Effects of dietary calcium compared with calcium supplements on estrogen metabolism and bone mineral density[1–4]

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ABSTRACT

Background: High calcium intake has been associated with both high bone mineral density (BMD) and high urinary estrogen metabolites. However, the role of dietary calcium and calcium supplements on estrogen metabolism and BMD remains unknown.

Objective: The objective was to investigate the importance of the source of calcium intake on estrogen metabolism and BMD.

Design: The average total daily calcium intake from supplements and diet, urinary estrogen metabolites, and spine and proximal femur BMD were studied in 168 healthy postmenopausal white women.

Results: Women who obtained calcium primarily from the diet or from both the diet and supplements had significantly ($P = 0.03$) lower ratios of nonestrogenic to estrogenic metabolites (2-hydroxyestrone 1/16α-hydroxyestrone) than did those who obtained calcium primarily from supplements. Adjusted BMD z scores were significantly greater in the subjects who obtained calcium primarily from the diet or from both the diet and supplements than in those who obtained calcium primarily from calcium supplements at the spine ($P = 0.012$), femoral neck ($P = 0.02$), total femur ($P = 0.003$), and intertrochanter ($P = 0.005$). This difference was evident especially in those who obtained calcium primarily from the diet, whose total calcium intake was lower than that in those who obtained calcium primarily from supplements.

Conclusion: Calcium from dietary sources is associated with a shift in estrogen metabolism toward the active 16α-hydroxyl metabolic pathway and with greater BMD and thus may produce more favorable effects in bone health in postmenopausal women than will calcium from supplements. *Am J Clin Nutr* 2007;85:1428–33.

KEY WORDS Calcium, estrogen metabolism, bone mineral density, osteoporosis

INTRODUCTION

Circulating estrogen is metabolized primarily via 2 mutually exclusive pathways: the 2-hydroxyl and the 16α-hydroxyl pathways (1, 2). The 2-hydroxylation pathway leads to formation of nonestrogenic metabolites, whereas the 16α-hydroxylation pathway leads to formation of metabolites that retain estrogenic properties (3, 4). It has been hypothesized that the balance between these 2 pathways may contribute to the overall "estrogen tone."

Disorders of estrogen metabolism have been implicated in the etiology of certain estrogen-dependent conditions, such as breast, endometrial, and ovarian cancers (5–7) and, recently, osteoporosis (8). For instance, it has been reported that women with increased 16α-hydroxylation, as indicated by a low ratio of 2-hydroxyestrone ($2OHE_1$) to 16α-hydroxyestrone ($16\alpha OHE_1$), have a high risk of breast cancer (5, 9), most likely because of a relatively higher estrogenic state. On the other hand, the same group of women are protected from bone loss and osteoporosis, presumably by the same underlying mechanism (8, 10), whereas the opposite is true when the 2-hydroxyl pathway predominates. These findings suggest that the manipulation of these pathways may alter the risk of certain hormone-dependent conditions.

There are several factors that modulate estrogen metabolism. Smoking (11) and high consumption of indole-containing vegetables (12) have been found to enhance 2-hydroxylation of estrogen. Medications such as cimetidine may inhibit estrogen hydroxylation (13), whereas thyroid hormones increase 2-hydroxylation (14). Obesity is associated with decreased 2-hydroxylation without any change in 16α-hydroxylation (15), and a family history of osteoporosis is associated with increased 2-hydroxylation (16). Very recently, we have added calcium to the list of agents that may influence estrogen metabolism by demonstrating that increasing calcium intakes are associated with increasing concentrations of estrogen metabolites (16). However, as we pointed out in that report, the significantly positive correlation between calcium intake and urinary metabolites was mainly observed in subjects whose calcium intake came from dietary sources. Meanwhile, the effect of different calcium sources on modulating estrogen hydroxylation and their consequence on bone metabolism remains undetermined. Considering that there are a variety of calcium sources available, it is possible that a differential effect on estrogen metabolism between the different sources exists and this may have an effect on BMD. The objective of this study, therefore, was to investigate the effect of the type of calcium intake on estrogen hydroxylation and bone density in a group of healthy postmenopausal women.

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SUBJECTS AND METHODS

Study population

This was a cross-sectional study conducted in community-dwelling, otherwise healthy women in the St Louis, MO, metropolitan area whose last menstrual period was ≥1 y ago. Participants were recruited through advertisements or direct mailing. This study was conducted in accordance with the guidelines in the Declaration of Helsinki for the appropriate treatment of human subjects. The protocol was approved by the Washington University School of Medicine Institutional Review Board, and written informed consent was obtained from each participant. Most of the participants in this study were participants in a previous related study (16), and the previous clinical and laboratory data in these women were used in this study. Those who were taking any medication known to affect bone metabolism, such as estrogen, selective estrogen receptor modulators (including raloxifene and tamoxifen), bisphosphonates (alendronate, risedronate, pamidronate, or zoledronate), aromatase inhibitors, GnRH analogs, glucocorticoids (>5 mg/d of prednisone or the equivalent for >1 mo), or phenytoin were excluded from the study. Intake of medications known to affect estrogen hydroxylation (phytoestrogens, cimetidine, thyroid hormones, and monooxygenase inhibitors) and drugs known to affect cytochrome P450 enzyme activity were also exclusionary criteria, as were diseases or conditions known to interfere with bone metabolism, including hyperthyroidism, osteomalacia, chronic liver disease, renal failure, hypercortisolism, malabsorption, immobilization, and alcoholism. Current tobacco users were excluded, whereas past smokers who stopped smoking for ≥6 mo were allowed into the study. Those who consumed more than one serving per day of vegetables containing high amounts of phytochemicals known to preferentially enhance 2-hydroxylation of estrogen, such as cabbage, cauliflower, Brussels sprouts, broccoli, and kale (12), were excluded from participation.

Clinical, dietary, and anthropometric data

Dietary calcium and vitamin D intakes were estimated from a 7-d dietary record, which was mailed to the participants ≥1 wk before the study visit. The 7-d dietary record contained a list and serving sizes of common dietary sources of calcium and the amount of calcium in each serving and a list and serving sizes of vegetables, fruit (mainly oranges), and fruit juices. The participants were asked to record the daily intake of these foodstuffs. They were also asked to record their daily intake of calcium from supplements, and their average daily intake from both diet and supplements was determined for 7 d. Because most of the individuals had a total calcium intake that came from a combination of sources, we arbitrarily divided the subjects into 3 groups on the basis of the predominant source of their average total daily calcium intake: *1*) Supplement group—those in whom most (≥70%) of the calcium intake was obtained from supplements and <30% was from the diet, *2*) Diet group—those in whom ≥70% of the calcium intake was obtained from dietary sources and <30% was from supplements, and *3*) Diet + Supplement group: those in whom the percentage contribution of diet and supplements fell between those 2 limits, ie, diet and supplements contributed >30% but <70% of calcium.

A separate diet history on the intake of foodstuffs, particularly vegetables, that may modulate estrogen hydroxylation was also obtained at study entry as part of the inclusion and exclusion criteria. Study participants were asked to estimate their average weekly intake of vegetables such as cabbage, cauliflower, Brussels sprouts, broccoli, and kale. Those who consumed more than one serving per day of these vegetables was not allowed into the study for the reasons cited previously. Alcohol intake was expressed as the average number of alcoholic drink-equivalents consumed over a 1-wk period. A can of beer (336 mL), a glass of wine (112 mL), and 28 mL of a heavy alcoholic beverage (ie, >40% proof alcohol) were considered one drink-equivalent. Previous smoking was expressed in pack-years and was estimated as the number of 20-cigarette packs smoked per day multiplied by the number of years of smoking. Physical activity was expressed as a numerical score and was defined as sedentary (score of 1: sitting or lying most of the day), moderately active (score of 2: being on one's feet more than half of a day), and very active (score of 3: engaging in regular physical exercise) (17).

The family history of osteoporosis was obtained through a questionnaire and was coded as positive if a diagnosis of osteoporosis, kyphosis, or fragility fractures in the absence of secondary causes had been made in a blood relative (first or second degree, eg, aunts and grandmothers). Data on estrogen exposure were assessed through a number of variables, such as age at menarche, average number of periods per year during the reproductive years, number of years of birth control pill use, total number of pregnancies, number of pregnancies to term, months of lactation, age at menopause, and years since menopause (YSM). Body mass index (BMI; in kg/m^2) was calculated as weight in kilograms divided by the square of height in meters. The waist-to-hip ratio was calculated as the ratio between waist circumference, taken at the umbilical level, and hip circumference, measured 6 inches (15.24 cm) below the anterior superior iliac spine.

Biochemical data

Urinary estrogen metabolites were measured in 24-h urine specimens with the use of ESTRAMET immunoassay kits (Immuna Care Corp, Bethlehem, PA). The ESTRAMET series of test kits are monoclonal antibody–based competitive enzyme immunoassays for estrogen metabolites in microtiter plate format. The antibodies and assays for urinary 2- and 16α-hydroxyestrogen have been described (18). The monoclonal antibody to 2-hydroxyestrogens recognizes the 2-hydroxy forms of E_1, E_2, and E_3 equivalently. Similarly, the monoclonal antibody to 2-methoxyestrogens recognizes the 2-methoxy forms of estrogen metabolites equivalently and exhibits <0.1% cross-reactivity with any other estrogen, including 2-hydroxyestrogens. The monoclonal antibody to E_3 exhibited <2% cross-reactivity with any other estrogen. All urinary estrogen assays were performed according to methods described previously (8). Briefly, urine samples were incubated with enzymes that deconjugated estrogen metabolite sulfates and glucuronides to their respective free forms. The amount of estrogen metabolite in the enzymic hydrolysate was determined by competition between deconjugated estrogen in the hydrolysate and estrogen-labeled alkaline phosphatase for binding to specific monoclonal antibodies attached to the microtiter plate; >90% of the metabolites in the urine exist as glucuronides and were recovered totally by this method. The inter- and intraassay CVs for these enzyme-linked immunoassays were <9% and 13% respectively. Each urinary metabolite value was corrected for 24-h

TABLE 1
Clinical features of the study population according to the primary source of calcium intake

Clinical features	Supplement (n = 33)	Diet (n = 70)	Diet + supplement (n = 65)	P^1
Total daily calcium intake (mg/d)	1032.6 ± 103[2]	829.7 ± 70	1619.9 ± 73	<0.01
Supplement	928.4.2 ± 78.0	223.8 ± 115.8	876.0 ± 55.6	
Diet	214.9 ± 94.8	784.9 ± 45.3	743.9 ± 47.0	
Age (y)	62.7 ± 0.6	64.2 ± 0.6	63.6 ± 0.85	0.01
BMI (kg/m^2)	27.6 ± 0.89	27.8 ± 0.59	27.1 ± 0.61	0.74
Waist-to-hip ratio	0.80 ± 0.01	0.82 ± 0.01	0.82 ± 0.01	0.41
Years since menopause (y)	8.89 ± 1.82	15.04 ± 1.27	16.13 ± 1.30	<0.01
Smoking history				
Past smokers (%)	56.3	47.9	31.2	0.04
Total past smoking (pack-years)	13.3 ± 2.83	7.21 ± 1.94	6.18 ± 2.0	1.2
Alcohol intake (drink-equivalents)	1.69 ± 0.49	1.57 ± 0.32	1.078 ± 0.33	0.46
Positive family history of osteoporosis (%)	42.8	42.9	49.9	0.68
Activity score	2.27 ± 0.10	2.34 ± 0.07	2.41 ± 0.07	0.59

[1] Chi-square test for categorical variables (family history of osteoporosis and previous smoking history) and one-factor ANOVA for continuous variables (all other variables).
[2] $\bar{x}$ ± SE (all such values).

urinary creatinine (mg/24 h) and was expressed in ng/mg creatinine.

Serum samples were collected in the nonfasting state. Serum estradiol was measured with an ultrasensitive radioimmunoassay technique (Diagnostic Systems Laboratory, Webster, TX). The inter- and intraassay CVs for serum estradiol was <10%.

Bone mineral density

Bone mineral density (BMD) of the lumbar spine and the proximal femur was measured by dual-energy X-ray absorptiometry with the use of Hologic QDR 4500 (Hologic Inc, Waltham MA). BMD of the lumbar spine was performed with the anteroposterior projection and was calculated as the average of the L1 to L4 vertebrae. The nondominant hip was used for proximal femur scans, and values were calculated for the total femur, femoral neck, trochanter, and intertrochanteric areas. The CV of this technique with the QDR 4500 densitometer is 1.09% for the lumbar spine and 1.2% for the total femur in our center (16).

Statistical analysis

The results are expressed as means ± SEs. Differences in clinical features were compared by using one-factor analysis of variance for continuous variables (eg, age and BMI) and a chi-square test for categorical variables (eg, family history of osteoporosis) as appropriate. $P < 0.05$ was considered statistically significant. Urinary metabolite values among the different calcium groups (stratified according to sources as defined in the Methods section) were compared by using analysis of covariance (ANCOVA) with age, YSM, BMI, and average total calcium intake as covariates. Age-adjusted BMD z scores among the different calcium groups were also compared by using ANCOVA adjusted for BMI, YSM, previous smoking history, and average total calcium intake. Post hoc analyses with multiple-range testing using Bonferroni's method were performed for P values in the ANCOVA of <0.05. Stepwise multiple regression analyses were performed for individual urinary metabolites, total metabolites, and metabolite ratios to determine the independent contributions of each of the following variables: age, YSM, BMI, previous smoking history, alcohol intake, total calcium intake, and separate entries for calcium intake from the diet and from supplemental sources. Data were managed by using EXCEL 2000 (Microsoft Corp, Redmond, WA) and were analyzed by using STATGRAPHIC PLUS 5.0 (Manugistic Inc, Rockville, MD).

RESULTS

One hundred eighty-three women participated in this study, most of whom were participants in a previously published study (16). Because of the well-known ethnic differences in estrogen metabolism (2), we limited our analysis to 168 white women who accounted for the majority of our subjects. The demographic features of the study population, stratified according to the primary source of their calcium intakes, as described in the Method section, are shown in **Table 1**. There were 33 women in the Supplement group, 70 women in the Diet group, and 65 women in the Diet + Supplement group. Women in the Supplement group were significantly younger and early postmenopausal. There was a significantly greater number of previous smokers in the Supplement group than in the Diet and Diet + Supplement groups. Dairy sources accounted for the majority of the dietary calcium intake among women in the Diet (92.7% of dietary calcium) and the Diet + Supplement (92.1% of dietary calcium) groups. Women in the Supplement group consumed significantly less fruit and fruit juices than did women in the Diet and Diet + Supplement groups (0.42 ± 0.5, 2.6 ± 0.3, and 2.8 ± 0.4 servings/wk, respectively; $P < 0.01$). There was no significant difference between the 3 groups in terms of vegetable intake, although women in the Supplement group consumed a serving less of vegetables per week relative to the Diet and Diet + Supplement groups (2.1 ± 0.6, 3.0 ± 0.4, and 3.3 ± 0.4 servings/wk, respectively; $P = 0.27$).

Urinary metabolites were compared according to the source of calcium intake adjusted for age, YSM, BMI, and amount of average total calcium intake, variables that had been found to be either significantly different between the groups or influence urinary metabolites by regression analyses. We found that

TABLE 2
Urinary metabolites stratified according to the primary source of calcium intake[1]

	Supplement ($n = 33$)	Diet ($n = 70$)	Diet + supplement ($n = 65$)	P
$2OHE_1$ (ng/mg Cr)	7.329 ± 0.95	8.500 ± 0.63	8.190 ± 0.68	0.59
$2MeOE_1$ (ng/mg Cr)	2.902 ± 0.45	4.337 ± 0.30	4.820 ± 0.33	<0.01
$16\alpha OHE_1$ (ng/mg Cr)	3.109 ± 0.61	4.813 ± 0.42	4.762 ± 0.45	<0.05
E_3 (ng/mg Cr)	6.856 ± 1.25	6.958 ± 0.83	7.662 ± 0.90	0.83
$2OHE_1/16\alpha OHE_1$ (ng/mg Cr)	2.823 ± 0.24	1.965 ± 0.16	1.981 ± 0.17	<0.01
$2MeOE_1/16\alpha OHE_1$ (ng/mg Cr)	1.091 ± 0.09	1.006 ± 0.06	1.179 ± 0.07	0.19
$2OHE_1 + 2MeOE_1 + 16\alpha OHE_1 + E_3$ (ng/mg Cr)	20.25 ± 2.62	24.61 ± 1.74	25.43 ± 1.89	0.28
Serum estradiol (pmol/L)	55.07 ± 5.12	54.56 ± 2.45	51.92 ± 2.56	0.72

[1] All values are $\bar{x}$ ± SE. $2OHE_1$, 2-hydroxyestrone; $2MeOE_1$, 2-methoxyestrone; $16\alpha OHE_1$, 16α-hydroxyestrone; E_3, estriol. Group comparisons were performed by ANCOVA and were adjusted for age, years since menopause, BMI, smoking history, and average total calcium intake. Serum estradiol was compared between groups by one-factor ANOVA.

women in the Diet and Diet + Supplement groups had significantly higher $2MeOE_1$ and $16\alpha OHE_1$ values (**Table 2**.). Furthermore, women in the Diet and Diet + Supplement groups had significantly lower mean ratios of $2OHE_1$ to $16\alpha OHE_1$ compared with those in the Supplement group. There was no significant difference in estradiol concentrations between the 3 calcium groups.

Age-adjusted BMD z scores in the different skeletal sites were compared between the 3 calcium groups by ANCOVA adjusted for YSM, BMI, and average total calcium intake. Significant differences in BMD were observed for all of the skeletal sites ($P < 0.05$), except for the trochanter ($P < 0.07$). Post hoc analysis by multiple-range testing showed that BMD in women in the Diet and Diet + Supplement groups had significantly greater BMD in the spine, total femur, femoral neck, and intertrochanter than did women in the Supplement group (**Figure 1**).

Multiple regression analyses were performed by stepwise selection of clinical variables (as described in the Statistical Analysis section) thought to predict values of each metabolite, the metabolite ratio, and total metabolites. BMI was found to be a significant independent predictor of $2OHE_1$, $2MeOE_1$, E_3, $2MeOE_1$:$16\alpha OHE_1$, and total metabolites ($P < 0.05$ for all) (**Table 3**). Total calcium intake, in contrast, was found to be a significant independent predictor of $2OHE_1$, $2MeOE_1$, $16\alpha OHE_1$, and total metabolites ($P < 0.05$ for all), whereas previous smoking history was a predictor of the ratio of $2OHE_1$ to $16\alpha OHE_1$. The type of calcium source and other clinical variables, such as age and YSM, were not found to predict levels of urinary metabolites or metabolite ratios.

Our post hoc power calculations indicated that a sample size of 33 subjects per group had 81% power for comparing $2OHE_1$:$16aOHE_1$ between the 3 groups with the use of the means reported in Table 2 and an estimated within-group variance of 0.24 and 82% power for comparing spine BMD between the 3 groups with the use of the means shown in Figure 1 and an estimated within-group variance of 0.24.

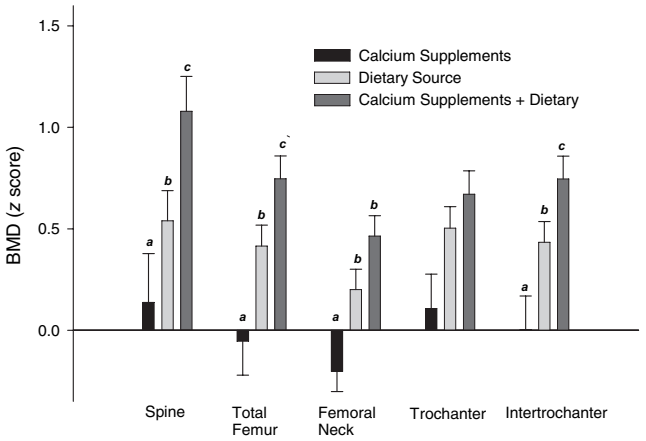

FIGURE 1. Mean (±SE) bone mineral density (BMD) normalized for age at different skeletal sites by primary calcium source in postmenopausal women. Group comparisons were made by ANCOVA and were adjusted for BMI, years since menopause, and average total daily calcium intake; $P < 0.05$ for all variables except trochanter ($P = 0.07$). Means with different lowercase letters are significantly different, $P < 0.05$ (post hoc analysis by multiple-range testing with Bonferonni's method).

DISCUSSION

Our results showed that women who obtained calcium primarily from dietary sources or from both diet and supplements had a lower ratio of inactive to active estrogen metabolites and a greater BMD than did the women who obtained calcium primarily from supplements. These findings suggest that dietary calcium is associated with a shift in the estrogen hydroxylation pathway favoring the production of active estrogen metabolites, which in turn may lead to a positive bone mineral homeostasis and greater bone density.

The current findings corroborate our previous report, suggesting that calcium may be an important modulator of estrogen hydroxylation (16). The lower ratio of $2OHE_1$ to $16\alpha OHE_1$ in women who consume a significant amount of calcium from their diet suggests that dietary calcium may promote a shift in estrogen metabolism to the active 16α-hydroxyl pathway. In contrast, it is possible that nutrients other than calcium, present in calcium-rich foods, may stimulate the 16α-hydroxyl pathway through unknown mechanisms preferentially. Alternatively, dietary sources of calcium may also contain active estrogenic compounds that may result in an apparent shift in estrogen metabolism. Because the main source of dietary calcium in our subjects was dairy products, the increase in 16α-hydroxyl metabolites in the women who consumed dietary calcium was perhaps related to the presence of predominantly active estrogenic metabolites in milk-derived products (19, 20). Some of these metabolites have been shown in previous studies to possess estrogen agonistic properties that are closer or even comparable with that of estradiol. For instance, administration of $16\alpha OHE_1$ prevented

TABLE 3
Results of multiple regression analysis of clinical predictors of urinary metabolite values[1]

Urinary metabolite and predictor variable	R^2	Regression coefficient	SE	CI
$2OHE_1$	10.6			
BMI		−0.26	0.08	−0.41 to −0.10
Total calcium intake		1.03×10^{-3}	0.54×10^{-3}	-0.03×10^{-3} to 2.10×10^{-3}
$2MeOE_1$	11.9			
BMI		−0.12	0.04	−0.20 to −0.04
Total calcium intake		0.71×10^{-3}	0.30×10^{-3}	0.20×10^{-3} to 1.24×10^{-3}
$16\alpha OHE_1$	3.40			
Total calcium intake		0.80×10^{-3}	0.35×10^{-3}	0.09×10^{-3} to 1.50×10^{-3}
E_3	5.06			
BMI		−0.26	0.09	−0.45 to −0.08
$2OHE_1/16\alpha OHE_1$				
Pack-years smoking	2.09	0.01	0.01	-1.0×10^{-3} to 22.6×10^{-3}
$2MeOE_1/16\alpha OHE_1$	3.71			
BMI		−0.02	0.01	-0.03 to 3.1×10^{-3}
Total metabolites	11.4			
BMI		−0.70	0.22	−1.13 to −0.27
Total calcium intake		3.5×10^{-3}	1.6×10^{-3}	0.46×10^{-3} to 6.6×10^{-3}

[1] Significance of predictor variables tested by multiple regression analysis. Possible predictor variables entered into the model were as follows: age, BMI, years since menopause, smoking history, alcohol intake, total calcium intake (mg/d), and separate entries for both calcium from diet and calcium from supplements.

ovariectomy-induced bone loss and reduced cholesterol concentrations to the same extent as administration of 17β-estradiol (4). In fact, it has been suggested that $16\alpha OHE_1$ may even be a more potent estrogen than estradiol, because of its binding to estrogen receptors and its low affinity to sex hormone–binding globulin (21). It is reasonable to speculate that dairy sources of calcium may generate a positive estrogenic environment, which in turn contributed to the greater bone densities in women who consumed significant amounts of dietary calcium. In support of this notion is the finding that subjects in the Diet group had a greater BMD despite a lower average total daily calcium intake relative to the Supplement group. Furthermore, the Diet + Supplement group had the highest BMD, perhaps suggesting an additive effect when both sources are used in combination.

Another possible mechanism is a better bioavailability of dietary calcium than of supplemental calcium. Calcium salts are available in a myriad of over-the-counter food supplement preparations, but their solubility and bioavailability are highly variable (22, 23). In addition, a large number of these supplements are sold without having been tested for calcium absorbability, which may be affected by the presence of other compounds included in the supplements. Although studies on the bioavailability of calcium salts present in supplements are available (24–26), few studies have compared the bioavailability of calcium from dietary and supplemental sources. In a report on 9 women and 3 men studied for a period of 6 wk, calcium from either milk or calcium-fortified orange juice produced equal bioavailability (27). Besides the small number of subjects, this study was conducted under ideal conditions, and supplements were administered at a time when absorption was believed to be optimum. In the "real world," an ideal time is not always possible; therefore, individuals who obtain most of their calcium from supplements may be at a disadvantage compared with those who obtain most of their calcium from the diet, which does not require particular timing for maximal absorption. Moreover, the high vitamin D content in dairy sources of calcium may have resulted in better calcium absorption and better BMD in the women who consumed most of their calcium from the diet. However, these factors may not be independent of one another, and the greater BMD in the women who consumed dietary calcium was a consequence of a favorable interaction between the better bioavailability of dietary calcium and the higher active estrogen metabolites associated with the intake of this type of calcium source.

Finally, it is likely that the greater BMD in subjects who consumed calcium from dietary sources might be a result of a lifelong healthier diet. Several investigators reported a greater BMD in patients who consumed a significant amount of fruit and vegetables (28, 29). Whether this is the consequence of a higher calcium content in these foodstuffs or because these individuals are also consuming an adequate amount of calcium and vitamin D from other sources remains to be determined. Indeed, in our study we found that women who consumed a significant amount of calcium from dietary sources, on average, consumed more servings of fruit and fruit juices per week than did the women who consumed calcium primarily from supplements. The role of fruit and vegetables on BMD is still unclear. In fact, some studies have shown that individuals who depend solely on vegetables as a source of calories have low BMD (30). Thus, if there is a positive effect of fruit and vegetables on BMD, it would possibly be on account of the so-called healthy diet (a diet that also includes dietary sources of calcium and vitamin D), which in most cases comes in conjunction with a healthy lifestyle (ie, avoidance of tobacco and alcohol in excess as well as regular exercise). In fact, our data seem to suggest this possibility because significantly fewer women who consumed calcium from dietary sources were past smokers.

Our study had limitations. First, because this study is cross-sectional, our findings may not reflect the lifelong calcium intake and dietary practices of our participants, especially around the time when peak bone mass is acquired. In addition, only one 7-d dietary record was used to assess the dietary habits of our subjects, which may not have been representative of the usual dietary

intake of these individuals. Second, although we excluded women with dietary and lifestyle habits and those taking medications known to modulate estrogen hydroxylation, we could not exclude the possibility that other food products present in dietary calcium sources may have caused the shift in estrogen metabolism toward the active pathway or that active estrogen metabolites themselves may be present in certain foodstuffs, such as dairy products. Third, we had a relatively limited number of participants in the Supplement group ($n = 33$) relative to the numbers in the other groups. However, the calculated power was adequate (81% for metabolites and 82% for spine BMD) to detect significant differences between groups with the current sample size.

In summary, our results suggest that calcium modulates estrogen metabolism, and a higher intake of calcium from dietary sources is associated with increased estrogen hydroxylation through the active pathway. Our findings also indicate that women whose calcium intake is primarily from the diet have greater bone densities than do those who obtain calcium mainly from supplements. Our results suggest that the type of calcium source may be an important determinant of estrogen metabolism, and the consumption of dietary calcium may have more positive effects on bone metabolism than does the consumption of calcium supplements and should be encouraged in patients at risk of bone loss.

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The authors' responsibilities were as follows—NN, JT, RC, and RCA-V: preparation and analysis of the data and writing of the manuscript; NN: recruitment of the participants, dietary assessment, blood and urine collection, and data entry; JT: dietary assessment and data entry; RCA-V (principal investigator): study design and supervision; RC: data interpretation. None of the authors had a personal or financial conflict of interest.

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