

# Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women<sup>1-4</sup>

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See corresponding editorial on page 1031.

## ABSTRACT

**Background:** Vitamin K has been associated with bone mineral density (BMD) and risk of hip fracture. The apolipoprotein (apo) E4 allele (*APOE\*E4*) has been associated with bone fracture through a putative effect on vitamin K transport in blood.

**Objective:** The objective was to determine the associations between vitamin K intake, apo E genotype, BMD, and hip fracture in a population-based cohort of elderly men and women.

**Design:** Dietary vitamin K intake was assessed with a food-frequency questionnaire in 335 men and 553 women (average age: 75.2 y) participating in the Framingham Heart Study in 1988–1989. Incidence of hip fractures was recorded from 1988 to 1995. BMD at the hip, spine, and arm was assessed on 2 separate occasions (1988–1989 and 1992–1993). Comparisons between apo E genotype and BMD were made relative to E4 allele status (at least 1  $\epsilon$ 4 allele compared with no  $\epsilon$ 4 allele).

**Results:** Individuals in the highest quartile of vitamin K intake (median: 254  $\mu$ g/d) had a significantly lower fully adjusted relative risk (0.35; 95% CI: 0.13, 0.94) of hip fracture than did those in the lowest quartile of intake (median: 56  $\mu$ g/d). There were no associations between vitamin K intake and BMD in either men or women. No association was found between the E4 allele and BMD, and there were no significant interactions between the E4 allele and phylloquinone intake and BMD or hip fracture.

**Conclusions:** Low vitamin K intakes were associated with an increased incidence of hip fractures in this cohort of elderly men and women. Neither low vitamin K intake nor E4 allele status was associated with low BMD. *Am J Clin Nutr* 2000;71:1201–8.

**KEY WORDS** Vitamin K, phylloquinone, osteoporosis, osteocalcin, apo E genotype, hip fracture, bone mineral density, elderly

## INTRODUCTION

Osteocalcin and matrix  $\gamma$ -carboxyglutamic acid (Gla) protein are 2 vitamin K–dependent proteins that have been identified in bone (1). Osteocalcin, the most abundant noncollagenous protein in bone matrix, is produced by osteoblasts during bone matrix formation. Osteocalcin is thought to act as a regulator of bone mineralization, although its precise function has yet to be elucidated (2). The Gla residues in vitamin K–dependent proteins, including

osteocalcin, bind to calcium ions. If sufficient vitamin K is not available because of dietary deficiency or antagonism by oral anti-coagulants, undercarboxylated proteins, which lack some or all of the Gla residues, are produced (3). With the loss of Gla residues, there may be a loss of function, although the degree of undercarboxylation required for a loss of function is not known for individual vitamin K–dependent proteins.

Undercarboxylated osteocalcin (ucOC) is a sensitive marker of vitamin K nutritional status (4). The circulating concentration of ucOC has been reported to be an indicator of hip fracture (5, 6) and a predictor of femoral bone mineral density (BMD) (7). In a 22-mo prospective cohort study of 7598 elderly women, ucOC predicted hip fracture risk independently of femoral neck BMD (8). Although these data raise the possibility that vitamin K insufficiency might be involved in the pathogenesis of osteoporosis, these data may also indicate overall nutritional inadequacy because diet was not assessed in these studies (1). More compelling was the recent report that dietary phylloquinone (vitamin K<sub>1</sub>) intakes <109  $\mu$ g/d were associated with an increased risk of hip fracture in 72327 women participating in the Nurses' Health Study (9).

Phylloquinone is transported in the triacylglycerol-rich lipoproteins (10) and there is a strong positive correlation ( $P < 0.001$ ) between plasma phylloquinone concentrations and triacylglycerol concentrations (11). Fasting plasma phylloquinone concentrations were also reported to be strongly influenced by the

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polymorphism of apolipoprotein (apo) E (12, 13). Phylloquinone concentrations were highest among those individuals with the *E2* allele, intermediate among those with the *E3* allele, and lowest among those with the *E4* allele. This distribution is in accordance with the relation between apo E genotype and the rate of hepatic clearance of chylomicron remnants from circulation, with the *E4* allele having the most rapid catabolism. It was also reported that individuals with the *E4* allele have a significantly higher history of bone fractures (14) and lower BMD (15) than do individuals without the *E4* allele. Collectively, these studies suggest a gene-nutrient interaction between the apo E genotype and vitamin K in the risk of hip fractures. However, these studies did not collect phylloquinone intake data.

The primary objective of the present study was to determine the associations between dietary phylloquinone intake and BMD measured at 6 anatomic sites (cross-sectionally and longitudinally) in a cohort of elderly men and women participating in the Framingham Heart Study. Associations between dietary vitamin K intakes and the incidence of hip fracture were also assessed in this cohort. Given the putative apo E–vitamin K interaction in fracture risk, these associations with BMD and incidence of hip fracture were also evaluated relative to *E4* allele status.

## SUBJECTS AND METHODS

### Subjects

The Framingham Heart Study cohort of 5209 men and women aged 28–62 y was established between 1948 and 1950 (16). Subjects have returned biennially for an extensive physical examination, for completion of comprehensive questionnaires, for anthropometric measurements, for blood chemistry measurements, and for assessment of cardiovascular and other risk factors by trained clinical personnel (17). The cohort is carefully followed for medical events, including hip fractures (18). The Framingham Osteoporosis Study began with bone measurements of subjects at the 20th examination (1988–1989). The protocol for this study was approved by the Institutional Review Board for Human Research at Boston University.

Of the ≈1300 survivors who participated in the 20th biennial physical examination (1988–1989), 335 men and 553 women had measurements from at least one BMD site, had completed a food-frequency questionnaire (FFQ), and were not current anticoagulant users. BMD measurements were repeated during the 22nd examination (1992–1993). At this time, 203 men and 376 women had completed the FFQ at the 20th examination, had 2 sets (20th and 22nd examinations) of BMD measurements from at least one site, and were not anticoagulant users at either examination. Current anticoagulant users at either examination (10 men and 9 women) were excluded from these analyses because of a potential warfarin–vitamin K interaction (19). As described in greater detail elsewhere (20), there were no significant differences in sex, education level, smoking status, or body mass index [BMI; in wt (kg)/ht<sup>2</sup> (m)] between subjects with and without complete baseline BMD and dietary intake data. However, subjects with complete data were younger (75.2 compared with 79.1 y;  $P < 0.001$ ) and more physically active (score: 33.5 compared with 31.3;  $P < 0.001$ ) than were those without bone measurements or a completed FFQ (20). Most of the subjects with no BMD measurements were homebound and thus were unable to have their BMD assessed in the clinic.

### Dietary assessment

Usual dietary intakes during the previous 12 mo were assessed at the 20th examination by using a 126-item semiquantitative FFQ as described elsewhere (21). This FFQ was validated for phylloquinone intake (9). Questionnaires were mailed to the subjects before the examination and once completed were returned to the examination site. Questionnaires with reported energy intakes  $<2.51$  and  $>16.74$  MJ/d (600 and 4000 kcal, respectively) or with  $>12$  food items left blank were considered invalid and excluded from further analysis. Information about use of vitamin and mineral supplements and specific types of breakfast cereal most commonly consumed was used in the estimate of total micronutrient intakes. Daily phylloquinone intakes were calculated by multiplying the phylloquinone content per serving of each food (22, 23) by the reported frequency of consumption and summing over all foods. The phylloquinone intakes reported include intakes from multivitamin and mineral preparations and other nutrient supplements.

### DNA isolation and genotyping

Leukocyte DNA was extracted from 5–10 mL whole blood with the method described by Miller et al (24). Apo E genotyping was performed in the cohort by using DNA samples collected during the 19th examination (1986–1987) as described by Hixson and Vernier (25). Briefly, a 244–base pair segment of the apo E gene, including 2 polymorphic sites, was amplified by polymerase chain reaction (PCR) in a DNA Thermal Cycler (PTC-100; MJ Research, Inc, Watertown, MA) with the oligonucleotide primers F4 and F6. Each reaction mixture was heated at 90°C for 2 min, followed by 35 cycles of amplification (94°C for 40 s, 62°C for 30 s, and 72°C for 1 min). The PCR products were digested with 5 U of the restriction endonuclease *HhaI*, and the fragments were separated by electrophoresis on an 8% polyacrylamide nondenaturing gel. After electrophoresis, the gel was treated with ethidium bromide for 30 min and DNA fragments were visualized by ultraviolet illumination. Of the 1078 persons who were genotyped, 266 men and 443 women also had BMD measurements and complete dietary data from the 20th examination.

### Bone mineral density

At the 20th examination, hip and spine BMD measures of the trochanter, femoral neck, Ward's area, and L2–L4 lumbar spine were taken by using dual-photon absorptiometry (DP-3; Lunar, Madison, WI). The BMD measures for the distal one-third site of the right radial shaft and ultradistal forearm were taken by using single-photon absorptiometry (SP2; Lunar). With this method, the CV for repeated measurements over 2 y in healthy young individuals was 2.65% for the femoral neck, 2.8% for the trochanter, and 4.16% for Ward's area (26). The CV for healthy, young control subjects was 3.9% for the distal one-third site of the radial shaft, 5.7% for the ultradistal forearm, and 2.2% for the lumbar spine. At the 22nd examination, BMD was measured in the hip and spine by using dual-energy X-ray absorptiometry (DXA) and in the right ultradistal forearm by using single-photon absorptiometry. The CVs for the DXA measurements of the hip at the 22nd examination were as follows: 1.7% for the femoral neck, 2.5% for the trochanter, 4.1% for Ward's area, and 0.9% for the lumbar spine. The CV for the distal one-third site of the radial shaft was 2% (27). To correct for measurement differences between dual-photon absorptiometry and DXA, equations

were derived by scanning a group of subjects with both machines (28). In the few cases in which different sides were measured across time, subjects were deleted from the change analysis ( $n = 17$  for the hip and 16 for the radius).

### Hip fractures

Osteoporotic hip fractures were defined as incident fractures of the proximal femur. Incident hip fractures were ascertained continuously in the cohort for 7 y (from the 20th examination to the end of the 23rd examination in 1995) by reviewing records from the Framingham Study, querying subjects directly (either at the examination or by telephone for nonattendees), and by reviewing death records. Hip fractures resulting from automobile accidents or other violent trauma ( $\approx 5\%$  of all cases) were excluded from the analyses. Hip fractures were confirmed and documented by hospital or nursing home discharge summaries, including emergency room notes, surgical records, and X-ray reports.

### Covariate information

As described in greater detail elsewhere (20), factors reported to affect BMD in this study population included age (26), BMI (29), alcohol use (30), smoking (31), and estrogen use by women (32). In this study, BMI was calculated from measurements of height at the first examination (1948–1950) before height might have been lost as a result of osteoporosis. Body weight was recorded at the 20th examination. Estrogen use was defined as current and continuous use for  $\geq 2$  y; evidence suggests that past estrogen use sustains no bone benefit (32). Physical activity was measured at the 20th examination with the Framingham physical activity index (33, 34). Smoking was defined as never, past, or current. Total grams of alcohol consumed per week were estimated and defined as none, light consumption, or heavy consumption, as described elsewhere (30). Dietary calcium, vitamin D, and caffeine intakes were assessed from the food-based section of the FFQ as described above. Use of calcium or vitamin D supplements, as recorded in the supplement section of the FFQ, were coded as dummy (yes or no) variables. Because most nutrients correlate with energy intake, adjustment for energy intake allowed an assessment of the independent effect contributed by the nutrient; adjustment also allows for differences in intake that may be due to body size or activity levels and corrects for some of the measurement error inherent in the FFQ (35).

### Statistical analyses

All statistical analyses were performed by using SAS release 6.12 (SAS Institute Inc, Cary, NC) on a VAX mainframe computer (Digital Equipment Corp, Maynard, MA). For cross-sectional analyses, measures of BMD at the femoral neck, trochanter, Ward's area, L2–L4 lumbar spine, distal one-third site of the radius, and ultradistal radius were regressed on total phylloquinone intake separately for men and women, with adjustment for potential confounders (age; BMI; alcohol use; smoking; estrogen use by women; physical activity; dietary calcium, vitamin, and caffeine intakes; energy intake; and calcium and vitamin D supplement use) in the general linear models procedure. The phylloquinone intakes used for analyses were a summation of intakes from both the diet and supplements. Of the entire study group, only 18 subjects reported a mean ( $\pm$ SD) intake of  $21 \pm 7.9$   $\mu$ g phylloquinone/d from supplement use. The mean ( $\pm$ SD) dietary phylloquinone intake of these 18 supplement users was  $133 \pm 114$   $\mu$ g/d, which was not significantly different from the mean dietary

**TABLE 1**  
Subject characteristics<sup>1</sup>

	Men	Women
Age (y)	75.1 $\pm$ 4.93 [335]	75.3 $\pm$ 4.83 [553]
BMI (kg/m <sup>2</sup> )	27.2 $\pm$ 3.99 [335]	26.3 $\pm$ 5.01 [553]
Dietary intake		
Phylloquinone ( $\mu$ g/d) <sup>2</sup>	143 $\pm$ 97 [335]	163 $\pm$ 115 [553]
Energy (MJ/d)	7.80 $\pm$ 2.63 [335]	6.87 $\pm$ 2.30 [553]
Caffeine (mg/d)	186 $\pm$ 161 [335]	200 $\pm$ 163 [553]
Calcium (mg/d)	743 $\pm$ 382 [335]	705 $\pm$ 330 [553]
Vitamin D ( $\mu$ g/d)	5.9 $\pm$ 4.2 [335]	5.2 $\pm$ 3.4 [553]
Supplement use (%)		
Phylloquinone <sup>2</sup>	1.8 [335]	2.2 [553]
Calcium	8.7 [335]	22.8 [553]
Vitamin D	22.7 [335]	29.8 [553]
Estrogen use (%)		
	—	5.1 [553]
Baseline BMD (g/cm <sup>2</sup> )		
Femoral neck	0.877 $\pm$ 0.147 [320]	0.719 $\pm$ 0.115 [527]
Trochanter	0.846 $\pm$ 0.151 [312]	0.625 $\pm$ 0.127 [523]
Ward's area	0.685 $\pm$ 0.173 [320]	0.559 $\pm$ 0.126 [527]
Spine L2–4	1.340 $\pm$ 0.223 [249]	1.066 $\pm$ 0.189 [422]
Radius <sup>3</sup>	0.719 $\pm$ 0.086 [329]	0.511 $\pm$ 0.090 [538]
Ultradistal radius	0.367 $\pm$ 0.062 [257]	0.242 $\pm$ 0.055 [439]
Change in BMD (g/cm <sup>2</sup> ) <sup>4</sup>		
Femoral neck	−0.010 $\pm$ 0.063 [203]	−0.025 $\pm$ 0.052 [376]
Trochanter	0.003 $\pm$ 0.086 [196]	−0.022 $\pm$ 0.061 [373]
Ward's area	0.000 $\pm$ 0.068 [203]	−0.025 $\pm$ 0.062 [376]
Spine L2–4	0.000 $\pm$ 0.104 [163]	−0.046 $\pm$ 0.095 [295]
Radius <sup>3</sup>	−0.028 $\pm$ 0.043 [200]	−0.027 $\pm$ 0.042 [388]
Ultradistal radius	−0.009 $\pm$ 0.038 [154]	−0.013 $\pm$ 0.033 [283]
APOE*E4 allele (%)		
Present	19.2 [266]	21.0 [443]
Absent	80.8 [266]	79.0 [443]

<sup>1</sup> $\bar{x} \pm$  SD;  $n$  in brackets. BMD, bone mineral density.

<sup>2</sup>Phylloquinone was the predominant form of dietary vitamin K.

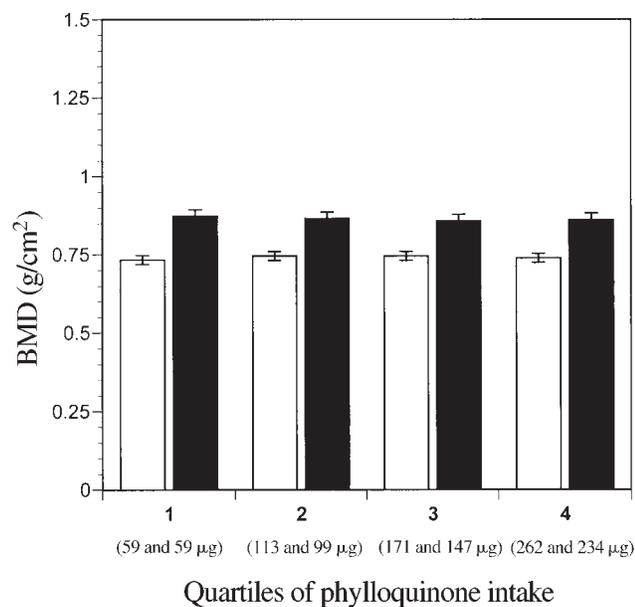
<sup>3</sup>Distal one-third site.

<sup>4</sup>Change over  $\geq 5$  y in between the 20th (1988–1989) and 22nd (1992–1993) examinations.

phylloquinone intake reported for the entire study group (Table 1). For the apo E analysis, cross-sectional models similar to those used for the analysis of phylloquinone intake were used. Apo E genotype was defined relative to E4 allele status, with individuals classified as either having or not having the E4 allele. Interactions between the E4 allele and phylloquinone intake were tested and removed from the model when not significant at  $P < 0.05$ .

Change in BMD was defined as BMD at the 22nd examination minus BMD at the 20th examination. These changes were regressed on total phylloquinone intake, on E4 allele status, and on the interaction between them for men and women separately. All potential confounders were used in the cross-sectional analyses as were the corresponding baseline measures of BMD at the 20th examination. This baseline BMD value was included because of the likelihood that a change in BMD might be related to the initial BMD. A series of models similar to those used in the cross-sectional analysis were used to evaluate the change in BMD. Statistically significant differences ( $P < 0.05$ ) across quartile groups were tested in the general linear models procedure in SAS and adjusted for potential confounders with  $t$  test comparison of the least-squares means.

Hip fracture analysis was conducted in all subjects. The number of fractures in the 7-y follow-up period was insufficient for



**FIGURE 1.** Mean ( $\pm$ SEM) femoral neck bone mineral density (BMD) measured at the 20th examination (1988–1989) in elderly women ( $\square$ ;  $n = 553$ ) and men ( $\blacksquare$ ;  $n = 335$ ) by quartiles of phyloquinone intake. There were no significant differences in BMD across quartiles of phyloquinone intake for either men or women. Median phyloquinone intakes of women and men, respectively, in each quartile are given in parentheses.

subset analysis. The Cox proportional hazards model was used to assess risk ratios and 95% CIs of incident hip fracture by quartile categories of baseline phyloquinone intakes; the lowest intake quartile was defined as the reference group. The linear trend across quartiles of phyloquinone intake was tested by using the median phyloquinone intake for each quartile. All potential confounders as well as the femoral neck BMD measurements from the 20th examination were adjusted for in the analysis. Similar analyses were conducted for apo E genotype. Those subjects classified as not having the *E4* allele were defined as the reference group. Interactions between *E4* allele status and phyloquinone intake were tested in the model. An age-adjusted Kaplan-Meier method, developed by Cupples et al (36), was used to estimate the probability of hip fracture during the 7-y follow-up period by quartiles of phyloquinone intake. The method involves the direct standardization of the Kaplan-Meier curve.

## RESULTS

### Subject characteristics

Characteristics of the study sample are presented in Table 1. Subjects had a mean age of 75 y at the first measurement (range: 68–94 y). Men had a higher BMD than did women at all skeletal sites.

### Dietary intakes

The mean ( $\pm$ SD) daily vitamin K intakes of the men and women were  $143 \pm 97$  and  $163 \pm 115$   $\mu$ g phyloquinone, respectively (Table 1), which were greater than the current recommended dietary allowance for vitamin K of 65–80  $\mu$ g/d (37), but consistent with dietary intakes reported for this age group (38).

Women with higher dietary phyloquinone intakes were younger than those with lower phyloquinone intakes (74.9 and 76.3 y in the highest and lowest quartiles, respectively;  $P = 0.04$ ). Average reported energy intakes were low for both men and women: 7.80 and 6.87 MJ (1865 and 1643 kcal), respectively. Average reported calcium and vitamin D intakes were below the recommended adequate intakes of 1200 mg/d and 10–15  $\mu$ g (400–600 IU)/d, respectively (39), but were consistent with average reported intakes in the United States (39).

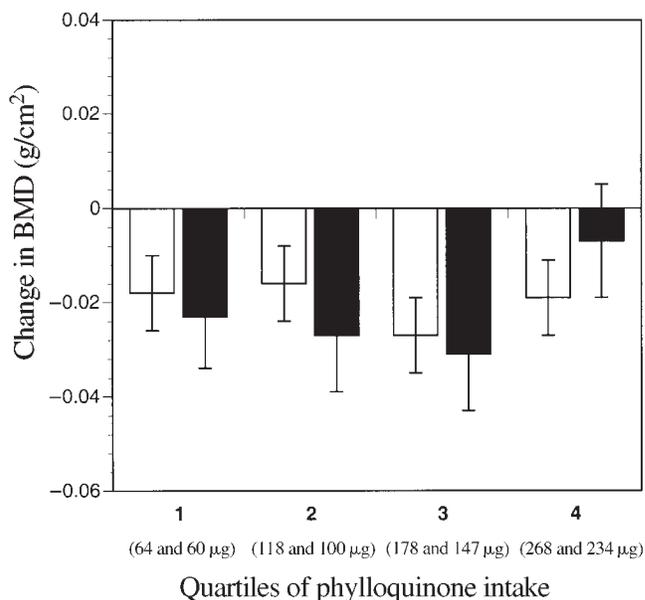
Only 2.0% of the study participants derived phyloquinone from supplements compared with a range of 8.7–29.8% who derived calcium and vitamin D from supplements (Table 1). Of those using supplements, the average phyloquinone intakes from supplements were  $20 \pm 9$  and  $23 \pm 6$   $\mu$ g/d for men and women, respectively.

### Bone mineral density

There were no significant associations between dietary phyloquinone intake and any of the BMD sites measured at the 20th examination for either men or women (Figure 1). When the changes in BMD measured between the 20th examination (1988–1989) and the 22nd examination (1992–1993) were stratified by quartiles of phyloquinone intake as estimated from the FFQ administered during the 20th examination, there were still no significant associations (Figure 2).

### Apo E genotype

The *E4* allele was identified in 19.2% and 21.0% of the men and women, respectively (Table 1). These distributions are similar to those reported for participants of the Framingham Offspring Study (40). When BMD measurements from the 20th



**FIGURE 2.** Mean ( $\pm$ SEM) change in femoral neck bone mineral density (BMD) between the 20th (1988–1989) and 22nd (1992–1993) examinations in elderly women ( $\square$ ;  $n = 413$ ) and men ( $\blacksquare$ ;  $n = 219$ ) by quartiles of phyloquinone intake. There were no significant changes in BMD across quartiles of phyloquinone intake for either men or women. Median phyloquinone intakes of women and men, respectively, in each quartile are given in parentheses.

**TABLE 2**Bone mineral density (BMD) measurements in elderly men and women by *APOE\*E4* allele status<sup>1</sup>

	Men		Women	
	<i>E4</i> allele ( <i>n</i> = 51)	No <i>E4</i> allele ( <i>n</i> = 215)	<i>E4</i> allele ( <i>n</i> = 93)	No <i>E4</i> allele ( <i>n</i> = 350)
	<i>g/cm<sup>2</sup></i>			
BMD at the 20th examination <sup>2</sup>				
Femoral neck	0.881 ± 0.027	0.869 ± 0.021	0.717 ± 0.017	0.729 ± 0.014
Trochanter	0.849 ± 0.028	0.842 ± 0.022	0.615 ± 0.019	0.630 ± 0.016
Radius <sup>3</sup>	0.708 ± 0.015	0.715 ± 0.011	0.540 ± 0.013	0.539 ± 0.011
Change in BMD <sup>4</sup>				
Femoral neck	-0.031 ± 0.016	-0.017 ± 0.011	-0.020 ± 0.010	-0.019 ± 0.008
Trochanter	-0.027 ± 0.019	-0.016 ± 0.014	-0.021 ± 0.011	-0.015 ± 0.010
Radius <sup>3</sup>	-0.032 ± 0.010	-0.034 ± 0.008	-0.028 ± 0.007	-0.024 ± 0.006

<sup>1</sup> $\bar{x} \pm$  SEM. There were no significant differences between the 2 genotype groups at any of the BMD sites measured for either men or women.<sup>2</sup>Adjusted for smoking status, calcium and vitamin D supplement use, alcohol consumption, BMI, age, energy intake, physical activity score, and vitamin D, calcium, and caffeine intakes.<sup>3</sup>Distal one-third site.<sup>4</sup>Change in BMD between the 20th (1988–1989) and 22nd (1992–1993) examinations. Adjusted for baseline BMD as measured in the 20th examination, smoking status, calcium and vitamin D supplement use, alcohol consumption, BMI, age, energy intake, physical activity score, and vitamin D, calcium, and caffeine intakes.

examination were stratified by the presence or absence of the *E4* allele, there were no significant differences between the 2 groups at any of the BMD sites measured in either men or women (Table 2). Likewise, when the analysis was repeated with use of the change in BMD between the 20th and the 22nd examinations, there were no significant differences between those with or without the *E4* allele. When men and women with the *E4* allele and low phylloquinone intakes (<80  $\mu\text{g}/\text{d}$ ) were compared with others without the *E4* allele and with higher phylloquinone intakes, there were no significant differences in BMD (data not shown). Furthermore, there were no significant interactions between *E4* allele status and phylloquinone intake on BMD measures.

### Hip fractures

In the 7 y of follow-up (between the 20th and the 23rd examinations, which ended in 1995), 8 and 36 hip fractures were reported among the 342 men (2.34%) and 558 women (6.45%), respectively. When the data for the men and women were combined, individuals in the highest quartile of vitamin K intake (median: 254  $\mu\text{g}/\text{d}$ ) had a significantly lower relative risk (RR: 0.35; 95% CI: 0.13, 0.94) of hip fracture than did those in the

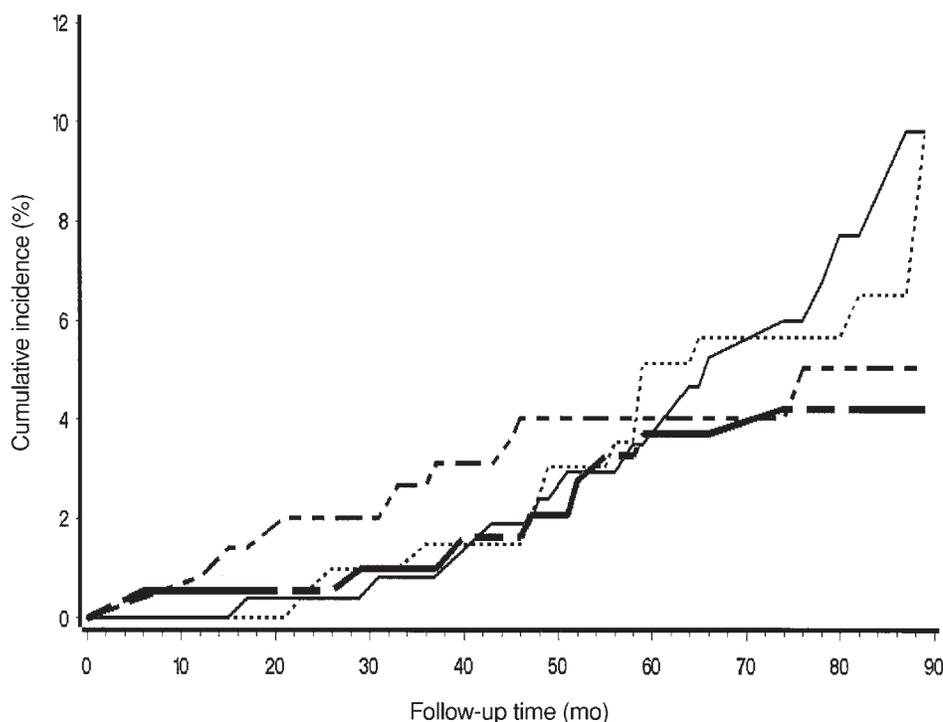
lowest quartile of intake (median: 56  $\mu\text{g}/\text{d}$ ) (Table 3). The percentage of subjects with incident hip fractures in the lowest quartile of phylloquinone intake (16/223; 7.2%) was significantly higher ( $P = 0.037$ ) than that in the highest quartile of phylloquinone intake (7/227; 3.1%). Furthermore, there was a significant linear trend ( $P = 0.047$ ) for a lower incidence of hip fractures at higher quartiles of phylloquinone intake. Adjustment for femoral neck BMD measured at the 20th examination did not attenuate this association. When the cumulative incidence of hip fracture was plotted against 7-y follow-up data by using the Kaplan-Meier method (Figure 3), there was a divergence in the cumulative incidence among quartiles of phylloquinone intake at 60 mo of follow-up. For the next 30 mo, there was a steep rise in incident hip fractures among the 2 lowest quartiles of intake but not among the 3rd and 4th quartiles of intake. Although not significant, the trend in these data lend further support to the observation that dietary phylloquinone intake is associated with incidence of hip fracture. When examined by the presence or absence of the *E4* allele, there were no significant differences in the incidence of hip fracture (data not shown). Likewise, there were no significant interactions between *E4* allele status and phylloquinone intake on incidence of hip fracture.

**TABLE 3**

Relative risks of hip fracture in elderly men and women by quartile of phylloquinone intake

Quartile	Number of participants with hip fractures	Median phylloquinone intake ( $\mu\text{g}/\text{d}$ )	Multivariate RR (95% CI) <sup>1</sup>	Multivariate RR (95% CI) adjusted for BMD <sup>2</sup>
1	16 of 223	56	1.00	1.00
2	11 of 222	105	0.53 (0.22, 1.28)	0.52 (0.20, 1.32)
3	10 of 228	156	0.59 (0.25, 1.39)	0.65 (0.25, 1.70)
4	7 of 227	254	0.35 (0.13, 0.94)	0.35 (0.12, 1.02)
<i>P</i> for trend <sup>3</sup>	—	—	0.047	0.054

<sup>1</sup>Adjusted for sex, smoking status, calcium and vitamin D supplement use, alcohol consumption, BMI, age, energy intake, physical activity score, and vitamin D, calcium, and caffeine intakes.<sup>2</sup>Adjusted for femoral neck bone mineral density (BMD), sex, smoking status, calcium and vitamin D supplement use, alcohol consumption, BMI, age, energy intake, physical activity score, and vitamin D, calcium, and caffeine intakes.<sup>3</sup>Linear trend across quartiles of phylloquinone intake, with median value per quartile.



**FIGURE 3.** Cumulative incidence of hip fracture over the 7-y follow-up period by quartiles of phylloquinone intake. Quartile 1, —; quartile 2, ·····; quartile 3, - - -; and quartile 4, - · - · -.

## DISCUSSION

Recent epidemiologic studies have implicated vitamin K as a potentially important dietary factor that can affect BMD and the risk of hip fracture (5, 6, 9). However the limited data on the relation between vitamin K and bone status in humans are inconsistent. Furthermore, the possible mechanisms whereby suboptimal vitamin K intake and status affect bone metabolism are poorly understood. In this study, we found a significant association between reported phylloquinone intakes and incidence of hip fracture in an elderly cohort of men and women. In contrast, we found no associations between reported dietary phylloquinone intakes and BMD in this elderly cohort, either in cross-sectional or longitudinal comparisons.

The mean age of the study participants was 75 y at the time baseline measurements of both dietary intakes and BMD were made. Participants who completed the FFQ and had BMD measurements at the 20th examination had fewer hip fractures (data not shown) and were younger and more physically active than were study participants who did not participate in either the dietary assessment or the BMD measurements (20). Despite these potential biases and the age of the study participants, both dietary (20) and nondietary factors, such as body weight (29), smoking (31), and estrogen use (18), have been associated with bone loss in this study group. The lack of association between phylloquinone intake and BMD in this elderly cohort does not imply that vitamin K is not important in determining BMD in earlier adulthood, before the acceleration of bone loss that occurs later in life.

Metabolic data suggest that dietary phylloquinone intakes <100  $\mu\text{g}/\text{d}$  do not appear to maximally support carboxylation of osteocalcin (41). There are few data on the relation between dif-

ferent dietary intakes of phylloquinone and corresponding ucOC concentrations, including the potential existence of a threshold effect. Although differences among quartiles of phylloquinone intake were significant for incident hip fracture, they were not associated with differences in BMD. Because there was only a 50- $\mu\text{g}$  phylloquinone/d difference between individual quartiles of intake, it is not known whether the differences were large enough to detect measurable differences in BMD. Because the FFQ is a semiquantitative instrument, the potential for misclassification of individuals by quartiles of phylloquinone intake also exists. Such a misclassification would attenuate our ability to detect associations between phylloquinone intake and BMD.

Alternatively, it is possible that any putative association between vitamin K and hip fracture is independent of BMD. In the EPIDOS study (8), which was a prospective study of risk factors for hip fracture among free-living elderly French women aged >75 y, ucOC concentrations predicted hip fracture risk independently of femoral neck BMD. Women with a high ucOC concentration and a low femoral neck BMD had a greater risk of hip fracture than did women with only 1 of the 2 risk factors. We report in this study that dietary phylloquinone intake was associated with risk of hip fracture in elderly men and women, independently of femoral neck BMD. Manipulation of dietary phylloquinone was shown recently to affect rates of bone turnover, as measured by total osteocalcin and cross-linked *N*-telopeptide of type I collagen (42). Suggested roles for vitamin K-dependent proteins in bone include the inhibition of bone formation (2) and calcification of cartilage (43). Clearly, more research is required to understand the mechanisms by which vitamin K may be an independent risk factor for hip fracture.

In this study, there were more incident hip fractures in men and women in the lowest quartile of phylloquinone intake than in those in the higher quartiles. These findings are consistent with those of Feskanich et al (9), who used the same FFQ and provided more evidence of a relation between dietary phylloquinone intakes and risk of hip fracture. In contrast with the Nurses' Health Study (9), we noted a significant trend toward a lower incidence of hip fractures associated with increasing quartiles of phylloquinone intake. One explanation for the discrepancy between the 2 studies is the difference in the range of phylloquinone intakes between the 2 populations. In the elderly cohort in the present study, median phylloquinone intakes across the quartiles ranged from 56 to 254  $\mu\text{g}/\text{d}$ , whereas in the Nurses' Health Study, median intakes across quintiles ranged from 109 to 242  $\mu\text{g}/\text{d}$  (9). The narrow range of dietary phylloquinone intakes in the latter study may have limited the ability to detect differences in the risk of hip fracture at the upper quintiles of intake. However, comparison of absolute phylloquinone intakes among different studies is of limited value because the FFQ used was developed to categorize or rank individual subjects and not to measure group means (44).

One consistent criticism of the epidemiologic data used to support an association between dietary vitamin K and fracture risk has been the potential confounding effect of overall poor nutrition (1). In the present study and in the Nurses' Health Study (9), dietary calcium and vitamin D intakes and supplements and dietary energy intakes were controlled for in the analysis of the association between phylloquinone intake and risk of hip fracture. However, the primary dietary sources of phylloquinone are green, leafy vegetables (38). High fruit and vegetable consumption was associated with greater BMD and less age-related bone loss in the same men and women that participated in this study (20). Although there was no reported association between phylloquinone intake and BMD, the findings of this study do not preclude the possibility that high vitamin K intakes may simply be a marker for an overall healthy diet that includes high vegetable consumption.

The role of triacylglycerols in phylloquinone transport has also been suggested as a nondietary confounder of ucOC (12). The *E4* allele has been associated with low BMD (15) and bone fracture (14), which has been attributed to a modulation of vitamin K transport. In the present study, we did not find an association between the *E4* allele and BMD or hip fracture in either men or women. However, it is plausible that individuals with low BMD or previous hip fractures had died before the 20th examination, even though the distribution of the apo E genotypes reported in this study is consistent with that reported for the younger offspring cohort of the Framingham Study (40).

In summary, there were significantly more incident hip fractures among elderly men and women in the lowest reported quartile of phylloquinone intake than in those in the highest quartile of intake. In contrast, we found no significant associations between dietary phylloquinone intake or *E4* allele status and BMD in this same cohort. Given the potential influence of diet, age, sex, and apo E genotype on both plasma phylloquinone concentrations and ucOC concentrations observed in metabolic and clinical studies, the associations between dietary phylloquinone and BMD and risk of hip fracture need to be studied in a healthy population of men and women younger than the subjects studied here. Inclusion of biochemical indexes of vitamin K, including ucOC, would further clarify any potential associations between vitamin K, apo E genotype, and bone. 

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