

The effect of different meals on the absorption of stable isotope-labelled phylloquinone

Kerry S. Jones*, Les J. C. Bluck, Laura Y. Wang, Alison M. Stephen, Celia J. Prynne and W. Andy Coward†
MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL, UK

(Received 23 October 2008 – Revised 24 March 2009 – Accepted 14 April 2009 – First published online 19 June 2009)

Few studies have investigated the absorption of phylloquinone (vitamin K₁). We recruited twelve healthy, non-obese adults. On each study day, fasted subjects took a capsule containing 20 µg of ¹³C-labelled phylloquinone with one of three meals, defined as convenience, cosmopolitan and animal-oriented, in a three-way crossover design. The meals were formulated from the characteristics of clusters identified in dietary pattern analysis of data from the National Diet and Nutrition Survey conducted in 2000–1. Plasma phylloquinone concentration and isotopic enrichment were measured over 8 h. Significantly more phylloquinone tracer was absorbed when consumed with the cosmopolitan and animal-oriented meals than with the convenience meal ($P=0.001$ and 0.035 , respectively). Estimates of the relative availability of phylloquinone from the meals were: convenience meal = 1.00; cosmopolitan meal = 0.31; animal-oriented meal = 0.23. Combining the tracer data with availability estimates for phylloquinone from the meals provides overall relative bioavailability values of convenience = 1.00, cosmopolitan = 0.46 and animal-oriented = 0.29. Stable isotopes provide a useful tool to investigate further the bioavailability of low doses of phylloquinone. Different meals can affect the absorption of free phylloquinone. The meal-based study design used in the present work provides an approach that reflects more closely the way foods are eaten in a free-living population.

GC/MS: Bioavailability: Dietary pattern analysis

The earliest discovered biological action of vitamin K was the control of blood coagulation and consequently dietary guidelines in the UK⁽¹⁾ and elsewhere⁽²⁾ are typically based on this function alone. However, it is now recognised that vitamin K has a much broader physiological role. Phylloquinone is the primary source of vitamin K in Western diets and average intakes in the UK are about 70 µg/d⁽³⁾. While these levels are sufficient for the maintenance of normal blood coagulation, they may be insufficient for maximal vitamin D-dependent carboxylation of osteocalcin^(4,5) and matrix Gla protein⁽⁶⁾. It has been suggested that higher phylloquinone intakes may be beneficial for bone health since they are associated with increased bone mineral density⁽⁷⁾ and lower fracture rates^(8,9); however, the conclusions from observational studies are generally not supported by more recent intervention data^(5,10–12).

Where intakes may be marginal, and in order to set evidence-based dietary recommendations, it is important to understand the relationship between intake and nutrient status. For phylloquinone, the relationship between intake and plasma concentration, although significant, is not very strong⁽¹³⁾. An important consideration for the intake–status relationship is the bioavailability, an understanding of which is essential for the determination of the dietary quantity of a nutrient required to ensure adequate status and hence

optimal health. In addition, information on phylloquinone bioavailability may be important in controlling coagulation in individuals on long-term anticoagulation treatment^(14–16).

Phylloquinone is found in a wide range of foods but vegetables provide over half of daily phylloquinone intake^(3,17). Green leafy vegetables are considered the major source of phylloquinone contributing about 20% to daily intake. Some types of oils and fats also contain high amounts of phylloquinone⁽¹⁸⁾, and due to their widespread use in many food products it is likely that these make an important contribution⁽³⁾. However, limited information is available on phylloquinone bioavailability from these different sources partly because of the difficulties associated with the analysis of phylloquinone. The majority of studies have compared absorption from different sources to standards, such as Konakion[®]^(19–21), but the results are difficult to compare because of differences in study protocols. The studies have been limited to comparisons of bioavailability from foods given to small numbers of subjects, and often using relatively large vitamin doses. Studies comparing the relative bioavailability of phylloquinone from vegetable and oil sources have produced conflicting results^(22,23), most likely due to the different methods used to measure absorption.

The use of stable isotope-labelled tracers potentially avoids some of these problems and provides a safe method

Abbreviation: AUC, area under the curve.

* **Corresponding author:** Dr Kerry S. Jones, fax +44 1223 437515, email kerry.jones@mrc-hnr.cam.ac.uk

† Dr Andy Coward passed away on 3 November 2007.

of investigating absorption and bioavailability^(24–26). The use of intrinsically labelled vegetables has been investigated for the measurement of phyloquinone absorption^(27–29), but this method of labelling is only suitable for single food items that are amenable to labelling in this way.

Bioavailability studies that focus on absorption from single food items may not reflect the true state of affairs in normal meal consumption since they do not take account of interactions between food components that may negate variation in bioavailability between single foods. Based on recent work in our laboratory^(30,31), we report here the development and application of a stable isotope-based method to measure phyloquinone absorption. Our initial aim was to determine the fraction of phyloquinone absorbed from test meals by using the labelled phyloquinone as a standard. However, because the meal significantly influenced absorption of the tracer we have presented the data as a meal effect, defined as the influence of the integrated meal on the absorption of free phyloquinone, and a matrix effect that describes the extraction efficiency or bioaccessibility of phyloquinone from the meal components. In order to provide an objective basis for the meal composition used in the present studies, the test meals were designed to reflect dietary clusters identified from the National Diet and Nutrition Survey of adults 2000–1⁽³²⁾.

Methods

Subjects

Twelve healthy, non-smoking subjects were recruited (five female and seven male). The subjects were aged 31.3 (SD 8.3) years (range 22–49 years), with a mean weight of 69.4 (SD 9.3) kg, and BMI of 23.1 (SD 2.3) kg/m². Ethical permission for the study was obtained from the Suffolk Local Research Ethics Committee and informed, written consent was obtained from all subjects.

Stable isotope-labelled phyloquinone

Methyl-¹³C-labelled phyloquinone was synthesised by ARC Laboratories (Apeldoorn, The Netherlands). Isotopic purity, assessed by GC/MS in our laboratory, was >98%. The molecular weight of naturally occurring, unlabelled phyloquinone is 450 Da and for the labelled material 451 Da. A stock solution of 83.8 mg in 100 ml of ethanol was prepared, divided into aliquots and stored at –18°C. To prepare the dose for volunteers, 48 µl of the standard solution was added to 1 ml of groundnut oil to give a solution of 40 µg/ml. Ethanol was evaporated from the oil by heating at 35°C under N₂ with a Pierce Reacti-therm heating block and Reacti-vap evaporator (Perbio Science, Erembodegem, Belgium). After vortexing for 1 min, 0.5 ml of groundnut oil containing 20 µg (44.4 nmol) of phyloquinone was transferred to a gelatine capsule (kindly provided by Capsugel, Colmar, France). Capsules were prepared fresh for each subject and stored, refrigerated, in amber medicine bottles.

Experimental protocol

Since recent phyloquinone intake has an effect on plasma phyloquinone concentration, before each study day,

volunteers were supplied with a standard evening meal and asked not to eat anything else. The meal consisted of a pizza containing no ingredients known to be high in phyloquinone. On three occasions, at least 2 weeks apart, volunteers attended the volunteer suite at MRC Human Nutrition Research after an overnight fast. An indwelling cannula was inserted into a forearm, and two baseline blood samples were collected into 7.5 ml EDTA S-monovettes[®] (Sarstedt Ltd, Leicester, UK). The volunteer then took the phyloquinone capsule (with water) immediately preceding consumption of one of three test meals. Previously prepared meals were defrosted overnight and reheated before consumption. In this three-period study, and to minimise sequence effects, subjects were randomised for the order they received the meals, with two subjects designated to each sequence. A snack (two slices of toast with sunflower spread and jam) was provided after 5 h and water was permitted throughout the study. Thirteen 7.5 ml blood samples were collected at 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 8.0 h post-dose. Samples were stored on ice and protected from light and within 1 h were centrifuged at 4°C for 20 min at approximately 2000g. Plasma was divided into aliquots and stored at –70°C until analysis.

Meal design

Each of the three meals was formulated to represent a meal from typical UK dietary patterns. Dietary pattern analysis of data from the National Diet and Nutrition Survey of adults 2000–1 has previously identified four dietary clusters for women and six for men, each characterised by their consumption of each of twenty-five food groups relative to the study population⁽³²⁾. Of these clusters, three showed similar patterns for both men and women and were chosen for the meal design. Fahey *et al.*⁽³²⁾ assigned a name to each of the dietary clusters to describe its defining characteristics. These names are repeated here in order to aid identification. The ‘convenience’ cluster is characterised by higher than average consumption of fast foods and refined cereals and lower than average consumption of fruit, vegetables and whole-grain cereals. The cosmopolitan cluster is characterised by higher than average consumption of fruits, vegetables, whole grains, fish and dairy foods and the animal-oriented cluster is characterised by higher than average consumption of red meat and saturated fat. The three test meals were designed to reflect the dietary characteristics of each cluster and, on the basis of food composition data contained equal amounts of phyloquinone (40 µg), energy (3200 kJ), energy from protein (20%), fat (40%) and carbohydrate (40%) and fibre (10.5 g). The meals were chicken pie with beans and chips (convenience), fish pie (cosmopolitan) and beef lasagne (animal-oriented). Dietary composition was estimated using the MRC Human Nutrition Research in-house suite of programs based on McCance and Widdowson’s *The Composition of Foods*, 4th edition⁽³³⁾, its supplements^(34,35) and the 6th edition⁽³⁶⁾. Phyloquinone values were derived from the 6th edition⁽³⁶⁾, published values⁽¹⁸⁾ and unpublished results (C Bolton-Smith and M Shearer). Meal composition is shown in Table 1. Each meal type was prepared in a single batch, cooked and frozen until required.

Table 1. Weight and nutritional information for the convenience (a), cosmopolitan (b) and animal-oriented (c) test meals

Ingredient	Weight (g)	Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Fibre (g)	Phylloquinone (μg)
(a) Convenience meal							
Chicken, light meat, raw	95	427	22.8	1.1	0.0	0.0	0.0
Baked beans	70	249	3.6	0.4	10.7	4.8	1.9
Milk, whole	65	179	2.1	2.5	3.1	0.0	0.4
Oven chips, frozen, baked	60	414	1.9	2.5	18.0	1.7	2.8
Bread, white	40	402	3.3	0.8	19.7	1.5	0.2
Flour, self-raising	35	491	3.1	0.4	26.5	1.4	0.3
Margarine (soya based)	25	677	Tr	18.3	0.1	Tr	19.5
Onions	20	19	0.2	Tr	1.0	0.3	0.1
Peas, frozen	18	52	1.0	0.2	1.7	1.3	5.2
Rapeseed oil	9	333	Tr	9.0	Tr	0.0	10.1
Total	437	3243	38.1	35.1	80.8	11.0	40.5
(b) Cosmopolitan meal							
Potatoes, boiled	260	796	4.7	0.3	44.2	3.6	2.4
Green beans, boiled	84	78	1.5	0.4	2.4	2.4	32.7
Bread, wholemeal	65	601	6.1	1.7	27.1	4.8	1.7
Milk, semi-skimmed	50	99	1.8	0.9	2.4	0.0	0.1
Haddock, smoked, raw	50	173	9.5	0.3	0.0	0.0	Tr
Salmon, raw	45	337	9.1	5.0	0.0	0.0	0.1
Cream, double	25	462	0.4	12.0	0.7	0.0	1.6
Cheese, cheddar	20	342	5.1	6.9	0.0	0.0	0.9
Fat (70%) spread	5	130	0.0	3.5	0.0	0.0	0.7
Butter	4	121	Tr	3.3	Tr	0.0	0.3
Flour, plain	4	58	0.4	0.1	3.1	0.1	Tr
Total	612	3197	38.6	34.4	79.9	10.9	40.5
(c) Animal-oriented							
Tomatoes, canned	160	110	1.6	0.2	4.8	1.3	9.6
White bread	95	955	7.9	1.9	46.9	3.5	0.4
Beef, lean, raw	95	490	19.2	4.4	0.0	0.0	0.3
Milk, whole	70	192	2.3	2.7	3.3	0.0	0.4
Lasagne, boiled	70	298	2.1	0.4	15.4	1.0	Tr
Peas, frozen	60	168	3.4	0.6	5.6	4.2	17.4
Onions	20	19	0.2	0.0	1.0	0.3	0.1
Olive oil	18	666	0.0	18.0	0.0	0.0	10.4
Tomato purée	10	29	0.5	0.0	1.3	0.0	0.8
Fat (70%) spread	5	130	0.0	3.5	0.0	0.0	0.7
Butter	4	121	Tr	3.3	Tr	0.0	0.3
Flour, plain	4	58	0.4	0.1	3.1	0.1	Tr
Total	611	3236	37.6	35.0	81.4	10.4	40.4

CHO, carbohydrate; Tr, trace.

Analysis of phylloquinone in meals

The extraction of phylloquinone from the meals was based on the method described by Booth *et al.* (37). Quantification was performed by the standard addition method. Chemicals were purchased from VWR (VWR International Ltd, Poole, UK). One meal from each batch was defrosted and heated in the same way as for the subjects' meals, then blended with an equal amount of warm purified water. A 30 g portion was homogenised using an IKA Ultra Turrax T25 basic homogeniser (Esstab, Essex, UK) and 2 g of the homogenised meal was transferred to a pestle and mortar and ground with 18 g of anhydrous sodium sulphate. One gram of the mixture was weighed into a 50 ml polypropylene centrifuge tube (Sarstedt Ltd) and 30 ml of 2-propanol–hexane (3:2, v/v) and 10 ml of purified water were added. At this stage, 3 ng of phylloquinone (Supelco, Poole, Dorset, UK) in 100 μl of hexane was added to half the tubes and to the remaining tubes was added 100 μl of hexane only. The tubes were vortexed for 3 min and then sonicated for 3 min with a Microsonix XL2000 model with 1/8 in. tapered microtip (Microsonix, New York, NY, USA). The tubes were further

vortexed for 3 min and then centrifuged at 2000 g for 5 min. From the top layer, 9 ml of hexane was removed and transferred to a disposable culture tube (16 \times 100 mm, Corning Ltd, Hemel Hempstead, UK) and evaporated under N_2 at 45°C. The contents of the tube were reconstituted in 300 μl of hexane and further purified by solid-phase extraction using 500 mg silica columns (Sep-Pak-RC™ 500 mg silica, Waters, Hatfield, Hertfordshire, UK). The columns were conditioned with 4 ml diethyl ether–hexane (3.5:96.5 v/v) and then 4 ml of hexane. The sample was added and washed with 6 ml of hexane before elution with 7 ml of diethyl ether–hexane (3.5:96.5, v/v). The use of a C18 solid phase extractor purification step was also investigated, but significant losses occurred during the washing steps and we found silica solid phase extractor alone provided sufficient purification. The sample eluent was collected into disposable glass tubes (13 \times 100 mm, Fisher Scientific Ltd, Loughborough, Leics, UK) and evaporated to dryness in a vacuum evaporator (Savant, New York, NY, USA). The samples were reconstituted in 200 μl of dichloromethane and 800 μl of methanol and analysed by HPLC. Extraction and analysis was performed in duplicate.

Quantitative analysis

Meal and total plasma phylloquinone were measured by HPLC with fluorescence detection after post-column reduction⁽³⁸⁾. Long-term reproducibility of quality control plasma samples was assessed by their analysis in parallel with the unknowns to ensure the quality assurance in routine analysis. The lower limit of detection of phylloquinone by the described HPLC method is 0.04 nmol/l⁽³⁸⁾. The inter-assay CV of lyophilised human plasma standards (Immunodiagnostik AG, Bensheim, Germany) were 4.3% (mean 0.48 nmol/l, *n* 8) and 1.7% (mean 1.50 nmol/l, *n* 8). The laboratory also participates in the vitamin K external quality assurance scheme run by the Human Nutristasis Unit at St Thomas' Hospital.

Isotope ratio analysis

Isotope ratio measurements of phylloquinone in plasma were performed by GC/MS after extraction and derivatisation as described previously⁽³⁰⁾. Briefly, sample extraction and clean-up was achieved by enzyme hydrolysis with lipase and cholesterol esterase, deproteination with ethanol, extraction with hexane and solid-phase extraction. Isotopic composition was determined in the pentafluoropropionyl derivative of phylloquinone. Analysis was performed on an Agilent GC/MS 5973N inert system (Agilent Technologies, Stockport, UK) comprising a 6890 GC with autosampler and equipped with on-column injection. The chromatography used a DB5-MS fused-silica capillary column (15 m × 0.25 mm inner diameter, 0.25 μm film thickness). Ions 598.4 *m/z* to 602.4 *m/z* (M to M + 4) were monitored in selected ion monitoring and high-resolution mode. Isotope ratios and thus tracer concentrations were calculated using the M + 1/M (599.4/598.4 *m/z*) ratio. The limit of quantification of the GC/MS method is 0.3 nmol/l of total phylloquinone (labelled + unlabelled (endogenous and from the meal) and inter-assay precision is about 3%⁽³⁰⁾. Isotopomer ratios were calculated using the fitting methods of Bluck & Coward⁽³⁹⁾ from the raw data generated by the GC/MS.

Calculations

Area under the curve (AUC) was used to assess absorption of the labelled phylloquinone tracer and was calculated using the trapezoid rule. Tracer AUC data assessed the effect of the meal on bioavailability of labelled phylloquinone from the capsule ('meal effect'). AUC values were checked for normal distribution by observing a quintile–quintile plot. AUC for tracer measurements were compared using linear regression with fixed effects for meal, subject and period.

Tracer concentration was calculated as

$$\text{Tracer concentration} = \frac{(R_{M+1}(t) - R_{M+1}(0))}{1 + (R_{M+1}(t) - R_{M+1}(0))} \times C(t),$$

where R_{M+1} is the ratio of M + 1 to M and at baseline (0) and subsequent time points (*t*). $C(t)$ is the concentration of total (i.e. labelled + unlabelled) phylloquinone, assessed by HPLC.

For comparisons of the bioavailability of phylloquinone from the meals, a different approach was taken. In each instance, if the absorption profile with time of phylloquinone from the meal were the same as that of the tracer, then the

relationship between them would be linear and with a 1:1 ratio after normalisation for the dose given by adjusting concentrations for the amount of phylloquinone provided as tracer or in the meal. By measuring the slope of regressions between normalised concentration of tracer and tracee from the meal, it was possible to measure the relative absorption of the phylloquinone from each meal compared with the tracer. This 'matrix effect' describes the extraction efficiency of phylloquinone from the meal. Data manipulation was performed in Excel (Microsoft, Seattle, WA, USA) and statistics were performed using STATA version 9.1 (StataCorp LP, College Station, TX, USA).

Results

Mean baseline plasma phylloquinone concentration was 0.35 (SD 0.30) nmol/l. Mean baseline intra-individual CV was 48%. The maximum total plasma phylloquinone increment in any subject was 2.5 nmol/l.

Phylloquinone in test meals measured by HPLC was 19.9 μg in the convenience meal, 26.3 μg in the cosmopolitan meal and 33.0 μg in the animal-oriented meal. The CV of results for duplicate extraction and analyses was <4%. For subsequent calculations, the measured meal phylloquinone content was used rather than that calculated from food tables.

The means of tracer AUC measurements were 0.88 (SD 0.43), 1.30 (SD 0.48) and 1.13 (SD 0.60) nmol/l × h for the convenience, cosmopolitan and animal-oriented meals, respectively. Individual values are shown in Table 2. Significantly more phylloquinone tracer was absorbed when consumed with the cosmopolitan and animal-oriented meals than the convenience meal ($P=0.001$ and 0.035 , respectively). We did not detect any significant difference between the cosmopolitan and animal-oriented meals ($P=0.120$). There were no significant differences in AUC between sexes, calculated using Student's *t* test (convenience $P=0.83$, cosmopolitan $P=0.88$, animal-oriented $P=0.81$). Calculation of AUC between 0–5 h and 5–8 h tested the possible influence of the 5 h snack on appearance of tracer phylloquinone in plasma. However, differences between meals in tracer absorption were similar before and after the snack. Profiles of plasma tracer against time are shown in Fig. 1.

Table 2. Tracer area under the curve (AUC) values for each individual and each meal

Subject	Tracer AUC (nmol/l × h)		
	Convenience	Cosmopolitan	Animal-oriented
A	0.81	1.11	0.85
B	0.64	0.94	0.84
C	1.04	1.71	1.31
D	2.04	1.87	2.44
E	0.94	2.03	1.51
H	0.30	1.11	0.45
I	0.97	0.94	0.96
J	0.51	0.83	0.57
K	0.82	1.02	1.59
L	0.78	1.99	1.49
M	0.69	0.64	0.27
N	1.03	1.40	1.23

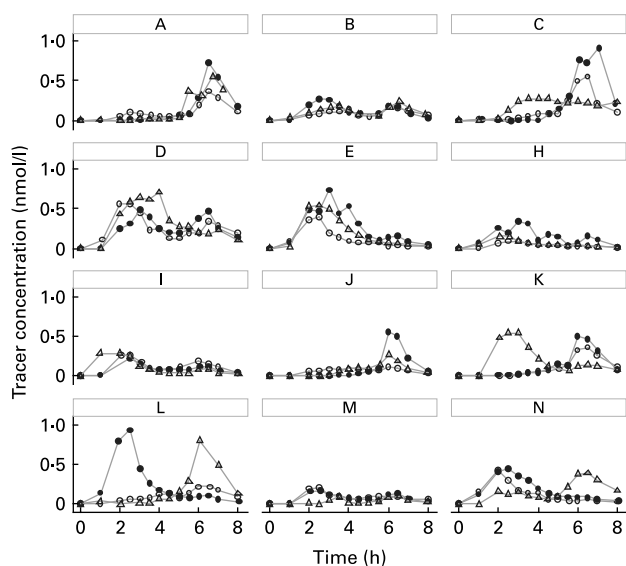


Fig. 1. (A–N) Plasma phylloquinone tracer concentration *v.* time profiles for each subject, after oral administration of 20 µg ¹³C-labelled phylloquinone consumed together with the convenience meal (○), cosmopolitan meal (●), or animal-oriented meal (△).

Means of the slopes of the relationship between tracer and meal tracee after adjustment for dose were 1.88 (SD 0.81), 0.59 (SD 0.32) and 0.43 (SD 0.40) for convenience, cosmopolitan and animal-oriented meals, respectively. Final relative bioavailability values (Table 3) between meals were calculated by multiplying values for the matrix and meal effects and are expressed relative to the convenience meal.

Discussion

The primary aim of the present study was to develop and apply a stable isotope-based method to measure phylloquinone bioavailability. We proposed a method using ¹³C-labelled tracer as a standard, taken at the same time as a test meal. Changes in plasma phylloquinone isotopic enrichment could then be used to calculate relative bioavailability of phylloquinone from the meals. However, dominance of the meal effect resulted in significant differences in tracer absorption between the meals. As a consequence, it was not possible to use the tracer as a global standard by which to compare phylloquinone absorption. An alternative approach was adopted where relative bioavailability of phylloquinone from the meals was assessed on the basis of the relationship between tracer and tracee absorption profile with time. Thus, two, separate but interacting, determinants to bioavailability can be considered.

Firstly, meal composition affects the bioavailability of free phylloquinone. This meal effect is the result of the meal modifying conditions within the gut and affecting digestion and/or absorption, and the potential enhancers or inhibitors of absorption. The second determinant, matrix effects or bioaccessibility, relate to the extraction of phylloquinone from the meal constituents. The appearance in plasma of ¹³C-labelled phylloquinone was used to determine meal effects, whereas the relationship between tracer and tracee phylloquinone was used to determine matrix effects. A final relative bioavailability value for each of the meals was determined as the product of the matrix and meal effects.

Absorption of phylloquinone from the capsule was significantly lower when taken with the convenience meal compared with the other meals. Such variation in absorption between meals has also been observed for stable isotope-labelled vitamin E⁽²⁶⁾. Various meal factors such as viscosity, particle size, meal volume, macronutrient content and energy density determine gastric emptying and intestinal transit⁽⁴⁰⁾, and these may affect phylloquinone bioavailability. To minimise differences in gastric emptying between the meals, all were balanced for fibre content, energy and percentage of energy from fat, protein and carbohydrate. However, the convenience meal had a 50% greater energy density that may have influenced phylloquinone absorption. Borel *et al.*⁽⁴¹⁾ investigated the effect of emulsified fat globule size on the absorption of vitamins A and E and concluded that the size of the particles does not affect absorption. Fat is required to stimulate bile secretion that is necessary for the absorption of phylloquinone⁽⁴²⁾. Butter increased phylloquinone absorption from spinach 3-fold⁽¹⁹⁾, but another study reported no difference in phylloquinone absorption between lettuce consumed with either 30 or 45% energy from fat⁽²⁰⁾. Meals in the present study contained similar amounts of fat, thus total fat is unlikely to explain the differences in absorption between meals. The fatty acid composition of a meal may affect absorption and plasma response by changing the physical characteristics of mixed micelles and/or influencing post-prandial lipid metabolism. Chylomicrons are the major carrier of postprandial phylloquinone⁽⁴³⁾ and chylomicron remnants transport phylloquinone to both liver and also bone⁽⁴⁴⁾. The size of mixed micelles may be important since longer chain fatty acids reduced phylloquinone absorption⁽⁴⁵⁾. PUFA decreased phylloquinone absorption *in vitro*⁽⁴⁵⁾ and rat⁽⁴⁶⁾ experiments, possibly through a greater affinity of the mixed micelle for phylloquinone decreasing transfer across the enterocyte. More recent studies in human subjects have shown that a PUFA-rich maize oil diet resulted in lower plasma phylloquinone compared with a diet enriched with

Table 3. Summary of meal and matrix effects and combined relative bioavailability values for the three test meals

Meal	Meal effect*	Meal effect (normalised)	Matrix effect†	Matrix effect (normalised)	Total effect‡
Convenience	0.88	1.00	1.88	1.00	1.00
Cosmopolitan	1.30	1.48	0.59	0.31	0.46
Animal-oriented	1.13	1.28	0.43	0.23	0.29

Values are normalised to the convenience meal.

* Meal effect refers to the effect of meal on absorption of tracer vitamin K₁ calculated from area under the curve measurements of plasma tracer concentration.

† Matrix effect refers to the bioavailability of vitamin K₁ from within the food matrix, relative to the tracer.

‡ The total effect is calculated as the product of the meal and matrix effects.

olive/sunflower oil⁽⁴⁷⁾. In the present study, the convenience meal contained more than 2-fold greater PUFA (37% of total fat) than both the cosmopolitan (19%) and animal-oriented meals (14%). Inhibition of phyloquinone uptake or altered postprandial metabolism by the high PUFA convenience meal could explain the lower absorption of phyloquinone from the capsule. Evidence suggests that vitamin E may affect phyloquinone status in rats^(48,49); however, other studies have shown that effects are limited to tissue status, not plasma status, both in animal⁽⁵⁰⁾ and in human studies⁽⁵¹⁾, thus it is likely that any effect is related to metabolism rather than absorption^(47,52). In the present study, the vitamin E content of each meal, calculated using food composition tables, was 11.0, 5.3 and 5.8 mg for the convenience, cosmopolitan and animal-oriented meals, respectively.

Observation of the tracer profiles in Fig. 1 reveals that in the majority of cases, an individual's plasma tracer concentration profile shows a similar pattern for each test meal, and suggests that the differences in tracer profiles reflect genetic or physiological differences between individuals, e.g. transfer of phyloquinone across the enterocyte or chylomicron metabolism (apo E genotype). High intra-individual consistency in absorption profiles of carotenoids has also been observed⁽⁵³⁾. Although in general tracer profiles within individuals were similar for the different meals, subjects K, L and N showed substantial intra-individual differences in tracer absorption profile between the meals that may have confounded the results. As far as practicable external influences on digestion and absorption were controlled (such as previous meals, fasting and seating position), it is possible that these may have affected the absorption profile. In addition, particularly where absorption was apparently delayed, tracer concentration remained elevated beyond the 8 h duration of the experiment thus similar future studies might consider increasing the sampling period. These observations demonstrate that consideration should be given to potential variability of the absorption profile in bioavailability studies, particularly where absorption is assessed on the basis of a measurement at a single time point⁽⁵⁴⁾, and further supports the importance of crossover studies. Although generally there was some consistency in the extent of tracer absorption between the different meals for the same individual (assessed by rank; Table 2), the potential variability in absorption of phyloquinone from the same meal eaten on different occasions for a given individual is unknown and may have influenced the data. A single study has reported the intra-individual variation of phyloquinone absorption, albeit of a very large dose of a pharmaceutical preparation of phyloquinone (2.2 μ mol Konakion). CV of the AUC measurements for three individuals each measured on three occasions were 18, 7 and 9%⁽¹⁹⁾.

In a number of individuals, tracer phyloquinone peaked between 5 and 8 h, or a second smaller peak was observed, apparently in response to the 5 h snack. Although there were no differences in AUC before and after the snack, the snack may influence the plasma tracer profile. In some individuals, the physiological response to the snack may be the release of partially absorbed phyloquinone into the circulation. Dueker *et al.*⁽⁵⁵⁾ reported a similar observation with a second plasma peak of labelled β -carotene. A comparable phenomenon has also been reported with fatty acids, where fatty acids consumed during an initial meal appear in the

circulation after a second meal a number of hours later^(56,57). An alternative explanation for the second peak is hepatic secretion of VLDL containing phyloquinone^(27,43,58).

In contrast to tracer absorption, and on the basis of the matrix effect alone, absorption of phyloquinone from the convenience meal was more than 3-fold than that from the cosmopolitan and animal-oriented meals. While the magnitude of this difference was reduced with the inclusion of the meal effect (tracer data), total relative bioavailability remained greater from the convenience meal. Greater absorption of phyloquinone from the convenience meal may be expected given the sources of phyloquinone. In the convenience meal, it can be estimated that more than 80% of the phyloquinone was in the oil phase, whereas in the cosmopolitan and animal-oriented meals only 10 and 20% of phyloquinone was in oil, respectively. Conversely, the majority of phyloquinone in the cosmopolitan and animal-oriented meals was sited within a vegetable matrix where it is tightly bound to the thylakoid membranes and may be less bioavailable because cell walls and membranes must be digested before absorption. Although it is generally assumed that fat-soluble vitamin absorption is greater from fat than from vegetables, there have been few direct comparisons⁽⁵⁹⁾. Previous studies to compare the bioavailability of phyloquinone from oil and vegetables have shown conflicting results. In one study, absorption, calculated by 24 h AUC, was reported to be significantly greater from oil than from broccoli⁽²³⁾ but an earlier study, which measured absorption of phyloquinone from multiple meals over 5 d, found no difference in absorption between these two sources⁽²²⁾. A number of caveats are applicable to our interpretation of the matrix effect data. Firstly, due to low phyloquinone content of the meals and low absorption, the plasma phyloquinone content attributable to phyloquinone from the meal was towards the lower end of analytical limits. Plasma phyloquinone concentration from the meal across the time course of the experiment was between 0.2 and 1 nmol/l. Secondly, and as discussed earlier in relation to the tracer data, there was variability in the pattern of phyloquinone appearance in plasma and in responses with the different meals, both of which may have influenced our interpretation of the matrix effect. Thirdly, although the mixed meal provides the opportunity to assess the combined impact of foods, interpretation of these effects is complicated by the many interacting factors.

Previous phyloquinone absorption studies have primarily tested single foods, either individually or in combination with untypical food combinations. The meal-based method described here was an attempt to use a less subjective approach to the design of test meals with the aim that they represent more accurately meals in a free-living situation in which food components may interact to negate effects observed in experiments with individual foods. The three meals in the present study were designed using the characteristics of dietary clusters identified in a national nutrition survey. The use of dietary analysis in this way provides a basis for the design of test meals in bioavailability experiments that can be used to probe relationships between dietary intake and plasma response and/or status. The meals were designed to contain equal amounts of phyloquinone, with the major difference between the meals, the sources of phyloquinone. However, direct analysis of meals revealed

variation in the measured value compared with that calculated from nutrient tables. The observed discrepancy however is not unusual; the phylloquinone content of foods is known to be highly variable and affected by heating⁽⁶⁰⁾. Furthermore, a comparison of the phylloquinone content of ten meals calculated by two nutrient databases compared with direct analysis showed variation of up to 89%⁽⁶¹⁾.

In summary, we have investigated a meal-based approach to measurements of absorption, which is more relevant to the consumption of foods in a free-living population. The apparent dominance of the meal effect showed that different meals affect absorption of phylloquinone, although the mechanism is unknown. Notwithstanding the potential limitations, the data also suggest that phylloquinone may be more bioavailable from oils and fats than from vegetable sources.

Acknowledgements

We would like to acknowledge the UK Food Standards Agency for funding this work (project number N05050). We would like to thank Louise McKenna for assistance in sample extraction and analysis, and the volunteers for participating in the study. We also thank Dr Adrian Mander and Mark Chatfield for statistical advice. There are no conflicts of interest. K. S. J., L. J. C. B., A. M. S., C. J. P. and W. A. C. all contributed to the design of the study. K. S. J. performed the actual study including meal design and preparation, volunteer work, GC/MS analysis of plasma, food extractions, and data analysis. L. Y. W. performed all the HPLC analysis. K. S. J. drafted the manuscript with input from all co-authors.

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