Dietary and Nondietary Determinants of Vitamin K Biochemical Measures in Men and Women

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ABSTRACT Few epidemiological studies that rely on the food frequency questionnaire (FFQ) for dietary assessment have measured biomarkers of vitamin K intake to independently confirm associations between self-reported dietary vitamin K intake and disease risk. Associations were examined between two sensitive biomarkers of vitamin K status, plasma phylloquinone and serum percent undercarboxylated osteocalcin (%ucOC), and self-reported usual phylloquinone intake as estimated from a FFQ. The influence of other dietary and nondietary factors on plasma phylloquinone concentrations was also examined. Dietary phylloquinone intake was estimated using a FFQ in 369 men and 468 women of the Framingham Offspring Study. The prevalence of high %ucOC concentrations (>20%), suggestive of a low vitamin K status, was 44% in men and 54% in women, respectively. After multivariate adjustment, the odds of a high %ucOC was 2.5 greater for women (odds ratio: 2.5; 95% confidence interval [CI]: 1.2–5.1) and almost three times greater for men (odds ratio: 2.8; 95% CI: 1.3–5.9) in the lowest dietary phylloquinone intake quintile category compared to the highest quintile category. Fasting triglyceride concentrations, smoking status and season were associated with plasma phylloquinone concentrations, independent of dietary phylloquinone intake. Phylloquinone and green vegetable intake was linearly associated with plasma phylloquinone, after adjustment for potential confounding factors. There were limitations in the use of the FFQ to predict phylloquinone, evident in an observed plateau effect and required nondietary adjustment factors. Despite these caveats, these findings support the use of a FFQ for a relative assessment of vitamin K status in population-based studies. J. Nutr. 132: 1329–1334, 2002.

KEY WORDS: dietary methods, biomarker, vitamin K, osteocalcin, Framingham Offspring Study

Few epidemiological studies have used biological markers of vitamin K intake to independently confirm estimates of self-reported usual dietary vitamin K intakes (1,2). Phylloquinone, also known as vitamin K-1, is the primary dietary source of vitamin K in the U.S. diet (3), and is found primarily in dark green vegetables and certain types of vegetable oils (4). Feeding studies have consistently demonstrated that plasma phylloquinone concentrations respond to recent dietary intakes (3). The diet-plasma phylloquinone association in free-living individuals is less consistent among studies but may be partially explained by different methods of dietary assessment used. Dietary phylloquinone intakes as estimated from diet records have been associated with plasma or serum phylloquinone concentrations (5–7), although Thane et al. (7) reported significant diet-plasma associations in elderly British women but not in men. In contrast, Schurges et al. (8) reported no association between usual dietary intake from a food frequency questionnaire (FFQ) 4 and serum phylloquinone concentrations in either men or women.

Another sensitive marker of overall vitamin K nutritional status is undercarboxylated osteocalcin (ucOC), expressed as a percentage of total osteocalcin (OC) (9). Low plasma phylloquinone concentrations, due to a low dietary intake (<10 μg vitamin K/d), have been associated with a greater percentage of the total OC that is not carboxylated (i.e., higher %ucOC) (10,11). Several feeding studies have shown that the percentage of ucOC decreases in response to an increased intake of phylloquinone, either in the form of foods (12) or supplements (10). However, there was no association between percent

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2 Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

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ucOC and self-reported usual dietary intakes in the National Health and Nutrition Examination Survey III (13).

Associations between self-reported dietary phylloquinone intakes and biomarkers of vitamin K status have been historically difficult to assess because accurate food composition data were limited for this nutrient. However, comprehensive information is now available on the phylloquinone content of foods commonly consumed in the U.S. diet (4,14,15). Using data from the Framingham Heart Study Offspring Cohort, the primary objective of this study was to assess the associations between concentrations of plasma phylloquinone and serum percent ucOC and self-reported usual phylloquinone intake as estimated from a FFQ. A second objective was to examine other dietary and nondietary factors that influence plasma phylloquinone concentrations.

SUBJECTS AND METHODS

Study population. The Framingham Heart Study, a longitudinal community-based study of cardiovascular disease, began in 1948 with a cohort of 5209 men and women aged 30–59 y (16). The offspring of these subjects and the offspring’s spouses (n = 6838) were invited to participate in the first Framingham Offspring Study, and 5135 eligible individuals participated in the examination (17). The Framingham Offspring Cohort has undergone repeat examinations approximately every 3–4 y.

The sixth examination of the Offspring Cohort began in January 1992 and was completed in December 1998. Of the 3532 participants who underwent a standardized medical history and physical examination at the sixth examination cycle of the Framingham Offspring Study, 3143 provided a valid FFQ. Of these 3143 participants, 917 (416 men and 501 women) had corresponding fasted blood samples that were randomly selected for analyses of vitamin K biochemical markers used for this study. Participants were excluded if they had missing covariate information (n = 8), were taking oral anticoagulants (n = 16), oral hypoglycemics (n = 33) or insulin (n = 15), or if they were severely hypertglycemic (fasting triglycerides ≥ 5.65 mmol/L; n = 8), reducing the final sample size to 837 (369 men and 468 women). This study was approved by the Institutional Review Boards for Human Research at Boston University and Tufts University.

Measurements. At the time of examination, information regarding medication use, medical history, smoking status, alcohol consumption and dietary intake was collected. Anthropometric measurements were taken at each examination and body mass index (BMI) [weight (kg)/height (m)²] was calculated. Individuals who regularly smoked at least one cigarette per day during the year before the examination were classified as current smokers. Postmenopausal status was defined as cessation of menses for at least 1 y, and postmenopausal women were further classified into those taking or not taking hormone replacement therapy.

Dietary assessment method. Usual dietary intake for the previous year was assessed at the sixth cycle using a semi-quantitative 126-item FFQ (18,19). The questionnaires were mailed to the participants before the examination and the participants were asked to bring the completed questionnaire with them to their appointment. The FFQ consisted of a list of foods with a standard serving size and a selection of nine frequency categories ranging from never or <1 serving/mo to >6 servings/d. Participants were asked to report their estimated frequency of consumption of each food item during the last year. Information about vitamin and mineral supplements use was also reported in the FFQ. Because there was no significant difference in phylloquinone intakes among supplement users compared with non-supplement users, intakes from multivitamin supplements were added to the calculated dietary intake of phylloquinone. Dietary information was judged as unreliable and excluded from further analysis if reported energy intakes were <2.51 MJ/d (600 kcal) or >16.74 MJ/d (4000 kcal/d) for women and >17.57 MJ/d (4200 kcal/d) for men, respectively, or if ≥12 food items were left blank.

Blood measurements. Fasting blood samples (≥10 h) were collected as part of the sixth examination cycle in EDTA-containing tubes and plasma was separated from blood cells by centrifugation (1600 × g, 10 min, 4°C). Plasma and serum samples were stored at −70°C for ≤2 years and were analyzed upon the first thaw. To examine effects of seasonal variation on biomarker concentrations, blood samples were classified as follows: Between December and February were classified as winter, between March and May as spring, between June and August as summer, and between September and November as fall.

Plasma phylloquinone was determined by HPLC with fluorescent detection as described elsewhere (20). Low and high control specimens had average values of 0.56 and 3.15 nmol/L, with total CV of 15.2% and 10.9%, respectively. Serum total OC and ucOC were analyzed by radioimmunoassay using procedures described by Gundberg et al. (9). This assay uses human osteocalcin for standard and tracer and a polyclonal antibody directed to intact human osteocalcin (21). It recognizes intact osteocalcin and the large N-terminal-mid molecule fragment (9). The ucOC was expressed as the percentage of OC not bound to hydroxyapatite in vitro (%ucOC) and normalized to the amount of total OC in a given sample by using equations described elsewhere (9). Total CV for the three control sera with average total OC results of 3.4, 7.1 and 11.9 µg/L were 22.3, 12.8 and 7.8%, respectively. Plasma 25-hydroxyvitamin D concentrations were measured using the DiaSorin (DiaSorin, Stillwater, MN) radioimmunoassay kit. Total CV for control values of 36 and 137 nmol/L were 8.5% and 13.2%, respectively. Triglyceride concentrations were measured enzymatically, as described elsewhere (22).

Statistical methods. All statistical analyses were performed using SAS (Version 8.1, SAS Institute, Cary, NC). To improve normality for statistical testing with continuous variables, natural logarithmic transformations were applied to plasma phylloquinone concentrations and a square root transformation to total OC concentrations. Adjusted geometric mean plasma phylloquinone and 95% confidence intervals (CI) were calculated by using least squares means. Because the distribution of %ucOC were highly skewed toward the lower values and deviated strongly from the assumption of normal distribution even after transformation, descriptive results are presented as medians and interquartile ranges.

To examine factors related to plasma phylloquinone concentrations, we created categories of BMI and alcohol intake and quintile categories of phylloquinone and green vegetable intakes (includes string beans, broccoli, cabbage/coleslaw, Brussels sprouts, cooked spinach, raw spinach, kale/mustard/chard, iceberg lettuce/head lettuce, romaine lettuce, leaf lettuce). We determined the multivariate adjusted geometric mean plasma phylloquinone, concentration and 95% CI within categories of lifestyle characteristics, using SAS PROC GLM, controlling for the effect of age, fasting triglyceride concentrations and dietary phylloquinone and energy intake. We report the P value for the comparison of each category of a variable with a reference category, when the F statistic from the ANOVA was significant (P < 0.05). Tests of linear trends were performed across categories of plasma phylloquinone using linear regression models with independent variables represented in regression models as continuous variables. Geometric mean plasma phylloquinone concentrations per decile category (median intake of each decile) of phylloquinone and green vegetable intake were plotted, after adjustment for sex, age, fasting triglyceride concentrations, energy intake, season and smoking status.

Based on feeding study data using the same assay for %ucOC (11,12), we have consistently observed that the %ucOC is <20% following constant dietary phylloquinone intakes equivalent to or greater than current dietary recommendations (23). Based on this observation, a cut-point of ≥20% ucOC was selected as suggestive of risk for dietary vitamin K insufficiency. Odds ratios (OR) and 95% CI for high (≥20%) UC were assessed by multivariate logistic regression analysis. The independent effects of the dietary phylloquinone or plasma phylloquinone concentrations on the risk of a high %ucOC were conducted separately for men and women. The following covariates were included in all multiple logistic regression analyses: age, BMI, alcohol intake, smoking, plasma 25(OH)D, season and menopausal status among women. In addition, analyses of vitamin K intake were adjusted for energy intake and analyses of plasma phylloquinone were adjusted for fasting triglyceride concentrations.

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RESULTS

Mean phylloquinone (µg/d) intake, plasma phylloquinone (nmol/L) and total OC (µg/mL) concentrations and the %ucOC are presented in Table 1. Because there was a positive correlation (r = 0.34) between plasma phylloquinone and triglyceride concentrations, the phylloquinone concentrations were adjusted for fasting triglyceride concentrations in the multivariate models. However, the determinants of plasma phylloquinone were similar, with or without adjustment for triglycerides (data not shown).

Because there was a significant interaction between age and sex observed for total OC (P = 0.03), total OC concentrations in each age group were presented separately for men and women. Mean daily phylloquinone intakes were higher (P < 0.001) in women than in men (151 and 115 µg/d, respectively); however, there were no sex-specific significant differences in mean plasma phylloquinone concentrations (0.94 and 0.99 nmol/L in women and men, respectively). There were no differences in dietary phylloquinone intake, plasma phylloquinone or total OC concentrations across age groups.

Potential nondietary and dietary determinants of the geometric mean plasma phylloquinone concentrations are shown in Table 2. Male smokers had lower plasma phylloquinone concentrations than non-smokers (0.82 nmol/L compared with 1.07 nmol/L, P = 0.02); no influence of smoking was found in women. Men and women examined during summer and fall seasons had higher concentrations of plasma phylloquinone compared with those examined during the winter (P for trend = 0.04 and 0.02 for men and women, respectively). There were no linear associations between plasma phylloquinone concentrations and age, BMI or alcohol intake in men or women.

Mean plasma phylloquinone concentrations showed a significant linear association with both total phylloquinone and green vegetable intake. When adjusted for age, sex, energy, triglyceride, season and smoking, there was a linear increase in plasma phylloquinone concentrations with greater dietary phylloquinone intake up to intakes of ~200 µg/d, at which point the plasma phylloquinone concentrations reached a plateau (Fig. 1). Similarly, a linear increase in plasma phylloquinone concentrations with greater intake of green vegetables was noted up to intakes of ~12 servings of green vegetables per week, after which there was an observed plateau effect (data not shown).

The distribution of %ucOC was highly skewed toward lower values, as shown in Figure 2. The prevalence of high %ucOC concentrations (≥20%), suggestive of a low vitamin K status, was 44% in men and 54% in women. The prevalence of a high %ucOC was 2.5 times greater for women and nearly 3 times greater for men in the lowest quintile of phylloquinone intake category compared with those in the highest quintile of phylloquinone intake (Table 3). The odds of a high %ucOC was also greater for those in the lowest quintile category of plasma phylloquinone (OR: 1.92; 95% CI: 0.98–3.76 in women and 4.11; 95% CI 1.81–9.34 in men) compared to those in the highest plasma phylloquinone category.

DISCUSSION

In this study, men and women with poor vitamin K status, as defined by either low dietary phylloquinone intake or low phylloquinone concentrations, had a higher prevalence of a high %ucOC, an independent risk factor for hip fracture (24). These findings are consistent with supplementation and feeding studies (10,11).

Although reported phylloquinone intakes were significantly higher among women compared with men, there were no corresponding sex-specific differences in plasma phylloquinone concentrations. However, the dependence of a single fasting plasma phylloquinone measurement on recent dietary intake limits its value as an indicator of vitamin K status (3). Interestingly, the unadjusted %ucOC was higher in women than in men, despite the higher reported intakes in the former. Current evidence does not suggest sex-specific differences in vitamin K absorption or function (12,25,26). An alternative explanation for the sex-specific discrepancy between intakes and biochemical markers is a potential overestimation by women of phylloquinone-rich foods, such as broccoli, cabbage and other greens (27).

Diet-plasma associations for phylloquinone were not affected by adjustment for triglyceride concentrations in this study. However, given that plasma phylloquinone concentrations are highly correlated with triglycerides in this study and others (28,29), plasma phylloquinone concent-

TABLE 1

<table>
<thead>
<tr>
<th>Dietary phylloquinone2</th>
<th>Plasma triglyceride</th>
<th>Plasma phylloquinone3</th>
<th>Total OC</th>
<th>%UCOC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/d</td>
<td>mmol/L</td>
<td>nmol/L</td>
<td>µg/L</td>
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<tr>
<td>Sex</td>
<td></td>
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</tr>
<tr>
<td>Men (n = 369)</td>
<td>115 (109–121)</td>
<td>1.27 (1.20–1.34)</td>
<td>0.99 (0.91–1.07)</td>
<td>4.34 (4.28–4.40)</td>
</tr>
<tr>
<td>Women (n = 468)</td>
<td>151 (143–158)</td>
<td>1.15 (1.09–1.20)</td>
<td>0.94 (0.88–1.02)</td>
<td>4.57 (4.52–4.62)</td>
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<tr>
<td>Age groups, y</td>
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<tr>
<td>&lt;50 (n = 147)</td>
<td>132 (120–144)</td>
<td>1.01 (0.93–1.11)</td>
<td>0.87 (0.77–0.99)</td>
<td>4.50 (4.36–4.65)</td>
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<tr>
<td>50–59 (n = 341)</td>
<td>135 (127–143)</td>
<td>1.21 (1.14–1.28)</td>
<td>0.95 (0.88–1.04)</td>
<td>4.37 (4.28–4.45)</td>
</tr>
<tr>
<td>60–89 (n = 226)</td>
<td>134 (125–144)</td>
<td>1.30 (1.21–1.39)</td>
<td>1.09 (0.98–1.20)</td>
<td>4.40 (4.29–4.50)</td>
</tr>
<tr>
<td>≥70 (n = 123)</td>
<td>132 (120–146)</td>
<td>1.24 (1.12–1.36)</td>
<td>0.90 (0.79–1.04)</td>
<td>4.07 (3.94–4.20)</td>
</tr>
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</table>

1 Least square mean for dietary phylloquinone and of plasma triglyceride and phylloquinone concentrations.
2 Analyses for total osteocalcin were done with the square root transformation. Untransformed means are presented here.
3 Adjusted for triglyceride concentrations.
4 Median (interquartile range).
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We previously reported that participants with the highest dietary intake of phylloquinone were less likely to smoke (30), an observation consistent with other studies (31). In this study, male smokers also had lower plasma phylloquinone concentrations than nonsmokers, independent of dietary phylloquinone intake. We currently do not have an explanation for this finding. There was no association between alcohol intake, green vegetable intake, and phylloquinone intake, and phylloquinone intake adjusted for triglyceride concentrations were lower in older subjects than in younger subjects.

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concentrations. In addition, analyses of vitamin K intake were adjusted for energy intake and analyses of plasma phylloquinone were adjusted for fasting triglyceride concentrations.

The seasonal effect on plasma phylloquinone may be due to regional or temporal differences in the phylloquinone content of the food supply, or due to the large intra-individual variation in both phylloquinone intakes and plasma concentrations (6).

We observed a significant association between plasma phylloquinone concentrations and both self-reported usual total phylloquinone intake and green vegetable intakes as estimated by an FFQ. However, there seemed to be a threshold effect at self-reported usual phylloquinone intakes of ~200 μg/d. There are no step-wise phylloquinone repletion feeding studies that have increased intakes from 200 to 400 μg/d, corresponding with the range of self-reported intakes in this study to confirm this observation. However, manipulation of dietary phylloquinone intakes from 100 μg/d to 400 μg/d in a feeding study has been shown to significantly increase plasma phylloquinone concentrations (12). An alternative explanation for this observed plateau may in part be due to the potential limitation of the semiquantitative FFQ to estimate high phylloquinone intakes and the inherent variability in the current food composition databases. Leafy green vegetables, the primary dietary source of phylloquinone, vary considerably in phylloquinone content. For example, the reported phylloquinone concentrations of a 100-g serving of raw spinach leaves range from 240-1220 μg (14). Intra-individual consumption of green vegetables also varies considerably (5) and whereas the FFQ estimates usual intake, the single plasma phylloquinone concentrations reported in this study reflect recent phylloquinone intake. Although the FFQ appears to be appropriate for ranking individuals by their phylloquinone intake across the entire range of intakes reported in this study, among others (1,2), the association between reported phylloquinone intakes and biomarkers may be attenuated at reported intakes > 170 μg/d. In The Netherlands’ study by Schurgers et al. (8), the reported mean dietary intakes were ~250 μg/d, so it is plausible that the lack of association between self-reported dietary intakes and serum phylloquinone concentrations was attributable to the use of an FFQ in a population with considerably higher average intakes than those reported in other population-based studies (1,2,7).

Consistent with other studies (10,34), plasma phylloquinone concentrations were inversely associated with %ucOC in

### TABLE 3

Multivariate odds ratios (95% CI) for %ucOC of ≥20% according to median quintile categories of dietary phylloquinone intake and plasma phylloquinone concentrations in men and women

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Serum ucOC (%)</th>
<th>Risk factor</th>
<th>Serum ucOC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin K intake, μg/d</td>
<td></td>
<td>Vitamin K intake, μg/d</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>2.51 (1.23–5.11) 0.01</td>
<td>54</td>
<td>2.75 (1.29–5.87) 0.009</td>
</tr>
<tr>
<td>109</td>
<td>1.57 (0.81–3.05) 0.15</td>
<td>91</td>
<td>2.10 (1.00–4.40) 0.05</td>
</tr>
<tr>
<td>146</td>
<td>1.22 (0.67–2.23) 0.43</td>
<td>119</td>
<td>1.87 (0.88–3.98) 0.11</td>
</tr>
<tr>
<td>193</td>
<td>1.58 (0.81–2.89) 0.14</td>
<td>163</td>
<td>1.18 (0.55–2.53) 0.68</td>
</tr>
<tr>
<td>307</td>
<td>1.0</td>
<td>254</td>
<td>1.0</td>
</tr>
<tr>
<td>Plasma phylloquinone, nmol/L</td>
<td></td>
<td>Plasma phylloquinone, nmol/L</td>
<td></td>
</tr>
<tr>
<td>0.40</td>
<td>1.92 (0.98–3.76) 0.06</td>
<td>0.43</td>
<td>4.11 (1.81–9.34) 0.0007</td>
</tr>
<tr>
<td>0.63</td>
<td>1.56 (0.79–3.10) 0.20</td>
<td>0.73</td>
<td>4.18 (1.91–9.11) 0.0003</td>
</tr>
<tr>
<td>1.02</td>
<td>1.08 (0.56–2.08) 0.83</td>
<td>1.07</td>
<td>2.31 (1.08–4.90) 0.03</td>
</tr>
<tr>
<td>1.57</td>
<td>1.12 (0.59–2.15) 0.73</td>
<td>1.67</td>
<td>1.75 (0.84–3.67) 0.14</td>
</tr>
<tr>
<td>2.65</td>
<td>1.0</td>
<td>3.02</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1 Adjusted for age, body mass index, alcohol, plasma 25(OH)D, smoking, season, multivitamin use and menopausal status for women only. In addition, analyses of vitamin K intake were adjusted for energy intake and analyses of plasma phylloquinone were adjusted for fasting triglyceride concentrations.
this study. Based on feeding study data using the same assay for %ucOC (11,12), we used a cut-point of ≥20% ucOC as suggestive of risk for dietary vitamin K insufficiency. Currently, there are widely divergent reports of the percentage of osteocalcin that is undercarboxylated in a healthy population. How the degree of carboxylation is assessed depends upon the amount of osteocalcin in the sample and the amount of hydroxyapatite used for binding (9). The current methodology precludes exact determination of undercarboxylated osteocalcin; instead it is a relative term, with the direction being critical and not the absolute percentages. Because low plasma 25(OH)D concentrations have also been associated with elevated %ucOC (35) and have a potential confounding effect on associations between %ucOC and hip fracture risk (36), serum %ucOC were adjusted for 25(OH)D concentrations in this study and we still observed a significant association. We also observed a significant association between self-reported usual phylloquinone intakes and risk of high %ucOC. For population-based studies, where collection of biological samples is impractical, use of an FFQ may be a valid alternative approach to the assessment of vitamin K status, particularly in populations consuming a wide range of phylloquinone intakes.

In summary, men and women with poor vitamin K status, defined by either low dietary phylloquinone intakes or low phylloquinone concentrations, had more prevalence of a high %ucOC, an independent risk factor for hip fracture (24). These findings support the use of an FFQ for a relative assessment of vitamin K status in population-based studies; however, there may be limitations to its use in populations with high phylloquinone intakes because of the observed plateau effect. Furthermore, triglyceride concentrations, smoking status and season were associated with plasma phylloquinone concentrations and should be accounted for in population-based studies assessing associations between plasma phylloquinone and disease risk.

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LITERATURE CITED