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Plasma phylloquinone (vitamin K_1) concentration and its relationship to intake in a national sample of British elderly people

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Plasma phylloquinone (vitamin K_1) concentration was examined according to season, socio-demographic and lifestyle factors and phylloquinone intake in a nationally representative sample of British people aged 65 years and over from the 1994–5 National Diet and Nutrition Survey. Values for both plasma phylloquinone concentration and phylloquinone intake were available from 1076 participants (561 men, 515 women). Eight hundred and thirty-four were living in private households, 242 in residential or nursing homes. Weighted geometric mean plasma phylloquinone concentrations were 0·36 (95 % CI 0·06, 2·01) and 0·24 (95 % CI 0·06, 0·96) nmol/l in free-living and institution samples respectively. Plasma phylloquinone concentrations did not generally differ between men and women, although values in free-living people were significantly lower during autumn and winter (October to March). Plasma phylloquinone concentration was not significantly associated with age. Plasma phylloquinone concentrations were positively correlated with phylloquinone intake in free-living men and women (r 0·18 and 0·30 respectively, both $P<0·001$). Stepwise multiple regression analysis found that 11 % of the variation in plasma phylloquinone concentration was explained by phylloquinone intake, season and plasma triacylglycerol concentration. After adjustment for age and corresponding nutrient intakes, plasma phylloquinone concentration was significantly associated (each $P<0·01$) with plasma concentrations of triacylglycerol, cholesterol, retinol and 25-hydroxyvitamin D in free-living women but not men, and with plasma concentrations of carotenes, α- and γ-tocopherols and lutein in free-living men and women. The possibility of concurrent low fat-soluble vitamin status in elderly populations may be a cause for concern.

Vitamin K status: Dietary intake: Fat-soluble vitamins

Interest in the functions of vitamin K has grown, almost exponentially, as the number of identified γ-carboxylated (vitamin K-dependent) proteins has increased (Ferland, 1998; Shearer, 2000*a*). Two of these proteins, osteocalcin (OC) (involved in bone mineralisation) and matrix Gla protein (involved in soft tissue, including vascular, calcification), may play major roles in the development of osteoporosis and cardiovascular disease respectively (Luo *et al.* 1997; Shearer, 1997, 2000*a*). As such, identification of vitamin K status in populations, particularly in the elderly, has become of public health importance.

Vitamin K occurs naturally in foods, mainly as phylloquinone (vitamin K_1), but also, to a much lesser extent, as menaquinones (MK; vitamin K_2) that occur in cheese, other fermented dairy foods, eggs and fermented soya-containing foods. Phylloquinone is the predominant form of vitamin K in the circulation and since it can be measured reliably by HPLC, plasma phylloquinone concentration can be used as a measure of vitamin K status. Although it is not a functional biomarker (such as undercarboxylated OC or undercarboxylated prothrombin), there is evidence that plasma concentrations broadly reflect tissue status and total body stores. Plasma phylloquinone concentration is considered to be a valid index of vitamin K status and has been shown to be very highly correlated with the carboxylation status of serum OC (Sokoll & Sadowski, 1996). Circulating phylloquinone concentrations are thought to be derived exclusively from the diet (Shearer, 2000*b*) and not by metabolic conversion from other forms of vitamin K. They are sensitive to changes

Abbreviations: MK, menaquinone; OC, osteocalcin.

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in recent dietary intake (Bolton-Smith *et al.* 1998) and have been shown to fall in response to diets that have been moderately (Suttie *et al.* 1988) or severely depleted (Ferland *et al.* 1993) of phylloquinone.

Only a few studies have reported plasma phylloquinone concentrations in elderly people (Sadowski *et al.* 1989; Booth *et al.* 1995), with none previously being available for the UK. The present study reports plasma phylloquinone concentrations in older adults, and the relationships between plasma phylloquinone concentration and season, socio-demographic and lifestyle factors. The relationships between plasma phylloquinone concentration and phylloquinone intake, together with other biochemical indices of nutritional status, were also investigated in the free-living sample in order to assess whether evidence of low or high vitamin K status was mirrored in the status of other, both water- and fat-soluble, micronutrients.

Subjects and methods

These analyses used data from a nationally representative sample of older adults living in mainland Britain, who participated in the 1994–5 National Diet and Nutrition Survey of people aged 65 years and over. Full details and methods are provided in the survey report (Finch *et al.* 1998), and so only a brief description is given here, together with details of the types of analyses used in the present study.

After obtaining ethical approval for the survey from the National Health Service Local Research Ethics Committees for each of the eighty postcode sectors involved, a stratified random sample of participants was obtained. Participants were living in private households ('free-living', 79 % of the total) or in institutions (i.e. residential and nursing homes, 21 % of the total), with both types of domicile represented in each postal sector. The survey fieldwork was conducted from October 1994 to September 1995, in four 'waves' corresponding to the four seasons. For statistical precision, predetermined numbers of men and women were recruited from each of three age groups: 65–74, 75–84 and 85+ years, requiring deliberate over-sampling of men and of the older age groups. Socio-demographic and lifestyle information was obtained by trained fieldworkers (Finch *et al.* 1998).

Blood samples and plasma phylloquinone concentration

In almost all cases, early morning venous blood samples were obtained, by venepuncture, after an overnight fast. Blood samples were taken to coincide with the period of dietary assessment as closely as possible, with the majority of blood samples taken within 7 d of the dietary assessment.

Plasma phylloquinone concentrations were analysed from heparinised plasma samples, which had been stored at −80°C for around 5 years, and undergone up to two freeze–thaw cycles. Plasma phylloquinone concentrations were determined using a three-stage procedure comprising: (1) lipid extraction; (2) solid-phase extraction; (3) analytical HPLC. An internal standard, MK-6, was used to compensate for losses of phylloquinone during the extraction process and subsequent chromatographic procedures. Plasma lipids from 0·5 ml samples of plasma were extracted into hexane after flocculation of proteins with ethanol (Shearer, 1991; McCarthy *et al.* 1997). The lipid extract was purified by solid-phase extraction using Sep-Pak silica cartridges (Shearer, 1991). Finally, the vitamin K-rich fraction was analysed by reversed-phase HPLC with detection by post-column reduction and fluorimetric detection as described by Haroon *et al.* (1986) and Davidson & Sadowski (1997) with slight in-house modifications. In this final stage, vitamin K compounds were resolved on a C18 Hypersil 3 μm base deactivated silica column, passed through a Zn column which, in the presence of Zn ions (provided by the mobile phase), reduced them to their fluorescent hydroquinones. The vitamin K hydroquinones of phylloquinone and the MK-6 internal standard were detected using a Jasco model FP-920 fluorescence detector (Jesco Corp., Tokyo, Japan) with excitation at 330 nm and emission at 430 nm. Using this method of analysis, no MK-6 or other menaquinones (MK-4 to MK-10) were detectable in plasma samples. Phylloquinone was quantified by the method of internal standardisation by reference to a calibration graph obtained by the injection of appropriate phylloquinone and MK-6 standards. This method had a lower limit of quantification of 0·11 nmol/l (0·05 ng/ml). Concentrations below this limit were arbitrarily assigned a value of 0·055 nmol/l (0·025 ng/ml), in order to include these detectable, but not reliably quantifiable, concentrations in the statistical analyses.

One of the authors' (MJS) laboratory runs the International Quality Assurance Scheme for phylloquinone analyses. For each set of plasma analyses, a number of quality control plasma samples (not less than 10 % of the unknowns) were analysed in parallel with the unknowns. For two different quality control plasma stocks used during the present study, intra-assay precisions (CV) were 7·1 % (*n* 12) and 9·7 % (*n* 18) respectively, while inter-assay precisions were 12·4 % (*n* 86) and 12·6 % (*n* 88) respectively.

Dietary assessment and phylloquinone intake

Details of the dietary assessment methodology and phylloquinone intake data have been reported elsewhere (Thane *et al.* 2002). Briefly, free-living participants kept a 4 d weighed record of all food and drink consumed. Phylloquinone intakes were estimated using both published (Bolton-Smith *et al.* 2000) and unpublished (MJ Shearer and C Bolton-Smith, unpublished results) phylloquinone content data (UK-specific wherever possible) for a comprehensive range of foods. Individual phylloquinone content values were assigned to the main contributors of phylloquinone intake (vegetables, fats and oils, cereals and cereal products), while average phylloquinone content values were used for the remaining eighty-seven subsidiary food groups. Phylloquinone contents of dietary supplements were also included, as obtained by direct analysis or from the manufacturers.

Data analysis

Data were weighted, using a population weighting factor,

to correct for disproportionate sampling (Finch *et al.* 1998). The resulting weighted samples then had similar profiles for gender, age and region of habitation as those of the population aged 65 years and over from the 1991 census data. Data from the two groups of participants (free-living or living in institutions) were analysed separately. Data reduction and analyses were carried out using Excel (Microsoft Corp., Redmond, WA, USA) and SPSS (SPSS Inc., Chicago, IL, USA) respectively.

Associations were examined between plasma phylloquinone concentration and a number of socio-demographic and lifestyle factors, including their associations with 'low' plasma phylloquinone concentration (defined as values in the lowest fifth of the distribution for each type of domicile). Plasma phylloquinone concentration was examined according to age group, region, season, occupational social class of head of household, level of household income, household composition, educational attainment, smoking and drinking habits for free-living participants; and age group, region, season, smoking and drinking habits for those living in institutions.

Associations were also examined between plasma phylloquinone concentration and phylloquinone intake, and with other biochemical indices of nutritional status, namely plasma triacylglycerol, cholesterol, retinol, carotenoids, 25-hydroxyvitamin D and tocopherols, which were further adjusted for intakes of total fat, saturated fatty acids, and vitamins A, D and E, as appropriate. Correlation analyses between plasma phylloquinone concentration and phylloquinone intake only included free-living participants who were well with eating habits unaffected and who provided a complete 4 d dietary record. Forward stepwise multiple regression analysis was used to assess the proportion of variation in plasma phylloquinone concentration associated with the variation in season, socio-demographic and lifestyle factors (see earlier), and phylloquinone and energy intakes. This was confined to the free-living sample since the dietary assessment procedure used for these participants was judged to be more robust than that used for the institution sample. The impact of oral anticoagulants (warfarin and/or heparin) on plasma phylloquinone concentration was discounted since they were reported to be used by less than 1 % of participants.

Due to the skewed distributions of both plasma phylloquinone concentrations and estimated intakes, geometric means (with 95 % CI) are given throughout. These were obtained by back-transformation of $\log_e$-transformed values and represent better 'average' measures for such non-normally distributed data. ANOVA (restricted to main effects with no interaction terms) with Scheffé tests, multiple linear and logistic regressions, χ^2-tests and two-tailed Pearson's correlation coefficients were performed, with $P<0{\cdot}01$ deemed significant. For the free-living sample, gender-specific multivariate analyses were adjusted for age group, region, occupational social class of head of household and smoking status; while age group, region and smoking status were adjusted for those living in institutions.

Results

Plasma phylloquinone concentrations were analysed from 859 free-living people, after excluding one outlier with an unfeasibly high value (15·0 nmol/l). Of participants, 10 % (87/859) had concentrations below the limit of quantification and were assigned a value of 0·055 nmol/l. Weighted plasma phylloquinone concentrations showed a positively skewed distribution (geometric mean 0·36 (95 % CI 0·06, 2·01) nmol/l), with no gender difference (Table 1). Of the 243 people living in institutions, 16 % (38/243) had concentrations below the limit of quantification and so were similarly assigned a value of 0·055 nmol/l. For both types of domicile, the weighted proportions of participants with plasma phylloquinone concentrations below the limit of quantification were not associated with season or any of the assessed socio-demographic or lifestyle factors ($P\geq 0{\cdot}01$, multiple logistic regression). Weighted plasma phylloquinone concentrations also showed a positively skewed distribution in the institution sample (geometric mean 0·24 (95 % CI 0·06, 0·96) nmol/l), again with no gender difference (Table 1).

The weighted geometric mean plasma phylloquinone concentration in the institution sample was significantly lower than that in the free-living sample (0·24 *v.* 0·36 nmol/l; $P<0{\cdot}001$). Although weighted plasma phylloquinone concentrations in men living in institutions did not differ from those of free-living men, values in women were significantly lower (0·23 *v.* 0·37 nmol/l, $P<0{\cdot}001$). The 97·5 percentile and maximum values in these women

Table 1. Plasma phylloquinone concentrations in British people aged 65 years and over

		Plasma phylloquinone concentration (nmol/l)*				
Category†	*n**	Geometric mean	95 % CI	Inner 95 % range‡	Arithmetic mean	SD
Free-living sample	878	0·36	0·34, 0·38	0·06, 2·01	0·54	0·56
Men	385	0·34	0·31, 0·37	0·06, 1·84	0·50	0·52
Women	493	0·37	0·34, 0·41	0·06, 2·06	0·56	0·58
Institution sample	225	0·24	0·21, 0·26	0·06, 0·96	0·33	0·27
Men	60	0·26	0·21, 0·32	0·06, 1·73	0·36	0·32
Women	165	0·23	0·20, 0·26	0·06, 0·89	0·32	0·25

* Weighted sample sizes (*n*) and statistics are presented to correct for non-proportional sampling (see p. 616).
† Plasma phylloquinone concentrations did not differ significantly ($P\geq 0{\cdot}01$) between men and women for the respective types of domicile, after adjusting for age group, region, occupational social class of head of household and smoking status in both samples (ANOVA).
‡ 2·5–97·5 percentile range. Values of 0·06 nmol/l indicate $<0{\cdot}11$ nmol/l (limit of quantification for HPLC).

were also lower than in their free-living counterparts (0·89 and 1·55 *v.* 2·06 and 5·37 nmol/l respectively), whereas in men only the maximum value was lower (1·88 *v.* 5·98 nmol/l).

Variation by socio-demographic and lifestyle factors

In the free-living sample, weighted plasma phylloquinone concentrations varied significantly only with season in both men and women (Table 2). Concentrations in samples taken during autumn were significantly lower than those taken during summer. More subtle seasonal differences in weighted plasma phylloquinone concentrations occurred by gender. Weighted mean plasma phylloquinone concentrations in those aged 85 years and over tended to be lower than those in the two younger age groups (65–74 and 75–84 years), although the differences were not statistically significant due to the wide variation (indicated by the 95 % CI) resulting from much smaller weighted sample sizes.

Proportions of free-living men and women with low plasma phylloquinone concentrations (i.e. in the lowest fifth of the range) were also assessed according to season, socio-demographic and lifestyle factors. Proportions were associated only with season, with higher proportions of both genders having low plasma phylloquinone concentrations during autumn and winter than during spring and summer (20–30 *v.* 10–13 %).

In contrast to the free-living sample, weighted plasma phylloquinone concentrations in people living in institutions did not differ with season or any of the assessed socio-demographic or lifestyle factors (Table 3). Similarly, the proportion of participants with low plasma phylloquinone concentrations was not related to any of the assessed factors.

Association between plasma phylloquinone concentration and phylloquinone intake

Plasma phylloquinone concentration was significantly correlated with phylloquinone intake in free-living men and women (r 0·18 and 0·30 respectively, both $P<0·001$). The relationship was stronger in women, with around 9 % of the variation in plasma phylloquinone concentration explained by that in phylloquinone intake, compared with only 3 % in men. Further adjustment for age, plasma concentrations of triacylglycerol and/or cholesterol had no significant impact on the strengths of the relationships for either gender.

Forward stepwise multiple regression analysis found that 11 % of the variation in plasma phylloquinone concentrations was explained by three variables (phylloquinone intake, season and plasma triacylglycerol concentrations), with phylloquinone intake explaining 6 % of the total variation. Other socio-demographic and lifestyle factors (see p. 618) and energy intake were not selected for entry into the final multiple regression equation as significant independent determinants of plasma phylloquinone concentration. Adjustment for the number of days between dietary assessment and date of giving the blood sample did not influence the relationship between plasma phylloquinone concentration and phylloquinone intake.

Table 2. Plasma phylloquinone concentrations in free-living British people aged 65 years and over, by gender and selected socio-demographic and lifestyle factors*

	Men			Women		
Socio-demographic or lifestyle factor	*n*	Geometric mean†	95 % CI†	*n*	Geometric mean†	95 % CI†
Age group						
65–74 years	255	0·36	0·32, 0·40	270	0·38	0·34, 0·42
75–84 years	107	0·32	0·28, 0·38	149	0·40	0·34, 0·46
85+ years	16	0·25	0·16, 0·39	36	0·30	0·23, 0·40
Region						
Scotland and North	125	0·32	0·27, 0·39	134	0·36	0·30, 0·42
Central, South-West and Wales	143	0·38	0·33, 0·44	166	0·35	0·35, 0·45
London and South-East	110	0·32	0·27, 0·38	155	0·32	0·32, 0·44
Season						
Autumn (Oct–Dec)	109	0·26[a]	0·22, 0·31	125	0·31[a]	0·26, 0·37
Winter (Jan–Mar)	84	0·29[ab]	0·24, 0·35	110	0·36[ab]	0·30, 0·42
Spring (Apr–Jun)	80	0·48[c]	0·39, 0·58	97	0·42[ab]	0·35, 0·50
Summer (Jul–Sep)	105	0·41[bc]	0·35, 0·48	123	0·44[b]	0·38, 0·52
Social class of head of household						
Non-manual	182	0·37	0·32, 0·42	218	0·42	0·37, 0·47
Manual	196	0·32	0·28, 0·36	237	0·35	0·31, 0·39
Smoking status						
Non-smoker	309	0·35	0·31, 0·39	383	0·39	0·35, 0·43
Smoker	69	0·31	0·25, 0·39	72	0·33	0·26, 0·41

[a,b,c]Mean values for categories within respective socio-demographic or lifestyle factors with unlike superscript letters were significantly different (Scheffé test, $P<0·05$), after adjusting for age group, region, occupational social class of head of household and smoking status, following $P<0·01$ in ANOVA (see p. 618).

*Weighted sample sizes (n) and statistics are presented to correct for non-proportional sampling (see p. 616).

†Geometric mean and CI both back-transformed from $\log_e$(plasma phylloquinone concentration) (nmol/l).

Table 3. Plasma phylloquinone concentrations in British people aged 65 years and over living in institutions, by gender and selected socio-demographic and lifestyle factors*†

	Men			Women		
Socio-demographic or lifestyle factor	*n*	Geometric mean‡	95 % CI‡	*n*	Geometric mean‡	95 % CI‡
Age group						
65–74 years	11	0·35	0·21, 0·56	13	0·24	0·14, 0·41
75–84 years	24	0·26	0·18, 0·36	75	0·23	0·19, 0·28
85+ years	25	0·23	0·15, 0·34	77	0·23	0·19, 0·27
Region						
Scotland and North	21	0·28	0·20, 0·41	63	0·23	0·18, 0·28
Central, South-West and Wales	25	0·26	0·18, 0·37	62	0·21	0·17, 0·26
London and South-East	14	0·22	0·12, 0·40	40	0·26	0·20, 0·35
Season						
Autumn (Oct–Dec)	11	0·26	0·13, 0·52	43	0·19	0·16, 0·24
Winter (Jan–Mar)	17	0·19	0·12, 0·30	39	0·21	0·17, 0·27
Spring (Apr–Jun)	20	0·32	0·23, 0·43	48	0·31	0·24, 0·39
Summer (Jul–Sep)	12	0·29	0·16, 0·53	35	0·20	0·14, 0·29
Smoking status						
Non-smoker	47	0·24	0·18, 0·32	148	0·23	0·20, 0·27
Smoker	13	0·33	0·23, 0·47	17	0·22	0·14, 0·32

* Weighted sample sizes (*n*) and statistics are presented to correct for non-proportional sampling (see p. 616).
† Plasma phylloquinone concentration was not significantly associated ($P \geq 0{\cdot}01$) with any of the selected socio-demographic or lifestyle factors or with alcohol intake in either men or women, after adjustment for age group, region and smoking status in ANOVA.
‡ Geometric mean and CI both back-transformed from $\log_e$(plasma phylloquinone concentration) (nmol/l).

Association between plasma phylloquinone concentration and other blood analytes

After adjusting for age (since plasma concentrations of some analytes are affected by this factor), plasma phylloquinone concentration was directly associated with plasma concentrations of retinyl palmitate, α- and β-carotenes, lutein, α- and γ-tocopherols, pyridoxal phosphate and urea in free-living men and women (Table 4). Significant positive associations were also found, in women only, between plasma phylloquinone concentration and plasma concentrations of triacylglycerol, cholesterol, retinol, α-cryptoxanthin, lycopene, 25-hydroxyvitamin D, erythrocyte glutathione reductase activation coefficient (as an index of riboflavin status), Se and albumin (Table 4). Associations were not altered significantly after further adjustment for the corresponding nutrient intakes or intakes of energy and protein.

Table 4. Associations between plasma concentrations of phylloquinone and those of selected plasma analytes in free-living British people aged 65 years and over*

	Associations† with plasma phylloquinone concentration‡			
	Men (*n* 355–384)		Women (*n* 468–491)	
Plasma analyte	Partial *r*	*P*	Partial *r*	*P*
Triacylglycerols‡	0·10	0·04	0·16	<0·001
Cholesterol	0·08	0·10	0·22	<0·001
Retinyl palmitate‡	0·20	<0·001	0·27	<0·001
Retinol‡	0·05	0·31	0·14	0·002
α-Carotene‡	0·13	0·01	0·21	<0·001
β-Carotene‡	0·15	0·004	0·20	<0·001
α-Cryptoxanthin‡	0·01	0·78	0·13	0·003
Lutein‡	0·33	<0·001	0·35	<0·001
Lycopene‡	0·05	0·33	0·19	<0·001
25-Hydroxyvitamin D‡	0·08	0·12	0·21	<0·001
α-Tocopherol‡	0·19	<0·001	0·31	<0·001
γ-Tocopherol‡	0·20	<0·001	0·19	<0·001
EGRAC‡	−0·05	0·35	−0·13	0·005
Pyridoxal phosphate‡	0·13	0·01	0·19	<0·001
Se	0·07	0·17	0·23	<0·001
Albumin	0·08	0·14	0·15	0·001
Urea‡	0·18	0·001	0·16	<0·001

EGRAC, erythrocyte glutathione reductase activation coefficient (index of riboflavin status).
* Weighted sample sizes (*n*) and statistics are presented to correct for non-proportional sampling (see p. 616).
† Adjusted for age.
‡ $\log_e$-transformed values.

Discussion

The present study provides unique normal ranges for plasma phylloquinone concentration in a representative sample of older British adults. This representative sampling probably accounts for the far greater variation in plasma phylloquinone concentrations in the inner 95 % of the distribution (30-fold for free living and 16-fold for participants living in institutions) than the 8-fold variation reported by Sadowski *et al.* (1989). It is unlikely that appreciable loss of phylloquinone occurred as a result of the storage of the blood samples for around 5 years at −80°C, hence it is of some public health concern that 10 and 16 % of these respective free-living and institution participants had plasma phylloquinone concentrations below the lower limit of quantification (0·11 nmol/l). Such low concentrations have been associated with sub-clinical and even clinical vitamin K deficiency (MJ Shearer, unpublished results). If extrapolated to the general population, vitamin K status (as indicated by plasma phylloquinone concentration) may be critically low in a sizeable minority of elderly British people. The lower plasma phylloquinone concentrations in the institution sample compared with the

free-living sample could be a reflection of poorer absorption or altered metabolism in these generally more infirm elderly participants.

Comparison of plasma phylloquinone concentrations between studies is not necessarily straightforward, for although an international quality assurance scheme is now operating, data may be reported as arithmetic or geometric means, from fasted or non-fasted samples, and with or without adjustment for concentrations of plasma lipids. Fasted blood samples were taken in the present survey to reduce the possibility of concentrations being influenced by post-prandial lipidaemia (Shearer, 1992). Compared with values in this National Diet and Nutrition Survey sample (Table 1), arithmetic mean plasma phylloquinone concentrations in sixty-five free-living Scottish adults (20–55 years) were 0·75 (SD 0·28) and 0·68 (SD 0·23) nmol/l in men and women respectively (Bolton-Smith *et al.* 1998). Mean National Diet and Nutrition Survey plasma phylloquinone concentrations were also lower than the few estimates in similar age groups from the USA (Sadowski *et al.* 1989; Booth *et al.* 1995) and the Netherlands (Schurgers *et al.* 1999), where mean values were greater than 0·92 nmol/l.

Seasonality in plasma phylloquinone concentration was found in the present study, and by Sadowski *et al.* (1989) in young and elderly people in New Mexico, USA, but has not been reported by others (Sokoll & Sadowski, 1996; Fenton *et al.* 2000). Both the existence and measurement of seasonality may depend on the population being studied and the methodologies used. The seemingly paradoxical differences in plasma phylloquinone concentrations but not phylloquinone intake by season and *vice versa* by region (Thane *et al.* 2002) may simply reflect the practical difficulties of standardised sample handling, storage and dietary assessment methods in large national studies.

Although the correlations between plasma phylloquinone concentration and phylloquinone intake were highly significant in free-living men and women (both $P<0·001$), the relationships were weak (r 0·18 and 0·30 for men and women respectively). Most other studies have shown significant correlations ($P<0·05$) between serum or plasma phylloquinone concentrations and dietary phylloquinone intakes (Booth *et al.* 1995, 1997; Bolton-Smith *et al.* 1998; Rock *et al.* 1999; Sakamoto *et al.* 1999), although these have not been shown by all ($P=0·22$, Schurgers *et al.* 1999). Weak or non-existent relationships may result from a lack of juxtaposition of the dietary and plasma assessments, since foods with a very high phylloquinone content tend to be consumed irregularly and plasma phylloquinone concentrations tend to reflect only the previous few days' intake (Sokoll *et al.* 1997).

The direct relationships between plasma concentrations of phylloquinone and plasma lipids and fat-soluble vitamins may be explained by their coexistence in foods (green vegetables for lutein and α- and β-carotenes, and vegetable oils for α- and γ-tocopherols), and their absorption and co-transport in lipoprotein particles (Kohlmeier *et al.* 1996; Lamon-Fava *et al.* 1998). The reasons for the far stronger associations in women than in men are currently unknown, although it may be speculated that hormonal differences or lipoprotein sub-types might influence transport and clearance differentially between the genders. The lack of relationship between plasma phylloquinone and cholesterol, and weaker relationship with triacylglycerol, in men compared with women has also been noted previously in a Scottish population (C Bolton-Smith and MJ Shearer, unpublished results).

The relationship between the plasma concentrations of phylloquinone and 25-hydroxyvitamin D in women was unexpected since they are transported by completely different mechanisms (phylloquinone by lipoproteins, 25-hydroxyvitamin D by a specific carrier protein) and their dietary sources are disparate. However, this finding is of particular interest in relation to bone health since a synergistic effect of phylloquinone (200 μg/d) and vitamin D (10 μg/d) plus Ca (1 g/d) supplementation was found in a 2-year intervention study of Scottish women aged 60–85 years (Bolton-Smith *et al.* 2001) with regard to an increase in bone mineral content at the ultra-distal radius site. A similar relationship has been reported previously, in a group of twenty-seven elderly Japanese men, in whom depressed concentrations of both analytes were suggested to participate concomitantly and cooperatively in their observed osteopenia (low bone mineral density) (Tamatani *et al.* 1998). Vitamin D regulates OC protein synthesis, and has been suggested to influence γ-carboxylation of OC (Binkley & Suttie, 1995). In a French study of elderly women undercarboxylated OC (a functional index of low vitamin K status) was negatively correlated with serum 25-hydroxyvitamin D, mirrored the circ-annual rhythm of 25-hydroxyvitamin D and was decreased by supplementation with vitamin D and Ca (Szulc *et al.* 1993).

Although suitably collected and stored plasma samples were not available for the analysis of OC in this population, plasma phylloquinone concentration was inversely correlated with serum undercarboxylated OC concentration in a study of 263 healthy American men and women aged 18–85 years (r −0·35, $P<0·001$) (Sokoll & Sadowski, 1996). Similarly, serum phylloquinone concentration was correlated inversely with serum undercarboxylated OC concentration (r −0·19, $P=0·005$) and directly with serum carboxylated OC concentration (r 0·13, $P<0·05$) in 244 healthy post-menopausal Scottish women aged 60–85 years (C Bolton-Smith and MJ Shearer, unpublished data). These results suggest that plasma phylloquinone concentrations at the population level are related to carboxylation status of OC in the circulation and indicate that plasma phylloquinone concentration may be a valid index of vitamin K status.

In addition to phylloquinone, vitamin K status may also be affected by MK which are derived from the diet and/or endogenously produced by gut bacteria, and by dihydrophylloquinone, which is produced by hydrogenation of vegetable oils. Neither were assessed in the current study. However, evidence suggests that the role of both may be small. The contribution of gut bacteria-derived MK (MK-7 to MK-9) to vitamin K status is questionable (Shearer, 1992; Conly *et al.* 1994; Suttie, 1995) and a recent study of Dutch elderly people (Schurgers *et al.* 1999) detected no diet-derived MK-4 in serum while MK-7 (from the diet and colonic bacteria) was detected in only 15 % (46/310) of serum samples. Booth *et al.*

(2001) reported very low activity of dihydrophylloquinone to γ-carboxylate OC, hence even if it is an appreciable component of total vitamin K intake (currently unknown in the UK) its impact is likely to be minor.

To conclude, an appreciable subset of the British elderly population may be at risk of low vitamin K status, as judged by plasma phylloquinone concentrations in this nationally representative sample. Current evidence suggests that phylloquinone is the most important form of vitamin K in relation to extrahepatic functions and, as such, may impact on bone and cardiovascular health. Plasma phylloquinone concentration was directly, although weakly, correlated with phylloquinone intake, and also with plasma concentrations of α- and β-carotenes, lutein, and α- and γ-tocopherols in free-living men and women, and with plasma 25-hydroxyvitamin D concentration in women. It appears that low circulating concentrations of one fat-soluble vitamin may be paralleled by low concentrations of others and that this is likely to reflect low dietary intakes of the micronutrient-rich (particularly green) vegetables. The possibility of concurrent deficiencies in fat-soluble vitamins in elderly populations may increase the risk of degenerative disease.

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