Chapter 4

Menaquinones, Bacteria, and Foods: Vitamin K2 in the Diet

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Additional information is available at the end of the chapter

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Abstract

Vitamin K2 is a collection of isoprenologues that mostly originate from bacterial synthesis, also called menaquinones (MKs). Multiple bacterial species used as starter cultures for food fermentation are known to synthesize MK. Therefore, fermented food is the best source of vitamin K2. In the Western diet, dairy products are one of the best known and most commonly consumed group of fermented products.

Although intensive research on metabolism and the biological effect of vitamin K2 continues today, data about vitamin K2 production and content in foods remain scarce. Dietary recommendations are still based on the classic role of vitamin K as an enzyme cofactor for coagulation proteins and do not consider differences in bioavailability and bioactivity between the various MKs and the possibly higher requirements for health effects apart from coagulation, such as bone or cardiovascular health. Here, we provide a global view of foods rich in vitamin K2 and their interactions together with other nutrients in selected health effects such as bone and cardiovascular health.

Keywords: Menaquinones, Bacteria, Food, Dairy, Health

1. Introduction

Vitamin K occurs naturally in two biologically active forms. Vitamin K1, also called phylloquinone (PK), is abundant in leafy green vegetables, such as cabbage, spinach, and lettuce [1]. The other form, vitamin K2, is called menaquinone (MK) and is predominantly of microbial origin [2, 3]. Vitamin K2 is mainly present in fermented food such as cheese and natto (fermented soybeans), but gut microbiota are also able to synthesize vitamin K2 [4]. One exception, menaquinone-4 (MK-4), is formed in humans and animals by tissue-specific conversion of PK and/or menadione [5]. However, in the literature, all MKs are mostly grouped under the term vitamin K2 resulting in the assumption that all MKs are similar in origin and function. Moreover, despite the knowledge that MKs are present in the food supply, little is known about their individual synthesis, growth conditions, and interactions of the producing bacteria and the total amounts of the different MKs in fermented foods. Regarding the findings that MKs play an important role in health aspects beyond coagulation, study of the interaction of MKs with other nutrients may lead to a better understanding of the effect of different food items on health aspects, such as bone health or cardiovascular health.

Such a global view could be essential for guiding the development of dietary intake recommendations for vitamin K.

2. Structure of vitamin K

Both vitamin K forms have 2-methyl-1,4-naphthoquinone, also called menadione or vitamin K3, as a common ring structure. However, they differ from each other in the length and degree of saturation of the polyisoprenoid side chain attached to the 3-position.

Phylloquinone (vitamin K1) possesses a phytyl side chain, which consists of four isoprene units, and one of them is unsaturated. Phylloquinone is found primarily in plants in association with chlorophyll, whereas menaquinone (vitamin K2) is principally synthesized by bacteria. Menaquinone contains side chains of varying length, for most the part of a polymer of repeating unsaturated 5-carbon prenyl units. Depending on the microorganism by which the chain is synthesized, the chain length generally ranges from 4 to 13 prenyl units. Menaquinones are classified according to the number of prenyl units. The number of units is given in a suffix (-*n*), that is, menaquinone-*n* and often abbreviated as MK-*n* [2, 6, 7]. Some bacteria produce isoprenologues in which one or more of the prenyl units are saturated. The additional hydrogen atoms are indicated with the prefix dihydro-, tetrahydro-, and so on and are abbreviated MK-*n*(H2), MK-*n*(H4), etc. [8].

Vitamin K is fat soluble. The melting points of menaquinones vary from 35°C to 62°C depending on the length of the multiprenyl side chain. Menaquinones are stable to heat and air but are very sensitive to alkali and ultraviolet (UV) irradiation [9].

3. Functions and biosynthesis of menaquinones in bacteria

The distribution of isoprenoid quinones has been studied in 900 microbial strains, 56 mold strains, and 88 yeast strains. About half of the studied bacteria contain menaquinone, but no menaquinones have been found in molds and yeast [10]. Menaquinone and demethylmenaquinones (DMKs) are found in the cytoplasmic membrane of bacteria. MKs and DMKs function as a reversible redox component of the electron transfer chain [11]. Additionally, reduced MKs exhibit antioxidant properties and can play a role in protecting cellular membranes from lipid oxidation [12]. Menaquinones are also necessary for sporulation and proper regulation of cytochrome formation in some Gram-positive bacteria such as *Lactobacillus subtilis* [13]. In particular, the food industry uses various lactic acid bacteria (LAB) as starter cultures to produce fermented milk products, meat products, and vegetables. As many LAB lack a heme biosynthesis pathway, which results in an incomplete electron transport chain, the addition of menaquinone to the media facilitates aerobic growth, improves yield, and reduces production costs [14].

Menaquinone synthesis has mostly been described in *Escherichia coli*, *Mycobacterium phlei*, and *Bacillus subtilis*. In *E. coli*, chorismate from the shikimate pathway is converted into the naphthoquinone ring by six enzymes (MenFDHCEB) [11, 15]. The isoprenoid side chain is synthesized separately and is joined to the naphthoquinone ring to form demethylmenaquinone. Prenylation and methylation catalyzed by polyprenyltransferase (MenA) and methyltransferase (MenG) are the last steps of the synthesis of menaquinone [16, 17]. An alternative pathway, called the futalosine pathway, was described in microorganisms that lack *men* genes. In this pathway, chorismate is converted to menaquinone with four enzymes encoded by *mqnABCD* genes and unknown enzymes [17–19]. The majority of the bacteria containing the classical menaquinone pathway are obligately or facultatively aerobic, and the majority of menaquinone in anaerobic bacteria is synthesized via the futalosine pathway [17]. For example, the metabolic pathway of *Lactoccocus lactis*, which is used as a cheese starter, can function through aerobic and anaerobic reactions, and the *men* genes for the synthesis of menaquinone were detected in its genome.

Menaquinones have side chains of different sizes in different organisms and sometimes even within the same organism. Depending on the growing conditions, the basic structure can be modified by demethylation of the naphthoquinone ring to reform DMK or by saturation of the isoprenoid side chain [2, 19].

4. Non-dietary sources of menaquinones

Bacterially synthesized menaquinones that contribute to human vitamin K2 requirements may be produced by the gut microbiota or by bacteria present in food. In humans, the most important genera of intestinal flora are *Bacteroides* and *Bifidobacteria*. However, only *Bacteroides* can synthesize menaquinone. The major forms produced by *Bacteroides* are MK-10 and MK-11. MK-6 produced by *Eubacterium lentum*, MK-7 produced by *Veillonella*, and MK-8 produced by *Enterobacter* were also found in isolates from intestinal flora [2, 7, 8, 20]. Most menaquinones are present in the distal colon, but the most promising site of absorption is the terminal ileum, where there are menaquinone-producing bacteria and bile salts that are needed for solubilization of menaquinones [7, 21]. Therefore, although intestinal microflora synthesize large amounts of menaquinones, the bioavailability of bacterial menaquinone is poor, and diet is the major source of functionally available vitamin K2 [3, 7, 8]. Recent studies also showed that a short-term decrease in dietary vitamin K intake is not compensated by intestinal menaquinones [22–24].

5. Dietary sources of menaquinones

Vitamin K2 is mostly synthesized by bacteria; therefore, the highest number of long-chain menaquinones is found in fermented dairy products, such as cheese and fermented vegetables, such as natto and sauerkraut [16]. One exception is MK-4, which is formed by a realkylation step from menadione present in animal feed or as a product of tissue-specific conversion directly from dietary phylloquinone [5]. The extent of the conversion to MK-4 is estimated to range from 5% to 25% of the ingested phylloquinone [25].

Searching for information about the concentration of vitamin K2 in food is not very fruitful. Out of more than 70 national food databases, only 12 provide the vitamin K content of food items. Only three of these food databases (the United States, the Netherlands, Turkey) specifically report the vitamin K2 concentration; all others publish only phylloquinone (PK) or total vitamin K or give no further information about the vitamin forms included in the given values. The comparison of the provided concentration of MK in these three databases is not possible because the values are based on different specifications and different processes. The data given in the US database are for MK-4. However, the Dutch database includes several types of menaquinones, ranging from MK-4 to MK-10. For the data from the Turkish database, there is no information concerning the definition of vitamin K2 [16, 26]. In countries where animals are supplemented with menadione as practiced in the United States [27] and the Netherlands [28], the MK-4 concentration is normally higher in food of animal origin. The supplementation practice used in Turkey is unknown. Last, the process and the bacterial strains used in the production of fermented food determine the concentration and forms of MK in products [16].

Scanning the literature for publications that report the results of vitamin K measurements in food provides additional separate values for different menaquinones. However, information about longer-chain menaquinones (MK-5 to MK-10) is very limited. **Table 1** summarizes the values of vitamin K2 for animal products such as dairy, meat, fish and eggs, and fermented vegetable products such as bread, sauerkraut, and legumes (natto).

Menaquinone content (μg/100 g; mean ± SD or range)								
Food	**MK-4**	**MK-5**	**MK-6**	**MK-7**	**MK-8**	**MK-9**	**MK-10**	**Source**
Dairy								
Whole milk	0.7–0.9	0.0–0.1	nd	nd	nd	nd	nr	[6]
Whole milk	0.8–1.0	nr	nr	nr	nr	nr	nr	[27]
Whole milk	2 ± 0.3	nr	nr	nd	nr	nr	nr	[32]
Whole milk	0.4–1.0	nr	nr	nr	nr	0–2	nr	[29]
Milk 1% fat	0.3–0.4	nr	nr	nr	nr	nr	nr	[27]
Milk 2% fat	0.4–0.5	nr	nr	nr	nr	nr	nr	[27]
Whipped cream	5.2–5.6	nd	nd	nd	nd	nd	nr	[6]

Menaquinone content (µg/100 g; mean ± SD or range)								
Food	**MK-4**	**MK-5**	**MK-6**	**MK-7**	**MK-8**	**MK-9**	**MK-10**	**Source**
Cream	8 ± 3	nr	nr	nd	nr	nr	nr	[32]
Butter	13.5–15.9	nd	nd	nd	nd	nd	nr	[6]
	21 ± 7	nr	nr	nd	nr	nr	nr	[32]
Fermented milk								
Whole milk, sour	0.6 ± 0.02	0.3 ± 0.002	0.2 ± 0.03	0.4 ± 0.04	2.0 ± 0.1	4.7 ± 0.2	nd	[31]
Buttermilk	0.2–0.3	0.1–0.2	0–0.2	0.1–0.3	0.5–0.6	1.2–1.6	nr	[6]
Mesophilic	nr	nr	4.2	5	25.9	100.8	8.5	[34]
Thermophilic	nd	nd	nd	nd	nd	nd	nd	[34]
Yogurt								
Whole	0.4–1.0	nr	nr	nr	nr	0–2.0	nr	[29]
Whole	0.5–0.7	0–0.2	nd	nd	nd	nd	nr	[6]
Whole	1 ± 0.1	nr	nr	0.1 ± 0.2	nr	nr	nr	[32]
Plain	0.4 ± 0.03	0.1 ± 0.006	nd	nd	nd	nd	nd	[31]
Skimmed	nd	nd	nd	nd	0–0.2	nd	nr	[6]
Cheese								
Curd	0.3–0.6	0–0.2	0.1–0.3	0.2–0.5	4.8–5.4	18.1–19.2	nr	[6]
Curd	2–10	nr	nr	nr	nr	40–70	nr	[29]
Hard	4.2–6.6	1.3–1.7	0.6–1.0	1.1–1.5	14.9–18.2	45.3–54.9	nr	[6]
Semi-hard	nr	nr	1.9	1.1	3.9	17.5	4.7	[34]
Soft	3.3–3.9	0.2 –0.4	0.5–0.7	0.9 – 1.1	10.7–12.2	35.1–42.7	nr	[6]
Soft	nr	nr	1.7	1.2	7.0	27.3	2.9	[34]
Processed	5 ± 2	nr	nr	0.3 ± 0.1	nr	nr	nr	[32]
Blue cheese	nr	nr	4.9	12.4	7.7	19.3	2.9	[34]
Appenzeller	4.3–5.2	nr	nr	nr	nr	nr	nr	[33]
Caerphilly	nr	nr	1.6 ± 0.1	nd	1.6 ± 0.1	32.4 ± 0.8	nd	[34]
Cheddar	10.2	nr	nr	nr	nr	nr	nr	[27]
Cheddar	nr	nr	2.2	2.1	3.2	12.9	5.2	[34]
Cheshire	nr	nr	1.6± 0.2	nd	5.8 ± 0.2	24.2 ± 0.4	nd	[34]
Comté	5.5–8.4	nr	nr	nr	nr	nr	nr	[33]
Comté	nd	nd	nd	nd	nd	nd	nd	[34]
Edam	3.3 ± 0.2	1.0 ± 0.1	0.6 ± 0.1	1.3 ± 0.1	10.5 ± 0.8	30.0 ± 2.6	0.9 ± 0.1	[31]
Emmental	8.1–8.6	nr	nr	nr	nr	nr	nr	[33]
Emmental	nr	nr	nd	nd	nd	nd	4.0	[34]

Menaquinone content (μg/100 g; mean ± SD or range)								
Food	**MK-4**	**MK-5**	**MK-6**	**MK-7**	**MK-8**	**MK-9**	**MK-10**	**Source**
Aged 90 d	5.2 ± 0.1	nd	trace	trace	nd	nd	nd	[31]
Aged 180 d	6.1 ± 0.5	nd	trace	nd	nd	nd	nd	[31]
Gamalost	1.0 ± 0.0	0.6 ± 0.0	0.3 ± 0.0	0.9 ± 0.1	4.8 ± 0.7	42.3 ± 7.0	2.1 ±0.4	[35]
Jarlsberg	8.4	nr	nr	nr	nr	nr	nr	[33]
Gruyère	8.1–9.6	nr	nr	nr	nr	nr	nr	[33]
Leicester	nr	nr	2.0 ± 0.1	2.1 ± 0.1	4.8 ± 0.2	16.2 ± 0.3	4.4 ± 0.2	[34]
Mozzarella	3.1–4.0	nr	nr	nr	nr	nr	nr	[27]
Mozzarella	nd	nd	nd	nd	nd	nd	nd	[34]
Norvegia	5.1 ± 0.9	nd	0.3 ± 0.1	1.3 ± 0.2	5.3 ± 0.5	29.6 ± 3.6	nd	[35]
Raclette	5	nr	nr	nr	nr	nr	nr	[33]
Swiss cheese	6.2–8.8	nr	nr	nr	nr	nr	nr	[27]
Meat								
Salami	8.2–10.1	nd	nd	nd	nd	nd	nr	[6]
Calf liver	1.1–8.9	nr	nr	nr	nr	nr	nr	[27]
Beef liver	0.4 ± 0.4	nr	nr	nr	nr	nr	nr	[27]
Bovine liver	6.8 ± 1.03	nd	9.44 ± 0.118	25.6 ± 0.59	13.8 ± 0.55	9.8 ± 0.7	14±1.7	[31]
Beef liver	0.8	nr	2.5	18.2	4.8	1.5	6.6	[30]
Pork liver	0.3–0.4	nd	nd	nd	nd	nd	nd	[6]
Pork liver	10.8 ± 1.44	nd	nd	16 ± 2.7	25 ± 5.2	6 ± 1.8	8±2.9	[31]
Pork liver	0.6	nd	0.04	0.6	0.5	0.3	0.5	[30]
Chicken liver	14.1 ± 2.0	nr	nr	nr	nr	nr	nr	[27]
Chicken liver	4	nr	0.03	nd	0.09	0.04	0.03	[30]
Beef kidney	2.1	nr	0.08	0.2	0.01	nd	0.1	[30]
Pork kidney	1.3	nr	0.02	0.07	0.05	0.22	0.24	[30]
Chicken kidney	5	nr	nd	nd	nd	nd	nd	[30]
Beef muscle	3.4	nr	0.03	0.03	nr	nr	nr	[30]
Pork thigh	6 ± 2	nr	nr	nr	nr	nr	nr	[32]
Pork steak	1.7–2.4	nd	nd	0.4–0.7	0.9–1.2	nd	nd	[6]
Pork chop	3.1 ± 0.46	nd	nd	0.12 ± 0.035	nd	nd	nd	[31]
Pork muscle	0.9	nr	0.03	0.03	nr	nr	nr	[30]
Chicken breast	6.4–11.3	nd	nd	nd	nd	nd	nd	[6]
Chicken leg	5.8–10.5	nd	nd	nd	nd	nd	nd	[6]
Chicken thigh	27 ± 15	nr	nr	nd	nr	nr	nr	[32]

Menaquinone content (µg/100 g; mean ± SD or range)								
Food	**MK-4**	**MK-5**	**MK-6**	**MK-7**	**MK-8**	**MK-9**	**MK-10**	**Source**
Chicken meat, leg and thigh	60 ± 8.2	nd	nd	nd	nd	nd	nd	[31]
Chicken muscle	8.9	nr	nd	nd	nr	nr	nr	[30]
Fish								
Rainbow trout, cultivated	3.1 ± 0.2	0.09 ± 0.019	nd	0.2 ± 0.058	nd	nd	nd	[31]
Pike perch	0.2 ± 0.025	0.05 ± 0.0044	0.05 ± 0.0008	0.5 ± 0.13	nd	nd	nd	[31]
Baltic herring	0.21 ± 0.002	nr	nd	nd	nd	nd	nd	[31]
Horse mackerel	0.6 ± 0.1	nr	nr	nd	nr	nr	nr	[32]
Mackerel	1 ± 0.2	nr	nr	nd	nr	nr	nr	[32]
Mackerel	0.3–0.5	nd	nd	nd	nd	nd	nr	[6]
Salmon	0.2–0.3	nr	nr	nr	nr	nr	nr	[27]
Plaice	0.1–0.3	nd	0.2–0.3	0.0–0.1	1.3–1.8	nr	nr	[6]
Eel	1.4–2.1	nd	0.0–0.2	0.2–0.6	nd	nd	nr	[6]
Salmon	0.4–0.6	nd	nd	nd	nd	nd	nr	[6]
Eggs								
Egg yolk	29.1–33.5	nd	0.6–0.8	nd	nd	nd	nr	[6]
Egg albumen	0.8–1.0	nd	nd	nd	nd	nd	nr	[6]
Whole egg	7 ± 3	nr	nr	nd	nr	nr	nr	[32]
Egg white	1 ± 1	nr	nr	nd	nr	nr	nr	[32]
Egg yolk	64 ± 31	nr	nr	nd	nr	nr	nr	[32]
Whole egg	5.6	nr	nr	nr	nr	nr	nr	[27]
Egg white	0.4	nr	nr	nr	nr	nr	nr	[27]
Egg yolk	15.5	nr	nr	nr	nr	nr	nr	[27]
Bread								
Bread	0	nr	nr	nr	nr	0.9–2	nr	[29]
Buckwheat	nd	nd	nd	1.0–1.2	nd	nd	nr	[6]
Plant products								
Sauerkraut	0.3–0.5	0.6–1.0	1.4–1.6	0.1–0.3	0.6–0.9	0.9–1.3	nr	[6]
Natto	nd	7.1–7.8	12.7–14.8	882–1034	78.3–89.8	nd	nr	[6]
	2 ± 3	nr	nr	939 ± 753	nr	nr	nr	[32]
Hikiwari natto (chopped natto)	nd	nr	nr	827 ± 194	nr	nr	nr	[32]

Menaquinone content (μg/100 g; mean ± SD or range)								
Food	**MK-4**	**MK-5**	**MK-6**	**MK-7**	**MK-8**	**MK-9**	**MK-10**	**Source**
Black bean natto	nd	nr	nr	796 ± 93	nr	nr	nr	[32]

nd, not determined; nr, not reported.

Table 1. Representative ranges of measured menaquinone concentration in food.

Values for MK-4 to MK-10 are available. MK-4 is found in all reported products except buckwheat, hikiwari natto, and black bean natto [6, 27, 29–35]. In non-fermented dairy products and in eggs, hardly any longer-chain menaquinones have been reported [6, 27, 29, 32]. Long-chain menaquinones are also rare in the muscle meat of beef, pork, and chicken [6, 30–32]. However, in offal, such as the liver and kidney, small-to-moderate concentrations of MK-6 to MK-10 have been detected [6, 27, 30, 31]. In fish, vitamin K2 concentrations are in general very low, and menaquinones other than MK-4 have been found in only a few fish species [6, 27, 31, 32]. These small amounts of longer-chain menaquinones are said to originate from the bacteria in decomposing organic material that serves as food for fish that live at the bottom of the sea such as eel and plaice [36]. In sour milk and buttermilk and in curd and hard and soft cheese, MK-8 and MK-9 mainly account for the total concentration of vitamin K followed by MK-6 and MK-7 [6, 27, 29, 31–35]. Fermented plant products are characterized by a high concentration of MK-7 (up to 1000 μg/100 g) [6, 32].

Almost no data are available about the stability and changes in vitamin K concentrations during storage of food in general and during ripening of fermented food in particular.

6. Production of different menaquinones by microorganisms in food

Fermentation is traditionally used to increase shelf life, to inhibit pathogens, and to improve organoleptic properties [37]. Additionally, the microbial production of vitamins provides a very attractive approach for improving the nutritional composition of fermented foods. A number of MK-producing species are commonly used in industrial food fermentation applications (**Table 2**). The main microorganisms used in fermented dairy products are lactic acid bacteria, which transform lactose into lactic acid. *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis*, and *Leuconostoc lactis* are used as starter cultures in semihard and soft cheeses. It was reported that these species produce menaquinone and MK-7 to MK-9 in particular for *Lactococcus* and MK-7 to MK-10 for *Leuconostoc* [2, 38]. For example, the starter cultures CHN211 and CHN22 from Hansen, which contain these species, produce MK-4 to MK-10; MK-9 is the main menaquinone with 472.4 ± 22.6 μg/100 g cells and 390.3 ± 10.4 μg/100 g cells, respectively [35]. Accordingly, the highest amounts of MK were detected in semihard and soft cheese and in Caerphilly and Cheshire, a crumbly cheese specialty, known for higher numbers of *Lactococcus* species (**Table 1**). In semihard cheese, menaquinones in amounts up to 29.1 μg/100 g have been detected. The main quantified form of menaquinone in dairy is MK-9 (usually more than 50%), and the second major form is MK-8. Manoury and coauthors also found a correlation between MK-9 and MK-8. For most dairy, the MK-9 level was four times higher than that of MK-8, and the authors suggested that microorganisms that produce MK-9 could also produce MK-8. Astonishingly, the level of MK-9 was not dependent on the fat level of the dairy products. Moreover, the authors found no link between pH and the MK-9 content. The highest amounts of MK-10 are usually found in hard cheese, with the exception of one semihard cheese [34].

Species/subspecies	**Food use**
Lactococcus lactis ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*	Cheese, buttermilk, sour cream, cottage cheese, cream cheese, kefir, yogurt
Lactococcus raffinolactis	Cheese
Leuconostoc lactis	Cheese
Leuconostoc mesenteroides	Vegetables, dairy
Brevibacterium linens	Cheese
Brochothrix thermosphacta	Meat
Hafnia alvei	Cheese
Staphylococcus xylosus	Dairy, sausage
Staphylococcus equorum	Dairy, meat
Arthrobacter nicotianae	Cheese
Bacillus subtilis "natto"	Natto (fermented soybean)
Propionibacterium shermanii	Cheese
Propionibacterium freudenreichii	Cheese

Adapted from Walther et al. [16].

Table 2. Menaquinone-producing bacteria in fermented food.

In Swiss Emmental cheese, *Propionibacterium* strains are added to the milk to improve the formation of holes inside the cheese body. During propionic acid fermentation, lactic acid is transformed into propionic acid, acetic acid, and carbon dioxide. Various studies showed the ability of *Propionibacterium* to produce menaquinone MK-9(4H) in anaerobic conditions [39, 40]. The highest amount of MK-9(4H) has been detected in Swiss Emmental (up to 31.4 μg/100 g MK-9(4H)) and Norwegian Jarlsberg (65.2 μg/100 g MK-9(4H)); both cheeses have a high propionic acid concentration. Smaller amounts are also found in Appenzeller (up to 2 μg/100 g), Comté (up to 6.0 μg/100 g), and Raclette (4.7 μg/100 g) [33].

In contrast, dairy products fermented with thermophilic lactic acid bacteria, such as Comté cheese, mozzarella, or yogurt products, contain only small amounts of menaquinone or none (**Table 1**). These thermophilic species include *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, and *Bifidobacterium*, and they are known to be non-vitamin K producers [2, 34].

In soft cheese, the average total menaquinones range 40.1 μg/100 g to 61 μg/100 g depending on the source, analytical method, and type of cheese (**Table 1**). Manoury and coauthors reported a soft cheese and a blue cheese with very high concentrations (up to 4.110 μg/100 g and 70 μg/100 g, respectively), but the researchers could not explain why these two cheeses are so rich in menaquinones [34].

One cheese with mold was also analyzed for menaquinone content. Gamalost, a Norwegian mold (*Mucor mucedo*) ripened autochthonous cheese, contains more menaquinone than Norvegia, a semihard Norwegian cheese, but the mold did not contribute to the production of vitamin K in Gamalost. The low pH in Gamalost and a higher fermentation rate may explain the differences in menaquinone content [35].

Some work has been conducted to improve the content of different menaquinones in dairy products. New research demonstrated that strains of *Lactobacillus fermentum* LC 272 isolated from raw milk could be a starter culture for fermented milk with a high level of vitamin K2 (MK-4) production [41]. This strain can produce 185 μg/L in Rogosa medium and 64 μg/L in reconstituted skim milk. Morishita and coworkers published a study in 1999 that showed the possibility of producing MK-8 and MK-9 with *Lactocouccus lactis* ssp. *cremoris* YIT2011 and MK-9 and MK-10 with *Lactococcus lactis* YIT 3001 (29–123 μg of menaquinone/L of the fermented medium) [38]. Additionally, several patents for *Lactococcus* capable of producing a significantly increased amount of vitamin K2 have been deposed.

In contrast to fermented animal products, fermented vegetable products contain mainly MK-7 (**Table 1**). Natto, a traditional Japanese food produced with *Bacillus subtili*s natto, contains the highest amount of menaquinone. The highest measured value is almost 1000 μg/100 g. *B. subtilis* natto is the key microorganism for industrial production of MK-7, and much work has been done to improve the production. Optimization of the fermentation medium, mutations of the strains, and biofilm formation have been described as means for improving the yield of MK-7 [42–46]. The use of organic solvents to extract vitamins is one of the major issues of the bulk production of MK-7. Berenjian and coworkers demonstrated that the addition of vegetable oil during a dynamic fermentation process could be a good process for producing an oil rich in MK-7. In that study, the oil contained 724 mg/L of MK-7, and they suggested using the oil in supplementary and dietary food products [47].

7. Dietary recommendations for menaquinone

Dietary recommendations for vitamin K are still based on knowledge of phylloquinone and its classic role as an enzyme cofactor for coagulation proteins. The recommendations do not consider the differences in bioavailability and bioactivity between the different forms of vitamin K or the possibly higher requirements for health effects apart from coagulation, such as bone or cardiovascular health [16].

Depending on country, sex, and age, the recommendations for vitamin K range from 50 to 120 μg per day for adults 19 years and older. These recommendations are generally presented as adequate intake or estimated values, and no tolerable upper intake level has been established for vitamin K [16, 25, 48]. Research for valuable biomarkers to measure the status of vitamin K in the population is ongoing. A recent study from Maastricht University compared the biomarkers for coagulation with those of bone and vascular health in 896 healthy volunteers. Whereas all coagulation proteins were completely carboxylated by vitamin K, and a high concentration of undercarboxylated Gla proteins (osteocalcin and matrix Gla protein) was found in the majority of the blood samples, indicating that most of the volunteers in this study had an inadequate supply of vitamin K [23]. As long as robust physiological endpoints are missing to differentiate the contribution of MKs to human health from that of PK, it is unlikely that specific dietary recommendations for MKs will be widely adopted in the near future. In the meantime, a preferred recommendation could be to consume a wide variety of foods which are good sources of PKs and MKs, respectively, such as green leafy vegetables and fermented dairy products [16, 49].

8. Dietary intake of menaquinones

As shown in **Table 1**, the most important sources of menaquinones are cheese, curd, offal, and fermented soybeans (natto). Based on regional differences in dietary patterns, the form and amount of specific menaquinones consumed may vary widely between populations. For example, in Japan, as a result of natto consumption, MK-7 is the most frequently consumed form of menaquinones. The contribution of MK-7 to total vitamin K intake is 25% among young women living in eastern Japan. Nearly all of the MK-7 intake originates from pulses, including fermented soybean natto [32]. The mean daily intake of MK-7 in this study was 57.4 μg with a range from 0 to 340 μg.

In countries with a traditional high intake of dairy products, such as the Netherlands, Germany, and the United Kingdom (UK), MK-7 to MK-10 contribute mostly to the menaquinone supply. Beulens and coauthors compiled the results from several European studies that estimated menaquinone intake using Food Frequency Questionnaires (FFQs). The self-reported mean daily intake of menaquinones in adults ranged from 20.7 μg for women in the Rotterdam Study to 43 μg in men in the UK National Dietary and Nutrition Survey. In all of these studies, cheese was the most important food source of menaquinones [49]. However, these data should be interpreted carefully because they were collected by FFQs that are designed to estimate the relative dietary intake of large populations but not to estimate absolute dietary intake. A seasonal survey in postmenopausal women in Tehran, Iran, used a monthly food record for 1 year. The researchers found a significantly higher intake of vitamin K in the spring, summer, and autumn compared to the winter. Unfortunately, these authors did not further specify vitamin K and did not provide any information about consumption of different food items containing vitamin K [50]. A study in older individuals to calculate the desired duration of a diet recording to estimate the individual vitamin K intake concluded that 13 24-hour recalls are ideal to record intraindividual variance. As this would not be realistic in most studies, the authors proposed a minimum of six nonconsecutive days of diet recording [51]. Another possible approach for estimating nutrient status is to use biomarkers. Biomarkers for menaquinones are undercarboxylated vitamin K-dependent proteins in the circulatory system. However, in addition to vitamin K availability, these biomarkers depend on the total amount of protein. To be sure that protein status does not confound vitamin K status, the measurements must be corrected for the total amount of the protein under study [52].

These limitations, together with the scarce and widely varying data on concentrations of different menaquinones in food items, show how fragmentary our knowledge of the supply of vitamin K2 in the general population remains.

9. Pharmacokinetics of menaquinones

Although the forms of vitamin K are classified as fat-soluble nutrients, the lipophilicity of the different forms changes with side-chain length. Whereas menadione is water soluble, phylloquinone and MK-4 are mildly lipophilic. Long-chain menaquinones are strongly lipophilic and soluble only in apolar organic solvents [36]. This lipophilicity also influences the absorption of vitamin K, which varies greatly depending on the food matrix. As long-chain menaquinones are found mainly in the fat fraction of dairy products, the absorption of these menaquinones is almost 100% in contrast to PK, where the poor uptake of only 5–10% from cooked vegetables can be improved only slightly by concomitant fat intake [6]. As a consequence, even the dietary intake of phylloquinone is much higher, menaquinones are equally important for vitamin K status, because of their better intestinal absorption. Independently of their form and origin, all K vitamins are transported to the liver, incorporated in triglyceride-rich lipoproteins. Unlike phylloquinone, which mostly remains in the liver to be used for clotting factor synthesis, menaquinones are released to the bloodstream incorporated in low-density lipoproteins and transported to the target tissue such as bone and arteries for Gla-protein carboxylation. Absorbability is further supported by a longer half-life, up to several days for long-chain menaquinones compared to phylloquinone, which normally disappears from the bloodstream after 8 hours. This longer postprandial presence in the bloodstream leads to a more constant circulating level of vitamin K2 and, as a consequence, longer availability of these long-chain menaquinones for uptake by extrahepatic tissues [36, 53]. Although there is some evidence that menaquinones with medium-chain length like MK-7 are better absorbed than short- (MK-4) or long-chain menaquinones (MK-8 and MK-9) [6], human data on the bioavailability, absorption, and kinetics of K2 vitamins from food are limited to MK-7 and MK-9 and have not been systematically tested for all menaquinones thus far [36, 49].

As researchers have found that MKs play an important role in health aspects beyond coagulation, the cooperation with other nutrients in vitamin K-rich food such as fermented dairy products may lead to a better understanding of the effect of different food items on health aspects, for example, bone health or cardiovascular health.

10. Bone health, menaquinones, and fermented dairy products

One of the most important research fields in the past and present is the study of the factors that influence the formation and conservation of strong bones. Osteopenia, including osteoporosis, is one of the most prevalent diseases in elderly individuals and is a large social, medical, and economic burden throughout the world. One out of three women and one out of five men older than 50 years are at risk of experiencing an osteoporotic fracture [54]. Low bone mineral mass is the main factor that causes osteoporotic fractures. Bone mass in later life is the result of the peak bone mass achieved during growth and the rate of age-related bone loss. Consequently, a high peak bone mass at maturity and a low bone loss during aging are the most promising factors in the prevention of osteoporosis and fractures. In addition to factors that influence bone health such as gender, age, body size, genetics, and ethnicity that are not changeable, other factors, especially lifestyle factors such as physical activity, smoking, alcohol consumption, and dietary patterns, can be modified [55]. Different dietary factors are known to positively influence bone health. They range from minerals (e.g., calcium, magnesium, phosphorus, potassium, and various trace elements) and vitamins (A, D, E, K, C, and certain B vitamins) to macronutrients such as proteins and fatty acids and finally to bioactive food components (e.g., peptides) that in recent years have been proposed to be beneficial for bone health [55]. All these elements are involved in bone metabolism. Currently, researchers are trying to identify and understand the mechanisms and interactions of these factors in relation to bone health [56].

Most studies that have investigated the relationship between dairy and bone health have shown a beneficial effect of dairy consumption, even if the reason for this link is still unclear [56, 57]. After many years of focusing on calcium as the beneficial element for bone health in dairy, recent evidence suggests that other macro- and micronutrients, as well as food components such as bioactive peptides, milk fat globule membrane, prebiotics, and probiotics present in milk and dairy products, play an important role in this health outcome [56]. Many of these nutrients support the bioavailability (phosphorus, vitamin D, magnesium, zinc, potassium), absorption (casein phosphopeptides, phosphorus, lactose, protein) and homeostasis (magnesium, potassium, vitamin D) of calcium and contribute to bone-building properties (phosphorus, magnesium, potassium, zinc, vitamin D, vitamin B12, and vitamin K) [56–58].

Most of these components are not or are positively affected by fermentation. That means their concentration remains the same in the fermented product compared with milk or even increases either by processing (i.e., fat-soluble vitamins in cheese) or by the activity of microorganisms (i.e., bioactive peptides, vitamin B12, or vitamin K2).

The role of vitamin K2 in bone health is strongly bound to osteocalcin (OC), a key regulator of calcium usage. This small Ca^{2+}-binding protein is involved in the mineralization of bones and teeth, and its potential to bind calcium is dependent on carboxylation with vitamin K2.

Only the fully carboxylated OC is able to strongly bind calcium and to consolidate calcification of the hydroxyapatite crystal lattice that requires a sufficient supply with vitamin K2 and other nutrients, such as retinoic acid and vitamin D, all involved in the regulation of osteocalcin production [59].

Fermented dairy products are vital for bone health because of their unique combination of various nutrients and microorganisms that support and maintain positive bone metabolism [57, 60]. Additionally, dairy matrix and nutrient composition may affect the delivery of menaquinones and improve vitamin K status [61].

11. Cardiovascular health, menaquinones, and fermented dairy products

Coronary artery calcification (CAC) is a predictor of cardiovascular disease (CVD) and mortality. Based on vitamin K's role in activating matrix Gla protein (MGP), a calcification inhibitor, vitamin K is proposed to play a preventive role in CAC and CVD [59, 62]. As recently reviewed, randomized controlled trials that examined the influence of vitamin K on the risk of cardiovascular disease are scarce [63]. The results of observational studies have shown an association between higher dietary menaquinone consumption and less calcification [64], decreased risk of coronary heart disease (CHD), CHD mortality, and all-cause mortality [65–67]. The results of a Dutch prospective cohort study suggested that of all MKs the long-chain menaquinones (MK-7 to MK-9) have the most beneficial effects on cardiovascular disease [67]. Although these results are promising, they must be interpreted with caution, because validated biomarkers for single MK intake are missing [16].

Complex milk fatty acid chemistry and several minerals, such as calcium, magnesium, phosphorus, and potassium provided in relevant concentrations, have been proposed to be involved in the complex mechanism of dairy products and their support to reduce CVD risk [68]. Among the high number of different fatty acids in dairy products, trans-palmitoleic acid, stearic acid, lauric acid, myristic acid, and oleic acid have been associated with beneficial effects on blood lipids and serum lipoprotein levels [56]. These assumptions are supported by the inverse association observed between CHD risk and the consumption of milk, cheese, and meat as the richest sources of MKs in the Western diet [6, 67].

12. Conclusion

Our knowledge of the consumption of menaquinones should be improved with weighed and extended food records [51] in combination with (multiple) biomarkers in the blood for vitamin K status [52] and the content of the various menaquinones in food items such as cheese, which contribute most to the supply with this vitamin.

As different lactic acid bacteria strains used in cheese production influence the expression of various MKs, analysis of a wide variety of different cheeses may be necessary for a representative overview of the vitamin K2 content in this food group. Although results from well-designed clinical trials investigating the association between menaquinones and bone health, as well as cardiovascular health, are rare, dairy products seem to be predestined to play a major role in the Western diet because of their nutrient density and matrix properties that improve the bioavailability of vitamin K2.

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