Plasma Transport of Vitamin K in Men Using Deuterium-Labeled Collard Greens

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The plasma transport of stable isotope-labeled phylloquinone at physiologic doses from food was studied. A single bolus of 100 g (396 ± 28 μg phylloquinone) deuterium-labeled collard greens was fed with a breakfast containing 24 g fat to 5 men (26 to 71 years). Eleven blood samples were obtained over 216 hours. Phylloquinone concentrations in plasma and lipoprotein subfractions were measured using high-performance liquid chromatography (HPLC), and the ion abundances of deuterated and endogenous phylloquinone were determined using gas chromatography/mass spectrometry (GC/MS). Plasma total phylloquinone concentrations peaked at 6 to 9 hours (10.51 ± 4.38 to 8.30 ± 4.64 nmol/L) and returned to baseline by 24 hours (1.26 ± 0.38 nmol/L). The triglyceride-rich lipoprotein (TRL) fraction was the major carrier of phylloquinone; low-density lipoprotein (LDL) and high-density lipoprotein (HDL) fractions contained smaller amounts. Maximum enrichment of plasma and TRL phylloquinone with deuterium (88% and 89%, respectively) was reached at 6 hours, respectively; $t_{1/2}$ was 22.8 hours (n = 3). Deuterated-phylloquinone was not detectable in plasma or TRL fraction at 72 hours. These results suggest rapid uptake and transport of physiologic doses of phylloquinone.

PHYLLOQUINONE (vitamin K_1) is a fat-soluble vitamin. Its mechanisms of action are mediated through the vitamin K-dependent γ-carboxylation of certain proteins. These proteins are involved in coagulation, regulation of bone formation, and potentially vascular calcification.[1] The main dietary sources are green leafy vegetables and oils, such as soybean, canola, olive, and cottonseed.[2] There are limited data on the metabolic fate of phylloquinone from these dietary sources. Given recent research that suggests an association between low dietary intake of phylloquinone and risk of hip fracture in older men and women,[3,4] it has become increasingly important to study phylloquinone absorption and transport at physiologic doses.

Much of our current understanding of vitamin K absorption and metabolism is based on studies using oral[5] or injected[6-8] doses of tritiated phylloquinone. Similar to other fat-soluble vitamins, phylloquinone is absorbed from the proximal intestine and the absorption is dependent on bile and pancreatic juice secretion.[6] In the intestine, phylloquinone is incorporated into nascent chylomicron particles that are secreted directly into the lymph and ultimately peripheral circulation. Phylloquinone remains associated with these particles during delipidation in circulation and subsequent uptake by the liver. It is estimated that approximately 80% of purified phylloquinone is absorbed.[6]

Vitamin K is not known to have a carrier protein; instead, triglyceride-rich lipoproteins (TRL), primarily chylomicron remnants, are thought to be the main transporters of phylloquinone.[9-11] Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particles may carry smaller amounts of plasma phylloquinone. Studies describing the transport of vitamin K in plasma lipids in the postprandial state to date have used pharmacologic doses (900 to 3,500 μg) of purified vitamin K rather than food-based preparations.[9,10] Likewise, under these conditions, the absorption of phylloquinone may have been different compared with that in the habitual diet because of the high amount of fat (1 g/1 kg body weight) provided with the phylloquinone.[9]

Phylloquinone absorption efficiency, assessed by the 24-hour area under the plasma phylloquinone curve, is higher from supplements compared with food sources and is enhanced when consumed with fat,[12,13] Similar studies suggest a greater absorption efficiency of phylloquinone from oils compared with equivalent phylloquinone intakes from green vegetables.[13,14] However, these recent studies on phylloquinone metabolism have been unable to differentiate between endogenous and exogenous forms of phylloquinone in circulation or control for the influence of additional meals and their nutrient composition on absorption.[12-14] Although successfully used in the past for the study of vitamin K absorption, current safety concerns limit the use of radioactive tracers.

Stable isotopes have been widely used in studies examining the bioavailability and bioefficacy of other fat-soluble vitamins (A and E),[15,16] whereas the use of stable isotope labeled phylloquinone is still novel. Stable isotopes are nonradioactive forms of elements that naturally occur in the environment and can be separated by mass and quantified by mass spectrometry (MS). In a pilot study, we described the use of deuterium-labeled phylloquinone from broccoli in the development of gas chromatography/MS (GC/MS) methodology for the study of phylloquinone absorption in a single subject.[17] The objective of

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Submitted May 23, 2003; accepted August 11, 2003.

Supported by federal funds from the US Department of Agriculture, Agricultural Research Service under Cooperative Agreement No. 58-1950-001 and No. 58-6250-6-001 and by grant from the Academy of Finland (to A.T.E.).

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

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0026-0495/04/5302-0044$30.00/0
doi:10.1016/j.metabol.2003.08.015

Table 1. Baseline Characteristics

Subject	Weight (kg)	BMI (kg/m^2)	Age (yr)	Total Cholesterol (mmol/L)	LDL Cholesterol (mmol/L)	HDL Cholesterol (mmol/L)	Total TG (mmol/L)
A	83	23.6	26	4.24	2.40	1.19	1.23
B	75	23.8	26	4.24	2.46	0.70	1.94
C	99	30.4	59	4.94	3.26	0.93	1.20
D	69	24.5	69	3.21	1.63	0.93	1.12
E	79	25.1	71	5.43	3.85	0.88	0.85
Mean (SD)	81 (11)	25.5 (2.8)	50 (23)	4.41 (0.84)	2.72 (0.86)	0.93 (0.18)	1.27 (0.41)

the current study was to examine phylloquinone uptake and transport in plasma and lipoprotein subfractions in men after a physiologic dose of deuterium-labeled phylloquinone obtained from collard greens. Collard greens were selected for their higher phylloquinone content and their shorter growth period, which reduced the deuterium required compared with broccoli.

MATERIALS AND METHODS

Subjects

Five healthy men (26 to 71 years) were recruited from the greater Boston area. All subjects fulfilled the following criteria: normal kidney, liver, thyroid, renal, and cardiac function; normal fasting glucose levels; normal clotting times; and were nonsmokers. Subjects were not taking the following medications: oral anticoagulants, antibiotics, anticonvulsants, barbiturates or phenobarbital-containing drugs, anti-inflammatory drugs, aspirin or aspirin-containing drugs, lipid-lowering medication, herbal preparations, or vitamin E supplements. Characteristics of the subjects upon entry into the study are shown in Table 1.

The study protocol was approved by the Institutional Review Board of New England Medical Center and Tufts University. All subjects gave their informed written consent for the study.

Preparation of the Deuterated Collard Greens

Collard greens (cultivar Georgia) were grown hydroponically at the USDA/ARS Children's Nutrition Research Center in Houston, TX, using a nutrient solution enriched with 31 atom-% deuterium oxide (D_2O).[18] The conditions of growing the plants were as previously described[17,19] with the following exceptions: the nutrient solution (20 plants/12 L solution) contained the following macronutrients in millimolars: KNO_3, 10; KH_2PO_4, 4; $Ca(NO_3)_2$, 3; $MgSO_4$, 3; and the following micronutrients in micromolars: $CaCl_2$, 25; H_3BO_3, 25; $MnSO_4$, 2; $ZnSO_4$, 6; $CuSO_4$, 0.5; H_2MoO_4, 0.5; $NiSO_4$, 0.1 and iron in chelated form as Fe(III)EDDHA (N,N′-ethylenebis[2-(2-hydroxyphenyl)-glycine]) at 10 μmol/L. The collard greens were maintained within an acrylic plastic enclosure (situated inside the growth chamber) until harvest at 6 weeks. This enclosure was supplied with supplemental carbon dioxide (CO_2) to maintain a concentration of approximately 400 ppm CO_2 for the plants (similar to external air). Water vapor in the atmosphere around the collard greens was maintained at approximately 31 atom-%.

At harvest, all leaves were packaged and shipped overnight on ice to the JM USDA Human Nutrition Research Center on Aging (HNRC) at Tufts University. The vegetables were weighed and steamed for 8 to 12 minutes until the leaves were completely cooked. Afterwards, they were pureed, portioned, and kept at −80°C until being analyzed, or used for the feeding studies.

Experimental Design

The subjects resided in the Metabolic Research Unit (MRU) at the JM USDA HNRC at Tufts University for 1 day. During the residency day (d1), subjects were provided with a breakfast at 8:30 AM, which included 100 g deuterated collard greens (396 ± 28 μg phylloquinone), a peach shake, whole milk, and an English muffin with butter and jelly. The breakfast yielded 570 kcal, 37% of calories from fat, 53% from carbohydrate, and 13% from protein. Furthermore, subjects were provided a lunch at 12 PM and a dinner at 5:15 PM. During d1 the mean calculated energy intake was 2083 kcal/d, 35.3% of calories was from fat, 15.9% from protein, and 52.1% from carbohydrates. Based on direct analysis, intake of vitamin K from sources other than the collards was low (27 μg phylloquinone and 16 μg dihydrophylloquinone). Subjects were free-living during days 2 through 10 and were instructed to consume their usual diets. Each volunteer was also instructed to consume a daily portion of green vegetables provided by the MRU to stabilize the intake of phylloquinone (100 g frozen broccoli or peas, containing 141 μg and 36 μg phylloquinone, respectively[20]).

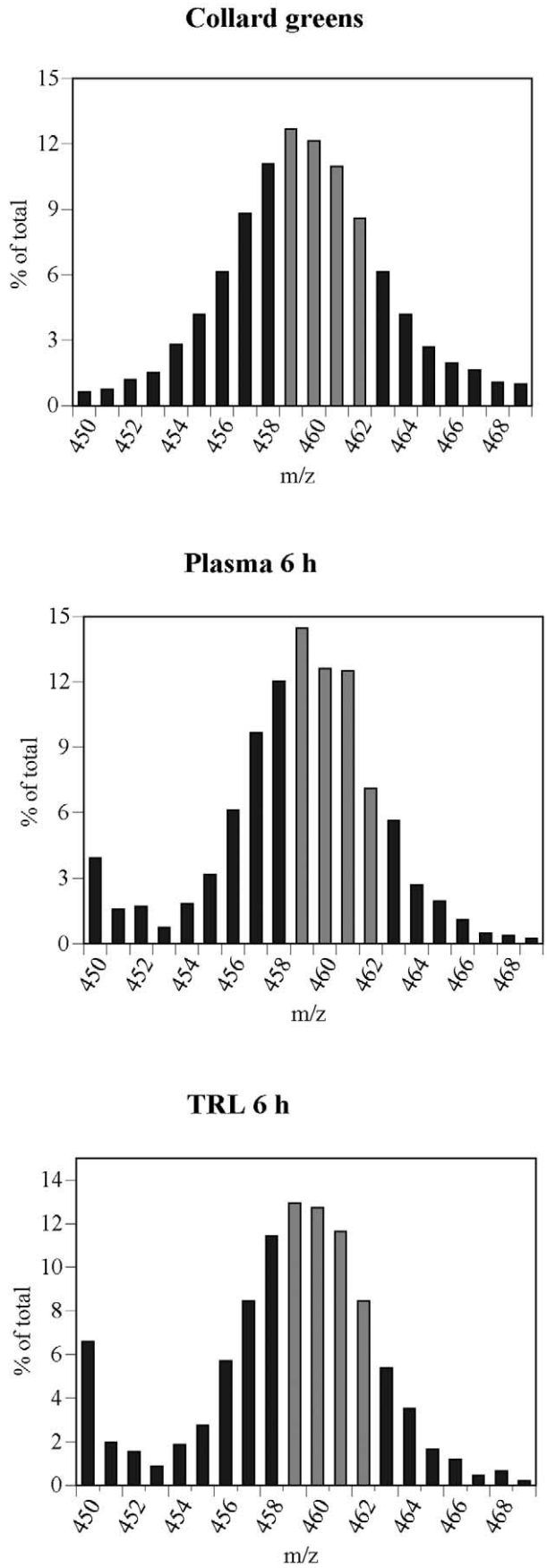
Fasting (>12 hours) blood was drawn at 0 hour on d1 before the ingestion of the deuterated collard greens, and 3, 6, 9, and 12 hours after the ingestion during d1. Fasting blood samples were subsequently collected at 8 AM on days 2, 3, 4, 5, 8, and 10 (corresponding to 24, 48, 72, 96, 168, and 216 hours).

Laboratory Measurements

Plasma lipids. Blood samples were collected in tubes containing EDTA (0.15% final concentration). Plasma was separated by centrifugation at 1,100 × g at 4°C. Lipoprotein fractions representing TRL (d < 1.006 g/dL), intermediate-density lipoprotein (IDL) (d = 1.003 to 1.019 g/dL), LDL (d = 1.019 to 1.063 g/dL), HDL (1.063 to 1.21 g/dL), and the lipid poor fraction (LPF) (d > 1.21) were isolated from plasma by sequential ultracentrifugation at 4°C.[21] Plasma and the lipoprotein fractions were assayed for total cholesterol, HDL-cholesterol and/or triglyceride with a Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, IN).[22] Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control, Atlanta, GA.

High-performance liquid chromatography determination of phylloquinone concentrations. Solvents used for extraction and chromatography were high-performance liquid chromatography (HPLC) grade (Fisher Scientific, Springfield, NJ). Working standards: phylloquinone (Sigma, St Louis, MO) and vitamin $K_{1(25)}$ (a gift from Hoffman La Roche, Basel, Switzerland) were prepared in HPLC grade methanol and were characterized spectrophotometrically and chromatographically before use. All standards were stored at 2°C to 8°C and protected from light. All operations were performed under yellow light. All glassware was rinsed before use with acetone to prevent contamination of samples with fluorescent material.

Phylloquinone was extracted and analyzed from collard greens and the duplicate portion of d1 diet as previously described.[23] Phylloquinone was extracted and analyzed from plasma and lipoprotein subfractions as described by Davidson and Sadowski.[24] The limit of detection of plasma phylloquinone was 0.13 nmol/L with HPLC.



Deuterated-phylloquinone analysis. For collard greens, plasma, and TRL samples dedicated for GC/MS analysis, the phylloquinone extraction and purification procedures were as previously described.[17] The limit of detection of phylloquinone was 5 pg per injection, equivalent to a plasma phylloquinone concentration of 0.09 nmol/L under the described conditions.

Phylloquinone isotopic ion abundance was determined using GC/MS equipped with an autoinjector (Agilent 6890 and 7683, Wilmington, DE). Five microliters of the sample was injected using cool on-column technique into a deactivated 0.53 mm fused silica column, which was connected by a zero-dead volume connector to a 5% phenyl polysiloxane carborane, HT5 column (30 m, ID 0.25 mm, film thickness 0.1 μm; SGE, Austin, TX). The temperature was programmed from 50°C to 300°C at 30°C/min and 300°C to 380°C at 10°C/min. Helium was used as a carrier gas. Methane negative chemical ionization was used to ionize phylloquinone, and the temperature of the ion source was 200°C.

The data were analyzed with MSD ChemStation software (Agilent, version G1701DA). Selected ion monitoring was used to detect the isotopomers of phylloquinone, the predominant isotopomers being m/z 450 for unlabeled phylloquinone and m/z 459-462 for deuterated-phylloquinone. The ions at m/z 459-462 were chosen for selected ion monitoring because they produced the highest signal to noise ratio for deuterated-phylloquinone. Ions at m/z 456-458 were not included because a compound of unknown structure from plasma interfered with the analysis of deuterated-phylloquinone at those masses. The ratio of the abundance of predominant labeled isotopomers to the abundance of all labeled isotopomers [ratio = (m/z 459-462)/(m/z 453-469)] was 0.37 in collard greens. This ratio was used in calculations to correct the abundance of deuterated-phylloquinone: Calc m/z 453-469 = (m/z 459-462)/0.37.

The scans of all labeled isotopomers in plasma and TRL samples were also analyzed showing that the isotopomer profiles and the most abundant isotopomer (m/z 459) corresponded to those in collard greens (Fig 1). There were small proportions of unlabelled phylloquinone in plasma and TRL samples, which are assumed to come from previous meals and/or body stores. However, there were high concentrations of unknown coeluting compounds in TRL and plasma in some samples that precluded the assessment of the ratio of predominant to all labeled phylloquinone isotopomers.

Percentage enrichment was calculated by using the following formula: %enrichment = [(calc m/z 453-469)/{(m/z 450) + (calc m/z 453-469)}] × 100.

Using %enrichment and total phylloquinone concentration as determined by HPLC, the concentration of deuterated-phylloquinone was calculated as follows: deuterated-phylloquinone, nmol/L = [%enrichment × (total phylloquinone, nmol/L)]/100.

Statistical Methods

The statistical analyses were performed with SAS for Windows, version 8.1 (SAS Cary, NC). Nondetectable values of phylloquinone in lipoprotein fractions were recoded as 0.05 nmol/L for the purposes of statistical analyses. The results are expressed as means ± SD. The changes in lipid, lipoprotein, and phylloquinone concentrations by time were analyzed with general linear models (GLM), and post hoc comparisons were made with Tukey-Kramer test. Correlation coefficients adjusting for multiple measurements were calculated between plasma phylloquinone and lipid concentrations.

Fig 1. Isotopomer distributions in collard greens, plasma, and TRL samples. Plasma and TRL samples were obtained 6 hours after the intake of deuterated collard greens (n = 1). The isotopomers that were measured (m/z 459-462) in selected ion monitoring are presented in gray bars.

Table 2. Total (labeled and unlabeled) Phylloquinone Concentrations in Plasma and Lipoprotein Fractions After the Intake of Deuterated Collard Greens

	0 h 8 AM	3 h 11 AM	6 h 2 PM	9 h 5 PM	12 h 8 PM	24 h d2	48 h d3	72 h d4	96 h d5	168 h d8	216 h d10	*P* Value for Trend
	B↓	L↓			D↓							
Plasma phylloquinone (nmol/L)	1.77[a] (1.47)	2.47[a] (1.08)	10.51[b] (4.38)	8.30[b] (4.64)	3.72[a] (2.29)	1.26[a] (0.38)	2.17[a] (2.12)	2.09[a] (2.90)	2.47[a] (1.83)	2.30[a] (1.42)	3.05[a] (2.40)	<.001
Plasma phylloquinone/TG (nmol/mmol)	1.29[a] (0.66)	1.64[a] (0.90)	4.52[b] (0.99)	3.72[ab] (1.53)	2.42[ab] (1.17)	1.00[a] (0.34)	1.96[ab] (2.27)	1.36[a] (1.42)	2.23[ab] (1.58)	1.91[ab] (1.53)	2.13[ab] (1.59)	.002
TRL phylloquinone (nmol/L)	0.88[a] (1.10)	1.14[a] (0.78)	5.48[c] (3.91)	4.71[bc] (3.14)	2.24[ab] (2.02)	0.44[a] (0.37)	0.85[a] (0.91)	1.30[a] (2.33)	1.26[a] (1.15)	1.00[a] (0.79)	1.75[a] (1.50)	<.001
TRL phylloquinone/TRL-TG (nmol/mmol)	0.89[a] (0.70)	1.00[ab] (0.32)	3.16[c] (1.46)	2.97[bc] (0.80)	1.89[abc] (0.68)	0.46[a] (0.22)	1.32[abc] (1.73)	1.22[abc] (1.74)	1.80[abc] (1.33)	1.15[abc] (0.90)	1.90[abc] (1.25)	.001
IDL phylloquinone (nmol/L)	ND[a]	ND[a]	0.36[b] (0.11)	0.13[a] (0.09)	0.14[a] (0.11)	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	0.15[a] (0.15)	<.001
LDL phylloquinone (nmol/L)	0.13[ab] (0.12)	0.13[ab] (0.13)	0.47[ab] (0.17)	0.53[b] (0.10)	0.31[ab] (0.16)	ND[a]	0.36[ab] (0.51)	0.20[ab] (0.09)	0.24[ab] (0.23)	0.30[ab] (0.15)	0.25[ab] (0.25)	.024
HDL phylloquinone (nmol/L)	ND[a]	ND[a]	0.56[b] (0.36)	0.46[ab] (0.22)	0.33[ab] (0.22)	ND[a]	0.23[ab] (0.26)	0.16[ab] (0.09)	0.25[ab] (0.17)	0.23[ab] (0.21)	0.34[ab] (0.21)	.004

NOTE. Values are means (SD), n = 5, except at time point 216, n = 4. Numbers without common superscripts are significantly different at $P < .05$.
Abbreviations: B, breakfast given at 8:30 AM; L, lunch given at 12 PM; D, dinner given at 5:15 PM; TRL, triglyceride-rich lipoprotein; ND, nondetectable.

The plasma %enrichment data (6 to 48 hours) was fitted to an exponential equation: $y = be^{kt}$, where y is remaining %enrichment, b is slope, k is rate of disappearance, and t is time. The k was determined for each subject (n = 3) to get the best fit to the above equation according to a least squares algorithm using Microsoft Excel (version 2000). All R^2 values comparing the actual and estimated y-values were greater than 0.95. Time required to reduce to enrichment by half was calculated as $t_{1/2} = 0.693/k$.[25]

RESULTS

Plasma total phylloquinone concentrations (mean±SD) peaked at 6 to 9 hours and returned to baseline by 24 hours, primarily in response to the intake of deuterated collard greens (Table 2). The TRL fraction was the major carrier of phylloquinone in plasma, also peaking at 6 to 9 hours in this fraction. Normalizing plasma and TRL phylloquinone concentrations to plasma triglyceride concentrations (nmol/mmol TG) and TRL-triglyceride concentrations (nmol/mmol TRL-TG), decreased the interindividual variability in postprandial concentrations, as indicated by smaller standard deviations. Although mean fasting phylloquinone concentrations in plasma and TRL appeared to increase from 24 to 216 hours, these changes were not statistically significant in post hoc analysis, possibly due to large interindividual variation in concentrations and small number of subjects. Phylloquinone concentrations increased in LDL and HDL fractions at 6 to 9 hours compared with baseline. LDL and HDL fractions carried smaller amounts of phylloquinone compared with the TRL fraction, even at the later time points when the clearance of the postprandial particles from the initial phylloquinone challenge in the TRL fraction was assumed to be complete. The mean phylloquinone concentration in the IDL fraction was nondetectable in most time points, except at 6, 9, 12, and 216 hours, reflecting the transient nature of the IDL fraction. No phylloquinone was detectable in the LPF fraction at any time point.

The percentage recovery of phylloquinone calculated by summing concentrations in all the lipoprotein fractions and dividing the sum by the total concentration in plasma was 45% to 100% over the different time points; recovery was below 50% when the total concentration of phylloquinone was less than 1.8 nmol/L. This finding highlighted a limitation of the current methodology. The percentage contribution of individual lipoproteins in the transport of phylloquinone at the different time points is not presented because of variable recoveries.

Total cholesterol, HDL-cholesterol, and IDL-cholesterol concentrations remained relatively stable during the study period, accompanied by a decline in LDL-cholesterol at 6 hours (Fig 2A). TRL-cholesterol concentration peaked at 6 to 9 hours in response to dietary intake. Triglyceride concentrations increased, reaching the highest concentrations of total and TRL-triglycerides at 6 hours and HDL-triglycerides at 9 hours. All triglyceride concentrations returned to baseline level by 24 hours and subsequent fasting samples showed similar concentrations (Fig 2B).

We encountered unanticipated analytical problems in measuring deuterated-phylloquinone in plasma and lipoprotein fractions of 2 subjects (subjects D and E) and in IDL, LDL, and HDL fractions of all the study subjects. In some subjects, other lipid soluble compounds, as yet unidentified, eluted from the HPLC at the same time as phylloquinone. When we injected these samples into the GC/MS, the unidentified components did not elute from the GC column, causing a blockage that interfered with the flow of subsequent samples through the column. Obtaining reliable GC/MS data for these samples was impossible despite further sample purifications with a repeated or modified HPLC run, or with a C18 column solid phase extraction, in addition to the silica column solid phase extraction.

Based on the 3 subjects with data on deuterated-phylloquinone, the peak enrichment of plasma and TRL with deuterated-

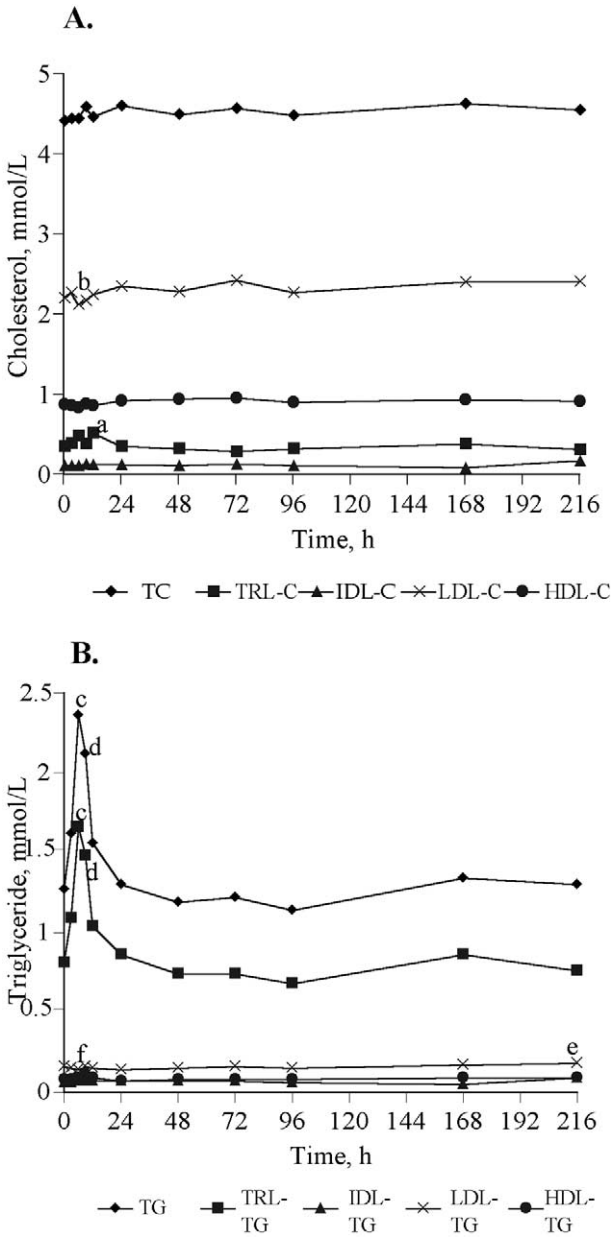


Fig 2. (A) Mean cholesterol and (B) triglyceride concentrations in plasma and lipoprotein fractions after the intake of deuterated collard greens. [a]*P* < .05 difference 6, 9 *v* 72 hours; [b]*P* < .05 difference 6 *v* 72 hours; [c]*P* < .05 different from time points 0, 3, 12, 24, 48, 72, 96, 168, and 216 hours; [d]*P* < .05 different from time points 0, 24, 48, 72, 96, 168, and 216 hours; [e]*P* < .05 difference 216 *v* 96, and 168 hours in IDL-TG; [f]*P* < .05 difference 9 *v* 0, 3, 24, 48, and 72 hours in HDL-TG.

phylloquinone (88% and 89%, respectively) was reached at 6 hours (n = 3) (Fig 3C and D). The highest concentration of deuterated-phylloquinone was also reached at 6 hours and then decreased rapidly from 6 to 12 hours (Fig 3A and B). The average $t_{1/2}$ was 22.8 hours (23.6, 24.6, and 20.3 hours in subjects A, B, and C, respectively). The average rate of disappearance of the enrichment was 0.030%-units/h (0.029, 0.028, and 0.034 %-units/h, respectively). Deuterated-phylloquinone was no longer detectable at 72 hours.

We also analyzed the individual concentrations and proportions of measured isotopomers (ie, m/z 450, 459-462) in plasma and TRL at different time points. Plotting the concentrations against time showed that the individual isotopomers m/z 459-462 overlapped and reached the maximum concentrations at the same time point. This implies that at the level of deuterium enrichment achieved for the phylloquinone in this study, there was no isotopic effect on the transport of phylloquinone.

DISCUSSION

The aim of this study was to assess vitamin K transport in response to ingestion of a physiologic dose of phylloquinone provided in collard greens endogenously labeled with deuterium. The intrinsic labeling and delivery method allowed us to track the exogenous phylloquinone derived from the test meal that included a green vegetable. The current study lends support to the potential widespread application of using stable isotopes in studying vitamin K bioavailability and bioefficacy, comparable to their use for other fat-soluble vitamins.[15]

Similar to that previously observed with the use of pharmacologic doses of vitamin K,[9-11] the TRL fraction was the main carrier of phylloquinone in plasma in response to physiologic doses attained from the diet (Table 2). Although it appeared that there was a high level of variability among study subjects, when plasma or TRL phylloquinone concentrations were normalized to plasma or TRL triglyceride concentrations, respectively, this variability was decreased. These data suggest that circulating phylloquinone concentrations were, in part, determined by circulating triglyceride levels reflected in the high degree of correlation between the 2 parameters ($r = .82$, $P < .001$) and consistent with population studies.[26] Plasma phylloquinone and cholesterol concentrations were not correlated ($r = .05$, $P = .72$). The potential relationship of circulating phylloquinone concentrations with plasma cholesterol has yet to be resolved.[7,27,28]

Increases in phylloquinone concentration in LDL and HDL in response to physiologic doses were delayed relative to the plasma and TRL fraction, as previously reported using pharmacologic doses.[9] Delayed incorporation into LDL suggests that some of the ingested phylloquinone is secreted from the liver via very–low-density lipoprotein (VLDL) and as a result of delipidation of VLDL by lipoprotein lipase, stays associated with its metabolic product, LDL. Similar to previous studies,[9,11] the HDL fraction carried smaller proportions of phylloquinone. Delayed incorporation into HDL may have occurred during the lipolysis of VLDL in plasma with excess surface disassociating to form a nascent discoidal HDL particle. Alternatively, transfer of phylloquinone from particles of intestinal origin to particles of hepatic origin could also account for phylloquinone in LDL and HDL, although no such exchange protein has been identified to date. Schurgers et al[10] observed that the maximum phylloquinone concentrations in plasma, TRL, LDL, and HDL occured at 4 hours after ingestion of a mixture of different forms of vitamin K (900 μg phylloquinone, 888 μg menaquinone-4, and 1,568 μg menaquinone-9). It is plausible that differences in polarity associated with the indi-

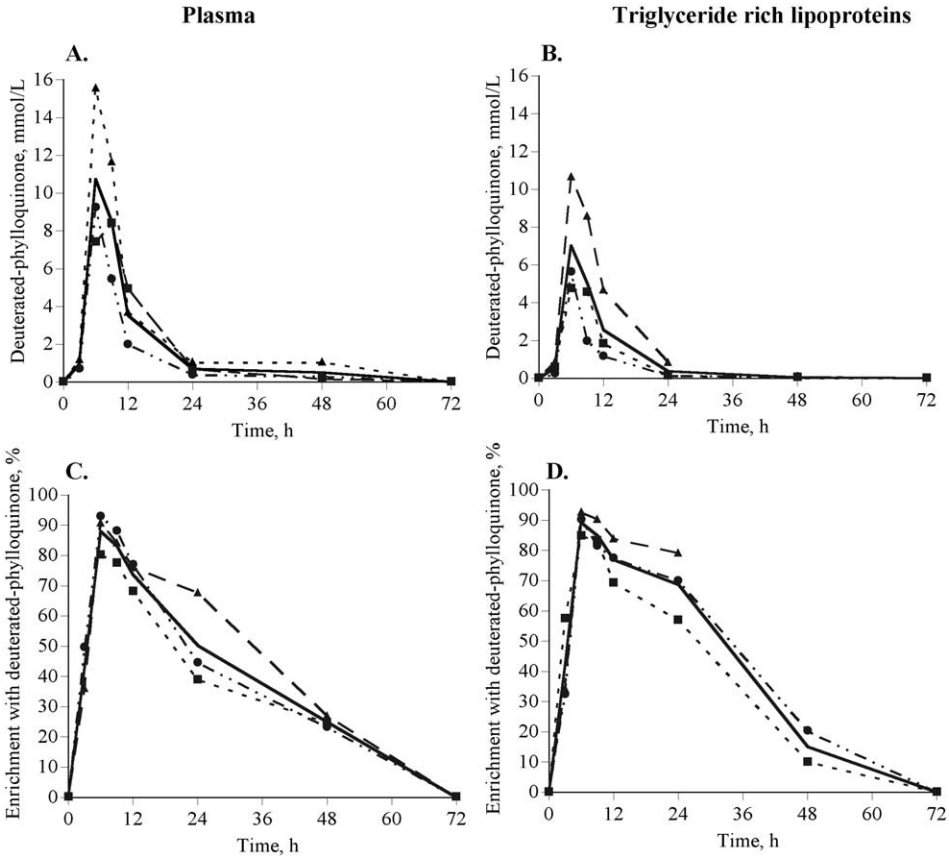


Fig 3. (A and B) Deuterated-phylloquinone concentration and (C and D) enrichment (%) in plasma and TRL after the intake of deuterated collard greens (dashed lines with symbols: ■, subject A; ▲, subject B; and ●, subject C; solid line, mean enrichment).

vidual side chains of the 3 vitamin K forms could account for these contrasting results. Because phylloquinone, the major form of vitamin K present in green plants, was the only form of vitamin K consumed by our study subjects, we cannot make direct comparisons between the 2 studies.

There was no phylloquinone detectable in the LPF in our study. Prior work has reported 1% to 2% of the total plasma phylloquinone in the LPF.[5,9] High supplemental doses of purified phylloquinone may have resulted in a temporary overloading of chylomicrons with vitamin K that became disassociated during ultracentrifugation due to the high g-forces used to isolate the lipoprotein fractions. It is also plausible that at physiologic levels used in this study the amount of phylloquinone in the LPF was below our detection limit.

The enrichment of plasma and TRL phylloquinone with deuterium had a strikingly similar pattern among the 3 subjects, for whom we could obtain data (Fig 3). Deuterated-phylloquinone rapidly displaced a high proportion (88%) of total plasma phylloquinone. This result and our previous result of a maximum enrichment of 80% after a dose of 168 μg deuterated-phylloquinone[17] suggest that the metabolism of phylloquinone differs from that of other fat-soluble vitamins with respect to rates and levels of plasma enrichment. For example, deuterium-labeled vitamin E, given at a dose of 5 times the Recommended Dietary Allowance (75 mg)[29] resulted in a lower maximum enrichment of plasma (~20%) than phylloquinone with greater delay after the ingestion (12 hours).[30] Deuterated-phylloquinone was no longer detectable in plasma at 72 hours, consistent with findings of low levels of radioactivity in plasma at 96 hours after oral dose[5] and at 72 hours after injected dose[6,7] of tritiated phylloquinone.

Normal circulating levels of phylloquinone are extremely low.[31] Although the individuals in this study had phylloquinone levels within normal range,[27] the percent recovery of phylloquinone from the subfractions was frequently less than 50%, suggesting that detection was limited by the assay sensitivity.

In summary, deuterated-phylloquinone is rapidly cleared from plasma. The TRL fraction is the major carrier of phylloquinone in plasma, whereas LDL and HDL fractions carry small amounts. Changes in deuterated-phylloquinone concentrations in plasma and TRL parallel changes in triglyceride concentrations, which suggests a close association with fat absorption. Further developments in the sample preparation and analysis are needed to more precisely assess phylloquinone metabolism.

ACKNOWLEDGMENT

We thank the volunteers who participated in this study, the MRU Staff, and the Nutrition Evaluation staff of the JM USDA HNRC at Tufts University for their contribution to the study and Dr Edward Saltzman for his medical assistance.

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