

6. Vitamin K

6.1 Introduction

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 synthesized 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 a bleeding tendency caused by the relative inactivity of the procoagulant proteins. Vitamin K-dependent proteins synthesized by other tissues include the bone protein osteocalcin and matrix Gla protein, though their functions remain to be clarified.

6.2 Biological role of vitamin K

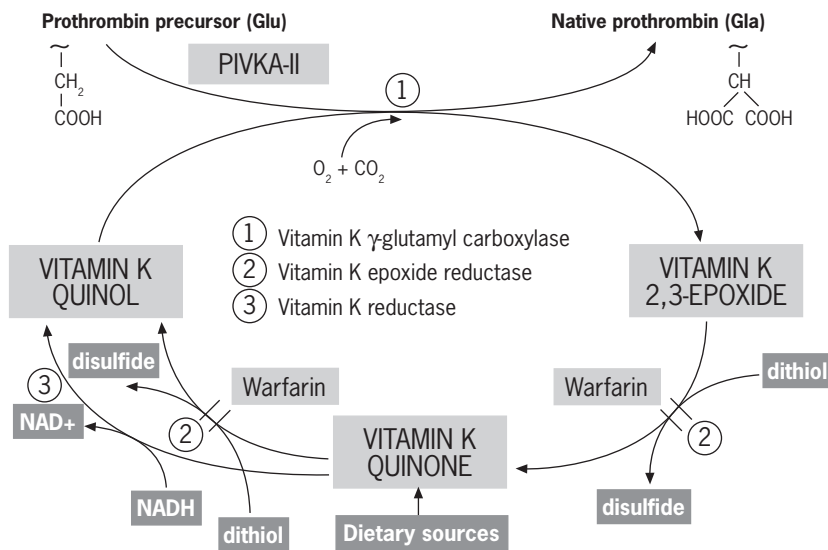
Vitamin K is the family name for a series of fat-soluble compounds which have a common 2-methyl-1,4-naphthoquinone nucleus but differ in the structures of a side chain at the 3-position. They are synthesized by plants and bacteria. In plants the only important molecular form is phyloquinone (vitamin K₁), which has a phytyl side chain. Bacteria synthesize a family of compounds called menaquinones (vitamin K₂), 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 synthesize 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 biological 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 6.1).

The four vitamin K-dependent procoagulants (factor II or prothrombin, and factors VII, IX, and X) are serine proteases that are synthesized in the liver and then secreted into the circulation as inactive forms (zymogens). Their biological activity depends on their normal complement of Gla residues, which are efficient chelators of calcium ions. In the presence of Gla residues 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

FIGURE 6.1
The vitamin K epoxide cycle



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 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 reaction 2 is dithiol dependent and is inhibited by coumarin anticoagulants such as warfarin. Dietary vitamin K may enter the cycle via an NADPH-dependent vitamin K reductase activity (reaction 3), which is not inhibited by warfarin.

yield the active protease clotting factors (1–3). Two other vitamin K-dependent proteins, 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. There is evidence that protein S is synthesized by several tissues including the blood vessel wall and bone and may have other functions besides its well-established role as a coagulation inhibitor. 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 characterized. Both are found in bone but MGP also occurs in cartilage, blood vessel walls, and other soft tissues. It seems likely that one function of MGP is to inhibit mineralization (6). Thus far, no clear biological role for osteocalcin has been established despite its being the major non-collagenous bone protein synthesized by osteoblasts (7–9). This failure to establish a biological 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).

6.3 Overview of vitamin K metabolism

6.3.1 Absorption and transport

Dietary vitamin K, mainly phylloquinone, is absorbed chemically unchanged from the proximal intestine after solubilization 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% (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). Although phylloquinone is

the major circulating form of vitamin K, MK-7 is also present in plasma, at lower concentrations and with 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.

6.3.2 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 (see section 6.2).

Human liver stores normally comprise about 90% menaquinones and 10% 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% of their initial levels after only 3 days (15). This high turnover of hepatic reserves of phylloquinone is in accord with the high losses of this vitamin through excretion (10).

Knowledge of hepatic stores of phylloquinone in different population groups is limited. Adult hepatic stores in a United Kingdom study were about 11 pmol/g (14) whereas in a study from Japan they were about two-fold 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 vitamin 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 may 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 also suggest that once in the liver, the lipophilic long-chain menaquinones are not easily mobilized (16, 18, 19).

6.3.3 Bioactivity

Very little information exists on the relative effectiveness of the different hepatic forms of K vitamins with respect to 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 (MK-7, -9, and -10) were more efficient than phyloquinone in reversing vitamin K deficiency when single doses were given parenterally and that their sustained effect on vitamin K status may be due to their slower hepatic turnover (18, 19). Groenen-van Dooren et al. (20) also observed a longer duration of the biological response of MK-9 compared with phyloquinone in vitamin K-deficient rats. On the other hand, Will and Suttie (21) showed that when given orally, the dietary requirement for MK-9 for the maintenance of prothrombin synthesis in rats is higher than that for phyloquinone. They also reported that the initial hepatic turnover of MK-9 was two- to three-fold slower than that of phyloquinone.

Suttie (18) emphasized 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, however, the development of subclinical signs of vitamin K deficiency detected in dietary phyloquinone restriction studies argues against this, especially when placed alongside the lack of change of hepatic menaquinone stores (15). One explanation is that many of the hepatic menaquinones are not biologically available to the microsomal γ -glutamyl carboxylase because of their different subcellular location; for instance, they may be located in the mitochondria and possibly other non-microsomal sites (18).

6.3.4 Excretion

Vitamin K is extensively metabolized in the liver and excreted in the urine and bile. In tracer experiments about 20% of an injected dose of phyloquinone was recovered in the urine whereas about 40–50% 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 μ g. It seems likely, therefore, that about 60–70% of the amount of phyloquinone absorbed from each meal will ultimately be lost to the body by excretion. These results suggest that the body stores of phyloquinone are being constantly replenished.

The main urinary excretory products have been identified as carboxylic acids with 5- and 7-carbon side chains, which are excreted 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 gastrointestinal tract, probably through deconjugation by the intestinal 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 side chain-shortened carboxylic acid metabolites are not biologically active.

6.4 Populations at risk for vitamin K deficiency

6.4.1 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. More recently, in order to give a better definition of the cause, it has been termed vitamin K deficiency bleeding (VKDB).

The time of onset of VKDB is now thought to be more unpredictable than previously supposed; currently three distinct syndromes are recognized: early, classic, and late VKDB (Table 6.1). 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 which is now termed late VKDB. In 1977 Bhanchet and colleagues (24), who had first described this syndrome, summarized their studies of 93 affected Thai infants, estab-

TABLE 6.1

Classification of vitamin K deficiency bleeding of the newborn infant

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

VKDB, vitamin K deficiency bleeding.
Source: reference (19).

lishing the idiopathic history, preponderance of breast-fed infants (98%), and high incidence of intracranial bleeding (63%). 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).

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 (26). 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).

Epidemiological 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 (27–29). For classic VKDB, studies using the detection of under-carboxylated prothrombin or proteins induced by vitamin K absence (PIVKA-II) as a marker of subclinical 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 practices.

For late VKDB other factors seem to be important because the deficiency syndrome occurs when breastfeeding is well established and mothers of affected infants seem to have normal concentrations of vitamin K in their milk (31). For instance, 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 an increased dietary requirement for vitamin K because of reduced absorption efficiency.

6.4.2 Vitamin K prophylaxis in infants

As 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 pro-

phylaxis 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 two-fold. 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 epidemiological 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 phylloquinone) given at birth. Although most of the subsequent epidemiological studies have not confirmed any cancer link with vitamin K prophylaxis, the issue is still not resolved (33, 34).

6.4.3 Vitamin K deficiency 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).

6.5 Sources of vitamin K

6.5.1 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), which contain 50–200 µg/100 g; other vegetable oils, such as peanut, corn, sunflower, and safflower, however, contain much lower amounts of phylloquinone (1–10 µg/100 g). The great differences between vegetable oils with respect to vitamin K content obviously present problems for calculating the phylloquinone contents of oil-containing foods when the type of oil is not known.

Menaquinones seem to have a more restricted distribution in the diet than does phylloquinone. Menaquinone-rich foods are those with a bacterial fermentation stage. Yeasts, however, do not synthesize menaquinones. In

the typical diet of developed countries, nutritionally significant amounts of long-chain menaquinones have been found in animal livers and fermented foods such as cheeses. The Japanese food *natto* (fermented soybeans) has a menaquinone content even higher than the phylloquinone content of 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. MK-4 may be synthesized in animal tissues from menadione supplied in animal feed. 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 has 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 intersubject variation, and levels are higher in colostrum 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). Currently most formulas are fortified; typical phylloquinone concentrations are about 50 µg/l.

6.5.2 Bioavailability of vitamin K from foods

Very little is known about the bioavailability 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% (39) compared with an estimated 80% 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 naphthoquinone plays a role in photosynthesis. In comparison, the bioavailability of MK-4 from butter artificially enriched with this vitamin was more than two-fold 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. those containing soybean and rapeseed oils) but in which the vitamin is not tightly bound

and its bioavailability likely to be greater. Even before bioavailability was taken into account, fats and oils that are contained in mixed dishes were found to make an important contribution to the phyloquinone content of the United States diet (40) and in a United Kingdom study, contributed 30% 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 phyloquinone, it is likely that the efficiency of their absorption, in the free form, is low, as has been suggested by animal studies (18, 21).

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

Intestinal microflora synthesize 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 synthesized by members of the anaerobic genus *Bacteroides*, and are found in large concentrations in 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 conclusive experimental evidence documenting the site and extent of 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. However, the balance of evidence suggests that the bioavailability of bacterial menaquinones is poor because they are for the most part tightly bound to the bacterial cytoplasmic membrane and also because the largest pool is present in the colon, which lacks bile salts for their solubilization (19, 23).

6.6 Information relevant to the derivation of recommended vitamin K intakes

6.6.1 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 subclinical 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 subclinical vitamin K deficiency (see also section 6.4.1). 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 are indirect in that they rely on the differential absorption of carboxylated and under-carboxylated forms to hydroxyapatite and are thus 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 individuals with vitamin K deficiency.

6.6.2 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 large disparity between intakes is reflected in plasma levels (Table 6.2).

Using the detection of PIVKA-II as a marker of subclinical deficiency, a study from Germany concluded that a minimum daily intake of about 100 ml of colostrum 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% of infants with

TABLE 6.2

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

Age (weeks)	Phylloquinone intake ($\mu\text{g}/\text{day}$)		Plasma phylloquinone ($\mu\text{g}/\text{l}$)	
	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 of phylloquinone averaged 0.86, 1.14, and 0.87 $\mu\text{g}/\text{l}$ at 6, 12, and 26 weeks, respectively.

^b All infants were fed a formula containing phylloquinone at 55 $\mu\text{g}/\text{l}$.

Source: reference (29).

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.

6.6.3 Factors of relevance to classical vitamin K deficiency bleeding

The liver stores of vitamin K in the neonate differ both qualitatively and quantitatively from those in adults. First, phylloquinone levels at birth are about one fifth those in adults and second, bacterial menaquinones are undetectable (14). It has been well established that placental transport of vitamin K to the human fetus is difficult (19, 22). The limited available data suggest that hepatic stores of menaquinones build up gradually after birth, 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 colonization 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. Immunoassays are the best diagnostic tool for determining the adequacy of vitamin K stores in neonates, as they detect levels of PIVKA-II. 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 absent in infants given formula milk (30, 48, 52) or prophylactic vitamin K at birth (48, 51, 52) shows that the detection of PIVKA-II reflects a dietary lack of vitamin K (see also section 6.4.1).

6.6.4 Factors of relevance to late vitamin K deficiency bleeding

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

Use of the most sensitive assays for PIVKA-II show 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).

6.6.5 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 United States 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 United States 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 6.3) (40). No studies have been conducted that assess functional markers of vitamin K sufficiency in children.

Intakes for adults in the Total Diet Study (Table 6.3) were also close to or slightly higher than the current United States 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 United States studies that older adults have higher dietary intakes of phylloquinone than do younger adults (55).

The results from the United States are very similar to a detailed, seasonality study conducted 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 United Kingdom study suggested that intakes were lower in people who work as manual labourers and in smokers, reflecting the lower intakes of green vegetables and high-phylloquinone content vegetable oil in these groups (57).

TABLE 6.3

Mean dietary intakes of phylloquinone from the United States Food and Drug Administration Total Diet Study (TDS) based on the 1987–88 Nationwide Food Consumption Survey compared with the recommended dietary allowance (RDA), by group

Group	No. ^a	Phylloquinone intake (µg/day)	
		TDS ^b	RDA ^c
<i>Infants</i>			
6 months	141	77	10
<i>Children</i>			
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
<i>Younger adults</i>			
Females, 25–30 years	492	59	65
Males, 25–30 years	386	66	80
Females, 40–45 years	319	71	65
Males, 40–45 years	293	86	80
<i>Older adults</i>			
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, 1990 (40).

^c Recommended dietary allowance, 1989 (54).

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% decrease in plasma phylloquinone (59). Similar evidence of a subclinical vitamin K deficiency coupled 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 conducted to date 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 80µg/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 10µg/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 45µg of 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% of the values determined 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 80µg/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 adults) and the plasma levels in the elderly group rose only 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 (to 90% of baseline) that was not seen in the elderly group; this suggested that the younger subjects were more susceptible to the effects of an acute deficiency than were the older subjects.

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 420µg/day for 5 days from a baseline intake of 100µg/day. Although total concentrations of osteocalcin were not affected, ucOC fell dramatically in response to the 420µg diet and by the end of the 5-day supplementation period was 41% lower than the baseline value. After the return to the mixed diet, the ucOC percentage rose significantly but after 5 days had not returned to pre-supplementation values. This study suggests that the carboxylation of osteocalcin in bone might require higher dietary intakes of vitamin K than those needed to sustain its haemostatic function.

6.7 Recommendations for vitamin K intakes

6.7.1 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, a policy that is

endorsed by the present Consultation (Table 6.4). The numbers of infants at risk without such a programme has a geographic component, the risk being more prevalent in Asia, 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 unrecognized, 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. As previously stated, intramuscular prophylaxis fell out of favour in several countries after the epidemiological report and subsequent controversy that this administration route may be linked to childhood cancer (32–34).

TABLE 6.4

Recommended nutrient intakes (RNIs) for vitamin K, by group

Group	RNI ^a (µg/day)
<i>Infants and children</i>	
0–6 months	5 ^b
7–12 months	10
1–3 years	15
4–6 years	20
7–9 years	25
<i>Adolescents</i>	
Females, 10–18 years	35–55
Males, 10–18 years	35–55
<i>Adults</i>	
<i>Females</i>	
19–65 years	55
65+ years	55
<i>Males</i>	
19–65 years	65
65+ years	65
<i>Pregnant women</i>	55
<i>Lactating women</i>	55

^a The RNI for each group is based on a daily intake of approximately 1 µg/kg body weight of phylloquinone.

^b This intake cannot be met by infants who are exclusively breastfed (see Table 6.2). To prevent bleeding due to vitamin K deficiency, it is recommended that all breast-fed infants 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 of life.

Infants who have been entirely fed with supplemented formulas are well protected against VKDB and on intakes of around 50µg/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 6.2). Clearly then, an optimal intake would lie below an intake of 50µg/day. Cornelissen et al. (64) evaluated the effectiveness of giving infants a daily supplement of 25µg 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 four-fold 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%) 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 breastfeeding 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 United States RDA for infants is 5µg/day for the first 6 months (the greatest period of risk for VKDB) and 10µg/day during the second 6 months (54). These intakes are based on the adult RDA of 1µg/kg body weight/day. However, if the vitamin K content of human milk is assumed to be about 2µg/l, exclusively breast-fed infants aged 0–6 months may ingest only 20% of their presumed daily requirement of 5µg (54). Whether a figure of 5µg/day is itself safe is uncertain. In the United Kingdom the dietary reference value for infants is set at 10µg/day, which in relation to body weight (2µg/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 breastfeeding does not mean that most infants are at risk of developing VKDB, as 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 artificial 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.

6.7.2 Infants (7–12 months), children, and adults

In the past, the requirements for vitamin K have only considered its classical function in coagulation; an RDA has been given for vitamin K in the United States (54, 58) and a safe and adequate intake level given in the United Kingdom (65). In both countries the adult RDA or adequate intake have been 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 woman 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 redress this information gap. The results of several dietary intake studies carried out 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 exceed the RDA; in 6-month-old infants the intakes exceeded the RDA of 10 µg by nearly eight-fold (40), reflecting the use of supplemented formula foods. Because there is no evidence of even subclinical 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 (Table 6.4).

The question remains 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. However, 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 at this time.

6.8 Toxicity

When taken orally, natural K vitamins seem free of toxic side effects. This apparent safety is borne out 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 purposes, especially for vitamin prophylaxis in neonates. Besides lacking intrinsic biological activity, the high reactivity of its unsubstituted 3-position has been associated with neonatal haemolysis and liver damage.

6.9 Recommendations for future research

The following are recommended areas for future research:

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

References

1. Suttie JW. Vitamin K. In: Diplock AD, ed. *Fat-soluble vitamins: their biochemistry and applications*. London, Heinemann, 1985:225–311.
2. Furie B, Furie BC. Molecular basis of vitamin K-dependent γ -carboxylation. *Blood*, 1990, 75:1753–1762.
3. Davie EW. Biochemical and molecular aspects of the coagulation cascade. *Thrombosis and Haemostasis*, 1995, 74:1–6.
4. Vermeer C. γ -Carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. *Biochemical Journal*, 1990, 266:625–636.
5. Ferland G. The vitamin K-dependent proteins: an update. *Nutrition Reviews*, 1998, 56:223–230.
6. Luo G et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix Gla protein. *Nature*, 1997, 386:78–81.
7. Vermeer C, Jie K-S, Knapen MHJ. Role of vitamin K in bone metabolism. *Annual Review of Nutrition*, 1995, 15:1–22.
8. Binkley NC, Suttie JW. Vitamin K nutrition and osteoporosis. *Journal of Nutrition*, 1995, 125:1812–1821.

9. Shearer MJ. The roles of vitamins D and K in bone health and osteoporosis prevention. *Proceedings of the Nutrition Society*, 1997, 56:915–937.
10. Shearer MJ, McBurney A, Barkhan P. Studies on the absorption and metabolism of phylloquinone (vitamin K₁) in man. *Vitamins and Hormones*, 1974, 32:513–542.
11. Shearer MJ, Barkhan P, Webster GR. Absorption and excretion of an oral dose of tritiated vitamin K₁ in man. *British Journal of Haematology*, 1970, 18:297–308.
12. Blomstrand R, Forsgren L. Vitamin K₁-³H in man: its intestinal absorption and transport in the thoracic duct lymph. *Internationale Zeitschrift für Vitaminsforschung*, 1968, 38:45–64.
13. Kohlmeier M et al. Transport of vitamin K to bone in humans. *Journal of Nutrition*, 1996, 126(Suppl.):S1192–S1196.
14. Shearer MJ et al. The assessment of human vitamin K status from tissue measurements. In: Suttie JW, ed. *Current advances in vitamin K research*. New York, NY, Elsevier, 1988:437–452.
15. Usui Y et al. Vitamin K concentrations in the plasma and liver of surgical patients. *American Journal of Clinical Nutrition*, 1990, 51:846–852.
16. Shearer MJ, Bach A, Kohlmeier M. Chemistry, nutritional sources, tissue distribution and metabolism of vitamin K with special reference to bone health. *Journal of Nutrition*, 1996, 126(Suppl.): S1181–S1186.
17. Hodges SJ et al. Detection and measurement of vitamins K₁ and K₂ in human cortical and trabecular bone. *Journal of Bone and Mineral Research*, 1993, 8:1005–1008.
18. Suttie JW. The importance of menaquinones in human nutrition. *Annual Review of Nutrition*, 1995, 15:399–417.
19. Shearer MJ. Vitamin K metabolism and nutrition. *Blood Reviews*, 1992, 6:92–104.
20. Groenen-van Dooren MMCL et al. Bioavailability of phylloquinone and menaquinones after oral and colorectal administration in vitamin K-deficient rats. *Biochemical Pharmacology*, 1995, 50:797–801.
21. Will BH, Suttie JW. Comparative metabolism of phylloquinone and menaquinone-9 in rat liver. *Journal of Nutrition*, 1992, 122:953–958.
22. Lane PA, Hathaway WE. Vitamin K in infancy. *Journal of Pediatrics*, 1985, 106:351–359.
23. Shearer MJ. Fat-soluble vitamins: vitamin K. *Lancet*, 1995, 345:229–234.
24. Bhançhet P et al. A bleeding syndrome in infants due to acquired prothrombin complex deficiency: a survey of 93 affected infants. *Clinical Pediatrics*, 1977, 16:992–998.
25. McNinch AW, Orme RL, Tripp JH. Haemorrhagic disease of the newborn returns. *Lancet*, 1983, 1:1089–1090.
26. von Kries R, Hanawa Y. Neonatal vitamin K prophylaxis. Report of the Scientific and Standardization Subcommittee on Perinatal Haemostasis. *Thrombosis and Haemostasis*, 1993, 69:293–295.
27. Haroon Y et al. The content of phylloquinone (vitamin K₁) in human milk, cows' milk and infant formula foods determined by high-performance liquid chromatography. *Journal of Nutrition*, 1982, 112:1105–1117.
28. von Kries R et al. Vitamin K₁ content of maternal milk: influence of the stage of lactation, lipid composition, and vitamin K₁ supplements given to the mother. *Pediatric Research*, 1987, 22:513–517.
29. Greer FR et al. Vitamin K status of lactating mothers, human milk and breast-feeding infants. *Pediatrics*, 1991, 88:751–756.

30. von Kries R, Becker A, Göbel U. Vitamin K in the newborn: influence of nutritional factors on a-carboxy-prothrombin detectability and factor II and VII clotting activity. *European Journal of Pediatrics*, 1987, 146:123–127.
31. von Kries R, Shearer MJ, Göbel U. Vitamin K in infancy. *European Journal of Pediatrics*, 1988, 147:106–112.
32. Golding J et al. Childhood cancer, intramuscular vitamin K, and pethidine given during labour. *British Medical Journal*, 1992, 305:341–346.
33. Draper G, McNinch A. Vitamin K for neonates: the controversy. *British Medical Journal*, 1994, 308:867–868.
34. von Kries R. Neonatal vitamin K prophylaxis: the Gordian knot still awaits untying. *British Medical Journal*, 1998, 316:161–162.
35. Booth SL, Davidson KW, Sadowski JA. Evaluation of an HPLC method for the determination of phyloquinone (vitamin K₁) in various food matrices. *Journal of Agricultural and Food Chemistry*, 1994, 42:295–300.
36. Booth SL et al. Vitamin K₁ (phyloquinone) content of foods: a provisional table. *Journal of Food Composition and Analysis*, 1993, 6:109–120.
37. Thijssen HHW, Drittij-Reijnders MJ. Vitamin K distribution in rat tissues: dietary phyloquinone is a source of tissue menaquinone-4. *British Journal of Nutrition*, 1994, 72:415–425.
38. Canfield LM, Hopkinson JM. State of the art vitamin K in human milk. *Journal of Pediatric Gastroenterology and Nutrition*, 1989, 8:430–441.
39. Gijsbers BLMG, Jie K-SG, Vermeer C. Effect of food composition on vitamin K absorption in human volunteers. *British Journal of Nutrition*, 1996, 76:223–229.
40. Booth SL, Pennington JAT, Sadowski JA. Food sources and dietary intakes of vitamin K-1 (phyloquinone) in the American diet: data from the FDA Total Diet Study. *Journal of the American Dietetic Association*, 1996, 96:149–154.
41. Fenton ST et al. Nutrient sources of phyloquinone (vitamin K₁) in Scottish men and women [abstract]. *Proceedings of the Nutrition Society*, 1997, 56:301.
42. Conly JM, Stein K. Quantitative and qualitative measurements of K vitamins in human intestinal contents. *American Journal of Gastroenterology*, 1992, 87:311–316.
43. Davidson S, Passmore R, Eastwood MA. *Davidson and Passmore human nutrition and dietetics*, 8th ed. Edinburgh, Churchill Livingstone, 1986.
44. Widdershoven J et al. Four methods compared for measuring des-carboxy-prothrombin (PIVKA-II). *Clinical Chemistry*, 1987, 33:2074–2078.
45. Vermeer C, Hamulyák K. Pathophysiology of vitamin K-deficiency and oral anticoagulants. *Thrombosis and Haemostasis*, 1991, 66:153–159.
46. Gundberg CM et al. Vitamin K status and bone health: an analysis of methods for determination of undercarboxylated osteocalcin. *Journal of Clinical Endocrinology and Metabolism*, 1998, 83: 258–266.
47. von Kries R et al. Vitamin K deficiency and vitamin K intakes in infants. In: Suttie JW, ed. *Current advances in vitamin K research*. New York, NY, Elsevier, 1988:515–523.
48. Motohara K et al. Relationship of milk intake and vitamin K supplementation to vitamin K status in newborns. *Pediatrics*, 1989, 84:90–93.
49. Kayata S et al. Vitamin K₁ and K₂ in infant human liver. *Journal of Pediatric Gastroenterology and Nutrition*, 1989, 8:304–307.
50. McDonald MM, Hathaway WE. Neonatal hemorrhage and thrombosis. *Seminars in Perinatology*, 1983, 7:213–225.

51. Motohara K, Endo F, Matsuda I. Effect of vitamin K administration on carboxy-prothrombin (PIVKA-II) levels in newborns. *Lancet*, 1985, 2:242–244.
52. Widdershoven J et al. Plasma concentrations of vitamin K₁ and PIVKA-II in bottle-fed and breast-fed infants with and without vitamin K prophylaxis at birth. *European Journal of Pediatrics*, 1988, 148:139–142.
53. Motohara K, Endo F, Matsuda I. Vitamin K deficiency in breast-fed infants at one month of age. *Journal of Pediatric Gastroenterology and Nutrition*, 1986, 5:931–933.
54. Subcommittee on the Tenth Edition of the Recommended Dietary Allowances, Food and Nutrition Board. *Recommended dietary allowances*, 10th ed. Washington, DC, National Academy Press, 1989.
55. Booth SL, Suttie JW. Dietary intake and adequacy of vitamin K. *Journal of Nutrition*, 1998, 128:785–788.
56. Price R et al. Daily and seasonal variation in phylloquinone (vitamin K₁) intake in Scotland [abstract]. *Proceedings of the Nutrition Society*, 1996, 55:244.
57. Fenton S et al. Dietary vitamin K (phylloquinone) intake in Scottish men [abstract]. *Proceedings of the Nutrition Society*, 1994, 53:98.
58. Suttie JW. Vitamin K and human nutrition. *Journal of the American Dietetic Association*, 1992, 92:585–590.
59. Allison PM et al. Effects of a vitamin K-deficient diet and antibiotics in normal human volunteers. *Journal of Laboratory and Clinical Medicine*, 1987, 110:180–188.
60. Suttie JW et al. Vitamin K deficiency from dietary restriction in humans. *American Journal of Clinical Nutrition*, 1988, 47:475–480.
61. Ferland G, Sadowski JA, O'Brien ME. Dietary induced subclinical vitamin K deficiency in normal human subjects. *Journal of Clinical Investigation*, 1993, 91:1761–1768.
62. Sokoll LJ et al. Changes in serum osteocalcin, plasma phylloquinone, and urinary γ -carboxyglutamic acid in response to altered intakes of dietary phylloquinone in human subjects. *American Journal of Clinical Nutrition*, 1997, 65:779–784.
63. Cornelissen M et al. Prevention of vitamin K deficiency bleeding: efficacy of different multiple oral dose schedules of vitamin K. *European Journal of Pediatrics*, 1997, 156:126–130.
64. Cornelissen EAM et al. Evaluation of a daily dose of 25 μ g vitamin K₁ to prevent vitamin K deficiency in breast-fed infants. *Journal of Pediatric Gastroenterology and Nutrition*, 1993, 16:301–305.
65. Department of Health. *Dietary reference values for food energy and nutrients for the United Kingdom*. London, Her Majesty's Stationery Office, 1991 (Report on Health and Social Subjects No. 41).