/d were associated with a 0.6% higher VOS, and in women, intakes of 110 and 172 compared with 40 mg/d were associated with 3.2–4.2% higher BUA. These differences in intakes were ~70 mg/d (quintile 4) and 136 mg/d (quintile 5), which is achievable through the usual diet (e.g., by consuming 2–4 kiwi fruit/d) (40). When we included vitamin C supplements in the dietary analysis, the findings did not differ markedly; hence, misclassification as a result of supplement use did not obscure the association. In our fracture study, vitamin C intake was not associated with fracture risk. However, hip fracture risk in men was significantly inversely associated with plasma vitamin C, and for men with plasma concentrations of 53–61 compared with 3–31 μmol/L, we observed significant reductions in fracture risk of 65% (hip) and 74% (spine). We found no effect of alcohol on the associations with vitamin C and BUA, VOS, or fracture risk when we included this variable in our statistical analyses.

Our findings of significant associations between vitamin C and heel ultrasound and fracture risk potentially reflect the crucial role of vitamin C in bone health (8–10). It has been well documented that vitamin C is an important cofactor in hydroxylation reactions within collagen fibers, thereby increasing collagen strength (8). Vitamin C may also mediate osteoblastogenesis via peroxisome proliferator–activated receptor-γ expression and osteoclastogenesis via receptor activator of nuclear factor κ-B ligand expression (9, 10).

Vitamin C intakes in men and women were shown to be associated with VOS and BUA, respectively. BUA is an indicator of the structural organization of bone, and VOS determines bone stiffness (41). Potential reasons for this are currently not known, but both ultrasound variables may be independently associated with osteoporotic fractures (37, 42, 43).

The current observational studies have several methodologic advances over previous studies. First, our investigations were based on more-accurately estimated dietary and supplemental

TABLE 3
Spine fracture risk after a median follow-up of 12.6 y stratified by quintiles of dietary vitamin C intake in 1808 men and 2334 women aged 39–79 y at baseline[1]

	Men					Women				
Quintile	Vitamin C intake, mg/d	*n*	Spine fracture, *n*	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[2]	Vitamin C intake, mg/d	*n*	Spine fracture, *n*	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[3]
1	0–45	362	19	1.00 (referent)	1.00 (referent)	0–48	467	36	1.00 (referent)	1.00 (referent)
2	46–62	362	12	0.59 (0.28, 1.24)	0.57 (0.26, 1.22)	49–67	467	20	0.60 (0.34, 1.06)	0.63 (0.35, 1.12)
3	63–86	361	15	0.75 (0.37, 1.51)	0.72 (0.36, 1.47)	68–91	467	21	0.62 (0.36, 1.09)	0.68 (0.38, 1.22)
4	87–120	362	11	0.52 (0.25, 1.12)	0.50 (0.23, 1.10)	92–124	467	21	0.61 (0.35, 1.07)	0.65 (0.36, 1.16)
5	121–471	361	21	1.10 (0.58, 2.08)	1.05 (0.52, 2.13)	125–405	466	26	0.81 (0.48, 1.38)	0.90 (0.50, 1.61)
P-trend	—	—	—	0.86	0.91	—	—	—	0.44	0.68

[1]Spine fracture risk was not associated with vitamin C intake in men and women. Spine fracture risk was calculated from Prentice-weighted Cox proportional HRs regressed on quintiles of vitamin C intake to determine differences between the upper quintiles referent to quintile 1 and *P*-trend values.
[2]Adjusted for age, family history of osteoporosis, BMI, smoking, physical activity, steroid medication, energy intake, dietary calcium intake, and calcium- and vitamin D–containing supplements.
[3]Adjusted for age, family history of osteoporosis, BMI, smoking, physical activity, steroid medication, energy intake, dietary calcium intake, calcium- and vitamin D–containing supplements, menopausal status, and hormone replacement therapy.

TABLE 4
Hip fracture risk after a median follow-up of 12.6 y stratified by quintiles of plasma vitamin C concentration in 1650 men and 2183 women aged 39–79 y at baseline[1]

	Men					Women				
Quintile	Vitamin C, μmol/L	*n*	Hip fracture, *n*	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[2]	Vitamin C, μmol/L	*n*	Hip fracture, *n*	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[3]
1	3–31	344	33	1.00 (referent)	1.00 (referent)	4–43	452	59	1.00 (referent)	1.00 (referent)
2	32–43	338	23	0.77 (0.43, 1.38)	0.77 (0.42, 1.43)	44–55	456	61	1.18 (0.78, 1.80)	1.20 (0.79, 1.84)
3	44–52	344	20	0.88 (0.47, 1.62)	0.76 (0.40, 1.42)	56–63	404	52	1.14 (0.74, 1.76)	1.09 (0.69, 1.72)
4	53–61	303	8	0.36 (0.16, 0.81)*	0.35 (0.16, 0.80)*	64–73	454	57	1.18 (0.78, 1.80)	1.13 (0.73, 1.77)
5	62–132	321	14	0.56 (0.29, 1.11)	0.52 (0.25, 1.06)	74–170	417	62	1.48 (0.97, 2.25)	1.46 (0.94, 2.27)
P-trend	—	—	—	0.025	0.016	—	—	—	0.11	0.18

[1]Hip fracture risk was calculated from Prentice-weighted Cox proportional HRs regressed on quintiles of plasma vitamin C concentrations to determine differences between the upper quintiles referent to quintile 1 and *P*-trend values. *$P < 0.05$ for significant differences between the 2 upper quintiles of plasma vitamin C referent to quintile 1.

[2]Adjusted for age, family history of osteoporosis, BMI, smoking, physical activity, steroid medication, energy intake, dietary calcium intake, and calcium- and vitamin D–containing supplements.

[3]Adjusted for age, family history of osteoporosis, BMI, smoking, physical activity, steroid medication, energy intake, dietary calcium intake, calcium- and vitamin D–containing supplements, menopausal status, and hormone replacement therapy.

vitamin C intakes from a 7dDD (26, 27) in contrast with FFQs used in previous studies (13, 15, 18, 19, 21–25). We also investigated prospective associations with fracture risk that had previously only been studied in one US study (19). To our knowledge, only 4 studies on bone health have previously been undertaken in men (14, 17–19); hence, our studies have provided more evidence that vitamin C sufficiency is especially relevant to men. We also addressed small sample sizes in British populations with the use of a random subcohort of 4000 participants (ultrasound study), representative of 25,639 people, and a case-cohort subset of 5319 participants (fracture study). One limitation of our study was its observational nature, which could not infer causality. Moreover, ultrasound measures are less precise than dual-energy X-ray absorptiometry is, although they have previously been shown to effectively predict osteoporotic fractures in population-based studies (37, 38, 42, 44). Moreover, these measurements were taken ∼3 y after those of diet and plasma were taken. In our fracture study, data to estimate vitamin C intake over the follow-up period were not available. The use of hospital-admission data may have underascertained spine fractures, which are commonly underdiagnosed (45), thereby limiting our power to detect any associations. Furthermore, we were unable to discriminate between low- and high-trauma fractures. However, because of the age profile of the population and the known association with the risk factors of age group and sex in our population, it is likely that only a very small proportion of fractures would have been compatible with a high-trauma cause. The variability in the top quintiles of vitamin C that were due to the small number of fractures may have inflated the associations. Although all analyses were adjusted for important covariates, residual confounding as well as unmeasured confounding may have occurred because variables such as sunlight exposure (46) were not measured.

TABLE 5
Spine fracture risk after a median follow-up of 12.6 y stratified by quintiles of plasma vitamin C concentration in 1619 men and 2024 women aged 39–79 y at baseline.[1]

	Men					Women				
Quintile	Vitamin C, μmol/L	*n*	Spine fracture, *n*	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[2]	Vitamin C, μmol/L	*n*	Spine fracture, *n*	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[3]
1	3–31	332	21	1.00 (referent)	1.00 (referent)	4–43	420	22	1.00 (referent)	1.00 (referent)
2	32–43	328	14	0.71 (0.35, 1.41)	0.68 (0.33, 1.40)	44–54	393	32	1.71 (0.96, 3.04)	1.83 (1.02, 3.29)
3	44–52	336	12	0.67 (0.32, 1.41)	0.61 (0.28, 1.32)	55–63	408	19	0.98 (0.52, 1.85)	1.03 (0.52, 2.03)
4	53–61	300	5	0.30 (0.11, 0.80)*	0.26 (0.10, 0.69)**	64–73	426	22	1.15 (0.62, 2.13)	1.24 (0.63, 2.42)
5	62–132	323	17	0.97 (0.50, 1.90)	0.90 (0.42, 1.90)	74–170	377	15	0.89 (0.45, 1.77)	1.01 (0.48, 2.11)
P-trend	—	—	—	0.49	0.38	—	—	—	0.41	0.71

[1]Spine fracture risk was calculated from Prentice-weighted Cox proportional HRs regressed on quintiles of plasma vitamin C concentrations to determine differences between the upper quintiles referent to quintile 1 and *P*-trend values. *,**Significant differences between the 2 upper quintiles of plasma vitamin C referent to quintile 1: *$P < 0.05$, ** $P < 0.01$.

[2]Adjusted for age, family history of osteoporosis, BMI, smoking, physical activity, steroid medication, energy intake, dietary calcium intake, and calcium- and vitamin D–containing supplements.

[3]Adjusted for age, family history of osteoporosis, BMI, smoking, physical activity, steroid medication, energy intake, dietary calcium intake, calcium- and vitamin D–containing supplements, menopausal status, and hormone replacement therapy.

In the ultrasound study, findings of a positive association between vitamin C intake and heel ultrasound were in agreement with those of previous studies that reported 3–5% higher BMD with increasing vitamin C intakes (13) and 1–2% higher BMD with every 100-mg/d increment in intake (14, 15). These studies predominantly used dual-energy X-ray absorptiometry for measurements, and none of the studies used heel ultrasound, and hence, we could not compare our effect sizes for BUA (3.2–4.2%) and VOS (0.6%) directly with those of previous investigations. However, the effect of diet on heel ultrasound compared with a 10-y increase in age was similar in women but was only small in men. The scale of the association for age was ~2 times greater than diet was for BUA (in women) and 14 times larger for VOS (in men). In contrast with diet, plasma vitamin C was not associated with heel ultrasound in this study. With consideration that vitamin C cannot be synthesized by humans, and the only source is from diet, not finding an association for plasma status may have suggested that dietary vitamin C may be a marker for other dietary factors that are positively associated with bone. To our knowledge, there has only been one previous cross-sectional study of vitamin C status and BMD in 13,080 US men and women (14). The results were nonlinear and showed that men with serum vitamin C of 28.4–56.8 μmol/L had higher hip BMD than did those with lower or higher concentrations, but no associations were shown in women. That study had a much-larger sample size than that of our study, and we hypothesize that we may have had similar findings if our investigations were undertaken in a larger population.

Our fracture-study findings of no associations between vitamin C intake and fracture risk in either sex are in agreement with the only comparable published cohort study (19), which also reported no association in 918 US men and women despite a follow-up of 15–17 y. In contrast, investigations between plasma vitamin C and fracture risk are lacking, which suggested that our finding of a significant inverse association in men but not women is novel, although the reasons for our sex-specific findings are unknown. We suggest that more-prospective fracture studies that investigate vitamin C intake and status are needed because current data are scarce.

Our investigations showed dietary vitamin C to be associated with heel ultrasound and plasma concentrations with fracture risk. Disagreements between diet and plasma status in association with bone health have been reported previously (14) and may be a result of an overestimation or underestimation of intake and homeostatic mechanisms that influence blood concentrations (28). It was not possible, in this exploratory analysis, to understand the mechanisms for these opposing findings, and thus, we propose that it is important for future studies to estimate both vitamin C intakes and blood concentrations when investigating relations with bone health.

In conclusion, the current study showed that higher intakes, but not plasma concentrations of vitamin C, were associated with higher heel ultrasound measures in both men and women. These findings may have important implications for fractures in the long term, although there were no associations between vitamin C intake and fracture risk in this population. In contrast, higher plasma vitamin C was a significant predictor of reduced fracture risk in men with the greatest reduction in risk shown at the spine. Our findings that vitamin C intake and status were inconsistently associated with bone health variables suggest that additional research is warranted.

The authors' responsibilities were as follows—HF: developed the research question, performed the data analyses, and drafted the manuscript; MAHL: prepared the dietary and supplemental data for the statistical analysis; K-TK: is principal investigator of the European Prospective Investigation into Cancer in Norfolk study; RNL: organized the data collection and implemented the record linkage; AAW: developed the research question, organized the data collection, and had primary responsibility for the final content of the manuscript; and all authors: were involved in the interpretation of the data, contributed to the writing of the manuscript, and read and approved the final manuscript. The authors received no specific grant from any funding agency in the commercial sectors for this research. The Faculty of Medicine and Health Sciences postgraduate funding office had no role in the design, analysis, or writing of this article. None of the authors reported a conflict of interest related to the study.

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