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A Single Measurement of Serum Phylloquinone Is an Adequate Indicator of Long-Term Phylloquinone Exposure in Healthy Older Adults[1–3]

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Abstract

Assessment of long-term phylloquinone exposure is challenging in studies investigating vitamin K in health. Data are equivocal as to whether a single measurement of circulating phylloquinone would be adequate. The primary purpose of the present study was to validate the use of a single measurement of serum phylloquinone as a surrogate for long-term phylloquinone exposure in healthy older adults. Using data from the Québec Longitudinal Study on Nutrition and Successful Aging, the objectives were to: *1*) determine the reproducibility of circulating phylloquinone over 2 y (n = 234); *2*) calculate how a single measurement would rank or classify individuals and attenuate the regression coefficient between circulating phylloquinone and a health outcome; and *3*) investigate the association of a single measurement of serum phylloquinone with long-term phylloquinone intakes assessed over the year prior to the blood draw (n = 228). The variance analysis based on 2 blood samples showed a fair to good reproducibility for serum phylloquinone (intra-class correlation = 0.49). The correlation coefficient between the ranking of individuals based on a single measurement of circulating phylloquinone and the ''true'' ranking would be 0.70. The multiple regression analysis showed that long-term phylloquinone intake was the strongest predictor of serum phylloquinone (t = 4.94; $P < 0.001$). The partial correlation coefficient (r = 0.32) was comparable with those reported in studies where blood sampling and diet recording were juxtaposed and/or multiple blood samples were used. The present study provides evidence that the use of a single measurement of circulating phylloquinone is adequate for assessing long-term phylloquinone exposure in healthy older adults. J. Nutr. 142: 1910–1916, 2012.

Introduction

There is a growing interest in the role of vitamin K in health, especially in aging populations. Given that phylloquinone is the primary K vitamer in diet and blood (1), phylloquinone intake and/or serum/plasma concentration have typically been used as indicators of vitamin K status (2–11). However, dietary phylloquinone intake is particularly challenging to assess, because intakes are characterized by a high day-to-day variability that requires ≥*5* d of measurement (12,13). Some FFQ have been used (2–4,6,7,10), but because they were designed to characterize overall diet they can lack accuracy when assessing a nutrient found in a limited number of foods such as phylloquinone (14). Dietary assessment is also complicated by the fact that phylloquinone bioavailability varies between food sources. Notably, phylloquinone from oil-based foods (vegetable oils, margarines, dressings) has been shown to be better absorbed than from vegetables (15,16).

In contrast, serum/plasma phylloquinone concentration is reliably determined by HPLC and accounts for differences in

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[3] Supplemental Figure 1 and Table 1 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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phylloquinone bioavailability. However, most epidemiological studies assessing circulating phylloquinone have used one blood sample per individual, whereas the data are equivocal as to whether a single measurement is a valid indicator of long-term phylloquinone exposure. On one hand, metabolic studies have shown circulating phylloquinone to be sensitive to short-term fluctuations in intakes (17–19), and one report found the within-person variance of plasma phylloquinone to be much higher than that between-person (20). On the other hand, 2 studies reported lower within-individual than between-individual variability (21,22) and 2 others found single-sampled plasma phylloquinone to be primarily determined by long-term phylloquinone intake (23,24). Although none of these studies were specifically designed to evaluate the validity of using one measurement of circulating phylloquinone, these conflicting findings would tend to suggest the need for multiple measurements as a precaution. However, such a strategy is invasive and can prove costly in large study samples. It is also an important limitation when using data from existing epidemiological studies where multiple blood samples may not be available.

The primary purpose of the present study was thus to evaluate the reliability of a single measurement of serum phylloquinone concentration as a surrogate for long-term phylloquinone exposure. Because vitamin K has been linked to many age-related outcomes such as bone health and vascular calcification (1), the study was conducted using samples of healthy older adults from the Québec Longitudinal Study on Nutrition and Successful Aging (NuAge)[14] cohort. Specifically, the objectives were to: *1*) determine the reproducibility of the serum phylloquinone concentration over 2 y; *2*) calculate the degree of accuracy to which a single measurement can rank or classify individuals and estimate the regression coefficient between circulating phylloquinone and a health outcome; and *3*) investigate the association of a single measurement of serum phylloquinone with a validated measure of long-term phylloquinone exposure.

Participants and Methods

Data source. The NuAge Study is a prospective cohort study of nutritional factors of successful aging. The methodology has been detailed elsewhere (25). Briefly, 1793 community-dwelling men and women aged 67–84 y were recruited in 2003–2005 from the Québec Medicare Database. At recruitment, participants were in good general health and free of disabilities in activities of daily living and of cognitive impairment (Modified Mini-Mental State Examination score >79). Follow-up interviews were performed annually up to 3 y after recruitment. The present study was conducted using an embedded subsample of 257 healthy adults taking part in a study on nutritional factors in cognitive aging (see **Supplemental Fig. 1** for a detailed flowchart). Objectives 1 and 2 were addressed using a variance component analysis including 234 participants for whom phylloquinone concentration was measured in serum samples at the 1- and 3-y NuAge follow-ups. The third objective was addressed using a multiple regression analysis including 228 participants having a serum phylloquinone concentration measured at the 1-y NuAge follow-up and six 24-h dietary recalls (24HR) collected in the year preceding the blood draw. All participants provided informed consent approved by the Ethics committees of both recruitment sites (Instituts universitaires de gériatrie de Montréal and Sherbrooke, QC Canada).

Serum phylloquinone concentration. Procedures of blood sampling, processing, and storing at −80°C have been described elsewhere (25). The phylloquinone concentration was measured on 12-h fasting sera (480 μL) using a well-established HPLC method (26,27). The intra-assay CV were 9.2 and 6.0% for phylloquinone concentrations of 0.2 and 0.8 nmol/L, respectively. The detection limit was 0.03 nmol/L and none of the serum samples were below this limit. Blood samples used in the present study were collected between May 2005 and May 2008. In Canada, over-the-counter supplements containing vitamin K were not available during that period and none of the participants reported taking vitamin K as a prescribed drug. Thus, circulating phylloquinone was from dietary sources only.

Dietary assessment. Prior analyses have shown that 6 d of diet recording is the minimum required to properly assess usual phylloquinone intake in older adults (13). Consequently, assessment of long-term phylloquinone intake was based on the 2 sets of 3 nonconsecutive 24HR that were collected 6 mo apart in the year following recruitment into the NuAge. Dietary interview procedures, 24HR processing, and computation of phylloquinone intake were previously detailed (13). Briefly, the multiple-pass method (28) was used by interviewers to limit memory bias and underestimation. Also, every effort was made to assign phylloquinone values to food items with missing data to lessen measurement errors. Energy (MJ), fat (expressed as percent energy), and total phylloquinone intakes were determined for each 24HR. To consider the varying phylloquinone bioavailability among different food sources, phylloquinone intake was also computed according to whether phylloquinone was in a plant food matrix or in an oil phase. Food items were thus divided into 3 food categories: "vegetables and fruits," "fats, oils, and dressings," and "mixed dishes and other food items" (e.g., meat products, dairy, food items based on recipes).

Other determinants of circulating phylloquinone. Phylloquinone in the bloodstream is mainly carried by TG-rich lipoproteins and, to a lesser extent, by the LDL and HDL fractions, making the blood lipid profile an important determinant of serum/plasma phylloquinone concentration (23,24,29–32). The overnight fasting concentrations of serum TG, total cholesterol, and HDL cholesterol (HDL-C) were determined at the Centre Hospitalier de l'Université de Montréal clinical biochemistry laboratory on a Roche/Hitachi Cobas c311 System analyzer using the TRIGL, CHOL2, and HDLC3 kits, respectively (Roche Diagnostic). The LDL cholesterol (LDL-C) concentration was estimated using the Friedewald equation (33). Other potential determinants included sex, age, race (Caucasian vs. others), BMI, smoking status (never vs. current/former smoker), alcohol consumption, and season of each individual blood draw (May to October vs. November to April). The mean daily consumption of wine, beer, and spirits (a standard drink providing 13.6 g of alcohol) during 12 mo was determined by a validated, semiquantitative, FFQ completed at NuAge baseline (34).

Statistical analyses. Participants' characteristics, serum phylloquinone concentration, and dietary variables are presented as geometric means (95%CI), arithmetic means (± SD), or percentage as appropriate. Student's *t* tests and χ^2 were used to compare the study sample with participants not selected from the NuAge cohort. The distribution of serum phylloquinone concentration was positively skewed. Thus, log-transformed data were used in both analytical approaches. Correlation coefficients between participants' characteristics and the 1-y follow-up serum phylloquinone concentration were determined by regression analysis.

The variance component analysis was based on multilevel modeling using iterative generalized least squares provided by MLwiN 2.20 software (35). Reproducibility of the serum phylloquinone concentration during the 2-y period was assessed by the intra-class correlation (ICC). An ICC <0.40 refers to poor reproducibility of the biomarker, ≥0.40 to <0.75 is fair to good reproducibility, and ≥0.75 refers to excellent reproducibility (36). The *r* between the ranking of individuals based on a single measurement of serum phylloquinone and the "true" ranking was calculated using the following equation (13,37):

$$r = \left[1 + \frac{\sigma^2_\varepsilon}{n\sigma^2_\mu}\right]^{-1/2},$$

where n is the number of independent blood samples and $\sigma^2_\epsilon/\sigma^2_\mu$ is the within-to-between variance ratio as determined by the variance

[14] Abbreviations used: 24HR, 24-h dietary recall; HDL-C, HDL cholesterol; ICC, intra-class correlation; LDL-C, LDL cholesterol; NuAge, Québec Longitudinal Study on Nutrition and Successful Aging.

component model. Finally, the percentage of attenuation (a) of the true regression coefficient was estimated as follows (13,37):

$$a = \left\{1 - \left[1 + \frac{\sigma_\varepsilon^2}{\sigma_\mu^2}\right]^{-1}\right\} \times 100.$$

The multiple regression analysis was conducted using PASW Statistics 18 software (version 18.0.3; SPSS). Mean daily intakes of energy, percent energy from fats, and phylloquinone (log-transformed) were calculated based on the 6 24HR. Two models were used to evaluate the association between long-term phylloquinone intake and serum phylloquinone, independent of other plausible determinants. In model 1, 2 blocks of variables were specified: block 1 entered sex, age, energy intake, and total phylloquinone intake (log-transformed); and block 2 entered stepwise smoking status, alcohol consumption, BMI, season, percent energy from fats, and serum TG (log-transformed), total cholesterol, LDL-C, and HDL-C concentrations. In model 2, block 1 included sex, age, energy intake, and phylloquinone intake provided by "vegetables and fruits," "fats, oils, and dressings," and "mixed dishes and other food items," and block 2 entered stepwise the same variables as in model 1. The adequacy of the 2 final models was evaluated by: *1*) examining added variable plots showing adjusted regression lines; *2*) comparing these lines with loess regression lines (Epanechnikov kernel function, 50% fit); *3*) assessing normality of residuals; and *4*) plotting residuals against predicted values. To evaluate the magnitude of effect in model 1, serum phylloquinone concentration-adjusted means (95%CI) were calculated at the 2.5th, 25th, 50th, 75th, and 97.5th percentiles of total phylloquinone intake. $P < 0.05$ was considered significant.

Results

The study sample was aged from 68 to 82 y, with fewer men than women, and was almost entirely Caucasian (99%) (**Table 1**). The BMI and the serum lipid profile were in the acceptable range for older adults (38,39). Usual phylloquinone intake varied from 13.2 to 302.0 μg/d (median, 70.7 μg/d), with 59 ± 20% provided by vegetables and fruits and 13 ± 10% (mean ± SD) by fats, oils, and dressings. The fasting serum phylloquinone concentration varied from 0.2 to 11.0 nmol/L and 0.1 to 23.4 nmol/L at the NuAge 1- and 3-y follow-ups, respectively. Participants included in the analyses were slightly younger and more educated than those who were not, but they did not differ for sex, race, smoking status, alcohol consumption, BMI, and dietary intakes including phylloquinone (**Supplemental Table 1**).

Variance component analysis. Based on log-transformed data, the within-individual and between-individual variances for serum phylloquinone were 0.044 (95% CI: 0.036–0.052) and 0.043 (95% CI: 0.031–0.055), respectively. The ICC was 0.49 ($\chi^2 = 66.19$; $P < 0.001$), indicating a fair to good reproducibility over 2 y for serum phylloquinone. Considering the variance ratio of 1.02, the ranking of individuals based on a single measurement of serum phylloquinone concentration would be moderately correlated with the "true" ranking ($r = 0.70$), i.e., ~53–58% of individuals would be correctly classified into the extreme fractions of fourths and fifths. The degree of attenuation of the "true" linear regression coefficient would be 50%. The degree of RR attenuation could be estimated using the formula provided by Rosner et al.: $\{RR_{observed} = \exp[ICC \times \ln(RR_{true})]\}$ (40). For example, the true RR of 0.75 would attenuate the observed RR to 0.87. Adjustment for serum TG (log-transformed) and total cholesterol significantly reduced the between-individual variances to 0.030 (95% CI: 0.020–0.040) such that the ICC decreased to 0.41.

Regression analysis. Regression modeling showed that sex, age, and energy intakes were not significant determinants of serum phylloquinone, although women tended to present slightly lower concentrations (**Table 2**). Stepwise entry of block 2 variables revealed that TG, total cholesterol, and alcohol consumption were independent determinants of circulating phylloquinone in both models. The addition of smoking status, BMI, serum LDL-C and HDL-C concentrations, season, and percent energy from fats did not significantly improve the model or change the regression coefficients (data not shown).

Usual dietary phylloquinone intake was the variable most strongly related to circulating phylloquinone (model 1: $t = 4.94$; $P < 0.001$) followed closely by TG. The partial correlation coefficient between serum phylloquinone concentration and phylloquinone intake was 0.32 and the simple correlation coefficient was 0.21 (Table 1), indicating that the regression model included important cofounders of the relationship. The magnitude analysis for model 1 showed that circulating phylloquinone rose with total phylloquinone intake but at a decreasing rate as phylloquinone intake increased (**Fig. 1**). When phylloquinone intake was modeled according to food sources (model 2), the adjusted R^2 increased, indicating that phylloquinone intake was better specified in model 2 than in model 1. The regression coefficient for phylloquinone intake from "fats, oils, and dressings" was approximately twice than that of phylloquinone intake from "vegetables and fruits" and "mixed dishes and other food items."

Discussion

To our knowledge, the present study is the first to provide comprehensive evidence that the use of a single measurement of circulating phylloquinone is adequate for assessment of long-term phylloquinone exposure in healthy older adults, as established by 2 analytical approaches. First, we observed a fair to good reproducibility of serum phylloquinone between 2 blood samples collected 2 y apart, indicating a relative consistency over time. Furthermore, we found that phylloquinone intake assessed during a 1-y period was the primary determinant of serum phylloquinone concentration determined on one nonjuxtaposed blood sample. As a practical guide for researchers, we also determined the degree of accuracy of a single measurement of serum phylloquinone concentration to rank or classify individuals according to their "true" circulating phylloquinone. Furthermore, we provide information on how a single measurement of serum phylloquinone concentration would attenuate the regression coefficient between circulating phylloquinone and a health outcome.

Variance components of serum phylloquinone. In the present study, intra-individual variability of serum phylloquinone concentration in healthy older adults was similar to the interindividual variability. Examination of the variance components of circulating phylloquinone is currently limited to data from 3 studies that reported inconsistent results (20–22). In a group of 28 hemodialysis patients, Kohlmeier et al. (21) observed a within : between variance ratio of 0.17, which is indicative of high reproducibility during the study period (3 wk) in these patients. A recent study conducted in 14 healthy adults recruited from laboratory staff and sampled weekly for 22 wk reported an intra-:inter-individual CV ratio of 0.88 (22), showing a variance partitioning similar to what we found. Conversely, Booth et al. (20) reported a within : between variance ratio of 5.4 in a group of 34 healthy volunteers recruited for a metabolic study (17 in each age group: 20–40 and 60–80 y), where participants were sampled 3 times during 20 wk. This variance ratio for phylloquinone is surprisingly high compared with ours, those discussed

TABLE 1 Description of study participants and correlations with the 1-y NuAge follow-up serum phylloquinone concentration[1]

Variable	*n*	Value	Correlation coefficient[2]	*P*
Female, *%*	257	54	0.01	0.93
Age, *y*	257	73.6 ± 3.9	0.01	0.93
Former and current smoker, *%*	257	44	0.07	0.29
Alcohol consumption, *drinks/d*	252	0.71 ± 1.05	0.08	0.20
BMI, *kg/m²*	257	27.6 ± 4.7	−0.01	0.91
Blood sample drawn from May to October, *%*	257	68	0.07	0.25
Serum lipid profile				
TG, *mmol/L*	240	1.41 (1.34–1.48)	0.27	<0.001
Total cholesterol, *mmol/L*	240	5.07 ± 0.97	0.23	<0.001
LDL-C, *mmol/L*	240	3.01 ± 0.83	0.18	0.006
HDL-C, *mmol/L*	240	1.36 ± 0.39	−0.06	0.34
Dietary intakes				
Energy intake, *MJ/d*	248	7.8 ± 1.8	0.03	0.63
Energy intake from fats, *%*	248	33 ± 4.9	0.01	0.84
Phylloquinone intake, *μg/d*				
Total (all food items)	248	72.4 (68.2–77.0)	0.21	0.001
Vegetables and fruits	248	49.1 (44.1–54.5)	0.13	0.04
Fats, oils, and dressings	248	8.3 (7.4–9.4)	0.31	<0.001
Mixed dishes and other food items	248	20.9 (19.6–22.4)	−0.01	0.82
Serum phylloquinone concentration, *nmol/L*				
1-y follow-up	257	0.96 (0.89–1.04)	–	–
3-y follow up	234	1.04 (0.95–1.14)	0.50	<0.001

[1] Values are percentages, means ± SD, or geometric means (95% CI), *n* = number of participants with available data. HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; NuAge, Québec Longitudinal Study on Nutrition and Successful Aging.
[2] Correlation coefficients were determined by regression analysis with the 1-y follow-up serum phylloquinone concentration (log-transformed) as dependent variable.

earlier, and even those reported for other fat-soluble vitamins (20,22). The fact that the data from the 2 age groups were pooled may explain the discrepancy. Nonetheless, in all 3 studies, generalization is limited by the small sample sizes and the nature of the participants per se, i.e., hemodialysis patients and nonrandomly selected volunteers.

Determinants of serum phylloquinone. Two studies previously examined the relationship between usual phylloquinone intake and single-sampled plasma phylloquinone (23,24). Consistent with our results, long-term phylloquinone intake and TG were found to be the primary determinants of circulating phylloquinone. They also found the relationship between dietary

TABLE 2 Parameter estimates from fitting multiple regression models testing predictors of log-transformed serum phylloquinone concentration in healthy older adults from the NuAge Study (*n* = 228)

	Model 1		Model 2	
Variables	β Coefficients (SE)	*P*	β Coefficients (SE)	*P*
Block 1: enter				
Women	−0.062 (0.040)	0.13	−0.073 (0.039)	0.06
Age, *y*	0.001 (0.004)	0.82	0.001 (0.004)	0.76
Energy intake (MJ/d)	−0.011 (0.011)	0.33	−0.016 (0.011)	0.16
Phylloquinone intake[1] (μg/d)				
Total (all food items)	0.423 (0.086)	<0.001	–	–
Vegetables and fruits	–	–	0.113 (0.046)	0.01
Fats, oils, and dressings	–	–	0.224 (0.041)	<0.001
Mixed dishes and other food items	–	–	0.095 (0.075)	0.21
Block 2: stepwise[2]				
Serum TG[1] (mmol/L)	0.504 (0.102)	<0.001	0.468 (0.100)	<0.001
Serum total cholesterol (mmol/L)	0.048 (0.018)	0.009	0.055 (0.018)	0.003
Alcohol consumption (drinks/d)	0.047 (0.018)	0.01	0.050 (0.018)	0.005
R^2 (adjusted-R^2)	0.23 (0.21)		0.28 (0.25)	

[1] Data were log-transformed. HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; NuAge, Québec Longitudinal Study on Nutrition and Successful Aging.
[2] Variables are presented in order of selection. Smoking status, serum LDL-C and HDL-C, percentage of energy from fats, BMI, and season were not selected in the final models.

intake and serum concentration to be a logarithmic function, which possibly mirrors the saturable energy-mediated mechanism responsible for the intestinal absorption of phylloquinone (41).

Neither of these 2 reports provided the partial correlation coefficients between long-term phylloquinone intake and the single-sampled plasma phylloquinone preventing an appreciation of the ranking agreement between these 2 measurement methods. In the present study, the partial correlation coefficient reported is quite high considering that: *1*) the relationship is mediated by digestion, absorption, uptake, utilization, metabolism, excretion, and homeostatic mechanisms (42); *2*) blood sampling and diet recording were not juxtaposed; and *3*) assessment of long-term phylloquinone intake is characterized by measurement errors attenuating the estimated correlation coefficient (13). Of note, the "deattenuated" partial correlation was estimated at ~0.40, considering an attenuation factor of 0.81 (13). Furthermore, a number of studies examining the relationship between short-term intake and plasma/serum concentration of phylloquinone, where blood sampling and diet recording were juxtaposed and/or multiple blood samples were used, reported correlation coefficients comparable with ours (r = 0.32–0.40 vs. 0.13–0.51) (20,43–45).

The present study reported a significant association between specific phylloquinone food sources and circulating concentrations in an observational design (12,45). Notably, we found that the strength of the association between phylloquinone intake from oil-based foods and serum phylloquinone concentration was twice as strong as the association between phylloquinone intake from fruits and vegetables and serum phylloquinone concentration. This finding is consistent with studies that examined phylloquinone bioavailability. Booth et al. (15) reported that phylloquinone bioavailability, measured as the 24-h AUC, was 1.56–1.66 times higher for a phylloquinone-fortified oil diet than for a broccoli diet. Similarly, a recent study showed phylloquinone bioavailability to be >3 times higher when provided in a meal with >80% of phylloquinone in the oil phase than in meals with >80% of phylloquinone in the vegetable matrix (16). Consistent with previous reports (21,43,44), final models also pointed to triglyceridemia as one of the main determinant of circulating phylloquinone. The significant association with total cholesterol likely originated from phylloquinone carried by the LDL-C and HDL-C fractions (30). The positive effect of alcohol consumption was not observed in previous studies (46,47). Because phylloquinone is soluble in ethanol, the presence of a moderate amount of alcohol in the intestinal lumen may improve phylloquinone solubility and bioavailability.

Implications for research. The present report provides important information for planning future population-based research. Collecting one serum or plasma sample per individual would be a cost-effective way to assess long-term phylloquinone exposure. Still, increasing the number of measurements to 2/individual would improve the ranking of individuals (r = 0.81) and reduce the attenuation of the regression coefficient to 34%. Results from our study also showed that phylloquinone intake from different food sources was not equally associated with circulating phylloquinone. This observation has important implications when interpreting results from studies using phylloquinone intake as an indicator of phylloquinone exposure. In nutritional epidemiology, where the expected effect size is usually small, the use of circulating phylloquinone could lessen the probability of false negative results by being a better estimate of the amount of phylloquinone directly available to tissues.

Although no earlier studies have specifically evaluated the validity of a single measurement of circulating phylloquinone to assess long-term phylloquinone exposure, previous reports have issued concerns about using a single measure, mainly because metabolic studies showed circulating phylloquinone to be sensitive to short-term fluctuations in intakes (17–19). However, these experimental conditions (e.g., 100 μg phylloquinone/d for 5 d followed by 420 μg/d for 5 d) were tested in the controlled environment of a metabolic ward and were not representative of the real-life context on which observational studies are based. In fact, older adults tend to present stable dietary habits that have been established throughout their life and do not drastically change their diet over time (48,49). Thus, from a nutritional epidemiologic point of view, the use of single serum/plasma sample should not be discarded simply on the basis of the above-mentioned metabolic studies. Furthermore, a recent study provided evidence that fasting serum phylloquinone integrates longer term phylloquinone status in addition to more recent intakes (50). Specifically, when 7 healthy adults ingested 70 μg of isotopically labeled phylloquinone in kale, 2 decay phases were observed, namely, one for plasma elimination (half-time = 8.8 h) and one for tissue elimination (half-time = 9 d). As a result, labeled phylloquinone was detected in fasting plasma for the entire study period (4 wk).

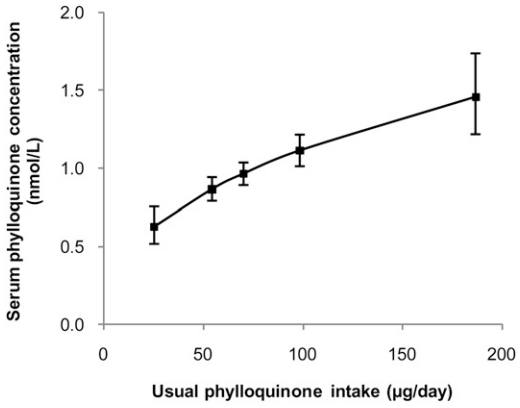

FIGURE 1 Fasting serum phylloquinone concentration as a function of long-term phylloquinone intake in 228 healthy older adults from the NuAge Study. Values are back-transformed means (95% CI) calculated for phylloquinone intake at the 2.5th, 25th, 50th, 75th, and 97.5th percentiles and adjusted for sex, age, energy intake, alcohol consumption, serum TG, and total cholesterol concentrations (model 1). NuAge, Québec Longitudinal Study on Nutrition and Successful Aging.

Strengths and limitations of the study. Our results are based on a powerful design, including 2 analytical approaches that independently validated the use of a single measurement of circulating phylloquinone. Incidentally, serum phylloquinone and usual phylloquinone intake were in accordance with previous studies conducted in similar populations (14,24,29,51). The NuAge Study sample included healthy older adults, mostly Caucasian, and excluded those who were frail or institutionalized, which could limit generalization. In the present study, a nonrandom convenience sample of the NuAge cohort was used. Nonetheless, the sample remained large and did not differ substantially from those who were excluded. Usual phylloquinone intake was assessed using the recommended 6 nonconsecutive 24HR (13). The multiple-pass method improved consistency across interviewers and decreased the potential memory bias. The regression analysis included the main plausible determinants of circulating phylloquinone. Still, we cannot rule out the possibility of other potential determinants of circulating phylloquinone such as the apoE genotype. However, the association between the apoE

genotype and circulating phylloquinone is controversial, with studies reporting apoEε4 carriers having a lower (21,52), higher (53), or similar (54) phylloquinone concentration than noncarriers. Finally, we cannot extrapolate our findings to the reproducibility of serum phylloquinone concentrations measured >2 y apart.

In conclusion, based on 2 comprehensive analytical approaches, the present study showed that a single measurement of fasting serum phylloquinone concentration is adequate to assess long-term phylloquinone exposure in healthy older adults. Notably, long-term phylloquinone intake was found to be the primary determinant of single-sampled serum phylloquinone concentration. Our findings also highlight phylloquinone food sources as an important modulator of circulating phylloquinone, pointing to serum concentration as an integrated measure of phylloquinone directly available to tissues. A single measurement of circulating phylloquinone could prove practical and cost-effective for ranking individuals and estimating the magnitude of associations in many observational studies involving healthy older adults.

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