# Chapter 10
# Vitamin K

Vitamin K is an essential fat-soluble micronutrient which is needed for a unique post-translational chemical modification in a small group of proteins with calcium-binding properties, collectively known as vitamin K – dependent proteins or Gla-proteins. Thus far, the only unequivocal role of vitamin K in health is in the maintenance of normal coagulation. The vitamin K – dependent coagulation proteins are synthesised in the liver and comprise factors II, VII, IX, and X, which have a haemostatic role (i.e., they are procoagulants that arrest and prevent bleeding), and proteins C and S, which have an anticoagulant role (i.e., they inhibit the clotting process). Despite this duality of function, the overriding effect of nutritional vitamin K deficiency is to tip the balance in coagulation towards a bleeding tendency caused by the relative inactivity of the procoagulant proteins. Vitamin K – dependent proteins synthesised by other tissues include the bone protein osteocalcin and matrix Gla protein; their functions remain to be clarified.

## Biological role of vitamin K

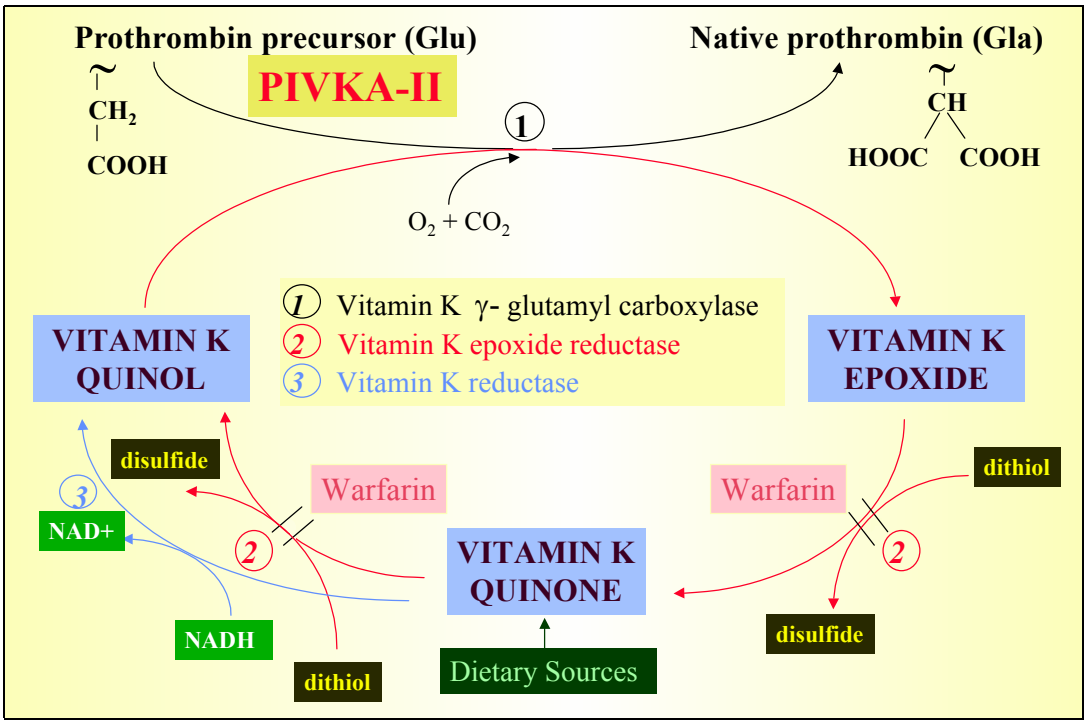
Vitamin K is the family name for a series of fat-soluble compounds, which have a common 2-methyl-1, 4-naphthoquinone nucleus but differ in the structures of a side chain at the 3-position. They are synthesised by plants and bacteria. In plants the only important molecular form is phylloquinone (vitamin $K_1$), which has a phytyl side chain. Bacteria synthesise a family of compounds called menaquinones (vitamin $K_2$), which have 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 synthesise menaquinones in which one or more of the double bonds is saturated. The compound 2-methyl-1,4-naphthoquinone (common name menadione) may be regarded as a provitamin because vertebrates can convert it to MK-4 by adding a 4-prenyl side chain at the 3-position.

The biologic role of vitamin K is to act as a cofactor for a specific carboxylation reaction that transforms selective glutamate (Glu) residues to γ-carboxyglutamate (Gla) residues (*1,2*). The reaction is catalysed by a microsomal enzyme, γ-glutamyl, or vitamin K – dependent carboxylase, which in turn is linked to a cyclic salvage pathway known as the vitamin K epoxide cycle (***Figure 11***).

Scheme shows the cyclic metabolism of vitamin K in relation to the conversion of glutamate (Glu) to γ-carboxyglutamate (Gla) residues for the coagulation protein prothrombin. A general term for the glutamate precursors of vitamin K-dependent proteins is proteins induced by vitamin K absence, abbreviated PIVKA. For prothrombin (factor II) the glutamate precursor is known as PIVKA-II. The active form of vitamin K needed for carboxylation is the reduced form, vitamin K quinol. Known enzyme reactions are numbered 1, 2, and 3. The carboxylation reaction is driven by a vitamin K-dependent carboxylase activity (*reaction 1*) which simultaneously converts vitamin K quinol to vitamin K 2,3-epoxide. Vitamin K 2,3-epoxide is reduced back to the quinone and then to the quinol by vitamin K epoxide reductase (*reaction 2*). The reductase activity denoted 2 is dithiol dependent and is inhibited by coumarin anticoagulants such as warfarin. Dietary vitamin K may enter the cycle via an NAD(P)H-dependent vitamin K reductase activity (*reaction 3*), which is not inhibited by warfarin.

*Figure 11*

**The vitamin K epoxide cycle**




The four vitamin K-dependent procoagulants (factor II or prothrombin, and factors VII, IX, and X) are serine proteases that are synthesised in the liver and then secreted into the circulation as inactive forms (zymogens). Their biologic activity depends on their normal complement of Gla residues, which are efficient chelators of calcium ions. In the presence of Gla and calcium ions these proteins bind to the surface membrane phospholipids of platelets and endothelial cells where, together with other cofactors, they form membrane-bound enzyme complexes. When coagulation is initiated, the zymogens of the four vitamin K-dependent clotting factors are cleaved to yield the active protease clotting factors (*1-3*). Two other vitamin K-dependent proteins called protein C and protein S play a regulatory role in the inhibition of coagulation. The function of protein C is to degrade phospholipid-bound activated factors V and VIII in the presence of calcium. Protein S acts as a synergistic cofactor to protein C by enhancing the binding of activated protein C to negatively charged phospholipids. Yet another vitamin K-dependent plasma protein (protein Z) is suspected to have a haemostatic role but its function is currently unknown.

Apart from the coagulation proteins, several other vitamin K-dependent proteins have been isolated from bone, cartilage, kidney, lungs, and other tissues (*4, 5*). Only two, osteocalcin and matrix Gla protein (MGP), have been well characterised. Both are found in bone but MGP also occurs in cartilage, blood vessel walls, and other soft tissues. There is evidence that protein S is synthesised by several tissues including the vessel wall and bone and may have other functions besides its well-established role as a coagulation inhibitor. It also seems likely that one function of MGP is to inhibit mineralisation (*6*). Thus far, no clear biologic role for osteocalcin has been established despite its being the major non-collagenous bone protein synthesised by osteoblasts (*7-9*). This failure to establish a biologic function for osteocalcin has hampered studies of the possible detrimental effects of vitamin K deficiency on bone health. Evidence of a possible association of a suboptimal vitamin K status with increased fracture risk remains to be confirmed (*7-9*).

## Overview of metabolism

### *Absorption and transport*

Dietary vitamin K, mainly as phylloquinone, is absorbed chemically unchanged from the proximal intestine after solubilisation into mixed micelles composed of bile salts and the products of pancreatic lipolysis (*10*). In healthy adults the efficiency of absorption of phylloquinone in its free form is about 80 percent (*10, 11*). Within the intestinal mucosa the vitamin is incorporated into chylomicrons, is secreted into the lymph, and enters the blood via the lacteals (*11, 12*). Once in the circulation, phylloquinone is rapidly cleared (*10*) at a rate consistent with its continuing association with chylomicrons and the chylomicron remnants which are produced by lipoprotein lipase hydrolysis at the surface of capillary endothelial cells (*13*). After an overnight fast, more than half of the circulating phylloquinone is still associated with triglyceride-rich lipoproteins, with the remainder being equally distributed between low-density and high-density lipoproteins (*13*). Phylloquinone is the major circulating form of vitamin K but MK-7 is present in plasma at lower concentrations and has a lipoprotein distribution similar to phylloquinone (*13*). Although phylloquinone in blood must have been derived exclusively from the diet, it is not known whether circulating menaquinones such as MK-7 are derived from the diet, intestinal flora, or a combination of these sources.

### *Tissue stores and distribution*

Until the 1970s, the liver was the only known site of synthesis of vitamin K-dependent proteins and hence was presumed to be the only significant storage site for the vitamin. However, the discovery of vitamin K-dependent processes and proteins in a number of extra-hepatic tissues suggests that this may not be the case.

Human liver stores normally comprise about 90 percent menaquinones and 10 percent phylloquinone (*14, 15*). There is evidence that the phylloquinone liver stores are very labile; under conditions of severe dietary depletion, liver concentrations were reduced to about 25 percent of initial levels after only 3 days (*15*). This high turnover of hepatic reserves of phylloquinone is in accord with the high losses of this vitamer through excretion (*10*). Knowledge of hepatic stores of phylloquinone in different population groups is limited. Adult hepatic stores in a UK study were about 11 pmol/g (*14*) whereas in a study from Japan they were about twofold higher(*15*). Such reserves are about 20 000–40 000-fold lower than those for retinol for relative daily intakes of phylloquinone that are only about 10-fold lower than those of vitamin A (*16*).

The relationship between hepatic and total-body stores of vitamin K is not known. Other sites of storage may be adipose tissue and bone; both are known to be sites where vitamin K-bearing chylomicrons and chylomicron remnants may be taken up. It has been reported that the predominant vitamer in human cortical and trabecular bone is phylloquinone; unlike the situation in liver, no menaquinones higher than MK-8 were detected (*17*).

In contrast to the hepatic preponderance of long-chain menaquinones, the major circulating form of vitamin K is invariably phylloquinone. The menaquinones MK-7 and possibly MK-8 are also present but the common hepatic forms MKs 9–13 are not detectable in blood plasma (*16, 18*). This might be a consequence of a different route of absorption (e.g., the possibility of a portal route for long-chain MKs *versus* the established lymphatic route for phylloquinone) but might suggest that once in the liver, the lipophilic long-chain menaquinones are not easily mobilised (*16, 18, 19*).

### *Bio-activity*

Very little information exists on the relative effectiveness of different hepatic forms of K vitamins for the coagulation function of vitamin K in humans. This information is important because of the preponderance of long-chain menaquinones in human liver. Early bioassay data from rats suggested that long-chain menaquinones (MKs-7, 9, and 10) were more efficient than phylloquinone in reversing vitamin K deficiency when single doses were give parenterally and that their sustained response may be due to their slower hepatic turnover (*18, 19*). A longer duration of the biologic response of MK-9 compared with phylloquinone in vitamin K-deficient rats was also observed by Groenen-van Dooren *et al.* (*20*). On the other hand Will and Suttie (*21*) showed that, when given orally, the dietary requirement of MK-9 for the maintenance of prothrombin synthesis in rats is higher than that for phylloquinone. They also reported that the initial hepatic turnover of MK-9 was two- to three-fold slower than that of phylloquinone.

Suttie (*18*) emphasised that the existence of a large pool of menaquinones in human liver does not necessarily mean that menaquinones make a proportionately greater contribution to the maintenance of vitamin K sufficiency. In humans the development of sub-clinical signs of vitamin K deficiency detected in dietary phylloquinone restriction studies argues against this, especially when placed alongside the lack of change of hepatic menaquinone stores (*15*). One explanation is that much of the hepatic menaquinones is not biologically available to the microsomal γ-glutamyl carboxylase because of a different sub-cellular location, especially location in the mitochondria and possibly other non-microsomal sites (*18*).

### *Excretion*

Vitamin K is extensively metabolised in the liver and excreted in the urine and bile. In tracer experiments it was found that about 20 percent of an injected dose of phylloquinone was recovered in the urine whereas about 40–50 percent was excreted in the faeces via the bile (*10*); the proportion excreted was the same regardless of whether the injected dose was 1 mg or 45 μg. It seems likely, therefore, that about 60–70 percent of the amounts of phylloquinone absorbed from each meal will ultimately be lost to the body by excretion. These results suggest that the body stores of phylloquinone are being constantly replenished.

Two major human excretion products have been identified: carboxylic acids with 5 and 7-carbon sidechains that are excreted in the urine as glucuronide conjugates (*10*). The biliary metabolites have not been clearly identified but are initially excreted as water-soluble conjugates and become lipid soluble during their passage through the gut, probably through deconjugation by the gut flora. There is no evidence for body stores of vitamin K being conserved by an enterohepatic circulation. Vitamin K itself is too lipophilic to be excreted in the bile and the sidechain-shortened carboxylic acid metabolites are not biologically active.

## Populations at risk

### *Vitamin K deficiency bleeding in infants*

In infants up to around age 6 months, vitamin K deficiency, although rare, represents a significant public health problem throughout the world (*19, 22, 23*). The deficiency syndrome is traditionally known as haemorrhagic disease of the newborn or more recently, to give a better definition of the cause, vitamin K deficiency bleeding (VKDB).

The time of onset of VKDB is more unpredictable than previously supposed and it is now useful to recognise three syndromes: early, classic, and late (***Table 26***). Until the 1960s, VKDB was considered to be solely a problem of the first week of life. Then, in 1966, came the first reports from Thailand of a new vitamin K deficiency syndrome that typically

presented between 1 and 2 months of life and is now termed late VKDB. In 1977 Bhanchet and colleagues (*24*), who had first described this syndrome, summarised their studies of 93 affected Thai infants, establishing the idiopathic history, preponderance of breast-fed infants (98 percent), and high incidence of intracranial bleeding (63 percent). More reports from South East Asia and Australia followed, and in 1983 McNinch *et al*. (*25*) reported the return of VKDB in the United Kingdom. This increased incidence was ascribed to a decrease in the practice of vitamin K prophylaxis and to an increased trend towards exclusive human milk feeding (*25*). Human milk has lower concentrations of vitamin K than do infant milk formulas (*26*).

Without vitamin K prophylaxis, the incidence of late VKDB (per 100,000 births), based on acceptable surveillance data, has been estimated to be 4.4 in the United Kingdom, 7.2 in Germany, and as high as 72 in Thailand (*27*). Of real concern is that late VKDB, unlike the classic form, has a high incidence of death or severe and permanent brain damage resulting from intracranial haemorrhage (*19, 22, 23*).

Epidemiologic studies worldwide have identified two major risk factors for both classic and late VKDB: exclusive human milk feeding and the failure to give any vitamin K prophylaxis (*19, 22, 23*). The increased risk for infants fed human milk compared with formula milk is probably related to the relatively low concentrations of vitamin K (phylloquinone) in breast milk compared with formula milks (*26, 28, 29*). For classic VKDB, studies using the detection of under-carboxylated prothrombin or proteins induced by vitamin K absence (PIVKA)-II as a marker of sub-clinical vitamin K deficiency have suggested that it is the low cumulative intake of human milk in the first week of life rather than an abnormally low milk concentration *per se* that seems to be of greater relevance (*30, 31*). Thus, classic VKDB may be related, at least in part, to a failure to establish early breast-feeding.

***Table 26***

**Classification of vitamin K deficiency bleeding of the newborn**

| Syndrome | Time of presentation | Common bleeding sites | Comments |
|---|---|---|---|
| Early VKDB | 0–24 hours | Cephalohaematoma, intracranial, intrathoracic, intra-abdominal | Maternal drugs a frequent cause (e.g., Warfarin, anti-convulsants) |
| Classic VKDB | 1–7 days | Gastrointestinal, skin, nasal, circumcision | Mainly idiopathic, maternal drugs |
| Late VKDB | 1–12 weeks | Intracranial, skin, gastrointestinal | Mainly iodiopathic, may be presenting feature of underlying disease (e.g., cystic fibrosis, α-1-antitrypsin deficiency, biliary atresia); some degree of cholestasis often present |

[a] VKDB, vitamin K deficiency bleeding.
Source: Shearer (19).

For late VKDB other factors seem to be important because the deficiency syndrome occurs when breast-feeding is well established and mothers of affected infants seem to have normal concentrations of vitamin K in their milk (*31*). Instead some (although not all) infants

who develop late haemorrhagic disease of the newborn are later found to have abnormalities of liver function that may affect their bile acid production and result in a degree of malabsorption of vitamin K. The degree of cholestasis may be mild and its course may be transient and self-correcting, but affected infants will have increased dietary requirements for vitamin K because of a reduced absorption efficiency.

***Vitamin K prophylaxis in infants***

Because bleeding can occur spontaneously and because no screening test is available, it is now common paediatric practice to protect all infants by giving vitamin K supplements in the immediate perinatal period. Vitamin K prophylaxis has had a chequered history but in recent years has become a high-profile issue of public health in many countries throughout the world. The reasons for this are twofold. First there is now a convincing body of evidence showing that without vitamin K prophylaxis, infants have a small but real risk of dying from or being permanently brain damaged by vitamin K deficiency in the first 6 months of life (*19, 22, 23*). The other, much less certain evidence stems from a reported epidemiologic association between vitamin K given intramuscularly (but not orally) and the later development of childhood cancer (*32*). The debate, both scientific and public, which followed this and other publications has led to an increase in the use of multiple oral supplements instead of the traditional single intramuscular injection (usually of 1 mg of phylloquinone) given at birth. Although most of the subsequent epidemiologic studies have not confirmed any cancer link with vitamin K, the issue is still not resolved (*33, 34*).

***Vitamin K in adults***

In adults, primary vitamin K–deficient states that manifest as bleeding are almost unknown except when the absorption of the vitamin is impaired as a result of an underlying pathology (*1*).

## Dietary sources

High-performance liquid chromatography can be used to accurately determine the major dietary form of vitamin K (phylloquinone) in foods, and food tables are being compiled for Western diets (*16, 35, 36*). Phylloquinone is distributed ubiquitously throughout the diet, and the range of concentrations in different food categories is very wide. In general, the relative values in vegetables confirm the known association of phylloquinone with photosynthetic tissues, with the highest values (normally in the range 400–700 μg/100 g) being found in green leafy vegetables. The next best sources are certain vegetable oils (e.g., soybean, rapeseed, and olive oils) which contain 50–200 μg/100 g. Some vegetable oils, such as peanut, corn, sunflower and safflower oils, have a much lower phylloquinone content (1–10 μg/100 g). The great differences between vegetable oils obviously presents problems for calculating the phylloquinone contents of oil-containing foods when the type of oil (or its storage condition) is not known.

Menaquinones seem to have a more restricted distribution in the diet than does phylloquinone. In the Western diet nutritionally significant amounts of long-chain menaquinones have been found in animal livers and fermented foods such as cheeses. Yeasts do not synthesise menaquinones and menaquinone-rich foods are those with a bacterial fermentation stage. The Japanese food *natto* (fermented soybeans) has a menaquinone content even higher than that of phylloquinone in green leafy vegetables.

The relative dietary importance of MK-4 is more difficult to evaluate because concentrations in foods may well depend on geographic differences in the use of menadione in animal husbandry, menadione from which MK-4 may be synthesised in animal tissues.

Another imponderable factor is the evidence that animal tissues and dairy produce may contain some MK-4 as a product of tissue synthesis from phylloquinone itself (*37*).

Knowledge of the vitamin K content of human milk has been the subject of methodologic controversies with a 10-fold variation in reported values of phylloquinone concentrations of mature human milk (*38*). Where milk sampling and analytical techniques have met certain criteria for their validity, the phylloquinone content of mature milk have generally ranged between 1 and 4 μg/l, with average concentrations near the lower end of this range (*28, 29, 38*). However, there is considerable intra- and inter-subject variation, and levels higher are in colostral milk than in mature milk (*28*). Menaquinone concentrations in human milk have not been accurately determined but appear to be much lower than those of phylloquinone. Phylloquinone concentrations in infant formula milk range from 3 to 16 μg/l in unsupplemented formulas and up to 100 μg/l in fortified formulas (*26*). Nowadays most formulas are fortified; typical phylloquinone concentrations are about 50 μg/l.

## Bio-availability of vitamin K from foods

Very little is known about the bio-availability of the K vitamins from different foods. It has been estimated that the efficiency of absorption of phylloquinone from boiled spinach (eaten with butter) is no greater than 10 percent (*39*) compared with an estimated 80 percent when phylloquinone is given in its free form (*10, 11*). This poor absorption of phylloquinone from green leafy vegetables may be explained by its location in chloroplasts and tight association with the thylakoid membrane, where this naphthoquinone plays a role in photosynthesis. In comparison, the bio-availability of MK-4 from butter artificially enriched with this vitamer was more than twofold higher than that of phylloquinone from spinach (*39*). The poor extraction of phylloquinone from leafy vegetables, which as a category represents the single greatest food source of phylloquinone, may place a different perspective on the relative importance of other foods with lower concentrations of phylloquinone (e.g., containing soybean and rapeseed oils) but in which the vitamin is not tightly bound and its bio-availability is likely to be greater. Even before bio-availability was taken into account, fats and oils that are contained in mixed dishes were found to make an important contribution to the phylloquinone content of the US diet (*40*) and in a UK study contributed 30 percent of the total dietary intake (*41*).

No data exist on the efficiency of intestinal absorption of dietary long-chain menaquinones. Because the lipophilic properties of menaquinones are greater than those of phylloquinone, it is likely that the efficiency of their absorption, in the free form, is low, as suggested by animal studies (*18, 21*).

## Importance of intestinal bacterial synthesis as a source of vitamin K

Intestinal microflora synthesise large amounts of menaquinones, which are potentially available as a source of vitamin K (*42*). Quantitative measurements at different sites of the human intestine have demonstrated that most of these menaquinones are present in the distal colon (*42*). Major forms produced are MK-10 and MK-11 by *Bacteroides*, MK-8 by *Enterobacter*, MK-7 by *Veillonella,* and MK-6 by *Eubacterium lentum*. It is noteworthy that menaquinones with very long chains (MKs 10–13) are known to be synthesised by members of the anaerobic genus *Bacteroides* and are major inhabitants of the intestinal tract but have not been detected in significant amounts in foods. The widespread presence of MKs 10–13 in human livers at high concentrations (*14, 15*) therefore suggests that these forms, at least, originate from intestinal synthesis (*16*).

It is commonly held that animals and humans obtain a significant fraction of their vitamin K requirement from direct absorption of menaquinones produced by microfloral synthesis (*43*), but hard experimental evidence documenting the site and extent of any absorption is singularly lacking (*18, 19, 23*). The most promising site of absorption is the terminal ileum, where there are some menaquinone-producing bacteria as well as bile salts. The evidence overall suggests that the bio-availability of bacterial menaquinones is poor because they are mostly tightly bound to the bacterial cytoplasmic membrane and the largest pool is present in the colon, which lacks bile salts for their solubilisation (*19, 23*).

## Evidence on which recommendations can be based

### *Assessment of vitamin K status*

Conventional coagulation assays are useful for detecting overt vitamin K-deficient states which are associated with a risk of bleeding. However, they offer only a relatively insensitive insight into vitamin K nutritional status and the detection of sub-clinical vitamin K-deficient states. A more sensitive measure of vitamin K sufficiency can be obtained from tests that detect under-carboxylated species of vitamin K-dependent proteins. In states of vitamin K deficiency, under-carboxylated species of the vitamin K-dependent coagulation proteins are released from the liver into the blood; their levels increase with the degree of severity of vitamin K deficiency. These under-carboxylated forms (PIVKA) are unable to participate in the normal coagulation cascade because they are unable to bind calcium. The measurement of under-carboxylated prothrombin (PIVKA-II) is the most useful and sensitive homeostatic marker of sub-clinical vitamin K deficiency. Importantly, PIVKA-II is detectable in plasma before any changes occur in conventional coagulation tests. Several types of assay for PIVKA-II have been developed which vary in their sensitivity (*44*).

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 under-carboxylated species of osteocalcin (ucOC) into the bloodstream. It has been proposed that the concentration of circulating ucOC reflects the sufficiency of vitamin K for the carboxylation of this Gla protein in bone tissue (*7, 45*). Most assays for ucOC have been indirect because they rely on the differential absorption of carboxylated and under-carboxylated forms to hydroxyapatite and are difficult to interpret (*46*).

Other criteria of vitamin K sufficiency that have been used are plasma measurements of phylloquinone and the measurement of urinary Gla. It is expected and found that the excretion of urinary Gla is decreased in vitamin K deficiency.

### *Dietary intakes in infants and their adequacy*

The average intake of phylloquinone in infants fed human milk during the first 6 months of life has been reported to be less than 1 μg/day; this is approximately 100-fold lower than the intake in infants fed a typical supplemented formula (29). This big disparity between intakes is reflected in plasma levels (***Table 27***).

Using the detection of PIVKA-II as a marker of sub-clinical deficiency, a study from Germany concluded that a minimum daily intake of about 100 ml of colostral milk (that supplies about 0.2-0.3 μg of phylloquinone) is sufficient for normal haemostasis in a baby of about 3 kg during the first week of life (*30, 47*). Similar conclusions were reached in a Japanese study which showed a linear correlation between the prevalence of PIVKA-II and the volume of breast milk ingested over 3 days (*48*); 95 percent of infants with detectable PIVKA-II had average daily intakes of less than about 120 ml, but the marker was not detectable when intakes reached 170 ml/day.

***Table 27***

**Dietary intakes and plasma levels of phylloquinone in human-milk-fed *versus* formula-fed infants aged 0–6 months**

| Age (weeks) | Phylloquinone intake (μg/day) | | Plasma phylloquinone (μg/l) | |
|---|---|---|---|---|
| | Human milk fed[a] | Formula fed[b] | Human milk fed | Formula fed |
| 6 | 0.55 | 45.4 | 0.13 | 6.0 |
| 12 | 0.74 | 55.5 | 0.20 | 5.6 |
| 26 | 0.56 | 52.2 | 0.24 | 4.4 |

[a]Breast-milk concentrations averaged 0.86, 1.14, and 0.87 μg/l of phylloquinone at 6, 12, and 26 weeks, respectively.

[b]All infants were fed a formula containing phylloquinone at 55 μg/l.

Source: Greer FR *et al*. (*29*).

### *Factors of relevance to classical vitamin K deficiency bleeding*

The liver stores of vitamin K in the newborn infant differ both qualitatively and quantitatively from that in adults. First, phylloquinone levels at birth are about one-fifth those in adults and second, bacterial menaquinones are undetectable (*14*). The resistance to placental transport of vitamin K to the human foetus is well-established (*19, 22*). Thereafter, the limited available data suggest that hepatic stores of menaquinones build up gradually, becoming detectable at around the second week of life but only reaching adult concentrations after 1 month of age (*14, 49*). A gradual increase in liver stores of menaquinones may reflect the gradual colonisation of the gut by enteric microflora.

A practical problem in assessing the functional status of vitamin K in the neonatal period is that there are both gestational and postnatal increases in the four vitamin K-dependent procoagulant factors which are unrelated to vitamin K status (*50*). This means that unless the deficiency state is quite severe, it is very difficult to interpret clotting factor activities as a measure of vitamin K sufficiency. The best diagnostic tool of the adequacy of vitamin K stores for neonates is by the detection of PIVKA-II by immunoassays. The use of this marker has clearly shown that there is a temporary dip in the vitamin K status of infants exclusively fed human milk in the first few days after birth (*30, 47, 48, 51, 52*). The fact that the degree of this dip is associated with human-milk intakes (*30, 47, 48*) and is less evident or abolished in infants given artificial feeds (*30, 48, 52*) or prophylactic vitamin K at birth (*48, 51, 52*) shows that the detection of PIVKA-II reflected a dietary lack of vitamin K.

### *Factors of relevance to late vitamin K deficiency bleeding*

The natural tendency for human-milk-fed infants to develop a sub-clinical vitamin K deficiency in the first 2–3 days of life is self-limiting. Comparisons between untreated human-milk-fed infants with others who had received vitamin K or supplementary feeds clearly suggest that this improvement in vitamin K–dependent clotting activity is due to an improved vitamin K status. After the first week, vitamin K–dependent clotting factor increases are more gradual, and it is not possible from clotting factor assays to differentiate between the natural post-natal increase in the synthesis of the core proteins from an improved vitamin K status leading to greater functional activity.

Use of the most sensitive assays for PIVKA-II shows that there is still evidence of suboptimal vitamin K status in infants solely fed human milk between the ages of 1 and 2

months (*52, 53*). Deficiency signs are less common in infants who have received adequate vitamin K supplementation (*52, 53*) or who have been formula fed (*52*).

***Dietary intakes in older infants, children, and adults and their adequacy***

The only comprehensive national survey of phylloquinone intakes across all age groups (except infants aged 0–6 months) is that of the US Food and Drug Administration Total Diet Study, which was based on the 1987–88 Nationwide Food Consumption Survey (*40*). For infants and children from the age of 6 months to 16 years, average phylloquinone intakes were above the current US recommended dietary allowance (RDA) values for their respective age groups, more so for children up to 10 years than from 10 to 16 years (***Table 28***) (*40*). There have been no studies of intakes in children in relation to functional markers of vitamin K sufficiency in children.

Intakes for adults in The Total Diet Study (***Table 28***) were also close to or slightly higher than the current US RDA values of 80 μg for men and 65 μg for women, although intakes were slightly lower than the RDA in the 25–30 years age group (*54*). There is some evidence from an evaluation of all the US studies that older adults have higher dietary intakes of phylloquinone than do younger adults (*55*).

The US results are very comparable with a detailed, seasonality study in the United Kingdom in which mean intakes in men and women (aged 22–54 years) were 72 and 64 μg/day, respectively; no significant sex or seasonal variations were found (*56*). Another UK study suggested that intakes were lower in manual workers and in smokers, reflecting their lower intakes of green vegetables and high-quality vegetable oil (*57*).

Several dietary restriction and repletion studies have attempted to assess the adequacy of vitamin K intakes in adults (*55, 58*). It is clear from these studies that volunteers consuming less than 10 μg/day of phylloquinone do not show any changes in conventional coagulation tests even after several weeks unless other measures to reduce the efficiency of absorption are introduced. However, a diet containing only 2–5 μg/day of phylloquinone fed for 2 weeks did result in an increase of PIVKA-II and a 70 percent decrease in plasma phylloquinone (*59*). Similar evidence of a sub-clinical vitamin K deficiency together with an increased urinary excretion of Gla was found when dietary intakes of phylloquinone were reduced from about 80 to about 40 μg/day for 21 days (*60*). A repletion phase in this study was consistent with a human dietary vitamin K requirement (for its coagulation role) of about 1 μg/kg body weight/day.

The most detailed and controlled dietary restriction and repletion study in healthy human subjects is that by Ferland *et al*. (*61*). In this study 32 healthy subjects in two age groups (20–40 and 60–80 years) were fed a mixed diet containing about 80 μg/day of phylloquinone, which is the RDA for adult males in the United States (*54*). After 4 days on this baseline diet there was a 13-day depletion period during which the subjects were fed a diet containing about 10 μg/day. After this depletion phase the subjects entered a 16-day repletion period during which, over 4-day intervals, they were sequentially repleted with 5, 15, 25, and 45 μg phylloquinone. The depletion protocol had no effect on conventional coagulation and specific factor assays but did induce a significant increase in PIVKA-II in both age groups. The most dramatic change was in plasma levels of phylloquinone, which fell to about 15 percent of the values on day 1. The drop in plasma phylloquinone also suggested that the average dietary intake of these particular individuals before they entered the study had been greater than the baseline diet of 80 μg/day. The repletion protocol failed to bring the plasma phylloquinone levels of the young subjects back above the lower limit of the normal range (previously established in healthy, free-living adults) whereas the plasma levels in the elderly group only rose slightly above this lower limit in the last 4 days. Another indication of

a reduced vitamin K status in the young group was the fall in urinary output of Gla (90 percent of baseline) that was not seen in the elderly group; this suggested that younger subjects are more susceptible to the effects of an acute deficiency than are older subjects.

***Table 28***

**Mean dietary intakes of phylloquinone from US Food and Drug Administration Total Diet Study based on 1987-88 Nationwide Food Consumption Survey *versus* US RDA**

| Group | No.[a] | Phylloquinone Intake (μg/day) 1990 TDS[b] | RDA[c] |
|---|---|---|---|
| Infants | | | |
| 6 months | 141 | 77 | 10 |
| Children | | | |
| 2 years | 152 | 24 | 15 |
| 6 years | 154 | 46 | 20 |
| 10 years | 119 | 45 | 30 |
| Females, 14–16 years | 188 | 52 | 45-55 |
| Males, 14–16 years | 174 | 64 | 45-65 |
| Younger adults | | | |
| Females, 25–30 years | 492 | 59 | 65 |
| Males, 25–30 years | 386 | 66 | 80 |
| Females, 40–45years | 319 | 71 | 65 |
| Males, 40–45years | 293 | 86 | 80 |
| Older adults | | | |
| Females, 60–65 years | 313 | 76 | 65 |
| Males, 60–65 years | 238 | 80 | 80 |
| Females, 70+ years | 402 | 82 | 65 |
| Males, 70+ years | 263 | 80 | 80 |

[a]The number of subjects as stratified by age and/or sex.
[b]Total Diet Study (*40*).
[c]Recommended Dietary Allowance, 1989 (*54*).

One important dietary intervention study measured the carboxylation status of the bone vitamin K-dependent protein, osteocalcin, in response to altered dietary intakes of phylloquinone (*62*). This was a crossover study, which evaluated the effect in young adults of increasing the dietary intake of phylloquinone to 420 μg/day for 5 days from a baseline intake of 100 μg/day. Although total concentrations of osteocalcin were not affected by either of the dietary treatments, ucOC fell dramatically in response to the 420 μg diet and by the end of the 5-day supplementation period was 41 percent lower than the baseline value. After the return to the mixed diet, the ucOC percent rose significantly but after 5 days had not returned to pre-supplementation values. This study suggested that the carboxylation of osteocalcin in bone may require higher dietary intakes of vitamin K than those needed to sustain its haemostatic function.

## Recommendations for vitamin K intakes

***Table 29***

**Recommended nutrients intakes for vitamin K**

| Age group | Recommended Nutrient Intake† μg /day |
|---|---|
| Infants and children | |
| 0–6 months | 5* |
| 7–12 months | 10 |
| 1–3 years | 15 |
| 4–6 years | 20 |
| 7–9 years | 25 |
| Adolescents, 10–18 years | |
| Females | 35-55 |
| Males | 35-55 |
| Adults | |
| Females, 19–65 years | 55 |
| 65+ years | 55 |
| Males, 19–65 years | 65 |
| 65+ years | 65 |
| Pregnancy | 55 |
| Lactation | 55 |

†The RNI for each age group is based on a daily intake of approximately 1 microgram/body weight of phylloquinone.
*This intake cannot be met by infants who are exclusively breast-fed.

To prevent bleeding due to vitamin K deficiency, the panel recommends that all breast babies should receive vitamin K supplementation at birth according to nationally approved guidelines. Vitamin K formulations and prophylactic regimes differ from country to country. Guidelines range from a single intramuscular injection (usually 1 mg of phylloquinone) given at birth to multiple oral doses given over the first few weeks (***Table 29***).

### *Infants 0–6 months*

Consideration of the requirements of vitamin K for infants up to age 6 months is complicated by the need to prevent a rare but potentially devastating bleeding disorder, which is caused by vitamin K deficiency. To protect the few affected infants, most developed and some developing countries have instituted a blanket prophylactic policy to protect infants at risk. The numbers of infants at risk without such a programme has a geographic component, being more prevalent in the Far East, and a dietary component with solely human-milk-fed babies having the highest risk (*22, 23, 27*). Of the etiologic factors, some of which may still be unrecognised, one factor in some infants is mild cholestasis. The problem of overcoming a variable and, in some infants, inefficient absorption is the likely reason that oral prophylactic regimens, even with two or three pharmacologic doses (1 mg phylloquinone), have occasionally failed to prevent VKDB (*63*). This makes it difficult to design an effective oral prophylaxis regimen that is comparable in efficacy with the previous "gold standard" of 1 mg phylloquinone given by intramuscular injection at birth. In several countries intramuscular prophylaxis fell out of favour after the epidemiologic report and subsequent controversy that this route may be linked to childhood cancer (*32-34*).

Infants who have been entirely fed with supplemented formulas are well protected against VKDB and on intakes of around 50 μg/day have plasma levels that are about 10-fold higher than the adult average of about 1.0 nmol/l (0.5 μg/l) (*29*) (***Table 29***). Clearly then, an optimal intake would lie below an intake of 50 μg/day. Cornelissen *et al*. (*64*) evaluated the effectiveness of giving infants a daily supplement of 25 μg phylloquinone after they had received a single oral dose of 1 mg at birth. This regimen resulted in median plasma levels at ages 4, 8, and 12 weeks of around 2.2 nmol/l (1.0 μg/l) when sampled 20–28 hours after the most recent vitamin K dose; this level corresponds to the upper end of the adult fasting range. In 12-week-old infants supplemented with this regime, the median plasma level was about fourfold higher than that in a control group of unsupplemented infants (1.9 *versus* 0.5 nmol/l). Also none of the 50 supplemented infants had detectable PIVKA-II at 12 weeks compared with 15 of 131 infants (11.5 percent) in the control group. This regime has now been implemented in The Netherlands and surveillance data on late VKDB suggest that it may be as effective as parenteral vitamin K prophylaxis (*63*).

The fact that VKDB is epidemiologically associated with breast feeding means that it is not prudent to base requirements solely on normal intakes of human milk and justifies the setting of a higher value that can only be met by some form of supplementation. The current US RDA for infants is 5 μg/day for the first 6 months (the greatest period of risk for VKDB) and 10 μg/day during the second 6 months (*54*). This is based on the adult RDA of 1 μg/kg body weight/day. However, if the vitamin K content of human milk is assumed to be about 2 μg/l, exclusively breast-fed infants aged 0–6 months may ingest only 20 percent of their presumed daily requirement of 5 μg (*54*). Whether a figure of 5 μg/day is itself safe is uncertain. In the United Kingdom the dietary reference value for infants was set at 10 μg/day, which in relation to body weight (2 μg/kg) is about double the estimate for adults (*65*). It was set with reference to the upper end of possible human milk concentrations plus a further qualitative addition to allow for the absence of hepatic menaquinones in early life and the presumed reliance on dietary vitamin K alone.

The association of VKDB with breast-feeding does not mean that most infants are at risk of developing VKDB, because this is a rare vitamin K deficiency syndrome. In contrast to measurements of PIVKA-II levels, comparisons of vitamin K–dependent clotting activities have shown no detectable differences between infants fed human milk and those fed artifical formula. The detection of PIVKA-II with normal functional levels of vitamin K–dependent coagulation factors does not imply immediate or even future haemorrhagic risk for a particular individual. The major value of PIVKA-II measurements in infants is to assess the prevalence of suboptimal vitamin K status in population studies. However, because of the potential consequences of VKDB, the paediatric profession of most countries agrees that some form of vitamin K supplementation is necessary even though there are widespread differences in actual practice.

### *Infants (7–12 months), children, and adults*

In the past, the requirements for vitamin K only considered its classical function in coagulation; an RDA was given for vitamin K in the United States (*54, 58*) and a safe and adequate intake level was given in the United Kingdom (*65*). In both countries the adult RDA or adequate intake was set at a value of 1 μg/kg body weight/day. Thus in the United States the RDA for a 79-kg man is listed as 80 μg/day and for a 63-kg women as 65 μg/day (*54*).

At the time previous recommendations were set there were few data on dietary intakes of vitamin K (mainly phylloquinone) in different populations. The development of more accurate and wide-ranging food databases is now helping to address this question. The results of several dietary intake studies in the United States and the United Kingdom suggest that the

average intakes for adults are very close to the respective recommendations of each country. In the United States, preliminary intake data also suggest that average intakes of phylloquinone in children and adolescents also exceed the RDA; in 6-month-old infants the intakes exceeded the RDA of 10 μg by nearly eightfold (*40*), reflecting the use of supplemented formula foods. Because there is no evidence of even sub-clinical deficiencies of haemostatic function, a daily intake of 1 μg/kg may still be used as the basis for the recommended nutrient intake (RNI). There is no basis as yet for making different recommendations for pregnant and lactating women.

A relevant question is whether the RNI should be raised to take into account recent evidence that the requirements for the optimal carboxylation of vitamin K–dependent proteins in other tissues are greater than those for coagulation. There is certainly evidence that the γ-carboxylation of osteocalcin can be improved by intakes somewhere between 100 and 420 μg/day (*62*). If an RNI for vitamin K sufficiency is to be defined as that amount necessary for the optimal carboxylation of all vitamin K-dependent proteins, including osteocalcin, then it seems clear that this RNI would lie somewhere above the current intakes of many, if not most, of the population in the United States and the United Kingdom. Because a clearly defined metabolic role and biochemical proof of the necessity for fully γ-carboxylated osteocalcin for bone health is currently lacking, it would be unwise to make such a recommendation.

### Toxicity

When taken orally, natural K vitamins seem free of toxic side effects. This safety is illustrated by the common clinical administration of phylloquinone at doses of 10–20 mg or greater. Some patients with chronic fat malabsorption regularly ingest doses of this size without evidence of any harm. However, synthetic preparations of menadione or its salts are best avoided for nutritional purpose, especially for vitamin prophylaxis in the newborn. Besides lacking intrinsic biologic activity, the high reactivity of its unsubstituted 3-position has been associated with neonatal haemolysis and liver damage.

### Future research

The following are recommended areas for future research:

- prevalence, causes, and prevention of VKDB in infants in different population groups;
- bio-availability of dietary phylloquinone (and menaquinones) from foods and menaquinones from gut flora;
- significance of menaquinones to human requirements for vitamin K;
- the physiologic roles of vitamin K-dependent proteins in functions other than coagulation; and
- the significance of under-carboxylated vitamin K-dependent proteins and sub-optimal vitamin K status to bone and cardiovascular health.

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