The Journal of Nutrition
Nutrient Requirements and Optimal Nutrition

Quantitation of [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-Tocopherol in Humans[1–3]

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Abstract

Half-lives of α-tocopherol in plasma have been reported as 2–3 d, whereas the Elgin Study required >2 y to deplete α-tocopherol, so gaps exist in our quantitative understanding of human α-tocopherol metabolism. Therefore, 6 men and 6 women aged 27 ± 6 y (mean ± SD) ingested 1.81 nmol, 3.70 kBq of [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol. The levels of ^{14}C in blood plasma and washed RBC were monitored frequently from 0 to 460 d while the levels of ^{14}C in urine and feces were monitored from 0 to 21 d. Total fecal elimination (fecal + metabolic fecal) was 23.24 ± 5.81% of the ^{14}C dose, so feces over urine was the major route of elimination of the ingested [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol, consistent with prior estimates. The half-life of α-tocopherol varied in plasma and RBC according to the duration of study. The minute dose coupled with frequent monitoring over 460 d and 21 d for blood, urine, and feces ensured the [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol (the tracer) had the chance to fully mix with the endogenous [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol (the tracee). The ^{14}C levels in neither plasma nor RBC had returned to baseline by d 460, indicating that the $t_{1/2}$ of [5-CH_3]-(2R, 4′R, 8′R)-α-tocopherol in human blood was longer than prior estimates. J. Nutr. 141: 1482–1488, 2011.

Introduction

Vitamin E is a collective term for lipid-soluble tocopherols and tocotrienols. Within the tocopherol subgroup, α-tocopherol is the most abundant in human tissues (1) due to the hepatic α-tocopherol transfer protein (α-TTP),[8] and it is the only form retained at high levels to meet the nutritional needs of humans. The antioxidant role of α-tocopherol is well known and it may have additional functions such as cell signaling, gene expression, and gene regulation (2).

Ingested vitamin E is incorporated into mixed micelles in the small intestine (3,4). The ingested vitamin E is then delivered to enterocytes by both protein-independent and -dependent mechanisms for absorption, tissue uptake, tissue efflux, and biliary secretion (5–13). In the enterocytes, α-tocopherol is packaged in chylomicra and endocytosed into the liver, where it selectively binds to the α-TTP. The α-TTP then routes α-tocopherol through export vesicles to the plasma membrane (14,15). From the plasma membrane, α-Tocopherol is secreted by a protein-dependent mechanism prior to association with lipoproteins and delivery to peripheral tissues (16–18). α-TTP regulates systemic α-tocopherol levels by directing its traffic within the hepatocyte (19). As these transport processes of vitamin E are better understood, they will elucidate the various mechanisms of this important vitamin. Once α-tocopherol biologic functions are stipulated, the quantitative understanding of metabolism elucidated from an oral dose of [5-$^{14}CH_3$]-RRR-α-tocopherol can determine the amount of intake necessary to sustain optimal health.

Half-lives of α-tocopherol in plasma have been reported as 2–3 d (20–23), whereas the Elgin Study required >2 y to deplete α-tocopherol (24,25). Although gaps exist in quantitative aspects of α-tocopherol metabolism, a recent study demonstrated the feasibility of quantifying the absorption, distribution, metabolism, and elimination of α-tocopherol with the use of a true tracer dose of [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopheryl acetate in a healthy man under steady-state conditions (26). Studies that also used labeled α-tocopherol have elucidated the following 3 features of α-tocopherol metabolism (27–29). First, most ingested α-tocopherol peaked in lymph between 2 and8 h and 21–28% of ingested α-tocopherol was absorbed via lymph; second, the ingested α-tocopherol appeared in plasma within 2–4 h, peaked in 5–14 h, and disappeared from the plasma with an apparent

[1] Supported by NIH grant R01 DK 081551, USDA Regional Research W-2002, the California Agricultural Experiment Station, and grant UL1 RR024146 from the National Center for Research Resources, a component of the NIH, and NIH Roadmap for Medical Research. Aspects of this work were performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344 and NIH National Center for Research Resources grant RR13461.
[2] Author disclosures: J. C. Chuang, H. D. Matel, K. P. Nambiar, S-H. Kim, J. G. Fadel, D. M. Holstege, and A. J. Clifford, no conflicts of interest.
[3] Supplemental Tables 1–6 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 jn.nutrition.org.
[8] Abbreviations used: AMS, accelerator MS; CCRC, Clinical and Translational Science Center's Clinical Research Center; C_{max}, maximum concentration; HNC, Human Nutrition Center; $t_{1/2}$ 0-t, half-life calculated using measured data points from 0-t d only; $t_{1/2}$ 0-t,∞, half-life calculated from 0-t d, extrapolated to infinity; Tbsp, tablespoon; T_{max} time to maximum; α-TTP, α-tocopherol transfer protein.
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Manuscript received January 24, 2011. Initial review completed March 15, 2011. Revision accepted May 11, 2011.
First published online June 29, 2011; doi:10.3945/jn.111.138925.

half-life of ~2 d; third, nearly 8% of ingested α-tocopherol was eliminated via urine in 3 d. The experimental duration of these radiolabeled α-tocopherol studies (27–29) was relatively short. Consequently, the ingested radiolabeled α-tocopherol (the tracer) may not have mixed fully with the deepest and slowest turning-over pools of α-tocopherol (the tracee) in the study participants. In addition, kinetic studies that used deuterated α-tocopherol often need to administer doses that are large (21,23,30,31) compared with the RDA of 15 mg/d (22.4 IU/d) of natural-source vitamin E. To obtain reliable estimates of the kinetic parameters of vitamin E metabolism, the study duration should be long enough for the tracer to fully mix with the tracee in the slowest turning-over α-tocopherol kinetic pools in the study participants. More importantly, the tracer dose should be small enough to not disturb the in vivo steady-state metabolism of the tracee. The present study reports the fate of a minute oral dose (1.81 nmol) of [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol in 12 healthy adults with blood sampled over 460 d and complete collections of urine and feces made over 21 d.

Materials and Methods

Synthesis of [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol. The [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol was synthesized as previously described (32) with 3 modifications. First, aqueous [^{14}C]-formaldehyde containing 1.85 MBq (Sigma-Aldrich) was used instead of paraformaldehyde to form the methyl morpholino derivative of (2R, 4′R, 8′R)-γ-tocopherol. Second, a 2-step, 1-pot reaction sequence was used instead of the 3-step reaction sequence. Third, reduction was conducted using sodium cyanoborohydride in isobutanol-benzene (5:2, v:v).

The final product was purified using a C-30 column (Develosil RP Aqueous, Phenomenex) with a mobile phase consisting of 90% ethanol and 10% water at 1 mL/min flow rate. Retention time for the final product was identical to α-tocopherol standard. The purity of [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol was 96.25%. The cold material was validated by ^{1}H NMR, ^{13}C NMR, and MS to be α-tocopherol. Characteristic signal peaks of the C5-CH_3 group were identified at 2.11 ppm in ^{1}H NMR and at 11.28 ppm in ^{13}C NMR. For the ^{14}C-labeled material, MS analysis was performed using a Sciex API 2000 triple-quadrupole mass spectrometer with positive ion atmospheric pressure chemical ionization and multiple reaction monitoring. The [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol at 0.05 μg/L in 0.1% formic acid in water:methanol (1:1, v:v) was infused into the MS at 5 μL/min. The transitions from *m/z* 433 ([M+H]$^+$) to *m/z* 167 (formed via removal of phytyl tail) and *m/z* 431 ([M+H]$^+$) to *m/z* 165 (nonlabeled α-tocopherol ions) were monitored and used for purity calculation. The isotopic purity of [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol was 81.87%. The ^{14}C-radioactivity was measured with a Wallac 1410 Liquid Scintillation Counter using [^{14}C] standards. The specific activity was 1.73 TBq/mol and each dose contained an aliquot of 3.70 kBq of [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol in ethanol.

Participants. The study was approved by Institutional Review Boards and was conducted at the UC Davis Clinical and Translational Science Center's Clinical Research Center (CCRC) and Ragle Human Nutrition Center (Ragle HNC). Study participants were recruited from UC Davis and from Yolo, Solano, and Sacramento counties. Informed consent was obtained from each of the 6 male and 6 female participants that were admitted into the study. Inclusion criteria were healthy, nonsmoking, not taking nutritional supplements for at least 3 mo prior to the dosing date, normolipemic, and BMI between19 and 29 kg/m^2. Exclusion criteria included a history of serious medical conditions, use of medications that may interfere with lipid metabolism, anemia, history of alcohol or drug abuse, and pregnancy or plans to become pregnant during the study. The volunteer's health status was verified by laboratory tests at the UC Davis Medical Center Pathology Department. The tests included complete blood count, plasma lipid panels, and plasma α-tocopherol levels. Participants completed the 2005 Block Dietary Questionnaire self-administered FFQ (33) 6 mo after dosing to assess consistency of dietary habits.

TABLE 1 Summary of participant characteristics, nutrient intakes, and baseline blood chemistries[1]

Variables	
Participant characteristics	
Age, *y*	27 ± 7
Weight, *kg*	67 ± 11
BMI, *kg/m^2*	22 ± 2
Packed RBC volume, *%*	41 ± 3
Baseline blood chemistries	
Plasma HDL-cholesterol, *mmol/L*	1.5 ± 0.4
Plasma LDL-cholesterol, *mmol/L*	2.9 ± 0.5
Plasma TG, *mmol/L*	0.9 ± 0.4
Plasma α-tocopherol	
−14 d prior to dosing, *μmol/L*	24 ± 3
−7 d prior to dosing, *μmol/L*	24 ± 3
−0 d prior to dosing, *μmol/L*	22 ± 3
RBC α-tocopherol	
−14 d prior to dosing, *μmol/L*	3.2 ± 0.6
−7 d prior to dosing, *μmol/L*	2.6 ± 0.6
−0 d prior to dosing, *μmol/L*	2.6 ± 0.4
Nutrient intake by FFQ	
Energy, *MJ/d*	8.8 ± 3.2
Total protein, *g/d*	81 ± 23
Fiber, *g/d*	18 ± 5
Saturated fat, *g/d*	27 ± 14
Monounsaturated fat, *g/d*	31 ± 13
Polyunsaturated fat, *g/d*	16 ± 7
(n-3) Fatty acids, *g/d*	2 ± 1
Cholesterol, *mg/d*	233 ± 90
α-Tocopherol, *mg/d*	7.6 ± 2.8
Vitamin C, *mg/d*	84 ± 21
Selenium, *mg/d*	121 ± 54

[1] Values are mean ± SD, *n* = 12.

Participants provided one 24-h urine sample and one fecal sample that served as baseline before admission into the CCRC. All urine was collected in Urisafe containers (Simport). Starting from the time of dosing, urine was collected at 6-h intervals until h 36 postdosing. From 36 to 48 h, urine was collected as one 12-h collection. From h 48 onward, all subsequent urine samples were collected in 24-h intervals until d 21 after dose. All fecal samples were collected in sterile, 4-m-thick stomacher bags (Thermo Fisher Scientific). Fecal samples were collected per bowel movement starting at d −1 until d 21. The month, date, and time of each fecal collection were recorded. All blood samples were drawn into tubes containing K_2EDTA (BD Vacutainer) and immediately placed on ice. All participants had ad libitum access to water throughout the study.

Participants had fasted for 12 h when they were admitted into the CCRC at 0700 h. The participants received a medical exam and then an i.v. catheter was installed in the forearm. A predose blood sample was drawn and the [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol–spiked milk was ingested at 0800 h (t = 0 h after dosing). The spiked milk was prepared by mixing [5-$^{14}CH_3$]-(2R, 4′R, 8′R)-α-tocopherol (0.78 μg, 3.7 kBq) with 60 g of milk (2% fat, lactose free, Lactaid, HP Hood). After consumption, the cup was rinsed with an additional 60 g of milk that was also ingested, and then breakfast was consumed promptly. The breakfast consisted of one-half (48 g) of a plain bagel (Sara Lee Foods and Beverages, Sara Lee) with 31 g [2 tablespoons (Tbsp)] of light cream cheese (Philadelphia, Kraft Foods Global). The breakfast provided 252 kcal (1056 kJ) and 8 g fat.

Lunch was served 5 h after dosing. Lunch consisted of grilled turkey breast in cranberry sauce (Healthy Choice, ConAgra Foods), 64 g of lettuce (Fresh Express Lettuce Trio, Fresh Express), 28 g (2 Tbsp) of Italian dressing (Wishbone, Unilever US), 230 g of cranberry juice (Ocean Spray, Ocean Spray Cranberries), and 1 banana. Lunch contained 623 kcal (2608 kJ) and 11 g of fat.

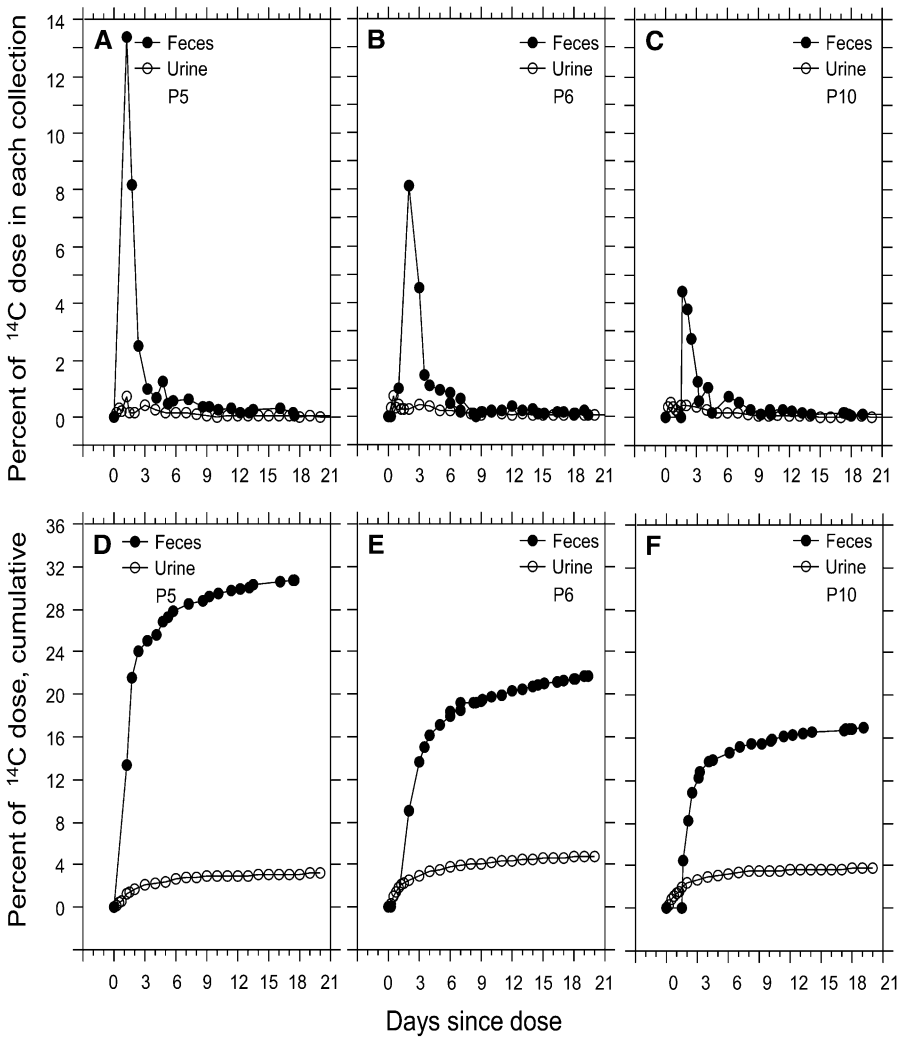

FIGURE 1 Elimination of ^{14}C in urine and feces of participants 5, 6, and 10.

A snack was served 7 h after dosing. The snack consisted of 1 chewy chocolate chip granola bar (Quaker, PepsiCo) and 1 snack pack of fat-free chocolate pudding (Hunts, ConAgra Foods). The snack contained 190 kcal (795 kJ) and 3 g of fat.

Dinner was served 11 h after dosing. Dinner consisted of 1 Glazed Chicken (Stouffers Lean Cuisine Café Classics, Nestle USA), 85 g of baby arugula (Earthbound Farm Organic, Earthbound Farm), 28 g (2 Tbsp) of Italian dressing (Wishbone, Unilever US), and 230 g of pineapple-orange juice (Dole, Duo Juice). Dinner contained 450 kcal (1884 kJ) and 11 g of fat.

An evening snack was served at 12 h after dosing. The snack consisted of 1 pack of candy (Skittles, Mars Snackfood US) and 1 orange. The snack contained 300 kcal (1256 kJ) and 3 g of fat.

The following morning, breakfast was served 24 h after dosing. Breakfast consisted of 1 bagel (95g) (Sara Lee Foods and Beverages, Sara Lee) served with 31 g (2 Tbsp) of light cream cheese (Philadelphia, Kraft Foods Global) and 120 g of milk (2% fat, lactose free, Lactaid, HP Hood). The subsequent lunch, dinner, and snack menus and serving times in the day were identical to those served on the preceding day.

After being admitted for 36 h, the participants were discharged and became outpatients at the Ragle HNC. Subsequent blood samples were drawn in a fasting state on d 3, 4, 5, 6, 8, 10, 20, 30, 40, 50, 60, 70, and 460 after dose. Final blood samples were checked for hemoglobin and packed cell volumes to confirm that participants were healthy at the end of the study. All subsequent collections of urine and feces were also received at the Ragle HNC.

Specimen analysis. Complete blood counts and plasma lipid panels were analyzed in the Clinical Pathology Laboratory at the UC Davis Medical Center, Sacramento, CA. Total cholesterol and total TG were analyzed on the Beckman Access autoanalyzer (Beckman Instruments). LDL-cholesterol concentrations were calculated by using the Friedewald equation. HDL-cholesterol levels were analyzed using the direct HDL-cholesterol assay. The inter- and intra-assay CV for cholesterol and TG assays were <4%. Homocysteine levels were measured by using the chemiluminescent immunoassay kit on a Siemens Immulite 2000 instrument (Siemens Immulite).

Aliquots of RBC, urine, and feces specimens were dried under vacuum and analyzed for total carbon in smooth wall tin capsules (Elemental Microanalysis) and analyzed for total carbon at the UC Davis Analytical Laboratory (34). Every 10th sample was measured in duplicate. The CV (%) of total carbon was 3.5% for RBC, 1.1% for feces, and 1.6% for urine. Finally, aliquots of plasma, RBC, urine, and feces specimens were measured for their levels of ^{14}C using accelerator MS (AMS) (35). Finally, blood α-tocopherol levels were analyzed (36).

Data analysis. Plasma and RBC profiles were plotted by time since dose on a natural log (ln) scale using Microsoft Excel. The decrease in plasma and RBC concentration over time followed apparent first-order kinetics. The AUC was calculated by trapezoidal rule and the area under the moment curve was calculated by PKSolver (37). The mean residence time was calculated as area under the moment curve/AUC and half-life was calculated as ln2 · mean residence time. Half-life designated as $t_{1/2}$ 0-t was calculated using measured data points from 0-t d only (not extrapolated to infinity). Half-life designated as $t_{1/2}$ 0-t, ∞ was calculated from 0-t d of data that is extrapolated to infinity. The apparent absorption was calculated as (^{14}C dose – fecal ^{14}C from 0 to 3 d)/^{14}C dose. The true absorption was calculated as [^{14}C dose – (fecal ^{14}C from 0

TABLE 2 Summary of ^{14}C mass balance, concentration maxima (C_{max}, % dose/L), time to maxima (T_{max}, d) in plasma (% dose/L), RBC (% dose/L), urine (% dose/collection), and feces (% dose/collection)[1]

Variables	Collection duration	Plasma	RBC	Total eliminated		Total retained
				Urine	Feces	Body (mass balance)[2]
				% of dose		
Present study: RRR-α-tocopherol	21 d urine, 21 d feces			4.26 ± 1.38	23.2 ± 5.8	72.5 ± 5.5
Prior study: RRR-α-tocopherol (26)	8 d urine, 7 d feces	–	–	6.32	27.9	65.8
Prior study: all-rac-α-tocopherol (26)	8 d urine, 7 d feces	–	–	16.5	31.5	52.0
Prior study: all-rac-α-tocopherol (28)	12 d urine, 12 d feces	–	–	<6.00	27.6	66.4
Prior study: all-rac-α-tocopherol (29)	3 d urine, 6 d feces	–	–	8.21	31.4	60.4
C_{max},		*% dose/L*		*% dose/collection*		
Present study: RRR-α-tocopherol		5.39 ± 1.58[a]	1.59 ± 0.31[b]	0.72 ± 0.41[c]	10.2 ± 3.64[d]	–
Prior study: RRR-α-tocopherol (21)		5.92 ± 1.90	1.09 ± 0.32	–	–	–
T_{max}		*d*				
Present study: RRR-α-tocopherol		0.58 ± 0.17[e]	0.94 ± 0.13[f]	1.42 ± 1.12[g]	2.12 ± 0.77[h]	–
Prior study: RRR-α-tocopherol (21)		0.53 ± 0.24	1.06 ± 0.41	–	–	–

[1] Values are mean ± SD. Letters indicate that the labeled means differ: [a-b, c-d] $P < 0.0001$; [e-f, g-h] $P < 0.0002$.
[2] Mass balance (%) = 100 – 4.26 (urine) – 23.2 (feces).

to 3 d – metabolic fecal ^{14}C)]/^{14}C dose. Metabolic fecal ^{14}C represented the amount of ^{14}C that was absorbed and subsequently eliminated in feces (after the first pass). The figures were drawn with StatView (SAS Institute, version 5.0.1) and transferred to Microsoft Office PowerPoint 2003. Simple regression analyses were used to assess the potential contribution of measured subject traits (BMI, HDL-cholesterol, LDL-cholesterol, etc.) to the observed inter-subject variation in outcome parameters [half-life, maximum concentration (C_{max}), percent retained, etc.] by using StatView (SAS Institute, version 5.0.1).

Results

The study included 12 participants whose characteristics, baseline blood chemistries, and nutrient intakes are summarized in **Table 1**. These data indicated a healthy group of participants. The characteristics, baseline blood chemistries, and nutrient intakes for each participant are included in **Supplemental Tables 1** and **2**.

The portions of ^{14}C that were eliminated via urine and feces from 3 representative participants (5, 6,and 10) are plotted in **Figure 1***A–C* and the cumulative collections are plotted in Figure 1*D–F*, respectively. Participant 5 (Fig. 1*D*) eliminated the most ^{14}C (30.8%) and participant 10 (Fig. 1*F*) eliminated the least ^{14}C (17%). **Table 2** includes a summary of the total amount of ^{14}C that was eliminated and its mass balance. Only 4.26 ± 1.38% was eliminated via urine, 23.2 ± 5.8% was eliminated via feces, and the mass balance was 72.5 ± 5.5% (100–4.26–23.2).

Table 2 also includes a summary of the C_{max} and the time to maximum (T_{max}) for plasma, RBC, urine, and feces. The plasma C_{max} was higher ($P < 0.0001$) than the RBC C_{max}. The plasma C_{max} was lower in males than in females ($P < 0.0111$). The urine C_{max} was lower than that of feces ($P < 0.0001$). Plasma T_{max} occurred sooner than did the RBC T_{max} ($P < 0.0002$). Urine T_{max} also occurred sooner than did the fecal T_{max} ($P < 0.0002$). Finally, the total amount of ^{14}C that was eliminated, its mass balance, the C_{max} and T_{max} for plasma, RBC, urine, and feces for each participant is included in **Supplemental Tables 3** and **4**.

The profiles of the ^{14}C tracer in plasma and RBC by time since dosing the 3 representative participants (5, 6, and 10) are plotted in **Figure 2***A–C*. The ^{14}C first appeared in plasma and RBC at ~0.05 and ~0.1 d, respectively. The ^{14}C peaked in plasma and RBC plasma at ~0.5 and ~1.0 d, respectively. The plasma AUC for participants 5 and 10 were similar to each other (~35% dose/L), whereas the AUC for participant 6 was twice as large (69% dose/L). RBC AUC for participants 5 and 10 were similar to each other (57% dose/L), whereas that for participant 6 was ~2.5 times larger (154% dose/L). Figure 2*D–F* showed that plasma $t_{1/2}$ 0–460,∞ for participants 5 and 10 were similar to each other (~104 d), whereas that for participant 6 was 24% longer (129 d). Figure 2*G–I* showed that RBC $t_{1/2}$ 0–460,∞ for participants 5 and 10 were similar to each other (~215 d), whereas that for participant 6 was twice longer (455 d). The AUC and $t_{1/2}$ for the ^{14}C in plasma and RBC from each participant over the following durations of time (0–2, 0–4, 0–5, 0–70, and 0–460 d) are included in **Table 3**. The detailed data on each participant are included in **Supplemental Tables 5** and **6**.

The ^{14}C tracer in participants 5, 6, and 10 first appeared in plasma between d 0.03 (min 40) and d 0.07 (min 100) and peaked there between d 0.42 (h 10) and d 0.83 (h 20), whereas the ^{14}C tracer first appeared in RBC at ~d 0.05 and peaked there between d 0.83 (h 20) and d 0.92 (h 22) (Fig. 2*A–C*). The ^{14}C levels in neither plasma (Fig. 2*D–F*) nor RBC (Fig. 2*G–I*) returned to baseline by d 460, indicating the $t_{1/2}$ of [5-CH_3]-(2R, 4′R, 8′R)-α-tocopherol in human blood was longer than prior estimates. The ^{14}C profiles in plasma until ~d 460 are illustrated in **Figure 3** and the AUC of each participant is calculated from Figure 3.

Discussion

The fate of the ^{14}C (the tracer) was quantified in urine and feces until d 21 and in blood (plasma and RBC) until d 460. The complete collections of urine and feces until d 21 and blood draws until d 460 were needed for accurate mass balance data, because the longer duration of the study, the greater the chance for the tracer to fully mix with the tracee in the slowest turning-over pools of α-tocopherol. Full mixing of tracer with tracee can become the key determinant of α-tocopherol metabolism late in studies. Short duration was a limitation of prior single-dose in studies (20–23,28,29).

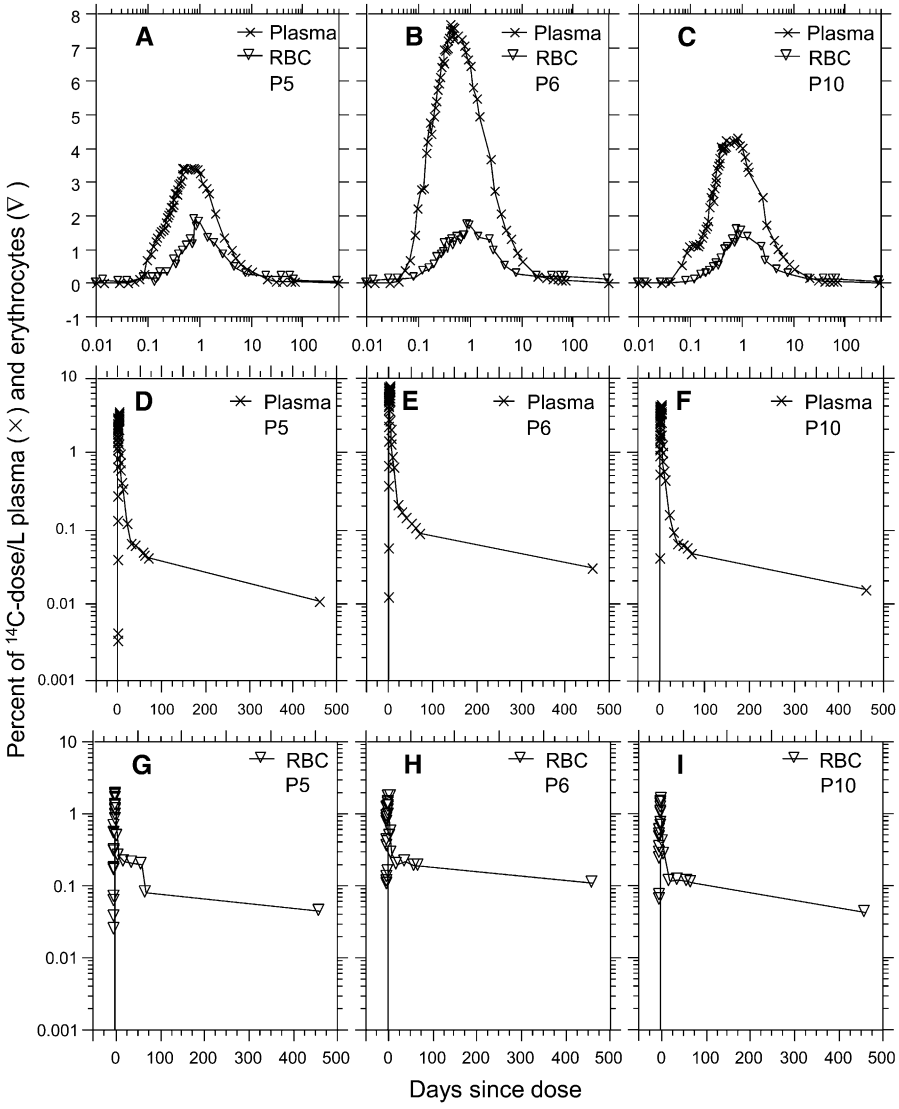

FIGURE 2 Profiles of ^{14}C in plasma (X) and RBC (▽) of participants 5, 6, and 10.

Baseline levels of α-tocopherol in plasma and RBC were reached at d 0 and −7, respectively (Table 1). The baseline blood levels of α-tocopherol were comparable to those of Americans ≥ 20 y old who did not use supplements or supplements containing vitamin E (38). In addition, the dietary intake of α-tocopherol was 7.6 ± 2.8 mg/d (Table 1), an intake close to the RDA (7.3 mg/d for men, 5.4 mg/d for women) and vitamin E intakes in the US (39). Finally, the ratio of vitamin E:PUFA in the diet was 0.48 ± 0.06, which was also close to the desirable ratio of ≥0.4 (40). So the participants were considered to be stable with respect to their α-tocopherol status and to be characterized as healthy and well nourished.

The amount of ^{14}C eliminated over a 21-d period via urine and feces were 4.26 ± 1.38% and 23.2 ± 5.8%, respectively. The amount retained as body mass balance [72.5 ± 5.5% (100–4.26–23.2%)] was close to intestinal absorption of α-tocopherol as previously reported (28,29,26) (Table 2). The major route of elimination for α-tocopherol is in feces, which is consistent with the current literature (41).

Prior tracer studies referenced in Table 2 administered ^{3}H- or ^{14}C-labeled α-tocopherol; 555–740 kBq ^{3}H in 0.46 μmol all-*rac*-α-tocopherol (28), 444–925 kBq ^{3}H in 2.32 mmol *all-rac*-α-tocopherol (29), and (in a test and retest design) 3.76 kBq ^{14}C in 1.82 nmol (2R, 4′R, 8′R)-α-tocopherol acetate and 3.70 kBq ^{14}C in 1.67 nmol all-rac-α-tocopheryl acetate (26). These 3 prior studies, whose duration was ≤12 d, reported that the apparent absorption of α-tocopherol was 72.4% with a range of 51–86% (28), 68.6% with a range of 55.0–78.6% (29), 77.5% for (2R, 4′R, 8′R)-α-tocopherol (26), and 77.5% for *all-rac*-α-tocopherol (26). Our study finding was consistent with these referenced studies of 79.2%. More details on the absorption are in Supplemental Table 3.

The C_{max} and T_{max} for plasma (5.39% of dose/L plasma and 0.58 d) and RBC (1.59% of dose/L RBC and 0.94 d) were consistent with prior values (27) referenced in Table 2. The C_{max} for plasma was 3-fold higher than for RBC. T_{max} for RBC was delayed for 12 h compared with that for plasma. The delay may reflect a transfer from plasma to RBC that may have involved more than simple diffusion (42,43). More details on the C_{max} and T_{max} are in Supplemental Table 4.

A total of 23.24% of the dose was eliminated in feces over 21 d, whereas only 4.26% was eliminated via urine. Only 0.03% (0.51 ± 0.25%/17 d) of the dose was eliminated per day via urine and only 0.14% (2.43 ± 0.87%/17 d) of the dose was eliminated per day via feces. The data agreed with prior studies that reported the body mass balance of α-tocopherol was not readily accessible for metabolism or elimination under normal conditions, because only a total of 0.17% (0.03% from urine + 0.14% from feces) of the dose was eliminated per day (44–46).

TABLE 3 Summary half-life and AUC of dose in plasma and RBC of present and prior study[1]

	AUC		$t_{1/2}$	
	Plasma	RBC	Plasma	RBC
	% dose/L·d		*d*	
Nonextrapolated				
0–2 d, 0-t	8.78 ± 2.68	2.49 ± 0.58	0.78 ± 0.07	0.87 ± 0.07
0–4 d, 0-t	11.9 ± 3.3	3.62 ± 0.85	1.12 ± 0.09	1.26 ± 0.10
0–5 d, 0-t	13.3 ± 3.7	4.32 ± 1.01	1.32 ± 0.07	1.50 ± 0.17
0–70 d, 0-t	24.2 ± 7.5	14.6 ± 3.6	7.46 ± 0.50	15.2 ± 3.0
0–460 d, 0-t	36.7 ± 9.5	42.4 ± 21.5	44.0 ± 12.0	95.7 ± 41.9
Extrapolated				
0–2 d, 0-∞	17.1 ± 6.0	5.52 ± 2.04	*2.18 ± 0.66*	2.64 ± 0.78
Prior study (21)			1.79 ± 0.88	2.13 ± 0.58
Prior study (20)			2.15 ± 0.73	
0–4 d, 0-∞	15.8 ± 4.7	5.44 ± 1.03	1.95 ± 0.21	2.47 ± 0.46
Prior study (23)			3.36 ± 0.80	9.3 ± 2.37
Prior study (22)			1.83 ± 0.78	
0–5 d, 0-∞	16.9 ± 5.3	5.56 ± 1.30	2.24 ± 0.26	2.48 ± 0.49
0–70 d, 0-∞	27.6 ± 8.4	32.9 ± 18.0	18.3 ± 5.2	96.2 ± 80.6
0–460 d, 0-∞	41.6 ± 10.7	63.8 ± 47.0	105 ± 67	217 ± 123

[1] Values are mean ± SD, n = 12.

The half-life based on measured data was always smaller than the half-life extrapolated to infinity over the same duration. This was especially obvious in the 0–70 and 0–460 d data. The differences between nonextrapolated and extrapolated half-lives demonstrated the importance of conducting studies long enough to capture the α-tocopherol metabolism in the slow turning-over pools. Thus, the longer the study duration, the better the estimate of the true half-life. The half-lives over 0–2, 0–4, 0–5, 0–70, and 0–460 d (Table 3) demonstrated the effect of duration on the calculation of α-tocopherol half-life.

The rate of disappearance of ^{14}C from plasma in Figure 2*D–F* and Table 3 showed that the nonextrapolated half-life from 0 to 70 d ($t_{1/2}$ 0–70) was 7.46 ± 0.50 d and the extrapolated half-life ($t_{1/2}$ 0–70, ∞) was 18.34 ± 5.24 d. These $t_{1/2}$ values were ~8 times greater than the prior values referenced in Table 3 (20–23). The $t_{1/2}$ 0–460 d and its extrapolated half-life ($t_{1/2}$ 0–460, ∞) were 44.03 ± 12.03 and 104.80 ± 67.63 d, respectively (Table 3). Based on the 0–70 d data, participant 7 had the shortest $t_{1/2}$ 0–70,∞ of 9.75 d and participant 9 had the longest at 26.81 d, a 2.7-fold difference between the 2 participants. The same study with a shorter analysis time had a 1.5-fold difference between these 2 participants.

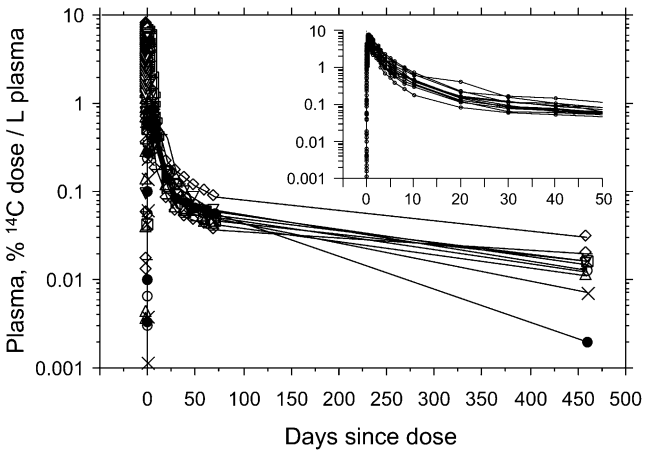

FIGURE 3 Plasma ^{14}C profile for all participants from 0 to 460 d and 0 to 50 d (insert).

In general, the half-life of ^{14}C-tracer for RBC and plasma shared similar trends, but the half-life was longer for RBC, especially over long periods of time. Based on 0–70 d of data, participants 9 and 10 had the shortest and longest RBC $t_{1/2}$ 0–70, ∞ of 10.19 and 219.78 d, respectively, which corresponded to a 22-fold difference.

The short half-lives based on ≤5 d may reflect the turnover of lipoprotein particles that (2R, 4′R, 8′R)-α-tocopherol relied on for transport. The α-tocopherol was packaged in chylomicra, endocytosed, and delivered to α-TTP to associate with lipoproteins for delivery to peripheral tissues (16–18). The lipoprotein half-lives of LDL apo-B particles, HDL apoA-I, and HDL apoA-II half-lives were 1.7, 2.7, and 3 d, respectively (47,48).

The long half-life values based on ≥20 d of data may reflect recycling of RRR-α-tocopherol to plasma from peripheral tissues such as adipose tissue, where ≤10% of adipocytes were removed annually (44). Very little α-tocopherol was mobilized from adipose tissue (45). Based on the present data, the half-life of plasma α-tocopherol seemed to be longer than prior estimates, primarily due to the long duration of the present study. Quantifying the fraction of the ^{14}C-dose in the plasma lipoprotein fractions over time since dosing and constructing a physiologically based pharmacokinetic model will provide new insights into RRR-α-tocopherol metabolism as it occurred in vivo in healthy humans.

Contributions of participant traits (LDL-cholesterol) to the ^{14}C outcome parameters were detailed as follows. High levels of LDL-cholesterol were associated with increased elimination of ^{14}C dose from 0 to 21 d in urine (r = 0.7897; P = 0.0022). High levels of HDL-cholesterol were associated with reduced elimination of the ^{14}C-dose over a 4- to 21-d period in feces (r = −0.7224; P = 0.0080) and a longer half-life of the ^{14}C-dose ($t_{1/2}$ 0–460, ∞) in plasma (r = 0.6386; P = 0.0469). Finally, the increased intakes of vitamin D were associated with a shorter half-life of the ^{14}C-dose ($t_{1/2}$ 0–70, ∞) in RBC (r = −0.7252; P = 0.0076). BMI and vitamin C and E intakes were not associated with the outcome parameters of ^{14}C in plasma, RBC, urine, or feces.

Acknowledgments

J.C.C. and A.J.C. designed research; J.C.C. conducted research; J.C.C., J.G.F., and A.J.C. analyzed and interpreted the data; A.J.C. and J.C.C. wrote the paper; A.J.C. had primary responsibility for the final content; S.K. advised on AMS target sample preparation for ^{14}C-AMS; and H.D.M., K.P.N., J.C.C., and D.M.H. synthesized and characterized the [5-$^{14}CH_3$]-RRR-α-tocopherol. All authors read and approved the final manuscript.

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