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Effect of food composition on vitamin K absorption in human volunteers

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The human vitamin K requirement is not known precisely, but the minimal requirement is often assumed to be between 0·5 and 1×10^{-6} g/kg body weight. In the present study we addressed the question to what extent circulating vitamin K concentrations are influenced by the form in which the vitamer is consumed. The experimental group consisted of five healthy volunteers who received phylloquinone after an overnight fast. On the first day of three successive weeks the participants consumed 1 mg (2·2 μmol) phylloquinone, either in the form of a pharmaceutical preparation (Konakion®), or in the form of spinach + butter, or as spinach without added fat. Circulating phylloquinone levels after spinach with and without butter were substantially lower (7·5- and 24·3-fold respectively) than those after taking the pharmaceutical concentrate. Moreover, the absorption of phylloquinone from the vegetables was 1·5 times slower than from Konakion. In a second experiment in the same five volunteers it was shown that relatively high amounts of menaquinone-4 enter the circulation after the consumption of butter enriched with this vitamer. It is concluded that the bioavailability of membrane-bound phylloquinone is extremely poor and may depend on other food components, notably fat. The bioavailability of dietary vitamin K (phylloquinone + menaquinones) is lower than generally assumed, and depends on the form in which the vitamin is ingested. These new insights may lead to a revision of the recommended daily intake for vitamin K.

Phylloquinone: Vitamin K: Blood: γ-Carboxyglutamate

Vitamin K is a generic name for a number of closely related compounds which may serve as a cofactor for the mammalian microsomal enzyme γ-glutamylcarboxylase. This enzyme catalyses the post-translational conversion of glutamate into γ-carboxyglutamate (Gla) residues at well-defined sites in a limited number of proteins (Suttie, 1985; Vermeer, 1990). If not specified further the term 'vitamin K' will be used for all compounds possessing cofactor activity for γ-glutamylcarboxylase. Gla-containing proteins have a regulatory function in blood coagulation (Furie & Furie, 1992), bone metabolism (Hauschka *et al.* 1989), and possibly also in cell growth (Manfioletti *et al.* 1993). Potential sources of vitamin K are the diet (Sakano *et al.* 1988; Hirauchi *et al.* 1989; Booth *et al.* 1993) and the intestinal flora (Conly & Stein, 1992), but accumulating data suggests that the direct absorption of menaquinones produced by bacteria in the colon is poor (Lipsky, 1988, 1994; Groenen-van Dooren *et al.* 1993, 1995). This is because bile salts (mainly present in the small intestine) are required for absorption of the various forms of vitamin K.

The main dietary sources of vitamin K are green vegetables (which exclusively contain phylloquinone) and dairy produce, in which a mixture of phylloquinone and menaquinones is found (Sakano *et al.* 1988; Hirauchi *et al.* 1989; Shearer *et al.* 1992; Booth *et al.* 1993). In this respect it is important to notice (a) that green leafy vegetables contain about 100

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times more vitamin K than does cows' milk (Booth *et al.* 1993), and (b) that in green plants vitamin K is tightly bound to the thylakoid membranes of the chloroplasts (Lichtenthaler, 1993), whereas in dairy produce it is dissolved in the fat fraction. The extent to which various forms of vitamin K are extracted in the digestive tract, and are absorbed in the intestinal mucosa, however, has remained unclear. In the present study we measured the serum concentrations of phylloquinone and menaquinone-4 as a function of time after consumption of various foods and a detergent-solubilized product.

MATERIALS AND METHODS

Experimental design

Expt 1. The three different vitamin K regimens described below were given on the first day of three successive weeks. Five healthy volunteers (three men and two women, all non-smokers, 25–45 years old) entered the experiments after an overnight fast. All five volunteers included in our experiment were subjected to the same nutritional regimen starting on the same day. In each case an oral dose of 1 mg (2·2 μmol) phylloquinone was taken at 08.00 hours, either in the form of a detergent-solubilized pharmaceutical concentrate (Konakion®, week 1), or as 227 g boiled spinach + 25 g butter (week 2), or as 227 g boiled spinach without added fat (week 3). No other food was allowed on the experimental day, except one meal which was low in both fat and vitamin K, and which was consumed at 12.00 hours. The meal consisted of four slices of toast with marmalade, one peeled apple, one banana, two glasses of orange juice, and water *ad libitum*. Food restriction was continued until 10 h after vitamin K ingestion. Blood (10 ml) was taken by venepuncture to prepare serum every hour during the first 10 h of the experimental day, and at 08.00 hours next morning (t = 24 h). The serum samples were subdivided into 1 ml portions and kept frozen and protected from light until vitamin K determination.

Expts 2 and 3. Expt 2 followed the same protocol as Expt 1, with the exception that 2·2 μmol menaquinone-4 was ingested in the form of 5 g butter enriched with this vitamer, in combination with one slice of toast. In Expt 3 the Konakion ingestion described in Expt 1 was repeated three times in three volunteers. The protocol of these studies was approved by the Medical Ethics Committee of the University Hospital – Faculty of Medicine, University of Limburg, Maastricht.

Materials

Detergent-solubilized phylloquinone (Konakion®) was from Hoffmann–La Roche (Basel, Switzerland), spinach was purchased as a commercially prepared, deep-frozen product (Iglo, shredded leaves) which was heated (without additions) until boiling, under constant stirring, before use. Menaquinone-6 (MK-6) was a kind gift from Roche. Silica Sep-pack cartridges were purchased from Millipore (Milford, MA, USA). Tetramethylammonium octahydrotriborate was from Johnson Matthey (Karlsruhe, Germany). All other solvents and chemicals were of the highest purity commercially available.

Serum measurements

Serum vitamin K was determined by fluorometric detection after electrochemical reduction according to Thijssen & Drittij-Reijnders (1993), with sample preparation and semi-preparative pre-purification as described by Hart *et al.* (1985). In short, after adding an internal standard (MK-6, 1 ng/ml serum), 1 ml of serum was extracted twice with 12 ml hexane, and the pooled hexane phases were evaporated under a constant stream of N_2 at 37°, dissolved in 2 ml hexane, and bound to a silica sep-pack column. Vitamin K was eluted from the silica with ether–hexane (3:97, v/v) and the fraction containing vitamin K was

concentrated by evaporating the hexane as described previously and adding 0·1 ml hexane to the residue. The total sample was then fractionated further on a semi-preparative silica normal-phase column (Phase Separations, Deeside, Clwyd) by HPLC in dichloromethane–hexane (5:95, v/v) with UV-detection at 254 nm. Fractions containing vitamin K were pooled and the solvent was evaporated as described previously. The residue was taken up in 25 μl isopropanol and used for the quantitative analysis of vitamin K: the total sample was applied to an Econosphere C_{18} reversed phase column (Alltech, Deerfield, IL, USA), which was eluted by isocratic HPLC using a mixture of methanol–isopropanol–water–tetramethylammonium octahydrotriborate (88·5:10:1·5:0·045, by vol.). The effluent of the column was reduced with a model 5010 analytical cell (Environmental Sciences Associates, Bedford, MA, USA), and analysed with a Jasco 821-FP spectrofluorometric detector (Separations Analytical Instruments, Hendrik Ido Ambacht, The Netherlands). The excitation wavelength was 246 nm, the emission wavelength was 430 nm. All measurements were made in duplicate, and mean values are used throughout this paper. The intra- and inter-run variations were 3·7 and 5·2% respectively.

Food analysis

For the extraction of vitamin K from spinach, 10 g boiled vegetable (wet weight) was mixed with 1 ml acetone and ground with 0·5 g sand. Subsequently 100 ml acetone was mixed with the homogenate and shaken for 30 min. After the addition of 100 ml water, the solution was extracted three times with 100 ml hexane; the pooled hexane fractions were evaporated under a constant stream of N_2 at 37°, and redissolved in 1 ml hexane. Quantitative analysis of phylloquinone in food extracts was performed by HPLC with post-column reduction and fluorescence detection, according to the same procedure as described for serum.

RESULTS

Before starting our experiments, the vitamin K content of various food items was assessed. The deep frozen spinach used in the present study was found to contain 9·8 nmol phylloquinone/g (wet weight), so that 227 g spinach provided 2·22 μmol phylloquinone. Butter contained 0·13 nmol phylloquinone/g; hence the contribution of phylloquinone in the 25 g butter (week 2) to serum vitamin K levels was regarded as negligible. Butter enriched with menaquinone-4 contained 0·44 μmol menaquinone-4/g. The phylloquinone content of Konakion was also checked, and found to be 44·4 mmol/l.

In the first experiment vitamin K was administered to the participants as a single oral dose, and in all cases circulating phylloquinone levels had returned to their starting levels after 24 h. Nevertheless a wash-out period of 6 d was scheduled between the three experimental days. During each of the experimental days one meal (12.00 hours) which was low in fat and vitamin K content was allowed. It is assumed that vitamin K absorption was not influenced by this meal. For each participant the serum phylloquinone concentration was established at 1 h intervals, and the mean values with their standard errors are given in Fig. 1. It is striking that very similar curves were obtained for all subjects: peak values occurred at 4 h (Konakion) and 6 h (spinach) after vitamin K ingestion, with only some interindividual variations in the peak heights. The absorption of phylloquinone from vegetables was slower than that from Konakion by a factor 1·5. Butter had an effect only on the peak height, not on the time at which the maximal serum concentration was reached. The recovery of phylloquinone from serum was considered to be similar to that of the internal standard, which was between 60 and 70% in all experiments.

In a second experiment among the same subjects the phylloquinone uptake from the spinach + butter regimen was compared with that from fat-solubilized menaquinone-4 (butter enriched with menaquinone-4). Each of the participants ingested 5 g butter

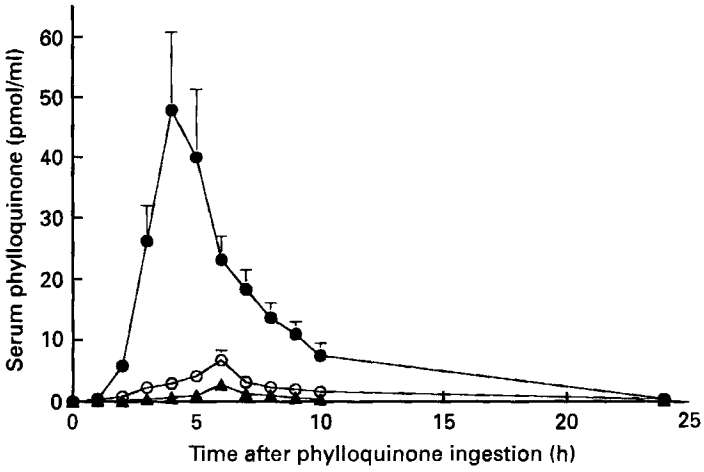

Fig. 1. Circulating phylloquinone concentration as a function of time and diet composition. Phylloquinone (1 mg; 2·2 μmol) was given as a single, oral dose to five fasting volunteers. Sources of phylloquinone were either Konakion (●), spinach + butter (○) or spinach without added fat (▲). The value at each time point is given as the mean of the five individual values with its standard error represented by a vertical bar. For details of procedures, see pp. 224–225.

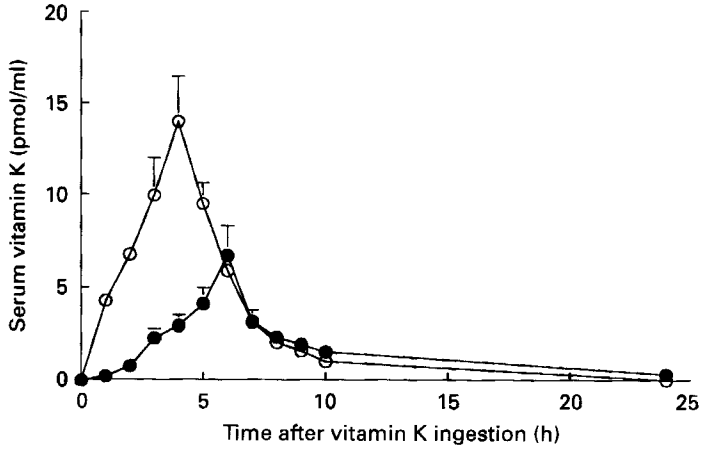

Fig. 2. Circulating menaquinone-4 concentration as a function of time after its ingestion. A single, oral dose of 1 mg (2·2 μmol) menaquinone-4 was given to the same five volunteers described in Expt 1. The menaquinone-4 was administered in the form of a butter-solubilized product (○) and the values are compared with those obtained for phylloquinone after the spinach + butter regime (●). For details of procedures, see pp. 224–225.

(containing 2·2 μmol menaquinone-4) with one slice of toast. As is shown in Fig. 2, the appearance of menaquinone-4 in serum was rapid, and reached a maximum at 4 h after ingestion. The peak height was approximately twice that of phylloquinone during the spinach + butter regimen.

The total increase of circulating phylloquinone and menaquinone-4 in Expts 1 and 2 was calculated for each participant from the area under the curve (AUC) between 0 and 10 h after the start of the vitamin K ingestion, and the values obtained for the various food items were expressed as a percentage of the value for Konakion. As can be seen in Table 1, the availability of phylloquinone from spinach alone was 4% that from Konakion, whereas the

Table 1. *Effect of vitamin K from different sources on serum phylloquinone or menaquinone levels in human subjects**

Subject			AUC† (nmol/l.h) (%)			
Code	Sex	Body wt (kg)	Konakion	Spinach + butter	Spinach − butter	MK-4 in butter
A	Male	78	310 (100)	18·4 (13·1)	5·8 (1·9)	81·8 (26·0)
B	Female	60	216 (100)	26·4 (12·3)	9·8 (4·6)	63·9 (29·3)
C	Male	64	201 (100)	17·6 (8·8)	6·9 (3·4)	59·7 (29·3)
D	Male	60	122 (100)	25·6 (21·0)	13·3 (10·9)	41·9 (33·9)
E	Female	82	117 (100)	18·2 (15·6)	4·2 (3·7)	42·8 (36·2)
Mean		69	193·1 (100)	25·8 (13·3)	8·0 (4·1)	58·1 (29·7)

AUC, area under the curve; MK-4, menaquinone-4.

* For details of sources and procedures, see pp. 224–225.

† Area under each individual absorption curve, calculated from the sum of the serum vitamin K concentrations at the various time points after subtraction of the starting values. The AUC values for the various nutritional regimens are expressed as a percentage of the corresponding Konakion experiment.

Table 2. *Intraindividual variation of dose–response curves after 2·2 μmol Konakion**

	AUC (nmol/l . h) of experiment no:					
	1	2	3	Mean	SE	(%)†
A	264	298	206	256	46·2	(12·0)
B	232	240	264	245	16·4	(4·3)
D	116	137	122	125	11·1	(2·9)

* Dose–response curves were prepared from serum phylloquinone after oral ingestion of 1 mg (2·2 μmol) Konakion. The curves were prepared as described in Fig. 1, and the area under the curve (AUC) was calculated from the first 10 h after vitamin K administration. The letters A, B and D represent the same individuals as in Table 1.

† Standard error expressed as a percentage of the mean.

effect of simultaneous consumption of butter improved this figure by not more than a factor of three. The uptake of fat-solubilized menaquinone-4 was more than two-fold higher than that of phylloquinone from spinach + butter, which was about one third that of detergent-solubilized phylloquinone. At 24 h after vitamin K ingestion the serum phylloquinone and menaquinone-4 levels had decreased to the starting levels, irrespective of the peak height on the day before. This shows that dietary influences on plasma vitamin K levels disappear completely after an overnight fast, which is consistent with the short half-life of vitamin K in the circulation (2 h).

In a final experiment we investigated to what extent intra-individual variations in vitamin K metabolism may have influenced the data obtained. For this purpose we recruited once more the volunteers designated as A, B, and D in Table 1, and for each of these subjects we made three dose–response measurements with 2-week intervals between each measurement. In this experiment vitamin K was administered by the oral ingestion of 2·2 μmol Konakion. The data are given in Table 2, and show that the day-to-day variations in the absorption patterns were small.

From the combined Expts 1, 2 and 3 there were twenty-nine starting values which were

obtained from five different subjects. The values were all within the normal range and varied from 0·7 nmol/l to 1·8 nmol/l with a mean value of 1·1 nmol/l. Within this range there was considerable week-to-week variation for each subject and none of the subjects could be classified as being either consistently high or low in the fasting serum level of vitamin K throughout the experimental period.

DISCUSSION

From the data presented in the present paper it is clear that phylloquinone is readily absorbed from Konakion, and circulated in blood with a peak time of 4 h. This is in accordance with previous observations of Shearer *et al.* (1974) who found a peak time between 2 and 4 h. The later peak time for phylloquinone from spinach indicates that its absorption from plant cells is a more time-consuming process, which may be influenced by digestive factors such as the rate at which phylloquinone is extracted from the cellular membranes. The concomitant intake of fat may increase the phylloquinone peak height, but obviously does not influence the rate of absorption. The effect of fat is probably due to its stimulation of bile secretion, which is known to be important for the absorption of hydrophobic compounds (Shearer *et al.* 1974).

Our results also indicate that the mean intestinal absorption of dietary phylloquinone is only a small fraction of the total phylloquinone content which may be extracted from the food using organic solvents (Booth *et al.* 1993). As was reported earlier, the efficiency of intestinal absorption of phylloquinone from Konakion is about 80% (Shearer *et al.* 1974). Based on this figure it may be estimated from our data that less than 10% of phylloquinone present in green vegetables is absorbed in the digestive tract. Although this is less than generally assumed, it leaves green vegetables as the main source of dietary phylloquinone. This conclusion implies that other sources of vitamin K may contribute significantly to human vitamin K status. It is well known, for instance, that substantial amounts of menaquinones are found in the fat fractions of dairy produce (yoghurt, cheese) and meat (Sakano *et al.* 1988; Hirauchi *et al.* 1989; Shearer *et al.* 1992), and the question is to what extent these menaquinones are absorbed from the diet. As a first attempt to obtain some insight into this problem we analysed the serum menaquinone-4 levels as a function of time after the ingestion of butter enriched with this vitamer. In all subjects we found a good response, with a mean AUC value of slightly more than 33% that for Konakion. This is the first demonstration that the absorption of K-vitamers from dairy products and fats is higher than that from plant membranes. Since dietary menaquinones may range from the moderately hydrophobic menaquinone-4 to the extremely lipophilic menaquinone-12, these results should be interpreted carefully: absorption characteristics and plasma concentrations may differ substantially from one menaquinone to the other. In the present study menaquinone-4 was used as an example, and the data obtained may not be extrapolated to other menaquinones without further investigation.

The results from Table 2 suggest that, at least for the pharmaceutical preparation, the interindividual variation of AUC values is greater than their intraindividual variation, which means that the efficacy of vitamin K-extraction may vary from one person to another. In this respect it is interesting to note that subject D had a relatively poor absorption of vitamin K from Konakion, but a very good uptake of vitamin K from spinach. This suggests that there are interindividual differences with respect to the amounts of vitamin K that can be extracted from various foods. Because of the hydrophobic nature of vitamin K it seems plausible that variation in bile secretion plays a role in these differences, although other mechanisms cannot be excluded at this time.

In conclusion, our results strongly suggest that there are substantial differences between the intestinal absorption and bioavailability of K vitamers from different foods.

Determination of dietary vitamin K intake alone (on the basis of total vitamin K contents of these foods) is of limited value, but should be accompanied by data on the bioavailability of the various K-vitamers.

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