

Dietary intake of vitamin K and risk of prostate cancer in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Heidelberg)¹⁻³

Katharina Nimptsch, Sabine Rohrmann, and Jakob Linseisen

ABSTRACT

Background: Anticarcinogenic activities of vitamin K have been observed in various cancer cell lines, including prostate cancer cells. Epidemiologic studies linking dietary intake of vitamin K with the development of prostate cancer have not yet been conducted.

Objective: We evaluated the association between dietary intake of phyloquinone (vitamin K₁) and menaquinones (vitamin K₂) and total and advanced prostate cancer in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition.

Design: At baseline, habitual dietary intake was assessed by means of a food-frequency questionnaire. Dietary intake of phyloquinone and menaquinones (MK-4–14) was estimated by using previously published HPLC-based food-content data. Multivariate-adjusted relative risks of total and advanced prostate cancer in relation to intakes of phyloquinone and menaquinones were calculated in 11 319 men by means of Cox proportional hazards regression.

Results: During a mean follow-up time of 8.6 y, 268 incident cases of prostate cancer, including 113 advanced cases, were identified. We observed a nonsignificant inverse association between total prostate cancer and total menaquinone intake [multivariate relative risk (highest compared with lowest quartile): 0.65; 95% CI: 0.39, 1.06]. The association was stronger for advanced prostate cancer (0.37; 0.16, 0.88; *P* for trend = 0.03). Menaquinones from dairy products had a stronger inverse association with advanced prostate cancer than did menaquinones from meat. Phyloquinone intake was unrelated to prostate cancer incidence (1.02; 0.70, 1.48).

Conclusions: Our results suggest an inverse association between the intake of menaquinones, but not that of phyloquinone, and prostate cancer. Further studies of dietary vitamin K and prostate cancer are warranted. *Am J Clin Nutr* 2008;87:985–92.

INTRODUCTION

Fat-soluble vitamin K evolves its essential function as a co-factor for the posttranslational γ -carboxylation of glutamate residues in vitamin K–dependent proteins (1). The term vitamin K refers to a group of compounds that have a 2-methyl-1,4-naphthoquinone ring in common but that differ in the length and structure of their isoprenoid side chain at the 3-position. The 2 forms of vitamin K that occur naturally in foods are phyloquinone (vitamin K₁) and the group of menaquinones (vitamin K₂, MK-n), which vary in the number of prenyl units. Whereas phyloquinone is abundant in green leafy vegetables and some vegetable oils, menaquinones are synthesized by bacteria; therefore, they mainly occur in fermented products such as cheese. Meat

and meat products also form a relevant source of menaquinones, especially MK-4, which is synthesized in the animals from either phyloquinone or the synthetic menadione (vitamin K₃) commonly added to animal foods (2). Because of the quinone structure, which poses as functional unit of several chemotherapeutics in cancer therapy, vitamin K has gained importance in the prevention and treatment of cancer (3). The synthetic menadione (vitamin K₃) inhibits carcinogenic cell growth, especially in combination with vitamin C, by oxidative processes leading to oxidative stress and depletion of cellular thiols (4, 5). Phyloquinone and menaquinones exert growth-inhibitory effects on cancer cells by acting as transcription factors of proto-oncogenes such as *c-myc*, *c-fos*, or *c-jun*, which foster cell-cycle arrest and apoptosis (6, 7). Antitumor activities of phyloquinone and menaquinones have been observed in various cancer cell lines, including liver, lung, stomach, and breast (5–11); in the case of menaquinones, several studies worked with MK-4 (5, 7–9) and one study worked with MK-1–3 (10), whereas others did not specify the type (6, 11). In a randomized trial of 43 women with viral cirrhosis of the liver, mega-doses of menaquinones (45 mg/d; type not specified) decreased the risk of hepatocellular carcinoma by \approx 80% compared with the control group (12). Concerning prostate cancer, menadione has been shown to reduce tumor growth rate in vitro (13) and in vivo (14), whereas K vitamins naturally occurring in the human diet were not investigated. To the best of our knowledge, epidemiologic studies linking the dietary intake of vitamin K with the development of prostate cancer have not been conducted so far. Here, we examine the hypothesis that dietary vitamin K intake is inversely associated with the incidence of prostate cancer in a prospective cohort study.

¹ From the Division of Cancer Epidemiology, German Cancer Research Centre, Heidelberg, Germany.

² Supported by contract no. 513943 from Environmental Cancer Risk, Nutrition and Individual Susceptibility (ECNIS), a network of excellence operating within the European Union's 6th Framework Program, Priority 5, "Food Quality and Safety," and by Graduiertenkolleg 793 scholarship from the Deutsche Forschungsgemeinschaft (to KN).

³ Reprints not available. Address correspondence to J Linseisen, German Cancer Research Center, Division of Cancer Epidemiology, Unit of Nutritional Epidemiology, Im Neuenheimer Feld 280, DE-69120 Heidelberg, Germany. E-mail: j.linseisen@dkfz-heidelberg.de.

Received August 23, 2007.

Accepted for publication October 25, 2007.

SUBJECTS AND METHODS

Study population

The Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Heidelberg) comprises 25 540 participants, of whom 11 928 are men aged 40–65 y. Recruitment from a random sample of the general population from Heidelberg and surrounding communities took place between June 1994 and October 1998, and the overall participation rate of invited subjects was 38.3% (15). At baseline, information on dietary and nondietary factors was assessed by self-administered questionnaires and a personal interview including questions on marital status, education, occupational status, smoking history, physical activity, use of medication, and history of disease. Anthropometric measurements including participant's weight and height were taken in the study center by trained interviewers following standardized methods. Follow-up was performed actively by mailed follow-up questionnaires ascertaining new cases of various diseases. The response rates in the 3 follow-up rounds completed to date were 93.5% (1st follow-up, 1998–2000), 91.8% (2nd follow-up, 2001–2004), and 92.0% (3rd follow-up, 2004–2007). The second and third follow-ups included questions on participation in prostate-specific antigen (PSA)-screening tests.

All participants gave written informed consent. The study was approved by the ethics committee of the Heidelberg Medical School.

Dietary data

Habitual dietary intake during the previous 12 mo was assessed by using a 145-item semi-quantitative food-frequency questionnaire (FFQ) at baseline. For each food item, participants specified typical portion size and consumption frequency, ranging from 1 time/mo to 6 times/d (16). Average daily food intake was calculated from the information on portion size and frequency of consumption for each food item. Calculation of the intakes of individual nutrients was carried out by using the German Food and Nutrition Database (BLS 2.3) (17). In this table, however, food content data on vitamin K is insufficient because the data sources are not exclusively based on HPLC-measured values, which are considered as the most reliable, and no data on menaquinones (vitamin K₂) are provided. Therefore, dietary intakes of phylloquinone and menaquinones were calculated by using previously published food content data based exclusively on HPLC. For the calculation of phylloquinone intake, data [(18); also: C Bolton-Smith et al, unpublished observations, 2000] that included values for ≈2000 foods were applied. The menaquinone (MK-4–14) content of relevant foods was derived from a Dutch publication (19) and supplemented with Japanese data on offal (20). Phylloquinone and menaquinone contents were assigned to the single foods contributing to the FFQ items either by direct matching, with adjustment for fat content where necessary (as described by Bolton-Smith et al), or on the basis of food similarities. Whereas some convenience products or mixed dishes, such as soups and cakes, are listed with their phylloquinone content in the database by Bolton-Smith et al, the phylloquinone content of some other combined foods consumed in EPIC-Heidelberg was calculated by means of standard recipes. Calculation of the menaquinone contents in “mixed” food, mainly cakes, was performed by recipe calculation.

Identification of prostate cancer cases

Identification of incident prostate cancer cases was based on self-reported primary prostate cancer during follow-up or on death certificates that were coded for prostate cancer as the underlying cause of death. All identified cases of incident prostate cancer—except the 8 most recent cases—were verified by medical records, death certificates, or both. Because of the high sensitivity of self-reports of prostate cancer as observed among the verified cases, the 8 cases based only on self-reports were included in the analysis. Information on stage and grade of prostate cancer was extracted by the study physician from pathology reports (procedures or tests conducted during the initial diagnosis), including tumor nodal metastasis (TNM) stage, Gleason histologic grade, and PSA level. Advanced prostate cancer was defined as prostate cancer with a Gleason sum score of ≥ 7 ; TNM staging score of T3/T4, N1–N3, or M1; PSA level at diagnosis of ≥ 20 ng/mL; or prostate cancer as the underlying cause of death. Although prone to detection bias, stage T1a cases were included in the analysis because their low number ($n = 4$, $< 2\%$ of all cases) was unlikely to affect the results.

Statistical analyses

The analytic cohort comprised 11 319 men after exclusion of subjects with missing dietary information or prevalent cancer (except for nonmelanoma skin cancer) ($n = 955$) and those in the top and bottom 1% of energy intake (ie, < 981 or ≥ 4815 kcal; $n = 230$). Individual person-time was calculated from the date of recruitment and the date of diagnosis of prostate cancer, the date of death from other causes, or the date of the last known contact, whichever came first.

The association between intakes of phylloquinone and total menaquinones (sum of MK-4 to MK-14) and the risk of total and advanced prostate cancer was analyzed by using Cox proportional hazards regression, calculating relative risks (RRs) (and 95% CIs). For both total and advanced prostate cancer, analyses were repeated after exclusion of cases diagnosed within the first 2 y after recruitment.

For the analyses, dietary intakes of phylloquinone and menaquinones were categorized into quartiles and entered simultaneously into the models. Tests for linear trends (P for trend) were performed by modeling the median values of phylloquinone and menaquinone quartiles as continuous variables. In addition, continuous models for intakes of phylloquinone and menaquinones were calculated per 10- μg increment. All models were stratified by age (in 1-y categories). Multivariate analyses were adjusted for potential confounders, including smoking status [never smokers, current cigarette smokers (1–14 or ≥ 15 cigarettes/d), former smokers who stopped < 10 or ≥ 10 y ago, or other smokers (pipe or cigar smokers or occasional smokers)], education (none or primary school, technical school, secondary school, or university degree), vigorous physical activity (none, < 2 h/wk, or ≥ 2 h/wk), energy from fat (kcal/d, in quartiles), nonfat-alcohol energy (kcal/d, in quartiles), alcohol (≤ 4.9 , 5–14.9, 15–30, or ≥ 30 g ethanol/d), calcium (mg/d, in quartiles), vitamin D (IU/d, in quartiles), tomato or tomato products (g/d, in quartiles), body mass index (in kg/m^2 , continuous), history of diabetes, and family history of prostate cancer. Other dietary and nondietary factors were examined but not included in the model when they neither were associated with prostate cancer nor confounded the association of vitamin K intake with prostate cancer.



Multivariate analyses were repeated with additional adjustment for consumption of vegetables, dairy products (including milk, cheese, and other dairy products), and meat (g/d, in quartiles). In a second approach, the intakes of menaquinones from major food sources (dairy products and meat or meat products) were modeled separately as continuous variables, after adjustment for phyloquinone and menaquinones from other sources in the multivariate model. Finally, menaquinones were modeled separately according to the length of the isoprenoid side chain (MK-4 compared with MK-5–9, continuous). All statistical analyses were performed with SAS software (version 9.1; SAS Institute, Cary, NC).

RESULTS

During a mean follow-up time of 8.6 y (97 731 total person-years), 268 incident cases of prostate cancer occurred, including 113 advanced cases (42% of all cases). Thirty-six cases (13% of all cases) were diagnosed during the first 2 y of follow-up. Median (25–75th percentile) intakes of phyloquinone and total menaquinones (MK-4–14) were 93.6 (70.9–123.5) and 34.7 (25.7–45.7) $\mu\text{g}/\text{d}$, respectively (**Table 1**).

Dietary phyloquinone was mainly provided by vegetables, soups or bouillon, fruit, and cereals or cereal products. Green leafy vegetables, including spinach, all varieties of lettuce, cabbage, Brussels sprouts, and broccoli, contributed 42% of phyloquinone intake. The main contributors of menaquinones were dairy products, meat or meat products, and cakes. Cheese was the greatest single food source, contributing 43% of total intake of menaquinones. The relatively high contributions of soups or bouillon to phyloquinone intake and of cakes to menaquinone intake can be explained by the vitamin K content of some ingredients. Such ingredients include vegetable oils and vegetables,

which contribute to the phyloquinone content of soups or bouillon, and eggs and dairy products (eg, butter and cream), which contribute to the menaquinone content of cakes. The subgroups of menaquinones differed with respect to food sources. Whereas the main food source of MK-4 (median intake 14.4 $\mu\text{g}/\text{d}$) was meat or meat products (37% of total intake), higher menaquinones MK-5–9 (median intake: 18.0 $\mu\text{g}/\text{d}$) were almost exclusively (85% of total intake) derived from dairy products. Menaquinones above MK-9 contributed little to total menaquinone intake (median intake: 0.8 $\mu\text{g}/\text{d}$) and were provided to 86% by meat or meat products, especially offal.

The upper quartiles of phyloquinone intake were associated with greater age, whereas the upper quartiles of intake of menaquinones were associated with lower age (**Table 2**). Subjects in the upper quartiles of phyloquinone and menaquinones had a lower body mass index, were more likely to have a university degree, and were more likely to practice vigorous physical activity >2 h/wk than were subjects in the lower intake quartiles. The smoking status of the participants did not differ distinctly between quartiles of vitamin K intake. A history of diabetes was more common in subjects in the upper quartiles of phyloquinone and less common in those in the upper quartiles of menaquinones. Participation in PSA screening was more common in subjects in the upper quartiles of phyloquinone (but not menaquinone) intake. A family history of prostate cancer was not associated with intake of phyloquinone or menaquinones. Total energy intake increased across quartiles of phyloquinone and menaquinones. Consequently, dietary intake of other energy-related nutrients and food groups increased by quartiles.

As shown in **Table 3**, dietary intake of phyloquinone was not associated with the incidence of either total or advanced prostate cancer. However, intake of menaquinones was nonsignificantly and inversely related to the risk of total prostate cancer (*P* for

TABLE 1
Median intakes of dietary phyloquinone and menaquinones and the main contribution of food group consumption to total intake in EPIC-Heidelberg¹

	Intake $\mu\text{g}/\text{d}$	Sources: food group and subgroup	Total intake of the specific nutrient
			%
Phylloquinone	93.6 (70.9–123.5) ²	Vegetables	62
		Green leafy vegetables	42
		Soups, bouillon	6
		Fruits	6
		Cereals, cereal products	5
Sum of menaquinones (MK-4–14)	34.7 (25.7–45.7)	Dairy products	60
		Cheese	43
		Meat, meat products	17
		Cakes	7
MK-4	14.4 (10.9–18.7)	Meat, meat products	37
		Dairy products	16
		Cakes	14
		Egg, egg products	11
MK-5–9 ³	18.0 (11.7–27.0)	Dairy products	85
MK-10–14 ³	0.8 (0.2–1.7)	Meat, meat products	86
		Offal	67

¹ *n* = 11 319. EPIC, European Prospective Investigation into Cancer and Nutrition.

² Median; interquartile range in parentheses (all such values).

³ Individual menaquinone intakes: MK-5, 0.3 (0.2–0.5); MK-6, 0.3 (0.2–0.5); MK-7, 0.8 (0.5–1.1); MK-8, 4.6 (3.1–6.7); MK-9, 11.9 (7.4–18.4); MK-10, 0.06 (0.01–0.13); MK-11, 0.12 (0.03–0.27); MK-12, 0.20 (0.04–0.42); MK-13, 0.40 (0.08–0.85); and MK-14, 0.02 (0.00–0.05) $\mu\text{g}/\text{d}$.

TABLE 2
Baseline characteristics of men of the EPIC-Heidelberg cohort by quartile (Q) of phyloquinone and menaquinone intake¹

Baseline characteristics	Phylloquinone (µg/d)				Menaquinones (µg/d) ²				P for trend ³	P for trend ³
	Q1 (<71)	Q2 (71–94)	Q3 (94–124)	Q4 (≥124)	Q1 (<26)	Q2 (26–35)	Q3 (35–46)	Q4 (≥46)		
Age at baseline (y)	51.4 ± 7.0 ⁴	52.0 ± 7.1	52.1 ± 7.1	52.3 ± 7.1	53.4 ± 6.9	52.0 ± 7.1	51.8 ± 7.1	50.6 ± 7.1	<0.0001	<0.0001
BMI (kg/m ²)	27.1 ± 3.5	27.0 ± 3.6	27.0 ± 3.6	26.8 ± 3.9	27.1 ± 3.5	27.0 ± 3.7	26.9 ± 3.5	26.8 ± 3.9	0.01	0.001
Body weight (kg)	83.4 ± 11.7	83.4 ± 12.1	83.7 ± 12.0	83.3 ± 12.8	82.8 ± 11.5	83.4 ± 12.1	83.7 ± 11.8	84.0 ± 13.0	0.65	0.002
Body height (cm)	175 ± 6.5	176 ± 6.7	176 ± 6.7	176 ± 6.8	175 ± 6.5	176 ± 6.5	176 ± 6.7	177 ± 6.9	<0.0001	<0.0001
Educational level (%)										
None or primary school	35	30	31	29	37	32	30	26		
Technical school	28	29	27	24	30	29	25	23		
Secondary school	5	6	6	6	5	5	5	7		
University degree	32	36	37	41	29	34	40	44		<0.0001
Smoking status (%)										
Never	29	31	31	30	29	31	31	29		
Former	43	45	44	44	47	43	44	43		
Current	28	24	25	26	24	27	25	28		0.14
Vigorous physical activity (%)										
None	37	37	34	34	39	36	33	33		
<2 h/wk	38	37	39	35	36	37	39	37		
≥2 h/wk	25	26	28	31	26	27	28	29		<0.0001
History of diabetes (%)	4	5	5	6	6	5	5	4		0.002
PSA screening (%)	46	49	49	49	49	49	49	46		0.02
Family history of prostate cancer (%)	4	4	3	3	3	4	4	3		0.55
Nutrient intake										
Total energy (kcal/d)	1889 ± 528	2106 ± 555	2286 ± 631	2513 ± 728	1724 ± 423	2047 ± 476	2294 ± 537	2729 ± 703		<0.0001
Total fat (g/d)	68.5 ± 23.5	78.5 ± 25.7	87.2 ± 29.8	98.7 ± 35.5	57.6 ± 15.2	74.3 ± 18.1	88.2 ± 23.4	112.7 ± 33.6		<0.0001
Alcohol (ethanol, g/d)	26.6 ± 28.3	25.1 ± 24.6	25.6 ± 26.1	26.0 ± 27.3	25.6 ± 26.9	26.2 ± 27.3	26.1 ± 26.8	25.3 ± 25.5		0.55
Calcium (mg/d)	676 ± 324	750 ± 315	821 ± 355	914 ± 383	550 ± 204	692 ± 241	827 ± 283	1093 ± 410		<0.0001
Phosphor (mg/d)	1166 ± 352	1302 ± 360	1422 ± 406	1572 ± 465	1055 ± 275	1254 ± 300	1421 ± 328	1732 ± 452		<0.0001
Vitamin D (IU/d)	122.0 ± 86.5	137.7 ± 82.6	153.8 ± 114.4	181.5 ± 147.3	110.1 ± 80.0	137.6 ± 91.1	154.2 ± 99.2	193.1 ± 151.3		<0.0001
Food intake (g/d)										
Vegetables	70.4 ± 26.4	98.8 ± 27.9	116.9 ± 35.6	161.9 ± 67.7	98.8 ± 48.8	107.6 ± 49.3	114.9 ± 50.2	126.9 ± 63.2		<0.0001
Green leafy vegetables	12.1 ± 6.5	20.7 ± 8.5	26.8 ± 10.9	42.1 ± 22.4	23.5 ± 16.1	24.8 ± 16.1	26.0 ± 16.6	27.5 ± 20.3		<0.0001
Dairy products	209.3 ± 228.3	219.0 ± 207.7	245.5 ± 240.7	263.9 ± 249.7	158.3 ± 148.7	205.5 ± 189.0	250.3 ± 228.9	323.6 ± 303.9		<0.0001
Cheese	25.6 ± 20.1	28.3 ± 20.9	30.0 ± 22.2	33.7 ± 25.8	12.4 ± 7.3	21.1 ± 10.0	30.7 ± 12.1	53.4 ± 28.2		<0.0001
Meat, meat products	105.8 ± 61.7	117.2 ± 64.6	124.6 ± 71.3	138.9 ± 86.9	89.1 ± 45.8	111.6 ± 55.7	128.2 ± 66.7	157.5 ± 95.1		<0.0001
Tomatoes, tomato products	18.7 ± 11.7	22.6 ± 12.7	24.7 ± 15.2	29.3 ± 18.2	18.8 ± 13.4	23.1 ± 13.6	24.7 ± 13.9	28.6 ± 17.5		<0.0001

¹ EPIC, European Prospective Investigation into Cancer and Nutrition; PSA, prostate-specific antigen.

² The sum of menaquinones MK-4 to MK-14.

³ Likelihood ratio test or Jonckheere-Terpstra test.

⁴ $\bar{x} \pm SD$ (all such values).

TABLE 3
Phylloquinone and menaquinone intakes and the relative risk (RR) of prostate cancer in men of the EPIC-Heidelberg cohort¹

	Phylloquinone ($\mu\text{g/d}$)				Menaquinones ($\mu\text{g/d}$) ²				P for trend	Continuous RR ³
	Q1 (<71)	Q2 (71–94)	Q3 (94–124)	Q4 (≥ 124)	Q1 (<26)	Q2 (26–35)	Q3 (35–46)	Q4 (≥ 46)		
Total prostate cancer										
Cases (n)	66	64	65	73	91	65	62	50		
Age-stratified RR	1.00	0.94 (0.66, 1.33) ⁴	0.97 (0.69, 1.38)	1.09 (0.77, 1.54)	1.00	0.81 (0.59, 1.11)	0.79 (0.57, 1.10)	0.73 (0.51, 1.04)	0.09	0.95 (0.88, 1.03)
Multivariate RR ⁵	1.00	0.88 (0.62, 1.25)	0.91 (0.63, 1.31)	1.02 (0.70, 1.48)	1.00	0.76 (0.53, 1.08)	0.71 (0.47, 1.06)	0.65 (0.39, 1.06)	0.10	0.94 (0.84, 1.05)
Multivariate RR ⁶	1.00	0.85 (0.59, 1.25)	0.90 (0.60, 1.36)	1.02 (0.64, 1.61)	1.00	0.75 (0.52, 1.07)	0.68 (0.45, 1.04)	0.61 (0.36, 1.02)	0.07	0.93 (0.82, 1.04)
Total prostate cancer, excluding cases occurring in the first 2 y after recruitment										
Cases	56	55	55	66	79	54	54	45		
Age-stratified RR	1.00	0.96 (0.66, 1.40)	0.99 (0.68, 1.44)	1.17 (0.81, 1.70)	1.00	0.76 (0.53, 1.07)	0.78 (0.55, 1.10)	0.73 (0.50, 1.07)	0.12	0.96 (0.88, 1.04)
Multivariate RR ⁵	1.00	0.91 (0.62, 1.33)	0.92 (0.62, 1.37)	1.09 (0.73, 1.62)	1.00	0.69 (0.47, 1.01)	0.66 (0.43, 1.02)	0.61 (0.36, 1.04)	0.10	0.94 (0.84, 1.06)
Multivariate RR ⁶	1.00	0.86 (0.57, 1.29)	0.87 (0.56, 1.36)	1.02 (0.62, 1.66)	1.00	0.68 (0.46, 1.00)	0.64 (0.41, 0.99)	0.57 (0.33, 0.98)	0.06	0.93 (0.82, 1.06)
Advanced prostate cancer										
Cases	27	33	28	25	42	29	27	15		
Age-stratified RR	1.00	1.19 (0.71, 1.99)	1.04 (0.61, 1.77)	0.98 (0.56, 1.70)	1.00	0.79 (0.49, 1.28)	0.76 (0.47, 1.25)	0.51 (0.28, 0.93)	0.03	0.87 (0.76, 0.99)
Multivariate RR ⁵	1.00	1.11 (0.66, 1.88)	0.97 (0.56, 1.71)	0.84 (0.46, 1.56)	1.00	0.79 (0.47, 1.34)	0.67 (0.36, 1.25)	0.37 (0.16, 0.88)	0.03	0.81 (0.67, 0.99)
Multivariate RR ⁶	1.00	1.18 (0.67, 2.06)	1.11 (0.59, 2.08)	1.09 (0.52, 2.27)	1.00	0.77 (0.45, 1.31)	0.63 (0.33, 1.19)	0.33 (0.14, 0.80)	0.01	0.76 (0.61, 0.95)
Advanced prostate cancer, excluding cases occurring in the first 2 y after recruitment										
Cases	22	26	21	20	34	20	22	13		
Age-stratified RR	1.00	1.16 (0.66, 2.06)	0.97 (0.53, 1.78)	0.96 (0.52, 1.79)	1.00	0.66 (0.38, 1.16)	0.75 (0.44, 1.30)	0.53 (0.27, 1.01)	0.07	0.89 (0.77, 1.04)
Multivariate RR ⁵	1.00	1.12 (0.62, 2.03)	0.92 (0.49, 1.74)	0.85 (0.43, 1.68)	1.00	0.62 (0.33, 1.14)	0.60 (0.30, 1.21)	0.34 (0.13, 0.86)	0.03	0.82 (0.65, 1.03)
Multivariate RR ⁶	1.00	1.16 (0.62, 2.19)	0.96 (0.47, 1.97)	0.94 (0.41, 2.17)	1.00	0.61 (0.33, 1.13)	0.57 (0.28, 1.16)	0.29 (0.11, 0.76)	0.02	0.79 (0.62, 1.00)

¹ n = 11 319, EPIC, European Prospective Investigation into Cancer and Nutrition; Q, quartile.

² Sum of menaquinones MK-4 to MK-14.

³ Per 10- $\mu\text{g/d}$ increment.

⁴ RR; 95% CIs in parentheses (all such values).

⁵ Cox proportional hazards models, adjusted for smoking (never, quit ≥ 10 y ago, quit <10 y ago, current ≤ 15 cigarettes/d, or other smoking), education (none or primary school, technical school, secondary school, or university degree), vigorous physical activity (none, <2 h/wk, or ≥ 2 h/wk), energy from fat, alcohol, nonfat-non-alcohol energy, calcium, vitamin D, tomato or tomato products (all nutrients entered as quartile-dummies), BMI, history of diabetes, and family history of prostate cancer.

⁶ Cox proportional hazards models as in footnote 5, with additional adjustment for the intake of vegetables, dairy products, and meat or meat products (quartiles).

trend = 0.06), showing significantly reduced RRs in the 3rd and 4th quartiles after exclusion of cases diagnosed within the first 2 y of follow-up and after further adjustment for food group intake. For advanced prostate cancer, the inverse relation with intake of menaquinones was even stronger and statistically significant in the multivariate-adjusted models. When advanced prostate cancer was alternatively defined separately according to stage (TNM stage T3/T4, N1–N3, or M1; $n = 50$) or grade (Gleason sum score ≥ 7 ; $n = 74$), obtained risk estimates were similar to those of the combined definition of advanced prostate cancer, but they were not significant, because of the low number of cases (data not shown). The multivariate adjustment and exclusion of cases diagnosed within the first 2 y of follow-up generally strengthened our results. Additional adjustment for intake of vegetables, dairy products, and meat changed the risk estimates only slightly.

The association between the intake of menaquinones from meat or meat products or of those from dairy products and the risk of prostate cancer is shown in **Table 4**. Only menaquinones from dairy products were associated with a significantly lower risk of advanced prostate cancer. Accordingly, the risk estimate for higher menaquinones (MK-5–9), predominantly (85% of total intake) derived from dairy products, was close to significance (RR: 0.82; 95% CI: 0.67, 1.02), whereas the risk estimate for MK-4 (37% of total intake derived from meat or meat products) was not significant (**Table 5**).

In our study population, 48% of all men had undergone ≥ 1 PSA test during follow-up; these tests had resulted in the detection of many occurrences of the early forms of prostate cancer, whereas similar malignant transformations remain undetected in men (noncases) who did not undergo PSA testing. When we restricted our cohort to men who had ≥ 1 PSA test, risk estimates were similar to those obtained in the total cohort (data not shown).

TABLE 4
Relative risk of prostate cancer according to dietary intake of menaquinones from major food sources in men of the EPIC-Heidelberg cohort¹

	Menaquinones from meat and meat products	Menaquinones from dairy products
Total prostate cancer ($n = 268$)		
Age-stratified	0.81 (0.62, 1.07)	0.96 (0.88, 1.06)
Multivariate ²	0.83 (0.59, 1.17)	0.94 (0.84, 1.06)
Advanced prostate cancer ($n = 113$)		
Age-stratified	0.86 (0.56, 1.32)	0.82 (0.69, 0.97)
Multivariate ²	0.84 (0.50, 1.41)	0.79 (0.63, 0.98)

¹ All values are continuous relative risk per 10- $\mu\text{g}/\text{d}$ increment; 95% CI in parentheses. EPIC, European Prospective Investigation into Cancer and Nutrition. $n = 11\ 319$.

² Cox proportional hazards models, adjusted for smoking (never, quit ≥ 10 y ago, quit < 10 y ago, current < 15 cigarettes/d, current ≥ 15 cigarettes/d, or other smoking), education (none or primary school, technical school, secondary school, or university degree), vigorous physical activity (none, $< 2\text{h}/\text{wk}$, or $\geq 2\text{h}/\text{wk}$), phyloquinone, menaquinones from other sources, energy from fat, alcohol, nonfat-non-alcohol energy, calcium, vitamin D, tomato or tomato products (all nutrients entered as quartile-dummies), BMI, history of diabetes, and family history of prostate cancer.

TABLE 5
Relative risk of prostate cancer according to intake of menaquinone-4 (MK-4) and MK-5–9 in men of the EPIC-Heidelberg cohort¹

	MK-4 ²	MK-5–9 ³
Total prostate cancer ($n = 268$)		
Age-stratified	0.89 (0.71, 1.12)	0.98 (0.88, 1.08)
Multivariate ⁴	0.80 (0.55, 1.17)	0.96 (0.85, 1.09)
Advanced prostate cancer ($n = 113$)		
Age-stratified	0.94 (0.66, 1.34)	0.82 (0.68, 0.99)
Multivariate ⁴	0.71 (0.40, 1.27)	0.80 (0.64, 1.00)

¹ All values are continuous relative risk per 10- μg increment; 95% CIs in parentheses. EPIC, European Prospective Investigation into Cancer and Nutrition. $n = 11\ 319$.

² Meat and meat products as the main contributors (37% of total intake).

³ Dairy products as the main contributors (85% of total intake).

⁴ Cox proportional hazard models, adjusted for smoking (never, quit ≥ 10 y ago, quit < 10 y ago, current < 15 cigarettes/d, current ≥ 15 cigarettes/d, or other smoking), education, vigorous physical activity (none, $< 2\text{h}/\text{wk}$, or $\geq 2\text{h}/\text{wk}$), phyloquinone, MK-10–14, energy from fat, alcohol, nonfat-non-alcohol energy, calcium, vitamin D, tomato or tomato products (all nutrients entered as quartile-dummies), BMI, history of diabetes, and family history of prostate cancer.

DISCUSSION

To the best of our knowledge, this report provides the first analysis of observational data on the association between dietary intake of vitamin K and the risk of prostate cancer. With higher intakes of menaquinones, prostate cancer risk decreased; this association was significant for advanced prostate cancer, especially with MK-5–9 provided by dairy products. In contrast, phyloquinone intake was not related to prostate cancer.

According to experimental data, the anticancer activity of the natural K vitamins phyloquinone and menaquinones is mediated by antiproliferative effects through induction of proto-oncogenes such as *c-myc* and *c-fos*, which foster cell cycle arrest and induce apoptosis in several cancer cell lines (3, 5). Most of these studies found distinct growth-inhibitory effects of menaquinones (5–9, 11), whereas substantially weaker (5, 11) or no (6, 9) effects were observed for phyloquinone. These findings seem to be reflected by our results, which showed a significant inverse association of total menaquinone intake with advanced prostate cancer and no associations for phyloquinone. Besides possible differential anticarcinogenic effects on the cellular level, the differing results obtained for phyloquinone and menaquinones may be related to differences regarding bioavailability, half-life, and tissue distribution (21). Phyloquinone, which is mainly provided by vegetables and other plant products, is tightly bound to the chloroplast membranes and therefore is less efficiently absorbed (5–15% bioavailable) than are menaquinones, which occur dissolved in the fat-fraction of dairy products or in meat (19, 22). Menaquinones have a substantially longer half-life in the blood circulation than does phyloquinone (19), and, therefore, they are available longer to develop anticancer activities. Finally, menaquinones are more likely to accumulate in extrahepatic tissues, because they, unlike phyloquinone, are redistributed by the liver via low- and high-density lipoproteins to extrahepatic organs (2, 23, 24). Although no data exist to date showing the concentrations of menaquinones in human or animal prostate, it is conceivable that menaquinones also accumulate in the prostate as they do in the pancreas, kidney, and brain (2, 24). However,

because of the tissue-specific conversion of phylloquinone to MK-4 (2, 23–25), the extent to which the menaquinone content of extrahepatic tissues is attributable to dietary intake of menaquinones or phylloquinone is unclear.

The inverse association between the intake of menaquinones and the risk of prostate cancer persisted after additional adjustment for main food sources of vitamin K, including dairy products and meat, which suggests that the observed effects are unlikely to be attributable to the coexistence of other nutrients with menaquinones in the same foods. We found menaquinones from dairy products to be more strongly associated with advanced prostate cancer incidence than were menaquinones from meat or meat products. Meat and meat products mainly contain MK-4 (and small amounts of menaquinones above MK-9), whereas the menaquinones MK-5–9 are almost exclusively found in fermented dairy products. This led us to the separate evaluation of MK-4 and MK-5–9 in relation to prostate cancer. The reduced RR of advanced prostate cancer was close to statistical significance for menaquinones MK-5–9 (provided to 82% by dairy products), which was not the case for MK-4 (main food source: meat or meat products, providing 37% of total intake). The differences in anticancer effects may be seen as a consequence of the longer half-life of MK-5–9 in the blood circulation than of MK-4 (25). Because menaquinones MK-10–14 contribute <4% of total menaquinone intake and differ with respect to the food source from MK-5 to MK-9, they were not separately analyzed.

Our findings of stronger associations of vitamin K intake with advanced than with total prostate cancer could be a hint that menaquinones play a role in tumor promotion and progression rather than in tumor initiation. The anticancer actions of the natural K vitamins are mediated by oncogene-associated cell cycle arrest and apoptosis, which are likely to play a major role in the promotion phase, a reversible process lasting several years and therefore most susceptible to the influence of cancer-preventive agents (26).

A limitation of the present study lies in the inaccuracies of dietary vitamin K intake estimation. The vitamin K content of a certain food may vary considerably, according to the type of plant, the various agricultural and manufacturer practices, seasonal variation, and maturity (27). In a food-composition database, this variation is usually subsumed in a single value for a certain food. Moreover, an FFQ is a dietary assessment instrument with limited ability to determine absolute intakes. However, these uncertainties would attenuate the observed association between dietary vitamin K intake and prostate cancer; ie, the true association would be even stronger. The database for the phylloquinone content in food that we used (18) includes \approx 2000 foods commonly consumed in Western Europe. It is based on data from laboratory analyses conducted by Bolton-Smith et al, other sources of direct HPLC analyses, and recipe calculation and assignment of values by food similarities. This database was used successfully for the estimation of phylloquinone intake in epidemiologic studies (28, 29), in which intake data were also compared with plasma vitamin K concentrations. Therefore, we applied the database of Bolton-Smith et al (18) to our data without further modification according to data on the phylloquinone content of foods published by other researchers (30–33).

Habitual dietary intake of menaquinones has rarely been calculated (34, 35). The menaquinone food table of Schurgers et al (19) comprises contents of MK-4 to MK-10 for \approx 30 food items,

although MK-10 was not detected in any of the samples. Menaquinones above MK-10 occur in detectable amounts only in some offal, and thus they play a minor role in human nutrition (20, 36). Nevertheless, we supplemented the menaquinone data of Schurgers et al with data on the content of MK-10–14 in some pork, beef, and chicken offal (20) that was eventually consumed in EPIC-Heidelberg. Other data on the menaquinone content of foods have been published (30–32). However, only the 2 chosen data sources analyzed the complete spectrum of MK-4 up to MK-10 or MK-14, respectively, and therefore allowed a valid calculation of total menaquinone intake.

The mean phylloquinone intake calculated from the EPIC-Heidelberg FFQ data was higher than the intakes in 2 studies using the database of Bolton-Smith et al (18), in which dietary intake was assessed by food records; those mean values in men were 70 and 84 μ g/d, respectively (27, 37). In a Dutch study (34) assessing intake of menaquinones by using an FFQ and the database of Schurgers et al (19), intakes in males (mean: 31 μ g/d) fit well with our results. Differences in the dietary assessment methods (38), differing age ranges, and food preferences of the populations may explain the slightly diverging results.

According to our data, dairy products contribute 60% to total intake of menaquinones, and it seems that menaquinones from dairy products have a more pronounced effect than those from meat products. These observations contrast with those from numerous prospective studies that suggest a positive association between the intake of dairy products, especially dairy calcium, and prostate cancer (39). However, in the present study, all multivariate analyses were adjusted for dietary calcium intake, and additional adjustment for dairy products did not affect the results significantly. Among the group of dairy products, cheese is the predominant source of menaquinones, whereas calcium is provided about equally by cheese and milk or milk products (40). A diet characterized by a moderate calcium intake, therefore, does not necessarily conflict with a diet high in menaquinones provided by cheese. The strengths of the present study include the prospective design, the ability to identify advanced prostate cancer cases, the information on PSA screening tests during follow-up, and the consideration of menaquinones, which are neglected in most studies on vitamin K because of their lower contribution to total vitamin K intake.

In summary, we found inverse associations between the dietary intake of menaquinones and the risk of prostate cancer. The associations were strongest for menaquinones from dairy products and in advanced cancer cases. Because the present study is presumably the first observational study on this topic, the results warrant confirmation by other studies.

We thank all participants of the EPIC-Heidelberg cohort study for their continuous collaboration.

The authors' responsibilities were as follows: KN: statistical analysis, interpretation of the data, and drafting of the manuscript; SR: critical revision of the manuscript; and JL: the study concept and design, acquisition of data, critical revision of the manuscript, and obtaining the funding for the study. None of the authors had any personal or financial conflict of interest.

REFERENCES

1. Shearer MJ. Vitamin K: metabolism and nutriture. *Blood Rev* 1992;6: 92–104.
2. Thijssen HH, Drittij-Reijnders MJ, Fischer MA. Phylloquinone and menaquinone-4 distribution in rats: synthesis rather than uptake determines menaquinone-4 organ concentrations. *J Nutr* 1996;126:537–43.

3. Lamson DW, Plaza SM. The anticancer effects of vitamin K. *Altern Med Rev* 2003;8:303–18.
4. Verrax J, Cadrobbi J, Delvaux M, et al. The association of vitamins C and K3 kills cancer cells mainly by autophagy, a novel form of cell death. Basis for their potential use as adjuvants in anticancer therapy. *Eur J Med Chem* 2003;38:451–7.
5. Nishikawa Y, Carr BI, Wang M, et al. Growth inhibition of hepatoma cells induced by vitamin K and its analogs. *J Biol Chem* 1995;270:28304–10.
6. Bouzahzah B, Nishikawa Y, Simon D, Carr BI. Growth control and gene expression in a new hepatocellular carcinoma cell line, Hep40: inhibitory actions of vitamin K. *J Cell Physiol* 1995;165:459–67.
7. Tokita H, Tsuchida A, Miyazawa K, et al. Vitamin K2-induced antitumor effects via cell-cycle arrest and apoptosis in gastric cancer cell lines. *Int J Mol Med* 2006;17:235–43.
8. Otsuka M, Kato N, Shao RX, et al. Vitamin K-2 inhibits the growth and invasiveness of hepatocellular carcinoma cells via protein kinase A activation. *Hepatology* 2004;40:243–51.
9. Wang Z, Wang M, Finn F, Carr BI. The growth inhibitory effects of vitamins K and their actions on gene expression. *Hepatology* 1995;22:876–82.
10. Okayasu H, Ishihara M, Satoh K, Sakagami H. Cytotoxic activity of vitamins K1, K2 and K3 against human oral tumor cell lines. *Anticancer Res* 2001;21:2387–92.
11. Wu FY, Liao WC, Chang HM. Comparison of antitumor activity of vitamins K1, K2 and K3 on human tumor cells by two (MTT and SRB) cell viability assays. *Life Sci* 1993;52:1797–804.
12. Habu D, Shiomi S, Tamori A, et al. Role of vitamin K2 in the development of hepatocellular carcinoma in women with viral cirrhosis of the liver. *JAMA* 2004;292:358–61.
13. Venugopal M, Jamison JM, Gilloteaux J, et al. Synergistic antitumor activity of vitamins C and K3 against human prostate carcinoma cell lines. *Cell Biol Int* 1996;20:787–97.
14. Jamison JM, Gilloteaux J, Taper HS, Summers JL. Evaluation of the in vitro and in vivo antitumor activities of vitamin C and K-3 combinations against human prostate cancer. *J Nutr* 2001;131(suppl):158S–60S.
15. Boeing H, Korfmann A, Bergmann MM. Recruitment procedures of EPIC-Germany. European Investigation into Cancer and Nutrition. *Ann Nutr Metab* 1999;43:205–15.
16. Bohlscheid-Thomas S, Hoting I, Boeing H, Wahrendorf J. Reproducibility and relative validity of energy and macronutrient intake of a food frequency questionnaire developed for the German part of the EPIC project. European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol* 1997;26(suppl):S71–81.
17. Al Delaimy WK, Ferrari P, Slimani N, et al. Plasma carotenoids as biomarkers of intake of fruits and vegetables: individual-level correlations in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Eur J Clin Nutr* 2005;59:1387–96.
18. Bolton-Smith C, Price RJ, Fenton ST, Harrington DJ, Shearer MJ. Compilation of a provisional UK database for the phylloquinone (vitamin K1) content of foods. *Br J Nutr* 2000;83:389–99.
19. Schurgers LJ, Vermeer C. Determination of phylloquinone and menaquinones in food. Effect of food matrix on circulating vitamin K concentrations. *Haemostasis* 2000;30:298–307.
20. Hiraiuchi K, Sakano T, Notsumoto S, et al. Measurement of K vitamins in animal tissues by high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* 1989;497:131–7.
21. Vermeer C, Schurgers LJ. A comprehensive review of vitamin K and vitamin K antagonists. *Hematol Oncol Clin North Am* 2000;14:339–53.
22. Gijsbers BL, Jie KS, Vermeer C. Effect of food composition on vitamin K absorption in human volunteers. *Br J Nutr* 1996;76:223–9.
23. Ronden JE, Thijssen HH, Vermeer C. Tissue distribution of K-vitamins under different nutritional regimens in the rat. *Biochim Biophys Acta* 1998;1379:16–22.
24. Thijssen HH, Drittij-Reijnders MJ. Vitamin K status in human tissues: tissue-specific accumulation of phylloquinone and menaquinone-4. *Br J Nutr* 1996;75:121–7.
25. Schurgers LJ, Vermeer C. Differential lipoprotein transport pathways of K-vitamins in healthy subjects. *Biochim Biophys Acta* 2002;1570:27–32.
26. Sun SY, Hail N Jr, Lotan R. Apoptosis as a novel target for cancer chemoprevention. *J Natl Cancer Inst* 2004;96:662–72.
27. Thane CW, Paul AA, Bates CJ, Bolton-Smith C, Prentice A, Shearer MJ. Intake and sources of phylloquinone (vitamin K1): variation with socio-demographic and lifestyle factors in a national sample of British elderly people. *Br J Nutr* 2002;87:605–13.
28. Thane CW, Bates CJ, Shearer MJ, et al. Plasma phylloquinone (vitamin K1) concentration and its relationship to intake in a national sample of British elderly people. *Br J Nutr* 2002;87:615–22.
29. Collins A, Cashman KD, Kiely M. Phylloquinone (vitamin K1) intakes and serum undercarboxylated osteocalcin levels in Irish postmenopausal women. *Br J Nutr* 2006;95:982–8.
30. Elder SJ, Haytowitz DB, Howe J, Peterson JW, Booth SL. Vitamin K contents of meat, dairy, and fast food in the U.S. *Diet J Agric Food Chem* 2006;54:463–7.
31. Ferreira DW, Haytowitz DB, Tassinari MA, Peterson JW, Booth SL. Vitamin K contents of grains, cereals, fast-food breakfasts, and baked goods. *J Food Sci* 2006;71(suppl):S66–70.
32. Koivu-Tikkanen TJ, Ollilainen V, Piironen VI. Determination of phylloquinone and menaquinones in animal products with fluorescence detection after postcolumn reduction with metallic zinc. *J Agric Food Chem* 2000;48:6325–31.
33. Koivu-Tikkanen TJ, Piironen VI, Henttonen SK, Mattila PH. Determination of phylloquinone in vegetables, fruits, and berries by high-performance liquid chromatography with electrochemical detection. *J Agric Food Chem* 1997;45:4644–9.
34. Geleijnse JM, Vermeer C, Grobbee DE, et al. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. *J Nutr* 2004;134:3100–5.
35. Schurgers LJ, Geleijnse JM, Grobbee DE, et al. Nutritional intake of vitamins K1 (phylloquinone) and K2 (menaquinone) in the Netherlands. *J Nutr Environ Med* 1999;9:115–22.
36. Shearer MJ, Bach A, Kohlmeier M. Chemistry, nutritional sources, tissue distribution and metabolism of vitamin K with special reference to bone health. *J Nutr* 1996;126(suppl):S1181–6.
37. Duggan P, Cashman KD, Flynn A, Bolton-Smith C, Kiely M. Phylloquinone (vitamin K1) intakes and food sources in 18–64-year-old Irish adults. *Br J Nutr* 2004;92:151–8.
38. Thompson FE, Subar AF. Dietary assessment methodology. In: Coulston AM, Rock CL, Mousen ER, eds. *Nutrition in the prevention and treatment of disease*. London, United Kingdom: Academic Press 2007:3–30.
39. Gao X, LaValley MP, Tucker KL. Prospective studies of dairy product and calcium intakes and prostate cancer risk: a meta-analysis. *J Natl Cancer Inst* 2005;97:1768–77.
40. Döring A, Honig-Blum K, Winkler G, Kammerlohr R, Fischer B, Keil U. MONICA Project Region Augsburg. Data book. Dietary surveys 1984/85 and 1994/95 in middle-aged men from the city of Augsburg. Neuherberg, Germany: GSF-Forschungszentrum, 1998.

