Effects of dietary calcium intake on body weight and prevalence of osteoporosis in early postmenopausal women\textsuperscript{1,2}

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ABSTRACT
Background: High calcium intakes seem to be ineffective at reducing bone loss in early postmenopausal women. However, the inverse relation between calcium intake and body weight can attenuate the negative effect of a low dietary calcium intake.

Objective: The objective was to assess the role of dietary calcium and body mass index (BMI) on osteoporosis, defined according to World Health Organization criteria as a lumbar bone density \(>2.5\) SD below the T score.

Design: This was a cross-sectional, retrospective, observational study conducted in 1771 healthy, early postmenopausal women, who were not taking calcium supplements at the first densitometric evaluation. Weekly frequency of dairy food consumption was used to estimate the relative intake of dietary calcium. Total dairy intake was classified into 4 categories by quartile cutoffs. Multiple logistic regression analyses were used to study this sample.

Results: BMI and prevalence of overweight showed significant inverse trends with increasing dairy intake. Calcium intake was not associated with osteoporosis when overweight was not considered. However, when overweight was considered in the analysis, women with the lowest calcium intake were more likely to have osteoporosis (odds ratio: 1.46; 95\% CI: 1.12, 1.89; \(P = 0.008\)) than were women with the highest calcium intake.

Conclusions: In early postmenopausal women, a low dietary calcium intake may increase the risk of osteoporosis, but its negative effect can be offset by the greater BMI found in women with a low calcium intake. Am J Clin Nutr 2007;86:639–44.

KEY WORDS Body weight, body mass index, dairy intake, early menopause, osteoporosis, women

INTRODUCTION
A predominant contributing factor to fracture risk is the substantial decline in bone mineral density (BMD) that occurs with age. Many investigations have examined the influence of calcium intake on rates of change in BMD. The positive effect of calcium in maximizing peak bone mass during childhood and adolescence (1, 2), in women during early adult life (3), in preventing vertebral bone loss in premenopausal women (4), and in the late postmenopausal period (5, 6) seems to be well documented, whereas calcium does not appear to prevent bone loss in the first years after menopause (7, 8). Many variables could have influenced the results of studies in early postmenopausal women, such as the time span of the observation, basal daily calcium intakes, the source of calcium (dairy products instead of supplements and the type of calcium salts), and the site of BMD measurement; trabecular sites are generally less responsive than are cortical sites; however, the general view is that the rapid phase of bone loss in early menopause is due to a loss in the direct action of estrogens on bone cells and that this phase is not responsive to higher calcium intakes.

Besides its role as a main component of the inorganic phase of bone, calcium plays a role as a second messenger in cell signaling processes and as an activator of intracellular functional proteins involved in a wide range of cellular activities. In recent years, several clinical and epidemiologic studies have reported a consistent inverse association between calcium intake and body weight (9–13). A possible physiologic mechanism explaining this relation was recently proposed by Zemel et al (14, 15), who showed that an increase in intracellular calcium concentrations in human adipocytes after stimulation with parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D [1,25(OH)\(_2\)D] is able to switch lipid metabolism from lipolysis to lipogenesis, which results in an increase in triacylglycerol storage. Consistently, 1,25(OH)\(_2\)D, and PTH have been found to be positively associated with body mass index (BMI) (16–18). Because serum PTH and 1,25(OH)\(_2\)D concentrations are regulated by calcium intake, this metabolic pathway would be responsible for the higher risk of overweight and obesity in subjects with a low calcium intake and for the weight loss after increases in dietary calcium intake. Because body weight affects both bone turnover and BMD through several mechanisms (19), the purpose of this study was to assess the role of dietary calcium by its effect on body weight in influencing BMD values in early postmenopausal women.

SUBJECTS AND METHODS
Study population and instruments
From March 1998 to February 2004, we recruited 1771 subjects from women referred to the open-access bone densitometry service of our hospital for their first densitometry evaluation. These women were all within 5 y of spontaneous menopause, as defined by the last episode of menstrual bleeding. For women
who reported amenorrhea for <12 mo, menopausal status was confirmed by testing for elevated concentrations of follicle-stimulating hormone (>50 mIU/mL). Women with early menopause (before the age of 45 y) were excluded because this condition has been shown to be associated with an increased rate of bone loss (20). Subjects were interviewed by a consultant using a structured questionnaire, and data were collected directly by using MEDLOG software (IAC, Mountain View, CA) tailored for medical data management. The questionnaire covered the following areas: socioeconomic status, reproductive variables, medical and drug use history, and lifestyle habits, including smoking, consumption of alcohol and coffee, and physical activity (21). Smoking was categorized as a dichotomous variable: nonsmokers (never smokers and ex-smokers, such as responders who stopped smoking ≥1 y before the study) and current smokers. Spare-time physical activity was assessed by inquiring about the reported number of 20-min sessions of leisure-time physical activity per week, and physically active behavior was defined as participation in >2 sessions/wk. Occupational physical activity was assessed for employed women and was classified as light (office clerks and other sedentary jobs) or heavy (manual work). Fracture incidence after menopause was investigated and confirmed, where possible, by medical record. All fracture sites were considered, but fractures after severe trauma (a fall from a height or a staircase and traffic accidents) were excluded. Altogether, 88% of self-reported fractures were confirmed by medical record.

All subjects completed a weekly food-frequency questionnaire, which was used in previous studies (22), to estimate calcium intake. On the basis of tables of nutrient values issued by the Italian National Institute of Nutrition (23), calcium intakes from some selected calcium-rich foods (milk and dairy products) were assessed with a 7-d food-frequency questionnaire. The foods checked represent the major sources of daily calcium intake in the Italian diet (24), including milk, aged cheese, soft cheese, cottage cheese, and yogurt. Portion sizes were quantified by using means of household measures (slices, cups, and glasses). To standardize the slice weight, 3 cardboard samples of different size were used (≈100, 50, and 25 g). The number of standardized servings was assessed, each containing ≈300 mg Ca (a glass of milk, a cup of yogurt, ≈100 g cottage cheese, a 50-g slice of soft cheese, and a 25-g slice of aged cheese). Women were categorized according to quartiles of weekly servings (≤7, 8–11, 12–15, and ≥16). Before the hypothesis testing began, we excluded 191 women who had diseases or who were taking drugs able to influence body weight or known to affect calcium metabolism, including women reporting a past or current use of calcium and vitamin D supplements in any form and women who were taking or had taken hormone replacement therapy. Subjects who reported a transmenopausal change in body weight or who reported consuming diets that induced a weight change >10% of the actual weight were excluded as well. The study was approved by the local Ethics Committee, and informed consent was obtained from all subjects.

Measures

Study personnel assessed height and body weight using previously calibrated conventional stadiometers. Body weight was measured to the nearest 0.1 kg with a calibrated balance beam scale. Height was measured with a vertical ruler to the nearest 0.5 cm. Subjects were measured while wearing light clothes and no shoes. BMI was calculated as weight (kg)/height² (m), and overweight was defined as a BMI ≥ 25 on the basis of international obesity classification (25). BMD measurements were carried out at the lumbar level (L2–L4) by dual-energy X-ray absorptiometry (Hologic QDR 4500, Waltham, MA). The CV in our laboratory is 0.5% in vitro and 1.0% in vivo. Osteopenia and osteoporosis were defined according to World Health Organization criteria (26) as a BMD below 0.909 and 0.759 g/cm², respectively, such as >1 SD and >2.5 SD below the T score. The methodology used to identify this value was described elsewhere (27).

Statistical analysis

After testing for normality of the distribution (Shapiro-Wilks test), the baseline variables were compared between quartile groups by one-factor ANOVA and by chi-square test for linear trend when dealing with continuous variables or categorical variables, respectively. To assess the role of recorded variables on the risk of being overweight (yes versus no), a stepwise multiple logistic regression analysis was performed. All the variables that were statistically significant in a univariate analysis, together with some basic variables such as age, age at menopause, and smoking, were considered. In a multivariable analysis, a generalized linear model was used to assess the predictors of BMD. To identify the factors associated with the probability of having osteoporosis, further stepwise multiple logistic regression analyses were performed. In the first model, all variables found to be significantly associated with osteoporosis in a univariate analysis as well as age, age at menopause, smoking, but not the overweight variable, were included; the second model included all of the variables included in the first model with the addition of the overweight variable.

In both models, the outcome variable was an ordinal multilevel response (osteoporosis, osteopenia, and normal) and an ordered logistic regression using the proportional odds model was then applied. All of the statistical tests were 2-sided at the 5% level and were performed by using SAS software (release 8.2; SAS Institute Inc, Cary, NC)

RESULTS

The demographic and clinical findings of the study population, by dairy intake quartiles, are shown in Table 1. No differences in mean age, age at menarche, and menopause were observed between the quartiles. Significant differences were observed when mean BMIs between the dairy intake quartiles were compared; significant decreases from the lowest to the highest quartile and the prevalence of overweight subjects in the lowest quartile (36.9) was nearly 3 times that in the highest quartile (13.5). Regarding health practice variables, no differences were found in the prevalence of current smokers or of high caffeine intake. No difference was found in occupational physical activity among the quartiles, whereas the proportion of women physically active in their leisure-time increased along with calcium intake. A significant positive trend was found between lumbar densitometric values and dairy intake, but the prevalence of osteopenia and osteoporosis as well as the number of subjects reporting low energy fractures after menopause did not show statistically significant differences between the quartiles.

The prevalence of overweight was assessed according to dairy intake quartiles and by BMD level (osteoporosis, osteopenia, or
normal. Both the calcium intake and the lumbar BMD variables were found to be statistically and inversely associated with overweight status. Within each BMD class, in fact, the proportion of overweight women increased as dairy intake decreased. This relation was nearly significant when women with osteoporosis were considered (P = 0.067) (Table 2). In the logistic regression analysis on the risk of being overweight (Table 3), spare-time physical activity and smoking were significant and independent explanatory variables for overweight. When the highest quartile of dairy intake was used as a reference category, the 2 lowest quartiles were significant predictors of overweight; the odds for individuals in the lowest quartile were 3.7-fold higher than those in the highest quartile.

A generalized linear model was then applied to identify independent predictors of lumbar BMD in our sample (Table 4). In this model, the level of dairy intake was a significant independent predictor of BMD together with age, age at menopause, and overweight, and the strength of the association increased along with a decreased consumption of dairy products.

In Table 5, results from the multiple logistic regression analyses are reported; in these models the outcome variable was lumbar BMD (classified as osteoporosis, osteopenia, or normal). In model 1, the overweight variable was not included: only age and age at menopause acted as significant predictive factors for osteoporosis compared with osteopenia or normal, and not the level of dairy intake. However, when overweight was examined together with the same variables (model 2), consistent with the results of the previous model, age and age at menopause were still significant predictors of osteoporosis compared with osteopenia or normal; little difference in the estimated odds was found. As

### TABLE 1
Demographic and health characteristics of 1771 early postmenopausal women by quartiles of dairy intake

| Dairy intake quartile (times/wk) | 1, ≤ 7 (n = 467) | 2, 8–11 (n = 440) | 3, 12–15 (n = 438) | 4, ≥ 16 (n = 426) | P

| Age (y) | 54.0 ± 2.7 | 54.0 ± 2.9 | 54.0 ± 2.8 | 54.0 ± 2.9 | 0.973
| Age at menarche (y) | 13.0 ± 1.5 | 12.9 ± 1.5 | 12.8 ± 1.6 | 12.9 ± 1.5 | 0.314
| Age at menopause (y) | 51.3 ± 2.2 | 51.4 ± 2.2 | 51.3 ± 2.1 | 51.3 ± 2.2 | 0.994
| Height (cm) | 160.3 ± 6.2 | 159.8 ± 5.7 | 160.3 ± 6.4 | 160.6 ± 5.6 | 0.260
| Weight (kg) | 62.1 ± 9.6 | 61.0 ± 9.1 | 60.1 ± 8.8 | 60.1 ± 9.3 | 0.005
| BMI (kg/m²) | 24.1 ± 3.4 | 23.9 ± 3.5 | 23.4 ± 3.3 | 23.2 ± 3.4 | 0.001
| Overweight [n (%)] | 155 (36.9) | 132 (30.1) | 108 (24.5) | 63 (13.5) | <0.001
| Spare-time physical activity [n (%)] | 61 (14.5) | 96 (21.9) | 98 (22.3) | 151 (32.3) | <0.001
| Workers [n (%)] | 149 (35.5) | 159 (36.3) | 167 (37.9) | 173 (37.0) | 0.990
| Light | 116 (68.7) | 123 (77.4) | 133 (79.6) | 134 (77.5) | 0.307
| Heavy | 33 (22.1) | 36 (22.6) | 34 (24.0) | 39 (22.5) | 0.908
| High caffeine intake [n (%)] | 40 (9.6) | 34 (7.8) | 34 (8.1) | 34 (7.5) | 0.307
| Current smokers [n (%)] | 79 (19.0) | 79 (18.2) | 86 (19.5) | 70 (15.0) | 0.179
| Lumbar bone mineral density (g/cm²) | 0.862 ± 0.12 | 0.869 ± 0.11 | 0.872 ± 0.11 | 0.890 ± 0.13 | 0.003
| Osteoporosis [n (%)] | 88 (20.9) | 79 (18.0) | 76 (17.3) | 82 (17.6) | 0.369
| Osteopenia [n (%)] | 182 (43.3) | 182 (43.3) | 180 (40.9) | 189 (40.5) | 0.369
| Patients with postmenopausal fractures [n (%)] | 17 (4.0) | 12 (2.7) | 8 (1.8) | 12 (2.6) | 0.137

P: One-factor ANOVA or chi-square test for linear trend.

### TABLE 2
Overweight distribution by quartiles of dairy intake and classes of bone mineral density

| Dairy intake quintile (times/wk) | 1, ≤ 7 (n = 467) | 2, 8–11 (n = 440) | 3, 12–15 (n = 438) | 4, ≥ 16 (n = 426) | P

| Osteoporosis | 18 (20.4) | 16 (20.2) | 14 (18.4) | 8 (9.8) | 0.067
| Osteopenia | 64 (35.2) | 55 (28.2) | 41 (22.8) | 23 (12.2) | <0.0001
| Normal | 73 (48.7) | 61 (37.2) | 53 (28.8) | 32 (16.3) | <0.0001

P: Chi-square test for linear trend.

### TABLE 3
Adjusted risks of overweight in 1771 early postmenopausal women

| | Odds ratio 95% CI | P

| Age (1- y increment) | 1.064 1.001, 1.132 | 0.048
| Age at menopause (1- y increment) | 0.950 0.877, 1.027 | 0.201
| Spare-time physical activity (no vs yes) | 1.458 1.100, 1.951 | 0.010
| Smoking (no vs yes) | 1.403 1.041, 1.914 | 0.029
| High caffeine intake (<4 vs >4 cups/d) | 0.939 0.636, 1.410 | 0.756
| Dairy intake | <0.0001
| Quartile 1 vs quartile 4 | 3.717 2.637, 5.238 | <0.0001
| Quartile 2 vs quartile 4 | 2.845 2.025, 4.036 | 0.004
| Quartile 3 vs quartile 4 | 2.070 1.456, 2.967 | 0.661

P: Stepwise multiple logistic regression.
expected, the overweight variable was a significant factor (OR: 1.95; 95% CI: 1.57, 2.41; $P < 0.0001$), and dairy intake was associated with osteoporosis compared with osteopenia or normal: women in the lowest quartile of dairy intake had an OR of 1.24 times that in women in the highest quartile of dairy intake (OR: 1.46; 95% CI: 1.12, 1.89; $P = 0.008$). No differences in risk of osteoporosis were found between the intermediate quartiles and the highest quartile of dairy intake.

**TABLE 5**
Predictors of lumbar bone mineral density in 1771 early postmenopausal women

<table>
<thead>
<tr>
<th>Predictor</th>
<th>$\beta$ Coefficient</th>
<th>$P^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (1-y increment)</td>
<td>-0.015</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Age at menopause (1-y increment)</td>
<td>0.013</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Overweight (no vs yes)</td>
<td>-0.044</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Spare-time physical activity (no vs yes)</td>
<td>-0.008</td>
<td>0.243</td>
</tr>
<tr>
<td>Smoking (no vs yes)</td>
<td>0.0004</td>
<td>0.955</td>
</tr>
<tr>
<td>High caffeine intake (&lt;4 vs &gt;4 cups/d)</td>
<td>0.013</td>
<td>0.198</td>
</tr>
<tr>
<td>Dairy intake</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Quartile 1 vs quartile 4</td>
<td>-0.037</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Quartile 2 vs quartile 4</td>
<td>-0.027</td>
<td>0.0006</td>
</tr>
<tr>
<td>Quartile 3 vs quartile 4</td>
<td>-0.024</td>
<td>0.0022</td>
</tr>
</tbody>
</table>

$^1$ Generalized linear model.

**DISCUSSION**

The results of our study suggest that the negative effect of a low dietary calcium intake on BMD values in the first years after menopause can be attenuated by a greater BMI found in women with a low calcium intake. The effect of calcium seems to vary with years after menopause. There is a general consensus that dietary calcium moderately reduces the rate of cortical bone loss in late menopause (5, 6, 28, 29), whereas it seems to be ineffective in the first years after menopause. In this period, a high dietary intake or calcium supplementation cannot offset the effect of estrogen deficiency that is considered the dominant mechanism for the accelerated phase of bone loss involving mainly trabecular bone (30). In these women, the benefits of an increased calcium intake on vertebral bone loss, if any, seem to be limited to the first year, but no added effect was seen in subsequent years (7, 31).

A positive association between bone density and BMI or body weight has been well documented in many large epidemiologic studies (32, 33). Bone-protective effects of BMI may increase weight bearing (34), increase aromatization of androgen to estrogen in adipose tissue (35), lower concentrations of sex hormone–binding globulin (36), or directly increase bone formation induced by high circulating concentrations of insulin (37) and other hormones secreted by the $\beta$ cells of the pancreatic islets (19). More recently, leptin and other hormones secreted by adipose tissue, such as adiponectin, have been investigated as possible links between adipose tissue and bone metabolism (38).

In recent years, a growing body of observational and clinical studies supports a role for dietary calcium and dairy foods in controlling body weight and excess adiposity. Several cross-sectional and longitudinal investigations have shown an inverse relation between calcium intake and body weight in children, in men, and in younger and older women (9–14). The inverse relation between body weight or BMI and the level of calcium intake (both dietary and from supplements) can also be inferred from the results of some cross-sectional (39) and longitudinal (40) studies focused on bone density. Davies et al (41) and Heaney et al (42) reanalyzed data from 9 studies (3 intervention and 6 observational studies) originally designed to evaluate the effect of calcium on a primary skeletal endpoint. These authors reported a negative association between calcium intake and body weight, body fat, and weight gain in young, middle-aged, and older women and calculated that a 300-mg increase in daily calcium intake was associated with a 3-kg difference in body weight.

As shown by the logistic regression analysis reported in Table 3, the level of dietary calcium intake had an independent predictive power toward overweight, with estimates quite similar to those found in the third National Health and Nutrition Examination Survey (14). The same variable also acted as an independent predictor of BMD values (Table 4), and women belonging to the lowest quartile of dietary calcium intake showed a significantly greater risk of having osteoporosis than did subjects in the highest quartile when the overweight variable was entered in the analysis (Table 5).

Data from the questionnaire used to evaluate dairy food intake indicated a median value of 12 servings/wk in the whole sample of 1771 women, which corresponded to 3600 mg Ca/wk. Because milk and dairy products account for 66–75% of the total
daily calcium intake in postmenopausal women living in northern Italy (24, 43), the mean daily calcium intake was estimated to be between 642 and 689 mg/d after correction for the percentages of extra-dairy calcium sources. These values are consistent with those identified in more accurate studies performed with the use of validated instruments (44, 45) and found in large epidemiologic studies of postmenopausal Italian women (46).

Even if the results of the generalized linear model shown in Table 4 were more consistent with a dose-response or linear effect of dietary calcium on BMD values, in the logistic analysis given in Table 5 only the lowest quartile of dairy intake showed a greater and significant risk of osteoporosis compared with the highest quartile. This result supports a threshold effect of calcium on bone mass, so that only intakes below a certain level would be expected to have a negative effect on bone metabolism (47, 48). Women in the lowest quartile can probably be identified with those with a daily calcium intake <400 mg, such as women who lose bone mass from the spine at a greater rate (5).

The possibility that different sources of calcium will have different effects remains to be determined. It has been suggested that the beneficial role of dietary calcium on both bone metabolism and body weight is markedly greater for dairy than for nondairy sources or calcium supplements. Because a decrease in PTH and 1,25(OH)2D concentrations is not influenced by calcium source, it has been hypothesized that other bioactive dairy substances could play a functional role in bone remodeling and in body weight regulation. Milk whey proteins seem to decrease the rate of lumbar bone resorption in postmenopausal women, independently of calcium intake (49). With regard to fat tissue metabolism, a greater reduction in BMI has been reported when calcium is derived from dairy products rather than from supplements (13).

Several metabolic mechanisms have been proposed to account for this difference. Milk contains conjugated linoleic acid, which has been shown to reduce body weight and adiposity in mice (50). In addition, inhibitors of angiogenesis I–converting enzyme that are recognized to be present in whey proteins (51) may inhibit angiogenesis II stimulation of adipocyte lipogenesis (52). Last, calcium itself and dairy proteins impair fat absorption, which increases fecal fat excretion and thereby reduces calorie intake and body weight (53).

Our study has some weaknesses. The main limitation is related to its retrospective and observational nature. Measurement of dietary calcium intake at a single time point may not reflect long-term exposure. On the contrary, if we assume that our sample had consolidated dietary habits, we cannot rule out the possibility that differences in dietary calcium intake from childhood to menopause account for BMD values in postmenopausal years. Similarly, even if we had excluded women who reported greater body weight changes, transmenopausal weight gain may have biased our results. Furthermore, nutritional studies showed that dairy consumption is associated with many other nutrients (54), and, in general, with healthier eating habits and perhaps healthier lifestyle habits, for example a greater level of physical activity (11), as we found in our sample in which the proportion of women physically active increased from the lowest to the highest quartile of dairy intake (Table 1).

In conclusion, our data suggest that, in a healthy sample of early postmenopausal women, a low dietary calcium intake may increase the risk of osteoporosis, but its negative effect can be offset by the greater BMI found in women with usual low dietary calcium intakes. Because the aim in these women is to prevent, rather than to treat, osteoporosis and safer alternatives to estrogen need to be sought, an adequate understanding of the role of potentially modifiable lifestyle factors such as nutrition must be addressed further. Because accumulating data support a beneficial role for dietary calcium in preventing not only osteoporosis, but also other diseases such as obesity, hypertension, and colon cancer, a relatively simple dietary modification at the population level could play an important role in reducing morbidity and mortality in postmenopausal women.

The authors’ responsibilities were as follows—MV: study concept, design, and writing of the manuscript; LB: analysis and interpretation of data; SC: collection of data; FZ: collection of data; and LS: critical revision of the manuscript for important intellectual content. The authors had no financial or personal conflict of interest.

REFERENCES