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Overview

Vitamin K Contents of Rodent Diets: A Review

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Adequate nutrient intake is critical in the maintenance of normal physiologic activity of rodents in biomedical studies. Vitamin K is an essential nutrient in rodent diets and functions as a cofactor for the γ-carboxylation of various proteins involved in blood coagulation and bone metabolism. Several forms of vitamin K are used in rodent diets, with a shift during the last decade towards the use of phylloquinone, the predominant form in human diets, and a concomitant increase in concentrations. This review summarizes current recommendations for vitamin K in rodent diets relative to our evolving knowledge about this fat-soluble vitamin.

Abbreviations: AIN, American Institute of Nutrition; Gla, γ-carboxyglutamic acid; MK, menaquinone; MSBC, menadione–sodium bisulfite complex; NAS-NRC, National Research Council of the National Academy of Sciences; NRC, National Research Council

Vitamin K is a family of structurally similar, fat-soluble 2-methyl-1,4-naphthoquinones that includes phylloquinone (vitamin K_1), menaquinones (vitamin K_2 or MKn), and menadione (vitamin K_3). Phylloquinone is present in green, leafy vegetables and various plant oils.[6] Menaquinones are synthesized endogenously primarily and have a different 3′ substituted hydrophobic side chain than that of phylloquinone. Nomenclature for individual menaquinones is based on the number of repeating isoprenoid units, such that the major forms are referred to as MK4 through MK10. MK4 is unique to the menaquinones in that it is alkylated from menadione present in animal feeds or is the product of animal tissue-specific conversion directly from dietary phylloquinone.[9,48,49] Menadione is a synthetic analogue that has no aliphatic side chain and acts as a provitamin (Figure 1).

Although vitamin K was identified in 1929 as a nutrient essential for adequate coagulation, approximately 40 y lapsed before its biochemical role was identified. Vitamin K is now known to be a necessary cofactor for the modification of glutamic acid residues to γ-carboxyglutamic acid (Gla) residues in specific proteins, known as vitamin K-dependent or Gla-containing proteins. The vitamin K-dependent coagulation proteins—factors II, VII, IX, and X and proteins C, S, and Z—are synthesized in the liver. The extrahepatic vitamin K-dependent proteins include osteocalcin (produced by osteoblasts), matrix Gla protein (produced in chondrocytes and vascular smooth muscle cells, among others), and GAS6 (a growth-arrest–specific protein thought to be involved in the stimulation of cell proliferation).[4,45] Through the vitamin K-dependent γ-carboxylation of these proteins, vitamin K has been implicated in hemostasis, bone metabolism, tissue calcification, and cell cycle regulation.[11,14,52] Moreover, through unknown mechanisms that are independent of its role as a cofactor for the γ-carboxylation reaction, vitamin K may also be involved in the inhibition of cancerous cell growth and synthesis of sphingolipids.[10,26]

New roles for vitamin K are actively being explored beyond its classic role as a cofactor, and comparative studies of the different forms of vitamin K in humans are emerging. These studies necessitate appropriate animal models using suitable dietary forms and concentrations of vitamin K. The following review addresses the history, requirement, absorption, and toxicity of vitamin K in rodent diets, with a focus on the concentrations and forms of vitamin K used in various formulations.

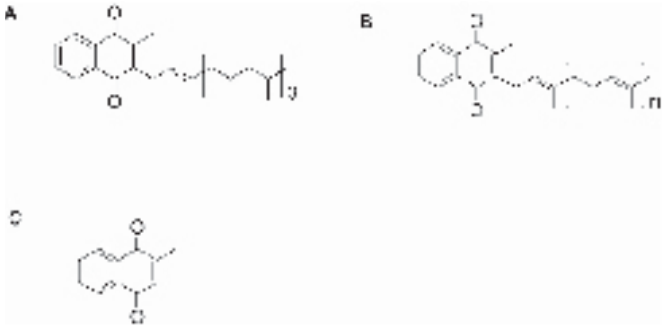

Figure 1. Chemical structures of (A) phylloquinone (vitamin K_1), (B) menaquinones (MK-n), and (C) menadione (vitamin K_3).

The Classification of Rodent Diets

Animal diets used in biomedical research are defined by the degree of ingredient refinement. The National Research Council (NRC) had identified 3 classes of diets, which (in ascending order of ingredient refinement) comprise the natural ingredient diets (also known as cereal-based diets), purified diets, and chemically defined diets.[30] Natural ingredient diets are used widely in biomedical research because of their low cost of production and well-established history of use. However, these diets can vary in nutrient content and lack the flexibility required for nutrient-specific studies. In contrast, purified diets allow for greater flexibility for manipulating the individual nutrients and have fewer contaminants as compared with the natural ingredient diets. Further, a review of the literature indicates greater repeatability of findings from animal studies using purified diets compared with natural ingredient diets.[7,9,19] Balanced against the high cost of chemically defined diets is the

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ability to establish the requirements for individual components, an example being individual essential fatty acids.[31] In addition, custom diets are widely available.

Forms of Vitamin K in Rodent Diets

Phylloquinone is the preferred form of vitamin K for human use in North America and Europe and is available as many forms, including colloidal suspension, emulsion, aqueous suspension, and tablets. Because of the expense of phylloquinone, various water-soluble forms of menadione are used for rodent diets, currently in the form of a menadione–sodium bisulfite complex (MSBC). This form has increased dietary stability compared with menadione and is widely used in the animal industry. Although in its pure form, menadione is about 10% as active as phylloquinone,[16] the water-soluble derivatives of menadione, such as MSBC, are absorbed much more readily than phylloquinone and have equal biologic activity.[42]

Rodent diets contain either phylloquinone or menadione and its derivatives, and there is evidence that the absorption of these various forms of vitamin K differs markedly. Absorption of dietary vitamin K from the proximal intestine occurs through the chylomicron and lymphatic pathway and is associated mainly with triacylglycerol-rich lipoproteins.[25,41,46] The liver rapidly accumulates ingested phylloquinone.[9,48] Although rapidly concentrated in liver, phylloquinone has a relatively short biologic half-life. Therefore, rats depend on a continuous dietary supply of vitamin K to support its role as a cofactor for γ-carboxylation of vitamin K-dependent proteins. Recent studies using germ-free rats showed that there is conversion of phylloquinone to MK4 in various extrahepatic tissues, such as the kidney, that is independent of the gut flora but not in the liver.[9,39] As a consequence, MK4 concentrations in these tissues exceed those of phylloquinone even when phylloquinone is the primary dietary form of vitamin K in the chow.[48,49]

Menadione is also known to be transformed into MK4.[47] As previously reviewed elsewhere, menadione is more polar than are phylloquinone and menaquinones and does not require bile salts for absorption. Instead menadione can be absorbed directly into the blood across the intestinal mucosa[46] and is rapidly metabolized.[50] In addition, this small lipophilic molecule readily passes through the blood–brain barrier and placenta, whereas phylloquinone does not.[44,48] These differences in absorption have implications when choosing the optimal dietary form for the study of vitamin K in rodent models of human disease. In particular, menadione has limited value in absorption studies because of its differences in absorption compared with that of forms present in the human diet.

Recommended Vitamin K Contents of Rodent Diets

Open-formula cereal-based diets. In 1972, NIH-07 was developed as an open-formula cereal-based diet that was satisfactory for reproduction, lactation, and maintenance of both rats and mice. The NIH-07 diet contained 3.1 mg vitamin K in the form of menadione per kg of diet.[23] From a series of rodent survival and bioassay studies, an open-formula nonpurified diet designated as NTP-2000 was formulated in 2000.[35] In the NTP-2000 diet, the amount of menadione was reduced to 1.0 mg per kg diet, with the use of a stabilized form of menadione (MSBC; Table 1). This reduction in menadione concentration, with concurrent change to a more biologically available form, was considered to have both met the requirement of vitamin K and overcome the problem of toxicity to animals during long-term studies.[35] Limited data are available regarding the stability of MSBC in diets that are subjected to standard diet manufacturing conditions, including steam injection and drying, or are autoclaved, all of which are known to reduce other forms of vitamin K. To compensate for potential losses during manufacturing, some companies use increased concentrations of vitamin K during the formulation process. Confirmation of the menadione content of these diets after manufacturing requires direct laboratory analysis, the assay for which is not widely available. Therefore rodent studies focused on vitamin K metabolism are best served by the use of purified diets that primarily contain phylloquinone, for which laboratory analysis is available for confirmation of actual intake.

Table 1. Vitamin K in cereal-based rodent diets: recommended intakes

	Diet	Menadione (mg/kg diet)
Rat and mouse	NIH-07	3.1
Rodent	NTP-2000	1.0[a]

[a]Provided as menadione–sodium bisulfite complex.

Purified diets. In 1972, the National Research Council of the National Academy of Sciences (NAS-NRC) recommended the provision of 0.05 mg of vitamin K in the form of menadione per kg of purified rat diet (Table 2). For mice, a lower intake of 0.02 mg menadione per kg of diet was recommended.[30] At the time, coprophagy was assumed to contribute sufficient endogenous production of menaquinones to meet the dietary vitamin K requirements of conventionally reared mice. Casein contains an average of 0.8 mg phylloquinone per kilogram, so with a recommended 20% casein content, an additional 0.16 mg of phylloquinone was assumed to present in these rodent chows.[37] In 1977, the American Institute of Nutrition (AIN) identified the AIN-76 rodent diet for nutritional studies, which standardized all rodent diets to an intake of 0.05 mg MSBC per kg diet, with an additional 0.16 mg of phylloquinone associated with the 20% casein content.[1,5] The greater incremental increase of the vitamin K content in the murine diet reflected, in part, the recognition that supplemental vitamin K was required for conditions in which endogenous menaquinones were not available, such as use of germ-free mice and those treated with antibiotics. Long-term rat studies using the AIN-76 diet challenged the adequacy of the vitamin K concentrations, particularly when concurrent with large doses of vitamin A and α-tocopherol.[38] Bieri[5] confirmed that the amount of vitamin K activity in the AIN-76 diet was suboptimal for Fisher rats when casein was replaced with other proteins. In the second report of the Ad Hoc Committee on Standards for Nutritional Studies (issued in 1980), the amount of vitamin K in AIN-76 rodent diet was increased 10-fold in the form of a stabilized form of menadione. This diet was designated AIN-76A.[2]

In 1988, an ad hoc committee was mandated to evaluate the appropriateness of the AIN-76A diet guidelines in light of concerns about both nutritional and technical problems with the diet.[5] On the basis of this evaluation, a new rodent diet was developed and designated AIN-93. In terms of vitamin K, there was a shift from menadione to phylloquinone as the preferred form in custom diets, with different concentrations according to the stage of the life cycle. The higher cost and lower stability of phylloquinone compared with menadione precluded a similar shift in commercially produced natural ingredient diets. The AIN-93G diet, which contains 0.75 mg of phylloquinone per kilogram of diet, and an additional 0.16 mg of phylloquinone associated with the 20% casein content, is recommended to sup-

Table 2. Vitamin K in purified rodent diets: recommended diet concentrations

		Vitamin K (mg/kg diet)				
	Diet	Phylloquinone	Menadione	Casein (%)	Additional phylloquinone (mg)	Data (reference)
NAS-NRC[a] recommendation						
Rat		0	0.05	0	0	1972 (25)
Mouse		0	0.02	0	0	1972 (25)
Rat		1.0	0	0	0	1995 (27)
Mouse		1.0	0	0	0	1995 (27)
Required dietary concentrations						
Rodent	AIN76[b]	0	0.05	20	0.16	1977 (1)
Rodent	AIN76A[b]	0	0.50[c]	20	0.16	1980 (2)
Rodent	AIN93G[b]	0.75	0	20	0.16	1993 (32)
Rodent	AIN93M[b]	0.75	0	14	0.11	1993 (32)

[a]National Academy of Sciences, National Research Council.
[b]American Institute of Nutrition.
[c]Provided as menadione–sodium bisulfite complex.

port growth, pregnancy, and lactation phases. The AIN-93M diet has a lower protein and fat content, contains the same amount of phylloquinone per kg diet as the AIN-93G diet, but contains an additional 0.11 mg of phylloquinone because of its lower (14%) casein content and is recommended for adult maintenance. Table 2 shows the NAS-NRC recommendations for the type and level of dietary vitamin K supplementation. For nutritional studies that are focused on vitamin K, investigators need to be aware that vitamin K forms and concentrations vary widely among commercially available rodent diets, thus reinforcing the need to conduct direct laboratory analysis on chows prior to use.

Adequacy of vitamin K in rodent diets. Dietary adequacy of vitamin K is often defined as the amount of vitamin K that is needed to maintain normal levels of plasma vitamin K-dependent clotting factors.[32] Continuous bleeding is the sign of frank vitamin K deficiency in rodents and can occur within 9 to 21 d in response to an absence of vitamin K in the diet.[27,34] Nevertheless, estimations of the requirements are difficult to establish because of the different forms of vitamin K used and lack of physiologic outcome measures other than coagulation times, which only change in response to severe deficiency. As discussed elsewhere, the definition of vitamin K deficiency is still dependent on coagulation assays.[22] In addition, dietary vitamin K requirements in animals are difficult to determine owing to intestinal floral synthesis of longer chain menaquinones and the degree of coprophagy; these 2 processes can contribute to overall vitamin K status and are an uncontrolled source of menaquinone intake.

The vitamin K requirement for most nonruminant animals ranges from 50 to 150 µg/kg diet.[42] Historically, vitamin K was not considered essential for mice reared under conventional conditions because of the substantial contribution from coprophagy. Unfortunately the dietary vitamin K requirements of mice have not been evaluated systematically, so their requirements currently are based on those for the rat. Both specific-pathogen-free CF1 mice and germ-free ICR/JCL mice were reported to die quickly from hemorrhagic diathesis when fed vitamin K-free diets.[8,12] Similarly, studies during the 1960s indicated that frank deficiency, as defined by prolonged blood clotting, was induced in rats within 14 d of being fed a soy protein diet void of vitamin K.[28] Kindberg and Suttie[22] subsequently demonstrated that 500 µg phylloquinone per kg diet did not sustain maximal activity of the hepatic γ-carboxylation in rats, whereas 1500 µg phylloquinone per kg diet did. No intermediate doses were tested, so based on extrapolation of the data from this single study, the 1995 NRC recommendations for vitamin K were set at 1 mg/kg diet, which represents a 20-fold increase when compared with the 1972 NRC recommended intakes.[22] More recently, higher intakes of vitamin K reportedly are required for accumulation in bone, compared with intakes required for accumulation in liver.[40]

The requirement for vitamin K also depends on gender, age, rodent strain, and factors influencing vitamin K absorption.[19,33] Female rats are more resistant to vitamin K deficiency compared to male rats, which is attributed to a facilitory action of estrogen on the intestinal absorption of vitamin K.[21] There is a trend towards higher phylloquinone concentrations in older rats, whereas MK4 concentrations decrease with age in the heart and kidney.[18] Knauer and colleagues[24] demonstrated that hepatic concentrations in Wistar rats are lower than those of Sprague-Dawley rats and are not explained by differences in absorption or turnover of vitamin K. Compared with normal rats, warfarin-resistant rats have much higher vitamin K requirements because they do not recycle vitamin K epoxide as efficiently.[15,17,43] Furthermore, high dietary concentrations of vitamins A and E accelerate the onset of signs of deficiency in rats fed vitamin K-deficient diets.[33,51]

Although the dietary requirement for vitamin K is poorly defined in rodents, particularly mice, the recommended amounts in rodent diets have increased consistently with each issue of the NRC nutrient requirements as more sensitive assays based on known vitamin K biochemical roles emerge. Vitamin K levels in the liver decrease rapidly after dietary depletion, with an estimated half-life of 10 h in the rat.[22] Therefore increases in the recommended intake of phylloquinone should not present challenges in creating a vitamin K-deficient state in a previously replete rodent. In addition, supplementation of vitamin K in the form of phylloquinone has not been identified with toxicity, so it can be argued that there are no detrimental consequences to erring on the high side with respect to setting the requirements for this nutrient. In a series of toxicity studies conducted during the 1940s, daily oral doses of as much as 2 g phylloquinone per kilogram of body weight fed to rats for 30 d had no reported effect.[29] In contrast, daily oral doses of 0.35 g menadione per kilogram of body weight resulted in anemia in rats and, when increased to 0.5 g/kg, resulted in 100% mortality.[29] Other cases of menadione toxicity have been reported, and this form is not used for human consumption.[3,20,36] The toxicity of menadione is assumed to be related to its role as quinone and not as a precursor of an active form of vitamin K.[7] Further, the reported toxic level of menadione (or its derivatives) in diet is about 1000 times the dietary requirement.[32]

Storage of Rodent Diets

Because it is particularly vulnerable to degradation by light, phylloquinone should be handled and stored in reduced light, and manufactured diet should be stored without excessive light exposure, at 4 °C, in plastic containers with tight-fitting lids. In contrast, menadione is considered to be more stable than other vitamins, such as vitamin A and thiamine. Despite this stability, diets containing menadione should not be stored for more than 6 mo, even under the best of conditions.[13] Moreover, deterioration should be monitored routinely.

Conclusion

Vitamin K is an essential nutrient in rodent diets and functions as a cofactor for the γ-carboxylation of various proteins involved in blood coagulation and bone metabolism. Little research in recent years has addressed the vitamin K requirements of rodents. One of the challenges in evaluating vitamin K requirements is the lack of a standardized assessment tool to define adequacy. The historic use of prolonged coagulation times to established requirements is now viewed as an insensitive measure of adequate intake to support the known biochemical role of vitamin K as a cofactor for the γ-carboxylation of various proteins. Further, tissue-specific conversion of dietary forms of vitamin K to another form, MK4, is now known. In response to these developments, there has been a shift to use of phylloquinone, which is the predominant form in human diets, in higher concentrations among purified rodent diets. Casein contributes to a substantial amount of additional phylloquinone in these diets. The current recommended intake of 1.0 mg/kg diet is extrapolated from data of a single study in rats and reflects a 20-fold increase over the last 25 y. No comparable studies have been done in mice, so their vitamin K requirements are now based on those of rats. Because these recommendations vary with the biochemical assay used, whether the 20-fold increase in recommended intake is warranted is unknown. However, phylloquinone supplementation is unknown to have toxic effects, and high intakes are assumed to have no detrimental effects. In addition, vitamin K deficiency can rapidly be induced in animals previously maintained on a diet that meets the recommended intakes. A synthetic form of vitamin K, menadione, is the most common form in cereal-based diets but has been reduced in content and modified in chemical form to address earlier concerns of absorption and toxicity. In contrast to phylloquinone, menadione has been associated with anemia and subsequent mortality when administered in doses at approximately 1000 times the current dietary requirements. Other functions for vitamin K are emerging and may necessitate additional evaluation of the forms and concentrations of vitamin K used in rodent diets.

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