Biodiscrimination of α-tocopherol stereoisomers in humans after oral administration¹-³

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ABSTRACT We investigated changes in the concentrations of the stereoisomers of α-tocopherol in serum and lipoproteins in seven normal, healthy women aged 21–37 y who had received oral administration of natural and synthetic α-tocopherol acetate. This study was conducted in three separate periods of 28 d each; there was a 3-mo washout period between each experimental period. During the first period the subjects were administered a daily dose of 100 mg RRR-α-tocopherol/d, whereas in the second and third periods 100 mg all-rac-α-tocopherol acetate/d and 300 mg all-rac-α-tocopherol acetate/d were given, respectively. Blood samples were collected 3 d before each treatment and 1, 3, 7, 14, and 28 d after treatment. α-Tocopherol stereoisomer concentrations in serum and lipoproteins (very-low-, low-, and high-density lipoproteins) were determined by the chiral HPLC method. The bioavailability of RRR-α-tocopherol was greater than that of all-rac-α-tocopherol acetate. When bioavailability was estimated from the increase in the concentration of RRR- or all-rac-α-tocopherol in serum, bioavailability of RRR-α-tocopherol administered at 100 mg/d was not different from that of all-rac-α-tocopherol acetate administered at 300 mg/d. 2R-Isomers and small amounts of 2S-isomers were detected in the serum lipoproteins of subjects administered all-rac-α-tocopherol acetate. Am J Clin Nutr 1997;65:785–9.

KEY WORDS RRR-α-tocopherol, all-rac-α-tocopherol, α-tocopherol stereoisomers, bioavailability, biodiscrimination, lipoprotein

INTRODUCTION

Synthetic α-tocopherol (all-rac-α-tocopherol) is used in many types of medical preparations and food additives and is composed of equal amounts of the eight different stereoisomers of α-tocopherol arising from the three asymmetric carbons (2-position in chroman ring and 4' and 8' positions in side chain tail). Of the eight stereoisomers, only the 2R,4'R,8'R (RRR) stereoisomer occurs naturally. Biological activities of the eight stereoisomers of α-tocopherol were determined by the rat fetal resorption assay to be as follows: RRR = 100%, RRS = 90%, RSS = 73%, SSS = 60%, RSR = 57%, SRS = 37%, SSR = 31%, and SSR = 21% (1). The biological activities of the four 2R-isomers are generally higher than those of the four 2S-isomers. Biological activity of the 4'R-isomers are higher than those of the corresponding 4'S-isomers. The 8'-configuration has the smallest influence on biological activity compared with the other two chiral positions of the α-tocopherol. Traber et al (2) and Ingold et al (3) synthesized deuterium-substituted RRR- and SRR-α-tocopherol, in which the number of deuterium atoms was varied to distinguish them, and the absorption, transportation, and biodiscrimination of RRR- and SRR-α-tocopherol were elucidated.

In the presence of bile acids and hydrolyzed products of fats produced by pancreatic lipase, α-tocopherol is absorbed from the small intestine into the lymph ducts in the form of chylomicrons (2, 4, 5). Chylomicrons are catabolized in the blood circulation by lipoprotein lipase, which forms chylomicron remnants, and the α-tocopherol incorporated in the liver is secreted from liver into plasma as very-low-density lipoprotein (VLDL). Hydrolysis of VLDL by lipoprotein lipase delivers fatty acids and α-tocopherol to tissues and yields low-density lipoprotein (LDL).

Moreover, with respect to the bioavailability and biopotency of α-tocopherol stereoisomers, several reports suggest differences among the absorption of various forms of vitamin E compounds in humans. Acuff et al (6) evaluated the relative bioavailability of RRR- and all-rac-α-tocopherol acetate using deuterium label (d3-RRR- and d6-all-rac-α-tocopherol acetate) and concluded that the ratio of bioavailability of RRR- to all-rac-α-tocopherol acetate is significantly greater than 1.36, the currently accepted recommended dietary allowance (RDA) in the United States. In these studies, the gas chromatography mass spectrometry–selected ion-monitoring method was used to distinguish these isomers.

Ueda et al (7) developed the newly revised chiral HPLC method to separate the eight stereoisomers of unlabeled α-tocopherol acetate into four peaks. With this method it is possible to separate all-rac-α-tocopherol acetate into 2R- and 2S-isomers. Using this method we reported previously the distribution of α-tocopherol stereoisomers in the tissues of rats fed diets containing different concentrations of all-rac-α-tocopherol acetate for 8 wk. The concentrations of 2R-isomers of α-tocopherol in the blood and tissues of rats fed all-rac-α-...
tocopheryl acetate were significantly higher than those of 25-isomers. In most tissues, the concentration of 25-isomers was in the following order: SRS > (SSS + SSR)/2 > SRR (8, 9).

In this experiment, we investigated the time-dependent changes in the concentration of α-tocopherol stereoisomers in serum and lipoproteins after oral administration of RRR- or all-rac-α-tocopherol acetate to normal, healthy humans by using the chiral HPLC method.

SUBJECTS AND METHODS

Subjects

Seven healthy female subjects (average body weight: 50.1 ± 6.3 kg) with no abnormalities of lipid or lipoprotein metabolism volunteered for the study. The subjects were aged 21–37 y and none used vitamin supplements. All subjects gave informed consent and the study was carried out under the guidelines established by the Center for Health Care of Ochanomizu University.

Protocol

The studies were performed in three separate 28-d periods. During the first 28-d period, subjects were administered orally one soft-gelatin capsule of 100 mg RRR-α-tocopherol/d after dinner, followed by a 3-mo washout period before the second period began. During the second 28-d period, subjects were administered orally two tablets each containing 50 mg all-rac-α-tocopheryl acetate/d after dinner (daily dose of 100 mg), followed again by a 3-mo washout period before the next period. During the third 28-d period, subjects were administered orally six 50-mg tablets/d: three tablets after lunch and three after dinner (300 mg all-rac-α-tocopheryl acetate/d).

During all periods, blood samples (20 mL) were collected in the morning 2 h after breakfast 3 d before treatment and 1, 3, 7, 14, and 28 d after treatment began. Serum was immediately ultracentrifuged at 600 × g for 10 min at 4 °C for the isolation of lipoproteins. Serum was stored at 4 °C until the lipoprotein fractions were isolated.

Methods

Lipoproteins [VLDL, LDL, and high-density lipoprotein (HDL)] were isolated with an L-80 ultracentrifuge (Beckman Instruments, Inc, Tokyo) by using a modification of the technique of Havel et al (10). Serum (6 mL) was overlaid with 2 mL plasma density buffer (d = 1.006 kg/L; 10 mmol Tris-HCl/L, 0.9% NaCl, 1 mmol Na2-EDTA/L, 0.01% NaN3, pH 7.4) in polycarbonate centrifuge bottles and centrifuged with a 50Ti rotor for 18 h at 100,000 × g in the L-80 ultracentrifuge at 4 °C; 1 mL of the upper VLDL fractions was collected (d < 1.006 kg/L). The bottom fraction was adjusted by adding solid potassium bromide and overlaid with 2 mL density buffer (d = 1.063 kg/L). These samples were then centrifuged at 106,000 × g for 18 h at 4 °C; 1 mL of the upper LDL fractions was collected (1.006 kg/L < d < 1.063 kg/L). The bottom fraction was adjusted by adding solid potassium bromide and overlaid with 2 mL density buffer (d = 1.21 kg/L) and the samples were then centrifuged at 106,000 × g for 18 h at 4 °C; 1 mL of the upper HDL fractions was collected (1.063 kg/L < d < 1.21 kg/L). The lipoprotein fractions were frozen and stored at −80 °C until analyzed. Cholesterol, triacylglycerols, and phospholipids in serum and each lipoprotein fraction were determined with a 7150 Auto-Analyzer (Hitachi Co, Tokyo).

The stereoisomeric composition of α-tocopherol in the serum and lipoproteins was analyzed by using the method of Ueda et al (7) as follows. Six percent ethanolic pyrogallol was added to each sample. After a 60% KOH solution was added to each of the sample tubes, the contents were saponified with occasional shaking at 70 °C for 30 min. After the sample was cooled in ice water, a 1% NaCl solution was added to the saponified medium and the medium was shaken vigorously with 10% ethyl acetate in hexane for 1 min. The mixture was then centrifuged at 600 × g for 5 min at 4 °C. The upper layer was collected to extract all stereoisomers of α-tocopherol. Extracted α-tocopherol was acetylated with dry pyridine and acetic anhydride at 50 °C for 15 min for chiral HPLC analysis. After the sample was cooled to room temperature, a 1% NaCl solution was added and the whole medium was extracted with hexane. The hexane layer was then collected and evaporated under a stream of nitrogen gas. The residue was dissolved in methanol:water (98:2, by vol) for determination of α-tocopherol stereoisomers by HPLC. Analysis of the obtained acetate of α-tocopherol stereoisomers was performed by HPLC. The HPLC apparatus consisted of a Waters LC Module 1 (Japan Millipore Ltd, Tokyo) and an SK-25 column oven (Nihon Seimitsu Kagaku, Co, Ltd, Tokyo). The analysis of α-tocopherol stereoisomers was performed at 38 °C by using a Chiralpak OP(+) column (250 × 4.6 mm internal diameter; Daicel Chemical Industries, Tokyo). The mobile phase was methanol:water (98:2, by vol) at a flow rate of 0.48 mL/min. The detection wavelength for tocopheryl acetate was 284 nm. For the determination of α-tocopheryl acetate, external calibration methods were used.

Statistical analysis

All results were expressed as means ± SDs. Paired Student's t tests were used for comparison of 25-isomers of α-tocopherol concentrations between LDL and HDL. Two-way analysis of variance (ANOVA) was used for comparison of 2R- and 25-isomers of α-tocopherol concentrations among each lipoprotein fraction. Differences were considered significant at P < 0.05.

RESULTS

Mean serum α-tocopherol concentration-time profiles are illustrated in Figure 1. The serum RRR-α-tocopherol concentration of all subjects increased up to 7 d during administration and reached a plateau after 7 d, an increase of ≈1.6-fold compared with baseline. In contrast, the serum all-rac-α-tocopherol concentrations of the subjects administered 100 mg all-rac-α-tocopheryl acetate/d did not change significantly during administration.

Serum concentrations of all-rac-α-tocopherol in the subjects administered 300 mg all-rac-α-tocopheryl acetate/d increased ≈1.6-fold by the 7th day after administration compared with baseline. This result suggests that the bioavailability of all-rac-α-tocopherol administered at a dosage of 300 mg all-rac-α-tocopheryl acetate/d was not significantly different from that of RRR-α-tocopherol given at 100 mg/d. The concentrations of total cholesterol, phospholipids, and triacylglycerols in serum
and lipoproteins were not significantly different during the experimental phase (data not shown).

Changes in the concentration of α-tocopherol stereoisomers in each lipoprotein of subjects administered 100 mg all-rac-α-tocopheryl acetate/d are shown in Figure 2. In all lipoproteins, only 2R-isomers were detected; no 2S-isomers were found. Of the serum lipoprotein fractions, HDL contained the largest proportion of circulating 2R-isomers.

As in subjects given 100 mg all-rac-α-tocopheryl acetate/d, the only α-tocopherol stereoisomers that were detected in the whole serum of subjects administered 300 mg all-rac-α-tocopheryl acetate/d were 2R-isomers; however, small amounts of 2S-isomers were also detected in separated lipoprotein fractions (Figure 3). Because we detected up to 20% of all α-tocopherol as 2S-isomers in the LDL and HDL fractions, we suspected that 2S-isomers were present in whole serum. However, the volume of whole serum specimens used (0.2 mL) may have been too small for determining 2S-isomers compared with lipoproteins separated from 6 mL serum. After oral administration, the concentration of 2S-isomers increased gradually but was significantly lower than that of 2R-isomers. The change in the ratio of α-tocopherol stereoisomers in LDL is shown in Figure 4. 2S-Isomers comprised ~4% of all-rac-α-tocopherol in LDL on the first day after administration. On day 28 after administration, the ratio occupied by 2S-isomers in whole serum increased to ~20% of α-tocopherol in LDL. The change in the ratio of α-tocopherol stereoisomers in HDL is shown in Figure 5. The concentration of 2S-isomers in HDL was significantly higher than that in LDL on days 1, 7, and 14 after administration (Table 1). In LDL and HDL the concentrations of 2S-isomers were in the order (SSS + SSR)/2 > SRS > SRR.

**DISCUSSION**

We investigated the relative bioavailability of RRR-α-tocopherol and all-rac-α-tocopheryl acetate and the changes in the concentrations α-tocopherol stereoisomers and lipoproteins in human subjects after oral administration of both these forms of tocopherol.

Several studies have reported the relative bioavailability of RRR- and all-rac-α-tocopheryl acetate in humans (11, 12). All results suggest that the bioavailability of RRR-α-tocopherol is significantly greater than that of all-rac-α-tocopheryl acetate. Our data (Figures 2 and 3) suggest that the difference between the bioavailability of RRR- and all-rac-α-tocopherol in serum is due to the limited utilization of the 2S-isomers of all-rac-α-tocopherol.

![Figure 1](image1.png)

**FIGURE 1.** Changes in serum concentrations of α-tocopherol in serum after oral administration of 100 mg RRR-α-tocopherol/d and 100 and 300 mg all-rac-α-tocopheryl acetate/d. I ± SD; n = 7 women.

![Figure 2](image2.png)

**FIGURE 2.** Changes in serum concentration of 2R- and 2S-isomers of α-tocopherol in lipoprotein fractions after oral administration of 100 mg all-rac-α-tocopheryl acetate/d. I ± SD; n = 7 women.
Although the concentrations of 25-isomers of α-tocopherol in LDL after oral administration (Figure 4) increased gradually, they were significantly lower than those of 2R-isomers. 25-Isomers comprised only ≈20% of the total up to 28 d after administration. Therefore, only small amounts of the 25-isomers of α-tocopherol were transported from liver into peripheral tissues.

Traber et al (13, 14) synthesized deuterium-substituted RRR- and SRR-α-tocopherol in which the substituted numbers of deuterium isomers were varied to distinguish them, and RRR- and SRR-α-tocopherol isomers were differentiated in the lipoprotein subfraction in blood of healthy humans and patients with familial isolated vitamin E deficiency. In VLDL, LDL, and HDL, the d6-RRR-fraction was significantly greater than the d3-SRR-fraction. They concluded that biodiscrimination of the α-tocopherol isomers might occur when VLDL is excreted to transport lipids with α-tocopherol from the liver and assumed that this mechanism may be facilitated by a tocopherol-binding protein. Sato et al (15) reported that they purified two isomers of rat tocopherol transfer proteins (α-TTP), which discriminate predominantly α-tocopherol among α-, β-, γ-, and δ-tocopherol analogues during transfer between liposomes and mitochondria. Arita et al (16) determined the presence of α-TTP in human liver by isolating its cDNA from a human liver cDNA library, which showed a 94% similarity in amino acid sequence to that of rat α-TTP.

Therefore, we also suggest that biodiscrimination of α-tocopherol stereoisomers is performed by α-TTP, preferentially recognizing 2R-isomers rather than 25-isomers in liver cytosol. The concentration of 25-isomers in HDL was significantly higher than that in LDL (Table 1). This result is supported by the report of Ogihara et al (17) that α-tocopherol in LDL is mostly distributed in lipoprotein fractions in males, whereas in females α-tocopherol in HDL is more common.

The bioavailability of RRR-α-tocopherol was similar to that of all-rac-α-tocopherol administered at three times the dose of RRR-α-tocopherol. The proportion of 25-isomers in LDL and HDL was in the order (SSS + SSR)/2 > SRS > SRR (Figures 4 and 5). This result is similar to the difference in biological activity of α-tocopherol stereoisomers. Because the RRR-, RRS-, RSR-, and RSS-isomers were not separated by our HPLC method, we could not compare the distribution of the RRR-, RRS-, RSR-, and RSS-isomers. Thus, it will be essential to investigate the concentration of isomers other than RRR-α-
**TABLE 1**

Concentration of 2S-isomers of α-tocopherol in lipoprotein fractions of subjects administered 300 mg all-rac-α-tocopheryl acetate/d on days 1, 3, 7, 14, and 28 after administration

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>0.30 ± 0.24</td>
<td>0.16 ± 0.21</td>
<td>0.68 ± 0.61</td>
<td>1.24 ± 0.56</td>
<td>1.51 ± 0.73</td>
</tr>
<tr>
<td>HDL</td>
<td>1.57 ± 1.20</td>
<td>1.37 ± 1.72</td>
<td>2.64 ± 0.82</td>
<td>2.59 ± 0.89</td>
<td>2.09 ± 1.02</td>
</tr>
</tbody>
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*Note: Data are given as mean ± SD; n = 7 women.*

α-tocopherol. Because it is clear from our data that RRR-α-tocopherol has a bioavailability almost three times higher than that of all-rac-α-tocopheryl acetate, RRR-α-tocopherol is preferable for the treatment and prevention of disease.

We conclude that the bioavailability of RRR-α-tocopherol was greater than that of all-rac-α-tocopheryl acetate because the 2R-isomers were preferentially incorporated in the serum lipoproteins of subjects administered all-rac-α-tocopherol. This result suggests that the tocopherol-binding protein in liver cytosol discriminates between 2R- and 2S-isomers and preferentially secretes 2R-isomers into VLDL.

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**REFERENCES**


