

Vitamin K Contents of Meat, Dairy, and Fast Food in the U.S. Diet

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The purpose of this study was to determine the contents of three forms of vitamin K [phylloquinone, dihydrophylloquinone, and menaquinone-4 (MK-4)] in representative samples (including different samples within the same food category) of meat ($n = 128$), dairy and eggs ($n = 24$), and fast foods ($n = 169$) common to the U.S. diet. The findings of our analysis indicate that no single food item in these categories is a rich dietary source of any one form of vitamin K. However, these foods are often consumed in large quantities; hence, they may be of importance in overall contribution to total vitamin K intake. The presence of MK-4 in meat, eggs, and dairy foods could be important as physiologic functions unique to MK-4 are identified.

KEYWORDS: Vitamin K; phylloquinone; menaquinone-4; meat; dairy; fast food; food composition

INTRODUCTION

Vitamin K is a fat-soluble vitamin found in both animal and vegetable foods, with the highest concentrations available in dark leafy greens (1). Dietary vitamin K exists as three major forms: phylloquinone (K₁), dihydrophylloquinone (dK), and menaquinones (MK- n). Phylloquinone, the predominant dietary form of vitamin K, is synthesized in plants, while dihydrophylloquinone is formed during the commercial hydrogenation of plant oils (2). Menaquinones are synthesized by bacteria, but there is also evidence that menaquinone-4 is synthesized from either dietary phylloquinone or menadione in certain animal tissues (3, 4).

Vitamin K acts as a cofactor for the carboxylation of certain glutamic acid residues in specific vitamin K-dependent proteins, to form γ -carboxyglutamic acid (Gla). The carboxylation of vitamin K-dependent proteins is known to be involved in hemostasis, bone metabolism, and cellular growth (5). Although the precise level of biological activity of menaquinones as compared to phylloquinone is still unknown, menaquinones do exhibit some vitamin K activity in both *in vitro* and animal systems (3). The tissue-specific abundance of MK-4 in specific animal organs (4), including the brain (6), suggests that MK-4 has physiologic functions that are not yet known.

With the discovery of new roles for different forms of vitamin K, it is important to quantify their contents in a variety of foods.

Although comprehensive databases for phylloquinone content of foods now exist, few studies have reported menaquinone content (7–9) and none have used a systematic sampling plan to obtain representative foods. Although meats contain relatively low amounts of phylloquinone (1), they may be a significant source of MK-4, as suggested by others (7–9). The purpose of this study was to determine MK-4 contents of representative samples of meat, dairy, eggs, and fast foods with corresponding phylloquinone and dihydrophylloquinone values, thereby providing the first comprehensive assessment of different forms of vitamin K among these common foods in the U.S. diet.

MATERIALS AND METHODS

The food samples used in this analysis were obtained from the U.S. Department of Agriculture Nutrient Data Laboratories as part of the National Food and Nutrient Analysis Program (NFNAP) (10, 11). Samples representative of foods consumed in the United States were purchased from retail outlets or fast food restaurants in 12 or 24 cities, respectively, around the country. The samples were shipped overnight to the Food Analysis Laboratory Control Center at Virginia Polytechnic Institute and State University (Blacksburg, VA) for preparation and processing. Ground beef, ham, bacon, liver, and pork were shipped to the Meat and Muscle Biology Lab at the University of Wisconsin (Madison, WI) for preparation and processing. In most cases, national or regional composites were prepared. In the case of some of the popular fast food burgers, regional composites were prepared. Aliquots of the composited, homogenized samples were then shipped frozen to the Vitamin K Laboratory at Tufts University (Boston, MA) and stored at $-80\text{ }^{\circ}\text{C}$ until they were assayed.

All solvents used in sample extraction and chromatography were of high-performance liquid chromatography (HPLC) grade (Fisher Scientific, Pittsburgh, PA). Phylloquinone (K₁), menaquinone-4 (MK-4),

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zinc chloride, and sodium acetate were purchased from Sigma Chemical Co. (St. Louis, MO); zinc (−200 mesh) was purchased from Johnson Matthey Electronics (Ward Hill, MA); purified 2',3'-dihydroxyphylloquinone (dK) was a gift from J. Pyrek, University of Kentucky Mass Spectrometry Facility; and the internal standard, K₁₍₂₅₎, was purchased from GLSynthesis Inc. (Worcester, MA). Primary and secondary stock solutions of standards were diluted to known concentrations in methanol for HPLC and in hexane for gas chromatography/mass spectrometry (GC/MS) and were characterized spectrophotometrically and chromatographically. Yellow light was used during extraction, purification, and analysis because vitamin K is sensitive to photooxidation.

The K₁, dK, and MK-4 contents of the meat samples were analyzed using a modified version of a reversed phase HPLC method described elsewhere (11, 12). Each NFAP sample was analyzed in duplicate. Samples were repeated if the coefficient of variance of duplicates was greater than 15% (except in samples with K₁, dK, or MK-4 concentrations of <5 µg/100 g of sample) or if a control sample (baby food, chicken, and vegetable flavor) for each batch of foods was outside the standard deviation of the established mean. A compilation of results from this control sample over a 6 month period gave a mean ± standard deviation (SD) result of 2.6 ± 0.3 µg/100 g for K₁ and 6.5 ± 0.5 µg/100 g for MK-4. Over this same period, the average within-run coefficient of variation for this sample was 8.3% for K₁ and 6.7% for MK-4. This method has a limit of detection of 14 pg per injection (equivalent to 0.06 µg/100 g of sample); however, concentrations less than 0.2 µg/100 g were reported as not detectable (ND).

The sample (0.1–0.2 g) was weighed directly into a 50 mL polypropylene centrifuge tube. Ten milliliters of water and an appropriate amount (equivalent to the approximate amount of phylloquinone projected for each sample) of internal standard were added, followed by 15 mL of 2-propanol:hexane (3:2 v/v). The mixture was vortexed for 3 min and then further dispersed by sonication (continuous output at 50% duty cycle, output control 4 for 60 s) using a Branson model 350 Sonifier Cell Disruptor with a 1/8 in. tapered microtip (Branson Ultra Sonics Corp., Danbury, CT). Finally, the samples were vortexed for another 3 min. Phase separation was achieved by centrifugation at 1800g for 5 min. The upper (hexane) phase was aspirated into a glass culture tube and evaporated to dryness under reduced pressure in a centrifugal evaporator (Savant Instruments, Farmingdale, NY, model Speed Vac SC210A). The residues were reconstituted with hexane. All hexane solutions were further processed by solid-phase extraction (SPE) on 500 mg Bond Elut silica columns (Varian Inc., Walnut Creek, CA). Each column was preconditioned by washing with 4 mL of hexane:diethyl ether (96.5:3.5 v/v) followed by 4 mL of hexane. After the sample was applied to the column, it was washed with 4 mL of hexane. The fraction that contained phylloquinone was eluted with an 8 mL wash of hexane:diethyl ether (96.5:3.5 v/v). The eluate was evaporated to dryness in the centrifugal evaporator. If, after silica SPE and drying, the residue appeared oily, C₁₈ SPE was performed to further purify the sample, as previously described (11). The final residue was reconstituted initially with 30 µL of methylene chloride followed by 170 µL of methanol with 10 mM ZnCl₂, 5 mM acetic acid, and 5 mM sodium acetate (5.5 mL of an aqueous solution of 2.0 M ZnCl₂, 1.0 M acetic acid, and 1.0 M sodium acetate was added to methanol to give a final volume of 1.0 L). The reconstituted residues were transferred to amber sample vials with glass inserts (300 µL) (manufacturing by Chromacol Ltd., distributed by SUN-SRI, Duluth, GA) and sealed with crimp caps. All vials were centrifuged for 5 min at 1800g before they were placed on the HPLC instrument.

The chromatographic system used to determine concentrations of MK-4, K₁, and dK in foods consisted of a 2695 Separations Module (Waters, Milford, MA) equipped with a vacuum degasser and a model RF-10AXL Shimadzu Fluorescence Detector (Shimadzu Instruments, Columbia, MD). The analytical column (150 mm × 3 mm) was packed with 5 µm BDS Hypersil C₁₈ (Keystone Scientific, Bellefonte, PA). Fluorescent derivatives of the injected quinones were produced on-line using a postcolumn reactor (2.0 mm × 50 mm) dry packed with zinc (−200 mesh). The excitation wavelength was 244 nm, and the emission wavelength was 430 nm. The mobile phase consisted of two solvents. Solvent A was methanol with 10 mM ZnCl₂, 5 mM acetic acid, and 5 mM sodium acetate prepared as described above. Solvent

B was methylene chloride. The 2695 was programmed to do the following gradient elution procedure: (i) pump a 90:10 (A:B) mixture at 0.60 mL/min from injection for the first 11.50 min; (ii) at 11.50 min, change the flow rate to 0.80 mL/min and the composition to 70:30 (A:B); (iii) at 19.50 min, change the composition to 90:10 (A:B); (iv) at 23.50 min, change the flow rate to 0.60 mL/min; and (v) at 24.0 min, end the cycle. This gradient elution procedure removed late-eluting lipophilic compounds that could interfere with subsequent sample analysis.

Standard curves were prepared from each calibrator injection. The fluorescence responses for MK-4, K₁, dK, and K₁₍₂₅₎ were linear with the slope of the lines bisecting zero. Therefore, we routinely performed single-point calibration, forcing the slope of the line through zero. Quantitation was achieved by direct comparison of peak area ratios [MK-4, K₁, or dK to K₁₍₂₅₎] generated from the calibration standard to those generated by the sample. Peak integration and sample concentration calculations were performed using Waters Millennium³² software, version 3.20.

To confirm that peaks corresponding to retention times of MK-4 were authentic, GC/MS was used to compare mass spectra of authentic MK-4 standard with HPLC fractions collected from chicken tender extracts. GC/MS confirmation of the peaks corresponding to K₁ and dK was previously described (2, 12). Chicken tender samples (*n* = 3) were prepared for quantitative HPLC, and fractions were collected from successive injections of the sample corresponding to the retention times of MK-4 and pooled. Pooled fractions containing the putative MK-4 were stored in hexane at −70 °C and protected from light until injection on the GC/MS. For injection, the pooled fraction was evaporated to dryness and brought to a concentration of approximately 100 µg MK-4/L of sample in hexane. The GC/MS was equipped with an Autoinjector (Agilent 6890 and 7683, Wilmington, DE).

Five microliters of the sample was injected using cool on-column technique into a deactivated 0.53 mm fused silica column, which was connected by a zero-dead volume connector to a 5% phenyl polysiloxane carborane, HT5 column (30 m, i.d. 0.25 mm, film thickness 0.1 µm; SGE, Austin, TX). The temperature was programmed from 50 to 300 °C at 30 °C/min and from 300 to 380 °C at 10 °C/min. Helium was used as a carrier gas. Methane negative chemical ionization was used to ionize MK-4, and the temperature of the ion source was 200 °C. The data were analyzed with MSD ChemStation software (Agilent, version G1701DA).

RESULTS AND DISCUSSION

To the best of our knowledge, this is the first report of the MK-4 contents of representative meat (*n* = 128), dairy (*n* = 24), and fast food (*n* = 169) samples from the U.S. food supply (Tables 1–3, respectively). Although the K₁ and dK contents of hot dogs, tuna, cheese, shakes, and certain fast foods were previously reported (13, 14), data for these forms of vitamin K are being presented again here for comparative purposes because MK-4 was determined in these same foods and in a larger sample size. The MK-4 standard analyzed by GC/MS produced a molecular ion at *m/z* 445. The extracts of chicken tenders produced mass spectra consistent with spectra of the MK-4 standard, which provided confirmation that the peaks corresponding to retention times of MK-4 in food samples analyzed were authentic MK-4.

In this study of representative U.S. foods, meat and dairy foods contained moderate amounts of MK-4. Chicken (Tables 1 and 3), cheddar cheese, and egg yolks (Table 2) contained the highest amounts of MK-4 in this study, with mean ranges of 6.3–22.1 µg/100 g of MK-4. Neither fat-free chicken broth nor chicken hot dogs contained any MK-4. Menadione is a common source of vitamin K in chicken feed, and it is assumed that the MK-4 present in the chicken products is formed from alkylation of menadione to MK-4. Alternatively, as suggested by Will et al. (15), chicks convert phylloquinone to MK-4. Meat and dairy foods, but not fast foods, generally contained more

Table 1. Vitamin K Contents of Meat

food	n	vitamin K contents ($\mu\text{g}/100\text{ g}$)								
		K ₁			dK			MK-4		
		mean	SD ^a	range ^b	mean	SD ^a	range ^b	mean	SD ^a	range ^b
beef, ground										
raw ground beef (low fat)	4	0.9	0.3	0.5–1.0	ND ^c			4.9	2.4	2.6–8.0
Raw ground beef (medium fat)	4	1.3	1.0	0.5–2.6	ND			8.1 ^d		7.6–8.6
raw ground beef (high fat)	4	2.4	0.5	2.1–3.1	ND			7.4 ^e	1.6	6.4–9.3
broiled ground beef (low fat)	4	1.2	1.0	0.5–2.6	ND			1.7	0.6	1.1–2.6
broiled ground beef (medium fat)	4	1.4	1.1	0.6–3.0	ND			7.2	1.5	5.5–8.6
broiled ground beef (high fat)	4	2.1	1.1	1.2–3.7	ND			5.1	2.6	1.9–8.2
beef, liver										
raw beef liver	4	3.1	1.4	1.9–4.6	ND			0.4	0.4	ND–0.9
pan-fried beef liver	4	3.9	2.5	2.0–7.5	ND			0.4 ^e	0.1	0.3–0.4
braised beef liver	4	3.3	1.8	1.7–5.8	ND			1.9	2.5	0.4–5.7
raw calf liver	2	1.0		0.8–1.1	ND			5.0		1.1–8.9
pan-fried calf liver	2	2.1		1.7–2.5	ND			6.0		5.9–6.1
braised calf liver	1	1.1			ND			1.1		
beef, hot dogs, regular fat	4	4.3	3.0	1.0–8.3	ND			5.7	0.5	5.0–6.1
(uncooked and cooked) ^f										
ham (with water or natural juices;	8	0.0	0.0	ND–0.1	ND			5.1	2.6	1.9–9.9
roasted and pan-broiled) ^f										
bacon (raw, pan-fried, microwaved,	15	ND	0.1	ND–0.4	ND			5.6	1.3	2.9–7.8
cooked, and baked) ^f										
pork, loin (raw, broiled, pan-broiled,	17	ND			ND			0.9	0.6	0.2–2.0
and braised) ^f										
meat franks, regular fat	4	2.5	3.8	0.5–8.1	ND			9.8	1.2	8.6–10.8
(uncooked and cooked) ^f										
chicken, liver										
raw chicken liver	4	ND			ND			14.1	2.0	12.8–17.1
pan-fried chicken liver	4	ND			ND			12.6	2.9	9.7–16.1
braised chicken liver	4	ND			ND			6.7 ^e	105	5.0–7.7
chicken, barbeque (uncooked	10	2.0	0.5	1.1–2.8	0.9	0.5	ND–1.7	22.1	6.2	13.6–31.6
and cooked) ^f										
chicken, hot dogs, regular fat	3	0.2	0.0	ND–0.2	ND			<i>g</i>		
(uncooked and cooked) ^f										
chicken, broth (99% fat free)	1	ND			ND			<i>g</i>		
crab (canned)	1	ND			ND			ND		
halibut, raw, Alaska wild	1	ND			ND			ND		
orange roughy fillets (raw	5	0.7	0.5	ND–1.3	ND			ND		
and baked) ^f										
shrimp (cooked and canned)	1	ND			ND			0.2		
salmon, raw, Alaska wild (Coho,	4	0.3	0.1	0.2–0.3	ND			0.3	0.0	0.2–0.3
Sockeye, Chum, and King)										
tilapia fillets (raw and baked) ^f	5	1.2	0.9	0.6–2.7	ND			ND		
tuna, light and packed in	3	2.3	3.7	0.1–6.6	ND			<i>g</i>		
water (canned)										

^a Standard deviation; no SD reported for analyses of ≤ 2 samples. ^b No range reported when values are equal. ^c Not detectable. ^d $n = 2$. ^e $n = 3$. ^f Cooking method did not influence content. ^g Data not available.

MK-4 than phylloquinone. This observation is consistent with previous findings, which reported higher levels of MK-4 as compared to phylloquinone in various meats, fish, milk, eggs, soft cheeses (9, 16), and animal organs (7). In one study from The Netherlands (9), hard cheeses contained more phylloquinone than MK-4, which is the opposite of what we report in this study. These differences may reflect differences in animal husbandry practices and/or the types of cheeses analyzed in the two studies. Likewise, the absolute amount of MK-4 and phylloquinone varies with species, as demonstrated by Indyk et al (17) in an interspecies comparison of the vitamin K content of raw milk. Depending on the biological activity of MK-4, intake of animal products could have an impact on vitamin K status, since meats and dairy are consumed in large quantities in the United States. A recent observational study from The Netherlands (18) indicated that total menaquinone intakes, including MK-4, of $> 33 \mu\text{g}/\text{day}$ conferred a protective effect on the incidence of coronary heart disease. However, the associations between MK-4 intakes and physiological outcomes are still relatively unknown. Furthermore, there are few studies

assessing usual MK-4 intakes, primarily because of the paucity of available MK-4 food composition data.

Although the precise level of biological activity of menaquinones as compared to phylloquinone is still unknown, menaquinones do exhibit some vitamin K activity in both in vitro and animal systems (3). It has been shown in animal models that vitamin K deficiency leads to dysfunctional sphingolipid metabolism (19, 20). MK-4 is the main form of vitamin K in the brain (21), which suggests that MK-4 may play a particular role in the function of sphingolipids. MK-4 may also have the potential to maintain or increase bone mineral density in osteoporotic women when administered in pharmacological doses (22). Any protective role of high dietary intakes of MK-4 on age-related bone loss is currently unknown. Long-chain menaquinones (MK-6 to MK-10) have been reported in select food items, such as certain cheese types and animal livers (8, 9, 23). However, it was beyond the scope of this study to measure menaquinones other than MK-4.

Consistent with previous literature (1, 8, 9, 23, 24), meat and dairy foods contained low levels of phylloquinone, ranging from

Table 2. Vitamin K Contents of Dairy Foods and Eggs

food	n	vitamin K contents ($\mu\text{g}/100\text{ g}$)								
		K ₁			dK			MK-4		
		mean	SD ^a	range ^b	mean	SD ^a	range ^b	mean	SD ^a	range ^b
milk										
1% milk	2	ND ^c			ND			0.4		0.3–0.4
2% milk (regular and chocolate) ^d	6	0.2	0.1	ND–0.3	ND			0.5	0.1	0.4–0.5
whole milk	4	0.3	0.0		ND			1.0	0.1	0.8–1.0
ice cream										
ice cream, regular fat (vanilla and chocolate) ^d	2	1.5		1.1–1.9	ND			2.6		2.4–2.8
cheese										
cheddar cheese	1	3.0			ND			10.2		
Swiss cheese	3	2.5	0.1	2.4–2.5	ND			7.8	1.4	6.2–8.8
mozzarella cheese (low moisture, part skim, shredded)	2	1.3		1.0–1.5	ND			3.6		3.1–4.0
eggs										
eggs, white, fresh, raw	1	ND			ND			0.4		
eggs, yolk, fresh, raw	1	0.7			ND			15.5		
whole eggs, fresh	1	0.3			ND			5.6		
whole eggs, fried	1	0.9			ND			9.0		
whole eggs, hard-cooked	1	0.4			ND			7.0		

^a Standard deviation; no SD reported for analyses of ≤ 2 samples. ^b No range reported when values are equal. ^c Not detectable. ^d Flavor did not influence the content of any form of vitamin K.

Table 3. Vitamin K Contents of Fast Foods

food	n	vitamin K contents ($\mu\text{g}/100\text{ g}$)								
		K ₁			dK			MK-4		
		mean	SD ^a	range ^b	mean	SD ^a	range ^b	mean	SD ^a	range ^b
hamburgers										
hamburger (2–4 oz)	17	5.9	2.2	1.1–10.1	ND ^c	0.6	ND–1.5	2.3	0.9	1.2–4.4
hamburger with cheese (2–4 oz)	5	6.0	2.1	4.2–9.3	ND	0.3	ND–0.7	2.9	1.6	1.5–5.6
hamburger with sauce (2–4 oz)	6	19.3	3.7	14.1–23.4	0.2	0.4	ND–1.0	1.4	0.5	1.1–2.4
hamburger with cheese and sauce (>4 oz)	4	13.5	5.4	6.9–19.1	ND	0.2	ND–0.3	2.3 ^d	1.3	1.4–3.8
sandwiches										
chicken sandwich	6	15.1	8.0	4.6–23.7	2.4	3.0	ND–7.7	2.7	1.3	1.1–4.7
fish sandwich	2	13.7		4.9–22.5	4.6		ND–9.1	0.3		0.2–0.3
chicken										
chicken nuggets	11	8.4	3.6	2.3–13.6	15.9	6.4	4.7–25.1	10.6 ^e	1.4	8.1–13.4
chicken tenders	8	7.9	6.4	3.8–23.5	20.1	16.7	ND–35.3	6.3	0.8	5.5–7.6
burritos										
burrito with bean	4	4.2	0.9	3.0–5.1	5.1	1.4	3.7–6.4	0.6	0.3	0.3–1.0
burrito with beef	8	5.7	1.0	4.7–7.8	4.2	1.6	2.6–6.8	0.9	0.2	0.8–1.4
burrito with chicken	4	5.3	0.5	4.8–5.9	3.8	1.0	2.6–4.7	2.7	0.6	2.0–3.4
tacos										
taco	4	15.4	4.1	9.8–19.7	6.6	2.7	4.1–9.9	1.4	0.3	1.1–1.9
taco with beef	8	16.0	5.4	9.3–22.4	4.5	1.0	3.5–5.9	1.0 ^f	0.3	0.7–1.7
taco with chicken	4	8.8	1.6	7.7–11.1	4.4	0.6	3.8–5.2	4.5	1.3	3.0–6.0
fast food pizza ^g										
cheese (regular, thin, and thick crust)	39	8.9	4.1	4.7–21.9	1.5	3.6	ND–13.2	1.8	0.7	0.6–3.3
pepperoni (regular, thin, and thick crust)	25	8.6	5.0	4.9–21.2	0.5	1.2	ND–4.0	2.1	0.3	0.8–4.1
meat and vegetable (regular, thin, and thick crust)	15	6.0	1.0	4.6–8.6	ND			1.9	0.3	1.3–2.4
shakes, chocolate and vanilla ^h	3	0.6	0.4	0.4–1.1	ND			3.4 ⁱ		2.3–4.4

^a Standard deviation; no SD reported for analyses of ≤ 2 samples. ^b No range reported when values are equal. ^c Not detectable. ^d $n = 3$. ^e $n = 10$. ^f $n = 7$. ^g The type of crust did not influence the content of any form of vitamin K. ^h Flavor did not influence the content of any form of vitamin K. ⁱ $n = 2$.

nondetectable levels in chicken liver, pork loin, some fish, and egg whites to $4.3\ \mu\text{g}/100\text{ g}$ in beef hot dogs. The phylloquinone content of meat and dairy foods is much lower than that of green leafy vegetables (1, 9, 23, 25). As reported earlier (13), fast foods, including hamburgers, tacos, fast food pizza, and fish and chicken sandwiches, contained higher amounts of phylloquinone than other meats and dairy foods, with concentrations

that ranged from 5.9 to $19.3\ \mu\text{g}/100\text{ g}$. The discrepancy in phylloquinone concentrations between store-bought meats and fast food meats suggests that the phylloquinone concentrations in the latter can be attributed to another source, primarily the plant oils (11) in which they were cooked or the condiments that were added to the fast food, such as lettuce, tomatoes, and sauce (25).

Overall, dihydrophyloquinone was ND in samples of meat and dairy foods but was present in low levels in most fast foods. Higher levels of dihydrophyloquinone were found in chicken nuggets and tenders, which contained 15.9 and 20 μg dK/100 g, respectively. Because dihydrophyloquinone is formed during the hydrogenation of plant oils (2), the partially hydrogenated oils used to cook fast foods probably account for these levels of dihydrophyloquinone. Dietary intake of dihydrophyloquinone from fast foods may contribute to vitamin K status in the U.S. population, depending on the biological activity of dihydrophyloquinone. There are limited data available regarding the biological activity of dihydrophyloquinone, although one human study suggests that it may not be equivalent to that of the parent form, phyloquinone (26).

The findings of our analysis of different forms of vitamin K in a variety of meats and dairy foods present in the U.S. food supply indicate that no single food item in these categories is a rich dietary source of vitamin K. However, these foods are often consumed in large quantities (27, 28); hence, they will be of importance in overall contribution to total vitamin K intake. The presence of MK-4 in meat and dairy foods could be important as physiologic functions unique to MK-4 are identified.

ACKNOWLEDGMENT

We express our appreciation to Greg Dolnikowski, Alison Tovar, Kristine Shedd, Reena Kanabar, and Dan Ferreira for their technical contributions.

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Received for review September 27, 2005. Revised manuscript received November 29, 2005. Accepted December 1, 2005. This material is based upon work supported by the Helmut Schumann Fund (S.J.E.), and federal funds from the U.S. Department of Agriculture, Agricultural Research Service, under Cooperative Agreement No. 58-1950-001 and the National Heart Lung and Blood Institute, National Institutes of Health, Agreement No. Y1-HV-8116-11. The Helmut Schumann Fund is thanked for support of S.J.E., and America's Beef Producers are thanked for support of the ground beef study. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.