Vitamin K Status May Be an Important Determinant of Childhood Bone Health
Kevin D. Cashman, PhD

There has been relatively little research emphasis on the effect of vitamin K on bone health during childhood. Recent interesting data from an observational study of healthy young girls (aged 3–16 years) in the United States suggests that better vitamin K status is associated with lower levels of markers of bone resorption and bone formation, suggesting a lower rate of bone turnover. However, in that study, vitamin K status was not consistently associated with bone mineral content or gain in bone mineral content over 4 years. There is a need for randomized phylloquinone supplementation trials to better understand the role of vitamin K on bone acquisition in growing children.

Key words: vitamin K status, bone mass, children

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INTRODUCTION

Vitamin K is a cofactor for vitamin K-dependent carboxylase, a microsomal enzyme that facilitates the post-translational conversion of glutamyl to γ-carboxyglutamyl residues.1 Its classic role in this respect involves the synthesis of several coagulation factors, including plasma pro-coagulants, prothrombin (factor II) and factors VII, IX, and X, and anticoagulants (proteins C and S).2–4 The maintenance of plasma prothrombin concentrations was the basis for the recommended dietary intake value of 1 mg/kg body weight per day set by the National Research Council in 1989 in the United States5 and by the Department of Health in 1991 in the United Kingdom.6 More recently, the identification of γ-carboxyglutamyl-containing proteins in bone, notably osteocalcin and matrix γ-carboxyglutamyl protein (also known as matrix Gla protein), has generated much interest in the role of vitamin K in bone metabolism and bone health.4,7,8,9 Furthermore, it has been suggested that dietary phylloquinone (vitamin K1) levels that are sufficient to maintain normal blood coagulation may be suboptimal for adult bone health.10–12

VITAMIN K AND ADULT BONE HEALTH

The circulating concentration of under-γ-carboxylated osteocalcin (ucOC), a sensitive marker of vitamin K nutritional status,13 has been reported to be a marker of hip fracture risk and a predictor of bone mineral density (BMD) (Table 1).14–23 Moreover, the findings of two large, prospective cohort studies: the Nurses’ Health Study21 and the Framingham Heart Study20 support an association between relative risk of hip fracture and vitamin K1 intake (Table 1). Furthermore, Booth et al.25 recently reported that adult women (from the Framingham Heart Study) in the lowest quartile of vitamin K1 intake (<70 μg/d) had significantly lower (P < 0.005) mean BMD of the femoral neck and spine than did those in the highest quartile of vitamin K1 intake (309 μg/d), an association that was not evident in men.

The mechanism of action of vitamin K on BMD and fracture remains unclear. The rate of bone turnover is a major determinant of BMD,24 and any factor that influences this rate may in turn impact BMD and fracture risk. Booth et al.25 showed that 15 days of dietary phylloquinone depletion (intake restricted to 11 μg/d) led to an increased rate of bone turnover in young adults, as assessed by serum total osteocalcin and urinary type I collagen cross-linked N-telopeptides; these markers were subsequently normalized by 10 days of phylloquinone repletion (approximately 200 μg/d). Binkley et al.26 showed that daily supplementation of healthy young and elderly adults with 1 mg phylloquinone for 2 weeks...
reduced serum total osteocalcin but did not alter other markers of bone turnover.

Phylloquinone supplementation (1 mg/d co-administered with minerals and vitamin D) of postmenopausal women has recently been reported to have no effect on markers of bone turnover at the end of 3 years, even though there was a significant reduction in total osteocalcin (but not other bone markers) after 1 year of supplementation.27

**VITAMIN K AND CHILDHOOD BONE HEALTH**

There has been much less research emphasis on the effect of vitamin K on bone health in earlier life.

<table>
<thead>
<tr>
<th>Study Design</th>
<th>Subjects (n)</th>
<th>Major Findings</th>
<th>Reference</th>
</tr>
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<tr>
<td>Cross-sectional</td>
<td>Children (14)</td>
<td>Ratio of serum carboxylated osteocalcin to serum intact osteocalcin was positively related to the ultrasound velocity of right tibia</td>
<td>Sugiyama et al.14</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>Japanese postmenopausal women (74)</td>
<td>Natto consumption was related to high serum menaquinone-7 concentrations and inversely associated with hip fracture incidence</td>
<td>Kaneki et al.15</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>Postmenopausal women (113)</td>
<td>ucOC was inversely correlated with metacarpal bone mass</td>
<td>Jie et al.16</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>Elderly women (98)</td>
<td>ucOC was inversely correlated with bone mineral density (BMD) of hip</td>
<td>Szulc et al.17</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>Adults (148)</td>
<td>Those with lower BMD had a mean vitamin K intake of 161 μg/d; those with higher BMD, 217 μg/d</td>
<td>Vermeer et al.18</td>
</tr>
<tr>
<td>Framingham Heart Study (cross-sectional)</td>
<td>Women (1479), men (1112) (mean age: 59 years)</td>
<td>Women in quartile 1 (mean vitamin K intake: 70.2 μg/d) had significantly lower BMD of femoral neck and spine compared with those in quartile 4 (mean vitamin intake: 309 μg/d); there was no significant association between vitamin K intake and BMD in men</td>
<td>Booth et al.19</td>
</tr>
<tr>
<td>Framingham Heart Study (prospective 7-year followup)</td>
<td>Women (553), men (335) (mean age: 75.2 years)</td>
<td>Those in quartile 4 (median vitamin K intake: 254 μg/d) had significantly reduced risk of fractures by 65% compared with quartile 1 (median vitamin intake: 56 μg/d); there was no significant association between vitamin K intake and BMD</td>
<td>Booth et al.20</td>
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<tr>
<td>Nurses’ Health Study (prospective 10-year followup)</td>
<td>Women (72, 327) (age range: 38–69 years)</td>
<td>The risk of a hip fracture was significantly reduced by 30% in women with vitamin K intake &gt; 190 μg/d</td>
<td>Feskanić et al.21</td>
</tr>
<tr>
<td>Prospective 3-year study</td>
<td>Elderly women (183)</td>
<td>The risk of hip fracture was 3.1-fold higher in women with raised ucOC levels at the start of the study</td>
<td>Szulc et al.22</td>
</tr>
<tr>
<td>Prospective</td>
<td>Elderly women (195)</td>
<td>Those patients who at the start of the study had higher ucOC values had a 5.9-fold increased relative risk of having a hip fracture during the observation period</td>
<td>Szulc et al.23</td>
</tr>
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</table>
Gaining an understanding of the role of vitamin K in bone metabolism and bone mass in early life is important, because finding new strategies to maximize the accretion of bone during growth may help to reduce the risk of osteoporosis in later life. Recently, Kalkwarf et al.28 investigated the effect of vitamin K intake and status on bone turnover and bone mass in healthy young girls in the United States. This was the first study to examine the relationship between vitamin K nutritive status and bone integrity in healthy children. The girls, aged 3 to 16 years (n = 245) and predominantly of Caucasian origin (97%), were participants in a longitudinal 4-year study of normal bone mass accretion during childhood and adolescence. Bone mineral content (BMC) and BMD measurements, together with measures of height, weight, and physical maturation (Tanner scores for breast and pubic hair development), were taken at baseline and then annually. Three-day food records were kept by the subjects with the help of their parents at each annual visit as well as at months 6 and 18. Physical activity was assessed annually by use of a questionnaire, again with the help of parents. Non-fasting blood samples were obtained from 222 of the 245 girls at baseline only. These were processed to serum and plasma and stored at −70°C until required for analysis of indices of vitamin K status and bone turnover. Bone turnover markers included serum levels of bone-specific alkaline phosphatase and total osteocalcin (both markers of bone formation),24 as well as cross-linked N-telopeptide of type I collagen (NTx), a marker of bone resorption.24 Biochemical indicators of vitamin K status included plasma concentrations of phylloquinone (measured by HPLC) and serum ucOC (measured by hydroxyapatite binding assay and expressed as the ratio of unadsorbed to total osteocalcin).

The aim of the study by Kalkwarf et al.28 was to assess two important research questions pertaining to the role of vitamin K in childhood bone health: 1) is greater phylloquinone intake, or better vitamin K status, associated with higher bone formation and lower bone resorption?, and 2) is greater phylloquinone intake, or better vitamin K status, is associated with higher bone mass? To address these questions, the biochemical and dietary data were used in four multiple regression models to assess four associations:

1. The association between dietary phylloquinone intake and biochemical indicators of vitamin K status (including energy intake as a covariate);
2. The association between dietary phylloquinone intake and indicators of vitamin K status and biochemical indicators of bone turnover (including covariates such as age, Tanner stage, height, weight, dietary calcium intake, and physical activity, and energy intake in models with phylloquinone intake);
3. The association between biochemical indicators of vitamin K status and BMC (adjusted for bone area), which was assessed at various skeletal sites: total hip, lumbar spine, total body, and total body minus the head; and
4. The association between biochemical indicators of vitamin K status at baseline and change in BMC of the total hip, lumbar spine, total body, and total body minus head over the 4-year study.

The investigators also performed longitudinal analyses to examine the relationship between dietary phylloquinone intake and BMC (which was adjusted for covariates including bone area, age, Tanner stage, height, weight, lean mass, dietary calcium intake, and physical activity).

The median dietary phylloquinone intake of the young girls (aged 3–16 years) was 45 mg/d (range 6–275 mg/d). The current US recommended intakes (Adequate Intake, or AI, values) for vitamin K for children aged 1 to 3 years, 4 to 8 years, 9 to 13 years, and 14 to 18 years are 30, 55, 60, and 75 mg/d, respectively.4 While the previous US and current UK recommendation of 1 μg/kg body weight/d5,6 is aimed at maintaining plasma prothrombin concentrations in adults and may not be appropriate for use in children and adolescents, the US Food and Nutrition Board established the above AI values based on median intakes for males and females reported by the NHANES III and rounding to the nearest 5 mg.5 Unfortunately, in the Kalkwarf et al. study,28 intake estimates were not reported by age groupings; rather, the estimate reflects the median intakes of girls aged 3 to 16 years. Furthermore, there is no information presented on what percentage of each age grouping (or of the entire group) failed to meet the relevant AI value.

There was no association between dietary phylloquinone intake and plasma phylloquinone concentration, whereas there was only a weak positive association ($R^2 = 0.02; P = 0.04$) between dietary phylloquinone intake and %ucOC, which became non-significant when data were adjusted for energy intake and age.28 Furthermore, there was no association between serum %ucOC and plasma phylloquinone concentration. The authors offer several potential reasons for this lack of association, including: 1) the acute change in plasma phylloquinone concentrations in response to intake, as well as the rapid (1–3 d) clearance of phylloquinone after ingestion; 2) the large day-to-day variation in phylloquinone intake; 3) the bioavailability of phylloquinone from vegetable sources as influenced by fat composition of the diet; and 4) the potential impact of dihydrophylloquinone.
the hydrogenated form of phylloquinone, and/or menaquinones (vitamin K₃) on vitamin K status. In addition, vitamin K intake was assessed by 3-day food records, which the authors acknowledge may not be adequate to reflect vitamin K nutriture in children. This might also explain the lack of association between intake estimates and vitamin K status indices.

In a recent preliminary study of vitamin K nutriture of young Irish girls, we chose to use the 14-day diet history to address the considerable day-to-day variability that is evident in vitamin K intake. In our study, there was a negative correlation between phylloquinone intake and serum ucOC concentrations in Irish girls aged 11 to 13 years (personal communication from Dr. Mairead Kiely).

In light of the lack of association between dietary phylloquinone intake and vitamin K status measures, it may not be entirely surprising that dietary phylloquinone intake was not associated with any of the markers of bone turnover (even after adjustment for age, Tanner stage, weight, height, physical activity, dietary calcium, or energy intake). On the other hand, after adjustment for covariates, the two biochemical measures of vitamin K status were significantly (plasma phylloquinone inversely and serum %ucOC positively) (P < 0.03) associated with serum N-telopeptide of type I collagen. Serum %ucOC, but not plasma phylloquinone, was positively associated (P < 0.004) with serum bone-specific alkaline phosphatase, while plasma phylloquinone was inversely associated (P < 0.04) with serum osteocalcin.

The mechanism(s) by which vitamin K status influences bone turnover is unclear, but the authors point to evidence from human bone marrow cell culture studies suggesting that vitamin K increases osteoblastogenesis and decreases osteoclastogenesis. Weber has referred to in vitro and/or animal data suggesting that vitamin K may positively influence calcium balance and inhibit production of prostaglandin E2 and interleukin-6, potent bone-resorbing agents, as additional possible mechanisms by which vitamin K may influence bone turnover. A reduction in the rate of bone turnover, arising from higher phylloquinone intake, would usually correspond to greater bone mass. However, this was not evident in the study by Kalkwarf et al.

Biochemical measures of vitamin K status were inconsistently associated with BMC and 4-year changes in BMC. Plasma phylloquinone and serum %ucOC were not associated with adjusted BMC of the total body, the total body minus the head, or the hip. On the other hand, surprisingly, lumbar spine BMC was inversely associated with plasma phylloquinone concentrations (P = 0.03) but was not associated with %ucOC.

Plasma phylloquinone concentrations were not associated with 4-year changes in BMC of the spine and hip, but were positively associated with changes in adjusted BMC of the total body (P = 0.056) and of the total body minus the head (P = 0.03). Serum %ucOC was not associated with 4-year changes in BMC of the hip, total body, or total body minus the head. However, again somewhat surprisingly, serum %ucOC was positively associated with 4-year changes in lumbar spine BMC (P = 0.001). It is not clear whether the associations between vitamin K status indices and BMC of the lumbar spine were true biological phenomena or spurious findings.

Schoon et al. demonstrated a significant independent and inverse correlation between serum ucOC and BMD at the lumbar spine of patients with longstanding Crohn’s disease (a group at increased risk of vitamin K deficiency), even though there was no relationship with BMD at the femoral neck or total body. The authors suggest that the trabecular bone of the spine is metabolically more active than the cortical bone of the hip and thus may be more susceptible to vitamin K deficiency. Furthermore, we have recently shown that serum ucOC is independently correlated with the rates of bone resorption and bone formation, and thus the rate of bone turnover (as assessed using sensitive and specific biochemical markers) in Crohn’s disease patients. While these markers reflect bone turnover throughout the skeletal system, the dominant source is the more metabolically active sites, including the spine. Thus, these findings, albeit in patients at increased risk of vitamin K deficiency, would cast some doubt on the associations between vitamin K status measures and lumbar spine BMC in healthy girls reported in the Kalkwarf et al. study.

Dietary phylloquinone intake was not found to be associated with BMC of the total body, total body minus head, or the lumbar spine, but was surprisingly inversely associated with BMC of the hip (P = 0.01). For illustrative purposes, the authors report examples of significant associations between the increase in vitamin K status measure/dietary phylloquinone intake (from the 10th percentile of the sample to the 90th percentile of the sample distribution) and percentage change in bone health index (Table 2).

The reasons for the apparent reduction in bone turnover rate with better vitamin K status and either the lack of effect or apparent negative effect on BMC of some skeletal sites are unclear. The authors suggest that one possibility is that the biochemical measures of vitamin K status reflect relatively acute effects of vitamin K on bone turnover, and longer-term effects of vitamin K are needed to result in measurable positive effects on bone mass. The findings of Braam et al. showing that phylloquinone supplementation daily for 3 years (co-administered with minerals and vitamin D) reduced post-
menopausal bone loss (femoral neck) may support this notion. However, phylloquinone supplementation for 3 years had no effect on markers of bone turnover in that study. Alternatively, the authors suggest that larger intakes of phylloquinone may be needed to significantly affect bone mass acquisition. Various vitamin K intakes have been reported at which risk of low BMD or fracture are significantly reduced in adults/elderly (Table 1). However, the vitamin K intake at which a reduction in risk is evident may differ by age group. It is also worth emphasizing that changes in the levels of biochemical markers of bone formation and bone resorption, which are reflective of the rate of bone turnover (bone remodeling) in adulthood, may be more difficult to interpret in early childhood due to the possible continuing process of bone modeling (i.e., growth and development).

The authors also mention that because serum osteocalcin was not fully γ-carboxylated in their study, this may suggest that the biochemical need for vitamin K was not met in the young girls. However, it still has not been established whether complete γ-carboxylation of osteocalcin is necessary, or desirable, for optimal bone health. Furthermore, Binkley et al. recently reported that phylloquinone intakes of 1000 or 2000 mg/d did not lead to complete γ-carboxylation of serum osteocalcin; they did, however, appear to lead to a plateau in γ-carboxylation.

**CONCLUSIONS**

The study of Kalkwarf et al. was the first to investigate the impact of vitamin K intake and status on indices of bone health in children and adolescents, important life stages in terms of bone mass accretion. Their findings suggest that better vitamin K status is associated with decreased bone turnover in healthy girls consuming a typical US diet. However, in that study, vitamin K status was not consistently associated with BMC. While this study was timely in that it addressed the important issue of vitamin K status and bone health in younger life, it was nonetheless observational in nature. This inevitably raises the issue of causal relationships and proof of causality. The authors acknowledged this fact, and posed a pertinent question of 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.

In terms of proof of causality, there is a general consensus that there is a need for well-designed, randomized phylloquinone supplementation trials in adults, and now also in children, to confirm observational findings and the role of vitamin K in bone metabolism and mass in healthy subjects. While we await such intervention studies, it is still advisable to develop dietary strategies that will allow adults and children to meet their dietary requirements. Phylloquinone is ubiquitously distributed in the diet; however, the range of concentrations in different food groups is very wide. We have found that vegetables and vegetable dishes, in particular green vegetables, were the main dietary sources of phylloquinone in the Irish girls (contributing about 53% to total intake; personal communication from Dr. Mairead Kiely). Thus, any advice on how to achieve the dietary requirement for phylloquinone during childhood will include the recommendation to consume adequate amounts of vegetables and, in particular, green vegetables.

**ACKNOWLEDGEMENT**

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**Table 2. Impact of Improving Vitamin K Intake/Status* on Bone Health Indices in Girls**

<table>
<thead>
<tr>
<th>Measurement Method</th>
<th>10th</th>
<th>90th</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma K(_1) (µg/mL)</td>
<td>0.25</td>
<td>1.42</td>
<td>21.9% decrease in serum NTx</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.8% decrease in serum tOc</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5% reduction in LS-BMC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5% greater 4-y gain in TB- and TB-H BMC</td>
</tr>
<tr>
<td>Serum %ucOC</td>
<td>9.0</td>
<td>20.6</td>
<td>21.7% increase in serum NTx</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22.0% increase in serum BSAP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19.0% greater 4-y gain in LS-BMC</td>
</tr>
<tr>
<td>Dietary K(_1) (µg/d)</td>
<td>21</td>
<td>89</td>
<td>1.0% decrease in TH-BMC</td>
</tr>
</tbody>
</table>

K\(_1\) = phylloquinone; NTx = type I collagen cross-linked N-telopeptides; tOc = total osteocalcin; LS-BMC = BMC of the lumbar spine; TB- and TB-H BMC = BMC of the total body and total body minus the head, respectively; BSAP = bone-specific alkaline phosphatase; TH-BMC = BMC of the total hip.

*Increasing from 10th to 90th percentile of sample distribution.
REFERENCES


