A feasibility study quantifying in vivo human α-tocopherol metabolism1–3

Andrew J Clifford, Fabiana F de Moura, Charlene C Ho, Jennifer C Chuang, Jennifer Follett, James G Fadel, and Janet A Novotny

ABSTRACT

Background: Quantitation of human vitamin E metabolism is incomplete, so we quantified RRR- and all-rac-α-tocopherol metabolism in an adult.

Objective: The objective of the study was to quantify and interpret in vivo human vitamin E metabolism.

Design: A man was given an oral dose of 0.001821 μmol [5-14CH3]RRR-α-tocopheryl acetate (with 101.5 nCi 14C), and its fate in plasma, plasma lipoproteins, urine, and feces was measured over time. Data were analyzed and interpreted by using kinetic modeling. The protocol was repeated later with 0.001667 μmol [5-14CH3]all-rac-α-tocopheryl acetate (with 99.98 nCi 14C).

Results: RRR-α-tocopherol acetate and all-rac-α-tocopheryl acetate were absorbed equally well (fractional absorption: ≈ 0.775). The main route of elimination was urine, and ≈90% of the absorbed dose was α-2(2’-carboxyethyl)-6-hydroxychroman. Whereas 93.8% of RRR-α-tocopherol flow to liver kinetic pool B from plasma was returned to plasma, only 80% of the flow of all-rac-α-tocopherol returned to plasma; the difference (14%) was degraded and eliminated. Thus, for newly digested α-tocopherol, the all-rac form is preferentially degraded and eliminated over the RRR form. Respective residence times in liver kinetic pool A and plasma for RRR-α-tocopherol were 1.16 and 2.19 times as long as those for all-rac-α-tocopherol. Model-estimated distributions of plasma α-tocopherol, extrahepatic tissue α-tocopherol, and liver kinetic pool B for RRR-α-tocopherol were, respectively, 6.77, 2.71, and 3.91 times as great as those for all-rac-α-tocopherol. Of the lipoproteins, HDL had the lowest 14C enrichment. Liver had 2 kinetically distinct α-tocopherol pools.

Conclusions: Both isomers were well absorbed; all-rac-α-tocopherol was preferentially degraded and eliminated in urine, the major route. RRR-α-tocopherol had a longer residence time and larger distribution than did all-rac-α-tocopherol. Liver had 2 distinct α-tocopherol pools. The model is a hypothesis, its estimates are model-dependent, and it encourages further testing. Am J Clin Nutr 2006;84:1430–41.

KEY WORDS α-Tocopherol, isomer, human, metabolism, radiocarbon, accelerator mass spectrometry

INTRODUCTION

Vitamin E includes 4 tocopherols and 4 tocotrienols. Of the 4 tocopherols, α-tocopherol has the highest vitamin E activity. Natural α-tocopherol is a single stereoisomer called RRR-α-tocopherol. Chemically synthesized α-tocopherol is an equal mix of 8 stereoisomers and is called all-rac-α-tocopherol. Biologic tissues distinguish among α-tocopherol stereoisomers, and thus isomer-specific metabolism is expected (1, 2). Qualitative effects of different α-tocopherol stereoisomers are well known, but gaps exist in the understanding of the quantitative aspects of their metabolism (3–5). We aimed to quantify and interpret the metabolism of RRR-α-tocopherol as to compare it with that of all-rac-α-tocopherol in an adult by using kinetic modeling. Kinetic models are built and used to obtain the most complete description possible of a metabolic system under study, to obtain values for critical parameters of the model, and to identify unique and testable hypotheses about the system.

The use of radiolabeled α-tocopherol enabled the quantification of key features of the metabolism of this important vitamin (6, 7). The investigators in those studies determined that α-tocopherol was absorbed via lymph, where it peaked 2–8 h after administration. They found that it first appeared in plasma in 2–4 h, peaked there in 5–14 h, and disappeared from plasma with a t1/2 of 53 h after administration. They found that net absorption was ≈70% and that ≈8% of α-tocopherol was eliminated via urine in 3 d. They found that almost all plasma 14C radioactivity was associated with intact α-tocopherol, but that the proportion in feces was uncertain. Finally, a new derivative of administered α-tocopherol, α-tocopheryl phosphate (α-TP), was discovered in rat liver (8).

The availability of α-tocopherols selectively labeled with deuterium (d3-, d6-, and d9-α-tocopherols) led to the following key discoveries. Acetate ester (ie, α-TAc), succinate ester, and free phenol forms of α-tocopherols were metabolized at the same rate (9, 10). Body α-tocopherol stores (d9-RRR-α-TAc) were preferentially replaced and augmented with d3-RRR-α-TAc alone.

1 From the Departments of Nutrition (AJC, FFM, CCH, JCC, and JF) and Animal Science (JGF), University of California, Davis, Davis, CA, and the Diet and Human Performance Laboratory, US Department of Agriculture, ARS, BHINRC, Beltsville, MD (JAN).
2 Supported by grant no. DK RO1 48307, National Center for Research Resources grant no. RR13461 from the National Institutes of Health, Contract no. W-7405-Eng-48 from the US Department of Energy (to Lawrence Livermore National Laboratory), and Hoffmann-LaRoche. Hoffmann-LaRoche donated the [C]α-tocopherols, and Robert Parker (Cornell University, Ithaca, NY) donated the α-CHEC reference standard.
3 Reprints not available. Address correspondence to AJ Clifford, 3147 Meyer Hall, Department of Nutrition, University of California, Davis, One Shields Avenue, Davis, CA 95616-8669. E-mail: ajc@ucdavis.edu. Received April 17, 2006. Accepted for publication August 1, 2006.
(noncompetitive uptake protocol) over a 1:1 mix of \( d_7\)-\(\text{RRR-}\alpha\)-Toc \( + d_7\)-\(\text{RR-}\alpha\)-Toc (competitive uptake protocol) in replacement diets (11). It was also discovered that liver played an important role (12), that \( \text{RRR-}\alpha\)-tocopherol disappeared from plasma much faster than did \( \text{RRR-}\alpha\)-tocopherol, and that this vitamin was in a state of rapid flow in and out of plasma (13). Because the absorption of \( \alpha\)-tocopherol (as measured from plasma concentrations of deuterated and protonated \( \alpha\)-tocopherol) was variable and low (14, 15), reassessment of that absorption for human nutritional purposes is appropriate (16).

All prior isotope studies except that of Acuff et al (17) were of relatively short duration, and thus a labeled tracer dose may not have equilibrated fully in slow-turnover \( \alpha\)-tocopherol pools. Large doses (ie, 1700 \( \mu\)mol/L) relative to the Recommended Dietary Allowance (RDA) of 23 \( \mu\)mol/L have been used (1), and they may have influenced \( \alpha\)-tocopherol metabolism were used. Because of biologic stereo selectivity, a true tracer-tracee relationship may not have existed when an \( \text{RRR-}\alpha\)-tocopherol tracer was used for an \( \text{RRR-}\alpha\)-tocopherol tracer. Therefore, we conducted a feasibility study in which we determined the fate of a true tracer dose of \([^{14}C]\text{RRR-}\alpha\)-Toc over a 63-d period; after a 3-mo washout period, we did the same for \([^{14}C]\text{all-rac-}\alpha\)-Toc in a crossover design.

SUBJECTS AND METHODS

Subject selection

The inclusion criteria stated that the subject or subjects must be 18–65 y old and healthy as determined by medical history and complete blood count and must have a body mass index (BMI; in kg/m\(^2\)) of 19–27, normal blood pressure, and a daily vitamin E intake of 7.5–12 IU/d (determined with a dietary questionnaire). Exclusion criteria were anemia; history of alcohol, drug abuse, or smoking; a serum cholesterol concentration \( \geq 5.44\text{ mmol/L} \) or an LDL-cholesterol concentration \( \geq 4.14\text{ mmol/L} \); use of any vitamin supplement during the 3 mo before the current study; and a serum \( \alpha\)-tocopherol concentration \( > 24\text{ \( \mu\)mol/L} \). Having diet supply all his \( \alpha\)-tocopherol (\( \text{RRR-}\alpha\)-isomer only) assured us that the \([^{14}\text{C}]\text{all-rac-}\alpha\)-Toc served as a true tracer, and his unlabeled \( \text{RRR-}\alpha\)-tocopherol served as a true tracee, even though this would not be the case with a very small dose of \( \text{all-rac-}\alpha[^{14}\text{C}]\text{CH}_3\)Toc.

Written informed consent was obtained from the volunteer under the guidelines of the Human Subjects Committee at the University of California, Davis. The study protocol was approved by that committee and by the Radiation Use Committee at the University of California, Davis. After those approvals were given, the protocol was reviewed and approved by the Lawrence Livermore National Laboratory Human Subjects Committee. The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Study design

The study had a crossover design. The subject was first given an oral dose of 0.001821 \( \mu\)mol \([^{14}\text{C}]\text{all-rac-}\alpha\)-Toc (with 99.98 nCi \( ^{14}\text{C} \)), and serial samples of blood were again collected for 63 d, of feces for 6 d, and of urine for 8 d after dosing. Three months from the date of \([^{14}\text{C}]\text{all-rac-}\alpha\)-Toc administration, the same subject was given an oral dose of 0.001667 \( \mu\)mol \([^{14}\text{C}]\text{all-rac-}\alpha\)-Toc (with 99.98 nCi \( ^{14}\text{C} \)), and serial samples of blood were again collected for 63 d, of feces for 7 d, and of urine for 8 d after dosing.

Meals were controlled for time and content on the days that each radiolabeled tocopherol was administered. Lunch was served 5 h after dosing and consisted of a frozen chicken dinner (Lean Cuisine; Nestle USA, Wilkes-Barre, PA), salad mix, and a banana; the meal had a total fat content of \( \approx 10.5\text{ g} \). The following snacks were consumed between lunch and dinner: chocolate chip granola bar (Quaker; Pepsico Beverages & Foods, Chicago, IL), fat-free chocolate pudding (Jell-O; Kraft Foods Global Inc, Glenview, IL), and cranberry juice (Old Orchard Brands, LLC, Sparta, MI); the total fat content of the snacks was \( \approx 10.5\text{ g} \). Dinner was served 12.1 h after the dose and consisted of a frozen chicken enchilada dinner (Healthy Choice; ConAgra Foods Inc, Omaha, NE), baby arugula blend salad (Portofino salad; Ready Pac Produce Inc, Irwindale, CA), 5 mL (1 tsp) Italian dressing (Wish-Bone “just2good”; Unilever US Inc, Englewood Cliffs, NJ), and 226.8 g (8 oz) pine-orange juice (Dole Food Company Inc, Chicago, IL); the total fat content was \( \approx 12\text{ g} \).

Test materials

The chemical and isotopic purity of \( \text{RRR-}\alpha[^{5-}\text{CH}_3\]Toc (56 Ci/mol) and \( \text{all-rac-}\alpha[^{5-}\text{CH}_3\]Toc (60 Ci/mol) were confirmed by reisolation on HPLC and analysis on a liquid scintillation counter. Ice-cold argon-degassed ethanol was prepared by bubbling argon through 50 mL ethanol (100%) for 15 min at \( \approx 2\text{ °C} \). Each radiolabeled tocopherol was taken up (dissolved) in ice-cold argon-degassed ethanol, transferred to an amber screwcap vial, covered with argon, and re-isolated on an Agilent 1100 HPLC with an Agilent C18 XDB column, with a 3.0 \( \times \) 150-mm stationary phase (Agilent Technologies, Avondale, PA) and a 95% degassed ethanol and 5% water mobile phase pumped at 0.35 mL/min. The eluent corresponding to vitamin E was collected, fortified with sodium ascorbate (final concentration: 50 mmol/L), degassed with argon for 10 min (to minimize oxidation), and stored at \( -70\text{ °C} \). HPLC chromatograms of the materials just before dosing showed only a single peak of \( ^{14}\text{C} \) that coeluted with \( \alpha\)-tocopherol, which indicated that the materials did not deteriorate during storage at \( -70\text{ °C} \) in argon-degassed ethanol containing 50 mmol ascorbate/L. Finally, the 2,7,8-trimethyl-2-(2-carboxyethyl)-6-hydroxychroman (\( \alpha\)-CEHC) reference standard was used.

Dose administration

Aliquots of the HPLC eluent with \( \approx 100\text{ nCi of } ^{14}\text{C} \) were added to a cup containing a whipped mix of 10.5 g olive oil and 30 g skim milk that supplied 4.5 \( \mu\)mol \( \alpha\)-tocopherol. In the case of the \([^{14}\text{C}]\text{all-rac-}\alpha\)-Toc, 140 \( \mu\)L eluent delivered 0.001821 \( \mu\)mol with 101.5 nCi \( ^{14}\text{C} \). In the case of the \([^{14}\text{C}]\text{all-rac-}\alpha\)-Toc, 260 \( \mu\)L eluent delivered 0.001667 \( \mu\)mol with 99.98 nCi \( ^{14}\text{C} \). Direct addition of eluent to the whipped mixture was chosen to avoid the oxidation of the dose that might occur if it were dried and resolubilized. The subject ingested the mixture, and the cup was rinsed twice with 60 g skim milk, which the subject also ingested immediately. Dosing was conducted under observation to ensure that the entire dose was consumed. Total fat intake on the day of dosing was 32.5 g (10 + 12 + 10.5 g). Administration of an \( \approx 100\text{-nCi dose of radiolabeled } \alpha\)-tocopherol to a healthy person was feasible because accelerator mass spectrometry
(AMS) quantifies labeled biochemicals to attomolar (10^{-18}) levels in milligram-sized samples, and, at this sensitivity, the radiation exposure is similar to that from a 1-h flight in a jet, which is a level that enables in vivo testing in normal persons.

**Specimen collection**

Blood was drawn (glass tubes containing EDTA) on the 12th and 6th days before each dosing to ensure that the subject’s plasma vitamin E status was in a steady state. Fifteen minutes before each radiolabeled tocopherol acetate was administered, the subject was fitted with an intravenous catheter in a forearm vein, and a baseline blood sample was drawn into a 10-mL tube that contained potassium EDTA (final concentration: 1.5 mg/mL). The dose was administered and additional blood was drawn every 30 min from 0 to 12 h, every 60 min from 12 to 16 h, and then at 18, 20, 22, 24, 28, 32, 36, 40, 44, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 216, 288, 336, 504, 672, 850, and 1512 h after dosing. The catheter was removed after the 48-h blood draw. After the first and second days since dosing, blood draws were taken in the fasting state.

Urine was collected in 4-L PolyPac containers (Fisher Scientific, Fairlawn, NJ) and the weight was recorded. Urine (24-h) was collected just before the administration of each radiolabeled tocopherol to serve as the baseline value. Urine was also collected from 0 to 8 h and from 8 to 24 h after dosing. All remaining collections were 24-h collections. A 40-mL aliquot of each collection was stored at −80 °C until they were analyzed.

Feces were collected in 4-mm-thick Stomacher bags (Fisher Scientific, Fairlawn, NJ), and the weight was recorded. The subject provided a feces collection on the day before the administration of each radiolabeled tocopherol; these samples served as the baseline values. All feces for the week after dosing were collected and weighed individually.

**Specimen processing**

Plasma was separated from whole blood by centrifugation for 10 min at 3300 rpm and 23 °C (Fisher Scientific, Fairlawn, NJ) and stored at −80 °C until analysis. The plasma was later analyzed for total α-tocopherol (18) to model the trace.

**Apportioning plasma 14C to α-tocopherol and 2,7,8-trimethyl-2-(2-carboxyethyl)-6-hydroxycroman**

Plasma lipoproteins were isolated by using a model RC-120 GX centrifuge, an AT2 rotor, and 2-mL polyallomer reseal tubes (all: Sorvall, Newton, CT). Three solutions were prepared: solution 1 was 0.195 mol NaCl/L for density = 1.006, solution 2 was 0.195 mol NaCl/2.44 mol NaBr/L for density = 1.063, and solution 3 was 0.195 mol NaCl/6 mol NaBr/L for density = 1.26. Solution 1 (600 μL) was added to the reseal tube, underlaid with plasma (1.2 mL), and centrifuged for 23 min at 120 000 rpm and 8 °C (in the Sorvall centrifuge). Chylomicra (from the top down to 2 mm from bottom of the tube) were aspirated into a fine-tip pipette (SAMCO Scientific Corporation, San Fernando, CA) and stored in Eppendorf tubes at 4 °C. The reseal tube was sliced (2.5 mm from the bottom), and the top was discarded to avoid contamination of the denser lipoproteins with chylomicra. The bottom portion, containing the denser lipoproteins, was injected beneath a 550-μL volume of solution 1 in a new reseal tube. The new reseal tube was topped off to its original volume (as needed) with solution 1 and centrifuged for 83 min at 120 000 rpm and 8 °C to float VLDL, which was then removed as described above for chylomicra. The procedure was repeated with solution 2, which was centrifuged for 125 min at 120 000 rpm and 8 °C to float LDL that was then removed, and then with solution 3, which was centrifuged for 210 min at 120 000 rpm and 8 °C to float HDL, that was then removed. The content in the remainder of this sliced reseal tube was saved as infranatant solution.

Isolated lipoprotein fractions were extracted by adding ≈200 μL CH2Cl2 containing 0.01% BHT to each fraction stored in an Eppendorf tube. The sample was mixed by vortex for 1 min and centrifuged at 5000 × g for 5 min at 23 °C (Fisher Scientific AccuSpin Micro, NJ), and the organic layer was transferred to a new Eppendorf tube. The extraction procedure was repeated twice, by using ≈100 μL CH2Cl2 (containing BHT) each time, to ensure that all lipid-soluble compounds were recovered. The CH2Cl2 was then evaporated under argon. The residue was re-suspended in 100 μL CH2Cl2 (containing BHT), mixed by vortex for 1 min, and centrifuged at 5000 × g for 5 min at 23 °C, and a 20-μL aliquot was dried at 23 °C to be used in AMS.

Urine from the third collection (24- to 48-h period after dosing with each radiolabeled α-TAc) was treated with glucuronidase and subjected to HPLC analysis (22), in which the eluent was collected in 500-μL fractions and analyzed for 14C by using AMS; coelution of the 14C peak with authentic α-CEHC identified the radiolabeled material in urine as [14C]-α-CEHC. Aliquots of urine from the fourth collection (48- to 60-h period after dosing with all-rac-α-[5-(14CH3)TAc] before and after they were treated with glucuronidase were also subjected to HPLC (22), in which the eluent was collected in 500-μL fractions and analyzed for 14C by using AMS; the appearance of a 14C peak only in the glucuronidase-treated sample identified the chemical nature of the 14C-labeled material in urine as [14C]-α-CEHC-glucuronide.

Each feces collection was diluted with 5 volumes of a 50/50 solution of 1 mol potassium hydroxide/isopropyl alcohol (KOH/IPA)/L, and the mix was dispersed by using a Stomacher laboratory blender (model 3500; Fisher Scientific) for 2 min at the high setting. The sealed bag was then heated for 2 h in an 80 °C water bath. After this treatment, samples were dispersed for a second time for 2 min. The samples were returned to the 80 °C water bath for another 2 h and again dispersed for 2 min by using the Stomacher. A 40-μL aliquot was then transferred to a 50-μL centrifuge tube (Falcon conical centrifuge tube; BD Biosciences, San Jose, CA) that contained ≈8 mL glass beads (6 mm; Fisher...
\textbf{\textit{α}}-tocopherol metabolism in humans

\section*{α-TOCOPHEROL METABOLISM IN HUMANS}

\section*{RESULTS}

The subject was a 36-year-old healthy man weighing 79.5 kg and 183 cm tall. His fasting plasma chemistries were 4.55 mmol/L (total cholesterol), 3.8 mmol/L (HDL cholesterol), and 0.70 mmol/L (triacylglycerol). The ratio of total to HDL cholesterol was 3.8, and 30.4 mg/dL (alpha tocopherol). During the 2-wk period immediately before each dose

\section*{CALCULATIONS, DATA ANALYSIS, AND PRESENTATION}

The plasma data were expressed as the fraction of dose per liter of plasma and plotted as a function of elapsed time since the administration of each \textit{\textit{\textit{\textit{14C}}}^{-}}-labeled stereoisomer. The use of this fraction also enabled the ratio of plasma \textit{\textit{\textit{\textit{14C}}}^{-}} from dosed \textit{\textit{\textit{\textit{14C}}}^{-}}[\textit{\textit{\textit{\textit{R}}} \textit{\textit{\textit{R}}} \textit{\textit{R}}}^{-}\textit{α-TAC}] to that from dosed \textit{\textit{\textit{\textit{14C}}}^{-}}[all-rac-α-TAC] to be plotted by time since dosing.

The elimination of \textit{\textit{\textit{\textit{14C}}}^{-}} via feces and urine was presented as the fraction of dose per collection, and cumulative elimination was presented as elapsed time since dose. An aliquot of the third urine collection (after each labeled isomer was administered) was treated with glucuronidase and analyzed by HPLC (22), in which 20-s fractions of eluent were collected and analyzed for \textit{\textit{\textit{14C}}} via feces and urine was presented as the fraction of dose per collection, and cumulative elimination was presented as elapsed time since dose. An aliquot of the third urine collection (after each labeled isomer was administered) was treated with glucuronidase and analyzed by HPLC (22), in which 20-s fractions of eluent were collected and analyzed for \textit{\textit{\textit{14C}}}^{-} by using AMS (21, 22). Urine (100 \textit{μL}) was diluted 1:10 with HPLC-grade H\textit{2}O. A 100-μL aliquot of the bead-beaten feces material was diluted 1:10 with the 50/50 KO\textit{H}/IPA solution. Fifty μL of a diluted solution of tributyrin in methanol (39 μL tributyrin added to 961 μL methanol) was added to all samples to ensure that each sample had 1 mg total carbon, the optimum for high quality graphite.

Carbon in the samples was converted to graphite (20, 21). \textit{\textit{\textit{14C}}} was measured at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory (Livermore, CA).

The subject was a 36-year-old healthy man weighing 79.5 kg and 183 cm tall. His fasting plasma chemistries were 4.55 mmol/L (total cholesterol), 3.8 mmol/L (HDL cholesterol), and 0.70 mmol/L (triacylglycerol). The ratio of total to HDL cholesterol was 3.8, and 30.4 mg/dL (alpha tocopherol). During the 2-wk period immediately before each dose

By comparing model-estimated and observed data sets for plasma, urine, and feces, we modified the model structure and parameter values in biologically relevant ways to optimize agreement of model-estimated with observed data sets by using WinSAAM kinetic analysis software [version 3.0.7; Internet: http://www.winsaam.com (33, 34)]. Tracer and tracee patterns were used for parameter estimates, and pathways between pools were assumed to be first order. Nomenclature for the kinetic model included rate constants in which \textbf{\textit{\textit{k}}}\textit{ij} was the delay rate to pool \textit{i} from pool \textit{j}, a delay in which \textbf{\textit{d}}\textit{ij} was the delay to pool \textit{i} from pool \textit{j} (in d), and flows in which \textbf{\textit{f}}}\textit{ij} was the flow to pool \textit{i} from pool \textit{j} (in μmol/d). Mean residence time (in d) was calculated by dividing the number 1 by the sum of the rate constants (reciprocal), leaving a particular donor compartment of interest.

A colon pool was added to provide a slight delay during which α-tocopherol in bile (delivered to the intestine but not reabsorbed) could transit to feces. On the basis of reports in the scientific literature (2, 19, 35–39), we set 3 parameters for the model: the delay \textit{d} to gastrointestinal, from diet) at 0.08 d; the absorption efficiency at 77.5%; and the renal clearance of α-CEHC at 50 d^{-1} (urine α-CEHC × urine volume/d (216 nmol/d)/(plasma α-CEHC (12 nmol/L times plasma volume (3.58 L))). The 3 parameters were incorporated into the WinSAAM software program. All model parameters were then optimized by using WinSAAM’s least-squares routine to minimize the difference between the model prediction and experimental data.

Once the model structure and parameter values provided good visual fits, the fits were optimized by using least-squares procedures. The rate constants and distributions of α-tocopherol among the various kinetically distinguishable pools were estimated. The steady-state rate constants were estimated from the plasma patterns of the \textit{\textit{\textit{14C}}} tracer by time since dose. Using these rate constants as we worked through the system (alternating between calculations for flows and masses), we measured the steady-state tracee masses (distributions) and in turn used them to compute tracer flows for the case of a constant daily α-tocopherol intake—i.e., 12, 15, and 35 μmol/d—that corresponded to low, intermediate, and RDA (40) for vitamin E. Finally, we compared the intakes of RRR-α-tocopherol and all-rac-α-tocopherol that were needed to sustain a steady-state plasma α-tocopherol concentration of 24 μmol/L.

We first modeled the data set from administered RRR-α-TAC alone to determine the parameters specific to this isomer to establish a true tracer-tracee relation in the RRR model development. Then, using the parameters established from the RRR model, we compared model prediction to experimental observation for the all-rac-α-TAC data set. We then unlinked pathways one-by-one according to the minimal principle, unlinking the fewest rate parameters to achieve good model fits to the experimental data (41). Because the RRR-α-TAC parameter set maintained the values determined from the initial fitting, the linked all-rac-α-TAC parameters matched the corresponding RRR-α-TAC parameters, and the unlinked all-rac-α-TAC parameters were used to improve the fitting of model prediction to the all-rac-α-TAC data set. The all-rac parameters identified for unlinking were chosen on the basis of visual observation of the all-rac model that was fitted to observed data and of previously published observations of α-tocopherol behavior both in vitro and in vivo.

Finally, in constructing the final model, we tested the hypothesis (among others) that 2 functionally distinct pools of α-tocopherol exist in liver, liver kinetic pool A (liver A) and liver kinetic pool B (liver B) (H\textit{a} = 1 pool against the alternate hypothesis, H\textit{a} = 1 pool). Other testable hypotheses we focused on were that RRR- and all-rac-α-tocopherol shared equal rate constants for transfer to extrahepatic tissues from plasma and vice versa, for transfer to plasma from liver B and vice versa, and for catabolism to α-CEHC.
administration, the subject maintained his usual dietary habits but consumed a diet that provided only 12 ± 4 μmol RRR-α-tocopherol/d, which stabilized his plasma α-tocopherol at ≤23.3 μmol/L, below the threshold (30 μmol α-tocopherol/L plasma) for α-CEHC transfer into urine (42). His plasma α-tocopherol was 22.9 ± 1.0 μmol/L when he was given each radiolabeled α-tocopherol. Normal plasma concentrations are 11–37 μmol/L (43), and thus this subject was considered a healthy study subject. His plasma α-tocopherol was 22.6 μmol/L 6 d before dosing and 23.3 μmol/L 6 d after the first dose ([14C]RRR-α-TAc), so he was in steady state with respect to his vitamin E status.

The [14C] tracer profiles in plasma from the administered [14C]RRR-α-TAc and [14C]all-rac-α-TAc in a crossover design with a 3-mo intervening washout period are shown in Figure 1A. The [14C] tracer first appeared after a delay of ≈2 h (0.08 d), peaked in 0.6 d when the [14C] concentration was 1.86 times that was 22.9 μmol/L 6 d before dosing and 23.3 μmol/L 6 d after the first dose ([14C]RRR-α-TAc), so he was in steady state with respect to his vitamin E status.

The [14C] tracer profiles in plasma from the administered [14C]RRR-α-TAc and [14C]all-rac-α-TAc in a crossover design with a 3-mo intervening washout period are shown in Figure 1A. The [14C] tracer first appeared after a delay of ≈2 h (0.08 d), peaked in 0.6 d when the [14C] concentration was 1.86 times that was 22.9 μmol/L 6 d before dosing and 23.3 μmol/L 6 d after the first dose ([14C]RRR-α-TAc), so he was in steady state with respect to his vitamin E status.

The distribution of [14C] among plasma lipoproteins at selected times since dosing is shown in Figure 2. Chylomicra were separated (from VLDL) from blood drawn at 4 and 6 h only. For purposes of presentation, VLDL was stacked atop the chylomicra (so that the height above and below the white stripe = VLDL: chylomicra). In general, chylomicra and VLDL labeling with [14C] were similar for both isomers. The VLDL:chylomicra tended to be smaller at 4 h than at 6 h. The largest effects between the isomers occurred after 12 h, when lipoprotein labeling from administered [14C]RRR-α-TAc was greater than that from administered [14C]all-rac-α-TAc. The profile of labeled lipoproteins matched that of the plasma shown in Figure 1. LDL cholesterol seemed to be the major carrier of the [14C], and VLDL + chylomicra ranking next; HDL appeared to be a minor carrier.

The elimination of the [14C] tracer in feces with the fraction of dose per collection shown in the insert is summarized in Figure 3. Approximately 22.5% of each administered [14C] tracer dose was recovered in the first 3 collections after dosing, and therefore the observed fractional absorption of the tracer for each isomer was ≈0.775 (range: 1.000–0.225). The model estimated fractional absorption or true digestibility ([f(3,1) – [f(5,25)] -
The fraction of the dose eliminated in urine was greater from 8 h after dosing and peaked in urine collected between 8 and 24 h. The observed tracer data (Figure 3) and model-derived tracee data matched exactly. The chemical identification of the $^{14}$C-labeled material in feces was not determined.

The elimination of the $^{14}$C tracer in urine is summarized in Figure 4. The tracer appeared in urine collected during the first 8 h after dosing and peaked in urine collected between 8 and 24 h. The fraction of the $^{14}$C dose per collection ($\ldots$) and as the cumulative fraction of the dose ($\ldots$) for both isomers. TAc, tocopheryl acetate.

When only all-rac-TAc isomer was given, this urine sample was analyzed both before and after treatment with glucuronidase to confirm the release of $^{14}$C-$\alpha$-CEHC. The non-hydrolyzed urine sample also shows a small $^{14}$C peak that eluted at $\approx 11$ min. We hypothesized that the small peak of $^{14}$C may well be 2,7,8-tetramethyl-2-(4′-carboxypentyl)-6-hydroxychroman ($\alpha$-CPHC), the direct precursor of $\alpha$-CEHC.

The final model that was tested and found to be consistent with the full range of our experimental data is shown in Figure 6. The numbers represent kinetically distinct pools of $\alpha$-tocopherol or its metabolites (if identified). The vectors represent material transfer (ie, flow) from a donor pool to a recipient pool. The initial model had 11 kinetically distinct pools, which were numbered 1–26 and tentatively identified anatomically in parentheses. Six of the 11 pools (ie, 6, 7, 9, 10, 25, and 26) were isomer specific. The sizes of pools 6, 7, 9, 10, 25, and 26 were isomer specific. Tracer and tracee patterns used for parameter estimates and pathways were assumed to be first order.

The plasma, feces, and urine data sets along with the model-fitted lines (model solutions) are shown in Figure 7. The lines are functions of model state variables and parameters that were mapped to the experimental data for direct comparison. Log scaling of the ordinate in Figure 7A enabled the plasma kinetic data to be examined closely. The model-fitted lines shown in Figure 7 indicate how well the model fitted the observed data. In Figure 7, model solutions for the plasma (A) and elimination (B) data were excellent, and therefore we accepted the alternate hypothesis ($H_2$ = 2 pools) that 2 functionally distinct pools of $\alpha$-tocopherol exist in liver—liver A and liver B (Figure 6). The plasma $^{14}$C concentrations were higher from administered $^{14}$C-$\alpha$-TAc than from administered $^{14}$C-$\alpha$-TAc via feces was indistinguishable, which shows that the absorption of the isomers was identical. Elimination of $^{14}$C from RRR-$\alpha$-TAc and all-rac-$\alpha$-TAc via feces was indistinguishable, which shows that the absorption of the isomers was identical. Elimination of $^{14}$C-$\alpha$-CEHC via urine was greater from $^{14}$C-all-rac-$\alpha$-TAc than from $^{14}$C-RRR-$\alpha$-TAc. This greater elimination could also be due to the shorter mean residence times.
of all-rac-α-TAc than of RRR-α-TAc in most pools, which would favor a more rapid elimination from administered [14C]all-rac-α-TAc than from [14C]RRR-α-TAc. Had we collected urine over a longer period, the cumulative elimination from both isomers should eventually match. This possibility needs testing.

Furthermore, we considered a liver A delay to better fit the model. A delay can speed up movement to chylomicra tocopherol from gastrointestinal tocopherol and can slightly improve the early upswing fit, especially for the [14C]all-rac-tocopherol over RRR-α-tocopherol. However, the upswings for [14C]all-rac-versus [14C]RRR-α-tocopherol were not well separated, and thus a delay did not improve identification of the new parameters because we tried to satisfy minimal difference and maximum simplicity.

The model-estimated daily rate constants of the 14C tracer are summarized in Table 1. The constant (k) that described the disposition of the [14C]α-TAc ranged from k = 0.013 for k(10,26) to k = 84.033 for k(7,4). The fractional SD (FSD) values ranged from 0.038 to 0.226, which indicated good statistical certainty, so that a substantial deterioration of curve fit would follow small changes in rate constants. Five rate constants were smaller for RRR-α-tocopherol than for all-rac-α-tocopherol. The largest difference between the isomers—k(9,6)—was 3.7-fold, whereas the smallest difference—k(25,7)—was only 1.08-fold. Eight rate constants were the same for the 2 isomers.

The model-estimated steady-state mass distributions and plasma concentrations of tracee for the case of a constant daily α-tocopherol intake (12, 15, or 35 μmol/d) that corresponded to low, intermediate, and RDA (40) for vitamin E are summarized in Table 2. The 12 μmol/d intake represents the subject’s intake during the study, 35 μmol/d represents the RDA, and 15 μmol/d represents about 1/2 the RDA. These 3 vitamin E intakes were used for simulation. The largest distributions were in pools 10, 26, and 6. Respective distributions in pools 10, 26, and 6 were 6.77, 2.71, and 3.91 times as great for RRR-α-tocopherol than for all-rac-α-tocopherol. Distributions in all remaining pools (pools 2, 7, 8-trimethyl-2-(2-carboxyethyl)-6-hydroxychroman.

Rate constants represent the fraction of a donor pool transferred to a recipient pool per day.

The model-estimated steady-state daily rate constants for disposition of an oral tracer dose of [14C]RRR- and [14C]all-rac-α-tocopheryl acetate in a crossover study design are summarized in Table 1.

<table>
<thead>
<tr>
<th>Rate constants from RRR dose</th>
<th>Formula</th>
<th>k^2</th>
<th>FSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>To EH α-tocopherol from plasma α-tocopherol</td>
<td>k(26,10)</td>
<td>0.863</td>
<td>0.039</td>
</tr>
<tr>
<td>To liver kinetic pool B from plasma α-tocopherol</td>
<td>k(6,10)</td>
<td>1.484</td>
<td>0.088</td>
</tr>
<tr>
<td>To plasma α-CEHC from liver kinetic pool B</td>
<td>k(9,6)</td>
<td>0.027</td>
<td>0.095</td>
</tr>
<tr>
<td>To plasma α-CEHC from liver kinetic pool A</td>
<td>k(9,7)</td>
<td>0.781</td>
<td>0.189</td>
</tr>
<tr>
<td>To colon from liver kinetic pool A</td>
<td>k(25,7)</td>
<td>2.537</td>
<td>0.226</td>
</tr>
</tbody>
</table>

Rate constants from all-rac dose

<table>
<thead>
<tr>
<th>Rate constants from all-rac doses</th>
<th>Formula</th>
<th>k^2</th>
<th>FSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>To EH α-tocopherol from plasma α-tocopherol</td>
<td>k(26,10)</td>
<td>2.155</td>
<td>0.038</td>
</tr>
<tr>
<td>To liver kinetic pool B from plasma α-tocopherol</td>
<td>k(6,10)</td>
<td>3.006</td>
<td>0.081</td>
</tr>
<tr>
<td>To plasma α-CEHC from liver kinetic pool B</td>
<td>k(9,6)</td>
<td>0.099</td>
<td>0.086</td>
</tr>
<tr>
<td>To plasma α-CEHC from liver kinetic pool A</td>
<td>k(9,7)</td>
<td>1.903</td>
<td>0.193</td>
</tr>
<tr>
<td>To colon from liver kinetic pool A</td>
<td>k(25,7)</td>
<td>2.752</td>
<td>0.195</td>
</tr>
</tbody>
</table>

Rate constants same from RRR and all-rac doses

<table>
<thead>
<tr>
<th>Rate constants from RRR and all-rac doses</th>
<th>Formula</th>
<th>k^2</th>
<th>FSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>To chylomicra from gastrointestinal</td>
<td>k(4,3)</td>
<td>0.841</td>
<td>0.047</td>
</tr>
<tr>
<td>To colon from gastrointestinal</td>
<td>k(25,3)</td>
<td>0.244</td>
<td>0.047</td>
</tr>
<tr>
<td>To liver from plasma chylomicra</td>
<td>k(7,4)</td>
<td>84.033</td>
<td>0.061</td>
</tr>
<tr>
<td>To plasma-α-tocopherol from liver kinetic pool A</td>
<td>k(10,7)</td>
<td>22.831</td>
<td>0.161</td>
</tr>
<tr>
<td>To feces from colon</td>
<td>k(5,25)</td>
<td>0.758</td>
<td>0.111</td>
</tr>
<tr>
<td>To plasma-α-tocopherol from EH</td>
<td>k(10,6)</td>
<td>0.013</td>
<td>0.058</td>
</tr>
<tr>
<td>To plasma-α-tocopherol from liver kinetic pool B</td>
<td>k(10,6)</td>
<td>0.403</td>
<td>0.115</td>
</tr>
<tr>
<td>To urine from plasma α-CEHC</td>
<td>k(8,9)</td>
<td>46.983</td>
<td>0.130</td>
</tr>
</tbody>
</table>

1 FSD, SD/mean; EH, extrahepatic; α-CEHC, [14C]2, 7, 8-trimethyl-2-(2-carboxyethyl)-6-hydroxychroman.

2 Rate constants represent the fraction of a donor pool transferred to a recipient pool per day.
estimates (46, 47). The mechanisms that limit the rise in plasma α-tocopherol may be triggered at concentrations of ≈25 μmol/L.

Calculated ratios of selected rate constants for the 14C tracer in addition to their ratio of RRR to all-rac (RRR:all-rac), ranked low to high, are shown in the right column in Table 3. The ratios indicate the proportions to various pools. The most striking differences were the RRR:all-rac of 0.3141 and 0.4321 for low fractional transfers of the RRR isomer from Liver A and liver B, respectively, to plasma α-CEHC (a catabolite), which indicated preferential degradation of the all-rac isomer by the α-hydroxylase and elimination via urine. Extraceheatic tissue α-tocopherol had the longest residence time (74.6 d), and it was the same for the RRR and all-rac isomers. The mean residence times of [14C]RRR-α-tocopherol in liver B and plasma were longer than those of [14C]all-rac-α-tocopherol in the same tissues. Plasma α-tocopherol had the shortest mean residence time, which was twice as long for the RRR as for the all-rac isomer.

The calculated daily flows at 3 levels of tracee intake and the flow RRR:all-rac (ranked low to high) are shown in Table 4. Highest flows were to liver B from plasma α-tocopherol and to plasma α-tocopherol from liver B. When the dietary tracee intake was 35 μmol/d, of the 382.5 μmol RRR-α-tocopherol/d that flowed to liver B from plasma α-tocopherol, 358.8 μmol/d (or 93.8%) returned to plasma α-tocopherol, whereas, of the 114.3 μmol all-rac isomer/d that flowed to liver B from plasma α-tocopherol, only 91.8 μmol/d (or 80.3%) returned to plasma α-tocopherol. The remaining 23.68 μmol RRR-α-tocopherol/d (only 6.19%) flowed to plasma α-CEHC, whereas 22.53 μmol

### Table 2
Model-estimated steady-state α-tocopherol tracee distributions and concentrations in plasma at 3 dietary intakes

<table>
<thead>
<tr>
<th>Intake</th>
<th>Pool</th>
<th>RRR-</th>
<th>all-rac-</th>
<th>RRR-</th>
<th>all-rac-</th>
<th>RRR-</th>
<th>all-rac-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12 μmol/d</td>
<td>15 μmol/d</td>
<td>35 μmol/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>88.359</td>
<td>13.035</td>
<td>110.449</td>
<td>16.294</td>
<td>257.713</td>
<td>38.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5697.04</td>
<td>2098.39</td>
<td>7121.30</td>
<td>2622.99</td>
<td>16 616.40</td>
<td>6120.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>305.26</td>
<td>78.076</td>
<td>381.58</td>
<td>97.598</td>
<td>890.34</td>
<td>227.71</td>
</tr>
</tbody>
</table>

### Table 3
Calculated ratios of steady-state rate constants and mean residence times of tracer

<table>
<thead>
<tr>
<th>Stereoisomer</th>
<th>Calculation</th>
<th>RRR-</th>
<th>all-rac-</th>
<th>RRR:all-rac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different for each isomer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>To plasma α-CEHC from liver kinetic pool B</td>
<td>k[9,6][k[9,6] + k[10,6]]</td>
<td>0.0619</td>
<td>0.1971</td>
<td>0.3141</td>
</tr>
<tr>
<td>To plasma α-CEHC from liver kinetic pool A</td>
<td>k[9,7][k[9,7] + k[10,7] + k[25,7]]</td>
<td>0.0299</td>
<td>0.0692</td>
<td>0.4321</td>
</tr>
<tr>
<td>To EH α-tocopherol from plasma α-tocopherol</td>
<td>k[26,10][k[26,10] + k[6,10]]</td>
<td>0.3677</td>
<td>0.4176</td>
<td>0.8805</td>
</tr>
<tr>
<td>To colon from liver kinetic pool A</td>
<td>k[25,7][k[25,7] + k[9,7] + k[10,7]]</td>
<td>0.0970</td>
<td>0.1001</td>
<td>0.9690</td>
</tr>
<tr>
<td>To plasma α-tocopherol from liver kinetic pool A</td>
<td>k[10,7][k[10,7] + k[9,7] + k[25,7]]</td>
<td>0.8371</td>
<td>0.8306</td>
<td>1.0512</td>
</tr>
<tr>
<td>To liver kinetic pool B from plasma α-tocopherol</td>
<td>k[6,10][k[6,10] + k[26,10]]</td>
<td>0.6323</td>
<td>0.5825</td>
<td>1.0855</td>
</tr>
<tr>
<td>To plasma α-tocopherol from liver kinetic pool B</td>
<td>k[10,6][k[10,6] + k[9,6]]</td>
<td>0.9381</td>
<td>0.8029</td>
<td>1.1683</td>
</tr>
<tr>
<td>Same for each isomer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal absorption (fractional absorption)</td>
<td>k[4,3][k[4,3] + k[25,3]]</td>
<td>0.775</td>
<td>0.775</td>
<td>1.0000</td>
</tr>
<tr>
<td>Mean residence times (d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EH α-tocopherol</td>
<td>1/[k[10,26]]</td>
<td>74,6269</td>
<td>74,6269</td>
<td>1.0000</td>
</tr>
<tr>
<td>Liver kinetic pool A</td>
<td>1/[k[10,7] + k[9,7] + k[25,7]]</td>
<td>0.0382</td>
<td>0.0364</td>
<td>1.0494</td>
</tr>
<tr>
<td>Liver kinetic pool B</td>
<td>1/[k[9,6] + k[10,6]]</td>
<td>2.3277</td>
<td>1.9920</td>
<td>1.1685</td>
</tr>
<tr>
<td>Plasma α-tocopherol</td>
<td>1/[k[26,10] + k[6,10]]</td>
<td>0.4261</td>
<td>0.1938</td>
<td>2.1986</td>
</tr>
</tbody>
</table>

α-CEHC, [14C]-α-2, 7, 8-trimethyl-2-(2-carboxyethyl)-6-hydroxychroman; EH, extraceheatic.
DISCUSSION

Despite its nutritional importance, quantification of human vitamin E metabolism is incomplete (3-5). Therefore, we conducted a crossover feasibility study and kinetically modeled the data set to quantify the behavior of RRR- and all-rac-α-tocopherol in a healthy man. We first followed the fate of administered [5,14C]α-Tocopherol in the subject’s plasma, plasma lipoproteins, urine, and feces over time. After a 3-mo washout period, we repeated the study with [5,14C]all-rac-α-Tocopherol. The subject’s dietary α-tocopherol intake was 12 ± 4 μmol/d, and his plasma α-tocopherol at 22.9 ± 1 μmol/L was below the threshold (30 μmol/L) for α-CEHC transfer into urine (42).

The 14C tracer profiles in plasma (Figure 1), feces (Figure 3), and urine (Figure 4) were consistent with prior estimates (7, 48). 14C tracer profiles in plasma lipoproteins (Figure 2) also were consistent with previous reports (49-53), and HDL had a low concentration of the 14C tracer, which reflects the key donor role for vitamin E (54, 55). Others already showed that the mechanisms of degradation and elimination were the same for all forms of tocopherol (42, 56), but the rates were higher for γ-tocopherol than for α-tocopherol (57). Others also showed that the all-rac-α-tocopherol isomer was preferentially degraded to α-CEHC over the RRR-α-tocopherol isomer (37, 58); our 3-fold elimination of the 14C tracer in urine in Figure 4 was consistent with the earlier findings. Finally, our confirmation of [14C]α-CEHC as a key (but not the only) metabolite of α-tocopherol in urine (Figure 5) fit well with prior reports (1, 2, 13, 17, 22, 42, 57, 59, 60). Therefore, our feasibility study yielded a data set that matched prior estimates well and that was suitable for kinetic modeling.

We built a kinetic model (Figure 6) to obtain values for critical parameters of the model and to identify unique and testable hypotheses about α-tocopherol metabolism. α-Tocopherol transfer protein not only catalyzes the intermembrane transfer of α-tocopherol to recipient from donor membranes (61, 62) but also retains and secretes α-tocopherol from hepatocytes to circulating lipoproteins for delivery to extrahepatic tissues (63-66).

TABLE 4

<table>
<thead>
<tr>
<th>Calculation</th>
<th>α-Tocopherol intakes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 μmol/d</td>
</tr>
<tr>
<td>Different for each isomer</td>
<td></td>
</tr>
<tr>
<td>To plasma α-CEHC from liver kinetic pool A</td>
<td>f(9,7)</td>
</tr>
<tr>
<td>To liver kinetic pool B from plasma α-tocopherol</td>
<td>f(6,10) = f(10,6) + f(9,6)²</td>
</tr>
<tr>
<td>To plasma α-tocopherol from liver kinetic pool B</td>
<td>f(10,6)</td>
</tr>
<tr>
<td>To EH α-tocopherol from plasma α-tocopherol and vice versa</td>
<td>f(26,10) = f(10,26)³</td>
</tr>
<tr>
<td>Same for each isomer</td>
<td></td>
</tr>
<tr>
<td>To chylomicra from gastrointestinal and to liver kinetic pool A from chylomicra</td>
<td>f(4,3) = f(7,4)⁴</td>
</tr>
<tr>
<td>To colon from gastrointestinal</td>
<td>f(25,3)</td>
</tr>
<tr>
<td>To urine from plasma α-CEHC</td>
<td>f(8,9)</td>
</tr>
<tr>
<td>To feces from colon</td>
<td>f(5,25)</td>
</tr>
<tr>
<td>To colon from liver kinetic pool A</td>
<td>f(25,7)</td>
</tr>
<tr>
<td>To plasma α-tocopherol from liver kinetic pool A and to plasma α-CEHC from liver kinetic pool B</td>
<td>f(10,7) = f(9,6)⁴</td>
</tr>
</tbody>
</table>

1 α-CEHC, [14C]α-2, 7, 8-trimethyl-2-(2-carboxyethyl)-6-hydroxycroman; EH, extrahepatic. Trace mass-transferred to recipient from donor pools in μmol/d.

2 At 35 μmol/d intake, 94% (358.8/382.5) of RRR-α-tocopherol flowing to liver α-tocopherol from plasma α-tocopherol, but only 80% (91.8/114.3) of all-rac-α-tocopherol did so; the remainder flowed to plasma α-CEHC and urine.

3 All trace (both isomers) delivered to chylomicra went to liver kinetic pool A. All trace (both isomers) flowing to EH α-tocopherol returned to plasma α-tocopherol.

4 All trace flowing to plasma α-tocopherol from liver kinetic pool A flowed to plasma α-CEHC and eventually to urine. The 12 μmol/d Intake represents the subject’s intake during the study. 35 μmol/d represents the Recommended Dietary Allowance, and 15 μmol/d represents approximately one-half the Recommended Dietary Allowance. The 3 vitamin E intakes were used for simulation.

all-rac-α-tocopherol/d (or 19.7%) did so. The flow to plasma α-CEHC from liver A was only 0.81 μmol/d for the RRR isomer but 1.878 μmol/d for the all-rac isomer, for RRR:all-rac of 0.431, which suggests that the all-rac isomer was preferentially degraded and eliminated as α-CEHC in urine.
the observed plasma data minus the 1-pool-model solution for it. That mean (±SE) difference, −0.005652 ± 0.001374 (n = 108), was significant (P < 0.0001), which showed the existence of 2 kinetically distinct pools in liver. Thus, with 2 distinct kinetic pools in liver, a delay δ(3,1), and a renal clearance for plasma α-CEHC, the final model (Figure 6) fit the very informative plasma, feces, and urine 14C data extremely well (Figure 7). We tentatively identified the pools as liver A and liver B.

The tracee distributions rose as α-tocopherol intake increased, and they rose more for RRR- than for all-rac-α-tocopherol (Table 2). The distributions and their relation to rising intake were consistent with the greater enrichment of mouse plasma (and tissues) with hexadeuterated RRR-α-tocopherol than with the trideuterated all-rac-α-tocopherol in mice (67). The tracee distributions also matched prior estimates of 6700 ± 860 μmol (68) and 2000–26000 μmol (69) in adipose tissue, a major store.

The only exception was the poor match to a physiological level for the plasma α-tocopherol; the model estimated 31 and 73 μmol/L for RRR-α-tocopherol intakes of 15 and 35 μmol/d, respectively. The 73 μmol/L concentration was higher than expected (46, 47), which suggested that the kinetics of α-tocopherol became nonlinear between intakes of 15 and 35 μmol/d. Because kinetic models enable plasma α-tocopherol concentrations to be simulated for varied intakes, we performed additional simulations to bring plasma α-tocopherol more in line with the prior estimate of 28 μmol/L for an RRR-α-tocopherol intake of 35 μmol/d. First, we increased the flow ([f(8,9)]; this step did not lower plasma RRR-α-tocopherol concentrations, which indicated that formation rather than elimination of α-CEHC has a rate-limiting effect. Second, we reduced fractional absorption, k(4,3)/k(4,3) + k(25,3), from 0.775 to 0.30; this step reduced the plasma α-tocopherol to 28 μmol/L, but the 0.30 value was inconsistent with prior estimates (7, 48). Third, we raised k(9,7) from 0.781 to 39.0, and that step reduced the plasma α-tocopherol to 29 μmol/L. Fourth, we raised k(9,6) from 0.027 to 0.081, and that step reduced plasma α-tocopherol to 27 μmol/L. Only the third and fourth simulations were effective, which indicates that they are rate-limiting pathways. The performance of the 4 additional stimulations shows how dose-response tracer modeling can identify control pathways.

In Table 3, the highest RRR-all-rac of 1.1683 showed a greater recycling of the RRR isomer to the plasma (70–73) from liver B (61, 67). In contrast, all-rac-tocopherol was preferentially degraded to plasma α-CEHC (0.1971 over 0.0619 and 0.0692 over 0.0299), to colon (0.1001 over 0.0970), and to extrahepatic tissue (0.4176 over 0.3677). The most striking difference was the rate-constant RRR-all-rac of 0.3141 and 0.4321 (Table 3) for high fractional transfers of the all-rac isomer from liver B to plasma α-CEHC. Thus, we quantified the degradation of the all-rac over the RRR isomer α-CEHC (74), and we do not support a trigger threshold for α-CEHC elimination (42) because 14C-α-CEHC was formed and eliminated well below the RDA intake of α-tocopherol.

It is important to note that, although tracer rate constant k(26,10) in Table 1 was smaller for RRR-α-tocopherol (0.863 ± 0.039) than for all-rac-α-tocopherol (2.155 ± 0.038), tracer flows f(26,10) in Table 4 were larger for RRR-α-tocopherol (76.3 μmol/d) than for all-rac-α-tocopherol (28.1 μmol/d). This occurred because of a larger tracer distribution of RRR-α-tocopherol (88.359 μmol) than of all-rac-α-tocopherol (13.035 μmol) (Table 2). The same happened with urine (Tables 2 and 3).

The larger distributions of RRR-α-tocopherol than of all-rac-α-tocopherol in pools 26 (5697 > 2098 μmol) and 6 (305 > 78 μmol) (Table 2), coupled with the smaller RRR-all-rac to plasma α-CEHC from liver B (0.3141) and liver A (0.4321) (Table 3), account for the greater amount of 14C tracer from all-rac-α-tocopherol than from RRR-α-tocopherol in urine. Furthermore, with an intake of 12 μmol/d, the RRR-α-tocopherol distribution in liver B was ~4 times that of all-rac-α-tocopherol. The proportion of tracee flowing from liver B to plasma α-CEHC for all-rac-α-tocopherol was ~3.2 times that for RRR-α-tocopherol, and thus the RRR-α-tocopherol 14C tracer experienced a greater dilution than did all-rac-α-tocopherol 14C tracer with the tracee. Consequently, less RRR-α-tocopherol 14C tracer than all-rac entered plasma α-CEHC, which forced the 14C tracer mass in urine to be greater for all-rac-α-tocopherol. It is important to notice that the tracee flows to urine were similar for the 2 isomers. This finding shows how the kinetic modeling of tracer data sets can explain or reconcile tracer and tracee data that may appear as conflicting steady-state flows.

In conclusion, we quantified the absorption, distribution, metabolism, and elimination of a true tracer dose of [14C]RRR-α-tocopherol in a healthy man under steady-state conditions and quantified α-tocopherol metabolism as it might occur in vivo. We found that liver has 2 kinetically distinct α-tocopherol pools, that α-CEHC is eliminated in urine in the absence of vitamin E supplementation, and that all-rac-α-tocopherol is preferentially degraded and eliminated in urine. Our approach, data set, and model-derived features of human vitamin E metabolism encourage further testing.

We thank Willy Cohn for his valuable input and discussions in designing the study, developing the model, and interpreting the results; John Vogel, Ted Ognibene, Bruce Buchholz, and Kurt Haack for the 14C measurements; and AJCN reviewers for their perceptive and helpful comments.

AJC designed the study and wrote the manuscript; FFM, CCH, and JCC participated in the sample preparation and analyses; JF participated in the diet analyses; JAN, JGF, and AJC developed the model and interpreted the results. None of the authors had a personal or financial conflict of interest.

REFERENCES


