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REVIEW

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Beyond Deficiency:

Potential benefits of increased intakes of vitamin K for bone and vascular health

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Summary Vitamin K is well known for its role in the synthesis of a number of blood coagulation factors. During recent years vitamin K-dependent proteins were discovered to be of vital importance for bone and vascular health. Recommendations for dietary vitamin K intake have been made on the basis of the hepatic requirements for the synthesis of blood coagulation factors. Accumulating evidence suggests that the requirements for other functions than blood coagulation may be higher. This paper is the result of a closed workshop (Paris, November 2002) in which a number of European vitamin K experts reviewed the available data and formulated their standpoint with respect to recommended dietary vitamin K intake and the use of vitamin K-containing supplements.

Key words vitamin K – gammacarboxy glutamate – osteoporosis – bone mineral density – cardiovascular disease

Introduction

Historically, compounds with vitamin K activity have been classified according to the chemical structure of the isoprenoid side chain at the 3-position of the 2-methyl 1.4 naphthoquinone nucleus. The major naturally occurring forms are phylloquinone (vitamin K_1; abbreviated K_1), and menaquinones (vitamin K_2). Menaquinones can be further sub-classified, depending on the length of their side-chain; the various forms are denominated as menaquinone-n etc. (abbreviated to MK-*n*), where n denotes the number of isoprenyl units in its side chain. Menadione (vitamin K_3) is a synthetic compound lacking a side chain but is biologically active by virtue of its conversion in the body to MK-4. The most nutritionally relevant menaquinones are MK-4 and MK-7 through MK-9. In the human diet, K_1 is predominantly found in leafy green vegetables and some vegetable oils and margarines [1, 2], whilst MKs are present in lower concentrations in meats, milk products, and eggs (MK-4), as well as in animal livers, cheeses, and natto (MK-5 to MK-13) [3]. There is evidence that K_1 can be endogenously converted to MK-4 [4].

In all cells that synthesise vitamin K-dependent proteins, dietary-derived vitamin K quinone is reduced to vitamin K quinol (KH_2), which acts as a cofactor for the posttranslational carboxylation of certain glutamate

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residues, producing gamma-carboxyglutamate (Gla). Hence vitamin K-dependent proteins are also known as Gla-proteins. During gamma-glutamyl carboxylation, the KH_2 cofactor is converted to vitamin K epoxide (KO); this metabolite is then salvaged and recycled to KH_2 via vitamin K by the warfarin-sensitive vitamin K-epoxide reductase (VKOR) [5]. Apart from their classical role in blood coagulation, Gla-containing proteins have a diversity of regulatory functions including blood coagulation, bone turnover [6, 7], vascular repair processes [8], the prevention of vascular calcification [9], cell cycle regulation and cell-cell adhesion, and signal transduction [10]. Vitamin K-deficiency will lead to the production of under-carboxylated (i. e., inactive) Gla-proteins, and thus to interference with the physiological processes mentioned above. Thus far, recommended levels of dietary vitamin K intake have been defined on the basis of the hepatic requirement for normal clotting factor synthesis. Accumulating evidence suggests that other tissues, notably bone and arteries, need higher vitamin K intakes for adequate carboxylation of the locally produced Gla-proteins. This paper is based on the proceedings from a closed workshop at which the authors reviewed new and existing data on the anti-osteoporotic and cardio-protective properties of K-vitamins, with the aim of establishing whether higher intakes (from diet or supplements) than currently recommended have appreciable health benefits. The principal opinions and the conclusions reached represent those of the majority of the workshop panel.

How to monitor vitamin K status

When equimolar doses of K_1, MK-4, and MK-9 (fat-solubilized pure compounds) are administered to healthy people, the highest plasma levels are obtained after K_1, lower concentrations after MK-4 (but appearing more rapidly), and still lower concentrations after MK-9. The plasma half-life of MK-9 is considerably longer than that of the other forms [11]. Bioavailability studies indicate that plasma levels attained after giving K_1 supplements are higher than those for equivalent amounts of dietary K_1 [3]. In population studies, plasma K_1 levels have been shown to be positively [12] and linearly [13] correlated with dietary K_1 intakes, but with an apparent plateau of plasma K_1 at intakes > 200 μg/day [13]. If administered as pharmacological, detergent-solubilized preparations, a linear dose-response relationship may continue to very high intakes [14]. The vitamin K studies carried-out in Dundee [15–17], and those from the UK National Diet and Nutrition Surveys [12] demonstrated that vitamin K intake was wide-ranging. The average intake in the younger population was found to be around 70 μg/day, whilst in older subjects typical values were between 80–120 μg/day. Similar data were obtained in population-based surveys in the USA [18], whereas in The Netherlands, a country with traditionally a high vegetable consumption, higher values were recorded [19]. These intake data alone cannot provide evidence of adequacy.

Population surveys have indicated that between 5 and 15% of healthy subjects have undetectable serum phylloquinone concentrations, suggesting that the vitamin K status of such individuals may be sub-optimal, especially if under-carboxylation of non-coagulation Gla proteins were shown to adversely influence health. Direct measurement of serum vitamin K levels is influenced by recent diet, and is prone to wide variations. Since K vitamins are carried by lipoproteins and are correlated to plasma lipids, blood sampling for analysis should be performed in subjects after an overnight fast. Serum vitamin K may be regarded as an overall marker for vitamin K status, and K_1 values of 150–200 pg/ml are considered to be the lower limit of the normal range [12]. Even though serum concentrations of individual menaquinones (MK-4 through MK-13) are generally below the lower detection limit of current analytical methods, the combined menaquinone fraction may form a substantial fraction of circulating and tissue vitamin K stores. A gap in our knowledge is that the relative efficacy of menaquinones as cofactors for the vitamin K-dependent carboxylase in different cells and tissues remains unclear. A potential alternative to serum vitamin K as a measure of vitamin K status is provided by measuring urinary vitamin K metabolites: both K_1 and K_2 vitamins are excreted in the urine as two side-chain-shortened metabolites (5C and 7C) [20]. Theoretically, analyses of these metabolites can help to define vitamin K status, because they provide insight into total vitamin K ($K_1 + K_2$) utilization. However, further studies are required to validate urinary metabolite excretion against other indices of vitamin K status.

Valuable as they are, assays for serum and urine vitamin K measurement do not provide information on adequacy at the tissue level. Therefore, the direct measurement of the gamma-carboxylation status of the Gla-proteins produced in the tissue of interest is ideally required. Since the Gla-coagulation proteins are synthesized in the liver, assays for them are appropriate for monitoring the hepatic vitamin K status. Severe vitamin K-deficiency may be diagnosed from coagulation assays, but such tests are insensitive for subclinical deficiency. Microtitre plate-based assays using conformation-specific monoclonal antibodies recognizing under-carboxylated species of prothrombin provide an alternative and sensitive tool for assessing hepatic vitamin K status [21]. Osteocalcin (OC) is a small Gla-protein uniquely synthesized in bone, and circulating under-carboxylated osteocalcin (ucOC) provides a measure of the vitamin K status of bone [22]. Population studies suggest a correlation between the levels of circu-

lating vitamin K, ucOC, and carboxylated osteocalcin (cOC), and all three parameters should ideally be measured to provide an accurate indication of vitamin K status and function. The ratio between ucOC and cOC is probably the most sensitive marker for bone vitamin K status. A wealth of evidence demonstrates that usual dietary intakes of vitamin K in healthy adults (together with an unknown fraction from bacterial synthesis of MKs in the gut) are more than sufficient to ensure almost complete gamma-carboxylation of the hepatic Gla-proteins involved in blood coagulation. However, it is equally clear that the dietary intakes are insufficient to ensure complete carboxylation of osteocalcin, around 10–30 % of which (the values are assay specific) occurs in an under-carboxylated state in the healthy adult population [23, 24]. Whether this has physiological significance with respect to bone health is presently unclear but it is a matter of potential concern that elevated serum ucOC was reported to correlate inversely with bone mineral density of the hip, and to correlate positively with hip fracture risk [22, 25, 26]. Population ranges for ucOC/cOC ratios may be determined either indirectly by using a differential binding assay to separate cOC and ucOC species prior to immunoassay or directly by using conformation-specific antibodies, which are available for both OC forms. Published values using indirect binding assays vary widely and it is important to standardise the many variables associated with this type of assay and make appropriate corrections for the basal level of OC [27]. Although the direct assays appear attractive, relatively little is known of the relative affinity of the ucOC and cOC antibodies for intact OC and the several OC fragments which may circulate either naturally, or which may arise as a consequence of underlying bone pathology or as artefacts due to inappropriate sample handling. Although ucOC is responsive to increased dietary vitamin K, a maximal response has only been achieved with pharmacological vitamin K intakes. For Gla proteins that are synthesised in tissues other than liver and bone, new biomarkers need to be developed. One Gla protein of major current interest is matrix Gla-protein (MGP). This protein is mainly synthesised by chondrocytes and vascular smooth muscle cells; hence under-carboxylated forms of MGP may reflect the vitamin K status of cartilage and arteries.

Physiology of bone metabolism

Bone is a living tissue that undergoes continuous turnover processes. Reconstruction comprises bone resorption by matrix-degrading osteoclasts and bone formation by matrix-forming osteoblasts. In the adult skeleton, new bone formation is primarily the result of bone remodelling. In both trabecular and cortical bone, bone remodelling results from the coupled bone resorption/formation activity of bone remodelling units that substitute old bone for new bone. Bone remodelling is a life-long process needed to maintain the mechanical integrity of the skeleton. In young adults, bone resorption and formation are coupled. Net bone resorption can however occur due to an uncoupling of the bone formation and resorption processes [28]. Skeletal integrity mainly depends on mechanical loading. Consequently, bone mass and strength are primarily influenced by mechanical forces. Except for trauma, muscle forces cause the largest loads on bones and the largest bone strains [29].

The threshold level for bone loss or bone accretion can be influenced by various local and systemic factors, known to modulate the bone remodelling process. Interestingly, up-regulation of pro-osteoclastogenic cytokines such as interleukin 6 results in the stimulation of bone resorption [30] and also promotes atherosclerosis [31]. During recent years various biomarkers of the activity of osteoblasts and osteoclasts have become clinically available [32]. Among them are collagen type I propeptides and degradation products, which are released into the circulation. Assays for intact or total OC, the most abundant noncollagenous protein in bone, are commonly used to assess bone formation while as already mentioned it is now possible to separately assess postranslational differences in the expression of cOC and ucOC. Bone markers reflect changes in bone metabolism and can thus provide insights in the physiology and pathophysiology of bone.

As expected, biomarkers of bone turnover are adversely affected by immobilisation [33] and microgravity [34]. However, the data obtained during spaceflights also indicate that some of the changes in bone turnover markers are not related to mechanical unloading but to lack of vitamin K [35]. Moreover, circadian variations [36], monthly fluctuations [30, 37] and a circannual rhythm [38] have been observed in bone turnover markers, which seem to be at least in part affected by differences in vitamin D status, calcium supply, and fluctuations in circulating sex hormones. All these observations are in line with the concept that the threshold values for net bone loss or net bone formation are not only influenced by mechanical loading or unloading of the bone but also by various exogenous factors including specific nutrients such as vitamin K.

Vitamin K is a potentially interesting contributory factor in the regulation of bone remodelling. Apart from the well-documented evidence linking under-carboxylation of osteocalcin to low bone mass, a deficiency of another Gla-protein, protein S, is also associated with low bone mass [39]. The precise role of osteocalcin in bone is still unknown. Osteocalcin-deficient mice appeared to have larger bones than their wild-type littermates, showing that osteocalcin is a negative regulator of bone formation [40]. On the other hand, the protein has

a role in the orderly deposition of hydroxyapatite, both in growing bone and during bone remodelling. Finally, the osteocalcin knock-out model showed strongly increased bone loss after ovariectomy, suggesting a protective role for osteocalcin after menopause. In human studies low serum vitamin K concentrations have been found in patients with osteoporotic fractures [44, 45], and the first randomised intervention studies indicate that increasing vitamin K intake may help reduce postmenopausal bone loss (see below).

MGP is of major importance to the prevention of premature calcification, and inhibits the deposition of extra-cellular calcium matrix in both cartilage and in arterial vessel walls. MGP-deficient mice were normal at birth, but developed massive calcifications in all large arteries in the weeks thereafter [41]. All animals died from rupture of the thoracic or abdominal aorta within 8 weeks after birth. The type of calcification in MGP-deficient mice is comparable to that found in association with aging and diabetes mellitus (also known as Mönckeberg's sclerosis) rather than to atherosclerosis. Whereas atherosclerosis is a disease of the arterial intima characterised by inflammation, macrophage infiltration, intima thickening and plaque formation, Mönckeberg's sclerosis is primarily a disease of the tunica media characterised by calcifications starting around the elastin fibers, followed by more elaborate calcifium salt depositions and vascular damage. On the basis of our present knowledge it is likely that increased vitamin K intake may protect vascular health via improved MGP carboxylation, and therefore may have a protective effect against age-related vascular stiffening rather than against classical atherosclerosis. The importance of vitamin K for bone and vascular health was also demonstrated in rats using coumarin antagonists to block vitamin K utilisation; in this model both bone malformation and aortic calcification were observed [42, 43].

Vitamin K intake, bone mass and fracture risk

Several studies have reported the relationship between vitamin K intake, bone mass, and hip fracture. The Nurses' Health Study recruited 72,327 women aged between 38 and 63 years, i. e. relatively young women who have a markedly lower incidence of hip fracture than elderly ones [46]. Their average baseline vitamin K intake was 192 µg/day. During the ten-year study period, there were 270 hip fractures (fracture incidence = 38.4/100,000 person-years). The study population was further divided into quintiles, according to dietary vitamin K_1 intake (Q1 received the lowest vitamin K intake, Q5, the highest). The incidence of hip fracture in women in Q2–Q5, relative to Q1, was lower, with a multiple adjusted relative risk of 0.70 (95% CI: 0.53–0.93). When these women were examined according to use of hormone replacement therapy (HRT) it was found that vitamin K intake had no effect on the hip-fracture risk of current or past HRT users. In contrast, it had a significant protective effect on subjects who had never used HRT (and were least protected against postmenopausal osteoporosis). Subjects in Q1 showed an incidence of 81.8 fractures per 100,000 person-years, whilst subjects in Q2–Q5 reported a much lower incidence of 54.8.

The Framingham study investigated vitamin K intake in older women (average age 75 years), in whom the average dietary vitamin K_1 intake was 155 µg/day [47]. There was no significant correlation between dietary K_1 intake and either bone mineral density (BMD) or bone loss. However, when the cohort was divided into quartiles according to K_1 intake (Q4 = highest K_1 intake), there was a significant reduction in hip fracture risk for Q4, compared to Q1 (RR = 0.35; 95% CI: 0.13–0.94). The authors concluded that vitamin K has a more pronounced effect on fracture than on BMD, and that dietary vitamin K intakes below 109 µg/d are associated with increased hip fracture risk. In another study in the Framingham Offspring cohort among younger women and men (average ages: 59 and 58 years respectively) there was a positive correlation between K_1 intake and BMD in women but not in men [48]. However in men, a positive correlation was found between BMD and blood parameters of vitamin K status (serum K_1 and ucOC) [49]. The physiological significance of this discordance between men and women remains to be elucidated.

Natto is a fermented soybean food particularly appreciated in eastern Japan (e. g. Tokyo), and it is extremely rich in vitamin K (notably MK-7). In a Japanese study, postmenopausal women in areas with a traditionally high natto intake were compared with women from western Japan (e. g. Hiroshima) where natto is not a common food. It was found that natto consumption was associated with markedly elevated serum levels of MK-7. Interestingly, hip fracture incidence is markedly higher in western Japan than in eastern Japan [50]. Thus, it was suggested that the high intake of natto-derived MK7 contributes to the lower hip fracture incidence in eastern Japan. Other studies investigated hip fracture incidence in two other populations. Elderly institutionalised persons have a much lower vitamin K and vitamin D intake and a much higher hip fracture risk as compared with home-dwellers, and several studies have identified low vitamin K intake as an independent risk factor [51, 52].

Whereas all these population-based studies provide an accumulating amount of evidence for high vitamin K intake as an independent factor decreasing the risk of postmenopausal osteoporosis and hip fracture, methodological imperfections and potential confounders mean that no firm conclusions can be drawn. Dietary ques-

tionnaires allow an evaluation of recent vitamin K intake whereas it is difficult to evaluate average intake over a life-time. In some cohort studies, evaluation of vitamin K intake has been repeated at different ages; however attrition of the less healthy and less dietary aware can result in a non-representative population. A high vitamin K intake usually depends on a high consumption of green vegetables and is often associated with a healthier lifestyle. Thus, final proof for the importance of vitamin K in bone health must come from well-designed intervention trials. Last but not least, mechanistic studies are needed to delineate the role of vitamin K and Gla-proteins in bone physiology at the molecular level.

Osteocalcin carboxylation, bone mass and fracture risk

The original stimulus for studying the relationship between dietary intakes of vitamin K and bone health had been some small patient-based studies suggesting a possible link between low serum K_1 and osteoporosis [44, 45] and even more convincing epidemiological evidence obtained by Szulc and co-workers showing a positive association between ucOC and fracture risk [25, 57] and an inverse association between ucOC and bone mass [26] in French elderly women. Another prospective study performed in elderly home-dwelling French women (EPIDOS) showed an increased hip fracture risk in the highest quartile of serum ucOC [58]. Similar associations linking an impaired carboxylation of OC with bone mass and fracture risk were subsequently reported from The Netherlands [22] and Finland [59]. Since, as already discussed, the carboxylation of OC is sensitive to vitamin K intakes in the usual dietary range, these associations with ucOC give credence to the hypothesis that adequate intakes of vitamin K are necessary to maintain healthy bones. Lately, this hypothesis has been reinforced by studies of patients with chronic gastrointestinal disorders in whom malabsorption of fat-soluble vitamins is common. Thus patients with primary biliary cirrhosis and Crohn's disease are known to be at high risk of osteoporosis and often have both low serum K_1 concentrations and a high circulating ucOC [53–56]. Importantly, the study by Schoon et al. [53] provided evidence of an inverse relationship between ucOC and BMD in Crohn's disease.

Vitamin K_1 intervention studies for bone health

The Dundee Bones and Vitamins Intervention Study (D-BAVIS) was a two-year intervention study involving vitamin K_1, vitamin D, and calcium [60]. The study aimed to determine whether vitamin K_1 and/or vitamin D plus calcium favourably influenced BMD in healthy older women, and whether vitamins K_1 and D had a synergistic effect on bone health outcomes. 244 women aged 60–87 years were recruited stratified by age into the study and randomly assigned to one of four supplement groups: [1] placebo, [2] vitamin K_1 200 μg/day, [3] vitamin D_3 10 μg/day and calcium 1 g/day, and [4] vitamin D_3 10 μg/day and calcium 1 g/day, and vitamin K_1 200 μg/day. Subjects were followed-up every six months with DXA bone scans, biochemical markers of bone turnover and serum 25-OH vitamin D and separate Glu and Gla-osteocalcin measurements for vitamin D and K status, respectively. In summary, the results were (i) there was evidence of sub-optimal vitamin D and K status at baseline, (ii) supplementation with vitamin D and vitamin K significantly raised serum 25-OH vitamin D and cOC concentrations, respectively, indicating improved vitamin status, (iii) vitamin D supplementation had no influence on gamma-carboxylation of osteocalcin, (iv) the sum of ucOC and cOC increased from baseline in all groups equivalently, (v) neither cross-linked N-telopeptides (NTX) nor bone-specific alkaline phosphatase (BAP) changed over time or between supplement groups, (vi) relative to baseline, there was a significant increase in bone mineral content and areal density at the ultra-distal radius site in the combined calcium plus vitamins K and D supplemented group, (vii) significant bone mineral loss only occurred at the mid-distal radius site, equivalent for all groups, and (viii) no significant loss occurred at the hip sites for any group (including placebo). The authors concluded that combined supplementation with vitamins K_1 and D_3 at dietary relevant intakes significantly improved BMD at the highest trabecular bone site measured, and that equivalent supplementation in high osteoporotic risk groups may be beneficial.

In the Maastricht osteostudy 188 postmenopausal women aged 50–60 years were recruited, and treated for three years with daily supplements [61]. The first group received placebo (maltodextrine), the second received minerals (500 mg/day calcium, 150 mg/day magnesium, and 10 mg/day zinc) and 8 μg/day vitamin D_3, and the third group received minerals plus vitamin D_3 and an additional 1 mg/day of vitamin K_1. Optimal osteo-protective effects were obtained at the site of the femoral neck if vitamin K was used in combination with minerals and vitamin D. Although no complete prevention of bone loss was achieved, the rate of bone loss had decreased by 35–40 % as compared to the placebo and minerals plus vitamin D groups. It may be calculated that if the observed effects continue over decades, lifelong supplementation could postpone fractures by up to ten years.

Vitamin K_1 intervention studies for vascular health

Accumulating evidence suggests that in many aspects arterial calcification mimics bone formation, which prompts interest in the effects of vitamin K on the vasculature. Previous population-based studies reported a significant reduction in aortic calcification with high vitamin K_1 [62] and vitamin K_2 intake [63], and a significant inverse correlation was found between vitamin K_2 intake, and the incidence of both ischaemic heart disease and cardiovascular mortality [63]. Based on these findings the effect of treatment on arterial characteristics was monitored in the Maastricht osteostudy. These unpublished findings clearly demonstrated that supplementation with vitamin K_1 can protect against vascular hardening and loss of arterial elasticity. High dose MK-4 also seems to have cholesterol lowering properties as shown in studies in rabbits [64] and humans [65].

MK-4 intervention studies for bone health

Extremely high doses (45–90 mg/day) of MK-4 have been used for the treatment of postmenopausal osteoporosis in Japan for several years [66, 67]. After the positive outcomes of the first clinical trials, the treatment is now used on a large scale; thus far, no adverse side-effects have been reported. A number of independent groups have claimed that this medication results in complete prevention of further bone loss in postmenopausal women, and in some women even a significant gain in BMD [68, 69]. The treatment was also reported to be successful in other groups at risk for bone loss such as haemodialysis patients and those treated with corticosteroids. It remains to be seen whether similar beneficial effects of high MK-4 intake will be observed outside Japan for populations whose predisposition to osteoporosis differs with respect to hereditary factors as well as lifestyle factors such as calcium intakes.

Potential adverse effects of vitamin K-antagonists (oral anticoagulants)

Vitamin K-antagonists are frequently used in the treatment and prevention of thromboembolic events. Their mode of action is that they interfere with the recycling of vitamin K-epoxide into the quinone form and thus rapidly deplete the vitamin K stores. During recent years, conflicting data have been reported on whether subjects on long-term oral anticoagulant treatment have low bone mass and increased risks for osteoporosis and fractures [70–74]. In a recent meta-analysis, Caraballo et al. concluded that long-term oral anticoagulation may be associated with a modestly increased bone fragility and osteoporotic fracture risk [75]. Most studies published thus far are retrospective and lack good control populations, however. Since anticoagulated patients form a diseased population which may differ from the general population in many aspects (lower mobility, more controlled diet, lower body mass index) these studies are not easy to interpret. At this time bone densitometry measurements or fracture risk assessment in a prospective randomised study in which subjects are anticoagulated with either coumarin-type (vitamin K-antagonists) or aspirin-type drugs has not yet been published.

A second potential adverse effect of oral anticoagulants is that they may promote vascular calcification. In animal model systems severe calcification has been reported after a relatively short treatment with warfarin [43, 76]. Remarkably, the warfarin-induced artery calcification could be completely blocked by vitamin K_2, but not vitamin K_1, suggesting a more prominent role for K_2 vitamins in the vasculature [76].

Immunological basis for high-dose vitamin K

The daily amounts of MK-4 administered in Japan as a prophylactic therapeutic agent are greatly in excess of those required to normalize gamma-carboxylation of vitamin K-dependent bone proteins, suggesting the possibility of alternative mechanisms of action in this specific use. Under normal dietary and physiological conditions, vitamin K is mainly catabolised by hepatic metabolism involving side chain shortening (most probably by the mitochondrial β-oxidation pathway) to two major urinary aglycones, a 5-carbon and 7-carbon carboxylic acid product with the 5-carbon metabolite predominating. The same two aglycones are excreted in greater amounts after the administration of pharmacological doses of K_1, MK-4 and MK-7 showing that there is a common pathway of catabolism for K vitamins [20, 77]. Whether the same oxidative degradation of vitamin K takes place in non-hepatic tissues is not known but the increased generation of vitamin K catabolites per se might explain some of the observed therapeutic effects of high-dose vitamin K intervention in ameliorating bone loss. It has been shown using cytohistochemistry that β-hydroxyacyl dehydrogenase activity (an enzyme of β-oxidation) is increased during bone resorption [78], suggesting the possibility that the catabolites of vitamin K exert unexpected biological activities. It has been shown, for instance, that in a rat paw oedema model the 7-carbon carboxylic acid catabolite of vitamin K has potent anti-inflammatory activity [79] mediated by modulation of cytokines [80].

It is widely appreciated that leukocytes can release a range of pro- and anti-inflammatory cytokines. Other cells can also release cytokines, including cells in bone,

which are in constant contact with the cells in the haemopoietic compartment, including, of course, leukocytes. Recent data have shown that the osteoblast-like cell line MG63 can be induced to release interleukin-6 (IL-6) in the presence of lipopolysaccharide (LPS) or $1,25(OH)_2$vitamin D_3 [81]. Furthermore, IL-6 has been recognised as a potent activator of bone resorption by inducing osteoclastogenesis [82]. Therefore, conditions that cause increased levels of IL-6 may increase bone resorption and decrease bone formation, whereas factors that suppress IL-6 may be expected to interrupt the biological pathways that lead to increased bone loss.

When the osteosarcoma MG-63 cell-line is challenged with LPS it can be induced to release IL-6 thus providing a cell model to investigate the biological properties of the vitamin K metabolites. If MG63 cells are co-challenged with LPS (from E. coli) and the 5-carbon vitamin K catabolite, an inhibition of IL-6 release was observed at high concentrations of this compound (10^{-5} M). However, the 7-carbon carboxylic acid catabolite was found to be a much more potent inhibitor of LPS-induced IL-6 release by MG63 cells in culture (10^{-7} – 10^{-8} M) [74]. This observation suggests the possibility that administration of high doses of vitamin K may also act on the skeleton through a modulation of cytokine-mediated events. In high dose vitamin K regimens the active 7-carbon carboxylic acid vitamin K catabolite may be able to prevent bone resorption by inhibiting cytokine-mediated osteoclastogenesis, and facilitate bone formation, while the vitamin K itself will ensure complete gamma-carboxylation of the vitamin K-dependent proteins in bone. Although speculative, the possibility that vitamin K may modulate physiological responses through manipulation of cytokine networks suggests a potentially interesting hypothesis and a novel avenue of research for therapeutic intervention in metabolic bone disease.

Which is the most important vitamin, K_1 or K_2?

There is no conclusive evidence that at nutritionally relevant doses, the physiological functions of vitamins K_1 and K_2 are different: both are capable of functioning as a cofactor for the gamma-glutamyl carboxylase, and differences may only be expected with respect to pharmacokinetics and tissue distribution. This may be the reason why some menaquinones seem to have a greater effect in preventing arterial calcification than K_1. In the western diet, K_1 from green vegetables forms around 80 % of the total vitamin K intake, but because of low bioavailability their contribution to total vitamin K status is likely to be over-estimated: vitamin K_1 from specific vegetable oils may be more efficacious, but more work is required in this area. If the potential specific anti-atherosclerotic effects of menaquinones are confirmed, then increased consumption of certain MK-containing foods (or high-MK food extracts or pure supplements) may be beneficial to health.

The only synthetic forms of vitamin K that are available for use in humans are K_1 and MK-4; both forms are well-absorbed. Published clinical trials with MK-4 have invariably used high doses (45–90 mg/day), whilst K_1 has been tested at relatively low doses. K_1 is the analogue used in nearly all K-containing food supplements and multivitamin preparations available on the western market. Nutritionally relevant doses are generally considerably less than 1 mg/day; unfortunately, no MK-4 studies have been published at or below this dose. Pharmacological doses of MK-4 are typically 45 mg/day, and no studies have been carried out with K_1 at this dosage level. Differences may become clearer if both analogues are used at comparable levels, and stronger comparisons could be made at dietary intakes up to 1 mg/day as well as at pharmacological doses up to 100 mg/day. Based on the available literature it is not yet possible to evaluate the efficacies of vitamins K_1 and MK-4 at similar dosage levels, and the execution of new, comparative trials should be encouraged. Bone and arterial vessel wall are potential target tissues for increased vitamin K intake, and both have the remarkable capacity to convert K_1 into MK-4 under physiological conditions [4]. To date there are no compelling clinical reasons to favour one vitamin K analogue over another, but from a dietary viewpoint, it may be preferable to investigate K_1.

In considering the potential efficacy of pharmacological doses of MK-4 it should be noted that there is evidence for a secondary function of this analogue over and above its role in glutamate carboxylation. The available evidence (mainly from cell culture experiments) suggests that MK-4 (but not K_1) may also be associated with production of interleukin-6, regulate the synthesis of PGE_2 [83], or inhibit the mevalonate pathway in a comparable way to bisphosphonates [84], but at present only preliminary data exist. Below we will restrict ourselves to the potential benefit of nutritionally relevant doses of vitamin K.

Consideration of recommended and supplemental levels of vitamin K in context to EU legislation

There is a growing awareness that vitamin K adequacy alone, and particularly when used as a food supplement, may have little effect on bone health if other nutrient inadequacies exist. Similarly, the long-term benefit of supplements containing only calcium and vitamin D is not always obvious: such supplements may show a transient effect on bone, but in studies of ≥3 years the beneficial effect relative to placebo is often lost [85]. Emerging data suggest that vitamin K supplements should also contain calcium and vitamin D, and perhaps other minerals such

as magnesium and zinc to have optimal osteogenic effects.

Dietary Adequate Intake (AI) values have only been defined in a limited number of countries, and are based on the intakes of vitamin K_1 needed to maintain hepatic synthesis of blood coagulation factors. The current guideline in the UK is 1 μg/day/kg body weight [86]. Average values for dietary vitamin K intake range from around 60–70 μg/day in several British and American studies [19, 87–89] to 245 μg/day in The Netherlands, a country known for high green vegetable intake. In all studies green vegetables were the main food source. Most studies report that one-half of the populations investigated had daily vitamin K intakes below the present guidelines. Based on consideration of bone and vascular health, there are as yet insufficient data to define tolerable upper limits, recommended daily intakes, or to distinguish between K_1 and K_2 requirements.

The bioavailability of vitamin K_1 from supplements is probably greater than that from most foods (e. g. possibly between 3 to 5 fold higher than that from green vegetables); hence the dietary-equivalent bioavailable K_1 from the supplements consumed, for example, by the D-BAVIS population is presumed to have been greater than 200 μg/d. Evidence from the Framingham study indicated that there was an increased risk of fracture at intakes below 109 μg/day. These data suggest that further studies are required to evaluate both the efficacy of higher food-based intakes and those of smaller supplemental doses, of the order of 100 μg/day before optimal intakes for bone health can be clarified. In the meantime, it seems fairly clear that higher dietary intakes than those generally consumed would be beneficial and that supplemental levels of around 100 μg/day would also improve vitamin K status in the majority of the population. European legislation exists defining the maximum supplementary dose of vitamin K_1 which can be administered, and in most countries, the addition of 100 μg/day is already allowed. The possibility for this to be increased by up to 50 % exists, if supportive clinical evidence were available. Currently in the EU countries synthetic MK-4 is not allowed as a food supplement because the supporting evidence for its independent role in health does not exist. The alternative routes for obtaining K_2 from the food chain are through eating natto (MK-7), cheeses (mainly MK-9) and menadione provided in animal feed, which enriches meat and eggs.

Any risks associated with relatively high consumption of either K_1 or K_2 appear minimal, with intakes up to 1 mg/d K_1 and 45 mg/d MK-4 often having been used without observed adverse events. Two possible exceptions exist. Firstly a potential problem relates to interference with oral anticoagulants. However, a systematic dose-response study among subjects on oral anticoagulant treatment demonstrated that the stability of anticoagulation was not significantly affected by vitamin K supplements at doses below 100 μg/day [14]. Secondly, preliminary studies have suggested that high vitamin K_1 supplementation (i. e. above 1 mg/day) can contribute to periodontal disease via a bacterial mechanism on gingival tissue (S. Hodges, unpublished data).

Conclusions

From the available dietary data, it would appear that daily intakes of between 200 and 500 μg/d of dietary vitamin K may be required for optimal gamma-carboxylation of OC, which may in turn benefit bone health. Available evidence of relative bioavailabilities suggests that the same benefits may be achieved with lower intakes of supplements, which need to be determined but may be of the order of 100 μg/d. There is growing evidence to suggest that vitamin K may be acting synergistically with vitamin D, calcium and possible other micronutrients to maximally influence bone mineral accretion and potentially inhibit vascular calcification. As such, health benefits may accrue from supplemental vitamin K being combined with vitamin D and minerals. Considerably more work is required in the area of vitamin K including understanding relative bioavailability, optimal tissue-specific status indicators and the relative importance of gamma-carboxylation status to the growing number of health outcomes that may be influenced by vitamin K inadequacy.

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