Dear Sir:

We read with great interest the publication of Kiyose et al. (1) concerning the biodiscrimination of a-tocopherol stereoisomers in humans after oral administration. Some readers may not be familiar with the stereochemistry of a-tocopherol. Its natural form consists of one stereoisomer, 2R,4′R,8′R-a-tocopherol (abbreviated RRR-a-tocopherol), whereas commonly used synthetic a-tocopherols have a 2RS,4′RS,8′RS configuration, also designated as all-rac-a-tocopherol. all-rac-a-Tocopherol and the corresponding esters contain 12.5% each of the RRR, RRS, RSR, RSR, RSS, SSS, and SSR stereoisomers. They have different relative biopotencies ranging from 100% for RRR to 21% for SSR (2). According to the US Pharmacopoeia (USP), RRR-a-tocopherol is 1.49 times more active and all-rac-a-tocopheryl acetate is 1.36 times more active than all-rac-a-tocopherol (3). We showed with rats that equipotent doses of all-rac-a-tocopheryl acetate or all-rac-a-tocopherol and the corresponding esters lead to equimolar plasma and tissue a-tocopherol concentrations; i.e., that the USP activity ratio is also valid as a tissue concentration ratio. Furthermore, the distributions of all eight individual a-tocopherol stereoisomers in plasma and tissues of rats treated with all-rac-a-tocopherol acetate were established (4,5).

Kiyose et al. (1) described the results of a study involving female volunteers treated daily with oral doses of 100 mg RRR-a-tocopherol, 100 mg all-rac-a-tocopherol acetate, or 300 mg all-rac-a-tocopheryl acetate for 28 days. In addition to serum concentrations of total a-tocopherol, a-tocopherol stereoisomers in different lipoprotein fractions were determined. However, the analytic method used did not permit the measurement of all stereoisomers individually. For example, the 2R stereoisomers (RRR, RRS, RSR, and SSR) are expressed only as sums. Accordingly, Kiyose et al. (1) could not measure which of the four 2R stereoisomers, and how much of each, was present in serum and lipoproteins. A comparison to lipoproteins of the group treated with RRR-a-tocopherol is not possible because the results are not shown.

Furthermore, Kiyose et al. (1) claimed that RRR-a-tocopherol has a bioavailability almost three times higher than that of all-rac-a-tocopherol acetate. This claim is based on the observation that treatment with 100 mg RRR-a-tocopherol (equal to 149 IU vitamin E) or 300 mg all-rac-a-tocopheryl acetate (equal to 300 IU vitamin E) resulted in similar final serum concentrations of total a-tocopherol ($\approx 50 \mu mol/L$), as shown in Figure 1 in reference 1. However, also shown in Figure 1 is that the mean pretreatment serum a-tocopherol concentrations were $\approx 35 \mu mol/L$ for the group receiving 100 mg RRR-a-tocopherol and $\approx 25 \mu mol/L$ for the group receiving 300 mg all-rac-a-tocopheryl acetate. In other words, the dose of 100 mg RRR-a-tocopherol increased mean serum a-tocopherol by $\approx 15 \mu mol/L$, whereas the 300-mg all-rac-a-tocopherol acetate dose raised serum concentrations by $\approx 25 \mu mol/L$.

Considering the relevant USP factor (1.49), 300 mg all-rac-a-tocopherol acetate should have led to a mean rise of serum a-tocopherol of $\approx 30 \mu mol/L$. The small difference between the theoretical and observed increases of serum a-tocopherol concentrations does not justify the above-mentioned claim of Kiyose et al. (1).

Furthermore, in contrast with animal studies with highly standardized experimental conditions, e.g., those in reference 4, bioavailability estimates from human data are hampered by a lack of relevant information such as the dietary habits, lifestyle factors, pretrial intakes, and tissue contents of a-tocopherol of the participants. Therefore, reliable bioavailability estimates require the application of sophisticated techniques to eliminate the influence of these unknowns. For example, Burton et al. (6) used differentially deuterated a-tocopherols and a-tocopheryl acetates and a gas chromatography-mass spectrometry combination to distinguish the effects of the deuterated compounds from those of nondeuterated a-tocopherol already present in the subjects. Cheeseman et al. (7) investigated the biokinetics of the free phenol, acetate ester, and succinate ester forms of RRR-a-tocopherol in humans, and Cohn (8) critically reviewed factors affecting the bioavailability of vitamin E.

Finally, the key data of Kiyose et al. (1) should have been presented as values, not simply as graphs, to permit readers a comprehensive evaluation of the claim. For example, Winklhofer-Roob et al. (9) published all the results required to show that increases of plasma a-tocopherol from baseline to week 6 were not significantly different after daily treatments with 400 IU RRR-a-tocopherol or two different all-rac-a-tocopheryl acetate preparations.

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of the eight stereoisomers of \( \alpha \)-tocopherol by chiral phase HPLC and capillary GC after isolation from tissues and plasma. Methods Enzymol 1994;234:302-10.


Reply to AW Kormann and H Bachmann

Dear Sir:

We thank Kormann and Bachmann for their comments on our manuscript (1). We conducted this experiment in 1994 using the analytic method of Ueda et al (2). This method measures \( \alpha \)-tocopherol stereoisomers as acetate derivatives with use of chiral phase HPLC. With use of this method, all-rac-\( \alpha \)-tocopheryl acetate was separated into four peaks. 2R isomers constituted the first peak and 2S isomers were separated into three peaks. Therefore, all-rac-\( \alpha \)-tocopheryl acetate was separated completely into 2R and 2S isomers. The main purpose of this study was to determine whether humans could discriminate between 2R and 2S isomers. Our animal data suggest that biodiscrimination between 2R and 2S isomers occurs in the liver, but not during absorption in the small intestine (3-5). According to our recent study (1), biodiscrimination of \( \alpha \)-tocopherol stereoisomers in humans was similar to that in rats.

Because each of the eight stereoisomers was not separated by Ueda et al’s method, we could compare the distribution of only 2R and 2S isomers in blood after oral administration. However, we consider measurement of the concentration of 2R and 2S isomers in human serum and lipoproteins to be valuable. Furthermore, we established that the bioavailability of RRR-\( \alpha \)-tocopherol administered in amounts of 100 mg/d was not different from that of all-rac-\( \alpha \)-tocopherol acetate administered in amounts of 300 mg/d when bioavailability was estimated from the increase in the concentration of RRR-\( \alpha \)-tocopherol and all-rac-\( \alpha \)-tocopheryl acetate in serum.

The published graph (Figure 1 in reference 1) was complex, and therefore might be misunderstood. However, the mean baseline concentration of \( \alpha \)-tocopherol in serum was 30.32 ± 3.32 \( \mu \)mol/L for the group receiving 100 mg RRR-\( \alpha \)-tocopherol and 26.22 ± 5.61 \( \mu \)mol/L for the group receiving 300 mg all-rac-\( \alpha \)-tocopherol acetate. After 7 d of treatment, serum \( \alpha \)-tocopherol concentrations increased to 47.33 ± 12.87 \( \mu \)mol/L for the group receiving 100 mg RRR-\( \alpha \)-tocopherol and to 41.55 ± 8.93 \( \mu \)mol/L for the group receiving 300 mg all-rac-\( \alpha \)-tocopherol acetate. Values in both the group receiving 100 mg RRR-\( \alpha \)-tocopherol and the group receiving 300 mg all-rac-\( \alpha \)-tocopherol acetate increased by 1.6-fold compared with baseline.

Moreover, the mean final serum \( \alpha \)-tocopherol concentrations (after 28 d) were 47.22 ± 13.48 \( \mu \)mol/L for the group receiving 100 mg RRR-\( \alpha \)-tocopherol and 48.29 ± 6.75 \( \mu \)mol/L for the group receiving 300 mg all-rac-\( \alpha \)-tocopherol acetate. This was an increase of ≈1.6-fold compared with baseline in the group receiving 100 mg RRR-\( \alpha \)-tocopherol. In contrast, this was an increase of ≈1.8-fold compared with baseline in the group receiving 300 mg all-rac-\( \alpha \)-tocopherol acetate. Consequently, we concluded that the increase in serum concentration in these women administered 100 mg RRR-\( \alpha \)-tocopherol/d was not significantly different from that in women given 300 mg all-rac-\( \alpha \)-tocopherol acetate/d; however, these data could not be used to accurately estimate the bioactivity of RRR-\( \alpha \)-tocopherol or of all-rac-\( \alpha \)-tocopherol acetate. However, we propose that the present ratio of bioactivity of all-rac-\( \alpha \)-tocopherol to RRR-\( \alpha \)-tocopherol (0.74) must be correct.

Recently, we published new data on the affinity of RRR- and SRR-\( \alpha \)-tocopherol to rat \( \alpha \)-tocopherol transfer protein (6). We found that the affinity of SRR-\( \alpha \)-tocopherol was 11% of that of RRR-\( \alpha \)-tocopherol. When it is possible to measure the affinity of each of the eight \( \alpha \)-tocopherol stereoisomers to \( \alpha \)-tocopherol transfer protein, new biochemical evidence of the biopotency of all-rac-\( \alpha \)-tocopherol will be obtained.

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Calcium: does it cause or prevent osteoporosis?

Dear Sir:

The recent review by Hatchock (1) suggests that dietary calcium could be effective in preventing bone loss and reducing the risk of fracture. Several years ago, Abelow et al (2) showed a positive association between calcium intakes and risk of hip fracture.