Vitamin E dose-response studies in humans with use of deuterated RRR-α-tocopherol1–3

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ABSTRACT
Background: Supplemental vitamin E does not raise plasma α-tocopherol concentrations more than ≈3-fold.
Objective: To elucidate the mechanism for the limitation in plasma α-tocopherol, we undertook human supplementation trials using incrementally increased doses of deuterated vitamin E.
Design: Plasma was obtained from 6 healthy, young adults (4 men and 2 women) during 3 sequential supplementation trials with doses of 15, 75, and 150 mg RRR-α-tocopheryl acetate labeled with deuterium (d3-RRR-α-tocopheryl acetate). A defined diet was provided on the day of deuterated vitamin E administration, but otherwise subjects ate ad libitum.
Results: The areas under the curves calculated from the plasma d3-RRR-α-tocopherol concentrations increased linearly with dose—a 10-fold increase in dose resulted in a 10-fold increase in area under the curve. d3-RRR-α-Tocopherol absorption and incorporation into plasma did not decrease with increasing dose. At 11 h, the 15-, 75-, and 150-mg doses resulted in 8 ± 4%, 21 ± 10%, and 37 ± 20% labeling, respectively, of plasma vitamin E. Plasma total (labeled plus unlabeled) α-tocopherol concentrations before supplementation were 12 ± 3 µmol/L and over the 96 h after the dose averaged 13.3 ± 2.6, 15.4 ± 3.0, and 16.7 ± 4.9 µmol/L for the 15-, 75-, and 150-mg doses, respectively.
Conclusions: d3-RRR-α-Tocopherol was incorporated into the plasma in preference to circulating plasma RRR-α-tocopherol. This could occur if the newly absorbed d3-RRR-α-tocopherol was preferentially used to replenish circulating vitamin E. Am J Clin Nutr 1998;68:847–53.

KEY WORDS Vitamin E supplements, lipoproteins, chylomicrons, stable isotopes, absorption, α-tocopherol transfer protein, adults, humans

INTRODUCTION
Plasma α-tocopherol concentrations in normal subjects can only be raised ≈2–3-fold, regardless of the duration, amount (>100 mg), or frequency of vitamin E supplementation (1–5). During oral vitamin E tolerance tests, in which 100 IU/kg body wt (≈5–7 g vitamin E) was administered, normal subjects’ serum α-tocopherol concentrations increased ≈4-fold above baseline at 6 h and decreased to 3-fold above baseline at 24 h (6, 7). This limitation in plasma vitamin E concentration could result if vitamin E absorption is limited. Studies in rodents suggest that only a fraction of the dose of tocopherol is absorbed and that the percentage absorption is inversely related to the size of the dose. For example, in studies of rats with cannulated thoracic ducts, ≈65% was absorbed when vitamin E was infused at a rate of 0.12 mg/h, whereas only 18% was absorbed when vitamin E was infused at higher rates (1.73 mg/h) (8). This limitation of vitamin E absorption occurred despite nearly complete absorption of simultaneously infused triacylglycerol (at a rate of 117 mg/h) (8).

Less information is available about vitamin E absorption in humans. In the early 1970s, vitamin E absorption was estimated to be 51–86%, measured as fecal radioactivity after ingestion of [3H]α-tocopherol (<1 mg unlabeled) (9, 10). However, when Blomstrand and Forsgren (11) cannulated the thoracic lymph ducts in 2 subjects with gastric carcinoma and lymphatic leukemia and measured vitamin E absorption, they found fractional absorption to be only 21% and 29%, respectively, of label from meals containing [3H]α-tocopherol and [3H]α-tocopheryl acetate, respectively. Methods of measuring vitamin E absorption are fraught with difficulties; it is uncertain whether complete collections were made. Fecal bacteria could have caused losses and increased apparent absorption, and incomplete collection of lymph probably also occurred. More recent studies comparing the bioavailability of α-tocopherol and α-tocopheryl acetate in humans using deuterium-labeled vitamin E have shown that deuterated α-tocopherol concentrations are similar irrespective of the form administered when given in equal doses, suggesting that there is no limitation of tocopheryl acetate hydrolysis (12).

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The mechanism for the limitation of plasma α-tocopherol concentrations observed in humans in response to increasing doses of supplemental vitamin E may not depend solely on decreased fractional absorption, but may involve, in addition, liver-mediated vitamin E transport. After absorption, vitamin E enters the circulation from the intestine in chylomicrons. During chylomicron lipolysis, vitamin E is distributed to all the circulating lipoproteins (13, 14). As the chylomicron contents are hydrolyzed, excess surface components are created and transferred to HDL. HDL can readily exchange surface components with other lipoproteins (15–17) and the plasma phospholipid transfer protein potentiates this activity (18). Simultaneously, lipoprotein lipase may act as a transfer protein for vitamin E by delivering it to tissues (19). Chylomicron remnants are taken up by the liver, which secretes the newly absorbed dietary lipids in VLDLs that are preferentially enriched in RRR-α-tocopherol (20).

The liver has a central role in maintaining plasma vitamin E concentrations (21) and in discriminating between the different homologues and stereoisomers of vitamin E. These functions are dependent on the hepatic α-tocopherol transfer protein (α-TTP) (22–25). A defect in the α-TTP gene is associated with) impaired incorporation of RRR-α-tocopherol into nascent VLDL (26), 2) impaired ability to discriminate between RRR- and SRR-α-tocopherols (27), and 3) a severe neurologic disorder resembling Friedreich ataxia (28, 29). Retinitis pigmentosa has also been observed in patients with α-TTP gene defects (30). This disorder, originally called familial isolated vitamin E deficiency, is now called ataxia with vitamin E deficiency. The disorder is due to a mutation in the gene for α-TTP on chromosome 8 (31). The most frequently reported α-TTP gene defect in patients with ataxia with vitamin E deficiency is a deletion of the terminal portion of the protein (31, 32).

Vitamin E biological activity has been suggested to be dependent on the ability of α-TTP to bind and transfer the various forms of vitamin E (33). Taken together with the role of α-TTP in maintaining plasma vitamin E concentrations, this suggests that α-TTP is also involved in limiting plasma vitamin E concentrations in response to supplemental vitamin E (21).

To evaluate the mechanism for the limitation of plasma α-tocopherol concentrations during vitamin E supplementation, 3 trials using increasing doses of 15, 75, and 150 mg RRR-α-tocopheryl acetate labeled with deuterium (d₃-RRR-α-tocopherol) were carried out sequentially in normal subjects. The unlabeled and deuterated α-tocopherol contents of plasma, chylomicrons, and lipoproteins were measured to evaluate the efficacy of supplementation.

### SUBJECTS AND METHODS

#### Deuterated tocophers

Deuterated vitamin E (2R,4'R,8'R-α-5[C₂H₃]α-tocopheryl acetate (d₃-RRR-α-tocopherol acetate) was synthesized by Eastman Chemical Products, Inc (Kingsport, TN). Plasma or lipoprotein vitamin E was extracted with heptane after saponification in the presence of 1% ascorbic acid in ethanolic potassium hydroxide at 70°C. The analysis of the deuterated and unlabeled α-tocopherols by gas chromatography–mass spectrometry after conversion to their trimethylsilyl ethers with quantitation by comparison with an added internal standard, 2-ambo-α-(5, 7, 8-[C₃H₃]₃)tocopherol, was described previously (13, 34–38) except that the column used was an Ultra 1 and an HP 5890 series II gas chromatograph connected to a 5970B mass-selective ion monitoring unit was used (all Hewlett-Packard, Palo Alto, CA).

#### Experimental protocol

This study was carried out with the approval of the Institutional Review Board of the New York University Medical Center and the Institutional Review Board of the National Institutes of Health. All subjects gave written, informed consent.

Six healthy, young adults (4 men and 2 women aged < 20 y) from the NIH Clinical Research Center Normal Volunteer Program, who were of normal height and weight without any known metabolic abnormalities, participated in this study (Table 1). The subjects were inpatients at the center during the study but their activities were unrestricted. Subjects ate ad libitum except for the metabolic diet they were fed on the day of deuterated vitamin E administration. The subjects were studied 2 at a time to permit timely lipoprotein separations. The 2 subjects were given the 15-mg dose, then plasma sampling was carried out. Two weeks later, the subjects were given the next higher dose, and so on for the highest dose. This was to guarantee that the deuterated plasma tocopherol concentrations decreased to unimportant amounts compared with the responses to the next higher dose.

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Subjects were given the dose with a standard breakfast containing 30 g fat. Blood samples (7 mL per time point) were drawn into EDTA-containing tubes (Becton Dickinson, Rutherford, NJ) at 0, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 21, 24, 27, 36, 48, 72, and 96 h after subjects consumed the deuterated α-tocopherol. Subjects were fitted with a peripheral intravenous line that was kept open by a slow infusion of saline and used for the repetitive blood drawing for the first 27 h. Breakfast was consumed between the 24- and 27-h blood samplings. Plasma was separated from blood cells by centrifugation at 2000 × g for 5 min at 4°C. An aliquot of plasma was immediately frozen at −70°C, while the remainder of the plasma was used for lipoprotein isolation as described previously (13).

Statistical analyses

Statistical analyses were carried out with SUPERANOVA (Abacus Concepts, Inc, Berkeley, CA). The statistical significance of the d3-α-tocopherol concentrations and the ratios of d3-α-tocopherol to total α-tocopherol in plasma and each of the lipoprotein fractions in response to the different doses was determined by using analysis of variance (ANOVA) with repeated measures [one factor (time) and one factor (dose) within groups]; main effects are reported. Areas under the plasma d3-α-tocopherol concentration curves (AUCs) were calculated by using KALAIDAGRAPH (Synergy Software, Reading, PA). For AUCs and rates, ANOVA with repeated measures [one factor (dose) within groups] and least-square mean analyses were used for comparisons. Results were considered to be significant at the 95% confidence level (P < 0.05).

RESULTS

Deuterated tocopherol concentrations in the subjects increased significantly (main effect, P < 0.001) in plasma with dose size (Figure 1) and in each of the lipoprotein fractions (Figure 2). To estimate the responses to increasing doses of deuterated vitamin E, the AUCs of the d3-RRR-α-tocopherol concentrations were calculated for each individual for each of the 3 doses (15, 75, and 150 mg) (Figure 3). On average, there was a linear increase with increasing dose—a 10-fold increase in dose resulted in a 10-fold increase in the AUC. Subject 3 was an exception and it is not clear why the AUC for the 150-mg dose did not increase appreciably in response to large doses of supplemental vitamin E. Calculation of the AUCs and rates, ANOVA with repeated measures [one factor (dose) within groups] and least-square mean analyses were used for comparisons. Results were considered to be significant at the 95% confidence level (P < 0.05).

FIGURE 4. Mean (±SD) plasma deuterated (d3-) RRR-α-tocopherol concentrations in 3 trials after subjects consumed single doses containing 15, 75, or 150 mg d3-RRR-α-tocopheryl acetate with breakfast; n = 6, except subject 1 dropped out of the 150-mg trial. Plasma d3-RRR-α-tocopherol was measured by gas chromatography–mass spectrometry as described in Methods. There were significant main effects of dose, P < 0.0001, and time, P < 0.0002, and a significant interaction, P < 0.05 (ANOVA).
this experiment did not allow estimation of the absorptive rate, the linear increase in chylomicron d3-RRR-\(\alpha\)-tocopherol AUC accords with this statement. These data suggest that incorporation of vitamin E into chylomicrons and subsequent secretion into plasma does not decrease with increasing dose size up to 150 mg. However, the observation that plasma total \(\alpha\)-tocopherol does not similarly increase shows that a limitation in the maximum plasma \(\alpha\)-tocopherol concentration exists (Figure 4). This maximum plasma \(\alpha\)-tocopherol concentration was also postulated on the basis of the urinary excretion of a vitamin E metabolite [2,5,7,8-tetramethyl-2(2'-carboxyethyl)-6-hydroxychroman], which was found to increase with supplemental vitamin E intakes >50 mg/d (39).

Plasma \(\alpha\)-tocopherol half-lives in normal, unsupplemented subjects were estimated to be 47 ± 22 h in our previous report (40). This relatively slow turnover was dependent on liver secretion of \(\approx 0.8 \pm 0.6\) plasma \(\alpha\)-tocopherol pools. We observed that the plasma \(\alpha\)-tocopherol concentration (25 \(\mu\)mol/L) of a typical, unsupplemented subject (70 kg) was calculated to be a plasma \(\alpha\)-tocopherol pool of \(\approx 100 \mu\)mol (or 40 mg) based on an estimated plasma volume of 4 L. The rate of secretion by the liver of 0.8 \(\alpha\)-tocopherol pools is \(\approx 74 \mu\)mol/d or \(\approx 32\) mg/d. This amount far exceeds the amount of vitamin E consumed [eg, the

FIGURE 2. Mean (±SD) chylomicron, VLDL, LDL, and HDL deuterated (d3-) RRR-\(\alpha\)-tocopherol concentrations after increasing doses of deuterated vitamin E. Plasma lipoproteins were isolated from subjects (n = 6, except for 150-mg dose when n = 5), and d3-RRR-\(\alpha\)-tocopherol was measured by gas chromatography–mass spectrometry as described in Methods. There were significant main effects of dose and time (ANOVA): chylomicrons: dose, \(P < 0.0001\); time, \(P = 0.002\); and interaction, \(P = 0.0001\); VLDL: dose, \(P < 0.0001\); time, \(P = 0.004\); and interaction, \(P = 0.01\); LDL and HDL: dose, \(P < 0.0001\); time, \(P = 0.0001\); and interaction, \(P = 0.0005\).

FIGURE 3. Plasma deuterated (d3-) RRR-\(\alpha\)-tocopherol areas under the curve (AUCs) after increasing doses (15, 75, or 150 mg d3-RRR-\(\alpha\)-tocopheryl acetate) of deuterated vitamin E. The data from the 6 subjects (symbols 1–6) described in Table 1 were used to calculate the AUCs for each dose. Plasma d3-RRR-\(\alpha\)-tocopherol AUC increased linearly with d3-RRR-\(\alpha\)-tocopheryl acetate dose size (\(r^2 = 0.999\)).
recommended dietary allowance of 10 mg α-tocopherol (41) and emphasizes the importance of hepatic α-TTP in salvaging and recycling vitamin E that is delivered to the liver.

Cohn et al (42) showed in intact rats and in isolated rat hepatocytes that vitamin E is secreted from the liver in VLDL. Previously, we also showed in isolated perfused monkey livers that VLDLs are secreted from the liver enriched in RRR-α-tocopherol (20). It is likely that VLDLs are the only lipoproteins that are involved in vitamin E secretion from the liver because LDLs are
of the circulating pool of vitamin E discussed above. Amin E was deuterated on the first day, 80% on the third day, and also for 8 consecutive days and found that 50% of the plasma vitamin E was found first in chylomicrons and then in VLDLs and other plasma lipoproteins. This could occur if newly absorbed deuterated vitamin E is preferentially transferred to the liver in chylomicron remnants and then in VLDLs and other plasma lipoproteins. The mechanism by which α-TTP carries out this function is unknown and appears to be independent of the direct incorporation of α-tocopherol in triacylglycerol-rich lipoproteins (46). However, α-TTP is required to maintain plasma vitamin E concentrations because patients with an α-TTP gene defect (31) have impaired α-tocopherol secretion in VLDL (26, 27) and become vitamin E deficient. Once the α-tocopherol–enriched VLDLs are secreted into the circulation, the normal mechanisms of lipoprotein metabolism, as well as the ready exchange of vitamin E between lipoproteins, distributes the vitamin E to all of the circulating lipoproteins, as discussed in the Introduction.

The finding that the percentage of deuterated vitamin E increased with increasing deuterated vitamin E dose (Figure 5) without an equivalent increase in the total α-tocopherol concentration (Figure 4), suggests that the new vitamin E replaces the old. The rapidity with which large doses of deuterated vitamin E become the predominant plasma vitamin E form was also observed by Burton et al (47), who administered 150 mg each of RRR- and all-rac-α-tocopheryl acetate to normal individuals for 8 consecutive days and found that 50% of the plasma vitamin E was deuterated on the first day, 80% on the third day, and then a rapid decrease in the percentage deuterated on cessation of dosing. These data are consistent with the daily replacement of the circulating pool of vitamin E discussed above.

Plasma deuterated α-tocopherol disappearance rates also increased with increasing dose (Figure 7). One possible explanation for these findings is that part of the plasma α-tocopherol increase after administration of large vitamin E doses is nonspecific, and that these higher plasma concentrations are not maintained. Thus, in response to large vitamin E doses, α-tocopherol is distributed during chylomicron catabolism to the circulating lipoproteins and raises plasma α-tocopherol concentrations nonspecifically; subsequently, during the normal course of lipoprotein metabolism as the α-tocopherol leaves the plasma and is taken up by the liver, only a limited amount can be resecreted into the plasma and plasma α-tocopherol concentrations fall (40).

Epidemiologic studies have shown increased protection against coronary artery disease in subjects consuming vitamin E in doses > 100 mg taken for ≥ 2 y (48, 49). Furthermore, large doses of vitamin E (400 or 800 mg) were found to decrease risk of heart attacks by 77% in patients with proven heart disease (50), whereas 50-mg doses were ineffective (51). However, 50-mg vitamin E supplements were found to be protective against prostate cancer (52). The present study, using deuterated α-tocopherol, suggests that the protective effects of supplemental vitamin E may be a result of overriding the effects of α-TTP, resulting in increased plasma incorporation of newly absorbed α-tocopherol. These observations merit further investigation.

In summary, this study shows that there is no apparent limitation in vitamin E absorption by humans consuming doses ≤ 150 mg RRR-α-tocopheryl acetate. Furthermore, the data show that new vitamin E replaces old. That is, plasma α-tocopherol concentrations do not increase linearly with dose size because the newly absorbed vitamin E in part replaces the α-tocopherol in circulating lipoproteins.

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REFERENCES


