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THE IMPORTANCE OF MENAQUINONES IN HUMAN NUTRITION

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Abstract

Bacterially produced menaquinones, 2-methyl-1,4-naphthoquinones with an unsaturated polyisoprenoid chain at the 3-position, are biologically active forms of vitamin K that are present in high concentrations in the human lower bowel. Menaquinones are found in human liver and circulate in human plasma at much higher concentrations than previously thought. Numerous case reports of antibiotic-induced, vitamin K–responsive hypothrombinemias have been taken as evidence that menaquinones contribute importantly to satisfying the

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human vitamin K requirement. However, more recent production of symptoms of vitamin K insufficiency in normal human subjects by dietary restriction of vitamin K argues against their nutritional significance. Current data support the view that menaquinones may partially satisfy the human requirement but that their contribution is much less than previously thought.

INTRODUCTION

Discovery of Menaquinones

Vitamin K was discovered as a result of a series of experiments conducted by the Danish nutritional biochemist Henrik Dam on the possible necessity of cholesterol in the diet of the chick. Dam (16) noted that chicks ingesting diets that had been extracted with nonpolar solvents to remove the sterols developed subdural or muscular hemorrhages and that blood taken from these animals clotted slowly. These observations were not unique. Similar responses were reported by other investigators when chicks were fed fish meal as a protein source. A series of investigations led by Dam (16) and by Almquist (2), who had continued the studies at Berkeley, established that the new essential nutrient was found in the nonsterol, nonsaponifiable portion of the lipid extracts of liver and a number of green vegetables. The Berkeley group also determined that much of the variability in reproducing the response was due to the use of different batches of fish meal as a protein source in these experiments. Drying the fish meal slowly and allowing bacterial action resulted in a protective response that was not obtained when the meal was dried rapidly. The term vitamin K was apparently first used by Dam (15a) in a short note in *Nature* concluding that the factor could not be identical to vitamin A, D, or E: "I therefore suggest the term vitamin K for the antihaemorrhagic factor." Dam prepared a crude plasma prothrombin fraction from chick plasma and demonstrated that its activity was decreased when it was obtained from vitamin K–deficient chicks. It was also shown in the late 1930s that the hemorrhagic condition resulting from obstructive jaundice or biliary problems resulted from poor utilization of vitamin K by these patients (16). These bleeding episodes were also attributed to a lack of plasma prothrombin.

Dam subsequently collaborated with Karrer of the University of Zurich, and by 1939 they had succeeded in isolating the vitamin as a yellow oil from alfalfa (16). Doisy's Washington University group (21) also purified vitamin K activity from both alfalfa and putrefied fish meal. These investigators were the first to characterize phylloquinone (vitamin K_1) as 2-methyl-3-phytyl-1,4-naphthoquinone and to synthesize this compound. They also isolated a form of the vitamin from putrefied fish meal, which, in contrast to the oil isolated from alfalfa, was a crystalline product. Subsequent studies demonstrated that

this compound, then called vitamin K_2, contained an unsaturated side chain, and it was characterized as the compound now known as menaquinone-6, 2-methyl-3-(*all-trans*-farnesylfarnesyl)-1,4-naphthoquinone. It was soon realized that the bacteria present in fish meal produced a number of homologues of the vitamin, with an unsaturated polyisoprenoid chain at the 3-position. These homologues were called menaquinones (MKs) and assigned a number indicating the number of isoprenoid units (usually 6–11) in the chain. The properties of menaquinones, with their long hydrophobic side chains, differ significantly from those of the smaller, less bulky, less hydrophobic phylloquinone (Figure 1).

The early history of the discovery of vitamin K has been well reviewed by the leaders in these efforts (8, 16, 21). The compound characterized by Doisy was assumed for many years to be the correct structure of the first menaquinone isolated. However, Isler et al (37) later demonstrated that a crystalline form of the vitamin isolated by Doisy's method contained seven, not six, isoprenyl units and was in fact 2-methyl-3-(*all-trans*-farnesylgeranylgeranyl)-1,4-naphthoquinone, or MK-7, not MK-6.

Metabolic Role of Vitamin K

The hemorrhagic condition that resulted from a dietary lack of vitamin K was initially shown to be associated with a decrease in plasma prothrombin activity. Later, as plasma clotting factors VII, IX, and X were discovered, it was shown that the synthesis of these factors, which are involved in the cascade resulting in activation of prothrombin to thrombin, was also depressed in the deficient state. These four plasma proteins (prothrombin plus factors VII, IX, and X) were collectively called the vitamin K–dependent clotting factors, and for years they were thought to be the only proteins that required the vitamin for synthesis. However, three other plasma proteins also need vitamin K for synthesis: proteins C, S, and Z. Proteins C and S act as anticoagulants, rather than procoagulants, in hemostasis, and the function of the protein Z is not known (17). A limited number of extrahepatic vitamin K–dependent proteins have also been identified. The most completely characterized of these are two proteins originally found in bone, osteocalcin or bone Gla protein (BGP), and matrix Gla protein (MGP). The physiological role of these proteins has not been clearly established and is the subject of another chapter in this volume (76). In its reduced, hydronaphthoquinone form, vitamin K is a substrate for an O_2-dependent enzyme localized in rough endoplasmic reticulum that catalyzes the posttranslational carboxylation of specific glutamyl residues in a limited number of newly synthesized proteins to form γ-carboxyglutamyl Gla residues. The properties of this enzyme, which is commonly called the vitamin K–dependent carboxylase or γ-glutamyl carboxylase, have been reviewed (65, 75). The coproduct of this reaction is vitamin K 2,3-epoxide. The normal

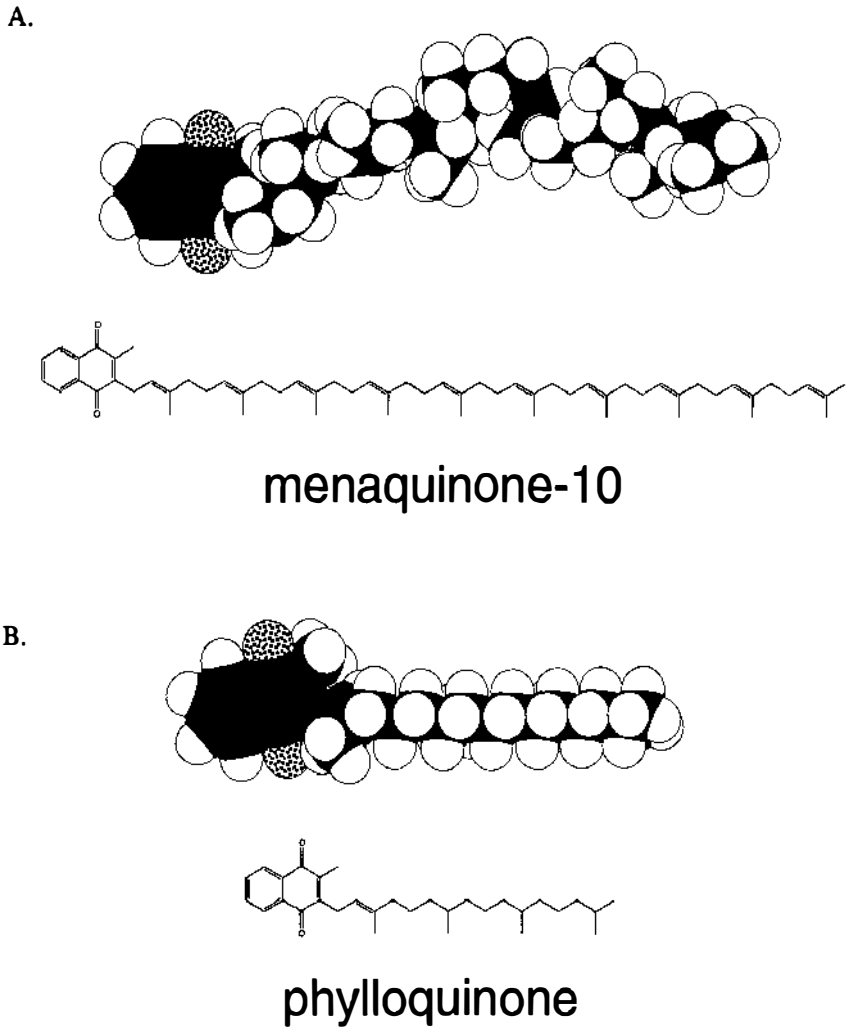

Figure 1 Structure of phylloquinone (vitamin K_1) and menaquinone-10 (MK-10). The energy-minimized structures indicate the extreme hydrophobic nature of the long-chain bacterial menaquinones.

recycling of this metabolite to the reduced vitamin is blocked by 4-hydroxy-coumarins (68) and is the basis for the acquired vitamin K deficiency achieved by oral anticoagulant therapy.

Biological Activity of Phylloquinone vs Menaquinones

Some indication of the physiological importance of menaquinones can be obtained from reports of attempts to determine the relative potencies of phyl-

loquinone and the various menaquinones. Using an 18-h oral dose curative bioassay that measured prothombin time responses in vitamin K–deficient chicks, Matschiner & Doisy (47) found long-chain menaquinones (eight isoprenoid units or longer) to be less active than phylloquinone. In a similar experiment, MK-7 was shown (77) to have lower activity than phylloquinone. However, when these homologues of vitamin K were administered intracardially to vitamin K–deficient rats and the change in prothrombin time was measured 18 h later, the long-chain menaquinones were up to 25 times more active than phylloquinone (48). The data suggest that the low activity of the long-chain menaquinones in the chick bioassay could be due to differences in absorption of these forms of vitamin K.

The relative activity of menaquinones vs phylloquinone has also been studied in crude preparations of the vitamin K–dependent carboxylase. In both intact (38) and solubilized (80) microsomal systems measuring the carboxylation of prothrombin precursors, only small differences in activity were observed between phylloquinone and the long-chain menaquinones. The activity of MK-1 and MK-2, which are not natural products, in the solubilized microsomal system was increased compared with that in an intact microsomal system or in intact animals, suggesting that the substituent on position 3 may interact selectively with the membranes. However, in another intact microsomal system (27), MK-2 had 10 times, and MK-3 80 times, the activity of phylloquinone. Using a partially purified carboxylase system and apparent kinetic constants for the carboxylation reaction, Buitenhuis et al (9) reported that MK-2 through MK-6 have activities similar to those of phylloquinone, whereas menaquinones with 7 or more isoprenoid units were less active than phylloquinone.

These studies have unequivocally established that menaquinones can exhibit vitamin K activity in both in vitro systems and animals. However, they provide little information about the physiological importance of menaquinones in human nutrition.

BIOSYNTHESIS AND DISTRIBUTION OF MENAQUINONES

Menaquinone Biosynthesis

Menaquinone biosynthesis, which has been extensively reviewed by Bentley & Meganathan (4, 5), has been studied mainly in *Escherichia coli, Mycobacterium phlei,* and *Bacillus subtilis.* As might be expected, the carbons of the aromatic naphthoquinone ring are furnished by shikimic acid, with chorismic acid as the next biosynthetic intermediate. All seven carbon atoms of shikimate are incorporated in the naphthoquinone ring, and the remaining three carbons

of the ring are furnished by α-ketoglutarate. The thiamine pyrophosphate–dependent addition of α-ketoglutarate to isochorismate results in the formation of the benzenoid derivative *O*-succinylbenzoate, which is converted to 1,4-dihydroxy-2-naphtholate in a coenzyme A–dependent reaction. Decarboxylation and prenylation result in the formation of a series of multiprenyl demethylmenaquinones. The final reaction in menaquinone synthesis is an S-adenosylmethionine–dependent methylation, which forms the biologically active form of the vitamin. Studies of *E. coli* mutants have identified five menaquinone biosynthetic genes. These are now being cloned, sequenced, and expressed to elucidate the remaining details of the pathway (59).

Menaquinones are produced by a large number of facultative and obligate anaerobes, and the particular homologue(s) of vitamin K produced by an organism has been used extensively as a taxonomic tool (11). Relatively few of the bacteria that comprise the normal intestinal flora are major producers of menaquinones. The obligate anaerobes of the *Bacteroides fragilis, Eubacterium, Propionibacterium,* and *Arachnia* groups are menaquinone producers, as are facultatively anaerobic organisms such as *E. coli.* Major intestinal organisms such as *Bifidobacterium, Lactobacillus,* or *Clostridium* species do not produce the vitamin (25, 54).

Intestinal and Stool Menaquinone Concentrations

The human gut contains an active flora of anaerobic, menaquinone-producing bacteria, leaving no doubt that the daily production of intestinal vitamin K greatly exceeds the required amount, which is rather small. Menaquinones extracted from stool samples, intestinal contents, plasma, or body tissues are readily separated using high-performance liquid chromography (HPLC), but analyses are complicated by large numbers of interfering substances that are often present in amounts greatly exceeding the amount of menaquinones. This problem undoubtedly contributes to the wide variation in the concentrations of menaquinones reported by different investigators. Reported concentrations of MK-4 should not be considered in the same context as those of longer-chain menaquinones. This vitamer is not a major bacterial product, and its tissue synthesis from menadione is well etablished (18, 19). Recent (29, 79) as well as past (6, 7) literature suggests that some animals can synthesize MK-4 from phylloquinone, but whether this conversion is mediated by tissue or by bacteria has not been determined.

Fujita et al (28) reported fecal concentrations of MK-4 through MK-10 in one-month-old formula-fed and breast-fed babies. Total menaquinone concentrations (geometric means and 95% confidence intervals) were 2.9 (1.3–6.7) nmol/g of dry feces for breast-fed babies and 9.0 (3.9–20.9) nmol/g of dry feces for formula-fed babies. In both groups, MK-7 and MK-8 were the predominant fecal forms. Kindberg (41) had difficulty quantitating a number

of the menaquinones in adult human stool samples because of interfering substances but reported the sum of MK-6, MK-9, and MK-10 as 1.58 ± 0.26 (mean ± SEM) nmol/g dry feces in 30 subjects on a normal diet and as 2.02 0.17 nmol/g dry feces after the same subjects consumed a liquid diet (Sustacal). This concentration is lower than that reported in infants but represents only a portion of the total menaquinone spectrum. Higher concentrations of menaquinones were reported in a more recent study by Conly & Stern (13), who analyzed stool samples from 10 subjects and found ~23 nmol menaquinone/g dry weight (calculated from a reported 19.9 ± 0.4 μg/g). MK-9 and MK-10 comprise ~70% of the total. In this study, intestinal contents from more proximal regions of the bowel were obtained from a limited number (two to five) of subjects via colostomies or ileostomies. Proximal jejunum samples were also obtained by nasojejunal intubations, and distal ileal samples were collected at the time of appendectomy. The total menaquinones (nmol/g dry weight) found in these regions of the gut were as follows: colostomy samples, 7; ileostomy samples, 2; terminal ileum, 10; and jejunum, 0.04. Total intestinal tract contents were also obtained from five individuals at the time of bowel preparation for colonoscopic examination. Mean total menaquinone content was 1.8 mg (~2100 nmol; range 0.3–5.1 mg). Given that the current recommended daily allowance (RDA) for vitamin K is ~70 μg (50) and that the amount needed to prevent an elevated prothrombin time is probably much less, the human digestive tract clearly contains a large reservoir of vitamin K.

Serum and Liver Menaquinone Concentrations

How much of the potentially useful gut vitamin K pool can be utilized has been more difficult to determine. Methodology for obtaining accurate analyses of plasma or serum phylloquinone has been developed over the past 10 years, and the range of values reported by most investigators has narrowed. Normal phylloquinone concentrations appear to range from 0.3 to 3.0 nM (~0.15–1.5 ng/ml). Early estimates (22) suggested that the total vitamin K in human liver was comprised of ~50% phylloquinone and 50% mixed menaquinones. On the basis of this information, most investigators thought that an individual menaquinone in serum might be below analytical detection limits. However, this is apparently not the case. Hirauchi et al (30) reported a mean of 0.21 ng/ml of MK-6, a mean of 0.37 ng/ml of MK-7, and a mean of 0.20 ng/ml of MK-8 in four human subjects. Shino (64) also reported means of 0.26 ng/ml of MK-6, 3.8 ng/ml of MK-7, and 0.26 ng/ml of MK-8 in five subjects. The very high MK-7 concentrations observed in this study may have been due to the consumption of natto, a fermented soybean product consumed by some of the Japanese population that contains a very high concentration of MK-7 produced by *Bacillus natto.*

Table 1 Plasma concentrations of phylloquinone, MK-7, and MK-8 in normal subjects

Number	Age in years	Plasma vitamin K (pg/ml ± SD)			References
		Phylloquinone	MK-7[a]	MK-8[a]	
11	27 ± 5	506 ± 372	293 ± 180	543 ± 316 (2)	33
17	69 ± 8	601 ± 283	328 ± 170 (1)	259 ± 156 (4)	33, 34
38	80 ± 5	585 ± 490	226 ± 178 (2)	161 ± 145 (9)	31

[a] Values in parentheses are the numbers that were below detection limits and that were not included in the means.

More recently, values from larger populations have become available. These are summarized in Table 1. These data suggest that the circulating concentrations of MK-7 and MK-8 are substantial in most individuals and that in many cases the total of these two vitamers exceeds that of phylloquinone. Two of these reports (31, 34) also assessed circulating phylloquinone and menaquinones in elderly patient populations with spine, femoral, neck, or hip fractures. These indices of poor bone health were associated with pronounced decreases in MK-7 and MK-8 as well as in phylloquinone. Whether these observations reflect a role of vitamin K in maintaining bone health or simply general low nutritional status in the debilitated population is not known.

Analyses of these traces of vitamin K in biological fluids pose a significant analytical problem, and until these analyses become more routine and verified, caution should be used in interpreting much of the published data. In most cases, peaks are identified by cochromatography with standards, but it appears that absolute identification of presumed peaks by mass spectral analysis seldom has been performed. Interestingly, substantial values for MK-6 have been reported in two studies (30, 64), whereas in others (31–33), MK-6 has not been found. Moreover, MK-6 has been used routinely by some investigators as an internal standard during analysis. At present, we know little about the clearance rate of this circulating menaquinone compared with phylloquinone. The data do, however, suggest that physiologically significant amounts of menaquinones circulate and are available to tissues.

A broad spectrum of menaquinones has also been detected in human liver. Table 2 summarizes data from three reports suggesting that MK-10 and MK-11 are major contributors to the hepatic menaquinone pool and that MK-7 and MK-8 may be present in more variable amounts in different populations. These data indicate that phylloquinone accounts for only ~10% of the total vitamin K in liver and that the remainder is comprised of a broad mixture of menaquinones. Usui et al (74) have reported that only ~3% of the total vitamin K in eight subjects who had consumed a diet low in phylloquinone (~5 μg/day) for 3 days was present as phylloquinone. The diet low in vitamin K did not affect

Table 2 Menaquinone content of human liver

n	Menaquinone (pmol/g liver)[a]									References
	MK-5	MK-6	MK-7	MK-8	MK-9	MK-10	MK-11	MK-12	MK-13	
7	12 ± 18	12 ± 13	57 ± 59	95 ± 157	2 ± 4	67 ± 71	90 ± 74	15 ± 13	5 ± 6	71
6	NR	NR	122 ± 61	11 ± 2	4 ± 2	96 ± 16	94 ± 26	21 ± 6	8 ± 3	73
7	NR	NR	34 ± 12	9 ± 2	2 ± 1	75 ± 10	99 ± 15	14 ± 2	5 ± 1	74

[a] Values are mean ± SEM. Data from References 71 and 73 have been recalculated from data presented as ng/g liver. NR, not reported.

hepatic menaquinone concentrations. McCarthy et al (49) reported that analyses of a few adult livers showed that menaquinones, namely MK-7, MK-8, MK-9, and MK-10, comprise 75–97% of the total vitamin K pool. Kayata et al (40) reported that the menaquinone content of five 24-month-old infants was approximately sixfold higher than that of three infants less than 2 weeks of age, and another study (62) failed to find menaquinones in neonatal livers. Substantial concentrations of MK-6, MK-7, and MK-8 have also been reported (32) in human cortical and trabecular bone.

These reports of human tissue menaquinone concentrations suggest that menaquinones are a potentially important source of vitamin K in humans. Large amounts of menaquinones are present in the gut and can be detected in serum. The liver, which is responsible for synthesis of most of the known vitamin K–dependent proteins, contains much more menaquinone than phylloquinone. Caution should be used nonetheless in assessing current values in the literature. Analytical techniques are still evolving, and currently reported values can vary considerably. However, the presence of menaquinones in body tissues and in large amounts in the gut is well established.

Menaquinone Absorption

The available data clearly show that large amounts of menaquinones are present in human liver and indicate that they can be detected in serum as well. The question of when and how menaquinones are absorbed has been addressed in recent reviews (44, 60). Early, rather nonphysiological experiments (35) demonstrated that MK-9 disappeared from the lumen of the isolated rat ileum or large intestine but did not document its appearance in plasma or lymph. Ichihashi et al (36) have shown that in the presence of bile, MK-9 is absorbed via the lymphatic pathway from rat jejunum but that in the absence of bile, no uptake of MK-9 from the colon to lymph or blood occurred within 6 h. A recent study (14) showed that oral administration of 1 mg mixed menaquinones (80% MK-8 and MK-10) to anticoagulated human subjects effectively decreased the extent of the acquired hypoprothrombinemia. This is a large dose of vitamin K, but it demonstrates that the human digestive tract can absorb these more hydrophobic forms of the vitamin when they are presented to the small intestine. Some menaquinones are present in fermented food products and cheese (60, 64) and may be absorbed by this route. Additionally, a small but nutritionally significant portion of the intestinal content of the vitamin is located not in the large bowel, but in a region where bile acid–mediated absorption could occur (13).

Phylloquinone is transported in chylomicrons and other low-density lipoprotein particles (60), and its clearance is affected by apolipoprotein E polymorphism (57). Little is known about menaquinone transport or clearance from the circulation. Interestingly, the reports of circulating menaquinones stress

quantitation of MK-6 and MK-7, whereas reports of hepatic and intestinal contents suggest that the longer-chain form predominates. One should also keep in mind that the vast majority of the gut menaquinone pool is located in bacterial membranes and thus is probably not available for absorption. However, some cellular lyses do occur, and some secretion may take place.

CLINICAL EVIDENCE OF MENAQUINONE IMPORTANCE

Vitamin K–Responsive, Antibiotic-Induced Hypoprothrombinemia

Vitamin K deficiency in the adult human, characterized by vitamin K–responsive hypoprothrombinemia, is a relatively rare condition. A number of miscellaneous disorders of intestinal lipid absorption have been reported (58) to be associated with an acquired vitamin K deficiency, but most reported cases have been associated with antibiotic administration (67). As early as the late 1940s and early 1950s, antibiotic therapy was recognized as a potential contributing factor in the development of potentially serious hypoprothrombinemia, and a vitamin K deficiency in hospitalized patients that is predominantly associated with antibiotic administration can contribute to morbidity and mortality. Numerous case reports of antibiotic-induced hypoprothrombinemia have been summarized (43, 52, 58, 63), and a review of the available data indicates that these cases of hypoprothrombinemia are not limited to the use of a single antibiotic. Penicillin, semisynthetic penicillins, and cephalosporins commonly have been involved, but the uses of aminoglycosides, trimethoprim, chloramphenicol, amphotericin B, erythromycin, and clindamycin have also been reported. These antibiotic-induced hypoprothrombinemias have historically been assumed to result from a decrease in the synthesis of menaquinones by gut organisms, with the underlying assumption that menaquinones are important in satisfying at least a portion of the normal human requirement for vitamin K. In nearly all of these case reports, evidence of decreased menaquinone synthesis in the presence of antibiotic treatment is lacking.

An apparent increase in the incidence of these responses in the early 1980s seemed to be associated with the use of a number of the newer β-lactam antibiotics, in particular cephalosporin, cefamandole, or the related oxa-β-lactam, moxalactam. Subsequent studies implicated a number of other cephalosporins, all of which contained an N-methylthiotetrazole (NMTT) side chain (8, 43, 63). The initial reaction to these reports was to assume that the β-lactam and oxacepham antibiotics inhibited menaquinone production. These antibiotics are administered intravenously, but through significant biliary excretion they can decrease the population of menaquinone-producing organisms in stool

cultures (12). However, the effect of these antibiotics on menaquinone production has not been clearly determined. Ramotar et al (53) found that moxalactam and ticarcillin or moxalactam and tobramycin significantly reduced the number of fecal *E. coli* and *B. fragilis* as well as fecal menaquinone (MK-4 through MK-10) concentrations in neutropenic patients. However, Suttie et al (69) found that only three of nine volunteers receiving NMTT-containing antibiotics (cefamandole, cefoperazone, or moxalactam) experienced a decrease in total fecal MK-6, MK-9, and MK-10, whereas five of nine experienced an increase in these menaquinones in feces. Those antibiotics whose clinical use has been associated most closely with severe hypoprothrombinemia can therefore affect both fecal flora distribution and menaquinone production, but the effects are variable and many non-NMTT-containing antibiotics yield similar results.

Hypotheses other than hypoprothrombinemia-decreased menaquinone production were also proposed to explain the observed response. Evidence to support or disprove a theory that either NMTT generated by metabolism of these antibiotics or an NMTT metabolite was a direct inhibitor of the vitamin K–dependent carboxylase has been reviewed (67). In none of the in vitro studies was the inhibition by NMTT reversed by increasing the vitamin K concentration of the incubation, whereas most cases of hypoprothrombinemia induced by NMTT-containing antibiotics appeared to be vitamin K responsive. A clearer understanding of the nature of the NMTT response was gained from the demonstration (3) of significant amounts of circulating vitamin K 2,3-epoxide following vitamin K administration to hypoprothrombinemic patients receiving moxalactam. The hypothesis that antibiotics containing NMTT are vitamin K epoxide reductase inhibitors was subsequently confirmed (15) in an animal model, and the importance of vitamin K status to the development of a hypoprothrombinemic response to NMTT-containing antibiotics was shown by the finding (10, 45, 61) that patients with low phylloquinone concentrations in circulating plasma and other indications of low nutritional status were susceptible to this response.

The results of these studies conclusively demonstrate that antibiotics containing a NMTT side chain or a methyl-thiadiazole-thiol (MTD) side chain (cefazoin) can cause an inhibition of the hepatic epoxide reductase, resulting in a coumarin-like response in the synthesis of vitamin K–dependent coagulation factors. However, these antibiotics are very weak anticoagulants, and an adverse response is seen only in those patients with low vitamin K status.

Efforts to Produce Vitamin K Deficiency by Restricting Dietary Vitamin K

Historically, vitamin K deficiency has been characterized by the presence of a vitamin K–responsive hypoprothrombinemia, as defined by a prolongation

of the Quick prothrombin time. However, this deficiency has been difficult to produce in human subjects, suggesting that the utilization of menaquinones is sufficient to meet the human vitamin K requirement or that the requirement is so low that the vitamin cannot be excluded sufficiently from the diet. Numerous reports of vitamin K deficiency associated with the interference of intestinal lipid absorption have been documented in an excellent review by Savage & Lindenbaum (58). Clearly, patients with bile flow obstruction or biliary fistulas, pancreatic insufficiency, sprue, or inflammatory bowel disease are at risk for vitamin K deficiency. The incidence of vitamin K deficiency during starvation or severe malnutrition is unclear and difficult to separate from an indirect effect of protein deficiency.

The most commonly cited study relating to the importance of menaquinones in the development of vitamin K deficiency is that of Frick et al (26). Ten patients with apoplexy were given parenteral 5% glucose (200–400 Cal/day), NaCl, KCl, and B vitamins for a period of 1 month. Seven of the patients also received unspecified antibiotics "affecting the intestinal flora" (26). These seven were judged to be vitamin K deficient after 1 month on the basis of a decline in Quick prothrombin times and in factor II, VII, and X assays. The three other subjects were not classified as deficient, although two of them had factor VII and X activities below 80% and a slight decrease in prothrombin time and factor II assays. No measurements of intestinal flora or menaquinone production are available from this study, and it should be recognized that the subjects had severe calorie, protein, and possibly essential fatty acid deficiency. Udall (72) achieved a statistically significant increase in prothrombin time in 10 subjects fed polished rice, black coffee, sugar, and a daily multivitamin capsule for 3 weeks. These results suggested that menaquinones were not as important as previously thought in preventing the development of vitamin K deficiency. Studies utilizing these drastic measures of dietary restriction in an attempt to limit vitamin K intake will not likely be repeated.

Two other early attempts to produce dietary vitamin K deficiency have been reported. Doisy (20) fed a chemically defined diet designed to provide <10 μg vitamin K/day to two normal subjects and was able to reduce prothrombin concentrations to <50% by ~20 weeks. Small doses of mineral oil were administered for half the study period, and neomycin was administered for 2 or 3 months in an attempt to decrease vitamin absorption and synthesis. These patients responded to the administration of ~0.5 μg vitamin K/kg per day, which rapidly restored clotting activity to normal. From this study, investigators concluded that ~1 μg vitamin K/kg per day was sufficient to maintain normal clotting factor synthesis in the normal adult human. O'Reilly (51) maintained four normal volunteers on a diet providing ~25 μg vitamin K/day and administered antibiotics (tetracycline or neomycin) in an attempt to decrease intestinal synthesis. Prothrombin activity was maintained at a range of

70 to 100% of normal during a 5-week period, with lower values observed near the end of the study. The Frick and O'Reilly studies point to the difficulty of producing a substantial effect on the synthesis of vitamin K–dependent clotting factors by dietary restriction of vitamin K alone, whereas the affect of antibiotic administration in the Doisy study is difficult to assess. However, vitamin K–responsive hypoprothrombinemia has been reported in some patients with a history of low vitamin K intake (39). Although no antibiotics were involved in these cases, other nutritional deficiencies were undoubtedly present and may have affected the expression of vitamin K deficiency.

Prothrombin time has been determined to be a very insensitive measure of vitamin K sufficiency (66), and as more sensitive assays have been developed, a limited number of more carefully controlled studies have been reported. Allison et al (1) maintained subjects for 13 days on a synthetic diet (Sustacal powder dissolved in skim milk) for 13 days. Vitamin K intake, by direct analysis of the diet, was 2–5 μg/day, and 30 of 33 subjects also received 1 of 10 antibiotics. Factor VII values <60% of normal were noted on at least 1 day in 7 subjects, and a measure of under-γ-carboxylated prothrombin (PIVKA II), the S/E ratio, was observed in 21 of 33 subjects. These responses were spread across all groups and were not related to subsequent effects of the various antibiotics on stool menaquinones (67). In a small study (70) of free-living college-aged male subjects in whom median vitamin K intake was reduced from ~80 to ~40 μg/day by diet alteration, a statistically significant decrease in urinary Gla excretion and functional prothrombin (S/E ratio) was observed after 21 days. These subjects did not receive any antibiotics, and the apparent responses were reversed by vitamin supplementation. A larger study utilizing a lower vitamin K intake (24) has produced similar results. Young (28 years) and elderly (70 years) subjects were fed a diet containing ~10 μg vitamin K/day in a metabolic ward setting. Both groups contained eight male and eight female subjects. After 13 days, urinary Gla excretion was decreased in the young but not in the elderly subjects, and an increase in nonfunctional PIVKA II was seen in both groups.

EXTENT OF HEPATIC MENAQUINONE UTILIZATION

Although the human hepatic vitamin K pool is predominantly composed of menaquinones rather than phylloquinone, the relative utilization of the two forms of the vitamin has not been determined. The distribution of menaquinones in rat liver is related to the intestinal distribution (42), and some information on menaquinone utilization in this species is available. In a study comparing the metabolism of MK-9 with that of phylloquinone in the rat (78), liver MK-9 was less efficient than phylloquinone in preventing indications of vitamin K deficiency. Although accurate half-lives for MK-9 could not be

determined, the initial turnover rate of phylloquinone appeared to be two to three times as fast as that of MK-9. In a subsequent study (56), the appearance of phylloquinone epoxide or MK-9 epoxide following warfarin administration to block vitamin K epoxide recycling was used in an attempt to quantitate the utilization of the two vitamers. These studies were complicated by the ongoing turnover and metabolism of both the vitamers and their epoxides, but 1 h after warfarin administration, more than four times as much phylloquinone epoxide as MK-9 epoxide was present in the liver. Equal amounts of the two vitamers were present before warfarin was administered. This study also demonstrated that a much higher fraction of the total hepatic MK-9 pool was located in the mitochondria compared with the microsomal fraction than was the case for phylloquinone. This mitochondrial localization was particularly evident for MK-9 epoxide. Studies of human liver samples (73) have also reported concentrations of MK-10 and MK-11 in mitochondria four times that of a microsomal fraction, but similar concentrations of phylloquinone were observed in the two fractions. The limited data available suggest that the large pool of menaquinone in human liver does not necessarily mean that gut menaquinones contribute significantly to the maintenance of vitamin K sufficiency. The pool size may represent to a large extent a very slow turnover of long-chain menaquinones, which, even if present, may not be as effective a form of the vitamin as phylloquinone.

INTERPRETATION OF AVAILABLE DATA

Standard nutrition texts often indicate that menaquinones furnish a significant portion (up to 50%) of the human daily vitamin K requirement. This view is based on the widespread reports of antibiotic-induced, vitamin K–responsive hypoprothrombinemia; on the difficulty in producing a dietary deficiency of the vitamin; and on the knowledge that a large fraction of the hepatic stores of the vitamin consists of menaquinones. However, the importance of these observations to the question of menaquinone utilization by humans has been called into question. A negative effect on intestinal menaquinone synthesis following antibiotic administration cannot be assumed, and many cases of antibiotic-induced hypoprothrombinemia may simply result from low food intake in a seriously ill population. A preliminary report (23) of a study of elderly hospitalized patients has indicated that 43% of patients not using "drugs known to interfere with vitamin K metabolism" had elevated concentrations of PIVKA II. These findings suggest that many patients have borderline dietary deficiency. The difficulty in producing clear-cut vitamin K deficiency in normal subjects is largely related to the lack of sensitivity of the classical assays used to detect deficiency. More recently, studies utilizing stricter dietary restriction have resulted in alterations in more sensitive assays. A limited number

of reports point to symptoms of vitamin K deficiency in individuals not taking antibiotics. Alterations in diet have been shown (46, 55) to have profound effects on intestinal menaquinone synthesis in the rat and also may have affected gut flora in some of the human case reports. Whether the relationship between a number of malabsorption syndromes and vitamin K deficiency helps elucidate the importance of menaquinones is difficult to determine without a better understanding of how and where in the gut menaquinones are absorbed.

The current answer to the question, Are menaquinones important in human nutrition? is therefore yes, but to a degree not yet determined. Most evidence suggests that they are less important than previously thought. The amount of vitamin K utilized per day can be calculated readily from urinary excretion of unmetabolizable γ-carboxyglutamic acid. However, the extensive recycling of the coproduct of the carboxylase reaction, vitamin K 2,3-epoxide, to the active vitamin, makes it difficult to relate this value to the amount of vitamin required per day. The difference between this physiological requirement and the dietary requirement would be the menaquinone contribution, but at this time these calculations cannot be made.

The available data point to a number of areas of fruitful research. Information on the hepatic turnover and utilization of the various long-chain hepatic menaquinones as carboxylase substrates would greatly augment our understanding of their importance. More in-depth elucidation of the origin, turnover rate, and individual menaquinone composition of the circulating menaquinone pool would also be of benefit to investigators. The degree to which the concentration of serum menaquinone varies among individuals, its relationship to the amount and distribution of menaquinones in the stool menaquinone pool, and the influences of antibiotics on the size of this pool are all questions open to direct study, the answers to which would clarify the current situation. As more data on the human dietary vitamin K requirement become available, our knowledge of the role of menaquinones will increase considerably.

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