α-Tocopherol disappearance rates from plasma depend on lipid concentrations: studies using deuterium-labeled collard greens in younger and older adults[1–4]

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ABSTRACT

Background: Little is known about α-tocopherol's bioavailability as a constituent of food or its dependence on a subject's age.

Objective: To evaluate the α-tocopherol bioavailability from food, we used collard greens grown in deuterated water (^{2}H collard greens) as a source of deuterium-labeled (^{2}H) α-tocopherol consumed by younger and older adults in a post hoc analysis of a vitamin K study.

Design: Younger (mean ± SD age: 32 ± 7 y; n = 12 women and 9 men) and older (aged 67 ± 8 y; n = 8 women and 12 men) adults consumed a test breakfast that included 120 g ^{2}H collard greens (1.2 ± 0.1 mg ^{2}H-α-tocopherol). Plasma unlabeled α-tocopherol and ^{2}H-α-tocopherol were measured by using liquid chromatography–mass spectrometry from fasting (>12 h) blood samples drawn before breakfast (0 h) and at 24, 48, and 72 h and from postprandial samples collected at 4, 5, 6, 7, 9, 12, and 16 h.

Results: Times (12.6 ± 2.5 h) of maximum plasma ^{2}H-α-tocopherol concentrations (0.82% ± 0.59% total α-tocopherol), fractional disappearance rates (0.63 ± 0.26 pools/d), half-lives (30 ± 11 h), and the minimum estimated ^{2}H-α-tocopherol absorbed (24% ± 16%) did not vary between age groups or sexes (n = 41). Unlabeled α-tocopherol concentrations were higher in older adults (26.4 ± 8.6 μmol/L) than in younger adults (19.3 ± 4.2 μmol/L; P = 0.0019) and correlated with serum lipids (r = 0.4938, P = 0.0012). In addition, ^{2}H-α-tocopherol half-lives were correlated with lipids (r = 0.4361, P = 0.0044).

Conclusions: Paradoxically, α-tocopherol remained in circulation longer in participants with higher serum lipids, but the ^{2}H-α-tocopherol absorbed was not dependent on the plasma lipid status. Neither variable was dependent on age. These data suggest that plasma α-tocopherol concentrations are more dependent on mechanisms that control circulating lipids rather than those related to its absorption and initial incorporation into plasma. This trial was registered at clinicaltrials.gov as NCT0036232. *Am J Clin Nutr* 2015;101:752–9.

Keywords: age, bioavailability, cholesterol, pharmacokinetics, triacylglycerides, vitamin E

INTRODUCTION

Vitamin E Dietary Reference Intakes were set in 2000 with the estimated average requirement (EAR)[5] equal to 12 mg/d (1). More than 90% of adults in the United States, who do not consume supplements, do not achieve α-tocopherol intakes equal to the EAR (2). Because most of the US population does not apparently suffer from symptoms of α-tocopherol deficiency, there is concern that the EAR is too high. An alternative explanation, on the basis of studies that used ^{14}C-α-tocopherol (3–5), is that the bioavailability of food α-tocopherol is higher than previously estimated by using deuterium-labeled α-tocopheryl acetate-fortified apples (6). As has been noted for vitamin A absorption by humans (7), there are limitations of the various methods for measuring vitamin E absorption. Ideally, a dual-isotope technique that uses an intravenous dose and an oral dose, as has been described for cholesterol (8), would be a useful approach for the quantitation of the fractional absorption of fat-soluble vitamins, but this technique requires that the fat-soluble vitamin be suspended in an intravenous dose that mimics chylomicrons (e.g., a lipid emulsion).

Because of difficulties in estimating the actual α-tocopherol absorption, relative bio-availabilities between various vitamin E forms have been investigated and shown to be useful for identifying α-tocopherol regulatory mechanisms (9–11). In addition, this technique has been used to examine apolipoprotein E-4 effects on vitamin E pharmacokinetics (12). Other than various causes of fat malabsorption that lead to poor α-tocopherol absorption and vitamin E deficiency (13), there is little information with regard to the effect of physiologic factors on vitamin E

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[5] Abbreviations used: C_{max}, maximum plasma concentration; d1, day 28 of the parent study; EAR, estimated average requirement; FDR, fractional disappearance rate; T_{max}, time of maximum plasma concentration.

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bioavailability. In addition, little information is available concerning the effects of age on vitamin E bioavailability.

Previous studies showed that collard greens grown in deuterated water contain sufficient ^{2}H-phylloquinone per serving to carry out investigations of absorption and transport of vitamin K (14). Because plants make and store both α-tocopherol and phylloquinone (15), we hypothesized that ^{2}H-α-tocopherol would also be present to allow measurements of α-tocopherol pharmacokinetics and bioavailability from a plant matrix. We further hypothesized that the bioavailability of α-tocopherol endogenously present in food would be greater than what we previously observed by using α-tocopheryl acetate (6), because α-tocopheryl acetate must be hydrolyzed before absorption (16). In addition, it is not known whether absorption of nutrients, especially those that require fat absorption and chylomicron secretion, are affected by aging. Potentially, older participants would not absorb vitamin E as effectively as younger people, as has been shown for lycopene but not α- or β-carotene (17) or vitamin K absorption (18, 19). To evaluate the bioavailability of ^{2}H-α-tocopherol from food, we used collard greens grown in deuterated water as a source of ^{2}H-α-tocopherol as part of a breakfast consumed by both older and younger adults.

METHODS

Materials

HPLC-grade methanol, hexane, and ethanol were obtained from Fisher Scientific. Unlabeled α- and γ-tocopherols, ascorbic acid, potassium hydroxide, and butylhydroxytoluene were from Sigma-Aldrich.

Deuterated collard greens

The cultivation and preparation of the ^{2}H collard greens for consumption was described previously (14). Briefly, collard greens (*Brassica oleracea* var. *acephala*, cultivar Georgia) were grown hydroponically by using a nutrient solution enriched with 31 atom% ^2H_2O. Collard greens were maintained within an acrylic plastic enclosure (situated inside an environmental growth chamber, model PGW36; Conviron) until harvest at 6 wk. At harvest, all leaves were packaged and shipped overnight on ice to the Jean Mayer USDA Human Nutrition Center on Aging at Tufts University. Vegetables were weighed and steamed for 8–12 min until the leaves were completely cooked. Afterward, the vegetables were pureed, portioned, and kept at −80°C until used for the feeding studies. An aliquot was also used for the determination of the ^{2}H-α-tocopherol concentration.

Participants

The Institutional Review Board of New England Medical Center and Tufts University approved the study protocol; all participants gave written informed consent for participation in the study. Only de-identified plasma samples were sent for analysis to the investigators at Oregon State University. This trial was registered at clinicaltrials.gov as NCT0036232.

This study was originally designed to determine dietary and nondietary factors that influence phylloquinone absorption, transport, and utilization (19). Within the parent study, ^{2}H collard greens were used to evaluate phylloquinone bioavailability and lipoprotein transport during periods of phylloquinone restriction and supplementation. The collard greens also contained ^{2}H-α-tocopherol; therefore, α-tocopherol-pharmacokinetics were also investigated by using the same plasma samples that were collected from participants who consumed the collard greens.

As described elsewhere, healthy ambulatory men and women participants in a younger age group (18–40 y) and an older age group (55–80 y) were recruited from the greater Boston area. Women in the older age group were postmenopausal for ≥3 y. All participants fulfilled the following criteria: normal kidney, liver, thyroid, renal, and cardiac function; normal fasting glucose concentrations; and normal clotting times. At the time of the study, participants were not users of the following medications: oral anticoagulants within the previous 12 mo; antibiotics within the previous 3 mo; anticonvulsants, barbiturates, or phenobarbital-containing drugs; herbal preparations; or vitamin E supplements. Participants consumed 600 mg elemental calcium and 10 μg (400 IU) cholecalciferol from 30 d before the study and throughout the study. Subjects consumed a baseline diet for 5 d and a vitamin K restricted diet for 28 d.

Study design

On day 28 of the parent study (d1), participants resided in the Metabolic Research Unit at the Jean Mayer USDA Human Nutrition Center on Aging at Tufts University for 1 d (19). During this residency day (d1), participants were provided with a breakfast, which contained 1 serving fruit yogurt (with 15 g wheat germ), toasted English muffin, butter, honey, skim milk, and decaffeinated coffee and a 120-g serving of ^{2}H collard greens as described previously (19). The breakfast contained 4.7 mg α-tocopherol (unlabeled), 450 kcal, and 14% fat. Over the course of d1, subjects consumed 30.7% kcal from fat and a total of 9.2 mg α-tocopherol from the breakfast and from a standardized lunch and dinner. Participants were free living on days 2, 3, and 4 but were provided with all meals and beverages to minimize interindividual variability.

Fasting (>12 h) blood samples were drawn at 0 h before the ingestion of ^{2}H collard greens. After the consumption of breakfast, blood samples were drawn at 4, 5, 6, 7, 9, 12, and 16 h. Fasting blood samples were subsequently collected at 0800 on days 2, 3, and 4 (corresponding to 24, 48, and 72 h). All blood samples were collected in tubes containing EDTA (0.15% final concentration); plasma was separated by centrifugation and stored frozen until analysis.

Measurement of deuterium-labeled vitamin E

Plasma and collard green α-tocopherols were extracted by using a modified method for saponification and vitamin E extraction (20). Briefly, plasma (50 μL) or collard greens (1 g) were added to ethanol (containing 1% ascorbic acid) and mixed thoroughly and H_2O and saturated potassium hydroxide were added. After the addition of a known amount of internal standard (α-tocotrienol), samples were incubated at 60°C for 30 min. After cooling and the addition of 1% ascorbic acid and butylhydroxytoluene, samples were extracted with hexane. An aliquot of the organic phase was dried under nitrogen and resuspended in 200 μL 1:1 ethanol:methanol (vol:vol) for injection into the HPLC system.

The HPLC system (Waters) consisted of a 2695 Separations Module that contained a cooled auto-injector (10°C), a 50-μL sample loop, and a column oven (30°C). The column was a Synergi Hydro-RP (250 mm L × 3.0 mm inside diameter, 4-μm particle size; Phenomenex) with a precolumn (AQ C18, 4 × 3 mm inside-diameter SecurityGuard; Phenomenex). The mobile phase consisted of 100% methanol delivered at 1 mL/min for 10 min. The HPLC was coupled to a ZQ 2000 single-quadrupole mass spectrometer (Micromass) with an atmospheric pressure chemical ionization source operated in negative mode. The corona voltage was set to 25 μA, and the sample cone voltage was set to −35 V. The source temperature was set to 120°C, and the probe temperature was set to 400°C. The desolvation gas (nitrogen) was set to 350 L/h, and the cone gas (nitrogen) was set to 20 L/h. Single-ion recording data were obtained at 429 *m/z* for unlabeled α-tocopherol and 423 *m/z* for α-tocotrienol; retention times for α-tocotrienol and α-tocopherol were 5.7 and 8.6 min, respectively.

To establish which ^{2}H-α-tocopherols were likely present in the participants' plasma, isotopic distribution patterns were determined for collard greens similarly to what was described for ^{2}H-phylloquinone (14, 21). The predominant isotopomers in the collard greens' α-tocopherols were *m/z* 435–446; no unlabeled α-tocopherol *m/z* 429 was detected (**Figure 1**). The ions at *m/z* 438–442 (equivalent to 9–13 ^{2}H atoms, respectively, on the α-tocopherol) were chosen for selected ion recording in plasma extracts because these ions encompassed ~60% of the total ion abundance for α-tocopherol from the collard greens. The concentration of collard green α-tocopherol was determined by using HPLC with electrochemical detection with the use of authentic standards as described (20). Collard greens were determined to contain 9.8 ± 0.7 mg ^{2}H-α-tocopherol/kg greens, which resulted in 1.2 ± 0.1 mg ^{2}H-α-tocopherol per 120-g serving or ~2.7 μmol administered to each participant (and no unlabeled collard green α-tocopherol). Subjects also consumed 4.7 mg (10.0 μmol) unlabeled α-tocopherol from the breakfast.

To estimate plasma α-tocopherols, peak-area data were collected for unlabeled α-tocopherol *m/z* 429 as well as peak areas for ions at *m/z* 438–442. These latter peak areas were summed to estimate the majority of ^{2}H-α-tocopherols in the plasma. Areas for α-tocopherol and ^{2}H-α-tocopherol were used to measure α-tocopherols by the ratio of their respective areas to the area internal standard. Deuterated α-tocopherols were corrected to the 100% ion abundance observed in the collard greens. The percentage of labeled to total (labeled and unlabeled) α-tocopherols was calculated for each time point for each participant.

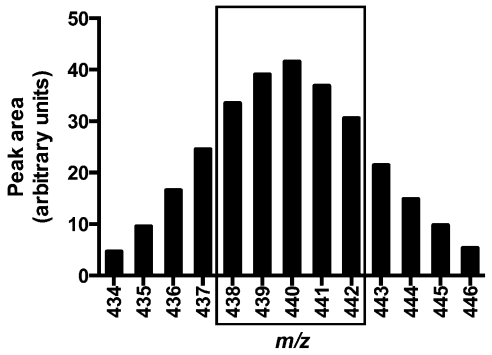

FIGURE 1 Distribution of ^{2}H-α-tocopherol isotopomers. Single-ion recording mass spectrometer data were used to obtain the isotopic distribution of ^{2}H-α-tocopherol isotopomers extracted from collard greens similarly to what was described for ^{2}H-phylloquinone (14, 21). The predominant isotopomers in the collard green α-tocopherols were *m/z* 438–442 as indicated by the box; these ions encompassed ~60% of the total α-tocopherol ion abundance. No unlabeled α-tocopherol (*m/z* 429) was detected. The ions at *m/z* 438–442 [equivalent to 9–13 deuterium (^{2}H) atoms, respectively, on the α-tocopherol) were chosen for selected ion recording in plasma extracts.

Mathematical and statistical analyses

The AUC of plasma ^{2}H-α-tocopherol concentrations for each person was calculated by using the trapezoidal rule. Maximal concentrations and the time of maximal concentration were identified by visual inspection of the data. α-Tocopherol fractional disappearance rates (FDRs) and half-lives were calculated from the ln of the plasma %^{2}H-α-tocopherol concentrations

$$\{[^2\text{H-}\alpha\text{-tocopherol}\,(\mu\text{mol/L})/[^2\text{H-}\alpha\text{-tocopherol} + \text{unlabeled }\alpha\text{-tocopherol}\,(\mu\text{mol/L})]\} \times 100\} \quad (1)$$

as well as the ln of ^{2}H- α-tocopherol concentrations (22). To calculate pools per day, the FDR was multiplied by 24 h. There were no differences in kinetic outcomes whether the actual concentrations or the percentage of ^{2}H-α-tocopherol was used for calculations; therefore, only variables calculated by using %^{2}H-α-tocopherol concentrations are shown. The FDR (the slope of the disappearance curve) was calculated for each individual by using the ln of %^{2}H-α-tocopherol concentrations (or ^{2}H-α-tocopherol concentrations) compared with time. The linest function (Microsoft Excel for Mac 2011, version 14.4.7) was applied by using concentrations from the time of maximum plasma concentration (T_{max}) to 72 h; outcomes were acceptable only if the r^2 of the fit was >0.9. Two participants had to be excluded because %^{2}H-α-tocopherol concentrations were too low to be fitted reliably; none of their data are included in this study.

The plasma ^{2}H-α-tocopherol concentration was extrapolated back to time zero from the linear regression analysis. This value and the estimated plasma volume were used to calculate the amount of absorbed ^{2}H-α-tocopherol. Plasma volumes (L) were calculated from each participant's body weight by using separate equations for men

$$(14.5 \times \text{body weight} + 2035) \div 1000 \quad (2)$$

and women

$$(24 \times \text{body weight} + 872) \div 1000 \quad (3)$$

as described (23). Fractional absorption was calculated from the estimated amount of ^{2}H-α-tocopherol hypothetically present in the circulation at time zero divided by the amount of ^{2}H-α-tocopherol administered (2.7 μmol).

Data are reported as means ± SDs. Statistical comparisons between groups were performed on logarithmically transformed data with GraphPad Prism software (version 6f; GraphPad Software). The significance of variables was evaluated by using a 2-factor ANOVA followed by Tukey's post hoc test when significant interactions or main effects ($P < 0.05$) were observed; most comparisons were not different. Spearman correlations were calculated with GraphPad Prism software.

TABLE 1
Participant characteristics at baseline[1]

	Younger		Older				
	Women	Men	Women	Men	*P*-interaction	*P*-women compared with men	*P*-younger compared with older
n	12	9	8	12	—	—	—
Age, y	32.9 ± 6.4	30.4 ± 7.2	67.3 ± 7.9	66.3 ± 8.1	NS	NS	0.0001
BMI, kg/m^2	25.5 ± 3.6	25.3 ± 3.3	25.6 ± 4.8	25.2 ± 4.9	NS	NS	NS
Triacylglyceride, mmol/L	0.70 ± 0.27^a	1.27 ± 0.35^b	1.27 ± 0.80^{a,b}	1.19 ± 0.66^{a,b}	0.0249	0.0435	NS
Total cholesterol, mmol/L	4.24 ± 0.94	4.18 ± 0.94	5.20 ± 0.96	4.39 ± 0.78	NS	NS	0.0442
LDL cholesterol, mmol/L	2.35 ± 0.81	2.51 ± 0.70	3.07 ± 0.66	2.58 ± 0.71	NS	NS	NS
Total lipids, mmol/L	4.94 ± 1.06^a	5.44 ± 1.02^{a,b}	6.47 ± 1.04^b	5.58 ± 0.78^{a,b}	0.0322	NS	0.0088

[1]All values are means ± SDs. The significance of variables was evaluated by using a 2-factor ANOVA followed by Tukey's post hoc test. For comparisons in a row that do not bear the same superscript letter, $P < 0.05$.

RESULTS

Baseline characteristics of participants

Participants were recruited with respect to age and sex; the groups included younger women (median age: 34.6 y; range 20.3–40.3 y; $n = 12$), younger men (median age: 30.6 y; range: 20.6–39.7 y; $n = 9$), older women (median age: 66.3 y; range: 55.7–66.3 y; $n = 8$), and older men (median age: 64.0 y; range: 56.8–82.6 y; $n = 12$; **Table 1**). With regard to BMI, there were no differences between age groups or between sexes. There were no differences between older and younger adults with respect to fasting serum triacylglycerides, but when these were summed with total cholesterol for each individual, older participants (5.94 ± 0.98 mmol/L) had, on average, ~15% higher lipid concentrations than those of younger participants (5.16 ± 1.05 mmol/L; $P = 0.0088$).

^{2}H-α-tocopherol kinetics

A plot of plasma labeled and unlabeled α-tocopherol concentrations from a representative participant who had kinetic variables similar to the average values of all participants showed that plasma unlabeled α-tocopherol concentrations did not vary appreciably over the course of the 72-h study. By contrast, ^{2}H-α-tocopherol concentrations increased to a maximum and then decreased at an exponential rate as ^{2}H-α-tocopherol left the plasma compartment (**Figure 2**).

The AUC of plasma ^{2}H-α-tocopherol concentrations was calculated for each individual; AUCs did not vary between the age or sex groups (**Table 2**). Indeed, none of the ^{2}H-α-tocopherol kinetic variables, such as T_{max}, the maximum plasma concentration (C_{max}), FDR, or half-life, varied between age groups or between sexes (Table 2). Overall average values were as follows: AUC, 6.5 ± 4.3 μmol ^{2}H-α-tocopherol/L × h; T_{max}, 12.6 ± 2.5 h; C_{max}, 0.82 ± 0.59%; FDR, 0.63 ± 0.26 pools/d; and half-life, 30.2 ± 11.1 h.

Age groups differed in plasma vitamin E status. Unlabeled α-tocopherol concentrations were greater in older than younger adults when examined at baseline ($T = 0$, $P = 0.0264$), averaged over all time points ($P = 0.0008$), at 24 h ($P = 0.0113$), averaged over fasting time points (24, 48, and 72 h; $P = 0.0016$), or at the average of the individual T_{max} (for ^{2}H-α-tocopherol concentrations, $P = 0.0011$; **Table 3**). When plasma ^{2}H-α-tocopherol concentrations were used to calculate kinetic variables, these values were not different between the 2 age groups (**Table 4**). The measured ^{2}H-α-tocopherol C_{max} from each individual was, on average, 0.17 ± 0.12 μmol/L, the hypothetical ($T = 0$) C_{max} was 0.23 ± 0.15 μmol/L, the estimated absorbed ^{2}H-α-tocopherol was 0.65 ± 0.41 μmol, and the fraction of ^{2}H-α-tocopherol absorbed was 24 ± 15%.

We investigated the reasons underlying the differences in unlabeled α-tocopherol concentrations between age groups and lack of similar differences in ^{2}H-α-tocopherol kinetic variables. Notably, there were significant differences in serum lipids between groups at baseline (Table 1), and serum lipids increased with age (Spearman $r = 0.4390$; 95% CI: 0.1427, 0.6631; $P = 0.0041$). Overall average unlabeled α-tocopherol concentrations were correlated with total lipids (Spearman $r = 0.5396$; 95% CI: 0.2695, 0.7311; $P = 0.0003$; **Figure 3**A).

Unlabeled α-tocopherol concentrations assessed at all the other times shown in Table 3 were also correlated with total lipids (data not shown). Calculated half-lives varied widely (Table 2), but these too were correlated with serum lipids (Spearman $r = 0.4361$; 95% CI: 0.1392, 0.6611; $P = 0.0044$; Figure 3B) as did logarithmically transformed FDR (data not shown). However, baseline serum lipids were not correlated with either the amount of estimated absorbed ^{2}H-α-tocopherol (not shown) or the percentage of the ^{2}H-α-tocopherol dose absorbed (Figure 3C).

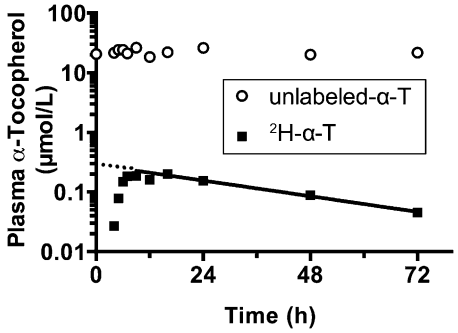

FIGURE 2 Representative participant plasma labeled and unlabeled α-tocopherol concentrations with an illustration of the curve fitting of ^{2}H-α-tocopherol concentrations postpeak (time of maximum plasma concentration). Plasma unlabeled-α-T (circles) and ^{2}H-α-T (squares) concentrations from a representative participant whose values were similar to average concentrations and kinetic variables are shown. The fractional disappearance rate calculated from the plasma % ^{2}H-α-tocopherol and plasma α-tocopherol concentrations were used to calculate estimated ^{2}H-α-tocopherol concentrations to generate the line shown; the dotted line denotes the extrapolation back to time zero to estimate the hypothetical ^{2}H-α-tocopherol at $t = 0$ shown in Table 4. ^{2}H-α-T, ^{2}H-α-tocopherol; unlabeled-α-T, unlabeled α-tocopherol.

TABLE 2
^{2}H-α-tocopherol kinetic variables[1]

	Younger		Older	
	Women	Men	Women	Men
n	12	9	8	12
AUC, μmol ^{2}H-α-tocopherol/L × h	5.9 ± 3.9	5.6 ± 4.5	7.7 ± 4.4	7.0 ± 4.8
T_{max}, h	12.2 ± 2.1	12.2 ± 2.5	11.9 ± 2.9	13.8 ± 2.5
C_{max}, %	0.94 ± 0.67	0.82 ± 0.74	0.79 ± 0.48	0.71 ± 0.48
y intercept, %	1.28 ± 0.87	1.03 ± 0.78	1.07 ± 0.62	0.98 ± 0.64
FDR, pools/d	0.68 ± 0.32	0.58 ± 0.18	0.66 ± 0.25	0.61 ± 0.27
Half-life, h	28.1 ± 9.8	30.6 ± 7.9	29.3 ± 12.3	32.6 ± 14.0
R^2	0.9565 ± 0.0322	0.9551 ± 0.0364	0.9602 ± 0.0343	0.9501 ± 0.0387

[1]All values are means ± SDs. C_{max} is expressed as the percentage of ^{2}H-α-tocopherol per plasma total α-tocopherol concentration; the *y* intercept and FDR were calculated for each individual from the percentage of ^{2}H-α-tocopherol compared with time curves; the R^2 is the average correlation coefficient for the fitting of the curves to the data. There were no significant differences for any of the variables shown (age, sex, or an age × sex interaction (2-factor ANOVA). C_{max}, maximum plasma concentration; FDR, fractional disappearance rate; T_{max}, time of maximum plasma concentration.

DISCUSSION

To our knowledge, this is the first time that α-tocopherol bioavailability from food with naturally incorporated ^{2}H-α-tocopherol was measured, and we showed that the small amounts of collard green ^{2}H-α-tocopherol were absorbed and could be detected after plasma incorporation. Strengths of this study include the large number of participants investigated (n = 41) and ranges of ages (20–82 y) and baseline circulating lipid concentrations (Figure 3). Limitations included the relatively low dose administered, which limited the length of time that the deuterated α-tocopherol could be measured, as well as the lack of measurement of lipids at the time of dose administration. In addition, the study was carried out in subjects consuming a phylloquinone-restricted diet. Nonetheless, it was unlikely that the dietary restriction limited the generalizability because these vitamin K intakes are reported in ~15–25% of the US adult population (24, 25).

We showed that the α-tocopherol naturally present in plants (e.g., synthesized by the plant) has similar rates of disappearance and half-lives as we observed when we previously tested α-tocopherol bioavailability from food by using exogenously added α-tocopheryl acetate (6, 22). These findings are consistent with Cheeseman et al. (26) who simultaneously compared differently deuterated α-tocopherol and α-tocopheryl acetate. The mean FDR (0.63 ± 0.26) and half-lives (30 ± 11) presented herein (Table 2) were consistent with our previous experiments, but the variability was greater in the current study, which was likely a result of the very low dose of ^{2}H-α-tocopherol administered (1.2 ± 0.1 mg ^{2}H-α-tocopherol/120 g serving collard greens), or perhaps there is greater variability inherent with releasing and absorbing α-tocopherol from a plant matrix. Nonetheless, the kinetic variables showed no major differences in α-tocopherol bio-availabilities between younger and older adults.

To our surprise, despite the lack of significant differences between age groups with respect to the α-tocopherol kinetic variables (Table 2) and ^{2}H-α-tocopherol concentrations (Table 4), plasma unlabeled α-tocopherol concentrations were uniformly higher in older than younger participants (Table 3). Not unexpectedly, plasma unlabeled (but not labeled) α-tocopherol concentrations were highly correlated with baseline serum total lipids (Figure 3A). These data were consistent with α-tocopherol transport in circulating lipoproteins, and differences in lipids concentrations were reflected in the plasma α-tocopherol concentrations (13). Previously, we showed that dietary fat consumption (0–21% in the test breakfast) altered AUCs but not α-tocopherol disappearance rates or half-lives (6). Therefore, what was unexpected in the current study was the significant correlation between α-tocopherol half-lives and serum total lipids (Figure 3B). Apparently, as serum lipids increase, α-tocopherol remains in circulation for a longer time likely because higher lipid concentrations are associated with slower lipoprotein

TABLE 3
Unlabeled α-tocopherol concentrations[1]

	Younger		Older				
	Women	Men	Women	Men	*P*-interaction	*P*-women compared with men	*P*-younger compared with older
n	12	9	8	12	—	—	—
Unlabeled α-tocopherol							
T = 0 h, μmol/L	18.7 ± 4.8	21.6 ± 5.7	28.3 ± 11.7	24.8 ± 8.6	NS	NS	0.0264
Average T = all times, μmol/L	18.6 ± 3.9	20.3 ± 4.7	28.8 ± 9.9	24.7 ± 7.7	NS	NS	0.0008
T = 24 h, μmol/L	18.6 ± 4.7	21.1 ± 6.2	27.9 ± 11.0	24.3 ± 6.8	NS	NS	0.0113
Average at 24, 48, and 72 h, μmol/L	19.7 ± 4.2	20.8 ± 5.7	30.3 ± 10.3	24.6 ± 7.6	NS	NS	0.0016
T_{max},[2] μmol/L	18.6 ± 4.7	19.4 ± 5.1	28.6 ± 11.9	25.5 ± 8.9	NS	NS	0.0011

[1]All values are means ± SDs. The significance of variables was evaluated by using a 2-factor ANOVA.
[2]T_{max}, time of maximum plasma concentration.

TABLE 4
^{2}H-α-tocopherol concentrations and estimated absorbed ^{2}H-α-tocopherol[1]

	Younger		Older				
	Women	Men	Women	Men	*P*-interaction	*P*-women compared with men	*P*-younger compared with older
n	12	9	8	12	—	—	—
C_{max}[2] ^{2}H-α-tocopherol, μmol/L	0.16 ± 0.11	0.16 ± 0.16	0.20 ± 0.10	0.17 ± 0.12	NS	NS	NS
T = 0 h, hypothetical max	0.22 ± 0.13	0.21 ± 0.16	0.24 ± 0.06	0.23 ± 0.17	NS	NS	NS
Plasma volume, L	2.51 ± 0.26	3.19 ± 0.17	2.49 ± 0.36	3.27 ± 0.15	NS	<0.0001	NS
Estimated absorbed ^{2}H-α-tocopherol, μmol	0.57 ± 0.38	0.67 ± 0.48	0.59 ± 0.09	0.75 ± 0.54	NS	NS	NS
Estimated fraction of ^{2}H-α-tocopherol absorbed, %	21 ± 14	25 ± 18	22 ± 3	28 ± 20	NS	NS	NS

[1]All values are means ± SDs. The significance of variables was evaluated by using a 2-factor ANOVA.
[2]C_{max}, maximum plasma concentration.

catabolism and uptake by tissues (27). However, the fraction of the dose absorbed was not correlated with serum lipids (Figure 3C).

To compare the current study with the various previously published studies, we showed that many studies reported the C_{max} as well as the dose and the amount of fat in the administered breakfast. The values, when expressed as the percentage of dose/L plasma, allowed comparisons of the various labeled dose sizes, which ranged from submicromolar to millimolar (**Table 5**). Similar values of the C_{max} were reported in the current study (6.4 ± 4.4%) and the ^{14}C-α-tocopherol study (5.4 ± 1.6%) (5) despite the widely disparate estimates of fractional absorption rates between the current study (24 ± 15%) and the ^{14}C-α-tocopherol study (81 ± 1%). In contrast, the C_{max} of the apple study (6) at the highest fat intake was 11.2 ± 2.5%, which suggested that fat may improve vitamin E absorption when administered as α-tocopheryl acetate and that additional studies are needed that use more-accurate methods to assess fractional absorption. Such measures are needed to estimate how much food vitamin E must be consumed to provide the amounts calculated from biokinetic studies (1). Another limitation of the current study was that estimates derived by using the method of extrapolating the decay curve back to time equal zero are meant for a one-compartment system and are less reliable than those derived for a multicompartment model, which ideally should be used for α-tocopherol pharmacokinetics over a longer time than 72 h.

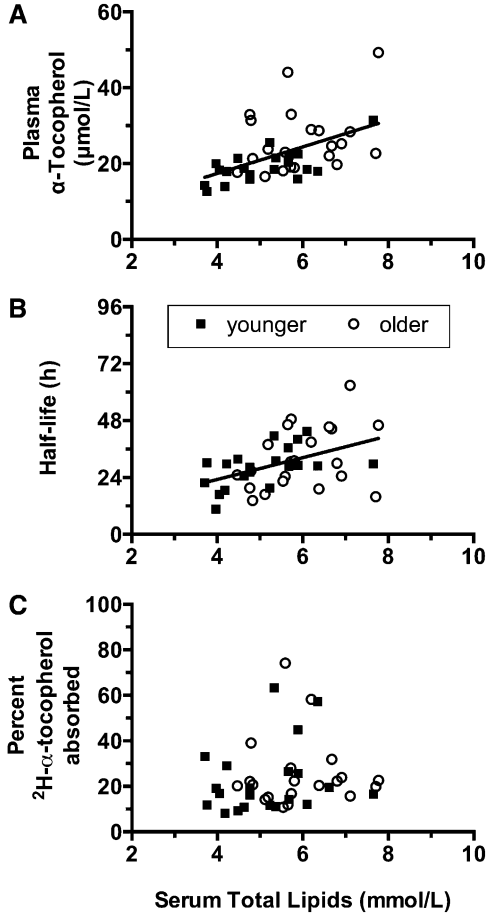

FIGURE 3 Correlation of overall average plasma α-tocopherol concentrations (A), half-lives (B), or fractional absorption of ^{2}H-α-tocopherol (C) with serum lipids. A: Overall average unlabeled α-tocopherol concentrations were correlated with total lipids (Spearman r = 0.5396; 95% CI: 0.2695, 0.7311; P = 0.0003). B: Calculated half-lives were also correlated with serum lipids (Spearman r = 0.4361; 95% CI: 0.1392, 0.6611; P = 0.0044). Correlations are shown as lines (generated by using a linear regression). C: The lack of relation is shown between serum lipids and the percentage ^{2}H-α-tocopherol absorbed from collard greens.

Currently, available estimates of vitamin E absorption are based on plasma concentrations of labeled α-tocopherol after oral administration (6), which can be considered a minimum absorption estimate, or estimates are based on labeled α-tocopherol present in fecal collections, which can be considered a maximum absorption estimate (4, 5, 28) with the true absorption rate likely between both estimates. The use of radioactive α-tocopherol also has some limitations, because the material has the potential to self-irradiate and, thereby, causes the formation of oxidized tocopherol. Indeed, there have been reports of 30–41% absorption by using estimates of fecal radioactive α-tocopherol, whereas simultaneously, no plasma radioactive α-tocopherol was detected (29). Because of observations that newly absorbed α-tocopherol replaces the endogenous α-tocopherol in human circulation (9), it seems unlikely that the newly absorbed radioactive α-tocopherol would not appear in the circulation especially because the role of the hepatic α-tocopherol transfer protein is to maintain plasma α-tocopherol concentrations. The fractional α-tocopherol absorption is likely to be as high as 55–79% in healthy participants but much-more limited during various forms of fat malabsorption (30).

Unlike the assessment of absorption, there is general agreement as to the kinetic variables for the fast turning-over pool of

TABLE 5
Comparison of concentrations at C_{max} from various published studies[1]

Study (reference)	*n* participants	Dose administered, μmol	Matrix	C_{max}, percentage of dose/L plasma[2]	Fat, g	Percentage of fat calories	Breakfast calories, kcal
Collard greens	41	2.7	Collard greens	6.4 ± 4.4	1.6	14	450
Apple study (6)	5	50	"Vacuum impregnation solution"	3.5 ± 1.8	0	0	47
				6.9 ± 1.5	2.4	6	380
				11.2 ± 2.5	11	21	471
Smoker study (10)[3]	22	116	Encapsulated	2.3 ± 1.2	18.7	30	560
RBC (37)	12	344	Encapsulated	6.0 ± 1.9	40	—	Not stated
ApoE4 participants (12)	10	344	Encapsulated	3.2 ± 0.5	40	—	Not stated
^{14}C-α-tocopherol (4)[4]	12	0.00181	2% milk	5.4 ± 1.6	10.4	32.6	319

[1]ApoE4, apolipoprotein E-4; C_{max}, maximum plasma concentration; RBC, red blood cell.
[2]All values are means ± SDs.
[3]Both smokers and nonsmokers, all of whom consumed vitamin C supplements.
[4]Fat = 8 g + 2.4 g milk fat; 28% + fat from milk; 252 kcal + 67 kcal from milk.

vitamin E. For example, a mean half-life of 53 h was reported in 1970 as estimated by using radioactive α-tocopherol (30). Of course, multiple pool models estimate longer half-lives for the slower turning-over pools such as adipose tissue or nervous tissues (e.g., spinal cord and brain) (5, 9, 31).

These estimates for vitamin E pharmacokinetics are very different from those of vitamin K. Vitamin K (phylloquinone), which was studied previously by using deuterium-labeled collard greens (14), showed a relatively fast plasma disappearance compared with that of vitamin E; ^{2}H-α-tocopherol persisted in the plasma with a half-life of 30 h ± 11 for the fast turning-over pool, whereas ^{2}H-phylloquinone had returned to baseline by 24 h. In this study, the T_{max} for ^{2}H-α-tocopherol was 12 ± 2 h, whereas the T_{max} was from 6 to 9 h for ^{2}H-phylloquinone (14). These findings emphasize the importance of the well-established role of the hepatic α-tocopherol transfer protein in maintaining plasma α-tocopherol concentrations in the fast turning-over pool (32) as well as a lack of a similar mechanism for maintaining phylloquinone concentrations (14). Differences in the kinetics of the plasma transport of the 2 vitamins are especially striking because of the similarity in their structures, both of which have a phytyl tail and both are absorbed in chylomicrons (33). Studies that used ^{2}H-phylloquinone showed that liver contains ^{2}H-phylloquinone, suggesting that the unmodified form is transported in triacylglyceride-rich lipoproteins during absorption (34, 35). Currently, specific comparisons between the pharmacokinetics of vitamins E and K are not available for participants in this study.

In conclusion, despite the lack of major differences between younger and older adults in the bioavailability of collard green ^{2}H-α-tocopherol, we showed that ^{2}H-α-tocopherol half-lives were correlated with serum total lipids. Thus, α-tocopherol remained in circulation longer at higher serum lipid concentrations, likely because higher lipid concentrations are associated with slower lipoprotein catabolism and uptake by tissues. These findings have important public health consequences because they highlight a limitation in assessing vitamin E status by using only plasma α-tocopherol concentrations. Lipids were only 15% higher in the older group, but plasma α-tocopherol concentrations were 25% higher, likely because the lipids allowed the plasma to carry more vitamin. A better biomarker of vitamin E status is needed such as the vitamin E metabolite α-carboxyethyl hydroxychromanol (36).

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