Multiple Vitamin K Forms Exist in Dairy Foods

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Abstract

Background: The plant-based form of vitamin K (phyllloquinone, vitamin K-1) has been well quantified in the US diet. Menaquinones (vitamin K-2) are another class of vitamin K compounds that differ from phylloquinone in the length and saturation of their side chain, but they have not been well characterized in foods.

Objectives: The objectives of this study were to 1) quantify phylloquinone and the different forms of menaquinones [menaquinone (MK) 4–MK13] in milk, yogurt, Greek yogurt, creams, and cheeses and 2) compare the menaquinone contents of full-fat, reduced-fat, and nonfat dairy products.

Methods: All dairy samples were either obtained from the USDA National Food and Nutrient Analysis Program or purchased from retail outlets. Phylloquinone and menaquinone concentrations in these dairy products were quantified by mass spectrometry technology.

Results: Full-fat dairy products contained appreciable amounts of menaquinones, primarily in the forms of MK9, MK10, and MK11. We also measured modest amounts of phylloquinone, MK4, MK8, and MK12 in these products. In contrast, there was little MK5–7 or MK13 detected in the majority of dairy products. The total vitamin K contents of soft cheese, blue cheese, semi-soft cheese, and hard cheese were (means ± SEMs): 506 ± 63, 440 ± 41, 289 ± 38, and 282 ± 5.0 µg/100 g, respectively. Nonfermented cheeses, such as processed cheese, contained lower amounts of vitamin K (98 ± 11 µg/100 g). Reduced-fat or fat-free dairy products contained 5–22% of the vitamin K found in full-fat equivalents. For example, total vitamin K contents of full-fat milk (4% fat), 2%-fat milk, 1%-fat milk, and nonfat milk were 38.1 ± 8.6, 19.4 ± 7.7, 12.9 ± 2.0, and 7.7 ± 2.9 µg/100 g, respectively.

Conclusions: To the best of our knowledge, this is the first report of menaquinone contents of US dairy products. Findings indicate that the amount of vitamin K contents in dairy products is high and proportional to the fat content of the product. Curr Dev Nutr 2017;1:e000638.

Introduction

Dietary sources of vitamin K are found in 2 natural forms: phylloquinone (vitamin K-1) and menaquinones (vitamin K-2). All forms of this fat-soluble vitamin share a common structure, 2-methyl-1,4-napthoquinone. The menaquinones differ in structure from phylloquinone in their 3-substituted lipophilic side chain and are designated by the number of isoprenoid units [i.e., menaquinone (MK)-n]. Menaquinones with ≤13 isoprenoid units have been identified (1). Whereas phylloquinone is widely distributed in the food supply, menaquinone forms appear to be limited to animal products and fermented foods (2). As an essential vitamin, vitamin K plays a role as an enzyme cofactor necessary for the modification of glutamic acid residues to γ-carboxyglutamic acid residues in specific proteins, referred to as vitamin K–dependent proteins (3). The vitamin K–dependent proteins matrix Gla protein, osteocalcin, and Gas-6 have been implicated in tissue calcification, bone metabolism, and cell cycle...
regulation (4–6). Vitamin K has multiple roles independent of its known biochemical function as an enzyme cofactor, such as anti-inflammatory (7), a ligand for steroid, and a xenobiotic receptor (8).

The current US recommendations for intakes of vitamin K are 90 and 120 µg/d for women and men, respectively. These guidelines are termed Adequate Intakes due to insufficient data with regard to vitamin K metabolism and the lack of a robust biomarker to generate precise dietary recommendations (9). The Adequate Intake is based on usual phylloquinone intakes and does not take into account the potential dietary contribution of other forms of vitamin K. Very little is known about the contribution of dietary menaquinones to overall vitamin K nutrition, and although it has been stated that ~50% of the daily requirement for vitamin K is supplied by gut bacteria through the production of menaquinones (10), there is little evidence to support this estimate. Estimated intakes of phylloquinone and menaquinones in dairy-producing countries in Western Europe suggest that between 10% and 25% of total vitamin K intake is provided by menaquinones, primarily from dairy sources (10, 11). However, menaquinones have not been systematically analyzed in US foods nor have menaquinones been included in total vitamin K intakes estimated in the US population, so these observations have yet to be substantiated outside of Western Europe.

The need to analyze menaquinones in commonly consumed foods is timely because observational data from dairy-producing countries in Europe suggest that intakes of menaquinones present in dairy products have stronger associations with heart health benefits than do phylloquinone intakes (12). Menaquinone data for commonly consumed foods from other countries are critical for determining if these observations are generalizable. Furthermore, the food-composition data applied to these few observational studies, almost exclusively from Netherlands, predominantly represent full-fat dairy products. Low-fat and nonfat dairy products are recommended as part of a healthy diet in the United States to reduce the risk of cardiovascular disease and associated comorbidities (13). The impact of reducing the fat content of dairy products on menaquinone content is unknown.

Advances in MS methodology have provided an ability to quantify multiple forms of vitamin K (phylloquinone and menaquinones) in various matrices, allowing us to explore, for the first time to our knowledge, the menaquinone content in the US food supply (14). The purpose of this study was to quantify the content of multiple forms of vitamin K in various dairy products, including yogurt, cheeses, milk, and milk-based products, and to examine the effect of fat content on the distribution and concentration of vitamin K forms in those products.

Methods

Fifty of the dairy samples used in this study were provided by USDA Nutrient Data Laboratory, which conducts the National Food and Nutrition Analysis Program (15). The nationally collected dairy samples were first delivered to the Food Analysis Laboratory Control Center at Virginia Tech in Blacksburg, Virginia, for preparation of aliquots, and then delivered frozen on dry ice to the Vitamin K Laboratory at Tufts University and stored at −80°C until analysis. The National Food and Nutrition Analysis Program infrastructure incorporates a nationally representative sampling approach (15, 16), approved analytical methods, and a rigorous quality assurance scheme. In addition, 148 dairy samples used in this study were purchased in 2016 from retail outlets that have substantial annual sales in order to capture the diversity of products available in the Boston (Massachusetts) area. Appropriate containers were used to maintain refrigeration during the transport to the laboratory. All of the samples collected by our laboratory were composited, placed in aliquots, and stored at −80°C before analysis. Shelf-life date, analysis date, brand name, and fat content were recorded. We used available information from the manufacturers to determine fat content (i.e., full-fat, reduced-fat, etc.).

The dairy products were grouped in categories on the basis of dairy type and fat content (Table 1): milk, yogurts, Greek yogurts, kefirs, creams, processed cheeses, fresh cheeses, blue cheeses, soft cheeses, semi-soft cheeses, and hard cheeses. Aside from processed cheese, all other types of cheeses included ≥2 different brands and different lots.

All of the cheese sample aliquots (~10 g) were freeze-dried to remove liquid nitrogen and manually ground into a powder by using a mortar and pestle. Approximately 0.05–0.2 g of each sample was used for analysis. The procedures for vitamin K extraction and sample purification have been previously described (14). Phylloquinone and MK4–MK13 concentrations were measured by LC-MS by using deuterium-labeled phylloquinone as an internal standard (Sigma Aldrich) and synthesized phylloquinone and MK4–MK13 as calibration standards (14).

The effects of dairy product fat content (full-fat, fat-free, or reduced-fat) on concentrations of total vitamin K, phylloquinone, and all detectable menaquinones were analyzed by 2-sample t test. Given the smaller sample size, the vitamin K content of cream products (heavy or whipping cream, half-and-half, and light cream) was examined by general linear model, with heavy or whipping cream used as the reference group. Significance was determined

<table>
<thead>
<tr>
<th>Dairy products analyzed</th>
<th>n</th>
<th>Type</th>
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</thead>
<tbody>
<tr>
<td><strong>Dairy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>43</td>
<td>Full-fat, 2%-fat, 1%-fat, nonfat</td>
</tr>
<tr>
<td>Yogurt</td>
<td>16</td>
<td>Full-fat and nonfat</td>
</tr>
<tr>
<td>Greek yogurt</td>
<td>16</td>
<td>Full-fat and nonfat</td>
</tr>
<tr>
<td>Kefirs</td>
<td>4</td>
<td>Low-fat</td>
</tr>
<tr>
<td>Cream</td>
<td>5</td>
<td>Heavy, light, half-and-half</td>
</tr>
<tr>
<td>Processed cheese</td>
<td>9</td>
<td>American cheese</td>
</tr>
<tr>
<td>Fresh cheese</td>
<td>12</td>
<td>Goat, feta, ricotta, Cotija, cottage, and mozzarella cheese</td>
</tr>
<tr>
<td>Blue cheese</td>
<td>12</td>
<td>Mozzarella part skim</td>
</tr>
<tr>
<td>Soft cheeses</td>
<td>10</td>
<td>Gorgonzola and blue cheeses</td>
</tr>
<tr>
<td>Semi-soft cheeses</td>
<td>14</td>
<td>Brie, camembert, crème fraîche, Limburger, mascarpone</td>
</tr>
<tr>
<td>Hard cheeses</td>
<td>12</td>
<td>Monterey Jack, Havarti, Fontina, Gouda, Swiss, and cream cheeses</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Reduced-fat cheddar</td>
</tr>
</tbody>
</table>

TABLE 1 Dairy products analyzed

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at $P < 0.05$, and all analyses were carried out by using SAS version 9.4 (SAS Institute). Data are reported as means ± SEMs.

**Results**

Dairy products obtained from the USDA Nutrient Data Laboratory and those purchased from retail outlets contained appreciable amounts of menaquinones, primarily in the forms of MK9, MK10, and MK11. Together these 3 menaquinones account for ~90% of total vitamin K in dairy foods.

The vitamin K content of different cheeses showed significant variability in total vitamin K concentrations, ranging from 40 μg/100 g to ≤850 μg/100 g (Figure 1). All forms of cheese contained MK9, MK10, and MK11. We also measured modest amounts of phylloquinone, MK4, MK7, MK8, and MK12 in these samples. In contrast, there was little MK5, MK6, or MK13 detected in the majority of cheese products. The total vitamin K content varied by cheese type, with soft cheese having the highest concentration, followed by blue cheese, semi-soft cheese, and hard cheese (means ± SEMs: 506 ± 63, 440 ± 41, 289 ± 38, and 282 ± 5.0 μg/100 g, respectively; Supplemental Table 1). Nonfermented cheeses, such as processed cheese, contained lower amounts of vitamin K (98 ± 11 μg/100 g). There was considerable diversity in vitamin K forms among fresh, semi-soft, blue, and soft cheeses but not in hard and processed cheeses. Soft cheeses and hard cheeses showed a similar vitamin K pattern, with high MK9 and MK10, with blue and semi-soft cheeses sharing a similar pattern dominated by MK9 and MK11.

Milk and yogurt products were also measured. The vitamin K concentrations of full-fat (4% fat), 2%-fat, 1%-fat, and fat-free

![FIGURE 1 Vitamin K content of different cheeses. MK, menaquinone; PK, phylloquinone.](https://academic.oup.com/cdn/article-abstract/1/6/e000638/4558638)
milk varied by fat content (Figure 2). Mean total vitamin K contents of full-fat milk, 2%-fat milk, 1%-fat milk, and fat-free milk were 38.1 ± 2.7, 19.4 ± 2.4, 12.9 ± 0.6, and 5.1 ± 0.9 μg/100 g, respectively. Both total vitamin K and individual menaquinone concentrations in the full-fat milk were significantly higher than in 2%-fat milk products (P < 0.05). Phylloquinone was only detected in full-fat milk. MK5–8 and MK12–13 were not detected in any milk samples. Fat-free milk contained only a minimal amount of MK9 and MK11.

Regular and Greek yogurt with full fat (mean ± SEM: 4.6% ± 0.5% and 4.0% ± 0.2%, respectively) contained similar vitamin K concentrations as full-fat milk (4% fat) (Table 2). Surprisingly, neither menaquinones nor phylloquinone were detected in fat-free yogurt. Low-fat kefir (n = 4) contained 10.2 ± 0.3 μg total vitamin K/100 g, of which only MK9 and MK11 were detected.

Additional dairy products were examined including cottage cheese, cheddar cheese, and cream (full-fat and reduced-fat) and were found to have unique distributions of vitamin K forms (Table 3). Four-percent-fat cottage cheese contained significantly higher MK8, MK9, and MK11 concentrations than reduced-fat cottage cheese. Reduced-fat cheddar cheese contained only 17% of total vitamin K content when compared with full-fat cheddar cheese. MK9, MK10, and MK11 concentrations in reduced-fat cheddar cheeses were lower by 87.1%, 96.5%, and 61.4%, respectively, compared with full-fat cheddar cheeses. Reduced-fat cream products showed less vitamin K content overall.

**TABLE 2**  Vitamin K contents of regular and Greek yogurt by fat content

<table>
<thead>
<tr>
<th></th>
<th>Regular yogurt</th>
<th>Greek yogurt</th>
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<tbody>
<tr>
<td></td>
<td>Full-fat (n = 9)</td>
<td>Fat-free (n = 7)</td>
</tr>
<tr>
<td>Vitamin K, μg/100 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phylloquinone</td>
<td>0.4 ± 0.1*</td>
<td>ND</td>
</tr>
<tr>
<td>MK4</td>
<td>0.7 ± 0.3</td>
<td>ND</td>
</tr>
<tr>
<td>MK5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MK6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MK7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MK8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MK9</td>
<td>13.2 ± 4.8*</td>
<td>ND</td>
</tr>
<tr>
<td>MK10</td>
<td>1.6 ± 0.6*</td>
<td>ND</td>
</tr>
<tr>
<td>MK11</td>
<td>8.4 ± 0.8*</td>
<td>ND</td>
</tr>
<tr>
<td>MK12</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MK13</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total</td>
<td>26.3 ± 6.4*</td>
<td>ND</td>
</tr>
<tr>
<td>Fat content, %</td>
<td>4.6 ± 0.5*</td>
<td>0.0</td>
</tr>
</tbody>
</table>

1Values are means ± SEMs. Concentrations were below the LLOD with the use of an LC-MS assay (LLOD: phylloquinone = 0.2, MK4 = 0.2, MK5 = 0.4, MK6–9 = 0.6, MK10 = 0.1, MK11 = 0.7, and MK12–13 = 0.8 μg/100 g). *Different from fat-free, P < 0.05. LLOD, lower limit of detection; MK, menaquinone; ND, not detectable.

2Sum of phylloquinone and MK4–MK13.

**Discussion**

Current dietary guidelines recommend a diet containing high-quality dairy foods (17). Dairy does not contain appreciable amounts of phylloquinone; hence, dairy has not historically been considered a rich dietary source of vitamin K. However, our data indicate that US dairy products are a good dietary source of menaquinone. MK9 was the major form quantified in the dairy samples, which is consistent with the findings of others (18, 19). However, through use of a sensitive LC-MS assay (14), we were able to extend that analysis to include measurement of MK11–MK13, and our data indicated 5- to 10-fold higher MK9 and MK10 contents in dairy products than previously reported (19), albeit in different dairy products and, in particular, artisan cheeses. We do not currently have an explanation for the higher concentrations reported here.

The large diversity of vitamin K forms among dairy products may be related to the microbial species used in the production of fermented dairy products. Menaquinone are synthesized by bacteria, including many found in fermented foods. In particular, lactic acid bacteria (LAB) are widely used in dairy and fermented-food industries (20, 21). LAB include a large number of cocci and bacilli, such as species of the genera *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* (22). Most of the cheese products contained LAB species as starters, which are reported to be the source of various menaquinone forms (23). *Staphylococcus*, *Hafnia*, and *Arthrobacter* and other bacteria that are used in the surface ripening of certain cheeses may be the reason for their corresponding high menaquinone values (2). However, the presence of menaquinones in nonfermented products such as milk is largely unexplained and could relate to the microbial content of the highly specialized ruminant digestive system (24). Kefir and yogurt have a short fermentation time, which may explain their low menaquinone content. Further investigation of the microbial composition of the different fermented dairy products is needed to interpret the diversity of menaquinone forms.
Our study shows that dairy products are a significant source of menaquinones and that the menaquinone content varies by fat content of the dairy product. This differs from the conclusions of Manoury et al. (19), who did not find an overall association between MK9 content and fat content of fermented dairy products. Whereas the latter study conducted a single correlation analysis between MK9 content and fat content across all fermented dairy products, we compared the individual MK contents with different contents of fat within the same dairy product, which may explain why we were able to detect a consistent reduction in menaquinone content with reduction in fat content. Currently, reduced-fat milk and yogurt are the most commonly consumed dairy products in the United States. In 2015, whole-fat milk accounted for 33% of milk sales, with the remaining 67% of milk purchased as reduced-fat milk (2%-fat, 1%-fat, and nonfat) (25). There is a recent trend of increasing full-fat milk and cheese consumption (25, 26), fueled by recent evidence that individuals who consume full-fat dairy products (measured by plasma dairy fat biomarkers) had a 43% lower risk of developing diabetes over the course of 15 y compared with persons who opted for low-fat dairy products (27, 28). Moreover, they found that greater intakes of high-fat dairy products, but not low-fat dairy products, were associated with less weight gain in the Women’s Health Study (29). The nutrient components contributing to these beneficial effects have yet to be identified, and our observations suggest that menaquinones warrant consideration.

Although menaquinone bioavailability has not, as we know, been studied with the use of stable isotopes, bacterially produced MK7 isolated from a food source (natto, a fermented soybean product) can be absorbed and is attributed to multiple health benefits, including bone and cardiovascular health (30, 31). More recently, studies have shown that the consumption of dairy products fortified with individual menaquinone forms are absorbed and may have greater bioactivity than menaquinones delivered in supplement form (30, 31). However, current understanding of fat-soluble menaquinone absorption, transport, and bioactivity is limited. As reviewed elsewhere (32), most menaquinone forms are not normally detected in circulation unless administered in supplement form. As our data indicate, menaquinone forms are more abundant in commonly consumed foods in the US diet than previously recognized. It is critical that a more complete understanding of menaquinone absorption and transport be developed in order to refine dietary recommendations for this nutrient. Collectively, these data highlight major gaps that still exist in our understanding of the role of menaquinone forms in vitamin K metabolism and its contribution to human health.

Our study was limited by the reliance on food labels for fat content instead of direct measurement of fat content. Whereas the samples obtained from the National Food and Nutrition Analysis Program were geographically representative of the US diet, those purchased in the Boston region were not. However, those purchased locally were selected from retail outlets that had national representation. Strengths of the study included the use of a highly sensitive and validated LC-MS method to quantify all menaquinone forms in a variety of dairy product types and direct comparison of full-fat and reduced-fat dairy products of the same brand.
and food type. Future studies are needed to compare the relative bioavailability and contribution of these individual menaquinones to health outcomes.

In summary, our results show that commonly consumed dairy products in the US diet contain appreciable amounts of multiple vitamin K forms that are directly related to fat content. Additional research is necessary to determine the role of microbes used in the production of dairy products, and their impact on menaquinone content. There is also a need to determine the relative bioavailability of all menaquinone forms given their abundance in the US diet.

Acknowledgments

The authors’ responsibilities were as follows—XF and XS: conducted the research; XF and SGH: analyzed the data and wrote the manuscript; XF, SGH, DBH, JPK, BEW, and SLB: reviewed the data, aided in the interpretation of results, and reviewed the manuscript; SLB: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

References