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Determinants of plasma dihydrophylloquinone in men and women

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Commercial hydrogenation results in the formation of *trans* fatty acids. An unintended consequence of the hydrogenation process is conversion of phylloquinone (vitamin K_1) to dihydrophylloquinone. Plasma dihydrophylloquinone concentrations have yet to be characterized in population-based studies. Dietary determinants of plasma dihydrophylloquinone were estimated using a semi-quantitative food frequency questionnaire in 803 men and 913 women in the Framingham Offspring Study. Geometric mean dihydrophylloquinone intake was 21·3 (95 % CI 20·4, 22·3) μg/d in men and 19·4 (95 % CI 18·5, 20·2) μg/d in women. Detectable (>0·05 nmol/l) plasma dihydrophylloquinone concentrations were measured in 41 % and 30 % of men and women, respectively. The multivariate odds ratio (OR) of detectable plasma dihydrophylloquinone from the lowest to the highest quartile category of dihydrophylloquinone intake were 1 (referent), 1·13 (95 % CI 0·83, 1·53), 1·66 (95 % CI 1·21, 2·26) and 1·84 (95 % CI 1·31, 2·58), *P* for trend <0·001, adjusted for sex, age, body mass index, triacylglycerols, season and energy intake. Higher *trans* fatty acid intake was associated with higher multivariate OR for detectable plasma dihydrophylloquinone (OR comparing extreme quartiles 2·41 (95 % CI 1·59, 3·64), *P* for trend <0·001). There were limitations in the use of plasma dihydrophylloquinone, evident in the high proportion of the population that had non-detectable dihydrophylloquinone concentrations. Despite this caveat, higher plasma dihydrophylloquinone was associated with higher dihydrophylloquinone intake and higher *trans* fatty acid intake.

Dihydrophylloquinone: Vitamin K: *Trans* fatty acids: Biomarker

Introduction

Plant oils are hydrogenated to alter the physical characteristics of the fat and increase chemical stability (Federal Register, 2003). This process increases the potential applications of the fat for commercial uses; hence the practice is widespread in many countries. The oils most commonly hydrogenated in the USA are soyabean and rapeseed, which are also rich in dietary sources of phylloquinone (also known as vitamin K_1; Booth & Suttie, 1998). An unintended consequence of the hydrogenation process is the saturation of the 2′,3′ double bond on the side chain of phylloquinone, resulting in the formation of 2′,3′-dihydrophylloquinone (Davidson *et al.* 1996). This change is of particular interest in light of prior work suggesting that while dihydrophylloquinone is absorbed from hydrogenated plant oils (Booth *et al.* 1996*a*, 2001), the biological activity of dihydrophylloquinone is less compared with its parent compound, phylloquinone (Booth *et al.* 2001).

Since 1990, there has been considerable interest in dietary *trans* fatty acid intakes because of potential adverse physiological consequence(s) associated with this class of fatty acids on plasma lipid and lipoprotein concentrations (Mensink & Katan, 1990; Lichtenstein *et al.* 1999). Estimation of dietary *trans* fatty acid intakes from self-reported data, especially from food frequency questionnaires (FFQ), has been hampered by uncertainty in accurately identifying the type of fats used for preparation of specific foods, especially commercially baked and fried foods or mixed dishes, and the completeness of the database for the *trans* fatty acid content of foods (Federal Register, 2003). For this reason, it would be useful to have a biological marker of *trans* fatty acid intake that was independent of the aforementioned

Abbreviations: FFQ, food frequency questionnaire; OR, odds ratio; ucOC, undercarboxylated osteocalcin.

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limitations. Because the sole source of dietary dihydrophylloquinone is from hydrogenated fat, and the majority of *trans* fatty acids comes from hydrogenated fat, plasma dihydrophylloquinone may serve as a surrogate marker for *trans* fatty acid intakes.

The first aim of this study was to investigate the feasibility of quantitating plasma dihydrophylloquinone in the Framingham Offspring Study (Kannel *et al.* 1979), and examining the associations between this biological marker and estimated dietary dihydrophylloquinone intakes. The second aim of this study was to assess the association between estimated *trans* fatty acid intake and plasma dihydrophylloquinone concentrations. The final aim of this study was to examine associations between plasma dihydrophylloquinone and biological markers of vitamin K status.

Methods

Study population

The Framingham Offspring Study is a longitudinal, community-based study of cardiovascular disease among the children and their spouses of the participants in the original Framingham Heart Study cohort (Kannel *et al.* 1979). The Offspring cohort (original *n* 5135) has undergone repeat examinations every 3 or 4 years since 1971. Data collection was performed between 1996 and 2000, during the sixth and seventh examination cycles of the Framingham Offspring Study. Subjects taking oral anticoagulants were excluded from the analyses (*n* 35). Valid FFQ data, covariate information and corresponding blood samples for phylloquinone and dihydrophylloquinone measurements were obtained from 1716 subjects. This study was approved by the Institutional Review Boards of Tufts-New England Medical Center and Boston University.

Dietary assessment

Usual dietary intake during the previous year was assessed using a semi-quantitative FFQ, as previously described (Willett *et al.* 1985; Rimm *et al.* 1992). The questionnaires were mailed to the participants before the examination, and completed questionnaires were returned at the examination. Dietary data were excluded if the subjects reported energy intake $<2{\cdot}51$ MJ/d (600 kcal/d) or $>16{\cdot}74$ MJ/d (4000 kcal/d) for women and $>17{\cdot}57$ MJ/d (4200 kcal/d) for men, or ≥ 12 food items were left blank. Daily dietary dihydrophylloquinone intake was calculated by multiplying the dihydrophylloquinone content per serving of each food (Booth *et al.* 1996*b*; Peterson *et al.* 2002; Dumont *et al.* 2003) by the reported frequency of consumption, and summing over all foods. The dihydrophylloquinone content of foods was based on recent laboratory analysis of geographically representative foods collected as part of the US Department of Agriculture's National Food and Nutrient Analysis Plan, as described elsewhere (Peterson *et al.* 2002). The FFQ included questions on the frequency of consumption of fried food consumed either out or in the home. The number of servings of bakery products was computed by summing the servings of crackers, cookies, brownies, doughnuts, cake, sweet rolls and pies. Servings of hot dogs, chicken/turkey dogs, French fries, pizza and tortilla were included in the category of fast food and servings of popcorn, potato chips and corn chips in the category of snacks. Data on *trans* fatty acid intake were available for 1238 of the 1716 subjects used in these analyses.

Blood measurements

Plasma phylloquinone and dihydrophylloquinone concentrations were determined by reverse-phase HPLC with use of post-column reduction and fluorometric detection (Davidson & Sadowski, 1997). The lower limit of detection for plasma phylloquinone and dihydrophylloquinone was 0·05 nmol/l. Serum total osteocalcin and undercarboxylated osteocalcin were measured by RIA (Gundberg *et al.* 1998). Undercarboxylated osteocalcin was expressed as the percentage of osteocalcin not bound to hydroxyapatite *in vitro* (%ucOC), and normalized to the amount of total osteocalcin in a given sample. Plasma triacylglycerols were measured using standardized enzymatic methods (McNamara & Schaefer, 1987).

Covariate measurements

Anthropometric measurements were taken at the time of the examination and BMI (kg/m^2) was calculated. Subjects were classified as abdominal obese if waist circumference was >88 cm in females and >102 cm in males (National Cholesterol Education Program Expert Panel, 2002). Data on regular smoking during the previous year (yes/no) (Y/N), menopausal status (cessation of menses for at least 1 year) and current use of hormone replacement therapy (Y/N) were collected at the time of the examination. Season (winter, spring, summer, autumn) was also used as covariate.

Statistical methods

All statistical analyses were performed using Statistical Analysis System, version 8.2 (SAS Institute, Cary, NC, USA). To improve normality for statistical testing, natural logarithmic transformations were applied to dietary dihydrophylloquinone intake and plasma phylloquinone. Adjusted geometric means and 95 % CI were calculated for these variables. Because plasma dihydrophylloquinone was non-detectable (defined as $\leq 0{\cdot}05$ nmol/l) in 64 % of the samples and the distribution was skewed toward lower values, the percentage of detectable (defined as $>0{\cdot}05$ nmol/l) concentrations, with 95 % CI, are presented, instead of actual concentrations. Because distribution of %ucOC was skewed toward lower values, the data are presented as percentage of subjects having ≥ 20 % ucOC, with 95 % CI, which is suggested to reflect low vitamin K intake, based on metabolic data using the same assay (Booth *et al.* 1999*a*, 2001).

Possible determinants of dietary intake and plasma concentrations $>0{\cdot}05$ nmol/l of dihydrophylloquinone included sex, age (<50, 50–59, 60–69, ≥ 70 years), BMI (<25, 25–30, ≥ 30 kg/m^2), abdominal adiposity (>88 cm for women, >102 cm for men), season (winter, spring, summer, autumn), postmenopausal status (Y/N) and current hormone replacement therapy (Y/N). Differences in geometric mean dihydrophylloquinone intake and proportion of detectable plasma dihydrophylloquinone across categories of these potential determinants were assessed using SAS PROC GLM and Scheffé's test for pairwise comparisons. In addition, geometric mean dihydrophylloquinone intake levels were adjusted for energy intake and the proportion of detectable plasma dihydrophylloquinone concentrations was presented with and without adjustment for plasma triacylglycerol concentrations. Analyses of plasma dihydrophylloquinone were adjusted for plasma triacylglycerols in the model because

previously metabolic studies suggest that the triacylglycerol-rich lipoprotein fraction is the main carrier of vitamin K in circulation (Lamon-Fava *et al.* 1998; Erkkilä *et al.* 2004). SAS PROC GLM was also used to examine the relation between quartile categories of dihydrophylloquinone intake and detectable levels of plasma dihydrophylloquinone and indicators of vitamin K status, including plasma phylloquinone and percentage of subjects with elevated %ucOC. In these analyses, geometric mean plasma phylloquinone concentrations and proportions of detectable plasma dihydrophylloquinone concentrations were adjusted for plasma triacylglycerol concentrations, and dihydrophylloquinone intakes were adjusted for energy intake.

Multivariate logistic regression analyses were used to calculate odds ratios (OR), with 95 % CI, for detectable plasma dihydrophylloquinone concentrations (defined as $>0{\cdot}05$ nmol/l) across quartile categories of dietary dihydrophylloquinone, *trans* fatty acids or foods rich in dihydrophylloquinone adjusting for significant correlates of either dihydrophylloquinone intake or plasma dihydrophylloquinone as well as energy intake and tertile categories of plasma triacylglycerols. Tests of linear trend across increasing categories of dietary dihydrophylloquinone, *trans* fatty acids or foods rich in dihydrophylloquinone were conducted by assigning the medians of intakes to categories that were treated as a continuous variable. Spearman correlation coefficients were calculated between plasma dihydrophylloquinone and triacylglycerols and between dihydrophylloquinone and *trans* fatty acid intakes. A value of $P<0{\cdot}05$ (two-tailed) was considered statistically significant.

Results

The mean plasma dihydrophylloquinone concentrations for all subjects were 0·31 (SD 0·76) and 0·19 (SD 0·41) nmol/l for men and women, respectively. The percentage of those with detectable plasma dihydrophylloquinone was higher in men than in women (41 % *v.* 31 %, $P<0{\cdot}001$; Table 1). There was a positive correlation between plasma dihydrophylloquinone and triacylglycerol concentrations (r 0·21, $P<0{\cdot}001$), so data are presented as adjusted and unadjusted for triacylglycerols in Table 1. Subjects with BMI >30 kg/m^2 or abdominal obesity were more likely to have detectable plasma dihydrophylloquinone. However, these associations became non-significant after adjustment for triacylglycerol concentrations. Among postmenopausal women, those who were taking hormone replacement therapy had less detectable plasma dihydrophylloquinone concentrations than those who were not. The percentage of subjects with detectable plasma dihydrophylloquinone was higher in summer than in spring.

Table 1. Mean percentage of subjects with detectable plasma dihydrophylloquinone concentrations ($>0{\cdot}05$ nmol/l) by categories of potential determinants (Mean values with their 95 % CI)

		% Detectable plasma dihydrophylloquinone, unadjusted for triacylglycerols			% Detectable plasma dihydrophylloquinone, adjusted for triacylglycerols		
	n	Mean	95 % CI	*P**	Mean	95 % CI	*P**
Sex							
Men	803	41·2	38·0, 44·5		40·7	37·5, 44·0	
Women	913	30·6	27·5, 33·6	$<0{\cdot}001$	31·0	27·9, 34·0	$<0{\cdot}001$
Age (years)							
<50	290	32·1	26·6, 37·6		34·0	28·6, 39·4	
50–59	690	33·9	30·3, 37·5		33·6	30·1, 37·1	
60–69	496	36·9	32·7, 41·1		36·3	32·1, 40·4	
≥ 70	240	41·7	35·6, 47·7	0·09	41·5	35·5, 47·5	0·15
BMI (kg/m^2)							
<25	512	29·9[a]	25·7, 34·0		32·2	28·0, 36·3	
25–30	703	35·7[ab]	32·2, 39·2		35·9	32·4, 39·4	
>30	499	41·3[b]	37·1, 45·5	$<0{\cdot}001$	38·7	34·4, 42·9	0·11
Abdominal obesity†							
No	815	32·8	29·5, 36·0		34·4	31·1, 37·7	
Yes	901	38·1	34·9, 41·2	0·03	36·6	33·5, 39·7	0·35
Current smoker							
No	1464	35·5	33·1, 38·0		35·6	33·2, 38·0	
Yes	252	35·7	29·8, 41·6	0·96	35·3	29·5, 41·2	0·95
Season							
Winter (Dec–Feb)	526	37·1[ab]	33·0, 41·2		37·7[ab]	33·6, 41·7	
Spring (Mar–May)	506	30·4[a]	26·3, 34·6		29·9[a]	25·8, 34·0	
Summer (June–Aug)	187	42·8[b]	35·9, 49·6		42·6[b]	35·9, 49·4	
Fall (Sep–Nov)	497	36·4[ab]	32·2, 40·6	0·02	36·4[ab]	32·3, 40·6	0·006
Postmenopausal status, women							
No	215	24·7	18·5, 30·8		26·8	20·6, 33·0	
Yes	698	32·4	29·0, 35·8	0·04	31·7	28·3, 35·1	0·18
Hormone replacement therapy, postmenopausal women							
No	439	32·2	28·6, 35·8		32·7	29·1, 36·3	
Yes	259	27·2	21·9, 32·5	0·13	26·1	20·9, 31·4	0·05

[a,b] Mean values without common letters are significantly different at $P<0{\cdot}05$ (Scheffé's test).

* Assessed using SAS PROC GLM.

† Abdominal obesity: waist >88 cm in women, >102 cm in men.

Geometric mean intakes of dihydrophylloquinone, adjusted for energy intake, were higher in men ($P=0{\cdot}004$), older adults ($P<0{\cdot}001$), those who had a BMI $>30\,kg/m^2$ ($P=0{\cdot}02$) and subjects with abdominal obesity ($P=0{\cdot}04$; Table 2). Among postmenopausal women, those who were taking hormone replacement therapy had lower intakes of dihydrophylloquinone than those who were not ($P=0{\cdot}02$). The major sources of dihydrophylloquinone for the study cohort are listed in Table 3. Bakery products contributed the majority (42·4 % and 41·3 % for men and women, respectively) of dihydrophylloquinone intake. Fast foods contributed 28·9 % and 23·6 % of the dihydrophylloquinone intake in men and women, respectively. The correlation between dietary dihydrophylloquinone and *trans* fatty acid intake was r 0·78, $P<0{\cdot}001$.

Multivariate OR for detectable plasma dihydrophylloquinone concentrations adjusted for sex, age, BMI, triacylglycerol, season and energy intake was 1·84 (95 % CI 1·31, 2·58) in the highest quartile category of dihydrophylloquinone intake as compared to the lowest quartile (P for trend $<0{\cdot}001$; Fig. 1). When analysed according to gender, the association was significant both in men and women (OR in increasing quartile categories 1 (referent), 1·37 (95 % CI 0·89, 2·09), 1·76 (95 % CI 1·13, 2·73) and 2·29 (95 % CI 1·41, 3·72), respectively, in men (P for trend 0·002); and 1 (referent), 1·15 (95 % CI 0·74, 1·79), 1·75 (95 % CI 1·12, 2·74) and 1·82 (95 % CI 1·13, 2·96), respectively, in women (P for trend 0·02)).

Compared with the lowest intake category of *trans* fatty acids, the odds of having a detectable concentration of plasma dihydrophylloquinone in the highest quartile category was three times higher in men and almost two times higher in women (Table 4). Higher intake of bakery products was associated with higher OR for detectable plasma dihydrophylloquinone in the whole sample ($P=0{\cdot}007$) and in men ($P=0{\cdot}03$); in women, the association did not reach significance ($P=0{\cdot}11$). There was a marginal trend ($P=0{\cdot}13$) for a higher OR for detectable plasma dihydrophylloquinone among those subjects who reported eating fried food out, but not fried food prepared at home, at least once a week.

Table 2. Geometric mean dihydrophylloquinone intake* by categories of potential determinants (Geometric mean values, adjusted for energy intake, with their 95 % CI)

		Dihydrophylloquinone intake (μg/d)		
	n	Mean	95 % CI	*P*†
Sex				
Men	803	21·3	20·4, 22·3	
Women	913	19·4	18·5, 20·2	0·004
Age (years)				
<50	290	20·1[a]	18·6, 21·7	
50–59	690	19·2[a]	18·3, 20·2	
60–69	496	20·3[a]	19·1, 21·5	
≥70	240	23·8[b]	21·9, 25·8	<0·001
BMI (kg/m^2)				
<25	512	18·9[a]	17·8, 20·0	
25–30	703	20·7[ab]	19·7, 21·7	
>30	499	21·1[b]	19·9, 22·4	0·02
Abdominal obesity‡				
No	815	19·5	18·7, 20·4	
Yes	901	20·9	20·0, 21·9	0·04
Current smoker				
No	1464	20·2	19·5, 20·8	
Yes	252	20·9	19·2, 22·6	0·45
Season				
Winter (Dec–Feb)	526	21·2	20·0, 22·4	
Spring (Mar–May)	506	20·1	18·9, 21·2	
Summer (June–Aug)	187	21·1	19·2, 23·2	
Fall (Sep–Nov)	497	19·2	18·1, 20·4	0·10
Postmenopausal status, women				
No	215	17·3	15·8, 19·0	
Yes	698	18·2	17·3, 19·1	0·39
Hormone replacement therapy, postmenopausal women				
No	439	18·7	17·7, 19·7	
Yes	259	16·6	15·4, 17·9	0·02

[a,b] Mean values without common letters are significantly different at $P<0{\cdot}05$ (Scheffé's test).
* As estimated from a food frequency questionnaire using published dihydrophylloquinone food composition (Booth *et al.* 1996*b*; Peterson *et al.* 2002; Dumont *et al.* 2003).
† Assessed using SAS PROC GLM.
‡ Abdominal obesity: waist >88 cm in women, >102 cm in men.

Table 3. Mean contribution of different food items to total dihydrophylloquinone intake*

	Mean contribution (%)	
Food	Men (*n* 803)	Women (*n* 913)
Total bakery products†	42·4	41·3
Cookies	19·7	20·1
Sweet rolls	7·2	7·7
Pies	6·7	5·5
Doughnuts	4·2	2·7
Total fast food‡	28·9	23·6
French fries	16·1	11·7
Pizza	12·0	11·4
Snacks§	4·0	5·1
Other foods		
Mayonnaise	4·9	7·1
Margarine	4·6	5·1
Coffee whitener	4·6	5·1

* As estimated from a food frequency questionnaire using published dihydrophylloquinone food composition (Booth *et al.* 1996*b*; Peterson *et al.* 2002; Dumont *et al.* 2003). Other food items contributed <2 % to total dihydrophylloquinone intake.
† Bakery products include crackers, cookies, brownies, doughnuts, cake, sweet rolls and pies.
‡ Fast food includes hot dogs, chicken/turkey dogs, French fries, pizza and tortilla.
§ Snacks include popcorn, potato and corn chips.

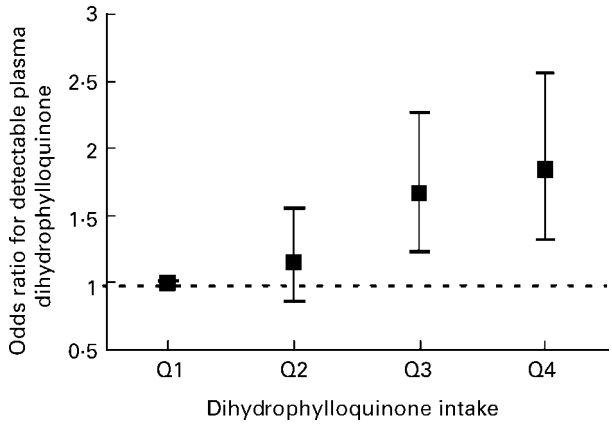

Fig. 1. Multivariate odds ratios (with 95 % CI) for detectable plasma dihydrophylloquinone concentrations (>0·05 nmol/l) according to median intake in quartile categories (Q1–Q4) of dihydrophylloquinone (8·2, 16·4, 26·7 and 49·1 μg/d, respectively) in 427, 432, 429 and 428 subjects, respectively, $P<0{\cdot}001$. The odds ratios are adjusted for sex, age (four groups), BMI (three groups), plasma triacylglycerols (tertiles), season and energy intake (continuous).

Table 4. Multivariate odds ratios (OR) for detectable plasma dihydrophylloquinone concentrations (>0·05 nmol/l) according to median intake categories of *trans* fatty acid intake (g/d) or sources of dihydrophylloquinone

All subjects					Men					Women				
Median intake	*n*	OR	95 % CI	*P* for trend	Median intake	*n*	OR	95 % CI	*P* for trend	Median intake	*n*	OR	95 % CI	*P* for trend
Trans fatty acids (g/d)														
1·1	312	1			1·3	141	1			1·0	166	1		
1·9	310	1·42	1·00, 2·05		2·1	135	1·76	1·05, 2·94		1·7	172	1·20	0·72, 2·01	
2·9	308	1·70	1·17, 2·46		3·2	143	2·39	1·41, 4·07		2·5	172	1·64	0·98, 2·75	
4·7	308	2·41	1·59, 3·64	<0·001*	5·2	144	3·01	1·66, 5·47	<0·001	4·1	165	1·85	1·03, 3·30	0·03
Bakery products (servings/week)†														
1·4	423	1			1·9	211	1			1·4	249	1		
3·9	418	1·25	0·93, 1·69		4·5	191	1·45	0·95, 2·21		3·8	211	1·24	0·82, 1·89	
7·8	446	1·48	1·10, 2·01		8·3	204	1·84	1·20, 2·81		7·4	229	1·24	0·81, 1·90	
19·4	429	1·66	1·19, 2·31	0·007	19·4	197	1·87	1·17, 2·98	0·03	19·9	224	1·52	0·96, 2·41	0·11
Fast food and snacks (servings/week)‡														
0·9	416	1			0·9	196	1			0·9	213	1		
1·9	441	1·07	0·80, 1·44		2·5	208	1·23	0·81, 1·87		1·9	268	1·27	0·84, 1·92	
3·9	427	1·20	0·89, 1·62		4·9	196	1·20	0·78, 1·85		3·4	213	1·39	0·89, 2·16	
8·0	432	1·31	0·94, 1·83	0·10	9·5	191	1·56	0·95, 2·54	0·11	7·4	219	1·30	0·81, 2·10	0·47
Fried food out (servings/week)														
0	1369	1			0	584	1			0	785	1		
2	345	1·23	0·95, 1·60	0·13	2	219	1·20	0·86, 1·69	0·29	2	126	1·20	0·78, 1·84	0·41
Fried food at home (servings/week)														
0	1384	1			0	623	1			0	761	1		
2	329	1·02	0·79, 1·32	0·89	2	178	0·86	0·60, 1·22	0·40	2	151	1·31	0·89, 1·93	0·17

* Models are adjusted for sex (when all subjects included), age (four groups), BMI (three groups), plasma triacylglycerols (tertiles), season and energy intake (continuous).
† Bakery products include crackers, cookies, brownies, doughnuts, cake, sweet rolls and pies.
‡ Fast food and snacks includes hot dogs, chicken/turkey dogs, French fries, pizza, tortilla, popcorn, potato and corn chips.

Geometric mean plasma phylloquinone concentrations were higher ($P<0{\cdot}001$) in those with detectable plasma dihydrophylloquinone (Table 5). The plasma phylloquinone–plasma dihydrophylloquinone association did not change when plasma triacylglycerols were removed from the model (data not shown). However, plasma phylloquinone concentrations were not associated with dihydrophylloquinone intake (Table 6). As a group, those who had higher dihydrophylloquinone intakes tended to have a higher percentage of elevated %ucOc ($P=0{\cdot}07$; Table 6). There was no significant sex interaction; however, the association between dihydrophylloquinone intake and percentage of elevated %ucOc was significant only in women. Somewhat surprisingly, the proportion of elevated %ucOC did not differ between those subjects who had non-detectable or detectable plasma dihydrophylloquinone (Table 5).

Discussion

To the best of our knowledge, this is the first report of a positive association between plasma dihydrophylloquinone concentrations and dihydrophylloquinone intake, as assessed with an FFQ, in a free-living population. A positive association was likewise observed between *trans* fatty acid intakes and detectable plasma dihydrophylloquinone concentrations.

There was a wide range of dihydrophylloquinone intakes in both the current study using the FFQ (0–140 μg dihydrophylloquinone/d), and in a previous study using the 14 d food diaries (range: 0–220 μg dihydrophylloquinone/d; Booth *et al.* 1999*b*). The geometric mean intakes of dihydrophylloquinone in men and women in the current study were in the same range as reported previously in 60–65-year-old men and women using 3 d dietary intake data (19 and 17 μg/d, respectively; Booth *et al.* 1996*b*), and in adult men and women using 14 d food diaries (19 and 15 μg/d, respectively; Booth *et al.* 1999*b*).

The large proportion of individuals with non-detectable concentrations of plasma dihydrophylloquinone was a limitation of the current study. Absolute reported intakes of dihydrophylloquinone were lower compared with those of phylloquinone previously reported for the Framingham Offspring Cohort (McKeown *et al.* 2002) so it is not unexpected that corresponding plasma dihydrophylloquinone concentrations were low. Dihydrophylloquinone may also be absorbed to a lesser extent or more rapidly metabolized and excreted compared with an equivalent amount of phylloquinone (Booth *et al.* 2001). In one metabolic study (Booth *et al.* 1996*a*), the plasma dihydrophylloquinone concentrations were all within detectable range (mean dihydrophylloquinone concentration of 0·56 nmol/l) in response to daily intakes of 23 μg dihydrophylloquinone. However, this study (Booth *et al.* 1996*a*) was limited to eight mildly hyperlipidaemic men and women, and it is plausible that the higher plasma dihydrophylloquinone concentrations were associated with higher triacylglycerol concentrations. It is assumed that dihydrophylloquinone would have a similar transport mechanism as the parent form phylloquinone, which is carried primarily by triacylglycerol-rich lipoproteins (Lamon-Fava *et al.* 1998; Erkkilä *et al.* 2004). Although there is a positive correlation between plasma phylloquinone and plasma triacylglycerols (McKeown *et al.* 2002), there is current controversy regarding the necessity to adjust plasma phylloquinone for plasma lipids (Traber & Jialal, 2000). Even less is known about correction of plasma dihydrophylloquinone for plasma lipids. Regardless of the mechanism(s), low plasma dihydrophylloquinone concentrations present analytical challenges when assessed in a free-living population that has a wide range of lipid concentrations and dihydrophylloquinone intakes.

Higher BMI and abdominal obesity were associated with higher dihydrophylloquinone intake, even after adjustment for energy intake. Whereas plasma dihydrophylloquinone was also positively associated with measures of obesity, further adjustment for plasma triacylglycerols resulted in a loss of statistical significance. It is not known whether the attenuation of associations with plasma dihydrophylloquinone following adjustment for plasma triacylglycerols were due to positive correlations between measures of obesity and plasma triacylglycerols (Grundy, 1998).

The hydrogenation process results in the formation of both *trans* fatty acids and dihydrophylloquinone (Davidson *et al.* 1996). The dihydrophylloquinone content in margarines has been reported to correlate with that of *trans* fatty acids (Koivu *et al.* 1999). However, sources of dihydrophylloquinone and *trans* fatty acids are not entirely the same. All dihydrophylloquinone comes from foods made with hydrogenated vegetable oils and is not naturally occurring. Whereas the majority of *trans* fatty acids (80 %) comes from foods made with hydrogenated vegetable oil, 20 % comes from endogenous production in ruminant animals which accumulates in the fat (Federal Register, 2003). Using data from 3 d food intake diaries collected as part of the Continuing Survey of Food Intakes by Individuals (CSFII 94-96; Federal Register, 2003), the major foods that contribute to *trans* fatty acid intake from hydrogenated fat include bakery products (42 % of the total *trans* fatty acid intake from products made with hydrogenated fat), margarine (21 %), French fries (11 %), potato and corn chips (6 %) and shortening (5 %). These contributions are similar to that observed for dihydrophylloquinone in this cohort, with the exception of margarine, which contributed less (5 %).

Having similar dietary sources, it was not unexpected that a positive association was observed between dihydrophylloquinone

Table 5. Concentrations of plasma phylloquinone and percentage of subjects with elevated (≥20 %) undercarboxylated osteocalcin (ucOC) according to categories of plasma dihydrophylloquinone concentrations

(Mean values with their 95 % CI)

	Plasma dihydrophylloquinone (nmol/l)*				
	≤0·05		>0·05		
	Mean	95 % CI	Mean	95 % CI	*P*†
Plasma phylloquinone (nmol/l)					
All	0·86‡	0·82, 0·91	1·14	1·06, 1·22	<0·001
Men	0·89	0·82, 0·97	1·16	1·06, 1·27	<0·001
Women	0·84	0·79, 0·90	1·12	1·02, 1·24	<0·001
ucOC (%)					
All	43·5§	40·6, 46·5	43·8	39·8, 47·9	0·91
Men	40·2	35·7, 44·7	40·4	35·0, 45·8	0·95
Women	46·2	42·2, 50·1	47·8	41·8, 53·7	0·66

* In ≤0·05 and >0·05 nmol/l categories, number of all subjects was 1106 and 610, respectively, for plasma phylloquinone, and 1087 and 599, respectively, for ucOC; number of men was 472 and 331 for plasma phylloquinone, and 467 and 323, respectively, for ucOC; number of women was 634 and 279, respectively, for plasma phylloquinone, and 620 and 276, respectively, for ucOC.

† SAS PROC GLM.

‡ Geometric means adjusted for plasma triacylglycerol.

§ Proportion of high (≥20 %) ucOC adjusted for plasma triacylglycerol.

Table 6. Concentrations of plasma phylloquinone and percentage of subjects with elevated (≥20 %) undercarboxylated osteocalcin (ucOC) according to quartile categories (Q) of dihydrophylloquinone intake*

(Mean values with their 95 % CI)

	Dihydrophylloquinone intake†								
	Q1 (low)		Q2		Q3		Q4 (high)		
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	P‡
Plasma phylloquinone (nmol/l)									
All	0·98§	0·90, 1·07	0·97	0·89, 1·05	0·97	0·89, 1·06	0·89	0·82, 0·98	0·48
Men	1·11	0·98, 1·27	0·96	0·85, 1·09	0·96	0·85, 1·08	0·95	0·83, 1·09	0·28
Women	0·90	0·80, 1·01	0·91	0·81, 1·01	1·03	0·92, 1·15	0·86	0·76, 0·96	0·13
ucOC (%)									
All	38·0‖	32·9, 43·1	44·5	39·8, 49·3	43·6	38·9, 48·4	48·4	43·3, 53·6	0·07
Men	36·4	29·1, 43·6	38·7	31·7, 45·6	41·1	34·2, 47·9	45·0	37·4, 52·6	0·49
Women	38·6[a]	31·5, 45·6	49·2[ab]	42·7, 55·8	44·5[ab]	37·9, 51·1	54·3[b]	47·2, 61·3	0·02

[a,b] Mean values without common letters are significantly different at $P<0·05$ (Scheffé's test).

* As estimated from a food frequency questionnaire using published dihydrophylloquinone food composition (Booth *et al.* 1996*b*; Peterson *et al.* 2002; Dumont *et al.* 2003).

† Median intakes in the quartile categories were 8·2, 16·4, 26·7 and 49·1 μg/d, respectively, in all subjects; 10·2, 19·4, 29·1 and 54·4 μg/d, respectively, in men; and 7·2, 14·1, 23·9 and 46·3 μg/d, respectively, in women. In quartile categories, number of all subjects was 427, 432, 429 and 428, respectively, for plasma phylloquinone, and 419, 426, 420 and 421, respectively, for ucOC; number of men was 200, 201, 199 and 203, respectively, for plasma phylloquinone, and 197, 200, 197 and 196, respectively, for ucOC; number of women was 228, 231, 229 and 225, respectively, for plasma phylloquinone, and 224, 225, 223 and 224, respectively, for ucOC.

‡ Assessed using SAS PROC GLM.

§ Geometric means adjusted for energy intake and plasma triacylglycerol.

‖ Proportion of elevated (≥20 %) ucOC adjusted for energy intake.

and *trans* fatty acid intakes in the current study. A positive association was likewise observed between *trans* fatty acid intakes and detectable plasma dihydrophylloquinone concentrations. When assessed on the basis of individual food categories, there was a positive association between the primary dietary source of dihydrophylloquinone, bakery products and detectable plasma dihydrophylloquinone. Estimating *trans* fatty acid intake from products made with hydrogenated fat has been challenging in population-based studies because of the lack of a comprehensive database on *trans* fatty acid contents in foods and variable concentration of *trans* fatty acids in food items within a food category (Federal Register, 2003). Furthermore, the analysis of individual *trans* fatty acids in plasma or serum is elaborate and time consuming. Dihydrophylloquinone intake may better reflect dietary intake of hydrogenated fat than *trans* fatty acids because hydrogenation is the exclusive source of dihydrophylloquinone. One caveat to this conclusion is that dihydrophylloquinone is only formed during hydrogenation of phylloquinone-rich oils, which include soyabean, rapeseed, cottonseed and olive (Peterson *et al.* 2002). For those food supply systems that use hydrogenated plant oils that are not rich in phylloquinone, such as corn, peanut and safflower, dihydrophylloquinone would not be an appropriate marker of *trans* fatty acid intake.

A high serum %ucOC is considered an indicator of poor vitamin K status (Sokoll & Sadowski, 1996; Gundberg *et al.* 1998; Booth *et al.* 2001). Higher dihydrophylloquinone intakes tended to be associated with a higher proportion of elevated %ucOC in the current study. These associations are consistent with prior metabolic data in humans (Booth *et al.* 2001). In contrast, plasma dihydrophylloquinone concentrations were not associated with %ucOC, and at this point, the inconsistencies between the associations of %ucOC with dietary versus plasma dihydrophylloquinone remain unresolved.

In summary, despite the limitations in the use of plasma dihydrophylloquinone, evident in the high proportion of the population that had non-detectable dihydrophylloquinone concentrations, plasma dihydrophylloquinone is positively associated with dihydrophylloquinone intake as measured using an FFQ. Furthermore, dihydrophylloquinone (both plasma and intake) are positively associated with *trans* fatty acid intake.

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References

Booth SL, Davidson KW, Lichtenstein AH & Sadowski JA (1996*a*) Plasma concentrations of dihydro-vitamin K1 following dietary intake of a hydrogenated vitamin K1-rich vegetable oil. *Lipids* **31**, 709–713.

Booth SL, Lichtenstein AH, O'Brien-Morse M, McKeoun NM, Wood RJ, Saltzman E & Gundberg CM (2001) Effects of a hydrogenated form of vitamin K on bone formation and resorption. *Am J Clin Nutr* **74**, 783–790.

Booth SL, O'Brien-Morse ME, Dallal GE, Davidson KW & Gundberg CM (1999*a*) Response of vitamin K status to different intakes and sources of phylloquinone-rich foods: comparison of younger and older adults. *Am J Clin Nutr* **70**, 368–377.

Booth SL, Pennington JA & Sadowski JA (1996*b*) Dihydro-vitamin K1: primary food sources and estimated dietary intakes in the American diet. *Lipids* **31**, 715–720.

Booth SL & Suttie JW (1998) Dietary intake and adequacy of vitamin K. *J Nutr* **128**, 785–788.

Booth SL, Webb DR & Peters JC (1999*b*) Assessment of phylloquinone and dihydrophylloquinone dietary intakes among a nationally

representative sample of US consumers using 14-day food diaries. *J Am Diet Assoc* **99**, 1072–1076.

Davidson KW, Booth SL, Dolnikowski GG & Sadowski JA (1996) The conversion of phylloquinone to 2′,3′-dihydrophylloquinone during hydrogenation of vegetable oils. *J Agric Food Chem* **44**, 980–983.

Davidson KW & Sadowski JA (1997) Determination of vitamin K compounds in plasma or serum by high-performance liquid chromatography using postcolumn chemical reduction and fluorimetric detection. *Methods Enzymol* **282**, 408–421.

Dumont JF, Peterson J, Haytowitz D & Booth SL (2003) Phylloquinone and dihydrophylloquinone contents of mixed dishes, processed meats, soups and cheeses. *J Food Compos Anal* **16**, 595–603.

Erkkilä AT, Lichtenstein AH, Dolnikowski GG, Grusak MA, Jalbert SM, Aquino KA, Petersen JW & Booth SL (2004) Plasma transport of vitamin K in men using deuterium-labeled collard greens. *Metabolism* **53**, 215–221.

Federal Register (2003) Food labeling: trans fatty acids in nutrition labeling, nutrient content claims, and health claims. Final rule. pp. 41433–41506. Food and Drug Administration, HHS.

Grundy SM (1998) Hypertriglyceridemia, atherogenic dyslipidemia, and the metabolic syndrome. *Am J Cardiol* **81**, 18B–25B.

Gundberg CM, Nieman SD, Abrams S & Rosen H (1998) Vitamin K status and bone health: an analysis of methods for determination of undercarboxylated osteocalcin. *J Clin Endocrinol Metab* **83**, 3258–3266.

Kannel WB, Feinleib M, McNamara PM, Garrison RJ & Castelli WP (1979) An investigation of coronary heart disease in families. The Framingham Offspring Study. *Am J Epidemiol* **110**, 281–290.

Koivu T, Piironen V, Lampi A-M & Mattila P (1999) Dihydrovitamin K_1 in oils and margarines. *Food Chem* **64**, 411–414.

Lamon-Fava S, Sadowski JA, Davidson KW, O'Brien ME, McNamara JR & Schaefer EJ (1998) Plasma lipoproteins as carriers of phylloquinone (vitamin K_1) in humans. *Am J Clin Nutr* **67**, 1226–1231.

Lichtenstein AH, Ausman LM, Jalbert SM & Schaefer EJ (1999) Effects of different forms of dietary hydrogenated fats on serum lipoprotein cholesterol levels. *N Engl J Med* **340**, 1933–1940.

McKeown NM, Jacques PF, Gundberg CM, Petersen JW, Tucker KL, Kiel DP, Wilson PW & Booth SL (2002) Dietary and nondietary determinants of vitamin K biochemical measures in men and women. *J Nutr* **132**, 1329–1334.

McNamara JR & Schaefer EJ (1987) Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. *Clin Chim Acta* **166**, 1–8.

Mensink RP & Katan MB (1990) Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N Engl J Med* **323**, 439–445.

National Cholesterol Education Program (NCEP) Expert Panel (2002) Detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* **106**, 3143–3421.

Peterson JW, Muzzey KL, Haytowitz DB, Exler J, Lemar L & Booth SL (2002) Phylloquinone (vitamin K_1) and dihydrophylloquinone content of fats and oils. *JAOCS* **79**, 641–646.

Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Libin LB & Willett WC (1992) Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol* **135**, 1114–1126.

Sokoll LJ & Sadowski JA (1996) Comparison of biochemical indexes for assessing vitamin K nutritional status in a healthy adult population. *Am J Clin Nutr* **63**, 566–573.

Traber MG & Jialal I (2000) Measurement of lipid-soluble vitamins – further adjustment needed? *Lancet* **355**, 2013–2014.

Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH & Speizer FE (1985) Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* **122**, 51–65.