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The UK food data-base for vitamin K and why we need it

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Abstract

The need to make accurate assessments of dietary intakes vitamin K has been driven by recent evidence that vitamin K-dependant (Gla) proteins are widely present in the body and have other important physiological functions other than coagulation. Two of these proteins, osteocalcin and matrix Gla protein (MGP), are thought to have a role in maintaining skeletal integrity while MGP acts as an inhibitor of calcification in arteries and cartilage. An added impetus has been the development of functional markers that enable vitamin K status to be assessed from a tissue-specific perspective and evidence that bone Gla-proteins require higher dietary intakes of vitamin K for optimal carboxylation than do hepatic coagulation Gla-proteins. The UK food data-base for vitamin K began on an ad-hoc basis to identify the major individual food contributors but recently has been expanded to include values based on recipe calculations and food similarities. To date the data-base is restricted to the plant form phylloqinone, the major food source of vitamin K. The food items used to calculate values for composite foods have all been analyzed by a validated HPLC procedure, some 170 by our London laboratory. Currently about 2000 food items have been assigned a provisional phylloquinone content. Although this food data-base has enabled the first assessments of dietary intakes of phylloquinone in UK populations, many assigned values are provisional and need to be validated by direct analysis. Further work is needed to assess issues such as inter-sample variability, storage losses and the content of bacterial menaquinones.

Keywords: Food databases; Vitamin K; HPLC; Phylloquinone

1. Introduction

Vitamin K is the family name for a series of fat-soluble compounds which have a common 2-methyl-1,4-naphthoquinone nucleus but differ in the structures of a side chain at the 3-position. They are synthesized by both plants and bacteria. In plants the only important molecular form is phylloquinone (vitamin K_1) with a phytyl side chain. Bacteria synthesize a family of compounds called menaquinones (vitamin K_2) with side chains based on repeating unsaturated 5-carbon (prenyl) units. These are designated menaquinone-*n* (MK-*n*) according to the number (*n*) of prenyl units. Some bacteria also synthesize menaquinones in which one or more of the double bonds is saturated.

The classical role of vitamin K is as an antihaemorrhagic factor needed for the synthesis in the liver of functional forms of prothrombin (factor II) together with factors VII, IX and X (Newman & Shearer, 1998). After secretion into the blood, these four vitamin K-dependent proteins function as procoagulants in the coagulation cascade that, once initiated, culminates in the conversion of fibrinogen to fibrin and the formation of a haemostatic plug. The biochemical role of vitamin K in the synthesis of these clotting factors was established in 1974 when it was found that vitamin K acted as a cofactor for a post-translational modification in which specific peptide glutamate (Glu) residues were converted to γ-carboxyglutamate (Gla) residues. The finding that vitamin K promoted the synthesis of a hitherto unknown and unique functional group in all four of these clotting proteins was a crucial development and facilitated the means to search for other vitamin K-dependent proteins by showing that an isolated protein contained Gla residues. This soon resulted in the discovery of two other vitamin K-dependent coagulation proteins with anticoagulant properties (proteins C and S) that play a role in the feedback inhibition and regul-atory control of the blood coagulation mechanism (Newman & Shearer, 1998).

Of greater interest from the nutritional point of view, however, has been the discovery of a diverse group of proteins that have no connection with blood coagulation (see Ferland, 1998; Newman & Shearer, 1998 and Table 1). Osteocalcin, also called bone Gla-protein, is the most abundant noncollagenous protein of bone. It is

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Table 1
Distribution and roles of vitamin K-dependent (Gla) proteins

Gla protein	Tissue	Role
Prothrombin (factor II), factors VII, IX and X	Liver (then plasma)	Procoagulants
Protein C	Liver (then plasma)	Anticoagulant
Protein S	Liver (then plasma), endothelium, bone	Anticoagulant role as cofactor for protein C. Role in bone unknown
Osteocalcin (bone Gla-protein)	Bone	Unknown, may be a matrix signal for osteoclasts
Matrix Gla protein	Bone, cartilage and most soft tissues	Evidence for a role as an inhibitor of calcification
Gas6	Not investigated	Unknown, may regulate cell growth

synthesized by osteoblasts and binds to the hydroxyapatite lattice but a fraction is released into the circulation where it can be assayed. Osteocalcin itself is a good marker of bone turnover but its precise role remains unknown. Matrix Gla-protein (MGP) is also found in bone but unlike osteocalcin is present in many soft tissues and seems to play an inhibitory role in the calcification process. More recently a growth arrest-specific gene 6 (*gas6*) was discovered that codes for a new Gla protein. The role of the Gas6 protein remains to be found but it may be involved in the regulation of growth and apoptosis (Newman & Shearer, 1998).

2. Effects of dietary deficiencies of vitamin K

With respect to the coagulation function of vitamin K, it is well known that the only members of any healthy population at risk of bleeding from a dietary deficiency are infants up to around six months of age. Vitamin K deficiency bleeding in infants is a serious worldwide problem that although rare has a high risk of mortality or permanent disability. The risk is associated with exclusive breast feeding and in most countries is tackled by giving all infants a program of vitamin K supplementation. In contrast, there are no concerns that healthy adults from any country have insufficient dietary intakes of vitamin K to meet their coagulation requirements. This is why in the past there was little incentive to study dietary vitamin K intakes in adults as opposed to young infants.

In the last few years evidence has grown that the carboxylation of non-hepatic Gla proteins such as osteocalcin is more susceptible to dietary influences than the hepatic coagulation proteins (Shearer, 1997; Vermeer, Jie & Knapen, 1995). Research on the effects of undercarboxylation of osteocalcin on bone health has been hampered by the lack of knowledge of the function of osteocalcin although it is known that the Gla residues are necessary for its calcium-mediated binding to hydroxyapatite. However, there have been several studies showing associations of various indices of vitamin K insufficiency with bone fractures or a reduced bone mineral density. A hypothesis that is being actively investigated is that a suboptimal vitamin K status may impact on the elderly population by contributing to the pathogenesis of osteoporosis (Shearer). In addition the recent exciting findings in MGP-depleted transgenic mice that strongly implicate MGP as an inhibitor of calcification of arteries (Luo et al., 1997) add further weight to an earlier hypothesis that dietary vitamin K deficiency may play a role in the pathogenesis of athero-sclerosis (Jie, Bots, Vermeer, Witteman & Grobbee, 1996).

3. New functional markers of vitamin K status

The need for accurate tables for the vitamin K content of foods has been highlighted by the discovery and validation of new and sensitive functional markers of vitamin K status. The most useful and physiologically relevant functional markers are those based on the detection of vitamin K-dependent proteins that have a depleted Gla content because this is a direct reflection of the only delineated biochemical cofactor function of vitamin K. In states of vitamin K deficiency, undercarboxylated (des-gamma-carboxy) species of the vitamin K-dependent proteins are released from the tissue site of synthesis into the blood; their levels increasing with the degree of severity of vitamin K deficiency. For the vitamin K-dependent coagulation proteins, the importance of undercarboxylated forms (also first known as PIVKA or Proteins Induced by Vitamin K Absence) as biochemical markers lies in both their biological relevance (they are known to be functionally inactive) and the sensitivity of modern assays for their detection [e.g. species of undercarboxylated prothrombin (PIVKA-II) are detectable in plasma well before any changes occur in conventional coagulation tests]. In the same way that vitamin K deficiency causes PIVKA-II to be released into the circulation from the liver, a deficit of vitamin K in bone will cause the osteoblasts to secrete undercarboxylated species of osteocalcin (ucOC) into the bloodstream. It has been proposed by Vermeer that the concentration of circulating ucOC reflects the sufficiency of vitamin K for the carboxylation of this Gla protein in bone tissue (Vermeer & Hamulyák 1991; Vermeer et al., 1995).

Other markers of vitamin K status that are also being used to evaluate the effects of dietary intakes of vitamin K are measurements of urinary Gla excretion and direct measurements of circulating phylloquinone.

4. Dietary intakes of vitamin K and functional markers

A major advantage of using the degree of undercarboxylation as a functional marker is the ability to assay specific undercarboxylated proteins that are synthesized by different tissues and therefore reflect the vitamin K availability in that tissue. Thus, measurements of PIVKA-II and ucOC provide very sensitive markers of vitamin K adequacy for the carboxylation status of vitamin K-dependent proteins in liver and bone, respectively. In theory, then, it should be possible to assess the adequacy of dietary intakes of vitamin K on a tissue basis.

A potential concern for optimal nutrition is that the ucOC fraction in ostensibly healthy people may be only partially carboxylated. Thus the proportion of circulating ucOC has been shown to be readily responsive to changes in dietary intakes of phylloquinone. One study showed that increasing intakes from 100 to 420 μg/day reduced circulating ucOC by 41% (Sokoll, Booth, O'Brien, Davidson, Tsaioun & Sadowski, 1997). The possible importance of such studies lies with the separate findings that high levels of ucOC correlate with both hip fracture risk and hip bone mineral density in elderly women (see Shearer, 1997) and that average dietary intakes of phylloquinone in a Scottish study were only around 70 μg/day (Bolton-Smith & Shearer, 1997).

5. Measurement of vitamin K in foods by HPLC

5.1. Early studies in the UK

Until the late 1970s the only food composition data for vitamin K were derived from bioassays, usually in the chick (Parrish, 1980). Their disadvantage was that they provided only a qualitative guide to the vitamin K contents of a few foodstuffs (Suttie, 1992). The first realistic physicochemical assay methods came with the development of HPLC technology in the 1970s which for the first time offered the prospect of measuring with confidence the often quite low levels of vitamin K in foods.

The fact that phylloquinone is only synthesized by plants and menaquinones are only synthesized by certain bacteria (yeasts do not synthesize menaquinones) suggested that phylloquinone would be the major food source of vitamin K. Some early work carried out in the UK assessed the suitability of HPLC with UV detection for measuring phylloquinone in some common vegetables and milk (Shearer, Allan, Haroon & Barkhan, 1980). The results confirmed the high levels in green-leafy vegetables (reflecting the location of phylloquinone in the chloroplast) but also showed that the vitamin was present in significant amounts in some non-leafy vegetables and was detectable in most vegetables. With an appropriate purification procedure it was possible with HPLC-UV to measure phylloquinone down to concentrations of about 1 μg/100 g with a precision of $< 10\%$. The measurement of phylloquinone in human and cows' milk, especially the low levels in human milk, proved more difficult by HPLC-UV but with minor adaptations provided the first values for these foods using a physicochemical technique (Haroon et al., 1982; Shearer, Allan et al., 1980).

5.2. Later studies in the UK

In the area of vitamin K and food analyses, our major priority in the 1980s was to establish dietary intakes in breast-fed human infants, a group who had been shown to be at risk of bleeding from vitamin K deficiency. For such studies a more sensitive and selective detection technique was required. We therefore took an HPLC method based on the detection of vitamin K by dual-electrode electrochemical detection in the redox mode that we had developed for plasma assays (Hart, Shearer & McCarthy, 1985) and adapted this for the accurate measurements of phylloquinone in human milk at different stages of lactation (von Kries, Shearer, McCarthy, Haug, Harzer & Göbel, 1987).

At this time, the determination of the phylloquinone content of other common foods in the UK continued at a somewhat leisurely pace and an ad hoc data-base was gradually built up. In the last decade it has become clear that, with modern methods of HPLC analysis, the problems of measuring phylloquinone in foods have been largely overcome. In the UK, the food data-base assembled in our London laboratory has been extended using the same redox electrochemical detection method used in our previous milk analyses (von Kries et al., 1987) while in the USA, postcolumn fluorescence detection has been preferred by the Boston USDA group (Booth, Davidson & Sadowski, 1994). The good overall agreement between the phylloquinone content of many common foods analyzed in London (Shearer, Bach & Kohlmeier, 1996) and Boston (Booth, Sadowski & Pennington, 1995; Booth, Sadowski, Weihrauch & Ferland, 1993) is a testimony to the reliability of both detection methods.

6. Compilation of a provisional extended food data-base for the UK

By the early 1990s, with the growing evidence that adequate dietary intakes of vitamin K may be important for bone health, there was more urgency to obtain a more comprehensive food data-base for the UK so that

accurate intakes could be computed and applied to appropriate population studies. Aided by funding from MAFF, a much larger but still provisional data-base of the phylloquinone content of foods in the UK has now been compiled (Bolton-Smith et al., British Journal of Nutrition, submitted). The extension of the data-base had two main components, further direct analyses and recipe-based calculations.

Direct HPLC analyses of roughly 70 food items were performed to add to the previous analyses of about 100 major food items. Some of these were for margarines and spreads assayed previously that needed to be re-assayed because of changes made by the manufacturers in their oil composition. Apart from our own measurements, we used two other sources of food values determined by direct HPLC analysis. Most were values for simple foods taken from publications from the Boston USDA laboratory (Booth et al., 1993, 1995; Booth & Ferland, 1993). The second source were a few vegetables analyzed by Langenberg, Tjaden, deVogel and Langerak (1986) but who also considered the effects of cooking, freezing, canning and gamma-irradiation on the vitamin K content.

Indirect calculations of the vitamin K content for nearly 600 foods were based on recipe-based calculations. The recipes were derived from three main sources:

1. McCance and Widdowson's *The Composition of Foods* (5th Edition and supplements, HMSO, London).
2. Recipes reported in weighed food diaries of volunteers in a dietary intake study.
3. Common cookery books in the UK.

In the case of the recipes from *The Composition of Foods*, a computer program, MICRODIET, facilitated the calculations and automatically adjusts for any weight loss or gain during the cooking process.

Some foods values were assigned with assistance of ingredient information from the manufacturers such as the proportions of fruits and vegetables. One problem was that ingredient proportions were often confidential, e.g. the oil composition of margarines. However, two manufactures were willing to perform the calculations for a range of products based on the values that we gave them for the raw ingredients.

Finally, some foods that had not been analyzed were assigned a value based on similar items that had been analyzed. Such calculations took into account the food type, the fat (including where possible the fat type) and water content, and where appropriate the degree of green pigmentation and edible portion. One example is that all white fish with a similar fat content to our analyzed value for cod were assigned the same value; other values for white fish were adjusted for their relative greater or lower fat contents.

7. The UK food data-base for vitamin K: scope, problems and limitations

Using the methods of calculation described above, a food data-base for phylloquinone has been compiled with values assigned for about 2000 foods. In compiling this data-base, only values for whole foods or their components that are based on a validated HPLC procedure and are appropriate to the UK diet have been used.

At the present time the data-base is provisional and has several limitations. Perhaps the main limitation is that relatively few food items have been analyzed directly. Follow-up work is clearly necessary to expand the range of foods analyzed, and to test the accuracy of calculated recipe-based values by direct HPLC analysis. Other issues need to be addressed in the UK context. For example values are needed for ethnic foods, and studies are needed to assess sample variability in raw foods, batch variations in manufactured products and losses during storage. Other foods with assigned values that are based on food similarities and educated guesswork may be particularly suspect. The food similarity approach is an interim measure that with a potential variation in the vitamin K content of foods of several thousand fold was still deemed useful. Thus the two most important variables determining the vitamin K content of a food is whether it is derived from photosynthetic plant tissue and if containing oils or fats, the type of these constituents.

Another potential source of error is that vitamin K is sensitive to light. It has been shown that the vitamin K content of oils exposed to both sunlight and fluorescent light is rapidly destroyed, although this can be prevented by storage in amber bottles (Ferland & Sadowski, 1992). Little is known about the effects on vitamin K content on perishable major food sources such as green vegetables that are often exposed to light and air for several days.

Another recently identified source of error for certain cooked or manufactured foods is the chemical reduction of the 2′, 3′ side chain double bond of phylloquinone during the hydrogenation process of vegetable fats and oils. This leads to the conversion of phylloquinone to dihydro-vitamin K which is also a potential source of vitamin K although little is known about its bioactivity in relation to phylloquinone. Values for foods containing hydrogenated fats and oils that have been *calculated* from the phylloquinone content of the ingredient oils are therefore likely to be underestimates. At the time most of our direct HPLC analyses were performed, we were unaware of the possible presence of the dihydro-derivative. Therefore assuming dihydro-vitamin K is biologically active, our data-base of *directly analyzed* items will also underestimate the total vitamin K content.

Table 2
Phylloquinone content of oils and fats (average values from UK analyses)

Oil or fat	Phylloquinone content (μ g/100 g)
Coconut	0.5
Groundnut	1
Corn	3
Safflower	3
Sunflower	6
Oleo (beef)	6
Butter	7
Palm	8
Sesame	10
Olive	30
Olive (extra virgin)	80
Rapeseed	123
Soybean	173

It has been calculated that about 30% of the total vitamin K intake in USA children is in the form of dihydrovitamin K (Booth, Pennington & Sadowski, 1996) so this issue is clearly important.

One major problem in calculating the phylloquinone content of certain oil- and fat- containing foods is the lack of information about the nature of the oil/fat component. Again in our experience and that of others (Ferland & Sadowski, 1992) there may be considerable variability between different batches of the same oil. The wide range of phylloquinone contents of oils and fats analyzed in the UK is shown in Table 2.

Finally the data-base does not include the possible contribution from bacterial menaquinones. Preliminary work suggests that nutritionally significant amounts of the long-chain forms (mainly MK-8 and MK-9) are found in all cheeses (thought to be derived from the bacterial starter) although they can be detected in low concentrations in yoghurt and milk (Shearer et al., 1996). One menaquinone, MK-4, is an exception to the general rule that menaquinones have a bacterial origin. It has been known for many years that MK-4 may be synthesized by mammalian tissues from menadione, a compound that is not per se a natural food constituent but is used widely as a vitamin K supplement in animal husbandry. Dairy produce and flesh from animals given menadione supplements are therefore a potential source of MK-4 and this form has indeed been shown to be present in these products in The Netherlands (C. Vermeer, personal communication). Animals have also been shown to be capable of converting dietary phylloquinone to MK-4 (Thijssen & Drittij-Reijnders, 1994), a route that may also account for the MK-4 content of certain foods. Further work is needed to establish the nutritional sources and significance of dietary menaquinones.

8. Population studies using the UK food data-base for phylloquinone

With funding from MAFF (ANO504) this new UK data-base for phylloquinone in foods has already been used to assess dietary intakes in Scotland. The objectives were to determine the first accurate dietary intakes in a UK population, to assess their variability (intra- and inter-individual, seasonal) and to relate these to plasma levels and functional indices of coagulation (Bolton-Smith & Shearer, 1997). An ongoing MAFF project (AN0525) is assessing dietary phylloquinone intakes in elderly women in relation to bone health and to the new functional markers of vitamin K status, PIVKA-II and ucOC.

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