Vitamin K, bone turnover, and bone mass in girls[1–3]

Heidi J Kalkwarf, Jane C Khoury, Judy Bean, and James G Elliot

ABSTRACT

Background: Vitamin K has been suggested to have a role in bone metabolism, and low vitamin K intake has been related to low bone density and increased risk of osteoporotic fracture.

Objective: The objective of this study was to determine whether phylloquinone (vitamin K_1) intake and biochemical indicators of vitamin K status are related to bone mineral content (BMC) and markers of bone formation and bone resorption in girls.

Design: Vitamin K status [plasma phylloquinone concentration and percentage of undercarboxylated osteocalcin (%ucOC)] was measured at baseline in a study of 245 healthy girls aged 3–16 y. Cross-linked *N*-telopeptide of type 1 collagen (NTx) breakdown, osteocalcin, and bone-specific alkaline phosphatase were measured to reflect bone resorption and formation. BMC of the total body, lumbar spine, and hip and dietary phylloquinone intake were measured annually for 4 y.

Results: Phylloquinone intake (median: 45 μg/d) was not consistently associated with bone turnover markers or BMC. Better vitamin K status (high plasma phylloquinone and low %ucOC) was associated with lower bone resorption and formation. Plasma phylloquinone was inversely associated with NTx and osteocalcin concentrations ($P < 0.05$), and %ucOC was positively associated with NTx and bone-specific alkaline phosphatase concentrations ($P < 0.05$). Indicators of vitamin K status were not consistently associated with current BMC or gain in BMC over the 4-y study period.

Conclusions: Better vitamin K status was associated with decreased bone turnover in healthy girls consuming a typical US diet. Randomized phylloquinone supplementation trials are needed to further understand the potential benefits of phylloquinone on bone acquisition in growing children. *Am J Clin Nutr* 2004;80:1075–80.

KEY WORDS Vitamin K, phylloquinone, osteocalcin, undercarboxylated osteocalcin, bone density, bone turnover, bone resorption, girls, dietary intake

INTRODUCTION

Vitamin K has been suggested to have a role in bone metabolism, and inadequate vitamin K intake has been hypothesized to increase the risk of osteoporotic fracture (1). Vitamin K is found naturally in 2 forms, both of which have vitamin K activity. Vitamin K_1 (phylloquinone) is the primary dietary source of vitamin K and is found in high concentrations in leafy, green vegetables and in some plant oils. Vitamin K_2 (menaquinone) comprises a family of naphthoquinones with differing numbers of isoprenoid residues and is synthesized by bacteria. Comprehensive information on food sources of vitamin K_2 is not available, but it is found in animal meats, dairy products, and fermented foods. The current recommended adequate intakes for vitamin K are 55–75 μg/d for children 4–18 y of age and 90–120 μg/d for adults (2).

Vitamin K is a cofactor for the vitamin K–dependent carboxylase that facilitates the posttranslational carboxylation of glutamyl residues in select proteins. Three vitamin K–dependent proteins, osteocalcin, matrix Gla protein, and protein S, are found in bone; osteocalcin is the most abundant (1). The amount of osteocalcin that is undercarboxylated is thought to be a sensitive indicator of vitamin K status. In adults, serum concentrations of undercarboxylated osteocalcin (ucOC) decrease rapidly in response to supplementation with 80 μg to 1 mg phylloquinone/d and 45 mg menaquinone/d (3–8).

The exact role of vitamin K in bone is unclear. Osteocalcin is made by osteoblasts, and fully carboxylated osteocalcin binds the calcium ion of the hydroxyapatite molecule (1). It has been hypothesized that vitamin K is needed for bone mineralization (1, 9). Vitamin K also has been shown to increase osteoblastogenesis and decrease osteoclastogenesis, thereby increasing bone formation and decreasing bone resorption (10).

Results of studies on vitamin K and bone in adults are conflicting. Some (11–15) but not all (15) studies showed a relation between phylloquinone intake or ucOC and bone density or risk of osteoporotic fracture. A few studies showed an increase in bone density with phylloquinone and menatetrenone supplementation (8, 16–20). Most supplementation studies used pharmacologic doses (ie, 45 mg/d) of menatetranone, and the significance of these findings for intakes that could be achieved through dietary sources is unclear.

Little is known about vitamin K status and bone metabolism in children. Gaining an understanding of the role of vitamin K in bone metabolism in children is important, because finding new strategies to maximize the accretion of bone during growth may help reduce the risk of osteoporosis many decades later. We aimed to address the following research questions. Is greater dietary phylloquinone intake, or better vitamin K status, associated with higher bone formation and lower bone resorption in girls? Is greater phylloquinone intake, or better vitamin K status, associated with higher bone mass in girls?

[1] From the Children's Hospital Medical Center, Cincinnati (HJK, JCK, and JB), and DSM Nutritional Products, Inc, Parsippany, NJ (JGE).

[2] Supported by the Cincinnati Children's Hospital Medical Center and DSM Nutritional Products, Inc.

[3] Reprints not available. Address correspondence to HJ Kalkwarf, Division of General and Community Pediatrics, Children's Hospital Medical Center, MLC-7035, Cincinnati, OH 45229-3039. E-mail: heidi.kalkwarf@cchmc.org.

Received December 18, 2003.

Accepted for publication April 13, 2004.

The American Journal of Clinical Nutrition

Downloaded from www.ajcn.org by on April 26, 2008

SUBJECTS AND METHODS

We used data from a longitudinal study of normal bone mass accretion during childhood and adolescence that included 245 girls aged 3.0–16.0 y ($\bar{x}$: 9.8 y). The racial composition of the group was as follows: 238 whites, 4 blacks, and 3 girls of mixed race. The girls were recruited from a variety of sources: advertisements placed around the medical center, presentations to medical center auxiliary groups, and lists of past study participants. Eligibility criteria included no chronic health conditions or chronic use of medications. Blood samples were obtained only at baseline, whereas we had longitudinal data on dietary phylloquinone intake and bone mass. The study protocol was approved by the Institutional Review Board at Cincinnati Children's Hospital Medical Center, and written informed consent was obtained from study subjects and their parents.

Four annual measurements of bone mineral content (BMC) and bone mineral density were obtained by using dual-energy X-ray absorptiometry (QDR 2000; Hologic, Waltham, MA) at study months 0, 12, 24, and 36 mo. Dual-energy X-ray absorptiometry scans were acquired of the total body and left hip in pencil beam mode. Data for the lumbar spine were determined from the regional analysis of the total body scan. The total body scans were analyzed with the enhanced total body software version V5.73, and the hip scans were analyzed with software version V4.76 according to the manufacturer's guidelines. Only the total hip region was used for analyses as recommended by McKay et al (21), because appropriate definition of hip subregions during growth is confounded by changes in hip geometry.

Height, weight, and physical maturation were measured annually at the time of the bone mass measurements. Height was measured with a wall-mounted stadiometer, and weight was measured with an electronic balance. Height and weight were measured in duplicate, and the average value was used. A single trained person assessed physical maturation according to Tanner Stage criteria for breast and pubic hair development.

With help from their parents, the subjects kept 3-d food records at each annual visit and midway between both the first and second study visits and the third and fourth study visits (ie, at study months 0, 6, 12, 18, 24, and 36). Completed food records were reviewed by trained personnel, and the nutrient content was determined with the use of the NUTRIENT DATA SYSTEM (NDS, version 2.91, food database 12A, nutrient database 27; University of Minnesota, Minneapolis). Phylloquinone intake was determined from the NDS supplemental database version 2.93. The NDS has been shown to provide accurate estimates of dietary phylloquinone content relative to direct laboratory analyses (22). Information on nutrient intake from supplements was obtained from the NDS and from nutrition labels. Nutrient intakes from the 0- and 36-mo food records were related to bone measurements made at study months 0 and 36, respectively. Nutrient intakes from the 6- and 12-mo food records were averaged and related to bone measurements at study month 12, and nutrient intakes from the 18- and 24-mo food records were averaged and related to bone measurements at study month 24.

Physical activity was assessed annually with the use of the Past Year Leisure Time Physical Activity Questionnaire, which was designed and validated for measurement of leisure-time physical activity in adolescents (23). We modified the questionnaire by adding activities common among younger children (eg, jumping rope, swim lessons, snow skiing, and playing on playground equipment). Using the questionnaire, the subjects and their parents were first asked to identify from a list of 32 activities those performed >10 times in the past year. Space was provided to write in other leisure-time activities that were not on the list but were frequently performed. Information on frequency and duration in the past year was obtained for all activities performed >10 times. For each of the selected activities, the subjects and their parents were asked to mark the months in the past year in which each activity was performed, the average frequency per week, and the approximate duration per episode. The subjects were encouraged to make 2 separate entries for those activities that were performed both on their own and as part of a team, eg, jogging or running compared with track. Activities were coded as bone loading (involved standing or walking), impact loading (involved jumping or running), or strengthening (eg, swimming and rowing). Physical activity was quantified as the number of hours per week spent on all activities and as the number of hours per week spent on each of the 3 categories mentioned above. The number of hours of activity was calculated as the average number of hours per week for each activity [(number of months in period × frequency/wk × 4.33 wk/mo × minutes/episode)/60 min/h]. To obtain the total number of activity hours, this was then summed across activities. This was done for each of the 3 categories.

Nonfasting blood samples were obtained at baseline from 222 of the 245 girls; blood samples were not obtained at the annual follow-up visits. The blood samples were processed within 30 min of collection, and aliquots of serum and plasma were kept frozen at −70 °C until analyzed. Three biochemical markers of bone turnover were measured in serum samples: bone-specific alkaline phosphatase (BSAP), osteocalcin, and cross-linked *N*-telopeptide of type 1 collagen (NTx) breakdown. BSAP and osteocalcin are proteins secreted by osteoblasts and are thought to reflect bone formation. BSAP was measured by using an enzyme immunoassay (Quidel/MetraBiosystems, San Diego). Osteocalcin was determined by using an immunoradiometric assay (ELSA-OSTEO kit; CIS-US, Bedford, MA). NTx, which is a marker of bone resorption, was measured by using a competitive-inhibition enzyme-linked immunosorbent assay (Osteomark sNTx kit; Ostex International Inc, Seattle).

We measured 2 biochemical indicators of vitamin K status: plasma concentrations of phylloquinone and serum concentrations of ucOC. Plasma phylloquinone concentrations were measured by using HPLC with fluorescence detection (24). Serum ucOC concentrations were determined by using a modification of the hydroxyapatite binding assay (5). Samples were treated with hydroxyapatite to bind the fully carboxylated osteocalcin, and the supernatant fluid was assayed for the remaining undercarboxylated osteocalcin as described above. The percentage of ucOC (%ucOC) was calculated as the ratio of unadsorbed to total osteocalcin. All laboratory measurements were performed in the laboratory of John Suttie at the University of Wisconsin-Madison.

The distributions of dietary phylloquinone intake, %ucOC, and concentrations of plasma phylloquinone and the bone turnover markers (BSAP, osteocalcin, and NTx) were skewed upwards, so transformed variables were used for all statistical analyses. Multiple regression was used to assess 4 associations. The first association was that between dietary phylloquinone intake and biochemical indicators of vitamin K status (plasma phylloquinone and %ucOC). Energy intake was included as a covariate

The American Journal of Clinical Nutrition

Downloaded from www.ajcn.org by on April 26, 2008

in these regression models to provide a measure of nutrient density. The second association was that between dietary phylloquinone intake and indicators of vitamin K status (plasma phylloquinone and %ucOC) and biochemical indicators of bone turnover (BSAP, osteocalcin, and NTx). Covariates that were considered in these models included age, Tanner stage, height, weight, dietary calcium intake, and physical activity because these factors could theoretically affect concentrations of bone turnover markers. Quadratic and cubic terms for age were included to better model the complex changes in bone turnover markers with age. Energy intake also was included in models with phylloquinone intake. Covariates were kept in the regression models for a given bone marker if they were significantly associated ($P < 0.05$) with that bone marker for either of the vitamin K status measures (ie, plasma phylloquinone and %ucOC). The third association was that between biochemical indicators of vitamin K status and BMC. Because of the known size-related effects on estimates of bone density by dual-energy X-ray absorptiometry, particularly in children (25), we chose to use BMC as the outcome variable and statistically adjust for bone area, by using the natural logarithm of both, as recommended by Prentice et al (26). Four BMC outcomes were considered: total hip, lumbar spine, total body, and total body minus the head. The latter was included because the proportion of the total body BMC accounted for by the head differs with age, and better predictive models can be obtained when the head is excluded (27). Potential covariates considered in the regression models of BMC included bone area, age, Tanner stage, weight, lean mass, fat mass, calcium intake, and physical activity. For all BMC analyses, regression models were first run with all relevant covariates, and then a reduced model that included only those variables that were associated with the BMC outcome at $P < 0.05$ was fitted. Because of the strong collinearity between weight and lean mass, only the variable with the strongest association with the outcome of interest in bivariate analyses was considered in developing regression models for that outcome. The fourth association was that between biochemical indicators of vitamin K status at baseline and change in the BMC of the total hip, lumbar spine, total body, and total body minus the head over the 4-y study. Potential covariates considered in the regression models included changes in bone area, lean mass, fat mass, height and Tanner stage, and the average (baseline and final) calcium intake and physical activity level. Baseline Tanner stage and age were also included to account for the complex changes in BMC that occurred over the 4-y period in the girls aged between 3 and 16 y at baseline.

Lastly, we performed longitudinal analyses to examine the relation between dietary phylloquinone intake and BMC. These analyses were performed by using mixed-effects regression models in which the subject was included as a random effect and all other variables were included as fixed effects. Variables considered as covariates in these analyses were bone area, age, Tanner stage, height, weight, lean mass, dietary calcium intake, and physical activity. Statistical analyses were performed with the use of JMP version 3 (SAS Institute Inc, Cary, NC) and SAS version 8.2 (SAS Institute Inc).

RESULTS

Descriptive characteristics of the study subjects, baseline measurements, and values for biochemical measures of vitamin K status and for bone turnover markers are given in **Table 1**. The

TABLE 1
Subject characteristics at baseline and values for biochemical measures of vitamin K status and for bone turnover markers[1]

	Median	Range
Age (y)	9.8	3.0–16.0
Weight (kg)	32.0	11.5–95.2
Height (cm)	137.6	85.8–173.6
Tanner stage (%)[2]		
1	49	—
2	14	—
3	12	—
4	11	—
5	13	—
Leisure-time physical activity (h/wk)		
Bone loading	1.0	0–10.6
Impact loading	1.4	0–20.7
Dietary phylloquinone intake (μg/d)	45	6–275
Energy intake (kcal/d)	1819	608–3635
Calcium intake (mg/d)	896	232–2166
Plasma phylloquinone (μg/mL)	0.67	0.02–2.81
Undercarboxylated osteocalcin (%)	13.6	5.8–27.3
Bone-specific alkaline phosphatase (U/L)	112	21–385
Osteocalcin (ng/mL)	97	11–230
NTx (nmol BCE/L)	80	6–215

[1] $n = 245$ except for the laboratory measurements [ie, plasma phylloquinone, undercarboxylated osteocalcin, bone-specific alkaline phosphatase, osteocalcin, and cross-linked *N*-telopeptide of type 1 collagen (NTx)], for which $n = 222$.

[2] The higher of breast stage and pubic hair stage.

median dietary phylloquinone intake in this study sample was 45 μg/d. The %ucOC and all of the bone turnover markers varied considerably as a function of age and maturation. Therefore, we adjusted for these effects in the analyses involving bone markers.

We examined the relation between phylloquinone intake estimated from the 3-d food records and biochemical indicators of vitamin K status. Phylloquinone intake was not associated with plasma phylloquinone concentrations ($R^2 = 0.01$, $P = 0.11$), even when adjusted for energy intake ($P = 0.70$). There was a weak but significant positive association between dietary phylloquinone intake and %ucOC ($R^2 = 0.02$, $P = 0.04$), but this relation disappeared once we adjusted for energy intake and age ($P = 0.15$). There was no association between serum %ucOC and plasma phylloquinone concentrations ($R^2 = 0.0007$, $P = 0.69$).

Dietary phylloquinone intake estimated from the 3-d food records was not associated with any of the markers of bone turnover, regardless of adjustment for age, Tanner stage, weight, height, physical activity, dietary calcium intake, or energy intake. The partial R^2 for vitamin K_1 was <0.01, and all P values were >0.20.

Biochemical measures of vitamin K status were significantly associated with bone turnover markers (**Table 2**); however, the total amount of variance in bone markers explained by measures of vitamin K status was small, ie, <3%. There was a significant inverse association between plasma phylloquinone and NTx concentrations when adjusted for age (cubic polynomial), Tanner stage, height, and weight. An increase in plasma phylloquinone concentration from the 10th percentile of the sample distribution (0.25 μg/mL) to the 90th percentile of the sample distribution (1.42 μg/mL) was associated with a 21.9% decrease

The American Journal of Clinical Nutrition

Downloaded from www.ajcn.org by on April 26, 2008

TABLE 2
Associations between biochemical markers of bone turnover and indicators of vitamin K status[1]

	Indicator of vitamin K status	
Bone turnover marker	Plasma phylloquinone	%ucOC[2]
NTx		
β (SE)	−1.5492 (0.5771)	0.6841 (0.3073)
P	0.007	0.03
Partial R^2	0.022	0.015
BSAP		
β (SE)	0.7114 (0.5366)	0.8113 (0.2798)
P	0.19	0.004
Partial R^2	0.006	0.026
Osteocalcin		
β (SE)	−0.7428 (0.3625)	—
P	0.04	—
Partial R^2	0.012	—

[1] The indicator of vitamin K status was the independent variable, and the bone turnover marker was the dependent variable. The regression coefficients were generated from transformed data. The square root transformation was used for all bone markers and indicators of vitamin K status. Direct interpretation of the coefficients requires back transformation to original units. All regression models included age (cubic polynomial), Tanner stage, height, and weight. %ucOC, percentage of undercarboxylated osteocalcin; NTx, cross-linked *N*-telopeptide of type 1 collagen; BSAP, bone-specific alkaline phosphatase.

[2] The regression model between %ucOC and osteocalcin was not run because of the numerical interdependence of the 2.

in serum NTx concentrations. In accordance, %ucOC was positively associated with serum NTx concentrations when adjusted for covariates ($P = 0.03$). An increase in %ucOC from the 10th percentile (9.0%) to the 90th percentile (20.6%) was associated with a 21.7% increase in NTx concentrations.

BSAP was not associated with plasma phylloquinone concentrations with or without adjustment for covariates ($P = 0.19$). However, BSAP was positively associated with %ucOC, and this relation remained significant ($P = 0.004$) when adjusted for age, maturation, weight, and height. An increase in %ucOC from the 10th to the 90th percentile was associated with a 22.0% increase in BSAP.

Osteocalcin was inversely associated with plasma phylloquinone concentrations when adjusted for covariates ($P < 0.04$). An increase in plasma phylloquinone concentration from the 10th to the 90th percentile was associated with a 9.8% decrease in osteocalcin concentrations. Regression models were not run between osteocalcin and %ucOC because of the numerical interdependence of the 2.

Indicators of vitamin K status were inconsistently associated with BMC and 4-y changes in BMC. Plasma phylloquinone and %ucOC were not associated with BMC of the total body, the total body minus the head, or the hip when adjusted for age, bone area, lean mass, pubertal maturation, physical activity, or dietary calcium intake ($P > 0.05$). In contrast with our hypotheses, lumbar spine BMC was inversely associated with plasma phylloquinone concentrations ($P = 0.03$) but was not associated with %ucOC ($P = 0.6$). An increase in plasma phylloquinone concentration from the 10th to the 90th percentile was associated with a 5.0% reduction in spine BMC when adjusted for bone area and other covariates.

Plasma phylloquinone concentrations were not associated with 4-y changes in BMC of the spine and hip but were positively associated with changes in BMC of the total body ($P = 0.056$) and of the total body minus the head ($P = 0.03$) when adjusted for covariates. An increase in plasma phylloquinone concentration from the 10th percentile to the 90th percentile was associated with a 5.0% greater 4-y gain in total body BMC and in BMC of the total body minus the head. Serum %ucOC was not associated with 4-y changes in BMC of the hip, total body, or total body minus the head. In contrast with our hypotheses, %ucOC was positively associated with 4-y changes in lumbar spine BMC ($P = 0.001$), and an increase in %ucOC from the 10th to the 90th percentile was associated with a 19.0% greater 4-y gain in lumbar spine BMC.

Dietary phylloquinone intake estimated from the food records was not associated with BMC of the total body, the total body minus the head, or the lumbar spine in the longitudinal analyses in which mixed-effects regression models were used. However, dietary phylloquinone intake was inversely associated with BMC of the hip ($P = 0.01$). An increase in dietary phylloquinone intake from the 10th percentile of the sample distribution (21 μg/d) to the 90th percentile of the sample distribution (89 μg/d) was associated with a 1.0% decrease in total hip BMC.

DISCUSSION

Maximizing accretion of bone mass during growth is increasingly being recognized as an important strategy for minimizing the consequences of age-related bone loss and decreasing osteoporotic fracture risk in the elder years. Dietary approaches to maximizing accretion of bone mass are attractive because of the potentially widespread applicability to both healthy and chronically ill children. Although calcium intake has been shown to affect accretion of bone mass (28–31), little information on the relation between the intake of other nutrients and bone mass in children is available. Vitamin K intake has been related to bone turnover (7, 11, 32–34), bone density (4, 13, 16, 35), and risk of hip fracture (11–15) in adults. To our knowledge, no published studies have examined the relation between dietary vitamin K intake and bone in healthy children. We found that better vitamin K status, as reflected by biochemical indicators, was associated with lower concentrations of biochemical markers of bone formation and bone resorption. The phylloquinone intake of our study subjects was intermediate between the intakes reported in the Total Diet Study and in the third National Health and Nutrition Examination Survey (NHANES III) for girls of similar age (2, 36), which makes our findings relevant to children consuming typical US diets. However, we did not find a consistent association between biochemical indicators of vitamin K status or dietary phylloquinone intake and bone mass in cross-sectional or longitudinal analyses.

We did not find an association between phylloquinone intake estimated from the 3-d food records and plasma phylloquinone concentrations or %ucOC. There are several potential reasons for this. Plasma phylloquinone concentrations change acutely in response to intake, and phylloquinone is cleared within 1–3 d after ingestion (3, 37, 38). In addition, day-to-day variability in phylloquinone intake is large (39). Some (40, 41) but not all studies (37) have shown that the bioavailability of phylloquinone from vegetable sources also varies with the fat composition of the diet. Dietary intake of dihydrophylloquinone, the hydrogenated form

The American Journal of Clinical Nutrition

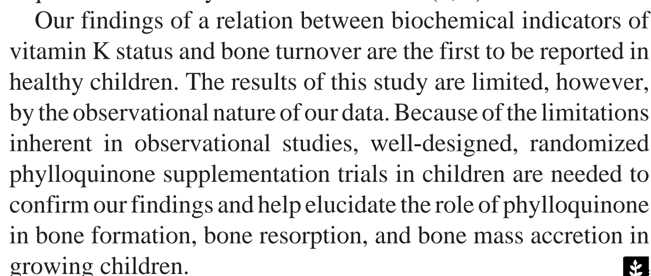
Downloaded from www.ajcn.org by on April 26, 2008

of phylloquinone, was not measured, and dihydrophylloquinone has been reported to constitute 30% of total phylloquinone intake in children (36). Although dihydrophylloquinone affects carboxylation of hepatic proteins, it has not been shown to affect carboxylation of osteocalcin or concentrations of bone turnover markers (7). Other studies that examined the relation between phylloquinone intake and plasma phylloquinone found either a weak association (39, 42, 43) or no association (44) between the 2. We also did not measure menaquinone intake, because comprehensive information on dietary sources of menaquinones is not available. However, milk, cheese, and meat contain menaquinones, and our study subjects commonly consumed these foods. In theory, menaquinone intake might have affected %ucOC. Food records used to estimate the nutrient intake of children may also be subject to error because parents do not observe all of their child's eating occasions, and children have differing abilities to recall foods and amounts eaten. Thus, it is possible that phylloquinone intake estimated from a 3-d food record may not be adequate to reflect vitamin K nutriture, particularly in children.

Because of the lack of association between dietary phylloquinone intake and indicators of vitamin K status, it was not surprising that we did not find an association between dietary phylloquinone intake and bone turnover markers or any consistent findings between phylloquinone intake and bone mass. Phylloquinone intake was not associated with total body or lumbar spine bone mass but was inversely associated with hip bone mass in longitudinal analyses. The mechanism for this relation is unclear because the purported roles of phylloquinone involve facilitating bone mineralization, increasing osteoblastogenesis, and decreasing osteoclastogenesis, all of which should result in a greater bone mass with higher phylloquinone intake (10). Thus, whether this inverse association was a biological phenomenon or a spurious finding is unknown. In our analyses, we statistically adjusted for factors that have consistently been shown to affect bone mass in children, but it is possible that there were other important factors that covaried with phylloquinone intake but that we did not measure.

We found that better vitamin K status was associated with lower bone turnover as concentrations of both markers of bone resorption and markers of bone formation decreased. Plasma phylloquinone was inversely associated with NTx and osteocalcin concentrations, and %ucOC was positively associated with NTx and BSAP concentrations. Although some studies in adults found that provision of supplemental phylloquinone reduces biochemical markers of bone resorption (7, 32, 33), other studies found no effect of phylloquinone supplementation on bone resorption markers (5, 6, 16). Inconsistent effects of phylloquinone supplemention on bone formation markers have also been reported. Osteocalcin concentrations have been reported to decrease (5, 7) or increase (6, 32, 33), and alkaline phosphatase concentrations have been reported to increase (33) or not change (5–7, 16). Bone formation and bone resorption are coupled processes. With the use of biochemical markers alone, it is unknown whether vitamin K status initially affects bone formation or bone resorption, which in turn results in comparable changes in the other, especially in children. In addition, BSAP concentrations, %ucOC, and total osteocalcin concentrations had complex and slightly different relations with age and pubertal maturation, which makes the interpretation of these markers in a cross-sectional study of children difficult. Despite our extensive statistical modeling efforts, we may not have fully accounted for these relations in our analyses. Whether vitamin K status improved in parallel with overall nutritional status, such that the association between vitamin K status and bone turnover was just a reflection of better nutritional status overall, is also unknown.

Although we found relations between biochemical indicators of vitamin K status and markers of bone formation and bone resorption, we did not find consistent associations between biochemical indicators of vitamin K status and bone mass. One possibility is that the biochemical measures of vitamin K status reflect relatively acute effects of the vitamin on bone turnover, and longer-term effects of vitamin K are needed to result in measurable positive effects on bone mass. Alternately, larger intakes of phylloquinone may be needed to significantly affect bone mass acquisition. The dietary phylloquinone intakes of our study subjects were similar to those of children in the US. The current recommended adequate intake of 55–75 μg/d for this age range was based on intakes of healthy children from NHANES III because little is known about the relation between phylloquinone intake and indicators of vitamin K status in children (2). The true requirement for phylloquinone may be higher than the current recommendation. Support for this comes from the fact that osteocalcin was not fully carboxylated in our study subjects; thus, the biochemical need for vitamin K was not met. A supplementation trial in postmenopausal women who were randomly assigned to receive 1 mg phylloquinone/d for 3 y showed a positive effect on bone density (16). Randomized trials using large doses of a menaquinone (ie, 45 mg MK-4/d) that are $\approx$500 times the recommended intake for adults (ie, 90–120 μg/d) also showed positive effects on bone density (8, 17–20). Whether phylloquinone and pharmacologic amounts of menaquinones operate through similar mechanisms to affect bone is unknown. Lastly, we did not have measures of vitamin D status in our study subjects. Vitamin D has important effects on bone, and studies have found that vitamin D, in addition to phylloquinone, may be required for carboxylation of osteocalcin (4, 6).



Our findings of a relation between biochemical indicators of vitamin K status and bone turnover are the first to be reported in healthy children. The results of this study are limited, however, by the observational nature of our data. Because of the limitations inherent in observational studies, well-designed, randomized phylloquinone supplementation trials in children are needed to confirm our findings and help elucidate the role of phylloquinone in bone formation, bone resorption, and bone mass accretion in growing children.

We thank Donna C Bianchi, who was responsible for subject recruitment and data collection, Cindy Deeks for calculating vitamin K_1 intake from the food records, and John Suttie and Jean Engleke for performing the laboratory analyses.

HJK was principal investigator of the study; was responsible for study design, interpretation of results, and manuscript preparation; and provided oversight for data collection and data analyses. JCK and JB were responsible for data analyses and interpretation. JGE was responsible for the study hypotheses and interpretation of the data. JGE is an employee of DSM Nutritional Products, Inc, which was one of the sponsors of this project. There were no other reported conflicts of interest.

The American Journal of Clinical Nutrition

Downloaded from www.ajcn.org by on April 26, 2008

REFERENCES

1. Vermeer C, Jie K-SG, Knapen MHJ. Role of vitamin K in bone metabolism. Annu Rev Nutr 1995;15:1–22.
2. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press, 2000.
3. Sokoll LJ, Booth SL, O'Brien E, Davidson KW, Tsaioun KI, Sadowski JA. Changes in serum osteocalcin, plasma phylloquinone, and urinary γ-carboxyglutamic acid in response to altered intakes of dietary phylloquinone in human subjects. Am J Clin Nutr 1997;65:779–84.
4. Schaafsma A, Muskiet FAJ, Storm H, Hofstede GJH, Pakan I, Van der Veer E. Vitamin D_3 and vitamin K_1 supplementation of Dutch postmenopausal women with normal and low bone mineral densities: effects on serum 25-hydroxyvitamin D and carboxylated osteocalcin. Eur J Clin Nutr 2000;54:626–31.
5. Binkley NC, Krueger DC, Engleke JA, Foley AL, Suttie JW. Vitamin K supplementation reduces serum concentrations of under-γ-carboxylated osteocalcin in healthy young and elderly adults. Am J Clin Nutr 2000; 72:1523–8.
6. Douglas AS, Robins SP, Hutchison JD, Porter RW, Stewart RW, Reid DM. Carboxylation of osteocalcin in post-menopausal osteoporotic women following vitamin K and D supplementation. Bone 1995;17:15–20.
7. Booth SL, Lichtenstein A, O'Brien-Morse M, et al. Effects of a hydrogenated form of vitamin K on bone formation and resorption. Am J Clin Nutr 2001;74:783–90.
8. Shiraki M, Shiraki Y, Aoki C, Miura M. Vitamin K_2 (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. J Bone Miner Res 2000;15:515–21.
9. Haffa A, Krueger D, Bruner J, et al. Diet- or warfarin-induced vitamin K insufficiency elevates circulating undercarboxylated osteocalcin without altering skeletal status in growing female rats. J Bone Miner Res 2000;15:872–8.
10. Koshihara Y, Hoshi K, Okawara R, Ishibashi H, Yamamoto S. Vitamin K stimulates osteoblastogenesis and inhibits osteoclastogenesis in human bone marrow cell culture. J Endocrinol 2003;176:339–48.
11. Szulc P, Chapuy M-C, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. J Clin Invest 1993;91:1769–74.
12. Hodges SJ, Akesson K, Vergnaud P, Obrant K, Delmas PD. Circulating levels of vitamin K_1 and K_2 decreased in elderly women with hip fractures. J Bone Miner Metab 1993;8:1241–5.
13. Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: The EPIDOS Study. J Clin Endocrinol Metab 1997;82:719–24.
14. Feskanich D, Weber P, Willett WC, Rockett H, Booth SL, Colditz GA. Vitamin K intake and hip fractures in women: a prospective study. Am J Clin Nutr 1999;69:74–9.
15. Booth SL, Tucker KL, Chen H, et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr 2000;71:1201–8.
16. Braam LA, Knapen MHJ, Guesens P, et al. Vitamin K_1 supplementation retards bone loss in postmenopausal women between 50 and 60 years of age. Calcif Tissue Int 2003;73:21–6.
17. Hidaka T, Hasegawa T, Fujimara M, Sakai M, Saito S. Treatment for patients with postmenopausal osteoporosis who have been placed on HRT and show a decrease in bone mineral density: effects of concomitant administration of vitamin K_2. J Bone Miner Metab 2002;20:235–9.
18. Sato Y, Honda Y, Kaji M, et al. Amelioration of osteoporosis by menatetrenone in elderly female Parkinson's disease patients with vitamin D deficiency. Bone 2002;31:114–8.
19. Ushiroyama T, Ikeda A, Ueki M. Effect of continuous combined therapy with vitamin K_2 and vitamin D_3 on bone mineral density and coagulofibrinolysis function in postmenopausal women. Maturitas 2002;41:211–21.
20. Shiomi S, Nishiguchi S, Kubo S, et al. Vitamin K_2 (menatetrenone) for bone loss in patients with cirrhosis of the liver. Am J Gastroenterol 2002;97:978–81.
21. McKay HA, Petit MA, Bailey DA, Wallace WM, Schutz RW, Khan KM. Analysis of proximal femur DXA scans in growing children: comparisons of different protocols for cross-sectional 8-month and 7-year longitudinal data. J Bone Miner Res 2000;15:1181–8.
22. McKeown NM, Rasmussen HM, Charnley JM, Wood RJ, Booth SL. Accuracy of phylloquinone (vitamin K-1) data in 2 nutrient databases as determined by direct laboratory analysis of diets. J Am Diet Assoc 2000;100:1201–4.
23. Aaron DJ, Kriska AM, Dearwater SR, Cauley JA, Metz KF, LaPorte RE. Reproducibility and validity of an epidemiologic questionnaire to assess past year physical activity in adolescents. Am J Epidemiol 1995;142: 191–201.
24. Haroon Y, Bacon DS, Sadowski JA. Liquid chromatographic determination of vitamin K_1 in plasma with fluorometric detection. Clin Chem 1986;32:1925–9.
25. Nelson DA, Koo WWK. Interpretation of absorptiometric bone mass measurements in the growing skeleton: issues and limitations. Calcif Tissue Int 1999;65:1–3.
26. Prentice A, Parsons TJ, Cole TJ. Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants. Am J Clin Nutr 1994;60:837–42.
27. Taylor A, Konrad PT, Norman ME, Karcke HT. Total body bone mineral density in young children: influence of head bone mineral density. J Bone Miner Res 1997;12:652–5.
28. Lloyd T, Andon MB, Rollings N, et al. Calcium supplementation and bone mineral density in adolescent girls. JAMA 1993;270:841–4.
29. Johnston CC Jr, Miller JZ, Slemenda CW, et al. Calcium supplementation and increases in bone mineral density in children. N Engl J Med 1992;327:82–7.
30. Dibba B, Prentice A, Ceesay M, Stirling DM, Cole TJ, Poskitt EM. Effect of calcium supplementation on bone mineral accretion in Gambian children accustomed to a low-calcium diet. Am J Clin Nutr 2000;71:544–9.
31. Bonjour JP, Carrie AL, Ferrari S, et al. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. J Clin Invest 1997;99:1287–94.
32. Craciun AM, Wolf J, Knapen MHJ, Brouns F, Vermeer C. Improved bone metabolism in female elite athletes after vitamin K supplementation. Int J Sports Med 1998;19:479–84.
33. Knapen MHJ, Jie K-SG, Hamulyak K, Vermeer C. Vitamin K-induced changes in markers for osteoblast activity and urinary calcium loss. Calcif Tissue Int 1993;53:81–5.
34. Sugiyama T, Kawai S. Carboxylation of osteocalcin may be related to bone quality: a possible mechanism of bone fracture prevention by vitamin K. J Bone Miner Metab 2001;19:146–9.
35. Booth SL, Broe KE, Gagnon DR, et al. Vitamin K intake and bone mineral density in women and men. Am J Clin Nutr 2003;77:512–6.
36. Booth SL, Pennington JA, Sadowski JA. Dihydro-vitamin K_1: primary food sources and estimated dietary intakes in the American diet. Lipids 1996;31:715–20.
37. Booth SL, O'Brien-Morse ME, Dallal GE, Davidson KW, Gundberg CM. Response of vitamin K status to different intakes and sources of phylloquinone-rich foods: comparison of younger and older adults. Am J Clin Nutr 1999;70:368–77.
38. Olson RE, Chao J, Graham D, Bates MW, Lewis JH. Total body phylloquinone and its turnover in human subjects at two levels of vitamin K intake. Br J Nutr 2002;87:543–53.
39. Booth SL, Sokoll LJ, O'Brien ME, Tucker K, Dawson-Hughes B, Sadowski JA. Assessment of dietary phylloquinone intake and vitamin K status in postmenopausal women. Eur J Clin Nutr 1995;49:832–41.
40. Gijsbers BL, Jie KS, Vermeer C. Effect of food composition on vitamin K absorption in human volunteers. Br J Nutr 1996;76:223–9.
41. Booth S, Lichtenstein A, Dallal G. Phylloquinone absorption from phylloquinone-fortified oil is greater than from a vegetable in younger and older men and women. J Nutr 2002;132:2609–12.
42. Booth SL, Tucker KL, McKeown NM, Davidson KW, Dallal GE, Sadowski JA. Relationships between dietary intakes and fasting plasma concentrations of fat-soluble vitamins in humans. J Nutr 1997;127:587–92.
43. McKeown N, Jacques P, Gundberg C, et al. Dietary and nondietary determinants of vitamin K biochemical measures in men and women. J Nutr 2002;132:1329–34.
44. Schurgers LJ, Geleijnse JM, Grobbee DE, et al. Nutritional intake of vitamins K_1 (Phylloquinone) and K2 (Menaquinone) in the Netherlands. J Nutr Environ Med 1999;9:115–22.

The American Journal of Clinical Nutrition

Downloaded from www.ajcn.org by on April 26, 2008