Dietary vitamin C and bone mineral density in postmenopausal women in Washington State, USA

Suzanne G Leveille, Andrea Z LaCroix, Thomas D Koepsell, Shirley A Beresford, Gerald Van Belle, David M Buchner

Abstract

Study objective—To examine the relationship between dietary vitamin C and hip bone mineral density (BMD) in postmenopausal women.

Design—This was a cross sectional study using retrospective diet and vitamin supplement data.

Setting—The Seattle area of Washington State.

Participants—Screenees for a clinical trial of a drug to prevent osteoporotic fractures; 1892 women aged 55-80 years who had hip bone densitometry and osteoporosis risk factor information.

Main results—Mean energy and dietary intake of vitamin C was 113 mg/day; including supplement use, mean intake was 407 mg/day. There were no differences in BMD according to diet-only vitamin C intake or combined dietary and supplemental vitamin C intake. Longer duration of vitamin C supplement use was associated with higher BMD in women who had not used oestrogen replacement therapy (trend p=0.02) and among women aged 55-64 years (trend p=0.01). Women aged 55-64 years who used vitamin C supplements for ≥10 years had a higher BMD than non-users aged 55-64 years (multivariate adjusted mean BMD 0.699 (0.017) g/cm² versus 0.655 (0.007) g/cm², p=0.02). Benefits were not evident in older age groups or in women who had used oestrogen in the past. Frequent intake of foods rich in vitamin C was not associated with BMD.

Conclusion—There was no evidence that vitamin C from the diet was associated with BMD, although long term use of vitamin C supplements was associated with a higher BMD in the early postmenopausal years and among never users of oestrogen.

Methods

A cross sectional design was used to examine whether dietary and supplemental vitamin C intake is associated with greater BMD. Subjects were screenees from the Seattle site of the fracture intervention trial (FIT), one of 11 sites participating in the clinical trial of alendronate, a potent amino-bisphosphonate, funded by Merck & Co, Inc. The design of the FIT study was described in detail previously. The study was approved by the human subjects review committees of the University of Washington and Group Health Cooperative of Puget Sound (GHC).

Subjects were postmenopausal women aged 55 to 80 years, and 80% were enrollees of GHC, a large Washington based health maintenance organisation (HMO), a comprehensive, prepaid healthcare delivery system which provides preventive, acute, and chronic healthcare services to its members. Study volunteers were excluded prior to clinic screening for the following reasons, as reported during a telephone interview: unexplained weight loss in the previous 12 months of >10% of weight, severe gastrointestinal disease, cancer in the past 10 years, bilateral hip replacement, recent use of medications that may influence bone turnover such as oestrogen, glucocorticoids, or etidronate, or use of a wheelchair or dependency on others for ambulation.

All GHC FIT screenees who completed the baseline clinic screening (n=2484) were sent questionnaires for the diet study; 1976 women...
(80%) completed and returned the forms. Eleven women who completed questionnaires were ineligible due to medical diagnoses that could influence bone status or unusual diets. An additional 73 subjects (3.7%) were excluded because of insufficient diet or screening data. The final study sample consisted of 1892 women.

DATA COLLECTION
The FIT clinic screening included a questionnaire on demographic characteristics, pertinent medical history including medication use, and osteoporosis risk factor information. Height and weight were measured by clinic study staff. BMD of the femoral neck was measured using dual energy x ray absorptiometry (DXA) (QDR-2000, Holologic, Inc, Waltham, Ma), performed by a trained technician.

FIT screenees were sent two questionnaires - a food frequency questionnaire (FFQ) and a vitamin supplement questionnaire. Dietary vitamin C intake was estimated using a 98 item food frequency questionnaire, a version of the National Cancer Institute/Block questionnaire, modified and analysed at the Fred Hutchinson Cancer Research Center. Dietary nutrient intake was calculated using the University of Minnesota’s Nutrition Coding Center database. The validity of the dietary vitamin C measure on the FFQ was demonstrated in a validation study of a very similar form in a sample of postmenopausal women; the correlation between vitamin C measured by the FFQ and from food records was 0.44 (R Patterson, Fred Hutchinson Research Centre, personal communication). Validation studies, demonstrating the validity of the instrument, have been conducted on earlier versions of the same FFQ.

Supplement intake
The vitamin supplement questionnaire was developed and pretested for this study. Subjects were asked to recall their usual supplement use for the 12 months prior to their screening visit for the FIT study. Questions on dose of individual vitamin C supplements used included the following categories: none, <300 mg, 300-700 mg, 701-1200 mg, and >1200 mg. The frequency categories on the questionnaire were as follows: rarely or never; once per week; 2-4 times per week; 5-7 times per week; and >7 times per week. In addition to dose and frequency questions, subjects were asked to identify duration of use for each supplement from among the following five categories: never, <1 year, 1-5 years, 5-10 years, and >10 years.

Supplement intake was determined to be greater than zero if reported use was at least for one year with a frequency of at least twice per week. For the calculation of average daily vitamin C supplement intake, dose was assumed to be the most common available dose in the reported dose category. For example, a reported vitamin C dose of “300-700mg” was assigned a dose of 500mg and the category of “more than 1200mg” was assigned a dose of 1500mg. In calculating the estimated intake, supplement use 2-4 times per week was equivalent to one half dose/day; 5-7 times per week was one dose/day; and 8 or more times per week was one and one half doses/day. Estimated amounts of vitamin C intake from the multivitamins were added to the supplement intake. We assigned the usual amounts of vitamin C in multivitamin supplements (60 mg, 100% RDA) according to the frequency of multivitamin use. For example, for one multivitamin use 2-4 times per week, daily vitamin C intake from the multivitamin was estimated at 30 mg, (one half dose per day). Average daily individual supplement intake was calculated as the product of the dose and frequency of use.

STATISTICAL ANALYSIS
Correlation coefficients between risk factors and total vitamin C intake (energy adjusted dietary intake plus supplement intake) were calculated using Pearson correlations for continuous variables and Spearman correlations for categorical variables. All analyses were conducted in SAS for mainframe v.6.08.

The analysis examined the relationship of hip BMD with total vitamin C intake, diet only intake, supplement intake, and vitamin C rich foods. Since energy intake was associated with BMD, we constructed a measure of diet only vitamin C intake that was adjusted for energy intake. The adjustment was done using the residual adjustment method. Dietary vitamin C intake was the dependent variable and total energy intake (kcal/day) was the independent variable in a linear regression model. The variables were log transformed for the regression and later exponentiated to derive the total intake amount. Residuals from the regression were subtracted from the predicted vitamin intake (as on the regression line) at the population mean of the total energy intake. The resulting measure of vitamin C intake was independent of energy intake. Total adjusted vitamin C intake was calculated as the sum of the energy adjusted dietary intake and the vitamin supplement intake.

The relationship between total and diet only vitamin C intake with BMD was examined using multiple linear regression adjusting for potential confounders. We examined a number of potential confounders that were known or suspected risk factors for osteoporosis; they included age, race (white and non-white), weight, height, number of reproductive years,
years since menopause (years from last natural menstrual period), number of live births, hormone replacement therapy (ever use and years of past use), surgical removal of both ovaries, hysterectomy, thiazide use, thyroid supplement use, smoking status (current, past, and never use), intake of calcium and vitamin D (intake from foods adjusted for energy intake and supplement intake), caffeine intake (mg/day), physical activity (number of blocks walked per day, number of hours of moderate and vigorous physical activity per week), self-rated health (fair or poor versus good, very good, or excellent), diabetes mellitus, and rheumatoid arthritis. Potential confounders related to dietary and supplemental vitamin C intake included energy intake (kcal/day), polyunsaturated fat intake (g/day), multivitamin use (yes/no), use of other single vitamin supplements, and alcohol intake (g/day). Confounders were selected based on their association with hip BMD in a model containing all potential confounders. In other words, the fully adjusted vitamin C/BMD models presented in this paper included all covariates that were independently associated with BMD in models containing all potential confounders (test of coefficient, p<0.05). This approach yielded a more precise estimate than either stepwise modeling or adding all possible confounders to the model either of which led to only slight variations in the coefficient for vitamin C.17

Log transformation of key exposure and outcome variables made no material difference in the findings, therefore, reported findings are from regressions using untransformed variables. Following energy adjustment, none of the nutrient intakes were found to be collinear, i.e., correlation coefficient > 0.60.18 Regression diagnostics included evaluation of the distribution of residuals for normality and residual plots for presence of outliers. Removal of the very few outliers did not materially change the findings, so they were retained in the analysis.

A separate analysis was conducted to evaluate the relationship between vitamin C supplement use and BMD. Interactions were evaluated by adding a cross product term to the regression models. Analysis of covariance was used to evaluate the association of duration of vitamin C use with BMD while controlling for multiple confounders. Pairwise t tests were performed to test for differences between adjusted means. Regression methods and χ² approximations were used to evaluate trends in duration of vitamin C use with BMD. Similar analytic approaches were used to evaluate associations between individual vitamin C rich foods and BMD.

**Results**

Means and percentages of demographic characteristics and osteoporosis risk factors are shown in table 1. Study participants had a mean age of 71.5 years, and were predominantly healthy, white (96.7%), women, many of whom were past users of oestrogen (41%). Total vitamin C intake correlated most significantly with calcium and vitamin D intakes (r 0.43 and 0.38, respectively), though correlations with vitamin C intake were also observed with age, weight, education, number of reproductive years, physical activity, past oestrogen use, thyroid use, diagnosis of rheumatoid arthritis, intakes of polyunsaturated fats and caffeine, and past smoking (range of correlation coefficients, 0.05-0.12; all were statistically significantly different from zero, p<0.05; data not shown).

Adjusting for energy intake resulted in very minor changes in the dietary vitamin C intake estimates (table 2). The mean dietary intake among women who were not taking vitamin C supplements was nearly the same as that of all respondents. The addition of supplemental intake to dietary intake of vitamin C increased the mean dietary intake fourfold, from 113 to 407 mg.

No association was found between total or dietary vitamin C intake and BMD (table 3). The results of the dietary analyses were not substantially different between the entire study group and the subgroup of non-users of supplements. The r² for the fully adjusted model of the total vitamin C intake was 0.29; two variables, age and weight, accounted for 25% of the variance in the full model.

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**Table 1** Subject characteristics and risk factors for osteoporosis

<table>
<thead>
<tr>
<th>Mean (SD)</th>
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<tbody>
<tr>
<td><strong>Age (y)</strong></td>
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<tr>
<td><strong>Weight (kg)</strong></td>
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<tr>
<td><strong>Education (y)</strong></td>
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<tr>
<td><strong>Reproductive years</strong></td>
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<tr>
<td><strong>Blocks walked per d</strong></td>
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<tr>
<td><strong>Vigorous activity (h/wk)</strong></td>
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<tr>
<td><strong>Vitamin D intake (μg/d)</strong></td>
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<tr>
<td><strong>Calcium intake (mg/d)</strong></td>
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<tr>
<td><strong>Energy intake (kcal/d)</strong></td>
</tr>
<tr>
<td><strong>Polyunsaturated fat (g/d)</strong></td>
</tr>
<tr>
<td><strong>Caffeine intake (mg/d)</strong></td>
</tr>
<tr>
<td><strong>Alcohol intake (g/d)</strong></td>
</tr>
<tr>
<td><strong>Race (% non-white)</strong></td>
</tr>
<tr>
<td><strong>Current smoker (%)</strong></td>
</tr>
<tr>
<td><strong>Former smoker (%)</strong></td>
</tr>
<tr>
<td><strong>Oestrogen past user (%)</strong></td>
</tr>
<tr>
<td><strong>Thyroid user (current) (%)</strong></td>
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<tr>
<td><strong>Thiazide diuretic (ever user) (%)</strong></td>
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<tr>
<td><strong>Diabetes mellitus (%)</strong></td>
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<tr>
<td><strong>Rheumatoid arthritis (%)</strong></td>
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<tr>
<td><strong>Bilateral oophorectomy (%)</strong></td>
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<tr>
<td><strong>Fair/poor self rated health (%)</strong></td>
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*Number of years from menarche to menopause.
†Total intake summed from energy adjusted dietary intake and supplement intake.

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**Table 2** Mean daily dietary, supplemental, and total vitamin C intakes

<table>
<thead>
<tr>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamin C (mg)</strong></td>
<td></td>
</tr>
<tr>
<td>Dietary intake *</td>
<td>115 (56)</td>
</tr>
<tr>
<td>Adjusted dietary †</td>
<td>113 (52)</td>
</tr>
<tr>
<td>Adjusted dietary w/o supplement users‡</td>
<td>108 (52)</td>
</tr>
<tr>
<td>Supplement intake§</td>
<td>294 (447)</td>
</tr>
<tr>
<td>Total intake¶</td>
<td>407 (454)</td>
</tr>
</tbody>
</table>

*Unadjusted dietary vitamin C intake calculated from food frequency questionnaire data.
†Energy adjustment calculated using residual adjustment method.
‡Excludes vitamin C and multivitamin supplement users (n=1068).
§Sum of individual and multivitamin supplement intake; average intake estimated for the year prior to study screening.
¶Sum of vitamin supplement intake and energy adjusted dietary intake.
ing for multiple potential confounders, including other vitamins, multivitamins, and calcium, did not change the findings.

The adjusted mean BMD increased slightly with years of vitamin C supplement use (fig 1). Among the women who had never used oestrogen, those who took vitamin C supplements for more than 10 years had higher BMD than women who had never taken vitamin C supplements (adjusted mean BMD 0.648 g/cm² versus adjusted mean BMD 0.628 g/cm², p=0.02). There was a significant trend for higher BMD with longer duration of vitamin C supplement use in the women who never used oestrogen (test for trend, p=0.02). The association was independent of other factors that could influence bone density, including calcium and vitamin D supplement use. Among the past users of oestrogen, there was no evidence of an association between duration of vitamin C supplement use and bone density. The test for interaction between duration of vitamin C use and status of past oestrogen use was of borderline statistical significance (p = 0.05).

To test the hypothesis that taking vitamin C supplements may be most beneficial during the perimenopausal period, the time of most rapid bone loss, we examined years of supplement use by age groups. After adjustment for several potential confounders, including years of calcium supplement use, women aged 55-64 years who had used vitamin C supplements for 10 or more years had 6.7% higher BMD than women who were non-users (adjusted mean BMD 0.699 g/cm² versus adjusted mean BMD 0.655 g/cm², p = 0.02) (fig 2). The association was not observed in the older age groups. There was a significant interaction between age and duration of vitamin C use (test of cross product term, p=0.002).

The frequency of intake of vitamin C rich foods varied widely among the study participants. Women who consumed more frequent servings of vitamin C rich foods did not have higher BMD than other women (table 4). Women who consumed oranges or citrus fruit five or more times per week had similar BMD to women who rarely ate oranges and citrus fruit (adjusted mean BMD 0.632 and 0.627, respectively). Results were similar for citrus juices, cantaloupe, broccoli, and tomatoes.

**Discussion**

Our findings do not show a consistent relationship between vitamin C intake and BMD in postmenopausal women. Supplemental vitamin C intake was associated with higher BMD in younger postmenopausal women and in women who had never used postmenopausal oestrogen replacement. Neither dietary vitamin C intake nor more frequent servings of foods rich in vitamin C were associated with BMD, regardless of multivariate adjustments.

Modest and inconsistent associations between dietary vitamin C and BMD have been observed previously in studies of broad groups of nutrients using multiple BMD sites. A study that reported positive vitamin C/BMD associations at one of three upper extremity sites in a cross sectional analysis, did not find the same results longitudinally, attributing the inconsistencies to problematic outliers in the longitudinal study. Vitamin C supplement

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**Table 3** Regression analysis of femoral neck bone mineral density with vitamin C intake* in all participants and in non-users of vitamin C supplements

<table>
<thead>
<tr>
<th>Models</th>
<th>Total vitamin C intake† among all participants</th>
<th>Dietary vitamin C intake among all participants</th>
<th>Dietary vitamin C intake among non-supplement users**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$ (SE)$^\dagger$</td>
<td>p value$^\dagger$</td>
<td>$\beta$ (SE)$^\dagger$</td>
</tr>
<tr>
<td>No covariates</td>
<td>0.004 (0.005)</td>
<td>0.44</td>
<td>0.019 (0.046)</td>
</tr>
<tr>
<td>Age &amp; weight</td>
<td>0.008 (0.005)</td>
<td>0.10</td>
<td>0.064 (0.040)</td>
</tr>
<tr>
<td>Full model: age, weight, height, reproductive years, thiazide use, thyroid use, energy intake, vigorous activity/wk, blocks walked/d, diabetes, past oestrogen use‡</td>
<td>0.006 (0.005)</td>
<td>0.22</td>
<td>0.019 (0.041)</td>
</tr>
<tr>
<td>Full model with vitamins D and E, calcium, and β-carotene intakes</td>
<td>0.005 (0.006)</td>
<td>0.42</td>
<td>0.010 (0.044)</td>
</tr>
</tbody>
</table>

*Total intake is the sum of energy adjusted dietary vitamin C intake and supplement intake.
†$\beta$ represents the unit change in bone mineral density (g/cm²) associated with 1g change in vitamin C intake.
‡Value from t test of regression coefficient.
§Number of years from menarche to menopause.
¶Past oestrogen use includes two variables: ever use (yes/no) and number of years of past use.
**Excludes multiple and individual vitamin C supplement users.

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**Figure 1** Adjusted mean bone mineral density (BMD) in relation to duration of vitamin C supplement use by all screening, and by oestrogen use and non-use in 1892 postmenopausal women. Means were adjusted for age, weight, height, energy intake, physical activity, thiazide use, number of years from menarche to menopause, thyroid supplement use, diabetes, past oestrogen use (among all screeners), vitamin D supplement use, and years of calcium supplement use.
use, compared with non-use, was associated with greater bone mineral content of the distal radius, but not at four other distal bone sites in older Japanese-American women living in Hawaii. The inconsistencies in the vitamin C findings observed both within and between studies examining the relationship between nutrient intake and bone density are probably a result of differences in the populations studied, the methods of nutrient measurement, and in the limited adjustment for confounders. Overall, the reported associations between bone density and vitamin C were not as strong as other osteoporosis risk factors, and measurement error in vitamin C intake assessment may have been a serious limitation to research in this area.

There are very few reports examining the vitamin C/BMD relationship by age strata in older women. Our finding that longer duration of vitamin C supplement use was associated with higher BMD only in women aged 55-64 is consistent with an earlier report showing a vitamin C/BMD association in subjects aged 51-60 (n=25 women and 5 men) but not in older participants. The mechanisms of bone loss during the perimenopausal and early postmenopausal years, the time of most rapid bone loss, may differ from that of later years, when the bone loss related to ageing is more gradual. Despite the lack of previous research on this issue, it is noteworthy that the observed vitamin C/BMD association parallels recent findings on oestrogen use, which show the greatest reduction in fracture risk in current oestrogen users who began oestrogen replacement therapy during the five years after menopause. Since we lacked information on age of initiation of vitamin C use, we were unable to determine whether older women who had taken vitamin C through their menopausal years had higher BMD than older women who began taking vitamin C supplements several years after menopause. This issue warrants further exploration as vitamin C may operate differently in early postmenopause versus later life.

The lack of a consistent supplement/BMD association in relation to strata of past and never use of oestrogen is difficult to interpret. Indeed, past users of oestrogens are likely to be women with greater risk for osteoporosis, shown in our data by their low BMD compared with never users of postmenopausal oestrogens. Although we adjusted for numerous osteoporosis risk factors, past users of oestrogen may differ from never users in ways that were unmeasured in our study. Also, the potent effects of oestrogen may obscure any likely more modest benefits of vitamin C. The inconsistency between the supplement and dietary vitamin C findings suggests that the supplement associations could be due to confounding, possibly from unmeasured characteristics distinguishing supplement users from non-users. However, in our previous work with the same study subjects, use of vitamin E and β-carotene supplements was not associated with BMD (unpublished findings). Controlling for the use of other vitamin supplements, including multivitamins, did not materially alter the vitamin C findings. If vitamin C intake has a role in limiting postmenopausal bone loss, it may require doses in excess of the high-
est dietary intake levels to exert any beneficial effect. A six ounce measure of orange juice contains approximately 60mg of vitamin C, a fraction of the most common dose reported by supplement users in our study (500 mg). With the high percentage of vitamin C supplement users in our cohort (36%), supplement use distinguished women with high vitamin C intakes from those with low intakes. In addition, the availability of duration of supplement use information was distinct from the dietary intake information which reflected average intake rather than duration of high intake. Considering these two factors, the high proportion of supplement users in the cohort and the available duration of use information, supplement use may have been the best measure for evaluating the relationship between vitamin C and BMD in this population.

Measurement of vitamin C intake with each of the study questionnaires had limitations. Although the FFQ was validated in a group of postmenopausal women, they were somewhat younger than the women we studied, thus it is unclear if recall of the older women may have been better or worse than the women in the validation study. Also, the vitamin supplement questionnaire was not previously validated and long term recall of past vitamin use could be subject to random error, which could have led to an underestimation of a vitamin C-BMD association. However, since multiple measures of vitamin C intake were used, the impact of measurement error on our findings was reduced. If measurement error was operating, for example, with the dietary vitamin C intake calculated from the FFQ data, it did not interfere with our detection of the expected associations between intake and subject characteristics and behaviours. Supplement intake, measured using a separate questionnaire, was not subject to the same errors of intake estimation as the FFQ. Another limitation to our study is the cross sectional design, however, since BMD is a measure that encompasses cumulative effects and because retrospective dietary and supplement information was obtained, the design was appropriate for this early investigation.

The mechanisms through which vitamin C may contribute to bone density remain to be studied. One possible mechanism is vitamin C's role in collagen formation and bone matrix development.11 Also, vitamin C is a potent antioxidant12 and antioxidants have been shown, in laboratory studies, to limit bone resorption.26,29 However, our own work exploring other dietary antioxidants, including vitamin E and β-carotene, showed no associations with BMD (unpublished findings). Other explanations that have been proposed for a vitamin C/BMD association include the actions of vitamin C on osteoblast growth or in promoting calcium absorption.13 Although our results suggest a modest association between vitamin C and BMD, the findings were inconsistent among supplement users, and no relationship was observed between dietary vitamin C and bone density. Available potent therapies such as oestrogen and alendronate, have been shown to reduce fracture risk, but these approaches may not be suitable for all postmenopausal women at risk for osteoporosis. Similar to previous studies of nutrients and bone density, our vitamin C findings are not definitive; however, interesting questions have been raised in this research. Further studies are needed to determine whether vitamin C offers a benefit in modifying postmenopausal bone loss.

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References

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