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Antioxidant Vitamin Supplements and Markers of Bone Turnover in a Community Sample of Nonsmoking Women

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ABSTRACT

Background: Whereas several epidemiological studies suggest that low dietary intake of vitamins C and E is linked to increased hip fracture in smokers and antioxidants (dietary and endogenous) are reduced in elderly osteoporotic women, none has demonstrated an effect of supplemental antioxidants on bone turnover.

Methods: In an observational study of 533 randomly selected women, we investigated the associations among the use of antioxidant supplements, vitamins C and E, serum levels of biochemical markers of bone turnover (C-telopeptide [CTx] and bone-specific alkaline phosphatase [BSAP]), and whole body bone mineral density (BMD).

Results: Twenty-two women were identified as current users of supplemental vitamin C or E. Duration of antioxidant supplement use was negatively associated with age-adjusted and weight-adjusted serum CTx, such that mean CTx levels (natural log transformed) were 0.022 units lower for each year of exposure. No significant differences were detected for adjusted serum BSAP or whole body BMD.

Conclusions: Our results suggest that antioxidant vitamin E or C supplements may suppress bone resorption in nonsmoking postmenopausal women. Coupling of bone formation and resorption may explain the absence of an effect on bone formation markers, given evidence of enhanced effects of antioxidants on osteoblast differentiation; this warrants further investigation. This work adds to the growing body of evidence that antioxidants may play a role in preventing osteoporosis.

INTRODUCTION

OXIDATIVE STRESS IS CAUSED BY exposure to oxidants that disrupt the reduction/oxidation (redox) status of cells. This process plays an im-

portant role in aging and has been implicated in the pathogenesis of degenerative diseases, including cancer,¹ diabetes mellitus,² atherosclerosis,³ neurodegenerative diseases,⁴ and rheumatoid arthritis.⁵ Osteoporosis is a common degenera-

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tive disease, yet there is limited evidence that it is characterized by oxidative stress.

Antioxidants attenuate the cytotoxic effects of oxidative stress by reducing reactive oxygen species (ROS) and other intracellular free radicals. Vitamins C and E function as antioxidant vitamins as they possess free radical-scavenging properties. Lower levels of these dietary and endogenous antioxidants have been found in elderly osteoporotic women compared with age-matched controls.⁶ Several epidemiological studies have found a positive association between dietary vitamin C intake and bone mineral density (BMD) in postmenopausal women,⁷⁻⁹ and low dietary intake of vitamins C and E has been linked to an increased risk of hip fracture in smokers.¹⁰

BMD and bone turnover are independent predictors of fracture risk.¹¹ Guinea pig studies have shown that low vitamin C intake in growing animals is associated with high rates of bone turnover.¹² Low intake of vitamin C has been associated with an increased rate of bone loss in postmenopausal women¹³; however, data concerning bone turnover in humans is lacking. We sought to evaluate the associations between the use of antioxidant supplements vitamins C and E and serum levels of biochemical markers of bone turnover, in conjunction with measurements of whole body BMD.

MATERIALS AND METHODS

Subjects

This community-based study is set in the Barwon Statistical Division surrounding Geelong in southeastern Australia and forms part of the Geelong Osteoporosis Study.¹⁴ From a total of 872 women randomly selected from electoral rolls, 816 over the age of 48 years were included in this analysis (median age 68.3 years, range 48-89 years). Fifty-six women were excluded because no blood sample was available for biochemical analysis. We have reported previously that the mean age of menopause for women living in the region is 48 years,¹⁴ and we used this as the minimum age for inclusion in the study. This study was approved by the Barwon Health Research and Ethics Advisory Committee. Written informed consent was obtained from all participants.

Data

Self-reported supplement and medication use, diet, and lifestyle were documented by questionnaire. Subjects were described as antioxidant supplement users if they were current users of vitamins C or E or both at the time of assessment. Duration of use was documented in years, but doses remained unspecified. Calcium intake was estimated from a validated food frequency questionnaire.¹⁵ Subjects were described as active if they reported at least "walking at brisk pace, performing normal housework or other work, and engaging in light exercise" on a regular basis; otherwise, they were described as sedentary. Blood samples were obtained after an overnight fast and stored at -80°C for random batch analysis. The serum bone resorption marker, C-telopeptide (CTx), was measured using an electrochemiluminescence immunoassay (Crosslaps, Roche, Germany), with an interassay coefficient of variation (CV) of 8.5%. Serum bone-specific alkaline phosphatase (BSAP), a marker of bone formation, was calculated from total alkaline phosphatase measured with a colorimetric assay (p-nitrophenyl phosphate with AMP buffer, Roche, Mannheim, Germany) after BSAP precipitation using wheat germ lectin as recommended by the manufacturer. The interassay CV was 11.3%. BMD was measured for the whole body (Lunar DPX-L, software version 1.31) in 801 women, with a short-term precision of 0.4%.

Statistics

Characteristics of supplement users and non-users were compared using 2-sample *t* test or Mann-Whitney for continuous and chi-square test for discrete data. Women who were smokers or users of hormone therapy (HT), bisphosphonates, calcitriol, oral glucocorticoids, calcium, or vitamin D supplements were excluded from the analyses investigating the association among antioxidant vitamin supplement use, markers of bone turnover, and BMD because of potential confounding. Analysis of variance (ANOVA), followed by the Tukey test of multiple comparisons, was used to test for differences in unadjusted serum bone turnover markers (serum CTx and BSAP), with duration of exposure to antioxidant vitamin supplements categorized into three groups: 0, <5 years, and 5+ years. Using continuous data, standard multiple regression tech-

niques were used to test the association among duration of supplement use, markers of bone turnover, and BMD. Both serum CTx and BSAP were natural log transformed (lnCTx and lnBSAP) in the regression models. All statistical analyses were performed using MINITAB (version 13).

RESULTS

Characteristics of total sample

Forty-four subjects were identified as users of vitamin C ($n = 26$) and/or vitamin E ($n = 22$), representing 5.4% of our sample. Median duration of use among the antioxidant supplement users was 4.0 years (interquartile range [IQR] 2.0–10.0). There were no differences in age, dietary calcium intakes, weight, or height between the groups (all $p > 0.05$); however, the supplemented group tended to have a lower body mass index (BMI) ($p = 0.05$). Unadjusted serum CTx values tended to be lower among antioxidant supplement users, but the difference was not significant: median CTx (IQR), antioxidant users vs. nonusers, 293 (163–490) vs. 376 (233–538) pg/mL, $p = 0.1$. No differences were observed for serum BSAP or whole body BMD ($p > 0.05$). There were no differences in the proportions of women who were smokers, physically active, or users of drugs known to affect bone metabolism (HT, bisphosphonates, calcitriol), but more of the antioxidant supplement users were also taking calcium or vitamin D: (38.6% ($n = 17$ of 44) vs. 11.8% ($n = 91$ of 772), $p < 0.001$). A small proportion (2.7%) of nonsupplement users and none of the supplemented group used glucocorticoids.

Duration of antioxidant supplement use, bone turnover markers, and BMD

Further analyses were performed for 533 women after excluding the following potential confounders: smokers ($n = 78$), users of HT, bisphosphonates, calcitriol, or glucocorticoids ($n = 159$), and users of calcium or vitamin D ($n = 108$). Characteristics of the antioxidant supplement users and nonusers in the reduced group ($n = 533$) are compared in Table 1. Antioxidant supplement users had lower weight and BMI. Among antioxidant supplement users, 68% had used them for < 5 years, and 32% had used them for ≥ 5 years. There was a pattern of decreasing CTx (unadjusted) with increasing duration of exposure (Fig. 1); CTx levels were lowest among women who had used them for at least 5 years ($p < 0.05$). No pattern was observed for serum BSAP or whole body BMD. Multiple regression analysis showed that duration of antioxidant supplement use was negatively associated with age-adjusted and weight-adjusted serum CTx, such that mean lnCTx levels were 0.022 units lower for each year of exposure (Table 2). No significant differences were detected for adjusted serum BSAP or whole body BMD.

Serum markers of bone turnover were correlated with each other and negatively correlated with whole body BMD: lnCTx and lnBSAP, $r = 0.3$; lnCTx and BMD, $r = -0.4$; lnBSAP and BMD, $r = -0.2$ (all $p < 0.001$). Duration of antioxidant supplement use was associated with BMD-adjusted lnCTx (slope = -0.047 , duration year, BMD g/cm^2 , $p = 0.003$) but not with BMD-adjusted lnBSAP. Among women with the same BMD, serum CTx levels were lower the longer the exposure to antioxidant supplements.

TABLE 1. CHARACTERISTICS OF ANTIOXIDANT SUPPLEMENT USERS AND NONUSERS: MEANS (\pm SD), MEDIAN (INTERQUARTILE RANGE), OR PERCENTAGE (n)

Characteristic	Supplement users $n = 22$	Nonusers $n = 511$	p value
Age (yr)	75.1 (56.3–82.3)	71.1 (60.7–80.8)	0.6
Weight (kg)	62.7 (\pm 17.2)	68.7 (\pm 13.7)	0.05
Height (m)	157.9 (\pm 8.0)	157.6 (\pm 6.3)	0.9
BMI (kg/m^2)	25.0 (\pm 5.7)	27.6 (\pm 5.2)	0.02
Dietary calcium (mg/day)	719 (\pm 366)	673 (\pm 362)	0.6
Active	36.4 (8)	48.1 (246)	0.3
CTx (pg/mL)	342 (216–603)	418 (279–571)	0.3
BSAP (U/L)	28 (20–36)	28 (20–37)	0.8
Whole body BMD (g/cm^2)	1.04 (\pm 0.16)	1.06 (\pm 0.12)	0.6

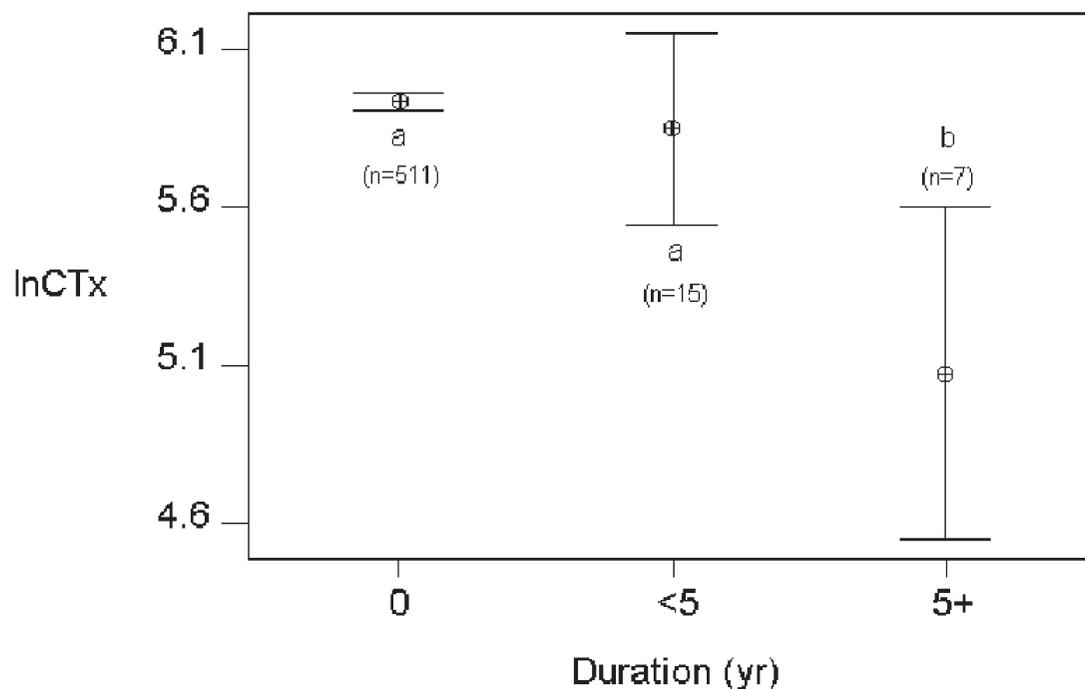


FIG. 1. Unadjusted serum C-telopeptide (pg/mL), natural log transformed (lnCTx) (mean \pm SE), with increasing duration of exposure to vitamin C or E supplements (a and b significantly different, $p < 0.05$).

DISCUSSION

To our knowledge, this is the first study to investigate the association between the use of antioxidant vitamin supplements and bone turnover in postmenopausal women. Our results suggest that use of vitamin C or E supplements is associated with decreased levels of serum CTx, a marker of bone resorption. No differences in serum levels of the bone formation marker BSAP or whole body BMD were detected. We observed that antioxidant vitamin supplement use is associated with reduced bone resorption in these women.

The close coupling of osteoclastic bone resorption and osteoblastic bone formation maintains bone mass in healthy young adults; bone loss oc-

curs when resorption exceeds formation. *In vitro* models demonstrate that ROS stimulate osteoclast differentiation¹⁶⁻¹⁸ and inhibit differentiation of bone osteoblastic cells.¹⁹ Thus, it is feasible that antioxidants may reduce the damaging effects of oxidative stress by reducing the upregulated osteoclastic differentiation and enhancing the downregulated osteoblastic differentiation. Our results suggest that antioxidant supplements reduce bone resorption. It is likely that bone formation would also be reduced through coupling, and this, in conjunction with increased osteoblastic differentiation in the presence of antioxidants, may result in no net change in serum markers of bone formation, as reported in this study. Further studies on the role of antioxidants

TABLE 2. MULTIPLE REGRESSION ANALYSIS WITH MARKERS OF BONE TURNOVER OR WHOLE BODY BMD AS DEPENDENT VARIABLE AND DURATION OF ANTIOXIDANT VITAMIN SUPPLEMENT USE (IN YEARS) AS INDEPENDENT VARIABLE OF INTEREST

Variable ^a	Adjustments	β -coefficient (slope)	p value
lnCTx	Age, weight	-0.022	0.05
lnBSAP	Age	-0.003	0.7
Whole body BMD	Age, weight	-0.001	0.6

^aUnits: CTx, pg/mL; BSAP, U/L; BMD, g/cm².

in the regulation of both osteoclastic bone resorption and osteoblastic bone formation are required to further explore this hypothesis.

Smoking is associated with increased levels of oxidative stress. Cigarette smoke is a source of free radicals and, moreover, can generate free radicals *in vivo*.²⁰ There is evidence to suggest that supplementation with antioxidants protects smokers from oxidative damage.²¹ Serum concentrations of both vitamins C and E are generally lower in smokers than in nonsmokers,^{21,22} and the protection against oxidative damage afforded by supplementation is more pronounced among smokers.²² Smoking is recognized as a risk factor for bone loss and fragile bones.^{23,24} Smoking alters the rate of bone turnover; cessation of smoking causes a reduction in the bone resorption marker, N-telopeptide, in postmenopausal women.²⁴ For these reasons, smokers were excluded from our detailed analysis, but there is a need to further explore, in a larger group, the hypothesis that the detrimental effect of smoking on bones is mediated through increased oxidative stress and that antioxidants may reduce the negative effects of cigarette smoking on bone.

There are some limitations to our study. First is the accuracy of self-reported antioxidant supplement use because of the possibility of recall bias and the potential irregularity of use. Second, the participants were on a free diet, and we have not analyzed dietary intakes of antioxidants. Third, it is possible that some of the subjects might have been deficient in antioxidant vitamins. Collagen cross-linking is reduced in vitamin C deficiency; thus, vitamin C nutrition may affect serum CTx concentrations.²⁵ Fourth, in our small study, no association was found between supplement use and whole body BMD, but small numbers may have limited our ability to detect an association. With $\alpha = 0.05$, we had 80% power to detect differences of 9.8% for BMD. Thus, we cannot exclude the possibility of a type II error. The observed difference in bone resorption is small, indicating that the antioxidant effect is likely to be limited in its size. Finally, as with all observational studies, our results could be biased by unrecognized confounders. The supplement users may be more health conscious, and although the supplemented group tended to have a lower BMI and a higher proportion were also supplemented with calcium and vitamin D, we detected no differences in dietary calcium intakes or in levels of activity.

CONCLUSIONS

Among nonsmoking postmenopausal women, we detected lower levels of the bone resorption marker, CTx, with increased exposure to vitamins C or E or both. Although no association was observed with BMD, the hypothesis that antioxidant supplements may suppress bone resorption warrants further investigation. This work adds to the growing body of evidence that antioxidants may play a role in preventing osteoporosis.

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