Response of vitamin K status to different intakes and sources of phylloquinone-rich foods: comparison of younger and older adults 1–4

Sarah L Booth, Maureen E O’Brien-Morse, Gerard E Dallal, Kenneth W Davidson, and Caren M Gundberg

ABSTRACT

Background: Phylloquinone, found in dark-green vegetables and certain plant oils, is the primary dietary source of the fat-soluble vitamin K. Limited data suggest that the relative bioavailability of phylloquinone from vegetables is lower than that from a supplement. This finding is relevant to the maintenance of optimal vitamin K status.

Objective: The objective of this study was to compare, in younger and older adults, the relative bioavailability of phylloquinone from a vegetable with that of a fortified oil.

Design: In a crossover design with three 15-d residency periods in a metabolic unit, younger and older men and women (n = 36) consumed a mixed diet containing 100 µg phylloquinone/day. During 2 residency periods, the mixed diet was supplemented for 5 d with either broccoli (377 µg phylloquinone/d; broccoli diet) or phylloquinone-fortified oil (417 µg/d; oil diet). The relative bioavailability of phylloquinone was defined by the difference in plasma phylloquinone, percentage serum undercarboxylated osteocalcin (%ucOC), and urinary γ-carboxyglutamic acid in response to 5 d of supplementation.

Results: For both younger and older adults, plasma phylloquinone concentrations were higher (P < 0.001) and %ucOC values were lower (P = 0.001) after the broccoli and oil diets than after the mixed diet only. Overall, the response to broccoli supplementation was not significantly different from the response to the fortified oil in either age group. Urinary γ-carboxyglutamic acid did not change in response to supplementation.

Conclusions: There was no significant difference in the relative bioavailability of phylloquinone, as evidenced by the lack of a significant difference in plasma phylloquinone and %ucOC between the 2 groups after either the broccoli or oil diets for younger and older adults. Am J Clin Nutr 1999;70:368–77.

KEY WORDS Osteocalcin, undercarboxylated osteocalcin, urinary γ-carboxyglutamic acid, phylloquinone, bioavailability, vitamin K, adults

INTRODUCTION

It is now recognized that coagulation assays, which were once the classic measure of vitamin K nutritional status, lack the sensitivity to assess changes in vitamin K status (1). More sensitive static and functional markers of vitamin K nutritional status have been developed and subsequently validated, including plasma phylloquinone concentrations, serum osteocalcin (OC) [total and the percentage undercarboxylated (%ucOC)], and urinary γ-carboxyglutamic acid (Gla) (2). Collectively, these measures have been assessed in relation to vitamin K deficiency (3–5), antagonism (6–8), and supplementation (9). Plasma phylloquinone concentrations have been correlated with recent dietary phylloquinone intakes, but have limited capacity as biochemical markers given their rapid (≤ 24 h) fluctuations in response to changes in dietary phylloquinone intakes (10).

Phylloquinone, the predominant dietary source of vitamin K, is found in both green vegetables and certain plant oils (10). Given that vitamin K is a fat-soluble vitamin, it is assumed that phylloquinone is more bioavailable from an oil than from a vegetable (11). Indeed, Gijsbers et al (12) and Garber et al (13) showed that the relative bioavailability of phylloquinone consumed in the form of an oil-based supplement is higher than the bioavailability of phylloquinone from different vegetables. In both of these studies, bioavailability was defined as the area under the curve for plasma phylloquinone. The effect of these differences in the relative bioavailability of phylloquinone from different foods on functional biochemical markers of vitamin K status is not known.

It is also not known whether these reported differences in the relative bioavailability of phylloquinone are consistent among different age groups. Absolute measures of vitamin K status are different in older adults than in older adults. For example, adults aged ≥ 60 y have higher plasma phylloquinone concentrations (14, 15) and higher urinary Gla-creatinine ratios (2) than do adults aged ≤ 60 y. Older adults also appear more resistant to

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Table 1

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<th>Younger adults</th>
<th>Older adults</th>
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<td></td>
<td>Men (n = 9)</td>
<td>Women (n = 9)</td>
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<tr>
<td>Age (y)</td>
<td>31.2 ± 1.9</td>
<td>30.7 ± 2.1</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Plasma phylloquinone (nmol/L)</td>
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<td>1.04 ± 0.10</td>
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<td>Dietary phylloquinone intake (μg/d)</td>
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1 ± SEM.
2 Average of 3 fasting plasma phylloquinone concentrations on day 1 for each subject, as reported previously (15). There was a significant main effect of age, but not of sex.
3 Average of three 4-d weighed dietary records maintained by each subject while in a free-living state, as reported previously (15).
4 n = 8 because of incomplete dietary records, as reported previously (15).

Subjects and Methods

Subjects

Thirty-six men and women were recruited from the New England region and stratified into 4 groups (9 subjects/group) according to age (younger adults: 20–40 y; and older adults: 60–80 y) and sex. A sample size of 9 per age-sex group was calculated based on a 0.05 level of significance and a power of 0.8 for detecting an estimated mean difference in plasma phylloquinone of 0.150 nmol/L, assuming a within-group SD of 0.075 nmol/L in response to dietary phylloquinone supplementation (9). These findings were part of a larger metabolic study to compare the relative bioavailability of phylloquinone from a vegetable with that of an equivalent amount of phylloquinone from an oil. In the larger metabolic study, which is reported here, relative bioavailability was defined as the difference in plasma phylloquinone concentrations, urinary Gla excretion, and %ucOC after phylloquinone supplementation in the form of broccoli or a fortified oil. We also tested the hypothesis that the change in vitamin K status in response to dietary phylloquinone supplementation is lower in older adults than in younger adults. Age-related differences in baseline plasma phylloquinone concentrations and their association with recent dietary intakes preceding admission into the metabolic study were reported elsewhere (15).

Response of Vitamin K Biochemical Measures

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Study design

In a crossover design, each study participant resided in the Metabolic Research Unit (MRU) at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University for 3 residency periods of 15 d each. There was a free-living period of ≥6 wk between each residency period, during which time each subject consumed a self-selected diet.

As described in greater detail elsewhere, the weight-maintaining diet (mixed diet) consumed throughout the 3 residency periods was a 3-d rotating plan based on foods that are commonly consumed in the American diet (17). The principal criterion in designing the mixed diet was to approximate the current recommended dietary allowance (RDA) for vitamin K by providing ≥80 μg phylloquinone/d (18). The mixed diet was also formulated to meet the current RDA for macro- and micronutrients as established for the older segment of the population, which is currently defined as >50 y of age (18).

For one residency period of 15 consecutive days, study participants consumed the mixed diet only (mixed-diet period). On days 6–10 of a second residency period (broccoli-diet period), each study participant consumed a 102.4-g serving of microwaved broccoli at both lunch and dinner (total of 204.8 g/d) in addition to the mixed diet. On days 6–10 of a third residency period (oil-diet period), the corn oil in the mixed diet was fortified with exogenous phylloquinone (Sigma Chemical Co, Inc, St Louis) at both lunch and dinner. The order of the residency periods was randomized for each study participant in this crossover design.

To control for potential variation in phylloquinone content, a single lot of each of the frozen vegetables was purchased for all study participants, protected from light, stored at −20°C, and microwaved for 90 s before consumption. The phylloquinone-rich food sources included green peas and beans, vegetable juice, and pumpkin muffins, and were distributed between the lunch and dinner meals. Intake of non-phylloquinone-containing foods, such as rice, bread, and carbonated beverages, was adjusted to fulfill the wide range of energy requirements among the study participants (7530–15070 kJ/d). The mixed diet contained a mean (±SD) of 25.6 ± 4.3% of energy as dietary fat. Study participants were allowed 1 cup of instant coffee/d, although consumption
was voluntary. No other supplemental foods or beverages were allowed during the residency periods, except for deionized water.

On multiple occasions, replicates of each meal were prepared and the entire contents of each single-day menu was homogenized. An aliquot of each single-day menu was then analyzed by HPLC to confirm the phylloquinone content of the diet. When these 3-d menus were prepared on 10 separate occasions over a period of 12 mo and the homogenates were analyzed by HPLC, the mean (±SD) phylloquinone concentration of the mixed diet was 100 ± 12 μg/d. A replicate of each study participant’s day 6 menu for both the broccoli and the oil diets was also analyzed by HPLC. During the oil-diet period, corn oil, which contains little endogenous phylloquinone, was fortified by using exogenous phylloquinone to maintain the fatty acid composition and total fat content of the mixed diet. Addition of the fortified oil increased the phylloquinone content of the diet to a mean (±SD) total of 417 ± 45 μg/d. The fortified oil was stored in opaque containers to prevent degradation of the phylloquinone. A single lot of frozen broccoli florets was purchased for the broccoli-diet period for the entire study group. Addition of the broccoli increased the mean (±SD) phylloquinone content of the diet to a total of 377 ± 46 μg/d. The mixed diet was calculated to contain a mean (±SD) calcium content of 947 ± 150 mg/d. During days 6–10 of the broccoli diet, the total calculated calcium content of the diet increased to a mean total intake of 1100 mg/d. As a group, the mean (±SD) dietary calcium intake was 837 ± 482 mg/d during the free-living period, as estimated from dietary records averaged over 4 d preceding admission into the MRU for each residency period (15).

Fasting blood samples were obtained between 0630 and 0800 on days 1, 2, 4, 6, 7, 9, 11, 12, 14, and 16 of each residency period. Plasma phylloquinone concentrations were assessed for all days on which blood samples were collected. Serum total and %ucOC measurements, PT, and APTT were assessed on days 1, 6, 11, and 16. Twenty-four-hour urine samples were collected daily throughout the residency periods for the measurement of urinary Gla, calcium, and creatinine. All samples were stored at −70°C and were protected from light and multiple freeze-thaw cycles until analyzed.

METHODS

PT and APTT were determined by photometric detection with an MLA Electra 800 automated clot timer (Medical Laboratory Automation, Inc, Pleasantville, NY) by using reagents from Dade Diagnostics (Miami). Phylloquinone (in food and plasma) was analyzed by reversed-phase HPLC by using postcolumn solid-phase chemical reduction of phylloquinone to its hydroquinone, followed by fluorometric detection (19, 20). Initially, we used a barium sulfate–binding radioimmunoassay (RIA) (7) to determine serum total and ucOC values in the first 9 study participants to complete the study (9). Blood samples from the entire group of study participants were subsequently reanalyzed for serum total OC and ucOC with a hydroxyapatite-binding RIA (21). ucOC was expressed as the percentage not bound (%ucOC) and normalized to the amount of total OC in a given sample by using equations described elsewhere (21).

Urinary Gla was determined by ortho-phthalaldehyde derivitization, followed by reversed-phase HPLC with fluorometric detection (22). Urinary Gla data were expressed as a percentage of baseline values and presented as means of 3-d moving averages from each study participant. Urinary calcium was analyzed by direct current plasma spectrometry with a Beckman Instruments (Fullerton, CA) Spectra-Span VI sequential direct current plasma spectrometer (23). Urinary creatinine was analyzed by a colorimetric method on a Cobas Mira analyzer (Roche Instruments, Belleville, NJ).

Statistics

Results were expressed as means ± SEMs unless otherwise specified. Results were considered statistically significant if the observed, two-sided significance level (P value) was ≤0.05. On day 1 of each residency period, associations were examined between OC (total and %ucOC) and PT plasma phylloquinone concentrations, 2) urinary Gla-creatinine ratios, and 3) dietary phylloquinone intakes during free-living periods (15). Associations were also examined between urinary Gla-creatinine ratios and J plasma phylloquinone concentrations, 2) osteocalcin (total and %ucOC), and 3) dietary phylloquinone intakes. These cross-sectional correlations on day 1 were analyzed by using the methods of Steiger (24) as implemented in MULTICORR, version 2.4 (J Steiger, University of British Columbia, Vancouver, Canada), to account for having measured the association on the same set of study participants at the start of 3 distinct residency periods in this crossover design. None of the preliminary tests of equal correlations across all 3 periods of the study yielded significant differences (all P > 0.10). Pearson correlation coefficients were used to measure the associations between urinary calcium and urinary Gla excretion (both absolute and normalized to creatinine excretion), in the manner of Bland and Altman (25, 26).

The effects and interactions of day, diet, age, and sex were assessed for all biochemical measures by using by-diet-measures analysis of variance comparing days 1, 6, 11, and 16 (days 1, 5, 10, and 15 for 24-h urine) across diet periods, as implemented in SYSTAT (version 7.0.1; SPSS Inc, Chicago). Diet and day were defined as within-subjects factors, and age group and sex were defined as between-subjects factors. If the day-by-diet interaction was significant (P < 0.05), Tukey’s honestly significant difference (HSD) test was used to establish differences within and between the 3 diet periods. The area under the curve (AUC) for phylloquinone from days 6 to 11 was analyzed by repeated-measures analysis of variance. Tukey’s HSD was used to compare the periods within each age group.

RESULTS

Coagulation measures

There were no significant changes in PT or APTT in response to diet. Mean PT and APTT on day 1 of all 3 study periods were 12.4 ± 0.04 and 29.2 ± 0.2 s, respectively. There were no significant changes in PT or APTT in response to consumption of J the mixed diet for 15 consecutive days (day 16: 12.5 ± 0.06 and 30.1 ± 0.4 s, respectively), 2) the broccoli diet for 5 consecutive days (day 11: 12.5 ± 0.01 and 29.9 ± 0.4 s, respectively), or 3) the oil diet for 5 consecutive days (day 11: 12.2 ± 0.06 and 30.1 ± 0.5 s, respectively). There were no significant age or sex effects on either PT or APTT.

Plasma phylloquinone

The older adults had higher plasma phylloquinone concentrations than the younger adults (P = 0.05), as reported elsewhere (15) (Table 1). There were no significant differences in plasma phylloquinone concentrations between men and women. For both the younger and older adults, there was a significant decrease in plasma
phyllloquinone concentrations between days 1 and 6 (0.26 ± 0.10 and 0.35 ± 0.13 nmol/L for younger and older adults, respectively), after which there was no significant change while consuming the mixed diet (Figure 1). The change in plasma phylloquinone concentrations from days 6 to 11 in the mixed-diet period (0.02 ± 0.07 and 0.003 ± 0.06 nmol/L for younger and older adults, respectively) was significantly less than in the broccoli-diet period (1.76 ± 0.34 and 2.01 ± 0.29 nmol/L for younger and older adults, respectively). Plasma phylloquinone concentrations on day 11 in the broccoli-diet period were not significantly different from those in the oil-diet period for either age group.

When the AUC for phylloquinone from days 6 to 11 was calculated, there was a significant diet-by-age interaction ($P = 0.045$), indicating that the effects of all 3 diets were significantly different between younger and older subjects (Figure 1). In the younger adults, AUCs in both the broccoli- and oil-diet periods were significantly different from those in the mixed-diet period ($P < 0.001$) and also from each other ($P = 0.044$), with the highest AUC measured in the oil-diet period. Older men had a significantly ($P = 0.008$) higher AUC than the older women. In contrast, there were no significant differences in AUCs between the younger men and women.

Serum total and $\%$ucOC

Mean total serum OC concentrations on day 1 were significantly correlated with plasma phylloquinone concentrations on day 1 ($r = 0.091$, $P = 0.034$). During each dietary period, mean $\%$ucOC values on day 1 were significantly correlated ($r = -0.194$, $P < 0.001$) with dietary phylloquinone intakes averaged over the 4 d preceding admission into the MRU, as described previously (15). No other cross-sectional correlations on day 1 between total or $\%$ucOC values and phylloquinone (plasma or diet) or urinary Gla excretion were significant. Because this study only had 36 study participants and CIs for each of the variables were wide, lack of significance in these day 1 associations may have been due to a lack of power as well as to the absence of any association.
Total OC concentrations were significantly lower on day 6 than on days 1, 11, and 16 of all 3 dietary periods ($P < 0.05$, Tukey’s HSD) and was more pronounced in the older adults than in the younger adults (Figure 2). However, each study participant had a catheter inserted at 0800 on day 6 of each residency period to study the diurnal variation in OC and other vitamin K biochemical measures (27). This led to red blood cell disruption in some samples, which could have initiated enzymatic degradation of OC (28). When the OC values from the 48 hemolyzed samples were removed from the day 6 analysis, there were no longer any significant effects of day or diet. Therefore, it is more plausible that the reported change in total OC on day 6 was an artifact of blood sample collection and not a response to the dietary manipulation of phylloquinone.

Overall, younger adults had significantly higher %ucOC values than did older adults ($P = 0.001$), but there were no significant differences between men and women. There were no significant changes in %ucOC in response to the mixed diet (Figure 3). When the changes in %ucOC from days 6 to 11 were compared between the 3 dietary periods, there was a significant diet period-by-day interaction ($P = 0.001$), with the oil and broccoli diets producing a dramatic drop in %ucOC on day 11 that was not observed with the mixed diet. On day 11, %ucOC values with the broccoli diet were not significantly different from those with the oil diet.

**Urinary Gla**

There was insufficient statistical evidence for an association between 24-h urinary Gla and plasma phylloquinone on day 1. The correlation was marginally significant ($P = 0.021$) for only 1 of the 3 dietary periods and did not remain significant after a Bonferroni adjustment for multiple tests. There were no other significant correlations between urinary Gla (absolute or normalized to creatinine) and phylloquinone (diet or plasma).

When expressed as a percentage of baseline, absolute urinary Gla excretion did not change significantly during the 15-d mixed-diet period (Figure 4). However, when normalized for creatinine and expressed as a percentage of baseline, there was a significant day-by-sex interaction during the mixed-diet period ($P = 0.001$), with the men having a greater decline in the urinary Gla-creatinine ratio between days 1 and 15 (day 15: 91 ± 4% and 91 ± 2% of baseline values for younger and older men, respectiv-
tively) than the women (day 15: 101 ± 2% and 99 ± 5% of baseline values for younger and older women, respectively). When the changes in urinary Gla excretion (expressed as a percentage of baseline) from days 5 to 10 were compared between the 3 diet periods, there was an overall diet period-by-day interaction ($P = 0.04$). The biological significance of this interaction is unclear because there were no significant differences in urinary Gla excretion between the 3 dietary periods on day 10 of each period (100.7 ± 1.0%, 101.6 ± 0.8%, and 100.7 ± 0.7% of baseline for the mixed-, broccoli-, and oil-diet periods, respectively).

Urinary calcium

Total urinary calcium was significantly correlated with urinary Gla excretion ($r = 0.19$, $P < 0.001$). Calcium normalized to creatinine was also significantly associated with the urinary Gla-creatinine ratio ($r = 0.38$, $P < 0.001$). Calcium excretion, either total or normalized to creatinine, was not correlated with plasma phylloquinone or serum OC (total or %ucOC). The urinary calcium-creatinine ratio showed a significant ($P < 0.001$) day effect, with the ratio being highest on day 6 of all 3 diets (Figure 5). This overall day effect was also noted during the mixed-diet period, with the urinary calcium-creatinine ratio being lowest on days 3–5 and 12–15 and highest on day 6. We have no explanation for this significant day effect. There were no significant differences when the urinary calcium-creatinine ratios from days 6 to 11 were compared among the 3 diets for either age group.

DISCUSSION

Results from this study suggest that there were no significant differences in the relative bioavailability of phylloquinone from broccoli and from a fortified oil in both younger and older adults. Bioavailability was defined by the change in plasma phylloquinone, %ucOC, and urinary Gla in response to the addition of these foods to a mixed diet. The total dietary intake of phylloquinone during supplementation was 377–417 µg/d. These findings have important implications because green vegetables are the primary dietary source of phylloquinone (10). Recent dietary stud-
ies suggest that the average American diet for younger adults does not meet the current RDA for vitamin K (10). If the relative bioavailability of phylloquinone from vegetables is not different from that of phylloquinone-rich oils, promotion of green vegetables for optimal vitamin K status is warranted. However, our findings are not consistent with those reported by others (12, 13). The relative bioavailability of phylloquinone may vary among plant species. Garber et al (13) reported recently that the relative bioavailability of phylloquinone from broccoli is significantly higher than that from spinach. Broccoli was selected in our study because it was ranked the primary dietary source of phylloquinone among postmenopausal women in the New England region (29). It is plausible that another vegetable, such as spinach, would have a lower relative bioavailability of phylloquinone than a phylloquinone-rich oil under our study conditions, although it is not known what other food components could contribute to this variation in bioavailability. Because there are regional differences in plant species ranked as primary dietary sources of vitamin K (10), comparison of the relative bioavailability of phylloquinone from different vegetables warrants further investigation.

Assessment of the bioavailability of a nutrient is complex and requires precise definition of the properties being measured (30, 31). The reported differences in the relative bioavailability of phylloquinone from a green vegetable compared with a phylloquinone-rich oil or supplement among the various studies may also reflect differences in the definition of bioavailability and the mode of administration. Previous studies have administered the phylloquinone-rich vegetable in a single dose after a 12-h fast and measured serial changes in plasma phylloquinone concentrations (12, 13). However, absorption of phylloquinone from a vegetable is increased in the presence of dietary fat (12). Also, intake of phylloquinone-rich foods on multiple eating occasions throughout the day mimics the American diet, but the absorption of phylloquinone under these conditions was not studied previously. In our study, the phylloquinone-rich foods were introduced for 5 consecutive days into both lunch and dinner meals, which were part of a mixed diet containing \(<25\%\) of dietary energy as fat. Under these conditions, plasma phylloquinone responded equally within 24 h to phylloquinone supplementation via either broccoli or fortified oil and returned almost to baseline values within 24 h of withdrawing the supplement. This rapid

![Figure 4](https://academic.oup.com/ajcn/article-abstract/70/3/368/4714853)

**FIGURE 4.** Mean (±SEM) response of urinary γ-carboxyglutamic acid (Gla) to consumption of a mixed diet (■), a mixed diet supplemented with broccoli (○), a mixed diet supplemented with phylloquinone-fortified oil (△), and a mixed diet supplemented with phylloquinone-fortified oil (△), and a mixed diet supplemented with phylloquinone-fortified oil (△) in 18 younger (top) and 18 older (bottom) men and women. In a crossover design, broccoli or oil was added to the mixed diet from the end of the day 5 urine collection to the end of the day 10 urine collection, as delineated by the vertical lines. There were no significant differences in urinary Gla excretion during the mixed-diet period, but there was a significant diet period-by-day interaction (P = 0.04) from days 5 to 10 of the 3 dietary periods.
response in plasma phylloquinone concentrations to dietary manipulation was consistent with the reported correlations between recent dietary intake of phylloquinone and corresponding plasma concentrations (15, 29, 32). There is no improvement in the diet-plasma correlation when dietary phylloquinone intakes are analyzed separately for vegetables and oils (29), which lends further support to our finding that there was no significant difference in relative bioavailability between the 2 primary dietary sources of phylloquinone.

In this study, there were significant diet and age effects when the changes in plasma phylloquinone supplementation were compared by using AUCs for phylloquinone. Older men and women had the greatest AUCs during the oil-diet period, which suggests that there is greater bioavailability of phylloquinone from oil than from broccoli. However, this difference in relative bioavailability was not observed among the younger adults. This disparity in response to phylloquinone supplementation between younger and older adults may explain the significantly higher baseline plasma phylloquinone concentrations consistently observed among older adults (2, 14, 15). Phylloquinone is transported by the triacylglycerol-rich lipoproteins (33), and there is a strong positive correlation between plasma phylloquinone and triacylglycerol concentrations. Because we did not measure concomitant plasma triacylglycerol concentrations in our study, it is not known whether the higher AUC for phylloquinone observed among older adults than among younger adults reflects increased triacylglycerol concentrations with age (34) or indicates a true age-related difference in phylloquinone absorption from broccoli than from oil.

In contrast with other studies, we also defined the relative bioavailability of phylloquinone by several functional biochemical indicators of vitamin K status, including %uOC and urinary Gla. Recent studies suggest that extrahepatic vitamin K–dependent proteins respond to vitamin K deficiency, supplementation, or antagonism differently than do hepatic vitamin K–dependent proteins (10). In this study, PT and APTT did not change in response to a range of dietary intakes (100–420 mg phylloquinone/d for 5–15 d). The lack of sensitivity of these coagulation measures to detect changes in response to manipulation of dietary phylloquinone have been reported by others (1, 4). In contrast, %uOC, a functional measure of extrahepatic vitamin K–dependent proteins,
significantly decreased in response to consumption of either broccoli or fortified oil.

There is some evidence, although not consistent, that there is a positive association between %ucOC and risk of hip fracture among postmenopausal women (35). Although %ucOC is a sensitive biochemical indicator of vitamin K nutritional status, it is not known whether the association with hip fracture reflects poor vitamin K status, as suggested by one epidemiologic study (36), or overall poor nutritional status, including vitamin D. Mean baseline %ucOC values for the participants in our study were 18–24%, despite usual dietary phyloquinone intakes that exceeded the current RDA for vitamin K (15). Extension of the phyloquinone supplementation period for > 5 d would provide more insight into the maximum level to which OC is optimally carboxylated through dietary supplementation.

We initially reported that %ucOC increased by an average of 28% in 9 younger adults after they consumed a mixed diet containing 100 µg phyloquinone/d for 15 d (9). When our sample size was expanded to include 18 younger (20–40 y) and 18 older (60–80 y) adults, %ucOC did not increase with the mixed diet in either younger or older adults. It is not known whether the previous report (9) of a significant response of %ucOC to the mixed diet among younger adults was a spurious result or reflected the different method used to measure %ucOC. Gundberg et al (21) determined recently that errors in the determination of ucOC were minimized by 1) expressing ucOC as a percentage of total OC in a given sample, and 2) correcting for the basal value of OC by using a polynomial equation derived from binding curves. Although we initially reported our values as %ucOC (9), we did not correct for the amount and preparation of barium salts added to the sample for binding of the OC.

The lack of response of urinary Gla to broccoli or fortified oil was expected because the supplementation period lasted only 5 d. The decrease in urinary Gla among younger adults while consuming the mixed diet for 15 d was also less in this study than originally reported for the first 9 younger adults (5% and 9%, respectively) (9). In the present study, there were no significant changes in urinary Gla among the older adults during consumption of the mixed diet. Urinary Gla data are inconsistent with those of %ucOC and plasma phyloquinone, both of which changed significantly after dietary supplementation with phyloquinone in the younger and older adults. Our laboratory reported previously that urinary Gla does not change in older adults in response to dietary depletion of phyloquinone, which suggests that the elderly are more resistant than are younger adults to vitamin K deficiency (4). Urinary Gla excretion is a measure of turnover of all Gla-containing proteins because free Gla is not further metabolized. Most urinary Gla originates from hepatic vitamin K–dependent proteins (4), and data from laboratory rats suggest that hepatic stores of phyloquinone increase with age (37). Greater hepatic stores among older adults may explain the disparity in responses between %ucOC and urinary Gla, suggesting that hepatic vitamin K–dependent proteins are not affected by dietary phyloquinone manipulation to the same extent as are extrahepatic vitamin K–dependent proteins, which appear to be equally responsive to dietary manipulation in younger and older adults.

Analysis of 4-d dietary records indicated that study participants were consuming <130 µg phyloquinone/d before admission into the metabolic study (15). The changes in plasma phyloquinone and urinary Gla from baseline after the consumption of a mixed diet containing 100 µg/d are consistent with previous findings that these biochemical indicators of vitamin K status are sensitive to dietary manipulation of phyloquinone (9). That plasma phyloquinone increased and %ucOC decreased in response to dietary supplementation of phyloquinone lend further support that %ucOC is a sensitive measure of vitamin K nutritional status.

In summary, the relative bioavailability of phyloquinone from broccoli was not found to be significantly different from that of a phyloquinone-fortified oil when given as part of a mixed diet to mimic usual patterns of phyloquinone consumption in the American diet. Whereas baseline plasma phyloquinone concentrations and %ucOC were significantly different between younger and older adults, plasma phyloquinone and %ucOC were not significantly different between the 2 groups in response to the mixed diet only or to phyloquinone supplementation (broccoli or oil). Furthermore, dietary intakes of ∼5 times the current RDA resulted in lower %ucOC and higher plasma phyloquinone values in both younger and older adults.

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REFERENCES