Noncompetitive Plasma Biokinetics of Deuterium-Labeled Natural and Synthetic \( \alpha \)-Tocopherol in Healthy Men with an apoE4 Genotype\(^1,2\)

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ABSTRACT Previous studies comparing the biokinetics of deuterated natural (\( RRR \)) and synthetic (all-\( rac \)) \( \alpha \)-tocopherol (vitamin E) used a simultaneous ingestion or competitive uptake approach and reported relative bioavailability ratios close to 2:1, higher than the accepted biopotency ratio of 1.36:1. The aim of the current study was to compare the biokinetics of deuterated natural and synthetic vitamin E using a noncompetitive uptake model both before and after vitamin E supplementation in a distinct population. Healthy men (\( n = 10 \)) carrying the apolipoprotein (apo)E4 genotype completed a randomized crossover study, comprised of two 4-wk treatments with 400 mg/d (\( RRR \)-\( \alpha \)-tocopheryl and all-\( rac \)-\( \alpha \)-tocopheryl acetate) with a 12-wk washout period between treatments. Before and after each treatment period, the subjects consumed a capsule containing 150 mg deuterated \( \alpha \)-tocopheryl acetate in either the \( RRR \) or all-\( rac \) form depending on their treatment regimen. Blood was obtained up to 48 h after ingestion, and tocopherols analyzed by LC/MS. After deuterated all-\( rac \) administration, plasma deuterated tocopherol maximum concentrations and area under the concentration vs. time curves (AUC) were lower than those following \( RRR \) administration. The \( RRR \)-all-\( rac \) ratios determined from the deuterated biokinetic profiles (maximum concentration; \( C_{\text{max}} \)) and AUCs were 1.35:1 ± 0.17 and 1.33:1 ± 0.18, respectively. The 4-wk supplementation with either \( RRR \) or all-\( rac \) significantly increased plasma \( \alpha \)-tocopherol concentrations (\( P < 0.001 \)), but decreased the plasma response to newly absorbed deuterated \( RRR \) or all-\( rac \) \( \alpha \)-tocopherol. Using a noncompetitive uptake approach, the relative bioavailability of natural to synthetic vitamin E in apoE4 males was close to the currently accepted biopotency ratio of 1.36:1. J. Nutr. 135: 1063–1069, 2005.

KEY WORDS: • tocopherol • natural • synthetic • biokinetics • bioavailability • apoE4

\( \alpha \)-Tocopherol is the most biologically active form of vitamin E, comprising \( \sim 90\% \) of vitamin E in the body (1). The potentially beneficial role of vitamin E in human health, especially with respect to heart disease, has received much attention (2–4). However, a full understanding of antioxidant supplementation (10) and merit investigation with respect to vitamin E bioavailability and requirements. Carriers of the apolipoprotein (apo)E4 allele, \( \sim 25\% \) of Western populations, are thought to be at significantly higher risk of cardiovascular disease relative to the common E3/E3 subgroup (5,6). Traditionally, this risk differential has been attributed to higher circulating plasma cholesterol (7) and apoB concentrations (8) in this subgroup. Data generated from the second Northwick Park Heart Study indicated hazard ratios (HR)\(^4\) for coronary heart disease (CHD) risk of 2.79, 0.85, and 1.47 in E4 smokers, E2 smokers, and E3 smokers, respectively, relative to a “never smoked” combined group, after correction for blood lipids and other CHD risk indicators (9). The authors suggested that the increase in HR in E4 smokers was potentially attributable to higher oxidative stress in this group, with the E4 protein having a lower antioxidant capacity than the E3 and E2 isoforms. Thus, apoE4 carriers represent a population that could potentially benefit from antioxidant supplementation (10) and merit investigation with respect to vitamin E bioavailability and requirements.

The \( \alpha \)-tocopherol molecule consists of a trimethylated chromanol ring and a saturated phytol side chain. There are 3 chiral centers at positions 2, 4’, and 8’. \( \alpha \)-Tocopherol is available from various food sources. In nature, e.g., vegetable oils and nuts, \( \alpha \)-tocopherol is found as a single stereoisomer, \( RRR \)-\( \alpha \)-tocopherol. Commercially available \( RRR \)-\( \alpha \)-toco-
erol is derived from deodorizer distillate, a by-product of soybean oil production, comprising primarily γ- and δ-tocopherol, which are chemically modified (by methylation) to RRR-α-tocopherol, or natural-source α-tocopherol. α-Tocopherol can also be synthesized chemically, yielding α-tocopherol consisting of 8 stereoisomers in equal proportions (RRR, RRS, RSR, RSS, SSS, SRR, SRS, and SSR) and is designated all-racemic α-tocopherol (all-rac). This form, or natural-source α-tocopherol, is commonly found in commercially available vitamin E supplements and fortified products. The relative biopotencies for each individual α-tocopherol stereoisomer were determined using the rat fetal resorption model (11) and range from 1064 for each individual α-tocopherol equivalent to 1.36 mg of synthetic α-tocopherol in preventing rat fetal resorption.

Regulation of plasma α-tocopherol is under the control of the hepatic α-tocopherol transfer protein (α-TTP) (14), whose affinity is closely related to the biological activity of each vitamin E homologue (14). This protein has high affinity for 2R forms of α-tocopherol, which explains the preferential systemic distribution of 2R forms of α-tocopherol in deuterium-labeled biokinetic studies (15–17). These studies confirmed that there is no discrimination between the forms of tocopherol during absorption, but there is selective enrichment of VLDL with 2R forms of α-tocopherol (15–17).

Human vitamin E biopotency data are lacking. Instead, biomarkers of bioavailability have been used. Bioavailability represents the rate and extent of a compound's appearance in the blood for which absorption is an important factor. This differs from biopotency, which is a measure of the biological effects exerted by an active compound. To monitor directly the specific form of vitamin E administered, stable-isotope labeled vitamin E is commonly used. These so-called biokinetic studies involve sampling over a period of time after administration of the label, and bioavailability is measured by monitoring the plasma response as maximum concentration (Cmax) and area under the concentration vs. time curve (AUC). Previously, such human studies consistently used a competitive uptake approach whereby both deuterated vitamin E and the opposite treatment. At the beginning (pre-) and end (post-) of each 4-wk supplementation period, the men underwent a 48-h biokinetic protocol with the corresponding deuterated form of α-tocopheryl acetate.

**FIGURE 1** The study design of a randomized, double blind, controlled, crossover trial in men. After a “run-in” period of vitamin C only, the men were randomly assigned to either natural or synthetic α-tocopheryl acetate for 4 wk. After a 12-wk washout period, participants crossed over to the opposite treatment. At the beginning (pre-) and end (post-) of each 4-wk supplementation period, the men underwent a 48-h biokinetic protocol with the corresponding deuterated form of α-tocopheryl acetate.

**SUBJECTS AND METHODS**

**Subjects.** Two equal cohorts were recruited by advertisement from the Universities and local communities in the Guildford and Reading area [~20 miles (32 km) apart]. Selection criteria were as follows: an apoE4 genotype (E3/E4, E4/E4); 25–55 yr old; BMI between 20 and 32 kg/m2; alcohol consumption ≤ 21 U/wk (where 1 U corresponds to 7.9 g alcohol); exercise ≥ 3 x 30 min aerobic sessions/wk; no diagnosed heart disease, diabetes, liver, or other endocrine dysfunction; no abnormalities of fat metabolism; and no use of antioxidant supplementation in the 6 mo before the start of the study. After screening, we recruited 10 subjects into the study. The study was approved by the University of Surrey and the University of Reading ethics committees and all participants gave written consent before participation.

**ApoE4 genotyping.** Genomic DNA was isolated from the white cell layer (buffy coats) of 10 mL EDTA blood using the Blood Spin™ DNA purification kit by Qiagen. The 2 ApoE polymorphic sites were amplified by PCR and digested with the HhaI restriction enzyme. The resultant fragments were characterized by gel electrophoresis as described by Hixon and Vernier (27).

**Study protocol.** The study was a randomized, double-blind, controlled, crossover trial (Fig. 1). Subjects consumed vitamin C (60 mg/d) for 4 wk before the start of the study and continued to do so throughout the study to standardize vitamin C status. After the 4-wk “run-in” with vitamin C only, the study subjects were randomly assigned to receive either natural (RRR-) or synthetic (all-rac-) α-tocopheryl acetate in the first arm and underwent a kinetic protocol with that deuterated form of α-tocopheryl acetate. The kinetic protocol lasted 49 h. On the morning of d 1, the men reported to the clinical investigation unit after an overnight fast (12 h), and a cannula was inserted in the antecubital vein of the forearm. A fasting baseline blood sample was collected and the subjects consumed a capsule containing 150 mg of deuterium-labeled RRR- (hexadeuterated) or all-rac- (trideuterated) α-tocopheryl acetate with a standard breakfast containing 40 g of fat to facilitate vitamin E absorption.

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Blood samples were subsequently taken at 3, 6, 9, 12, 24, and 48 h after the ingestion of the capsule. On d 1, standard meals and drinks were provided but for d 2 of the kinetic protocol, the subjects were free-living and consumed their normal diets. At the completion of the kinetic protocol, subjects started a 4-wk supplementation period with 400 mg/d of either RRR- or all-rac-\(\alpha\)-tocopheryl acetate depending on their randomization, followed by a second kinetic protocol carried out as described with the assigned form of deuterated \(\alpha\)-tocopheryl acetate. The subjects then underwent a 12-wk washout period after which 2 more kinetic protocols and a 4-wk supplementation regimen were carried out with the other form of \(\alpha\)-tocopheryl acetate. Compliance was assessed by capsule count.

**Deuterated tocopherols.** RRR-[5,7-Methyl-\(\text{\textsuperscript{2}H}_4\)]-\(\alpha\)-tocopheryl acetate and all-rac-[5-methyl-\(\text{\textsuperscript{2}H}_5\)]-\(\alpha\)-tocopheryl acetate were kind gifts from Cognis Nutrition and Health. Purities of the acetates were 98.9% for both species. Isotopic purity was determined to be \(>99.9\%\) by LC-MS. The deuterated tocopheryl acetates were encapsulated as described (30).

**Sample preparation.** Plasma was separated from whole blood by centrifugation at 1750 \(\times\) g for 10 min in a refrigerated centrifuge (\(4^\circ\)C). Plasma for tocopherol analysis was pipetted (1 mL) into cryovials containing 10 \(\mu\)L of BHT (1000 g/L in absolute ethanol) and snap-frozen in liquid nitrogen to prevent oxidation. Plasma for ascorbic acid analysis was pipetted using a method we developed recently (28). Amounts of deuterium-labeled natural (\(\text{rac}\)) or synthetic (all-\(\text{rac}\)) \(\alpha\)-tocopherols in extracted plasma samples were calculated from standard curves using the Mass Lynx version 4.0 software. Labeled \(\alpha\)-tocopheryl concentrations were corrected for the contribution of isotopic contamination and isotopic purity essentially as described (30).

**Blood lipids.** Plasma total cholesterol and triacylglycerides were determined using Randox\textsuperscript{\textregistered} enzymatic kits on an automated SPace biochemical analyzer.

**Ascorbic acid analysis.** Plasma ascorbic acid was determined by paired-ion reversed-phase HPLC coupled with electrochemical detection as described (31).

**Pharmacokinetics analysis.** Noncompartmental pharmacokinetic parameters associated with plasma concentration-time data after single oral dosing were determined using the PK Solution Version 2.0 software (Summit Research Services). Parameters obtained were \(C_{\text{max}}\), maximum time, AUC, rate of elimination, half-life, and volume of distribution (Vd).

**Statistical analysis.** This biokinetic study was part of a larger study comparing markers of antioxidant status, and monocyte gene expression in smokers and nonsmokers; thus the sample size calculations were performed for the whole study. Using a within-group variation of 20% which corresponds to variation in biokinetic studies, e.g., (32), and a difference to detect between treatments of 20%, a sample size of 16 would allow us to demonstrate a significant difference between groups with \(0.05\) probability and 80% power. In total, 10 subjects were recruited for the biokinetic part of the study. The software package GraphPad InStat (GraphPad Software) was used for all statistical analysis. This program automatically tests for normality. All data were found to be normally distributed; thus, parametric tests were used. Repeated-measures ANOVA was used to test for differences in deuterated tocopherol concentration over time and between treatments (natural vs. synthetic). Tukey’s post hoc test was used when a significant result was found (\(P < 0.05\)). Unpaired and paired \(t\) tests were used to test differences in kinetic parameters between natural and synthetic and pre- and postsupplementation. Results were considered significant at the 95% confidence level (\(P < 0.05\)). Values are shown as means \(\pm\) SEM.

**RESULTS**

**Subjects.** At the start of the study, the subjects’ age, BMI, total cholesterol, total triacylglycerides, and plasma ascorbic acid were 46 \(\pm\) 3.5 y, 26.5 \(\pm\) 1.3 kg/m\(^2\), 7.3 \(\pm\) 0.4 mmol/L, 2.2 \(\pm\) 0.3 mmol/L, and 50.8 \(\pm\) 4.6 \(\mu\)mol/L, respectively. These variables were not affected during the study.

**Baseline plasma endogenous \(\alpha\)-tocopherol.** Plasma \(\alpha\)-tocopherol concentrations before and after the 4-wk ascorbic acid supplementation regimen were 29.6 \(\pm\) 1.7 and 30.7 \(\pm\) 1.6 mmol/L, respectively. After the 4-wk supplementation with 400 mg/d of natural \(\alpha\)-tocopherol acetate, plasma \(\alpha\)-tocopherol concentrations increased from 31.6 \(\pm\) 1.6 to 45.5 \(\pm\) 2.9 mmol/L (\(P < 0.001\)). After the 4-wk supplementation with 400 mg/d of synthetic \(\alpha\)-tocopherol acetate, plasma \(\alpha\)-tocopherol concentrations also increased from 30.4 \(\pm\) 1.3 to 38.8 \(\pm\) 2.4 mmol/L (\(P < 0.01\)). Plasma \(\alpha\)-tocopherol concentration postsupplementation did not differ between natural or synthetic \(\alpha\)-tocopherol acetate ingestion.

**Plasma deuterium labeled \(\alpha\)-tocopherol.** After ingestion of deuterium-labeled natural (RRR) and synthetic (all-\(\text{rac}\)) \(\alpha\)-tocopherol acetate (Fig. 2) there was a significant increase in labeled \(\alpha\)-tocophers with time (\(P < 0.001\)). Deuterated \(\alpha\)-tocopherol appeared in plasma 3 h after dosing, and reached a maximum plasma concentration at 12 h postsupplementation (RRR and all-\(\text{rac}\)) and 9 and 6 h for RRR and all-\(\text{rac}\) postsupplementation, respectively. The plasma \(C_{\text{max}}\) of deuterated tocopherol was higher after RRR than with all-\(\text{rac}\) both pre- and postsupplementation; it was 11.2 and 8.8

**FIGURE 2** Plasma unlabeled and deuterium labeled \(\alpha\)-tocopherol concentration vs. time profiles in men after administration of either deuterium-labeled natural (RRR) or synthetic (all-\(\text{rac}\)) \(\alpha\)-tocopherol acetate, both pre- (A) and post- (B) 4 wk supplementation with either natural or synthetic \(\alpha\)-tocopherol acetate. Values are means \(\pm\) SEM, \(n = 10\).
μmol/L with RRR and all-rac, respectively, at 12 h presupplementation. At the same time point postsupplementation, there was a significant difference between the concentration of deuterated RRR and all-rac (9.2 ± 1.3 vs. 5.8 ± 0.8 μmol/L, P < 0.05).

In contrast to the increase in labeled α-tocopherol concentration, plasma unlabeled α-tocopherol concentration decreased with time from 0 to 12 h both pre- (P < 0.001) and postsupplementation (P < 0.01), after both deuterated RRR- and all-rac-α-tocopheryl acetate ingestion (Fig. 2).

**Percentage of α-tocopherol in the labeled form.** After ingestion of deuterated RRR, the percentage of labeled α-tocopherol had increased to 30% at maximum (12 h) presupplementation, but was only 22% postsupplementation (Fig. 3A). The percentage of labeled α-tocopherol postsupplementation at 12 and 24 h was lower (P < 0.05) than that before supplementation. Similarly, after ingestion of deuterated all-rac (Fig. 3B), the percentage of labeled α-tocopherol increased to 23% at maximum (12 h) presupplementation, but was only 17% postsupplementation (6 h).

**Kinetic parameters.** Noncompartmental kinetic parameters for labeled and unlabeled tocophers were derived from concentration vs. time profiles after administration of labeled α-tocopheryl acetate. Rates of absorption or elimination, half-lives, and Vd did not differ between deuterated RRR and all-rac both pre- and postsupplementation (Table 1). AUCs from deuterated α-tocopherol concentration profiles were lower after all-rac compared with RRR, and were lower postsupplementation compared with presupplementation with both forms. The AUC for deuterated all-rac α-tocopherol after 4 wk supplementation with synthetic α-tocopheryl acetate was lower (P < 0.05) than that of deuterated RRR α-tocopherol 4 wk postsupplementation with natural α-tocopheryl acetate. The deuterated Cmax was lower after deuterated all-rac than deuterated RRR, and was also lower postsupplementation than presupplementation. The rate of elimination of undeuterated (endogenous) α-tocopherol after 4 wk supplementation with synthetic α-tocopherol acetate was greater (P < 0.05) than before supplementation. Furthermore, the Cmax of undeuterated α-tocopherol was greater after 4 wk supplementation with either natural (P < 0.001) or synthetic (P < 0.01) α-tocopheryl acetate. Similarly, the AUC of undeuterated α-tocopherol was greater after 4 wk supplementation with either natural (P < 0.001) or synthetic (P < 0.01) α-tocopheryl acetate. Also, the AUC for undeuterated α-tocopherol after 4 wk supplementation with synthetic α-tocopherol acetate was lower (P < 0.05) than that of undeuterated α-tocopherol after 4 wk of supplementation with natural α-tocopherol acetate.

**Ratio of natural to synthetic α-tocopherol.** The ratio of RRR:all-rac was calculated from deuterated α-tocopherol concentration-time profiles (Fig. 2), and from deuterated tocopherol kinetic parameters (Table 1). Before supplementation, the ratio RRR:all-rac increased to a maximum of 1.35 after 12 h and remains relatively constant over the remaining time period (Fig. 4). After vitamin E supplementation the ratio RRR:all-rac increased to a maximum of 1.52, also after 12 h, and again remained essentially constant over the study period. The mean ratio of RRR:all-rac from Cmax and AUC, which are important determinants of bioavailability, were 1.35 ± 0.17 and 1.33 ± 0.18, respectively, before supplementation, and 1.21 ± 0.16 and 1.43 ± 0.19, respectively, after vitamin E supplementation (Table 2). Individual responses for deuterated α-tocopherol Cmax and ratios of RRR:all-rac derived from Cmax and AUC parameters showed large interindividual variation (Table 3). Maximum concentrations of deuterated RRR and all-rac ranged from 2.8 to 19.2 and 3.5 to 11.7 μmol/L.

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**TABLE 1**

Noncompartmental kinetic parameters for deuterated RRR and all-rac, and endogenous undeuterated α-tocopherol derived from concentration vs. time profiles after administration to men of labeled α-tocopheryl acetate at baseline (pre-) and after 4 wk supplementation (post-) with natural (N) and synthetic (S) α-tocopheryl acetate

<table>
<thead>
<tr>
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<th>Deuterated RRR</th>
<th>Deuterated all-rac</th>
<th>Undeuterated2</th>
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<tr>
<td></td>
<td>Pre-N</td>
<td>Post-N</td>
<td>Pre-S</td>
</tr>
<tr>
<td>Cmax, μmol/L</td>
<td>11.2 ± 1.9</td>
<td>9.2 ± 0.6</td>
<td>8.8 ± 1.3</td>
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<tr>
<td>Tmax, h</td>
<td>12.3 ± 1.4</td>
<td>11.4 ± 2.2</td>
<td>9.6 ± 0.7</td>
</tr>
<tr>
<td>AUC, (μmol·h)/L</td>
<td>387 ± 66</td>
<td>326 ± 20</td>
<td>291 ± 44</td>
</tr>
<tr>
<td>Elimination rate, μmol/(L·h)</td>
<td>0.027 ± 0.004</td>
<td>0.019 ± 0.002</td>
<td>0.020 ± 0.002</td>
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<tr>
<td>Half-life, h</td>
<td>33.8 ± 6.8</td>
<td>42.6 ± 6.3</td>
<td>41.3 ± 6.4</td>
</tr>
<tr>
<td>Vd, L/kg</td>
<td>0.35 ± 0.08</td>
<td>0.36 ± 0.02</td>
<td>0.48 ± 0.07</td>
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</table>

1 Values are means ± SEM, n = 10. a Different from Post-N, P < 0.05; b different from Pre-S, P < 0.05; c different from Pre-S, P < 0.01; d different from Pre-N, P < 0.001.

2 The values for undeuterated α-tocopherol Cmax are derived from time 0.
and synthetic tocopherol concentration-time profiles of newly absorbed deuterated tocopherols, encompasses the process of absorption and first-passage liver metabolism that reaches the systemic circulation intact; thus, it is termed dose that reaches the systemic circulation intact; thus, it encompasses the process of absorption and first-passage liver metabolism. Therefore, by measuring the plasma concentration vs. time profiles of newly absorbed deuterated tocopherols, it is possible to calculate the relative bioavailability of natural and synthetic tocopherol acetate. Previously, studies of this type administered deuterated natural and synthetic tocopherol acetates simultaneously in equal amounts, the so-called competitive uptake method (18–23). These studies reported ratios of natural:synthetic tocopherol close to 2:1 in plasma (19,20,23), leading to conclusions that natural-source vitamin E had twice the availability of synthetic vitamin E (20). In the present study, we assessed the relative bioavailability of natural and synthetic vitamin E using a noncompetitive model, in which the 2 forms were administered on separate occasions because this is the usual method for measuring bioavailability when studying the pharmacokinetics of drugs (33). The ratios of natural to synthetic tocopherol from concentration vs. time profiles, Cmax, and AUC analysis, which are important parameters of bioavailability in single-dose studies (24), were 1.35:1 ± 0.22, 1.35:1 ± 0.17, and 1.33:1 ± 0.18, very close to the currently accepted biopotency ratio of 1.36:1 derived from animal studies (11,13) and clearly in contrast to the findings of previous studies in which the competitive uptake approach was used (19,20,23). Our study is also the first to report a number of kinetic parameters for deuterated natural and synthetic tocopherol determined using a noncompetitive model. Our data essentially show no difference in the kinetic behavior of the administered natural and synthetic tocopherol acetates, except for AUC and Cmax. The deuterated tocopherol concentration vs. time profiles in the present study are consistent with those reported previously after a single dose (20,32,34). Both our deuterated tocopherol Cmax (23,34) and calculated half-lives (35) for all-tocopherol are also similar to those reported previously using the same dose of deuterated RRR-tocopherol acetate.

It has been argued that the competitive uptake model results in bias against all-rac, because when RRR and all-rac are given simultaneously in equal amounts, the actual dose would contain 75% of 2R-forms to compete with 25% of 2S-forms (24,26). Because the 2R forms are preferentially selected by α-TTP in the liver (no discrimination at the site of absorption) for systemic distribution (14), increasing the abundance of 2R-forms lowers the probability of α-TTP binding to 2S-forms (26), leading to discrimination against all-rac (24,26). However in the present study, in which all-rac was administered separately from RRR, there would be 50% each of the 2R- to 2S-forms in all-rac, and hence no such discrimination would occur. This could explain our findings of a mean ratio of 1.34:1 using this noncompetitive model, whereas ratios close

**TABLE 2**

| Ratio of RRR:all-rac obtained from deuterated α-tocopherol kinetic parameters after administration to men of deuterium-labeled natural and synthetic α-tocopherol acetates at baseline (pre-) and after 4 wk supplementation (post-) with natural and synthetic α-tocopherol acetates
<table>
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<tr>
<td></td>
<td>Presupplementation</td>
<td>Postsupplementation</td>
<td></td>
</tr>
<tr>
<td>Cmax, μmol/L</td>
<td>1.35 ± 0.17 (0.33)</td>
<td>1.21 ± 0.16 (0.32)</td>
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<tr>
<td>AUC, (μmol · h)/L</td>
<td>1.33 ± 0.18 (0.36)</td>
<td>1.43 ± 0.19 (0.38)</td>
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<tr>
<td>Elimination rate, μmol/(L · h)</td>
<td>1.38 ± 0.57 (1.11)</td>
<td>0.94 ± 0.19 (0.39)</td>
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<tr>
<td>Half-life, h</td>
<td>0.82 ± 0.40 (0.78)</td>
<td>0.81 ± 0.14 (0.27)</td>
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<tr>
<td>Vd, L/kg</td>
<td>0.74 ± 0.34 (0.67)</td>
<td>0.91 ± 0.22 (0.43)</td>
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1 Values are means ± SEM (95% CI), n = 10.

**DISCUSSION**

This study is the first to compare the bioavailability of labeled natural (RRR) and synthetic (all-rac) vitamin E using a noncompetitive approach in a distinct human population. Bioavailability, per definition, is the fraction of the administered dose that reaches the systemic circulation intact; thus, it encompasses the process of absorption and first-passage liver metabolism. Therefore, by measuring the plasma concentration vs. time profiles of newly absorbed deuterated tocopherols, it is possible to calculate the relative bioavailability of natural and synthetic tocopherol acetate. Previously, studies of this type administered deuterated natural and synthetic tocopherol acetates simultaneously in equal amounts, the so-called competitive uptake method (18–23). These studies reported ratios of natural:synthetic tocopherol close to 2:1 in plasma (19,20,23), leading to conclusions that natural-source vitamin E had twice the availability of synthetic vitamin E (20). In the present study, we assessed the relative bioavailability of natural and synthetic vitamin E using a noncompetitive model, in which the 2 forms were administered on separate occasions because this is the usual method for measuring bioavailability when studying the pharmacokinetics of drugs (33). The ratios of natural to synthetic α-tocopherol from concentration vs. time profiles, Cmax, and AUC analysis, which are important parameters of bioavailability in single-dose studies (24), were 1.35:1 ± 0.22, 1.35:1 ± 0.17, and 1.33:1 ± 0.18, very close to the currently accepted biopotency ratio of 1.36:1 derived from animal studies (11,13) and clearly in contrast to the findings of previous studies in which the competitive uptake approach was used (19,20,23). Our study is also the first to report a number of kinetic parameters for deuterated natural and synthetic α-tocopherol determined using a noncompetitive model. Our data essentially show no difference in the kinetic behavior of the administered natural and synthetic α-tocopherol acetates, except for AUC and Cmax. The deuterated α-tocopherol concentration vs. time profiles in the present study are consistent with those reported previously after a single dose (20,32,34). Both our deuterated α-tocopherol Cmax (23,34) and calculated half-lives (35) for all-tocopherol are also similar to those reported previously using the same dose of deuterated RRR-α-tocopherol acetate.

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to 2:1 were found in competitive uptake studies (19, 20, 23). Similarly, it may explain the similar kinetic properties of RRR- and all-rac when administered separately because in competitive studies, the 2S form was eliminated faster than RRR (35). In their review of the literature, Hoppe and Krennrich (24) found that the ratio in plasma responses comparing supplement doses of unlabeled RRR- or all-rac-α-tocopherol in 9 studies matched the presently accepted biopotency ratio of 1.36, whereas only recent bioavailability studies applying the competitive approach of simultaneous administration with deuterated α-tocopherol acetates, found ratios > 1.36:1, and closer to 2:1 (24).

The ratio RRR:all-rac increased to a maximum at 12 h. At time points before this, ratios close to 1:1 represent nondiscrimination between forms of vitamin E during absorption and chylomicron transport before first pass through the liver (15). Hepatic processing of vitamin E forms represents the major route of discrimination; however, because vitamin E can transfer between lipoproteins (36), especially during chylomicron metabolism, then it is possible that some forms bypass this discriminatory process and persist in the plasma, adding variation to the results.

It is important to note that in the present study, we chose to investigate subjects carrying the apoE4 genotype. This common polymorphism, present in 25% of the population, is associated with increased risk of cardiovascular disease (5, 6) which has been attributed to a reduced antioxidant status in these individuals. It was suggested that there is value in genotyping subjects for apoE in relation to vitamin E supplementation because apoE4 carriers could potentially benefit from such supplementation (10). We initially hypothesized that prospectively choosing only apoE4 individuals would have the advantage of potentially limiting interindividual variation in vitamin E responses. However, a large interindividual variation remained in individual biokinetic responses and bioavailability ratios, with certain subjects displaying similar or even increased bioavailability for the synthetic form. It is likely that a number of physiologic and/or genetic factors are important in influencing vitamin E status as we suggested recently (37, 38).

The present study consisted of two 4-wk supplementation regimens with either natural or synthetic α-tocopherol acetate (400 mg/d) in a crossover design, separated by a 12-wk washout period. This washout period was clearly sufficient, given that the baseline unlabeled concentrations were similar before each regimen (Table 1). After supplementation, the total plasma unlabeled α-tocopherol concentration increased significantly after both RRR- and all-rac-α-tocopherol acetate supplementation. However, postsupplementation α-tocopherol concentrations did not differ. Plasma vitamin E concentrations are limiting, in that they reach saturation kinetics and cannot be raised further than 2 to 3-fold regardless of the supplementation regimen (34, 39, 40). This can be explained in part by the rapid displacement of existing or α-tocopherol by newly absorbed α-tocopherol (34). This was shown in the present study as a decrease in unlabeled α-tocopherol concentrations in response to the increase in deuterated α-tocopherol concentration (Fig. 2). The present study design allows, for the first time, a direct comparison of the uptake of newly absorbed α-tocopherol both at baseline and after vitamin E supplementation. The supplementation regimen used (400 mg/d for 4 wk) should be sufficient to demonstrate steady-state plasma vitamin E concentrations after plasma vitamin E saturation, which reaches Cmax after 5 half-lives or 10–15 d (24). For example, Dimitrov et al. (40) supplemented subjects with 440, 880, or 1320 mg/d of synthetic α-tocopherol acetate consecutively for 28 d. Steady-state plasma α-tocopherol saturation occurred after 10, 5, and 5 d, respectively. Biokinetic studies supplementing 300 mg/d of a combination of natural and synthetic α-tocopherol acetate for either 8 (20) or 10 (19) consecutive days resulted in steady-state concentrations after 6 d (19, 20). Even though a half-life of 34–45 h, as found in the present study, appears to represent a slow plasma turnover of α-tocopherol, Traber et al. (35) demonstrated that there is rapid recycling of α-tocopherol due to the constant resecretion in VLDL which is on the order of 1.4 pools/d (∼74 μmol/d) and this results in the longer half-life (35). We found that after vitamin E supplementation, the plasma has a diminished ability to take up newly absorbed α-tocopherol. This was shown by the decreased deuterated α-tocopherol concentrations postsupplementation, the decreased extent of labeling postsupplementation, and the decreased area under the labeled α-tocopherol concentration vs. time curves (Table 1).

It is clear that α-TTP plays an important role in regulating plasma concentrations of vitamin E. It is likely that the decreased bioavailability of newly absorbed α-tocopherol after vitamin E supplementation observed in the current study is due to the saturation of α-TTP, such that less is available for systemic distribution via VLDL, and presumably more is metabolized. Because this effect was observed after both natural and synthetic supplementation and biokinetics, this shows that synthetic vitamin E contained enough of the preferred 2R-forms to saturate α-TTP. Indeed, this explains the observation that supplementation with synthetic vitamin E can result in similar plasma vitamin E concentrations compared with supplementation with natural source vitamin E, as observed in the present study and by others (41, 42). However, because the ratio of RRR:all-rac increased to 1.5:1 postsupplementation with vitamin E, it appears that the body discriminates inversely with natural α-tocopherol in saturating conditions, i.e., at high intakes. It is also possible that the dose of deuterated α-tocopherol administered influences biokinetics. Traber et al. (34) found a linear relation in labeled α-tocopherol dose (up to 150 mg) and AUC, suggesting that increasing dose does not decrease incorporation into plasma. However, higher doses may influence incorporation into plasma if the dose saturates α-TTP. This would also reduce the amount of newly absorbed released into the circulation as was observed after the supplementation regimen in the present study.

Alternatively, the concept that vitamin E status regulates α-TTP protein concentrations or activity might also explain this observation. In vitamin E-deficient rats, supplementation with both α- and δ-tocopherol increased hepatic concentrations of α-TTP mRNA (43). Unfortunately, there is very little information on rate of tocopherol-binding proteins, but it is certainly an area for further investigation.

In summary, our findings demonstrate that when studied with a noncompetitive approach under basal conditions, natural and synthetic α-tocopherol are kinetically equivalent and their relative bioavailability conforms to the currently accepted biopotency ratio of 1.36:1 in men with an apoE4 genotype. We also demonstrated that vitamin E biokinetics are influenced by vitamin E status, which becomes limiting after high vitamin E intakes, independently of the form used. In light of the findings of our study and those of others, we feel that the noncompetitive approach is an appropriate method with which to study the relative bioavailabilities of natural and synthetic α-tocopherol under normal conditions. However, caution should be observed in drawing conclusions on relative biopotency based on relative bioavailability rather than on in vivo surrogate markers of vitamin E status.
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LITERATURE CITED


