See corresponding editorial on page 4.

Nutritional associations with bone loss during the menopausal transition: evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids[1–4]

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ABSTRACT

Background: The menopausal transition is characterized by rapid bone loss. Few data exist on the role of nutrition.

Objective: The objective of the study was to ascertain which dietary factors influence perimenopausal skeletal loss.

Design: A longitudinal study was conducted of 891 women aged 45–55 y at baseline and 50–59 y at follow-up 5–7 y later. Bone mineral density (BMD) was measured by using dual-energy X-ray absorptiometry at the lumbar spine and femoral neck (FN). Nutrient intakes were assessed after the baseline visit and 5 y later, by using the same food-frequency questionnaire.

Results: After adjustment for energy intake and other confounders, higher intakes of calcium were correlated with change in FN BMD (ie, reduced loss) ($r = 0.073$, $P < 0.05$), and the intake of modest amounts of alcohol was associated with less lumbar spine bone loss ($P < 0.01$ for quartile of alcohol intake). Greater FN BMD loss was associated with increased intake of polyunsaturated fatty acids ($r = -0.110$, $P < 0.01$), monounsaturated fatty acids ($r = -0.069$, $P < 0.05$), retinol ($r = -0.067$; $P < 0.05$), and vitamin E ($r = -0.110$; $P < 0.01$). The latter 2 nutrients were highly correlated with polyunsaturated fatty acids. For premenopausal women, calcium and nutrients found in fruit and vegetables (vitamin C, magnesium, and potassium) were associated with FN BMD, and calcium, vitamin C, and magnesium were associated with change in FN BMD.

Conclusions: Although menopausal status and hormone replacement therapy use dominate women's bone health, diet may influence early postmenopausal bone loss. Fruit and vegetable intake may protect against premenopausal bone loss. *Am J Clin Nutr* 2004;79:155–65.

KEY WORDS Menopause, bone loss, calcium, fatty acids, alcohol, fruit and vegetables, bone mineral density

INTRODUCTION

The influence of diet on perimenopausal and early postmenopausal bone loss is not well understood. Most work has focused on calcium and vitamin D or a few isolated nutrients, but little work has focused on the diet in its entirety. Furthermore, results from older studies are less reliable because those studies used less precise techniques to measure bone mass (single-photon absorptiometry) and assess diet (24-h recall). Many earlier studies did not adjust for dietary energy intake (EI), which is an important confounder in studies of diet and disease (1).

Nutrients associated with fruit and vegetable intake (in particular, potassium and magnesium) have been associated with greater bone mineral density (BMD) in late premenopausal women (2, 3) and elderly men and women (4) and with less bone loss in elderly men (4). A number of other nutrients—vitamin C and niacin for postmenopausal women (5), iron, and magnesium (together with zinc for premenopausal women; 6)—were associated with greater forearm bone mineral content, and protein, phosphorous, zinc, and folate were associated with reduced postmenopausal bone loss (5). A placebo-controlled trial in which postmenopausal women were supplemented with zinc, copper, and manganese in addition to calcium resulted in a small increase in BMD over 2 y (7).

Controversy continues to surround the issue of calcium intake: 700 mg Ca/d is considered adequate for postmenopausal women in the United Kingdom (8, 9), whereas 1500 mg Ca/d is considered adequate for postmenopausal women in the United States, and there is disagreement among nutritionists as to whether there should be a guideline for calcium (10, 11). For much of the developing world, calcium intake is much lower (344 mg/d) than that in the developed world (850 mg/d) (12), and the developing world's diet is also high in substances (oxalate, phytate, and possibly fiber) that hamper the absorption of calcium. Yet those populations do not have higher prevalences of osteoporosis (13). Besides genetic differences, possible explanations include differences in vitamin D status

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(14), salt intake (15), and animal protein intake (16) and a general lack of alkali-forming metabolites in the typical Western diet, which could be remedied by increased intakes of fruit and vegetables (3, 17). Another explanation, not yet considered in this context, is the differences in fat intake between populations. The high fatty acid content of the Western diet may be a risk factor for osteoporosis, possibly through reduced absorption of calcium as a result of the formation of insoluble calcium–fatty acid soaps. The purpose of this study was to investigate which nutrients are associated with BMD and recent BMD loss around the time of the menopause, taking into account confounders such as dietary EI and physical activity level (PAL).

SUBJECTS AND METHODS

Subjects

A subset of 1064 healthy, mainly premenopausal women aged 45-54 y who took part in the Aberdeen Prospective Osteoporosis Screening Study and had a bone scan between 1990 and 1993 went on to complete food-frequency questionnaires (FFQ) according to season in 1993 (2, 3). This study is a population-based screening program for osteoporotic fracture risk involving >5000 women drawn at random from Community Health Index records from within a 40-km radius of Aberdeen, a city with a population of 250 000 in the northeastern part of Scotland (18, 19). Between 1997 and 1999, women were recalled for a second bone scan with the use of the most recent list of addresses obtained from Primary Care, Grampian Health Services. A total of 907 women from our subset returned, of whom 896 again completed the FFQ, according to season. At baseline, women were selected who were not taking any medication or did not have any condition likely to affect their bone metabolism. At the follow-up visit, 3 women were excluded from the analysis because they were receiving bisphosphonate therapy. An additional 2 women were also excluded: one was a wheelchair user and the other had dietary calcium intake that was an outlier; thus, 891 women underwent the final analysis. Written informed consent was obtained from all of the women, and the study was approved by the Grampian Research Ethics Committee.

Anthropometric and bone density measurements

The women were weighed without shoes and while wearing light clothing with the use of scales that were calibrated to 0.05 kg (Seca, Hamburg, Germany). Height was measured with the use of a stadiometer (Holtain Ltd, Crymych, United Kingdom). The BMD measurements of the left proximal femur (the femoral neck, or FN) and the lumbar spine (LS; L2–4) were performed by using dual-energy X-ray absorptiometry with the use of 2 Norland scanners (CooperSurgical Inc, Trumbull, CT). Most of the women (92.8%) were measured at baseline and follow-up using an XR26 scanner (CooperSurgical Inc), and the remainder (7.2%) of the women were measured with the use of an XR26 scanner at baseline and an XR36 scanner (CooperSurgical Inc) at follow-up. Calibration of the machines was performed daily, and quality assurance was performed by measuring the manufacturer's phantom at daily intervals and a hologic phantom at weekly intervals. The in vivo precision (CV) at our unit of the XR36 scanner is 1.20% for LS and 2.32% for FN. For the XR26 scanner, the corresponding values were 1.95% and 2.31% (LS and FN, respectively). These values were determined by duplicate measurements in 8 women aged 49–65 y (mean: 54 y) on the XR36 scanner and in a separate set of 8 women aged 49–63 y (mean: 53 y) on the XR26 scanner. A comparison between the XR26 and XR36 scanners was made by using 50 phantom measurements from each machine. It appeared that the XR36 scanner ($\bar{x} \pm$ SD: 0.7963 ± 0.0068 g/cm^{-2}) was giving slightly higher measurements than was the XR26 scanner (0.7771 ± 0.0054 g/cm^{-2}). BMD measurements from the XR36 scanner were therefore corrected by dividing by 1.02478509, the mean difference between the values obtained with the 2 machines. The influence of diet was examined by using BMD and, more important, the percentage of BMD change that had occurred since the first scan. Because the time elapsed between each scan will be different for each woman, this value was standardized to annual percentage of change in BMD, which, for simplicity, is referred to as *BMD change* in the text. Although data were available for other hip sites (trochanter and Ward's triangle), which showed similar trends, only results for FN BMD are presented because they have better precision.

Usual dietary intake

Usual dietary intake (over previous 12 mo) was assessed by using the same FFQ as was used in the baseline studies (2). The women were sent the FFQ by mail along with their dual-energy X-ray absorptiometric scan appointment details. Most of the women brought the completed FFQ to their appointments, and thus it could be checked for any missed questions that the subject could answer while still present. A similar questionnaire, based on the Caerphilly FFQ (20), was used for the Scottish Heart Health Study (21, 22). The FFQ containing 98 foods or food groups was validated against 7-d weighed records and biochemical markers of antioxidant status (23, 24), and its short- and long-term reproducibility was tested (25). The FFQs were coded and analyzed with the use of the Rowett Research Institute software program (RONA; Rowett Research Institute, Aberdeen, United Kingdom), which uses data originating from McCance and Widdowson's food composition tables and supplements (26) that were provided in database form by the Royal Society of Chemistry and the Ministry of Agriculture Fisheries and Food, now the UK Food Standards Agency. Alcohol intake was the amount consumed in the previous week. Details on dietary supplement use—including the type of supplement, the manufacturer, and the frequency of usage—were ascertained at baseline and follow-up visits. From this information, the amounts of the nutrients obtained from supplemental sources could be calculated. Because the FFQ was completed on 2 occasions, the 2 measurements for each nutrient represent replicate measurements; for those women whose nutrient intake had changed, the change occurred sometime between completion of the first and second FFQs. Therefore, the mean of the 2 results was taken. Basal metabolic rate (BMR) was calculated from the weight of each woman by using Schofield's equations (8), and the ratio of EI to BMR was used as a measure of satisfactory completion of the FFQ.

Nutrients were expressed either in terms of crude intake or after adjustment for EI (an important confounder in nutritional epidemiologic studies) by using the residual method (27). For ease of interpretation of coefficients (unstandardized) in the

regression analysis, nutrients were standardized to 8 MJ EI (nutrient divided by energy in MJ and multiplied by 8 to reach the estimated average requirement for women aged 50–59 y). Carotene is given as β-carotene equivalents (the sum of β-carotene intake and one-half of the α-carotene and α- and β-cryptoxanthins intakes) (26). Vitamin A was calculated as retinol equivalents by using the sum of retinol intake and one-sixth of the β-carotene equivalents, as is the usual practice (8). However, according to the more recent recommendations of the Institute of Medicine, we also used the sum of retinol intake and one-twelfth of the β-carotene equivalents (28).

Lifestyle questions

Physical activity levels (PALs) were obtained by using the same questions as were used in the Scottish Heart Health Study, which asks about usual activities over the previous year (29). The PAL is calculated from the numbers of hours in a 24-h period spent doing heavy, moderate, or light activities and the numbers of hours in the same period spent sleeping or resting in bed. These questions were asked separately for workdays and nonworkdays. PAL is normally defined as the ratio of overall daily energy expenditure to BMR. Seated work that allows or requires a person to move around, but with little or no strenuous activity, is consistent with a PAL of 1.6–1.7, and standing work (eg, that of a housewife or salesclerk) is consistent with PAL of 1.8–1.9 (30).

Statistical analyses

SPSS statistical software (version 10.0; SPSS Inc, Chicago) was used for all statistical analyses. Pearson's correlations and partial correlations (with adjustment for confounders) were obtained between BMD or BMD change and nutrients (by using the natural log-transformed variable if required). Linear multiple regression was undertaken to identify independent predictors of BMD change. Two separate variables were used for menopausal status and hormone replacement therapy (HRT) use. Three categories were used for menopausal status: premenopausal (regular menses), perimenopausal (irregular menses), and postmenopausal (defined as having had no periods for 6 mo) or HRT user. The HRT users included women of unknown menopausal status, because many women started taking HRT when they were still menstruating. For HRT use, the subcategories were nonuser, past user, and present user. One-way analysis of variance (with Scheffe post hoc analysis) and analysis of covariance with adjustment for confounders were used to analyze categorical variables (eg, menopausal status or HRT use and quartiles of highly skewed dietary variables such as alcohol intake). Although at baseline the women did not have any disease that was likely to affect their bone metabolism, a number of subjects had since developed conditions such as osteoarthritis, thyroid disease (mainly underactive thyroid that was being treated), and breast cancer. Osteoarthritis was controlled for in the analysis ($n = 83$). However, the numbers of subjects with thyroid disease ($n = 38$) or breast cancer ($n = 8$) were small, and a sensitivity analysis was carried out; that is, the analysis was carried out both with and without these women.

RESULTS

Response rate

A total of 907 women (85.2%) returned for a repeat bone scan; 477 (52.6%) did so between April and September (summer group), and 430 (47.4%) did so between October and March (winter group). Sixty-six women (6.2%) were unreachable, and a further 91 women (8.6%) did not want to take part in this part of the study. In comparing the returners with the nonreturners, we found no differences with regard to age, mean baseline BMD, dietary EI, PAL, and height. There were small, nonsignificant differences in mean weight (returners: 64.1 $\pm$ 11.0 kg; nonreturners: 65.9 $\pm$ 12.2 kg; $P = 0.060$), body mass index [(BMI; in kg/m^2) returners: 24.6 $\pm$ 4.0; nonreturners: 25.2 $\pm$ 4.2; $P = 0.080$], and mean daily alcohol intake (returners: 6.71 $\pm$ 7.9 g; nonreturners: 8.01 $\pm$ 8.3 g; $P = 0.069$).

Hormonal status and hormone replacement therapy use

Most (90%) of the women were premenopausal at the baseline visit, and none had taken HRT. At the follow-up visit, only 16% were still menstruating (6% premenopausal, 11% perimenopausal), 39% were postmenopausal (none of whom had taken HRT), and 45% had taken HRT (two-thirds of this group were still taking HRT). Only 3 women failed to answer the question about menopausal status and HRT use. Of the current HRT users, 229 women (out of 239) gave dates of usage: 9.6% of the 229 had been taking HRT for <1 y, and the rest had been taking HRT for a median of 4.4 y (mean $\pm$ SD: 4.5 $\pm$ 2.1 y). Of the 86 past HRT users (out of 98) who gave dates of HRT use, 58% had taken HRT <1 y. The other 42% had taken HRT for a median of 3 y (mean $\pm$ SD: 3.3 $\pm$ 1.6 y).

Anthropometric measurements and dietary changes

The anthropometric data collected at baseline and at the follow-up visit (**Table 1**) showed increases in mean weight and BMI and decreases in BMD. Despite the weight gain, mean EI had decreased (**Table 2**). There was also a small decrease in PAL that may in part explain the increase in weight. The BMD change was found to be dependent on menopausal status and HRT use: the greatest BMD loss occurred in postmenopausal women who were not taking HRT or had not taken HRT in the past (**Figure 1**).

Over 10% of the women ($n = 104$) claimed to be on a weight-reducing diet. There was no difference in mean weight gain between the women who were on a diet and those who were not, but those on a diet were heavier than the nondieters (71.8 $\pm$ 11.1 and 66.8 $\pm$ 11.7 kg, respectively; $P < 0.001$). Although there was no difference in daily EI at baseline (8.2 $\pm$ 2 MJ for both groups) or follow-up (7.7 $\pm$ 2.3 MJ for the dieters and 7.9 $\pm$ 2.2 for the nondieters; $P = 0.478$), the dieters ate less in terms of EI per kilogram of body weight than did the nondieters (109 $\pm$ 33 and 121 $\pm$ 38 kJ/kg, respectively; $P = 0.001$).

The ratio of EI to calculated BMR also had decreased since the baseline visit (Table 2). At the follow-up visit one woman had a high ratio of 3.92; if this subject was removed, the upper range decreased to 3.07, with no change to the mean and median. There was evidence of underreporting at both baseline and follow-up: 11.5% and 18.7% of the women at baseline had EI:BMR <1.0 and <1.1, respectively, and these proportions

TABLE 1
Characteristics of the study population at baseline and at follow-up[1]

	Baseline	Follow-up
Age (y)	47.5 ± 1.5 (44.9–53.5; 47.5)	53.9 ± 1.6 (50–59; 53.9)
Weight (kg)	64.1 ± 11.0 (37.0–124.5; 62.0)	67.4 ± 11.7 (42.0–123.0; 65.0)[2]
Height (cm)	161.4 ± 5.7 (135.9–178.8; 161.2)	160.6 ± 5.8 (133.8–178.2; 160.5)[2]
BMI (kg/m^2)	24.6 ± 4.0 (16.1–44.2; 23.8)	26.1 ± 4.4 (17.3–45.0; 25.2)[2]
Physical activity level[3]	1.88 ± 0.31 (0.90–3.14; 1.82)	1.86 ± 0.33 (1.04–3.22; 1.8)[4]
Bone mineral density (g/cm^2)		
Lumbar spine	1.064 ± 0.16 (0.633–1.958; 1.054)	0.998 ± 0.17 (0.591–2.078; 0.976)[2]
Femoral neck	0.886 ± 0.13 (0.588–1.660; 0.875)	0.833 ± 0.12 (0.571–1.818; 0.819)[2]

[1] $\bar{x}$ ± SD; range and median in parentheses. n = 891 women.
[2,4] Significantly different from baseline (Student's t test; natural log-transformed variables used if required): [2] $P < 0.001$, [4] $P = 0.025$.
[3] Ratio of overall daily energy expenditure to basal metabolic rate.

increased to 14.8% (EI:BMR <1.01) and 25.5% (EI:BMR <1.1) at follow-up. Eleven women at baseline and 5 women at follow-up had intakes <4 MJ. Overall, the mean EI:BMR was similar to that in other studies involving dietary assessment throughout Europe (31)—eg, 1.28 for women in the Scottish Heart Health Study (21) and 1.43 for the European Prospective Investigation of Europe (32)—but less than the ratio of 1.53 suggested for satisfactory completion of an FFQ (33). However, excluding underreporters would excessively bias the data. Therefore, the initial analysis included all women; the analysis was then repeated without the top and bottom 10% of EI:BMR.

There were small but significant differences in mean nutrient intake between the 2 visits (Table 2) that may reflect errors inherent in the dietary method rather than a real (albeit limited) dietary change. The proportion of women whose quartile classification had changed by >1 quartile was <15% for most

TABLE 2
Nutrient intakes at baseline and at follow-up[1]

Nutrient	Baseline	Follow-up	United Kingdom dietary reference values[2]
Energy (MJ)	8.2 ± 2.3 (3.2–16.8; 7.9)	7.9 ± 2.2 (3.5–21.0; 7.5)[3]	8.0
EI:BMR[4]	1.44 ± 0.40 (0.61–3.06; 1.38)	1.36 ± 0.39 (0.60–3.92; 1.29)[3]	NA[5]
Carbohydrate (g)	246 ± 72 (75–534; 239)	241 ± 71 (76–604; 233)[3]	235
Fat (g)	74.3 ± 28.7 (18.5–196.6; 70.8)	69.3 ± 26.8 (22.4–248.7; 63.8)[3]	71.4
Fiber (g)	16.3 ± 5.7 (4.9–40.6; 15.7)	16.1 ± 5.6 (4.3–43.4; 15.4)	18.0
Protein (g)	81.4 ± 22.5 (19.8–230.6; 78.3)	79.4 ± 21.4 (28.2–217.0; 76.4)[6]	46.5
Calcium			
(mg)	1055 ± 330 (174–2511; 1007)	1032 ± 315 (283–2514; 985)	700
Total calcium (mg)[7]	1070 ± 344 (174–2511; 1016)	1061 ± 333 (283–2514; 1001)	—
Magnesium (mg)	315 ± 84 (109–606; 306)	307 ± 82 (109–606; 306)[8]	270
Iron (mg)	12.7 ± 4.1 (2.3–34.6; 12.1)	12.2 ± (3.7–30.4; 11.5)[3]	8.7
Zinc (mg)	10.1 ± 2.9 (2.4–23.7; 9.7)	9.5 ± 2.6 (3.2–22.0; 9.2)[3]	7.0
Potassium (mg)	3358 ± 787 (1475–6549; 3281)	3329 ± 789 (1501–7941; 3256)	3500
Sodium (mg)	2689 ± 872 (560–7594; 2568)	2590 ± 842 (862–7159; 2440)[3]	1600
Phosphorous (mg)	1484 ± 399 (548–3391; 1435)	1455 ± 388 (544–3533; 1407)[9]	550
Vitamin C			
(mg)	119 ± 64 (17.7–441; 107)	121 ± 59 (13–484; 114)[10]	40
Total vitamin C (mg)[7]	131 ± 118 (17.7–2166; 112)	145 ± 119 (13–1183; 122)[3]	—
Vitamin D			
(μg)	3.9 ± 2.5 (0.2–34.1; 3.4)	4.1 ± 2.4 (0.2–18.9; 3.5)[8]	0/10[11]
Total vitamin D (μg)[7]	4.5 ± 3.1 (0.2–34.1; 3.7)	5.5 ± 3.8 (0.2–26.5; 4.3)[3]	—
Vitamin E			
(mg)	6.5 ± 2.2 (2.3–18.0, 6.3)	6.6 ± 2.2 (1.6–16.5; 6.3)	NA
Total vitamin E (mg)[7]	8.3 ± 10.1 (2.3–180.5; 6.5)	13.3 ± 32.0 (1.3–314; 7.0)[3]	—
Retinol			
(μg)	820 ± 602 (39–4354; 588)	665 ± 513 (70–5237; 480)[3]	600
Total retinol (μg)[7]	924 ± 666 (85–4354; 702)	882 ± 654 (70–5237; 627)	—
Folic acid (μg)	293 ± 87 (76–659; 281)	294 ± 89 (93–945; 284)	200

[1] $\bar{x}$ ± SD; range and median in parentheses. n = 891 women.
[2] Reference nutrient intakes were used for most nutrients; for energy, estimated average intake was used; and for carbohydrate and fat, population averages based on percentage daily total energy intake were used.
[3,6,8–10] Significantly different from baseline (Student's t test of natural log-transformed variables): [3] $P < 0.001$, [6] $P = 0.011$, [8] $P = 0.006$, [9] $P = 0.030$, [10] $P = 0.024$.
[4] Ratio of energy intake to calculated basal metabolic rate.
[5] Not available.
[7] Including supplement use.
[11] Normal lifestyle/confined indoors.

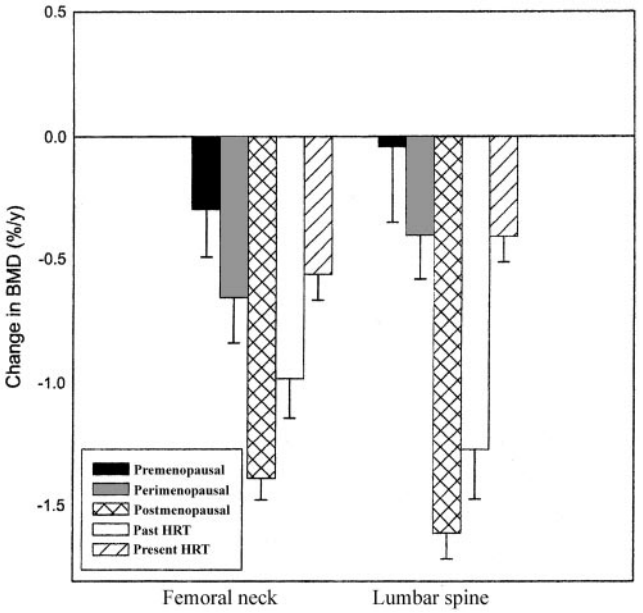

FIGURE 1. Mean (± 2 SEMs) annual percentage change in bone mineral density (BMD) according to mutually exclusive menopausal status and hormone replacement therapy (HRT) use at follow-up. Premenopausal (never used HRT), $n = 50$; perimenopausal (never used HRT), $n = 96$; postmenopausal (never used HRT), $n = 346$; past HRT use (previously used HRT), $n = 111$; present HRT use (taking HRT at time of visit), $n = 285$. Premenopausal and perimenopausal women and present HRT users significantly different from postmenopausal women and past HRT users, $P < 0.001$ (ANOVA with Scheffe comparison); postmenopausal women significantly different from past HRT users, $P < 0.001$ (ANOVA with Scheffe comparison).

nutrients; for iron, vitamin C, and vitamin D, however, the proportion was 18%.

Associations between nutrients and BMD or BMD change

When we examined the data from all of the women, there was no evidence of an association between nutrient intake (crude or energy-adjusted) and BMD. However, energy-adjusted calcium intake from diet alone was positively correlated with FN BMD change ($r = 0.066$, $P < 0.05$; **Table 3**). This remained significant after adjustment for age, height, weight, annual percentage weight change, PAL, annual percentage change in physical activity since the baseline visit, smoking, menopausal status, and HRT use ($r = 0.088$, $P < 0.05$). There was no evidence of an association between energy-adjusted calcium intake and LS BMD change ($P = 0.62$).

Total fat and saturated fatty acid intakes did not appear to be associated with either BMD or BMD change. However, polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) were negatively correlated with FN BMD change, and this negative correlation was significant after adjustment for confounders (Table 3). When the women were divided according to tertiles of calcium intake, the relation between PUFA intake and BMD change was significant only in those in the lowest third of calcium intake (after adjustment for confounders: $r = -0.213$, $P < 0.001$ for the lowest third of calcium from diet only, and $r = -0.201$, $P = 0.001$ for the lowest third of total calcium intake).

Vitamin E, retinol, and vitamin A intakes were also negatively correlated with FN BMD change (Table 3). Only vitamin E intake from diet alone was negatively correlated with LS BMD change. Vitamin E intake was highly correlated with PUFA (Pearson's correlation for log-transformed variables: $r = 0.822$, $P < 0.001$), but the relation was less strong between PUFAs and vitamin A intake ($r = 0.541$, $P < 0.001$). As might be inferred, vitamin E and vitamin A were also correlated with each other ($r = 0.560$, $P < 0.001$). When supplements were added to dietary vitamin E and vitamin A intakes, no relation was observed between these nutrients and BMD change. However, the addition of vitamin E supplements highly skewed the data on total vitamin E intake.

Alcohol intake data were positively skewed because 22% of women at baseline and 27% of women at follow-up consumed no alcohol at all. We used the mean of the 2 intakes and standardized it to 8 MJ of energy (the estimated average requirement for women aged 30–59 y), rather than using the residual method for energy adjustment, because the latter method depends on adhering to the normal rules of regression (27), and, clearly, alcohol is a highly skewed variable. Women in the lowest quarter of energy-standardized alcohol intake had greater bone loss at the LS than did those in the top quarter (**Figure 2**). This remained significant after adjustment for confounding variables. A similar trend was seen at the FN, although it was not significant ($P = 0.092$).

Regression analysis

The effect of each nutrient on BMD change was considered separately. For calcium, energy-adjusted calcium intake was found to be a significant predictor of FN BMD change, with or without dietary supplements (**Table 4**). Sensitivity analysis, carried out by excluding first women with breast cancer and then those with thyroid disease, did not affect the outcome. Multiple linear regression analysis was also repeated by leaving out the top and bottom 10% of EI:BMR (which may contain underreporters and overreporters, respectively), and calcium was still found to be a significant predictor of FN BMD change. When we examined the effect of calcium at different intakes of PUFAs, calcium intake was found to be a significant predictor of FN BMD change only at the highest third of PUFA intake, where it accounted for 2–4% of the total variation in FN BMD change (coefficient: 7.79×10^{-4}; 95% CI: 2.98×10^{-4}, 12.59×10^{-4}). There was a significant interaction between calcium intake and PUFAs with regard to FN BMD change ($P = 0.016$).

A number of other energy-adjusted nutrients had a negative effect on FN BMD change (Table 4). Fatty acids such as PUFAs and MUFAs and dietary vitamin E, retinol, and vitamin A (from diet only) were weak but significant predictors of FN BMD change, accounting for 0.3%–0.9% variation with coefficients for standardized PUFA, MUFA, vitamin E, retinol, and vitamin A intakes of -0.042, -0.019, -0.060, -1.73×10^{-4}, and -1.24×10^{-4}, respectively. PUFAs accounted for 3.3% of the variation in FN BMD loss in women in the lowest third of calcium intake (compared with 0.9% of the variation in the full group): the coefficient was -0.070 (95% CI: -0.108, -0.031).

Including the nutrient intake from dietary supplements, the relation between FN BMD change and retinol, vitamin A, or vitamin E was no longer significant. However, because vitamin E was particularly skewed as a result of adding the nutrient intake from supplements, the relation between BMD change and quartile of nutrient intake was examined. Although the

TABLE 3
Pearson's correlation coefficients between change in femoral neck bone mineral density (FN BMD) and change in lumbar spine BMD (LS BMD) with fat and fatty acids, vitamin E, and vitamin A[1]

	Change in FN BMD		Change in LS BMD	
Energy-adjusted nutrient	Unadjusted	Adjusted[2]	Unadjusted	Adjusted[2]
Calcium	%/y		%/y	
(diet only; mg)	0.066[3]	0.088[4]	0.015	0.042
Total calcium (mg)	0.063	0.075[3]	0.010	0.034
MUFA (g)	−0.066[3]	−0.069[3]	−0.005	−0.022
PUFA (g)	−0.088[4]	−0.105[4]	−0.002	−0.019
SFA (g)	0.017	0.037	0.058	0.044
Total fat (g)	−0.037	−0.029	0.004	0.001
Vitamin E				
(diet only; mg)	−0.101[4]	−0.110[4]	−0.084[4]	−0.100[4]
Total vitamin E (mg)[5]	0.026	0.019	0.031	0.034
Retinol				
(diet only; μg)	−0.072[3]	−0.067[3]	−0.021	−0.036
Total retinol (μg)[5]	−0.071[3]	−0.032	−0.019	−0.004
Vitamin A[6]				
(diet only; μg)	−0.090[4]	−0.090[4]	−0.041	−0.061[7]
Total vitamin A (μg)[5]	−0.004	−0.012	−0.029	−0.032
Vitamin A[8]				
(diet only; μg)	−0.087[4]	−0.084[3]	−0.034	−0.054
Total vitamin A (μg)[5]	−0.002	−0.007	−0.026	−0.026
β-carotene equivalents (μg)	−0.047	−0.060[7]	−0.046	−0.062[7]

[1] MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
[2] Adjusted for age, weight, annual percentage change in weight, height, smoking status, socioeconomic status, physical activity level, baseline BMD measurement at appropriate site, menopausal status, and hormone replacement therapy use.
[3] $P < 0.05$.
[4] $P < 0.01$.
[5] Including supplement use.
[6] Vitamin A calculated as retinol intake plus one-sixth of the β-carotene equivalents.
[7] $P < 0.08$.
[8] Vitamin A calculated as retinol intake plus one-twelfth of the β-carotene equivalents.

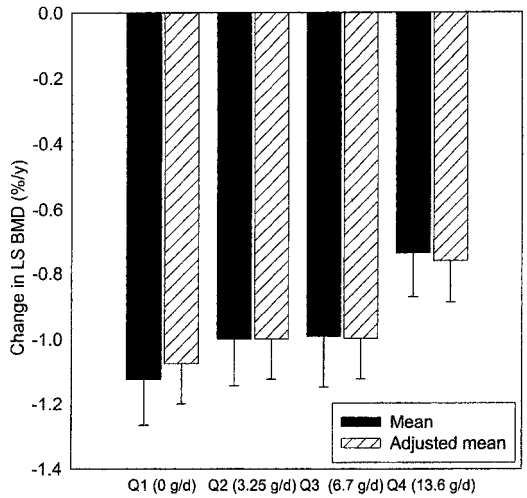

FIGURE 2. Mean (± 2 SEMs) annual percentage change in lumbar spine bone mineral density (LS BMD) by quartile (Q) of alcohol intake standardized to 8 MJ total energy intake, before and after adjustment for confounders, in all women ($n = 891$). Median alcohol intake per quartile is given in parentheses. Annual percentage change in LS BMD before adjustment significantly different, $P = 0.002$ (ANOVA). Q4 significantly different from Q1, $P = 0.001$ (Scheffe comparison). The differences remained significant after adjustment for age, weight, annual percentage change in weight, height, smoking status, socioeconomic status, physical activity level, baseline BMD measurement at appropriate site, menopausal status, and hormone replacement therapy (HRT) use, $P = 0.003$ (ANCOVA).

relation between total vitamin E and BMD was significant at both FN and LS sites [$P < 0.01$ (analysis of variance), $P < 0.04$ (analysis of covariance)], quartile 4 was no longer significantly different from quartile 1 for total vitamin E intake by Scheffe post test. However, there was a significant difference between quartiles 3 and 1 (**Figure 3**). For all of the women, alcohol intake (in energy-standardized quartiles) was the only nutrient intake found to be a significant predictor for LS BMD change, and it was associated with less bone loss (**Table 5**).

Correlation between nutrient intake and BMD or BMD change in premenopausal and perimenopausal women

A subset of women ($n = 146$) who were still menstruating and had never taken HRT were examined separately, because the confounding factors of HRT use and hormonal upheavals associated with the menopause should not affect this group. Significant positive associations were found between FN BMD change and energy-adjusted intakes of calcium, phosphorus, potassium, magnesium, folate, and vitamin C, and negative associations were found between FN BMD change and total fat, PUFAs, and MUFAs (**Table 6**). Linear regression analysis showed that the association with dietary calcium and total calcium accounted for 5.0% and 3.4%, respectively, of the total variation in FN BMD change and that the association with dietary vitamin C and total vitamin C accounted for 3.7% and 2.5%, respectively, of the variation in FN BMD change. In

TABLE 4
Results of multiple regression analyses to identify the effect of calcium intake and other nutrients on change in femoral neck bone mineral density (FN BMD)[1]

Independent factors	Variation explained	Coefficient (95% CI)	*P*
	%		
Intercept		−1.100 (−3.656, 1.455)	0.398
ln Baseline FN BMD	2.2	−0.720 (−1.144, −0.296)	0.001
Age	1.4	−0.029 (−0.057, 0.016)	0.262
Annual percentage weight change	0.9	0.0737 (0.029, 0.119)	0.001
Height	0.4	0.0132 (0.003, 0.023)	0.009
HRT use (none, past, present)	6.2	0.388 (0.320, 0.455)	<0.001
Menopausal status (premenopausal, perimenopausal, and postmenopausal or HRT user)	9.2	−0.595 (−0.710, −0.479)	<0.001
Dietary variables added separately to the regression model			
Energy-adjusted calcium intake (mg × 10^{-4})			
Dietary calcium intake[2]	0.6	3.51 (0.85, 6.17)	0.010
Total calcium intake[2]	0.5	2.69 (0.31, 5.07)	0.027
PUFA (g)[2]	0.9	−0.042 (−0.067, −0.017)	0.001
MUFA (g)[2]	0.4	−0.019 (−0.034, −0.003)	0.017
Vitamin E (diet only) (mg)[2]	0.4	−0.060 (−0.110, −0.010)	0.018
Retinol (diet only) (mg × 10^{-4})[2]	0.4	−1.73 (−3.20, −0.30)	0.018
Vitamin A (diet only) (mg × 10^{-4})[2]	0.3	−1.24 (−2.47, 0.17)	0.047

[1] Adjusted for independent prognostic factors, from age, weight, height, smoking status, weight change/y, socioeconomic status, presence of osteoarthritis, consuming a weight-reducing diet, baseline FN BMD, physical activity level (PAL), PAL change/y, menopausal status, and hormone replacement therapy (HRT) use. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

[2] Standardized to 8 MJ energy intake.

addition, for this subgroup, but not for the rest of the study population, there were significant correlations (after adjustment for confounders) between follow-up FN BMD, calcium, potassium, magnesium, and vitamin C (Table 6). Women taking vitamin C supplements ($n = 8$ for both visits) did not have significantly greater BMD or less BMD loss than did women who did not use vitamin C supplements. Calcium intake was also associated with greater LS BMD after adjustment for confounders, and the relation with LS BMD tended toward significance for phosphorous ($P < 0.08$) and magnesium ($P < 0.10$).

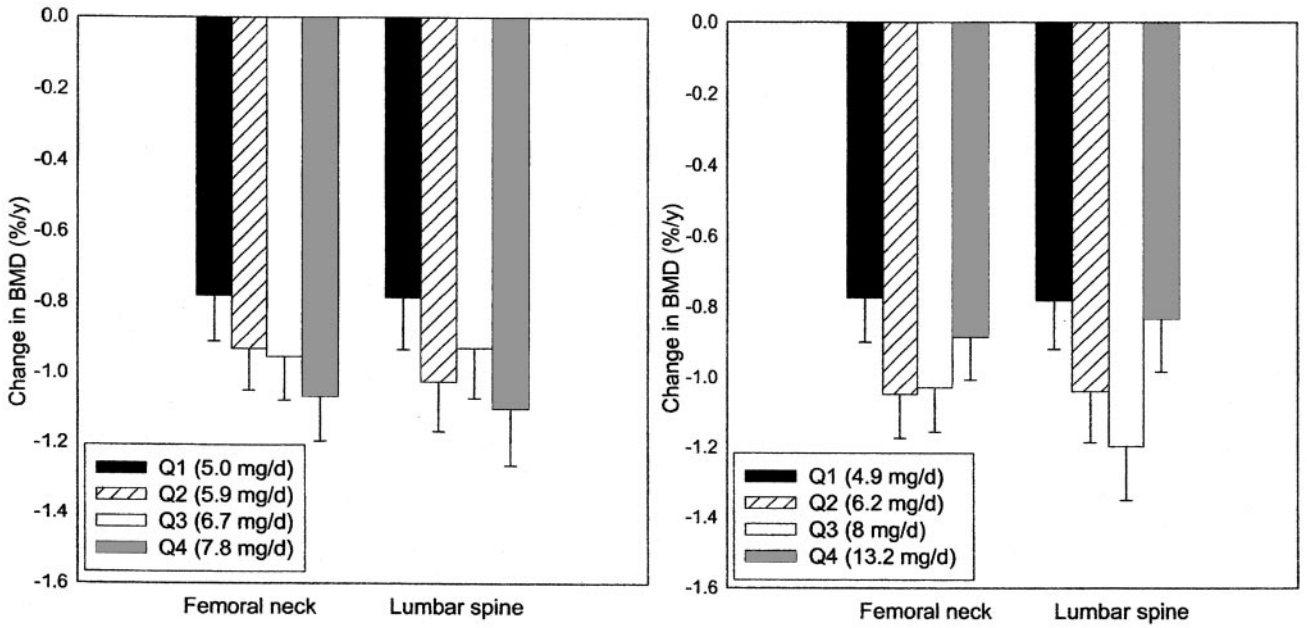

FIGURE 3. Mean (± 2 SEMs) annual percentage change in bone mineral density (BMD) by quartile (Q) of energy-adjusted dietary vitamin E intake (left) and by quartile of energy-adjusted total vitamin E intake (diet and vitamin supplements; right) for all women ($n = 891$). Median vitamin E intake per quartile is given in parentheses. Q1 of energy-adjusted dietary vitamin E intake significantly different from Q4: overall, $P < 0.05$ (Scheffe comparison); femoral neck, $P = 0.015$ (ANOVA); lumbar spine, $P = 0.019$ (ANOVA). Q1 of energy-adjusted total vitamin E intake significantly different from Q3: overall, $P < 0.01$ (Scheffe comparison); femoral neck, $P = 0.005$ (ANOVA); lumbar spine, $P = 0.001$ (ANOVA). The differences remained significant after adjustment for age, weight, annual percentage change in weight, height, smoking status, socioeconomic status, physical activity level, baseline BMD measurement at appropriate site, menopausal status, and hormone replacement therapy (HRT) use. For dietary vitamin E only: femoral neck, $P = 0.024$ (ANCOVA); lumbar spine, $P = 0.036$ (ANCOVA). For total vitamin E (diet and supplement): femoral neck, $P = 0.031$ (ANCOVA); lumbar spine, $P = 0.001$ (ANCOVA). Adjusted mean values (not shown) were similar to the unadjusted means.

TABLE 5
Results of multiple regression analyses to identify the effect of alcohol intake on change in lumbar spine bone mineral density (LS BMD)[1]

Independent factors	Variation explained	Coefficient (95% CI)	*P*
	%		
Intercept		−3.137 (−5.706, −0.569)	0.017
Weight	1.3	1.034 (0.661, 1.408)	<0.001
Age	1.3	0.0007 (−0.047, 0.034)	0.738
HRT use (none, past, present)	11.6	0.594 (0.521, 0.667)	<0.001
Menopausal status (premenopausal, perimenopausal, and postmenopausal or HRT user)	15.4	−0.888 (−1.016, −0.761)	<0.001
Alcohol intake (quartiles)[2]	0.7	0.0893 (0.034, 0.145)	0.002

[1] Adjusted for independent prognostic factors, from age, weight, height, smoking status, weight change/y, socioeconomic status, presence of osteoarthritis, consuming a weight-reducing diet, baseline LS BMD, physical activity level (PAL), PAL change/y, menopausal status, and hormone replacement therapy (HRT) use.

[2] Standardized to 8 MJ energy intake.

DISCUSSION

Calcium

Calcium was found to be a significant predictor of FN BMD change. In a calcium supplementation trial, calcium influenced LS bone loss in late menopausal women but not in early menopausal women (34). However, calcium appeared to slow cortical bone loss in early postmenopausal women (35). In agreement with results from another study in the United Kingdom (36), we did not find an association with calcium intake and absolute BMD. The calcium intake in our study was relatively high compared with that in other studies (mean: >1000 mg/d). It is uncertain whether this is a phenomenon in women in northeastern Scotland in general, or whether the women in the osteoporosis screening program became more aware of their diet in relation to bone health.

Fat intake and influence on calcium absorption

Both MUFAs and PUFAs were negatively associated with FN BMD change. Their intakes may reflect a dietary pattern low in important nutrients (37) or they may directly influence bone health. In support of our findings, other studies have found negative associations between fat intake and BMD (38, 39). Dietary fat was also associated with increased fracture risk (40), for which the authors suggested a number of possible mechanisms: eg, hyperinsulinemia induced by high-fat or high-sucrose diets may lead to a negative calcium-magnesium balance, or a high-lipid diet may reduce the efficiency of calcium absorption through the formation of calcium soaps or may contain high concentrations of retinol, which would increase bone resorption. In healthy individuals with a normal diet, fat is assumed to have no effect on calcium absorption (41, 42),

TABLE 6
Pearson's correlation coefficients between energy-adjusted nutrients and follow-up bone mineral density (BMD) and change in BMD in premenopausal and perimenopausal women who had never taken hormone replacement therapy (HRT)[1]

	FN BMD		FN BMD change		LS BMD	
	Unadjusted	Adjusted[2]	Unadjusted	Adjusted[3]	Unadjusted	Adjusted[2]
Calcium						
Diet only (mg)	0.163[4]	0.172[4]	0.249[5]	0.229[5]	0.188[4]	0.228[5]
Total calcium (mg)	0.136	0.164[6]	0.215[5]	0.203[4]	0.160[6]	0.211[4]
Phosphorous (mg)	0.141	0.160[6]	0.268[5]	0.244[5]	0.116	0.156[6]
Potassium (mg)	0.139	0.182[4]	0.193[4]	0.160[6]	0.074	0.123
Magnesium (mg)	0.127	0.167[4]	0.239[5]	0.199[4]	0.071	0.143
Zinc (mg)	0.097	0.081	0.085	0.057	0.065	0.061
Folate (mg)	0.123	0.095	0.183[4]	0.131	0.090	0.069
Vitamin C (mg)	0.120	0.195[4]	0.200[5]	0.199[4]	0.040	0.104
MUFA (g)	−0.043	−0.091	−0.232[5]	−0.213[4]	−0.023	−0.093
PUFA (g)	−0.044	−0.071	−0.193[4]	−0.203[4]	−0.058	−0.098
SFA (g)	0.016	0.011	−0.119	−0.075	0.068	0.053
Total fat (g)	−0.087	−0.124	−0.216[5]	−0.182[4]	−0.037	−0.088

[1] $n = 146$. FN, femoral neck; LS, lumbar spine; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. There was no significant relation between any nutrient and LS BMD change.

[2] Adjusted for age, weight, annual percentage change in weight, height, smoking status, physical activity level, and socioeconomic status.

[3] Adjusted for age, weight, annual percentage change in weight, height, smoking status, physical activity level, socioeconomic status, and baseline FN BMD.

[4] $P < 0.05$.

[5] $P < 0.01$.

[6] $P < 0.08$.

although the type of fat (43), chain length, degree of saturation (44), and position on the triacylglycerol molecule (45) all have an influence on calcium absorption through soap formation. It is possible that the degree of oxidation of unsaturated fatty acids may also be important. There is evidence dating from the early 1900s (46) that fats interfere with calcium absorption by forming calcium soaps, but later studies gave conflicting results (47, 48). Although discrepancies regarding the effect of fats on calcium absorption have been recognized, there was general agreement on the converse effect, that excess calcium reduces fat absorption (49). Later reports (50, 51) again suggested that calcium intake reduces fatty acid absorption, but the corollary that fat intake reduces calcium absorption has largely been ignored. There is interest in the recent discovery that leptin is a regulator of bone formation independent of its influence on body weight (52), but whether dietary fatty acids play any role in this scenario is not known. We did not observe a detrimental effect for saturated fatty acids, which may in part be explained by the fact that milk and milk products, which are sources of saturated fatty acids, are beneficial for bone health. In contrast to our work, dietary fat was positively associated (and dietary fiber negatively associated) with fractional calcium absorption (53). However, compared with the results in our study, mean dietary fat intake was much lower in the study by Wolf et al (53)—4l.4 and 67.3 g/d, respectively—as was EI—5.6 and 8.1 MJ/d, respectively.

Vitamin E, vitamin A, and retinol

Dietary vitamin E appeared to be a negative predictor of FN BMD change. It is possible that vitamin E may antagonize the action of vitamin K because increased clotting time is observed in patients who have had myocardial infarction and are being treated with 300 mg vitamin E/d (54). However, as that amount far exceeds normal intakes of vitamin E, a more likely explanation is that vitamin E is highly correlated with PUFAs and may simply be a surrogate marker for them. The correlation between PUFAs and vitamin A was less marked than that between PUFAs and vitamin E. There is evidence to suggest that vitamin A increases fracture risk (55, 56) and is associated with reduced BMD (57), although no effect was seen on radial BMD (58). Our own data show that, although dietary vitamin A (or retinol) appeared to worsen bone loss, there was no relation between BMD loss and either retinol or vitamin A intake when the vitamin A from supplements was added. Most of the supplement used was in the form of cod liver oil, and because this oil also contains vitamin D and long-chain n−3 fatty acids, it may be a confounder. A detrimental effect of vitamin A on bone loss cannot be ruled out, but, as with vitamin E and PUFAs, we believe that covariance between nutrients may be occurring, and further research is required to fully elucidate the role of these nutrients.

Alcohol

A positive association between modest alcohol intake and BMD has been found in postmenopausal women (59), elderly women (60), and our own baseline work (2, 3). Possible explanations are that alcohol stimulates the adrenal production of androstenedione and its conversion to estrone (61) or that alcohol may stimulate the secretion of calcitonin (62); both effects would favor an increase in bone mass. Alcoholic drinks may be a source of estrogenic substances (63), although no association between alcohol intake and serum estrone or estradiol concentrations was found in perimenopausal women (64). Wine contains antioxidants, and there is speculation that boron, of which red wine is a rich source, may play a role in bone metabolism (65). There may be some underreporting of alcohol intake. In addition, the FFQ gives a snapshot of alcohol intake within a specific time frame. By combining the results of the baseline and follow-up visits, however, it is likely that spurious results would be minimized. No other nutrient was found to reduce LS bone loss.

Fruit and vegetable nutrients

In the subgroup of women who were still menstruating and were not affected by the consequences of estrogen withdrawal, a number of nutrients related to fruit and vegetable intakes were associated with greater BMD and reduced bone loss. Vitamin C is important for collagen hydroxylation. Positive associations have been reported both between dietary vitamin C and BMD (2, 5) and between the use of vitamin C supplements and greater BMD (66, 67). Only 4% of the women in our study reported vitamin C supplement use at both visits, so perhaps it is not surprising that we did not observe any difference in BMD between supplement users and nonusers. In our own baseline studies (2, 3) and in the Framingham Study cohort (4), magnesium and potassium were associated with greater BMD. Low serum magnesium concentrations were found in women with osteoporosis (68), and the iliac crest trabecular bone of osteoporotic women contained less magnesium than was found in healthy subjects (69). The beneficial effect of potassium on BMD and the link with fruit and vegetable intakes are consistent with the theory that a diet rich in alkaline salts protects bone by balancing the acidic metabolites produced from dietary protein so that the need for release of alkaline salts from the bone is eliminated (17, 70). Potassium salts have been shown to improve calcium balance (71) and reduce bone turnover (72). However, potassium and vitamin C could simply be markers for fruit and vegetable intakes, and it may be other components of fruit and vegetables that are responsible for helping to protect bone, such as vitamin K, which is associated with decreased fracture risk as a result of undercarboxylation of osteocalcin (73).

Conclusions

The results of this study suggest that, although menopausal status and HRT use are the overriding factors affecting bone loss in women in their early fifties, dietary calcium may help reduce bone loss at the hip, and modest intakes of alcohol (equivalent to 1–2 glasses of wine/d) may help reduce bone loss at the spine. However, intakes of MUFAs and PUFAs (and possibly vitamin A) appear to worsen bone loss, and the detrimental effect of PUFAs is more pronounced at lower calcium intakes. This finding supports a link between PUFAs and reduced calcium absorption in which a possible mechanism is the formation of calcium–fatty acid soaps. For women who are still menstruating, nutrients associated with fruit and vegetable intakes appear to be protective, possibly because of their beneficial effect on acid-base balance or because they are sources of nutrients that are important for bone health.

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HMM carried out the study and was responsible for the data analysis and writing the manuscript; SAN was involved in the design of the dietary study and review of the manuscript; MHN gave nutritional advice throughout the study and suggested the hypothesis that fatty acids may be a reason for the difference in calcium requirements between different populations; MKC gave statistical advice and reviewed the manuscript; and DMR was responsible for the study design of Aberdeen Prospective Osteoporosis Screening Study and also critically reviewed the manuscript. None of the authors had financial or commercial interest in any company or organization sponsoring the research.

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