Does high vitamin K₁ intake protect against bone loss in later life?

Kevin D Cashman and Eibhlis O’Connor

The findings of a number of cross-sectional studies suggest benefits of high phylloquinone (vitamin K₁) intake on bone health in later life. Until recently these observational data were supported by the findings of an intervention study that showed a protective role for vitamin K₁ (together with calcium, magnesium, zinc, and vitamin D₃) on bone loss over 3 years in early postmenopausal women. Over the last 18 months, two further important intervention studies have been published, which investigated the effect of vitamin K₁ on bone loss in older subjects. These two studies add to the evidence-base but cast some doubt on the benefits of high vitamin K₁ intake on bone health in later life.

© 2008 International Life Sciences Institute

INTRODUCTION

Vitamin K is a cofactor for the vitamin K-dependent carboxylase, a microsomal enzyme that facilitates the post-translational conversion of glutamyl to γ-carboxyglutamyl residues.¹ 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.²⁻⁵ Furthermore, it has been suggested that dietary phylloquinone (vitamin K₁) levels that are sufficient to maintain normal coagulation (the basis for the recommended dietary intake value of 1 μg/kg body weight per day, and which still exists as the recommendation in the United Kingdom⁶ and the European Union⁷) may be suboptimal for adult bone health.⁸⁻¹⁰ An overview of the cross-sectional studies forming the evidence-base supporting a role for vitamin K and bone health appeared in this journal previously as a brief critical review by one of the present authors (KDC).¹¹ While preparing the present work, yet another cross-sectional study of the association between vitamin K₁ intake and bone mineral density (BMD) and bone turnover in early postmenopausal (Scottish) women was published.¹² MacDonald et al.¹² found some differences in BMD of the femoral neck and lumbar spine, as well as in urinary pyridinium crosslinks (markers of bone resorption), but not markers of bone formation, in women stratified by quartile of energy-adjusted intake of vitamin K₁. Interestingly, from a vitamin K and childhood-bone-health perspective, the novel cross-sectional findings of Kwalkwarf et al.,¹³ which were highlighted in the earlier brief critical review¹¹ and suggested an association between bone metabolism and vitamin K status in healthy young (3–16 years) girls in the United States, has been extended by our recent cross-sectional findings in young Danish girls; these findings showed a significant inverse association between serum percentage undercarboxylated osteocalcin (%ucOC, a sensitive marker of vitamin K status, with higher percentages being more reflective of poor status) and bone mineral content of the total body and lumbar spine in peri-pubertal Danish girls.¹⁴ One of the conclusions of the earlier brief critical review¹¹ was that in terms of proof of causality, there was a need for well-designed, randomized, phyloquinone supplementation trials in adults as well as in children, to confirm observational findings and the role of vitamin K in bone metabolism and mass in healthy subjects. This is a particularly important need in terms of informing nutrition policy makers and in setting nutrient recommendations. Until recently, only one intervention study

Affiliations: KD Cashman and E O’Connor are with the Department of Food and Nutritional Sciences, University College Cork, Cork, Ireland. KD Cashman is also with the Department of Medicine, University College Cork, Cork, Ireland.

Correspondence: KD Cashman, Department of Food and Nutritional Sciences, University College Cork, Cork, Ireland. E-mail: k.cashman@ucc.ie, Phone: +353-21-4901317, Fax: +353-21-4270244.

Key words: bone health, intervention studies, later life, phyloquinone, vitamin K₁
of the effect of vitamin K₁ supplementation on bone health indices in older subjects had been published, and the findings of that study supported a protective role for vitamin K₁ (together with calcium, magnesium, zinc, and vitamin D₃) on bone loss over 3 years in early postmenopausal women. Over the last 18 months, two further important intervention studies have been published, which investigated the effect of vitamin K₁ (alone or together with other micronutrients) on bone loss in older subjects. These two studies add to the evidence base but cast some doubt on the benefits of high phylloquinone intake on bone health in later life, as suggested from the findings of cross-sectional studies.

**INTERVENTION STUDIES OF VITAMIN K₁ SUPPLEMENTATION ON BONE LOSS IN LATER LIFE**

While there have been a number of Japanese studies of the effect of high-dose vitamin K₂ (menaquinone-4) on bone loss in healthy or osteoporotic postmenopausal women, until recently the only intervention study that investigated the effect of vitamin K₁ supplementation on loss of BMD over time in postmenopausal women was that by Braam et al., conducted in the late 1990s (Table 1). This was a double-blind, placebo-controlled, intervention study in healthy (Dutch) postmenopausal women, aged 50–60 years, who were randomized to receive one of the following regimens: 1) a daily supplement containing maltodextrine (placebo group); 2) calcium (500 mg), magnesium (150 mg), zinc (10 mg), and vitamin D₃ (8 μg) (mineral + vitamin D group); or 3) the latter formulation with additional vitamin K₁ (1000 μg) (mineral + vitamin D+K group) as a tasteless powder (one sachet to be mixed with water) or chocolate-coated tablets (3/day) with a crunchy malt core. These supplements were to be taken during the evening hours, preferably after the meal. The percentage of subjects using the powder or tablets was distributed equally across the three groups. The study duration was 3 years and BMD of the femoral neck and lumbar spine (L₂–L₄) were measured by dual energy x-ray (DEXA) at baseline and at 1, 2, and 3 years after intervention commencement. Fasting blood and 2-h morning urine samples were collected at baseline, 3, 12, and 36 months to measure biochemical markers of bone metabolism (i.e., serum total osteocalcin and bone-specific alkaline phosphatase; markers of bone formation, urinary deoxypyridinoline (Dpyr) and calcium, both standardized to creatinine; markers of bone resorption) as well as markers of vitamin D (serum 25-hydroxyvitamin D [25(OH)D]) and vitamin K (serum ucOC) status. Subjects were enrolled between November and the following March. Dietary vitamin K was not estimated, or at least not reported. While loss of BMD at the femoral neck occurred in all three treatment groups over the 3-year period, the mineral + vitamin D+K group showed reduced bone loss, with the differences in %BMD loss from baseline being 1.7% between the mineral + vitamin D+K group and the placebo group and 1.3% between the mineral + vitamin D+K group and the mineral + vitamin D group; these differences were statistically significant (P < 0.05) following adjustment for baseline BMD, age, BMI, and number of years since menopause. No significant differences were observed among the three groups with respect to change in BMD at the lumbar spine over 3 years. The concentration of ucOC dramatically (74%) declined by year 1 (first measurement point for this parameter) in the mineral + vitamin D+K group, but not the other two groups (0–15%). There were significant increases in serum 25(OH)D concentrations by month 3 of treatment in the mineral + vitamin D and the mineral + vitamin D+K groups (−10 and 20%, respectively); an effect not seen in the placebo group (−5%). These elevated serum 25(OH)D concentrations were maintained throughout the study (except in the mineral + vitamin D+K group at month 36). In general, urinary Dpyr and calcium were unaffected by intervention over the 3 years, irrespective of intervention. Serum total osteocalcin and bone-specific alkaline phosphatase responded variably within the mineral + vitamin D and mineral + vitamin D+K groups in the first 12 months, but no significant effects were evident in these bone formation markers after 3 years.

More recently, Bolton-Smith et al. performed a 2-year double-blind, placebo-controlled trial in healthy (Scottish) women aged 60 years and older (Table 1). Women were randomized to one of four groups who received either 1) placebo, 2) 200 μg/d vitamin K₁, 3) 10 μg vitamin D₃ plus 1000 mg Ca/d, or 4) 200 μg vitamin K₁, 10 μg vitamin D₃, and 1000 mg Ca/d. DEXA scans of the hip (femoral neck, femoral trochanter, and femoral Ward’s) and wrist/forearm (mid-distal radius and ulnar-ultradistal radius), as well as biochemical markers of bone turnover and vitamin K status (except serum vitamin K₁) were assessed at baseline and at 6-month intervals over the 2-year period of the intervention. Serum vitamin K₁, 25(OH)D and PTH were measured at baseline and at yearly intervals. Baseline measurements of all subjects were made in winter. Dietary intake was assessed by a validated food-frequency questionnaire (FFQ). While significant bone mineral loss (between 1 and 2%) occurred over the 2-year period at the mid-distal section of the radius in women in all four treatment groups, women who took combined vitamin K₁, vitamin D₃, and calcium (group 4) had a significant increase in BMD at the ultradistal radius (0.8% per year); an effect not seen in women in the other three groups (those taking placebo, vitamin K₁ alone, or vitamin D₃ and calcium). There were no
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>Subjects (n)</th>
<th>Duration (y)</th>
<th>Intervention</th>
<th>Outcome measures</th>
<th>Major bone findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braam et al. (2003)</td>
<td>Randomized, double-blind, placebo-controlled trial</td>
<td>Postmenopausal Dutch women, aged 50–60 y (155)</td>
<td>3</td>
<td>Randomized to 3 groups: 1) placebo, 2) 500 mg Ca + 10 mg Zn + 150 mg Mg + 8 μg Vit D3/d, 3) 500 mg Ca + 10 mg Zn + 150 mg Mg + 8 μg Vit D3/d + 1 mg Vit K1/d.</td>
<td>DEXA of femoral neck and lumbar spine (L2–L4); vitamin K and D status markers; bone turnover markers.</td>
<td>Loss of BMD at femoral neck in all groups over 3 years. Significantly reduced bone loss at femoral neck in group no. 3 compared to other two groups. No difference in loss of lumbar spine among three groups. In general, markers of bone turnover unaffected by intervention.</td>
</tr>
<tr>
<td>Bolton-Smith et al. (2007)</td>
<td>Randomized, double-blind, placebo-controlled trial</td>
<td>Healthy Scottish women, aged ≥60 y (244)</td>
<td>2</td>
<td>Randomized to 4 groups: 1) placebo, 2) 200 μg Vit K1/d, 3) 10 μg Vit D3 + 1000 mg Ca/d, 4) 200 μg Vit K1 + 10 μg Vit D3 + 1000 mg Ca/d.</td>
<td>DEXA of: femoral neck, femoral trochanter, femoral Ward’s; mid-distal and ultradistal radius, vitamin K and D status markers; bone turnover markers.</td>
<td>Significant loss of BMD at mid-distal radius and no significant difference between groups. Women in group no. 4 had significantly increased BMD of ultradistal radius compared to baseline; an effect not seen in the other three groups. No significant changes from baseline in BMD of three femoral sites. No effect of intervention on markers of bone turnover.</td>
</tr>
<tr>
<td>Booth et al. (2008)</td>
<td>Randomized, double-blind, controlled trial</td>
<td>Healthy US men and women, aged 60–80 y (452)</td>
<td>3</td>
<td>Randomized to 2 groups: 1) multivitamin* + 600 mg Ca and 10 μg Vit D3 + placebo/d, 2) multivitamin* + 600 mg Ca and 10 μg Vit D3 + 500 μg Vit K1/d.</td>
<td>DEXA of femoral neck, lumbar spine (L2–L4) and total body; vitamin K and D status markers; bone turnover markers.</td>
<td>No significant differences in changes in BMD of femoral neck, spine or total body between two groups. No significant differences in bone turnover marker levels between the two groups. Results similar when data from men and women combined, and from men and women separately, were used in the statistical analysis.</td>
</tr>
</tbody>
</table>

* Multivitamin containing 1.6 mg vit B1, 1.8 mg vit B2, 2.1 mg vit B6, 3 μg vit B12, 75 mg vit C, 12 mg vit E, 6 mg pantothenic acid, 20 mg niacin, 160 μg folate, 30 μg biotin per effervescent tablet.
significant changes from baseline in BMD of any of the three femoral sites (including femoral neck) after 2 years in any of the four groups. Over the 2 years of the study, serum vitamin K_1 significantly increased (151% on average) and %ucOC significantly decreased (51% on average) in the groups receiving vitamin K_1 alone (group 2) and in combination with vitamin D_3 and calcium (group 4). Serum 25(OH)D increased in groups 3 and 4 (16.8% on average), whereas %ucOC did not change in the placebo group and increased slightly (15.5%) in the vitamin D_3 and calcium group. Serum 25(OH)D decreased over the 2-year period in both the placebo and vitamin K_1 alone group (−17.5% on average). There was no effect of intervention with vitamin K_1, or indeed with vitamin D_3 and calcium, on serum bone-specific alkaline phosphatase and urinary N-telopeptides of type I collagen (NTx), markers of bone formation and resorption, respectively.

Very recently, Booth et al. reported the findings of their 3-year randomized, double-blind, controlled vitamin K_1 supplementation study in older men and women (aged 60–80 years) in Boston, USA (Table 1). A total of 452 men and women were randomized equally to receive a multivitamin containing vitamin B_1 (1.6 mg), vitamin B_2 (1.8 mg), vitamin B_6 (2.1 mg), vitamin B_12 (3 μg), vitamin C (75 mg), vitamin E (12 mg), pantothenic acid (6 μg), niacin (20 mg), folate (160 μg), and biotin (30 μg) together with 500 or 0 μg phyloquinone (vitamin K_1 supplemented and non-supplemented groups, respectively), as an effervescent tablet to be taken each morning in a 5- to 6-ounce glass of water. In addition, all study participants received a second daily effervescent tablet that contained 600 mg elemental Ca (as calcium carbonate) and 10 μg vitamin D_3 to be taken at the same time as the multivitamin tablet.DEXA measurements of the femoral neck, lumbar spine (L2–L4), and of the total body, as well as biochemical markers of bone turnover and of vitamins D and K status, were assessed every 6–12 months. Baseline dietary data were assessed using a validated FFQ. There were no significant differences in changes in BMD at the femoral neck, lumbar spine, and total body between the vitamin K_1-supplemented and non-supplemented groups. Vitamin K status improved in the vitamin K_1-supplemented group, as evidenced by a significant increase in plasma vitamin K_1 (107% in men and 209% in women) and a significant reduction in %ucOC (51% in men and 44% in women) compared to levels in the vitamin K_1 non-supplemented group. Plasma 25(OH)D, 1,25-dihydroxyvitamin D_3, and total osteocalcin, as well as urinary NTx did not differ between the two groups. Furthermore, when the statistical analyses were conducted separately for men and women, the results were similar to those reported for men and women combined.

Taking the findings of these three intervention studies together may lead to some clear and some more ambiguous conclusions in relation to the effect of vitamin K_1 supplementation on bone loss in older subjects. While BMD of the lumbar spine seems unresponsive to vitamin K_1 supplementation in postmenopausal women and older men, there are mixed findings in relation to its effect on femoral neck BMD; one study suggested a beneficial effect of vitamin K_1 supplementation in postmenopausal women but the other two studies did not. While it was only measured in one of the three studies, BMD of the total body and in one site in the forearm (mid-distal radius) seemed unresponsive and BMD at another site in the forearm (ultradistal radius) appeared responsive to vitamin K_1 supplementation in older women. Vitamin K_1 supplementation on its own had no benefit on bone health indices; when it did have an effect on bone health indices, it was only when given together with other micronutrients. Interestingly, two of the studies showed that supplementation with the same micronutrients on their own had no effect on BMD. It is also clear that any effects of vitamin K_1 supplementation on long-term (2–3 year) bone loss appear to be unrelated to changes in bone turnover, as all three studies failed to detect an effect of long-term vitamin K_1 supplementation on markers of bone turnover, albeit with different markers in some studies.

**DIFFERENCES AMONG INTERVENTION STUDIES POSSIBLY EXPLAIN DIFFERENCES IN BONE OUTCOMES**

While all three studies were performed in older subjects, two were in postmenopausal women and the third was in postmenopausal women and older men. However, the study by Booth et al. analyzed the men and women separately and found no gender effects. There are a number of other differences among the studies that might go some way towards explaining why such different bone outcomes, especially at the femoral neck, were seen.

The dose of vitamin K_1 provided to the women differed among the three studies, ranging from 200 μg/d through 500 μg/d to 1000 μg/d. It is possible that the higher dose of vitamin K_1 led to a more optimal bone vitamin K status. The circulating concentration of %ucOC is not only a sensitive marker of vitamin K nutritional status, but it has also been reported to be a marker of hip fracture risk and a predictor of BMD. Binkley et al. showed in their short-term dose-related vitamin K_1 supplementation study in healthy adults that a supplemental intake of 1000 μg vitamin K_1/d was needed to maximally suppress %ucOC. While intakes of supplemental vitamin K_1 above this level (e.g., 2000 μg/d) had no extra benefit in terms of reducing %ucOC, lower intakes (250, 375, and 500 μg/d) were even less effective.
The findings by Binkley et al. might suggest that the dose used by Braam et al. would have induced the greatest decline in %ucOC and thus lead to optimal γ-carboxylation of osteocalcin, which may have underpinned the effect on femoral neck BMD. Unfortunately, however, only two of the intervention studies assessed %ucOC and even at that some caution is needed in comparing estimates of reduction in %ucOC arising from vitamin K supplementation. The method to assess %ucOC can have a bearing on percentage values obtained. The two studies used different methodology, namely antibody assays against γ-carboxylated osteocalcin and under γ-carboxylated osteocalcin, and a hydroxyapatite-based assay. It is not clear whether this assay difference could explain, at least to some extent, why the percentage reduction in %Glu in the women supplemented with 500 mg/d in the Booth et al. study was 43.7%, while a 50.8% reduction was achieved by a lower dose (200 mg/d) in the Bolton-Smith et al. study. The women in both studies did appear to have similar average baseline %Glu levels (47.5% and 42.2%, respectively), despite habitual vitamin K intakes of about 85 and 175 μg/d, respectively. This difference in intake estimates may also be due to different FFQs being administered in the two studies. On the other hand, if these intake estimates are a true reflection of the dietary vitamin K levels, the US cohort has intakes well above the current US recommendations, whereas nearly two-thirds of the UK group of women failed to meet the US recommendations.

While not assessing %ucOC, Braam et al. did measure the concentration of ucOC in serum, using the same antibody assay as Bolton-Smith et al. The women in the Bolton-Smith et al. study had higher average baseline serum ucOC (5.2 ng/ml) compared to women in the study by Braam et al. (2.8 ng/ml). Supplementation with vitamin K, in both studies induced a 27% (at 200 mg/d) and 74% (at 1000 mg/d) reduction in serum ucOC after 12 months. Thus, it is difficult to assess the exact impact of vitamin K supplementation on bone vitamin K status in these three intervention studies other than to suggest the possibility that the high dose seemed to be the most effective in suppressing the degree of under-γ-carboxylation of osteocalcin, which is in line with the findings of Binkley et al.

Another important consideration in relation to the dose is whether the levels of vitamin K supplementation used in the three studies could be achieved by dietary means. Bolton-Smith et al. specifically chose to use 200 μg vitamin K/d as they believed this could be achieved by dietary means. Indeed, the authors point to data showing that their supplemental vitamin K was equivalent to the 95% percentile of vitamin K intake in younger Scottish adults. Furthermore, we have shown that the 95% percentile of intake for Irish men and women, aged 51–64 years, who participated in our national dietary survey, was 188 and 270 μg vitamin K/d, respectively. The total intake of vitamin K (from food and supplements) of the women in the Bolton-Smith et al. study was approximately 285 μg/d. Booth et al. used a higher level (500 μg vitamin K/d; total intake of 676 μg/d) of supplementation, which, while not impossible, would undoubtedly be more difficult to achieve by dietary means, as would the 1000 μg vitamin K/d used by Braam et al. (total intake not known as habitual intake of vitamin K was not reported). The median vitamin K intake in the top tertile of a group of Dutch elderly subjects was 278 μg/d, whereas the 95th percentile of vitamin K intake in the US NHANES cohort was 254–309 μg/d. Thus, attainment of these higher levels of vitamin K intake would more likely require the use of vitamin K supplements. However, a cursory examination of supplements currently available on the Irish market suggests that the level of vitamin K is relatively low, in the range of 30–60 μg/d.

Another major difference among the three intervention studies is the nutrient composition of the co-supplement used with vitamin K. While two of the studies coadministered equivalent amounts of vitamin D (10 μg/d), the third used slightly less (8 μg/d). Interestingly, there was no major difference in baseline serum/plasma 25(OH)D concentrations (23–25 μg/l) among the three studies. There was also a difference in the amount of supplemental calcium provided among the three studies (500 mg/d, 600 mg/d, and 1000 mg/d, respectively). Furthermore, while one study limited the co-administered micronutrients to calcium and vitamin D, another additionally included zinc and magnesium, and the third included multiple vitamins in addition to the vitamin D and calcium.

While the study duration was similar (3 years) in the studies of Braam et al. and Booth et al., the study by Bolton-Smith et al. was for 2 years. It is noteworthy that the effect of vitamin K supplementation (together with other micronutrients) on femoral neck BMD was not evident after 2 years in the study by Braam et al., which might have implications for interpretation of the findings of Bolton-Smith et al. However, it does not explain the lack of effect on femoral neck BMD in the study by Booth et al., which was for 3 years.

Surprisingly, the study of Braam et al. was the only one that reported power calculations to estimate the number of subjects needed per group. Although the numbers per group (49–56) in the study by Bolton-Smith et al. are similar to that used by Braam et al., Booth et al. had much higher numbers per group (223–229 combined gender; 133–134 women only). The characteristics of the women in the different studies may have been
different though. While the women not receiving vitamin K supplementation in the study by Braam et al.\textsuperscript{15} lost BMD at the femoral neck over the 3 years of the study (−5% change from baseline), the women in the Bolton-Smith et al.\textsuperscript{16} and Booth et al.\textsuperscript{17} studies had no significant loss. Even the women in the placebo group in the study by Bolton-Smith et al.\textsuperscript{16} did not have a significant loss of BMD at the femoral sites. The reason for this difference among studies is unclear. However, the women in the Braam et al.\textsuperscript{15} study were significantly younger (mean age, 55 years) than the women in either the study by Bolton-Smith et al.\textsuperscript{16} (mean age, 68 years) or Booth et al.\textsuperscript{17} (mean age, 68.5 years), and thus were in an earlier postmenopausal stage. This may have impacted the outcome of the study.

CONCLUSION

Based on the studies described here, it would appear that vitamin K\textsubscript{1} supplementation does not protect against loss of bone mineral in some skeletal sites (lumbar spine, total body, mid-distal radius) in older subjects. Furthermore, the evidence-base for bone health benefits at the femoral neck from vitamin K\textsubscript{1} supplementation is mixed and may require further research. In particular, the inconsistent findings in relation to the effects of vitamin K\textsubscript{1} supplementation on BMD of the hip do not explain the mechanism underpinning the protective effect of high vitamin K intake/status against hip fracture observed in a number of prospective cohort studies.\textsuperscript{21,23–25} More research is needed on whether vitamin K may be lowering the risk of fractures via other mechanisms, such as effects on bone-quality parameters.

The finding that relatively low-dose vitamin K\textsubscript{1} supplementation improved BMD of the forearm (ultradistal radius) of postmenopausal women\textsuperscript{16} is interesting even though it was only investigated in one study. Bolton-Smith et al.\textsuperscript{16} suggest that the ultradistal forearm has a higher metabolic turnover rate than predominantly cortical bone and they hypothesize that it may be more responsive to dietary intervention. The authors\textsuperscript{16} point to evidence from a meta-analysis of studies in which BMD changes were measured following exposure to oral anticoagulants (vitamin K antagonists). The meta-analysis shows only a trend towards bone loss at most sites, while a significant decrease in BMD was observed at the ultradistal radius.\textsuperscript{34} Thus, while antagonizing vitamin K activity (via oral anticoagulants) appears to decrease BMD of the ultradistal radius, enhancing vitamin K activity (via vitamin K\textsubscript{1} supplementation together with vitamin D and calcium) appears to increase BMD. While fractures of the distal forearm occur with a different pattern than hip and vertebral fractures, they are, nonetheless, common in older women. For example, while much lower than the incidence of vertebral fractures, the incidence of fractures of the distal forearm in elderly females (65+ years) are similar to, or exceed, that of the hip.\textsuperscript{35} Patients with any type of fragility fracture are at increased risk of other types of fracture. Thus, confirmation of these bone-sparing effects of relatively low-dose vitamin K\textsubscript{1} supplementation at the distal forearm in a second independent study is important.

REFERENCES

1. Esmon CT, Sadowski JA, Suttie JW. A new carboxylation reaction. The vitamin K\textsubscript{1}-dependent incorporation of H\textsubscript{4}CO\textsubscript{3}- into prothrombin. J Biol Chem. 1975;250:4744–4748.


