Biodiscrimination of α-tocopherol stereoisomers in humans after oral administration\textsuperscript{1-3}

Chikako Kiyose, Riho Muramatsu, Yoshiko Kameyama, Tadahiko Ueda, and Osamu Igarashi

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-tocopheryl 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 \textit{RRR}-α-tocopherol/d, whereas in the second and third periods 100 mg \textit{all-rac}-α-tocopherol acetate/d and 300 mg \textit{all-rac}-α-tocopheryl 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 \textit{RRR}-α-tocopherol was greater than that of \textit{all-rac}-α-tocopherol acetate. When bioavailability was estimated from the increase in the concentration of \textit{RRR}- or \textit{all-rac}-α-tocopherol in serum, bioavailability of \textit{RRR}-α-tocopherol administered at 100 mg/d was not different from that of \textit{all-rac}-α-tocopheryl acetate administered at 300 mg/d. \textit{2R}-Isomers and small amounts of \textit{2S}-isomers were detected in the serum lipoproteins of subjects administered \textit{all-rac}-α-tocopherol acetate. \textit{Am J Clin Nutr} 1997;65:785–9.

KEY WORDS \textit{RRR}-α-tocopherol, \textit{all-rac}-α-tocopherol, α-tocopherol stereoisomers, bioavailability, biodiscrimination, lipoprotein

INTRODUCTION

Synthetic α-tocopherol (\textit{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 \textit{2R,4'R,8'R} (\textit{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: \textit{RRR} = 100%, \textit{RRS} = 90%, \textit{RSS} = 73%, \textit{SSS} = 60%, \textit{RSR} = 57%, \textit{SRS} = 37%, \textit{SRR} = 31%, and \textit{SSR} = 21% (1). The biological activities of the four \textit{2R}-isomers are generally higher than those of the four \textit{2S}-isomers and among \textit{2R}-isomers the biological activities of the \textit{4'R}-isomers are higher than the corresponding \textit{4'S}-isomers. The \textit{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 \textit{RRR}- and \textit{SRR}-α-tocopherol, in which the number of deuterium atoms was varied to distinguish them, and the absorption, transportation, and biodiscrimination of \textit{RRR}- and \textit{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 \textit{RRR}- and \textit{all-rac}-α-tocopheryl acetate using deuterium label (\textit{d3-RRR}- and \textit{d6-all-rac}-α-tocopheryl acetate) and concluded that the ratio of bioavailability of \textit{RRR}- to \textit{all-rac}-α-tocopheryl 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-tocopheryl acetate into four peaks. With this method it is possible to separate \textit{all-rac}-α-tocopheryl acetate into \textit{2R}- and \textit{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 \textit{all-rac}-α-tocopheryl acetate for 8 wk. The concentrations of \textit{2R}-isomers of α-tocopherol in the blood and tissues of rats fed \textit{all-rac}-α-
tocopheryl acetate were significantly higher than those of 2S-
isomers. In most tissues, the concentration of 2S-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-tocopheryl 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 metabo-
lism volunteered for the study. The subjects were aged 21–37
and none used vitamin supplements. All subjects gave in-
formed consent and the study was carried out under the guide-
lines 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 adminis-
tered orally six 50-mg tablets/d: three tablets after lunch and
two 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 tech-
nique 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 106 000 × 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
overlayered 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 ser-
um 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 and the medium was 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 layers were subsequently collected and evaporated
under a stream of nitrogen gas. The residue was dissolved in
methanol:water (98:2, by vol) for determination of α-tocoph-
erol 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-tocoph-
erol stereoisomers was performed at 38 °C by using a Chiral-
pack 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
r tests were used for comparison of 2S-isomers of α-tocopherol
concentrations between LDL and HDL. Two-way analysis of
variance (ANOVA) was used for comparison of 2R- and 2S-
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 concen-
tration 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 \(\alpha\)-tocopherol stereoisomers in each lipoprotein of subjects administered 100 mg \(\text{all-\text{rac}}\)-\(\alpha\)-tocopherol 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 \(\text{all-\text{rac}}\)-\(\alpha\)-tocopherol acetate/d, the only \(\alpha\)-tocopherol stereoisomers that were detected in the whole serum of subjects administered 300 mg \(\text{all-\text{rac}}\)-\(\alpha\)-tocopherol 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 \(\alpha\)-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 \(\alpha\)-tocopherol stereoisomers in LDL is shown in Figure 4. \(2S\)-Isomers comprised \(\approx 4\%\) of \(\text{all-\text{rac}}\)-\(\alpha\)-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 \(\approx 20\%\) of \(\alpha\)-tocopherol in LDL. The change in the ratio of \(\alpha\)-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 \(\text{RRR-} \alpha\)tocopherol and \(\text{all-\text{rac}}\)-\(\alpha\)-tocopherol acetate and the changes in the concentrations of \(\alpha\)-tocopherol stereoisomers and lipoproteins in human subjects after oral administration of both these forms of tocopherol.

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

![Figure 1](https://academic.oup.com/ajcn/article-abstract/65/3/785/4655297)  
**FIGURE 1.** Changes in serum concentrations of \(\alpha\)-tocopherol in serum after oral administration of 100 mg \(\text{RRR-} \alpha\)-tocopherol/d and 100 and 300 mg \(\text{all-\text{rac}}\)-\(\alpha\)-tocopherol acetate/d. \(\bar{x} \pm \text{SD}; n = 7 \text{ women.}\)

![Figure 2](https://academic.oup.com/ajcn/article-abstract/65/3/785/4655297)  
**FIGURE 2.** Changes in serum concentration of \(2R\)- and \(2S\)-isomers of \(\alpha\)-tocopherol in lipoprotein fractions after oral administration of 100 mg \(\text{all-\text{rac}}\)-\(\alpha\)-tocopherol acetate/d. \(\bar{x} \pm \text{SD}; n = 7 \text{ women.}\)
Although the concentrations of 2S-isomers of α-tocopherol in LDL after oral administration (Figure 4) increased gradually, they were significantly lower than those of 2R-isomers. 2S-isomers comprised only ~20% of the total up to 28 d after administration. Therefore, only small amounts of the 2S-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-TPP 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-TPP.

Therefore, we also suggest that biodiscrimination of α-tocopherol stereoisomers is performed by α-TTP, preferentially recognizing 2R-isomers rather than 2S-isomers in liver cytosol. The concentration of 2S-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 2S-isomers in LDL and HDL was in the order (SSS + SSR)/2 > SRS > SSR (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-...
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-tocopherol 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-tocopherol 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


