Factors associated with calcium absorption efficiency in pre- and perimenopausal women[1–3]

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ABSTRACT

Background: The amount of calcium ingested by an individual may affect several chronic conditions, including osteoporosis, hypertension, and colon cancer. However, individuals vary in their ability to absorb the calcium they consume.

Objective: The purpose of this study was to examine sources of interindividual variation in the efficiency of calcium absorption in women.

Design: Fractional calcium absorption was estimated in 142 healthy pre- and perimenopausal women. Dietary habits, lifestyle factors, calciotropic hormones, and vitamin D receptor gene polymorphisms were also assessed.

Results: Calcium absorption values averaged 35% and ranged from 17% to 58%. Fractional calcium absorption was positively associated with body mass index ($r = 0.22$, $P = 0.007$), dietary fat intake ($r = 0.29$, $P = 0.001$), serum 1,25 dihydroxyvitamin D [1,25$(OH)_2$D] concentrations ($r = 0.23$, $P = 0.006$), and parathyroid hormone concentrations ($r = 0.21$, $P = 0.015$). Fractional calcium absorption was inversely associated with total calcium intake ($r = -0.18$, $P = 0.030$), dietary fiber intake ($r = -0.19$, $P = 0.028$), alcohol consumption ($r = -0.14$, $P = 0.094$), physical activity ($r = -0.22$, $P = 0.007$), and symptoms of constipation ($r = -0.16$, $P = 0.059$). In stepwise regression analysis, dietary fat, dietary fiber, serum 1,25$(OH)_2$D, and alcohol consumption emerged as independent predictors of calcium absorption, explaining 21.02% of the observed variation. Women in the lowest tertile of the ratio of dietary fat to fiber had 19% lower fractional calcium absorption values than did women in the highest tertile of ratio of dietary fat to fiber (test of trend, $P < 0.001$).

Conclusions: There is a wide range of calcium absorption values in healthy women. The amount of dietary fat consumed relative to dietary fiber appears to have an important role in determining differences in calcium absorption performance among individuals. *Am J Clin Nutr* 2000;72:466–71.

KEY WORDS Calcium absorption efficiency, variation, premenopausal women, perimenopausal women, vitamin D receptor polymorphisms, parathyroid hormone

INTRODUCTION

Inadequate calcium intake influences bone loss and the risk of osteoporosis (1). The results of recent epidemiologic studies suggest that hypertension and colorectal cancer may also be linked to low calcium intake (2, 3). Yet the health benefits to be expected from calcium depend not only on how much calcium is consumed but also on the body's ability to absorb the ingested calcium. Most studies have been limited to measures of dietary calcium intake without consideration of the amount of calcium absorbed by the body, which can vary widely from <10% to >60% in relatively healthy women (4).

Factors that determine why some women absorb calcium poorly whereas others absorb it efficiently are not entirely clear. We showed previously that age, estrogen status, and usual calcium intake explained only a small amount of the variability in the efficiency of calcium absorption in a healthy population of women (5). We found that calcium absorption decreases by ≈0.0021/y after age 40 y and that estrogen withdrawal is responsible for a drop of ≈0.022. Calcium absorption efficiency is also inversely related to usual calcium intake; however, differences in the usual calcium intake explain only ≈26% of interindividual variation (5). In addition, serum 25-hydroxyvitamin D [25(OH)D], mouth-to-cecum transit time, and urinary calcium have been found to be important sources of variation, together explaining 44% of the observed variation in calcium absorption efficiency (6). Yet to date, a large portion of the interindividual variation in absorption performance remains unexplained, particularly in community-dwelling women.

The purpose of the present study was to expand on these previous findings and explore additional presumed sources of variation in calcium absorption efficiency. More specifically, we examined a variety of dietary constituents, lifestyle factors, calciotropic hormones, and vitamin D receptor (VDR) gene polymorphisms that may affect calcium absorption efficiency in a sample of healthy pre- and perimenopausal community-dwelling women.

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[2] Supported by the National Heart, Lung, and Blood Institute (grant NIH HL 45167-03), Medco Containment Services, the National Osteoporosis Foundation, and the National Institute on Aging (training grant T32 AG00181).

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Received July 7, 1999.

Accepted for publication February 23, 2000.

SUBJECTS AND METHODS

Subjects

Calcium absorption was measured between March 1995 and April 1996 in a cross-sectional sample of women enrolled in the Women's Healthy Lifestyle Project (WHLP) (7). In brief, WHLP is an ongoing randomized clinical trial involving 535 healthy women and designed to test whether elevations in cardiovascular risk factors can be prevented with dietary and behavioral intervention. At baseline, the women were randomly assigned to either an assessment-only control group (n = 275) or a lifestyle intervention group (n = 260). The aim of the intervention was to reduce total dietary fat to 25% of daily energy, saturated fat to 7% of daily energy, and dietary cholesterol to 100 mg/d. In addition, participants who were assigned to the intervention group were given a modest weight-loss goal to prevent weight gain and were asked to increase their physical activity to 4180–6270 kJ/wk by increasing moderate-intensity aerobic activity, such as walking.

A detailed description of the calcium absorption test was mailed to the 286 white WHLP participants who were found to have the VDR genotypes *BB* (n = 97) or *bb* (n = 189). Women were not eligible if they were postmenopausal, had used hormone replacement therapy within the previous month, or were currently using prescription medications known to influence calcium metabolism. The 142 participants who volunteered for the calcium absorption measurement were similar in demographic and health characteristics to the WHLP participants who did not volunteer for the study. The protocol was approved by the Human Institutional Review Board and the Radiation Safety Committee at the University of Pittsburgh. Written, informed consent was obtained from each subject.

Assessment of fractional calcium absorption

All measurements were conducted at the participant's 18- or 30-mo follow-up clinic appointment, which was ≈1.5–2.5 y after the baseline WHLP examination. The calcium absorption test was completed in the morning after an overnight fast. Fractional calcium absorption was estimated from the appearance of ^{45}Ca in the blood after the ingestion of 50 g labeled calcium-fortified apple juice (containing 63 mg Ca) and 120 g additional unlabeled calcium-fortified apple juice (Speas Farm; Sundor Brands, Inc, Mt Dora, FL) containing 152 mg Ca. The total calcium load was 215 mg. The quantity of ^{45}Ca used in each test was ≈370 kBq (10 μCi)/dose. Labeled ^{45}Ca was prepared by the Osteoporosis Research Center, Creighton University, Omaha, and then shipped to the University of Pittsburgh for mixing with the unlabeled juice. Actual dosing occurred midway during the consumption of a standard light test meal consisting of 4 graham cracker squares and 1 teaspoon of margarine. Blood was drawn into a serum separator tube exactly 3 h after ingestion of the tracer and was allowed to clot at room temperature. Serum was separated within 2 h of collection and frozen at −70 °C. Frozen serum samples were later shipped on dry ice by overnight delivery to Creighton University for estimation of fractional calcium absorption using the single oral isotope method, as described elsewhere (8, 9). The test was reproducible with a CV of 9% for a small sample of 8 assessment-only control women measured twice 1 y apart.

Assessment of dietary and lifestyle factors

Dietary calcium intake was estimated by self-report using a modified Block semiquantitative 10-item food-frequency questionnaire (10). Use of calcium supplements was determined by asking the women their dose and frequency of use of multivitamins with minerals, specific vitamin and mineral supplements, and antacids. Total calcium intake was calculated by adding daily calcium intake from the diet plus calcium intake from supplements. Total dietary energy (in kJ), fat, fiber, protein, and phosphorus were estimated by using a Block food-frequency questionnaire (11). Physical activity was assessed by a trained interviewer using a modified Paffenbarger physical activity questionnaire, which estimated energy expended over the previous week on the basis of blocks walked per day, flights of stairs climbed per day, and participation in sports or recreational activity (12). Self-reported current alcohol consumption, caffeine consumption (from coffee, tea, and cola), and cigarette use were determined. In addition, the women were asked whether they had had symptoms of constipation within the 2 wk before their calcium absorption measurement.

Other measurements

The women were classified as premenopausal or perimenopausal. A woman was considered premenopausal if she reported menses within the 3 mo before her examination and perimenopausal if she reported no menses within the 3 mo before her examination. If a woman was amenorrheic for ≥12 mo, she was considered postmenopausal and was excluded from the study. Fasting blood samples were collected for measurement of serum calcium, parathyroid hormone (PTH), and 1,25 dihydroxyvitamin D [1,25$(OH)_2$D]. Serum calcium was measured by using atomic flame spectroscopy and intact PTH (iPTH) was measured by using a 2-site immunochemiluminometric method (Endocrine Sciences, Inc, Calabasas Hills, CA). Serum 1,25$(OH)_2$D was measured by radioreceptor assay using a procedure similar to that of Reinhardt et al (13) and modified by Endocrine Sciences. Modifications to the Reinhardt procedure were that tritiated hormone was purchased from NEN-Dupont (Boston) and repurified by HPLC before use and Bondelute (Varian, Harber City, CA) eluates are dispensed as aliquots for duplicated assay and recovery check. Solvent was dried in a rotary evaporator (Savant, Holbrook, NY) under vacuum. Body weight was measured with a balance-beam scale and height was measured with a stationary vertical height board (Perspective Enterprises, Inc, Kalamazoo, MI). Body mass index (BMI; in kg/m^2) was calculated. High-molecular-weight DNA was extracted from peripheral leukocytes by the salting-out procedure of Miller et al (14). Genotypes for the VDR *Bsm*I, *Apa*I and *Taq*I polymorphic restriction sites were determined by using polymerase-chain-reaction methods described in Zmuda et al (15).

Bone mineral density (BMD) of the total hip was measured by using dual-energy X-ray absorptiometry (DXA) with the array mode (HOLOGIC QDR 2000; Hologic Inc, Waltham, MA). DXA measurements were made by a certified technician who was blinded to the treatment groups. Standardized procedures for patient positioning and use of the DXA software were used. The scans were analyzed with HOLOGIC software, version 7.10 with the compare feature. The within-subject CVs with a similar protocol were 1.3% for the femoral neck (16). T scores were calculated by using the mean (±SD) total femur BMD (in g/cm^2) for a 20–29-y-old female reference population using data from the third National Health and Nutrition Examination Survey (NHANES III), 1988–1994 (17). We used the World Health Organization criteria to define women with a T score at the total

TABLE 1
Characteristics of the study subjects

Characteristic	
Anthropometric variables	
Age (y)	48.9 ± 1.8 (45.8–53.6)[1]
Weight (kg)	64.9 ± 9.4 (46.7–89.8)
Height (cm)	163.2 ± 5.9 (149.8–177.8)
BMI (kg/m^2)	24.4 ± 3.2 (18.3–35.1)
Bone mineral density	
Total hip (g/cm^2)	0.92 ± 0.11 (0.62–1.3)
Osteopenic at hip	18 (12.7)[2]
Osteoporotic at hip	0 (0)
Menopausal status	
Premenopausal	133 (93.7)
Perimenopausal	9 (6.3)
Randomization group	
Assessment only	72 (51)
Intervention	70 (49)
Education	
High school diploma	28 (19.7)
0–4 y of college	69 (48.6)
Advanced degree	45 (31.7)
Calciotropic hormones	
Serum calcium (mmol/L)	2.3 ± 0.08 (2.1–2.5)
Serum 1,25 dihydroxyvitamin D (pmol/L)[3]	150.72 ± 39.12 (72–280.8)
Serum PTH (pmol/L)[4]	3.58 (0.74–11.68)[5]
Fractional calcium absorption	0.35 ± 0.08 (0.17–0.58)
Calcium intake	
Dietary calcium (mg/day)	537 (47.0–1508.2)[5]
Calcium supplement use	73 (51.4)[2]
Total calcium (mg/d)	824.9 (47.0 –3955.0)[5]
Nutrient intake	
Total energy (kJ/d)[6]	5625.03 ± 2011.8 (1886.4–13824.9)
Dietary fat (g)[7]	41.4 (4.9–174.5)[5]
Dietary fiber (g)[7]	11.0 (4.0–26.7)
Protein (g)[8]	55.1 (14.0–170.7)
Phosphorus (g)[7]	980.3 (279.4–3827.0)
Other lifestyle factors	
Physical activity (kJ/wk)	5500.88 (234.08–28252.62)
Current caffeine use	102 (71.8)[2]
Current alcohol use	115 (81.0)
Current smoking	8 (5.6)
Reporting symptoms of constipation in the previous 2 wk	38 (27.1)

[1] $\bar{x}$ ± SD; range in parentheses; n = 142.
[2] n; percentage in parentheses.
[3] n = 140.
[4] n = 138.
[5] Median; range in parentheses.
[6] n = 137.
[7] n = 129.
[8] n = 130.

femur between −1.0 and −2.5 as being osteopenic and a T score <−2.5 as being osteoporotic (18).

Statistics

All analyses were performed by using SPSS (version 4.1; SPSS Inc, Chicago). Descriptive characteristics are presented as means and SDs unless otherwise indicated. Total calcium intake, physical activity, dietary variables (fat, fiber, protein, and phosphorus), and iPTH were log transformed to conform more closely to a normal distribution. The medians of the untransformed values are presented for ease of interpretation. Some of the numbers in the tables vary because of missing data and outliers. Pearson product correlation coefficients were calculated to examine the direction and magnitude of the relation between measured variables and fractional calcium absorption; *t* tests and analysis of variance (ANOVA) were used to test unadjusted means for differences in fractional calcium absorption across VDR genotypes. Stepwise regression analysis showed which factors emerged as the most important independent predictors of fractional calcium absorption when variables that were found to be univariately associated with fractional calcium absorption ($P < 0.10$) were entered together. To further explore the relation between fat, fiber, and efficiency of calcium absorption, we calculated a ratio of fat to fiber for each individual [dietary fat (in g/d) divided by dietary fiber (in g/d)]. ANOVA was used to examine the relation between mean fractional calcium absorption values and tertile of the ratio of fat intake to fiber intake.

RESULTS

Characteristics of the subjects

All of the women were white and ranged in age from 46 to 54 y ($\bar{x}$: 49 y) (**Table 1**); most were premenopausal (93.7%). The participants were highly educated working women and almost one-third (31.7%) had received advanced degrees after college. The mean weight of the women in the sample was 64.9 kg and the mean BMI was 24.4. Women from both randomization groups of the parent study (intervention group versus assessment-only control group) were equally represented in the sample. BMD of the hip averaged 0.92 ± 0.11 g/cm^2; 12.7% and 0% of the women were considered to have osteopenia and osteoporosis, respectively. The median dietary calcium intake averaged 537 mg/d, and 51.4% of the women reported that they received additional calcium from a multivitamin with minerals or from a specific vitamin or mineral supplement. The median total calcium intake of the study sample was estimated to be 824.9 mg/d. Median intakes of other dietary factors were comparable with NHANES III estimates for women in a similar age group (19), except for dietary fat intake, which was lower than US averages (20). Few participants smoked and nearly three-fourths consumed caffeine from coffee, tea, or cola beverages at the time of the study. The median total energy expenditure for leisure-time activity was 5501 kJ/wk. More than one-quarter of the sample reported symptoms of constipation within the previous 2 wk. Mean values and ranges for serum calcium, PTH, and 1,25(OH)$_2$D all fell to within normal limits for adults.

Factors associated with fractional calcium absorption

The mean (±SD) value of fractional calcium absorption was 0.35 ± 0.08 and spanned a wide range from 0.17 to 0.58. Fractional ^{45}Ca absorption was significantly and positively correlated with BMI (r = 0.22, P = 0.007), dietary fat (r = 0.29, P = 0.001), serum 1,25(OH)$_2$D concentrations (r = 0.23, P = 0.006), and iPTH (r = 0.21, P = 0.015) (**Table 2**). Fractional ^{45}Ca absorption was inversely correlated with total calcium intake (r = −0.18, P = 0.03), dietary fiber (r = −0.19, P = 0.03), alcohol consumption (r = −0.14, P = 0.09), physical activity (r = −0.22. P = 0.007), and symptoms of constipation (r = −0.16, P = 0.06). There was no significant association between fractional calcium

TABLE 2
Pearson correlation coefficients (*r*) for measured variables and fractional calcium absorption

Characteristic	*r*	*P*
Anthropometric variables		
Age (y)	−0.06	0.455
Weight (kg)	0.23	0.006
Height (cm)	0.05	0.538
BMI (kg/m^2)	0.22	0.007
Calciotropic hormones		
Serum calcium (mmol/L)	−0.11	0.180
Serum 1,25 dihydroxyvitamin D (pmol/L)	0.23	0.006
Parathyroid hormone (pmol/L)	0.21	0.015
Calcium intake		
Dietary calcium (mg/d)	−0.09	0.267
Calcium supplement use (mg/d)	−0.17	0.040
Total calcium (mg/d)	−0.18	0.030
Nutrient intake		
Dietary fat (g)	0.29	0.001
Dietary fiber (g)	−0.19	0.028
Protein (g)	−0.02	0.855
Phosphorus (g)	−0.07	0.995
Lifestyle factors		
Physical activity (kJ/wk)	−0.22	0.007
Current caffeine use (0 = no, 1 = yes)	0.11	0.204
Current alcohol use (0 = no, 1 = yes)	−0.14	0.094
Currently smoking (0 = no, 1 = yes)	0.01	0.941
Report symptoms of constipation in the previous 2 wk (0 = no, 1 = yes)	−0.16	0.059

absorption and age, dietary protein, dietary phosphorus, caffeine consumption, or smoking. There were no significant differences in the mean values of fractional absorption across VDR genotypes.

In a stepwise regression analysis, dietary fat, dietary fiber, alcohol consumption, and serum 1,25(OH)$_2$D emerged as significant and explained 21.02% of the observed variation in calcium absorption (**Table 3**). Dietary fat (g/d) and dietary fiber (g/d) emerged as the 2 most important independent predictors of fractional calcium absorption. These 2 variables, taken together, explained 13.8% of the variability in fractional calcium absorption. Serum 1,25(OH)$_2$D explained another 4.3% and alcohol consumption explained another 3.0% of the variation in fractional calcium absorption. BMI, PTH, total calcium intake, physical activity, and symptoms of constipation did not have an independent effect on fractional calcium absorption once dietary fat, dietary fiber, alcohol consumption, and serum 1,25(OH)$_2$D were included in the model.

Women in the lowest tertile of the ratio of fat to fiber (tertile 1) had significantly lower mean fractional calcium absorption values than did women in the highest tertile (tertile 3) (test of trend, $P < 0.001$) (**Figure 1**). This amounted to a 19% lower fractional calcium absorption for women in tertile 1 than for women in tertile 3 after adjustments for randomization group, BMI, calcium intake, serum 1,25(OH)$_2$D, PTH, alcohol consumption, and physical activity ($P < 0.05$).

DISCUSSION

We found a wide range of fractional calcium absorption values in this sample of premenopausal and early perimenopausal women. We identified several factors associated with the efficiency of calcium absorption. Dietary fat, dietary fiber, serum 1,25(OH)$_2$D, and alcohol consumption were independent determinants of calcium absorption efficiency, explaining approximately one-fifth (21.02%) of the total variability in calcium absorption. In particular, women who reported consuming diets with the lowest ratio of fat to fiber had 19% lower mean fractional calcium absorption values than did women who consumed diets with the highest ratio of fat relative to fiber.

Interestingly, at the time of the calcium absorption measurement, the women who had been randomly assigned to the intervention group had significantly lower mean fractional calcium absorption values than did the women who were assigned to the assessment-only control group (0.34 compared with 0.36, $P < 0.001$). This was the direction of difference we expected, because we found lower dietary fat intakes to be significantly associated with lower calcium absorption values, and the women in the intervention group had significantly lower dietary fat intakes than did the women in the assessment-only control group at the time of the calcium absorption measurement.

Of importance, the calcium absorption measurement was done cross-sectionally at the participant's 18- or 30-mo follow-up appointment, which was ≈1.5–2.5 y after the women were assigned to groups. The length of the lifestyle intervention was 5 y; however, the most intensive portion of the intervention was within the first 6 mo. Thus, data were pooled from both randomization groups for the calcium absorption study. We were limited in that we did not have a baseline calcium absorption measurement to enable us to examine how changes resulting from the lifestyle intervention (eg, in diet, physical activity, and weight) related to changes in calcium absorption efficiency.

We speculate that a potential link between a low-fat, high-fiber diet and poor calcium absorption is related to intestinal transit time. We showed previously that a faster mouth-to-cecum transit time is associated with poorer efficiency of calcium absorption, suggesting that gut motility has a large effect in healthy subjects (6). Fat intake may alter gut motility and affect

TABLE 3
Stepwise regression model for fractional calcium absorption (criterion variable) and measured variables found to be significant ($P < 0.10$) in univariate analysis (predictor variables)[1]

Variable	B coefficient (SE)	Partial R^2	Cumulative R^2	*P*
Dietary fat	0.044 (0.01)	0.076	0.076	0.001
Dietary fiber	−0.055 (0.02)	0.062	0.138	0.003
Serum 1,25 dihydroxyvitamin D	8.88 E-04 (3.7 E-4)	0.043	0.181	0.011
Alcohol consumption	−0.034 (0.02)	0.030	0.210	0.032

[1] Measured variables found to be significantly correlated with fractional calcium absorption were entered into the model: BMI, 1,25 dihydroxyvitamin D, parathyroid hormone, total calcium intake, physical activity, dietary fat, dietary fiber, and symptoms of constipation.

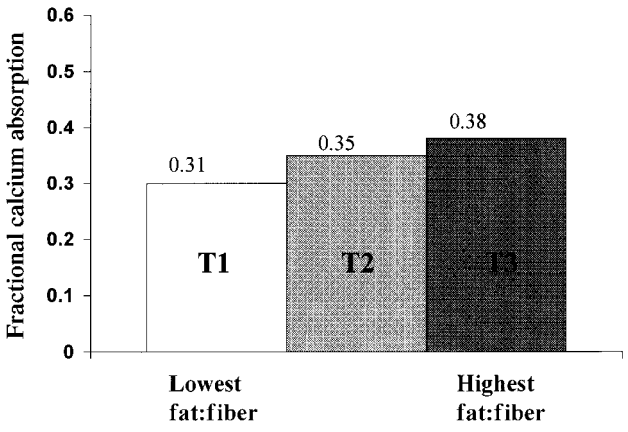

FIGURE 1. Tertile of the ratio of fat to fiber and fractional calcium absorption. We calculated a fat-to-fiber ratio for each subject [dietary fat (g/d) divided by dietary fiber (g/d)]. Tertiles are defined as follows: tertile 1 (T1), fat:fiber < 2.9; tertile 2 (T2), 2.9 < fat:fiber < 4.8; tertile 3 (T3), fat:fiber = 4.8. Mean fractional calcium absorption values were adjusted for randomization group, BMI, total calcium intake, serum 1,25 dihydroxyvitamin D concentration, parathyroid hormone concentration, alcohol consumption, and physical activity. Overall $P < 0.001$.

absorption by slowing transit time and increasing the duration of contact with the absorptive surface (21). Increased fiber intakes may inhibit calcium absorption by increasing the bulk of intestinal contents, speeding the transit time of the stool, and in theory allowing less time for absorption to occur (21). Future studies are necessary to enable us to better understand the physiologic mechanisms for the interaction between dietary fat, dietary fiber, calcium intake, and calcium absorption in the gut.

It will also be important to understand the type of fat and fiber that affect the efficiency of calcium absorption. The results of some studies suggested that only the fiber in wheat bran, and not the fiber in green leafy vegetables such as kale, broccoli, and bok choy, is detrimental to calcium absorption performance, although the reasons remain unclear (22–24). Experiments with baby formulas suggested that the length of the fatty acids (eg, long- versus medium-chain fatty acids) may differentially influence the amount of calcium that gets absorbed by the gut (25). However, we found the direction and magnitude of the relation among fiber, fat, and fractional calcium absorption to be similar whether we stratified by saturated and unsaturated fatty acids or by soluble and insoluble fiber. We were limited in that we could not disentangle the association between calcium absorption and the different types of fiber within these groups (eg, bran fiber compared with psyllium fiber). Examination of these various types of fiber and their association with calcium absorption is important but was beyond the scope of this study.

The inverse relation between fractional calcium absorption and alcohol consumption was unexpected, particularly given that the amount of alcohol consumed was modest. To date, no data on the effects of moderate alcohol consumption on calcium absorption in humans are available for comparison. In general, it is thought that long-term consumption of alcohol, particularly in high amounts, probably has an adverse effect on calcium-regulating hormones, although the mechanisms remain to be established (26). In our study, alcohol consumption was not associated with serum concentrations of calcium, PTH, or $1,25(OH)_2D$, making it difficult to speculate as to the mechanism for the modest, yet significant, inverse relation between moderate alcohol consumption and the efficiency of calcium absorption observed in our sample.

Our findings regarding serum $1,25(OH)_2D$ and calcium absorption efficiency were expected and were similar to those recently published by Wishart et al (27), who reported that serum $1,25(OH)_2D$ accounted for 5% of the variance in calcium absorption efficiency in perimenopausal women. 25(OH)D measurements were not available in our sample to show whether the relation between $1,25(OH)_2D$ and fractional calcium absorption was attenuated after we controlled for 25(OH)D concentrations as suggested by Barger-Lux et al (6). We did stratify women who were studied in the summer, fall, winter, and spring as a potential indicator of level of vitamin D nutrition but did not find significant differences in fractional calcium absorption between groups. Although it is generally believed that circulating $1,25(OH)_2D$ is the principal regulator of calcium absorption, the small variance explainable by $1,25(OH)_2D$ in our sample suggests that other factors play a more important role.

Our results did not support the idea that VDR gene polymorphisms produce physiologically significant effects on calcium absorption. Failure to find a relation between VDR gene polymorphisms and fractional calcium absorption is consistent with the results of 2 studies that found no effect of VDR gene polymorphism on calcium absorption (28, 29). Our findings do contradict 3 studies that found a significant role of VDR genotypes on calcium absorption efficiency (27, 30, 31). We considered the fact that the effect of VDR gene polymorphisms on calcium absorption efficiency could be masked by confounding factors such as calcium intake, as suggested by Dawson-Hughes et al (31). However, in our study we were unable to find any interaction between VDR genotypes (*BB* or *bb*) and low versus high calcium intakes. Thus, when we combine our findings with those of others, it still remains unclear whether VDR genotypes have a role in calcium absorption. Furthermore, differences among studies in calcium test loads, the menopausal status of the subjects, sample size, sex of the subjects, and consideration of potential confounders all contribute to the difficulty in reaching a consensus as to whether VDR genotypes affect calcium absorption efficiency. Future studies should consider the effect of more functional VDR gene polymorphisms, such as those at the *Fok*I restriction site and their potential association with fractional calcium absorption, although our recent data (published as yet only in abstract form) did not show any association with the *Fok*I genotype and the efficiency of calcium absorption (32).

This study had several limitations. We had reduced power to adequately examine the association between fractional calcium absorption and smoking because <6% of our sample ($n = 6$) smoked cigarettes. In addition, the age range of the subjects was narrow, which prevented meaningful analysis of absorption as a function of age. We were also limited in our assessment of constipation status because we did not specifically ask for the time between bowel movements. Participants may have interpreted "constipation in past 2 wk" differently. Our assessment of menopausal status was also limited in that it was based on the number of months without menses and not on hormonal blood markers, such as estradiol and follicle-stimulating hormone concentrations. Although there was the possibility of misclassification of our subjects, our results were similar when analyzed with or without the 9 women in our study who were considered to be

perimenopausal. Another limitation relates to the potential generalizability of our data. As a group, the subjects were highly educated white women with relatively low fat intakes and high use of calcium supplements. In addition, our subjects were all enrolled in a larger randomized clinical trial, and although both randomization groups were equally represented in our sample and adjustments for randomization group did not alter the results, it will be important to confirm these results in other populations. Finally, we were limited in that we did not have measures of hydration status or transit time in our sample to help explain the potential mechanisms for the interaction between dietary fat, dietary fiber, and calcium absorption in the gut.

The wide range of calcium absorption efficiencies found in a healthy sample of women raises questions as to whether more serious consideration should be given to evaluating calcium absorption performance before making calcium intake recommendations. These results raise interesting physiologic questions about the interrelation among dietary fat, dietary fiber, and calcium in the body. Further research is necessary to enable us to better understand the extent to which widespread public health recommendations for a low-fat, high-fiber diet may affect calcium absorption performance, bone, and other chronic disorders in which calcium may have a protective role.

We are indebted to June Bierman at Creighton University for her assistance with the preparation of the calcium absorption test. We thank Alhaji Buhari and Steven Wisniewski for their statistical support and Amy Flaugh for her technical assistance. Our appreciation goes to the staffs of the Women's Healthy Lifestyle Project and the Department of Epidemiology's Health Studies Office for their ongoing contributions to the project.

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