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[1]DO DIETARY ANTIOXIDANTS PREVENT POSTMENOPAUSAL BONE LOSS?

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ABSTRACT

The role of dietary antioxidants in osteoporosis has not been well explored. The objective of this study was to examine the relationship between the dietary antioxidants, vitamin E and beta-carotene, and hip bone mineral density (BMD) in postmenopausal women. Subjects were 1892 screenees, aged 55-80 years, who were volunteers for a clinical trial. Bone densitometry and osteoporosis risk factor information was obtained during screening. Dietary and supplement information was obtained by mailed food frequency and vitamin supplement questionnaires. We found no evidence of an association between dietary and/or supplemental vitamin E and bone density of the femoral neck. Dietary beta-carotene, adjusted for age and weight was positively associated with hip BMD ($\beta=1.5x10^{-6}$ gm/cm^2, p=0.05). Further adjustment for osteoporosis risk factors diminished the association ($\beta=0.7x10^{-6}$gm/cm^2, p=0.38). Neither total nor supplemental beta-carotene intake was found to be associated with BMD. We did not find that vitamin E or beta-carotene was associated with femoral neck bone density in postmenopausal women, however, the potential role of antioxidants and other nutrients in postmenopausal bone loss warrants further study, including research of other bone sites.
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Key words: Antioxidants, Beta-carotene, Diet, Epidemiology, Osteoporosis, Vitamin E

INTRODUCTION

Osteoporosis is a major public health problem, causing an estimated 90% of all hip fractures in older adults.(1) Recent evidence suggests that free radicals may have a role in the

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development of osteoporosis.(2-6) Antioxidant-like compounds have been shown to inhibit free radical production and bone resorption in laboratory studies.(5,6) Antioxidants, which scavenge free radicals, have been found to have protective effects in other chronic diseases, including cancer and heart disease.(7-9)

One of the very few clinical studies examining antioxidants and bone status showed a correlation between serum levels of glutathione reductase, an endogenous antioxidant, and bone mineral density (BMD) in older women.(2) A Finnish study found no association between serum osteocalcin, a marker of bone formation, and serum antioxidants in men aged 65-84 years.(10)

The role of selected dietary factors in the maintenance of bone health, such as calcium and vitamin D, has frequently been studied, but little research has looked specifically at antioxidants. A recent report from Australia examining the association between dietary carotenoid intake and bone mineral status found no significant association in postmenopausal women.(11) In studies of broad groups of nutrients, inconsistent findings have been reported for dietary vitamin C and retinol with bone density in women.(12-14) Inconsistencies have also been observed in studies comparing vegetarians to omnivores for fracture rates and bone density.(15-19) Contrary to expectations, vegetarians in these studies did not consistently consume higher amounts of beta-carotene and vitamin E. The relative contribution of vitamin supplements, which are used regularly by one-third to one-half of older people, has rarely been addressed.(20)

Nutrients such as calcium and vitamin D have been shown in controlled clinical trials to be protective for osteoporosis.(21,22) These and other potentially beneficial nutritional factors for which research is lacking could have a significant public health impact in slowing or preventing bone loss. The purpose of this study was to assess whether dietary and/or supplemental intake of vitamin E or beta-carotene was associated with greater BMD in a large cohort of postmenopausal women.

METHODS

Subjects were screenees from the Fracture Intervention Trial (FIT), a multi-center randomized clinical trial of a recently approved medication to treat osteoporosis, alendronate. The design of the FIT study was described in detail previously.(23) Briefly, Group Health Cooperative (GHC) in Seattle is one of 11 sites for the study, funded by Merck and Co., Inc. Subjects recruited at the Seattle FIT site were postmenopausal women (more than 2 years post-menopause) aged 55 to 80 years from the Seattle metropolitan area. Approximately 80% of the screenees were GHC enrollees, the remaining volunteers were recruited from the community. Volunteers were excluded during a telephone screening if they had unexplained weight loss, severe gastrointestinal disease, cancer in past 10 years, bilateral hip replacement, recent use of medications that may influence bone turnover such as estrogen, glucocorticoids, or etidronate, and inability to walk independently. Of the 2484 screenees who were sent dietary questionnaires for our study, we received complete dietary information from 1892 women.

Characteristics, pertinent medical history, and osteoporosis risk factor data were collected by questionnaire at the clinic screening visit for FIT, and height and weight measurements were obtained. BMD of the femoral neck was measured using dual energy x-ray absorptiometry (DXA) (QDR2000, Hologic, Inc., Waltham, MA), a highly precise measure (precision 1%) with very low radiation exposure (3 mRem). All BMD scans were performed by a trained densitometry

technician under the supervision of study investigators.

Additional dietary data was collected for this study using a mailed 98-item food frequency questionnaire (FFQ) and vitamin supplement questionnaire. Subjects were asked to recall dietary intake for the 12 months prior to their baseline screening visit for the FIT study and vitamin supplement use for the previous 10 years. The FFQ was a version of the National Cancer Institute/Block questionnaire,(24) modified at the Fred Hutchinson Cancer Research Center (FHCRC) to assess antioxidant intake. Questionnaires similar to the current form have been validated in previous studies.(26,27)

The vitamin supplement questionnaire was developed for this study to assess dose, duration and frequency of supplement use. Supplement intake was estimated for vitamins that reportedly were taken for at least one year with a frequency of two or more times per week. Respondents were asked to identify their intake by categories of frequency and duration of use. Estimated usual amounts of vitamin E intake from multivitamins (30 i.u., 100% RDA) were calculated and added to the total supplement intake. Beta-carotene was only recently added to multivitamin supplements, therefore, no contribution was assigned for beta-carotene from multivitamin use.

Statistical Analysis

The relationship between each of the antioxidants with hip BMD was assessed using 3 measures of intake: 1) total antioxidant intake (combined diet and supplement intake), 2) dietary-only antioxidant intake; 3) supplement-only antioxidant intake.

In calculating total intakes of each antioxidant, we adjusted the dietary antioxidant intake for energy intake prior to adding the supplement intake. Using the residual-adjustment method (28), we controlled for energy intake because it was likely to be associated with BMD and, in part, to correct measurement error due to over- and under-reporting of intake on the FFQ. The residual-adjustment method had nearly the same effect as adjusting for energy intake in the antioxidant model; slight differences in the estimates were due solely to log-transformations.

Adjusted least square means of BMD were calculated by quintiles of vitamin E and beta-carotene intake. Quintile cutpoints for vitamin E (mg) were: <6.2; <8.0; <28.2; <302.8; ≥ 302.8. Quintile cutpoints of beta-carotene (mcg) were: <2482; <3433; <4633; <6755; ≥ 6755. Multiple linear regression was used to examine the relationship between antioxidant intake and BMD, controlling for potential confounders. Potential confounders that were evaluated included factors known to influence bone density: age, weight, height, number of reproductive years, years since menopause (years from last natural menstrual period), number of live births, ever use and years of past use of hormone replacement therapy, surgical removal of both ovaries, hysterectomy, thyroid supplement use, thiazide use, smoking status (current, past, and never use), intake of calcium and vitamin D, caffeine intake (mg/day), physical activity (number of blocks walked per day; number of hours of moderate and vigorous physical activity per week), diagnosis of diabetes or rheumatoid arthritis. Other potential confounders were related to dietary and supplemental antioxidant intake: total energy intake, polyunsaturated fat intake (gm/day), multivitamin use (yes/no), alcohol intake (gm/day). Covariates that were independently associated with BMD were included in antioxidant/BMD models (test of coefficient, $p<0.05$). Stepwise modeling procedures were then used to select variables from the full models in order to derive the most efficient models (forward selection at $p<0.15$; backward elimination at $p<0.15$). Log-transformation of variables made no material difference in the findings; regression results using untransformed variables only are presented in this paper. The relationship between antioxidant

supplement use and BMD was evaluated by analysis of covariance and by comparing adjusted means using t-tests.

RESULTS

Independent predictors of bone density included age, weight, number of reproductive years, past estrogen use and years of past use, energy intake, diabetes, use of thiazide diuretics, and physical activity. Age and weight were the most strongly associated with BMD (Pearson correlation coefficient for BMD with age r = -0.22; BMD with weight, r = 0.49).

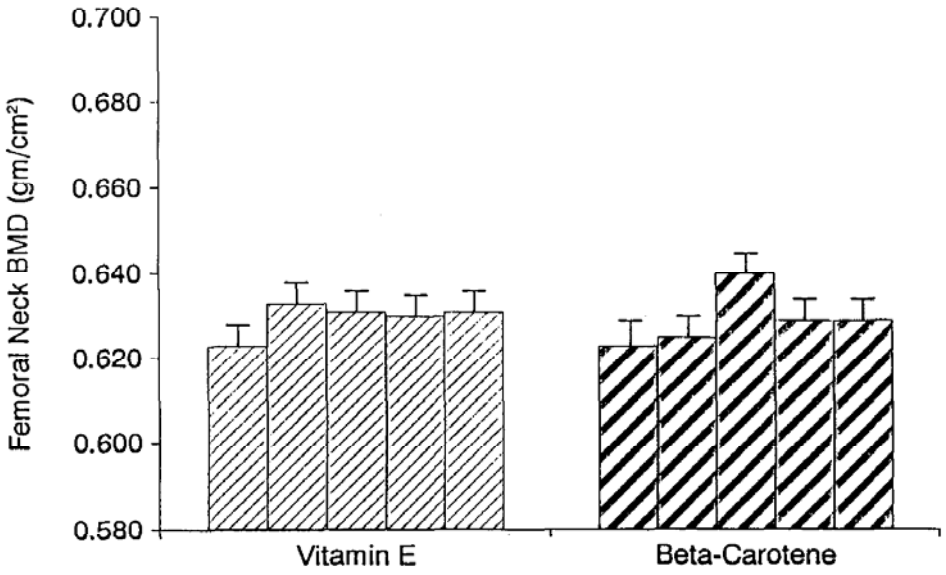

FIG.1 Mean femoral neck BMD by quintiles of total daily dietary and supplemental vitamin E and Beta-carotene intakes, adjusted for age, weight, height, energy intake, number of reproductive years, diabetes, thiazide use, smoking, bilateral ooporectomy, number of live births, years of past estrogen use, hours of vigorous activity per week, and number of blocks walked per day.

The total average daily intake (including supplements) of vitamin E was 107.5mg (range 2.7 - 1151.5) and for β-carotene, the average was 5339.6mcg (range 437 - 31461). The age-adjusted mean BMD did not vary across quintiles of total vitamin E intake (Figure 1). There was no clear trend for increasing BMD with increased intake of beta-carotene, though women in the middle quintile had higher BMD than those who consumed the lowest quantities of beta-carotene. After adjusting for mulitple confounders, subjects who consumed higher total amounts of vitamin E and beta-carotene through diet and supplements combined were not found to have higher hip BMD (Table 1). Although the association between dietary beta-carotene and BMD seemed stronger after controlling for age and weight, full multivariate adjustment diminished the association. Dietary vitamin E showed almost no relationship with BMD in any of the models. Controlling for other antioxidants produced no material changes in the findings.

The age and weight adjusted means for BMD were higher for vitamin E and beta-carotene supplement users compared to non-users, however, after adjusting for multiple confounders, including vitamin D and calcium supplement use, the mean BMD was nearly the same in supplement users versus non-users of each supplement (adjusted mean BMD: vitamin E user = 0.630 gm/cm^2, non-user = 0.629gm/cm^2; beta-carotene user = 0.628gm/cm^2, non-user = 0.629gm/cm^2). Essentially, no association with BMD was found with longer duration of Vitamin E or beta-carotene supplement use, nor was dose of vitamin E found to be associated with BMD (data not shown).

We considered whether the total number of fruits and/ or vegetables consumed per day might be associated with bone density. The total number of vegetable servings was weakly associated with BMD after adjusting for age, weight, and energy intake ($p = 0.05$, data not shown), however, this effect diminished and was no longer evident after controlling for multiple confounders. Subjects who reported eating more fruit servings per day or more servings of fruit and vegetables combined were not found to have higher hip bone density.

TABLE 1

Expected Change in Hip Bone Mineral Density (g/cm^2) by Unit Change in Total and Kilocalorie-Adjusted Dietary Vitamin E and Beta-Carotene Intake

Models	Total Vitamin E β (S.E.)*	P	Total Beta-carotene β (S.E.)*	P
No covariates	0.005 (0.013)	0.69	0.0002 (0.0005)	0.70
Age and weight	0.007 (0.011)	0.57	0.0007 (0.0005)	0.15
Full model#	0.002 (0.012)	0.85	0.0003 (0.0005)	0.54
Full model adjusted for other antioxidant intakes**	-0.008 (0.015)	0.59	0.0002 (0.0005)	0.76
	Kcal-adj. diet vitamin E β (S.E.)*	P	Kcal-adj. diet β-carotene β (S.E.)*	P
No covariates	-1.4 (0.9)	0.11	0.0002 (0.0009)	0.78
Age and weight	-0.5 (0.7)	0.52	0.0015 (0.0008)	0.05
Full model#	-0.2 (0.8)	0.82	0.0007 (0.0008)	0.38
Full model adjusted for other antioxidant intakes**	-0.3 (0.8)	0.73	0.0005 (0.0009)	0.57

* Regression coefficients and standard errors were multiplied by 10^3.

Full model included age, weight, height, energy intake, number of reproductive years, diabetes, thiazides, smoking, bilateral oophorectomy, number of live births, past estrogen use & years of use, vigorous activity (hrs/wk), blocks walked/day.

** Adjustments included vitamins C, E and beta-carotene.

DISCUSSION

Our findings showed essentially no association between total intake of vitamin E or beta-carotene and hip bone density among postmenopausal women. Separate examinations of the two sources of the dietary antioxidant vitamins, ie. from diet and supplements, resulted in similar findings. Neither measure of nutrient intake would have been adequate for evaluating the two antioxidants. Beta-carotene has only recently become widely available as a supplement, precluding an examination of long-term effects of supplement use. However, high quantities of the antioxidant can be found in food sources, such as carrots and other yellow-orange vegetables. In contrast, vitamin E has been widely available in supplement form for many years, and the average daily intake of vitamin E from foods is low compared to that of supplements (7 mg versus 100 mg).

Ours is one of the first epidemiologic studies to address the influence of dietary antioxidants in postmenopausal osteoporosis. Research into the role of antioxidants in bone remodelling has primarily been laboratory-based. Studies have demonstrated that oxygen-derived free radicals are associated with increased osteoclastic bone resorption, and that the resorptive process is inhibited by antioxidants.(3,5,6) There is speculation that the role of free radicals in bone resorption may be related to the action of cytokines (4), which are increasingly becoming a focus in osteoporosis research. Although the role of dietary antioxidants in bone resorption has rarely been explored, a recent animal study found that a vitamin E-enriched diet promoted trabecular bone formation.(29) Our own research was limited to two of several dietary antioxidants and one major bone site, leaving many questions to consider surrounding the complex issue of antioxidants and postmenopausal bone loss. Additional studies could explore the potential role of other substances found in fruits and vegetables such as isoflavinoids and phytoestrogens, in the preservation of bone density in older women.

The one brief epidemiologic report with which we can compare our findings examined dietary carotenoids and bone density in a population of men and women aged 27-86 years (n=205).(11) Although no significant carotenoid-BMD association was found in the small population of postmenopausal women studied, the authors found evidence that beta-carotene intake may be associated with BMD of the lumbar spine. The authors reported only limited adjustment for potential confounders.

The lack of a beta-carotene or vitamin E/ BMD association could be attributed to nondifferential measurement error related to use of the food frequency questionnaire, biasing our findings toward the null. However, with vitamin E, the intake from the diet was small compared to supplements and no effect was observed with either nutrient source. An additional concern in observational studies of vitamin supplements is multicollinearity. We found that very few women took both supplemental beta-carotene and vitamin E (5%) and many vitamin E users did not take beta-carotene supplements (21% of all participants). Multivitamin use, another source of multicollinearity, was not associated with bone density in our data and none of the nutrients we studied were highly correlated. Furthermore, adjustments for other micronutrients did not materially alter our findings.

While there are limitations to the generalizability of our findings because of our population profile, the study volunteers, who were predominantly white and well-educated women, were very responsive to our survey (80% response rate) and were perhaps better able to complete the study questionnaires, particularly the detailed food frequency form. This trade-off may have enhanced

our ability to study our hypothesis by limiting measurement error in our data collection.

We found no evidence that vitamin E or beta-carotene has a beneficial impact on postmenopausal femoral neck bone density. However, the potential role of antioxidants and other nutrients in postmenopausal bone loss warrants further study, including research of other bone sites, such as the trabecular bone of the spine.

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